

21ST ANNUAL
SYMPOSIUM
OF THE

INTERNATIONAL CANNABINOID
RESEARCH SOCIETY

PHEASANT RUN
ST. CHARLES, IL. USA
JULY 05 - 10, 2011

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PROGRAMME AND ABSTRACTS

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Research Triangle Park, NC
USA

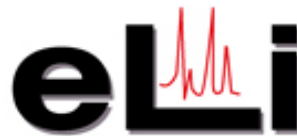
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REGISTRATION: JULY 5TH, 2011 (16.00 – 19.00)

PHEASANT RUN

WELCOME RECEPTION: 18.30 – 20.00

PHEASANT RUN ATRIUM

DAY 1

WEDNESDAY, JULY 6TH

7.30	BREAKFAST		
8.45	WELCOME AND OPENING REMARKS		
ORAL SESSION 1. LEARNING, MEMORY AND PAIN CHAIRS: ANDREA HOHMANN AND RUTH ROSS			
9.00	Robert Hampson and Sam Deadwyler	CANNABINOIDS ALTER NEURAL ENCODING BUT NOT RECALL OF MEMORY	1
9.15	Marcoita Gilbert and Ken Soderstrom	CANNABINOID AGONISM REDUCES SONG PERCEPTION-INDUCED ARC EXPRESSION IN AN AUDITORY REGION OF ZEBRA FINCH TELENCEPHALON	2
9.30	Ruth Ross, Gemma Halliday, Scott Davidson, Lynne Shanley, Marissa Lear and Alasdair MacKenzie	ALLELIC VARIANTS OF AN INTRONIC CB1 ENHANCER SHOW DIFFERENTIAL RESPONSES IN HIPPOCAMPAL AND HYPOTHALAMIC NEURONES; IMPLICATIONS FOR CANNABIS USE	3
9.45	Jing Han, Philip Kesner and Xia Zhang	CANNABINOID IMPAIRMENT OF SPATIAL WORKING MEMORY VIA HIPPOCAMPAL ASTROCYTE- MEDIATED SYNAPTIC LONG-TERM DEPRESSION	4

10.00	M. Jerry Wright Jr., Sophia Vandewater and Michael Taffe	CANNABINOID MODULATION OF VISUAL LEARNING AND MEMORY IN RHESUS MACAQUES	5
10.15	COFFEE BREAK		
10.45	Josee Guindon, Spyridon Nikas, Ganesh Thakur, V. Kiran Vemuri, Alexandros Makriyannis and Andrea Hohmann	CANNABINOID CB2 RECEPTORS ACTIVATION SUPPRESSES NEUROPATHIC PAIN EVOKED BY THE CHEMOTHERAPEUTIC AGENT CISPLATIN IN RATS	6
11.00	Barbara Costa, Diana Russo, Dominique Ronzulli and Francesca Comelli	EXPERIMENTAL OSTEOARTHRITIS IN RATS IS ATTENUATED BY ORAL ADMINISTRATION OF PALMITOYLETHANOLAMIDE	7
11.15	James Burston, Lee Shipman, Bright Okine, Stephen Woodhams, Elizabeth Stockley, Andrew Bennett, Gareth Hathway and Victoria Chapman	CARRAGEENAN-INDUCED PAIN BEHAVIOUR IS ASSOCIATED WITH PPAR α POSITIVE IMMUNE CELL RECRUITMENT, AND IS MODULATED BY PALMITOYLETHANOLAMIDE	8
11.30	Sharleen Yuan and Brian Burrell	PRESYNAPTIC AND POSTSYNAPTIC MECHANISMS MODULATING ENDOCANNABINOID-DEPENDENT NOCICEPTIVE SIGNALING	9
11.45	Aron Lichtman, Steven Kinsey, Laura Wise, Jonathon Long, Dana Selley and Benjamin Cravatt	CB1 RECEPTOR FUNCTION IS MAINTAINED FOLLOWING CHRONIC ADMINISTRATION OF LOW DOSES OF THE MONOACYLGLYCEROL LIPASE (MAGL) INHIBITOR JZL184	10
12.00	LUNCH		

ORAL SESSION 2. GASTROINTESTINAL AND CANCER

CHAIRS: LINDA PARKER AND CRISTINA SÁNCHEZ

14.00	Kitty CM Verhoeckx, Michiel GJ Balvers, Henk FJ Hendriks, Heleen M Wortelboer and Renger F Witkamp	PRESENCE AND FORMATION OF N-ACYL-SEROTONINS IN THE INTESTINE	11
14.15	Erin Rock, Daniele Bolognini, Cheryl Limebeer, Maria Cascio, Sharon Anavi-Goffer, Paul Fletcher, Raphael Mechoulam, Roger Pertwee and Linda Parker	CANNABIDIOL MAY ATTENUATE VOMITING AND NAUSEA BY ACTING AS AN INDIRECT 5-HT _{1A} AGONIST	12
14.30	Steven Kinsey, Scott O'Neal, Daniel Normura, Jonathan Long, Benjamin Cravatt, John Grider and Aron Lichtman	MAGL OR FAAH INHIBITION PROTECTS AGAINST NSAID-INDUCED GASTRIC MUCOSAL DAMAGE	13
14.45	Mireille Alhouayek, Didier Lambert, Nathalie Delzenne, Patrice Cani and Giulio Muccioli	INCREASING 2-ARACHIDONOYLGLYCEROL LEVELS COUNTERACTS COLITIS AND RELATED SYSTEMIC INFLAMMATION	14
15.00 - 17.00	POSTER SESSION 1 COFFEE		P1
17.00	María M Caffarel, Eduardo Pérez-Gómez, Clara Andradas, Gema Moreno-Bueno, Marta Muñoz Fernández de Legaria, Juana M Flores, Manuel Guzmán and Cristina Sánchez	ROLE OF THE CB ₂ CANNABINOID RECEPTOR IN THE PATHOGENESIS OF BREAST CANCER	15

17.15	Eduardo Perez-Gomez, Clara Andradas, Miguel Quintanilla, Manuel Guzman and Cristina Sanchez	A ROLE FOR GPR55 IN MULTISTAGE MOUSE SKIN CARCINOGENESIS	16
17.30	Alessia Ligresti, Luciano De Petrocellis, Aniello Schiano Moriello, Roberta Verde, Pierangelo Orlando and Vincenzo Di Marzo	MECHANISMS OF THE ANTI-CANCER EFFECTS OF CANNABIDIOL AND OTHER NON-PSYCHOTROPIC CANNABINOIDS ON HUMAN PROSTATE CARCINOMA	17
17.45	Liliana Soroceanu, Chandani Limbad, Ryuichi Murase, Juanita Allison, Isabel Adrados, Rumi Murase, Arash Pakdel, Pierre-Yves Desprez and Sean McAllister	CANNABIDIOL INHIBITS GLIOBLASTOMA DISPERSAL AND EXPRESSION OF STEM CELL MARKERS IN PATIENT-DERIVED PRIMARY CULTURES	18
18.00	Gabriella Aviello, Barbara Romano, Francesca Borrelli, Raffaele Capasso, Fabiana Piscitelli, Pierangelo Orlando, Vincenzo Di Marzo and Angelo Antonio Izzo	CARCINOGENIC-INDUCED COLON CANCER IN MICE: ROLE OF THE ENDOCANNABINOID SYSTEM AND CHEMOPREVENTIVE EFFECTS OF CANNABIDIOL	19

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DAY 2
THURSDAY, JULY 7TH

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9.00	Yanan Zhang, Alan Fulp, Katharine Bortoff, Herbert Seltzman and Rangan Maitra	RATIONAL SYNTHESIS OF CANNABINOID RECEPTOR 1 ANTAGONISTS FOR PERIPHERAL SELECTIVITY	20
9.15	Han Zhou, Richard W. Mercier, Lingling Shen, Patricia H. Reggio and Alexandros Makriyannis	AM994-BINDING ARCHITECTURE OF HUMAN CANNABINOID RECEPTOR 2 USING LIGAND-ASSISTED PROTEIN STRUCTURE FOLLOWED BY MOLECULAR DYNAMICS	21
9.30	Daniele Bolognini, Maria Grazia Cascio and Roger G. Pertwee	EVIDENCE THAT AM630 IS A HUMAN CB2 RECEPTOR PROTEAN AGONIST	22
9.45	Rangan Maitra, Katharine Bortoff, Huaqin Pan, Patricia Reggio and Herbert Seltzman	INHIBITION OF ALCOHOLIC HEPATIC STEATOSIS BY A TYPE 1 CANNABINOID RECEPTOR NEUTRAL ANTAGONIST	23
10.00	Vladimir Poltoratsky, Genevieve LaRoche, Emily Oestreich, Patrick GiGuere, Shailendra Devkota, Haroon Tariq, David Siderovski and Somnath Mukhopadhyay	REGULATION OF CANNABINOID RECEPTOR SIGNALING BY RGS (REGULATORS OF G-PROTEIN SIGNALING) PROTEINS: A NEW PARADIGM FOR MODULATING CB1 AND CB2 RECEPTOR-MEDIATED SIGNALOSOME	24

10.15	Haleli Sharir, Evangelia Kotsikorou, Dow P Hurst, Narasinga Rao, Larry Barak, Yushi Bai, Marc Caron, Herbert H. Seltzman, Susanne Heynen-Genel, Loribelle B. Milan, Russell Dahl, Thomas D.Y. Chung, Patricia H. Reggio and Mary E. Abood	FUNCTIONAL IDENTIFICATION OF NEW AGONISTS AND ANTAGONISTS AT GPR55	25
10.30	Evangelia Kotsikorou, Dow Hurst, Haleli Sharir, Narasinga Rao, Larry Barak, Marc Caron, Herbert Seltzman, Susanne Heynen-Genel, Loribelle Milan, Thomas Chung, Mary Abood and Patricia Reggio	NOVEL AGONISTS AND ANTAGONISTS OF THE GPR55 RECEPTOR	26
10.45	COFFEE BREAK		
ORAL SESSION 4. CARDIOVASCULAR CHAIRS: ALLYN HOWLETT AND ROGER PERTWEE			
11.30	Christopher Stanley, Gillian Manning and Saoirse O'Sullivan	CHARACTERISATION OF 2-ARACHIDONOYLGLYCEROL- INDUCED VASORELAXATION IN HUMAN MESENTERIC ARTERIES	27
11.45	Linda E. Klumpers, Marianne Fridberg, Jan Freijer, Marieke L. De Kam, Paul Little, H. Daniel Kleinloog, Christian E. Elling and Joop M.A. van Gerven	DEMONSTRATING PERIPHERAL RESTRICTION OF CB1 ANTAGONIST TM38837 IN HUMANS	28
12.00	PRESIDENTIAL PLENARY SPEAKER "HUNTINGTIN FROM EVOLUTION TO PATHOLOGY " ELENA CATTANEO, PH.D. <i>Department of Pharmacological Sciences and Center for Stem Cell Research Università degli Studi di Milano, Milan, Italy</i>		

13.00	LUNCH		
ORAL SESSION 5. METABOLISM CHAIRS: ITAI BAB AND CECILIA HILLARD			
14.00	Richard Wilson, Marie Smith, Saoirse O'Sullivan and Susan Anderson	THE EFFECTS OF ENDOCANNABINOIDS ON HUMAN OSTEOBLAST GROWTH IN VITRO	29
14.15	Nicole Bowles, Matthew Hill, V. Kiran Vemuri, Ilia Karatsoreos, Zhiying Li, Alexandros Makriyannis and Bruce McEwen	PERIPHERAL CANNABINOID CB1 RECEPTOR BLOCKADE REDUCES DEVELOPMENT OF GLUCOCORTICOID- MEDIATED METABOLIC SYNDROME	30
14.30	Elad Wasserman, Gila Maor, Joseph Tam, Alon Bajayo, Vardit Kram, Raphael Mechoulam and Itai Bab	CANNABINOID REGULATION OF BODY GROWTH	31
ORAL SESSION 6. NEUROBIOLOGY & NEURODEGENERATIVE DISORDERS CHAIRS: MARY ABOOD AND JAVIER FERNANDEZ-RUIZ			
14.45	Daniel Liput, Mark Prendergast and Kimberly Nixon	ENDOCANNABINOID MODULATION ATTENUATES ETHANOL-INDUCED NEURODEGENERATION DURING WITHDRAWAL	32
15.00	Haiying Zhang, Xia Li, Qing-Rong Liu, Hong-Ju Yang, Eliot Gardner and Zheng-Xiong Xi	EXPRESSION AND CELLULAR DISTRIBUTIONS OF CANNABINOID CB2 RECEPTORS IN MOUSE BRAIN	33
15.15	Chu Chen, Huizhi Du, Xiaolei Chen and Jian Zhang	ENDOCANNABINOID 2-ARACHIDONOYLGLYCEROL SUPPRESSION OF COX-2 EXPRESSION IS MEDIATED VIA PPAR-gamma	34

15.30 - 17.00	POSTER SESSION 2		P2
COFFEE			
ORAL SESSION 7. PSYCHIATRIC			
CHAIRS: SACHIN PATEL AND JENNY WILEY			
17.00	Erik Oleson and Joseph Cheer	2-ARACHIDONOYLGLYCEROL MODULATES CUE-EVOKED DOPAMINE RELEASE DURING REWARD-SEEKING	35
17.15	Daniela Hauer, Roland Toth, Florian Weis, Patrizia Campolongo and Gustav Schelling	DEPRESSION IN CARDIAC SURGERY PATIENTS IS ASSOCIATED WITH REDUCED PERIOPERATIVE PLASMA ENDOCANNABINOID LEVELS	36
17.30	Mateus Bergamaschi, Marcos Chagas, Danielle Oliveira, Bruno Martinis, Regina Queiroz, Jaime Hallak, Antonio Zuardi and José Crippa	CANNABIDIOL REDUCED ANXIETY IN TREATMENT-NAÏVE SOCIAL PHOBIC PARTICIPANTS	37
17.45	Sharon Anavi-Goffer, Shimon Rabichev, Ken Mackie, Tibor Harkany, Ruth A. Ross and Ester Fride	POSTNATAL NMDA-INHIBITION INDUCES ALTERATIONS IN CANNABINOID CB2 RECEPTOR EXPRESSION	38
18.00	Sachin Patel and Joshua Sumislawski	STRESS-INDUCED BEHAVIORAL ADAPTATION IS ASSOCIATED WITH ENHANCED 2-ARACHIDONOYLGLYCEROL SIGNALING IN THE AMYGDALA AND PREVENTED BY CHRONIC ENDOCANNABINOID AUGMENTATION	39

18.15	Andrea Dlugos	ACUTE STRESS INCREASES CIRCULATING N-ACYLETHANOLAMINES, INCLUDING ANANDAMIDE, IN HEALTHY HUMANS	40
18.30	Jenny Wiley	CHRONIC ANTIPSYCHOTIC ADMINISTRATION DURING ADOLESCENCE ALTERS LATER THC EFFECTS ON COGNITION	41

NOTES:

DAY 3
FRIDAY, JULY 8TH

7.30	BREAKFAST		
ORAL SESSION 8. CANNABIS, HUMAN USE & ADDICTION CHAIRS: ARON LICHTMAN AND DANIELA PAROLARO			
9.00	Garry Milman, David Schwope, David Gorelick and Marilyn Huestis	CANNABINOID PHARMACOKINETICS IN EXPECTORATED ORAL FLUID FOLLOWING CONTROLLED SMOKED CANNABIS	42
9.15	Raul Gonzalez, Randi Schuster and Inga Salija	INHIBITORY CONTROL IN YOUNG CANNABIS USERS: RELATIONSHIPS WITH CANNABIS USE AND SYMPTOMS OF CANNABIS ADDICTION	43
9.30	Tiziana Rubino, Fabiana Piscitelli, Pamela Prini, Simona Speziali, Alessia Ligresti, Vincenzo Di Marzo and Daniela Parolaro	THE EFFECT OF ADOLESCENT THC TREATMENT ON THE ENDOCANNABINOID SYSTEM MATURATION IN SPECIFIC BRAIN AREAS AND NEURAL REFINEMENT	44
9.45	M. Imad Damaj and Aron Lichtman	NICOTINE REWARD: A ROLE FOR CB2 RECEPTORS?	45
10.00	COFFEE BREAK		
10.30	Robert E. Vann and D. Matthew Walentiny	PRECLINICAL EVALUATION OF MARIJUANA'S APPETITIVE, REWARDING AND PSYCHOACTIVE PROPERTIES	46

10.45	Brian Thomas, Peter Stout, Jeri Roper-Miller, Anderson Cox, Richard Daw, Poonam Pande, Kenneth Davis and Megan Grabenauer	ADVANCED ANALYTICAL METHODS FOR THE SURVEILANCE AND DETECTION OF SYNTHETIC CANNABINOIDS IN HERBAL FORMULATIONS AND BIOLOGICAL MATRICES	47
11.00	Jonathan Page, Harm van Bakel, Atina Cote, Jake Stout and Tim Hughes	THE GENOME OF <i>CANNABIS SATIVA</i> L.	48
11.15 – 12.00	NIDA CAREER INFO LUNCHEON “EFFECT OF CHANGING CONTROLLED SUBSTANCE SCHEDULES ON CANNABINOID RESEARCH”		
11.15	LUNCH		
12.00 – 16.00	ICRS / CCIC / UCSF PRESENTS A CONTINUING MEDICAL EDUCATION COURSE CANNABINOIDS IN CLINICAL PRACTICE: CHALLENGES AND OPPORTUNITIES CHAIRS: DONALD ABRAMS, M.D. AND MAURO MACCARRONE, PH.D. 3.75 AMA PRA CATEGORY 1 CREDITS		
12.00	INTRODUCTION AND OVERVIEW CECILIA HILLARD, PH.D.		CME1
12.35	NAUSEA AND EMESIS LINDA PARKER, PH.D.		CME2
13.10	NEUROPATHIC AND PALLIATIVE PAIN DONALD ABRAMS, M.D.		CME3

13.45	COFFEE BREAK	
14.00	PSYCHIATRY SACHIN PATEL, PH.D.	CME4
14.35	NEUROLOGY DIEGO CENTONZE, PH.D.	CME5
15.10	CANNABINOIDS IN HEALTHCARE WILLIAM NOTCUTT, M.D.	CME6
15.45	CLINICAL REVIEW AND CLOSING REMARKS MARK WARE, MBBS MRCP, M.SC.	CME7
16.00	BREAK	
16.30 – 17.30	KANG TSOU MEMORIAL LECTURE “ENDOCANNABINOID SIGNALING IN PERIIMPLANTATION BIOLOGY” SUDANSU K DEY, PH.D. Lova Riekert Chair and Professor of Pediatrics, Cancer and Cell Biology Director, Division of Reproductive Sciences University of Cincinnati College of Medicine	

DAY 4
SATURDAY, JULY 9TH

7.30	BREAKFAST		
ORAL SESSION 9. ENDOCANNABINOID SIGNALING CHAIRS: MATTHEW HILL AND BELA SZABO			
9.00	Xiaofei Sun, Huirong Xie, Bliss Magella and Sudhansu Dey	ENDOCANNABINOID SIGNALING ORIGINATING IN THE LUMINAL EPITHELIUM IS CRITICAL FOR STROMAL DECIDUALIZATION	49
9.15	Michiel Balvers, Kitty Verhoeckx, Heleen Wortelboer, Jocelijn Meijerink and Renger Witkamp	FATTY ACIDS, N-ACYL ETHANOLAMINES AND INFLAMMATION: TISSUE, TIME AND COMPOUND SPECIFIC EFFECTS	50
9.30	Bela Szabo and Mario Lederer	ROLE OF CALCIUM RELEASE FROM INTRACELLULAR STORES IN ENDOCANNABINOID PRODUCTION AND RETROGRADE SYNAPTIC SIGNALING IN THE CEREBELLAR CORTEX	51
9.45	Brian C. Shonesy, Tyler Rentz, Anthony J. Baucum II, Danny G. Winder, Sachin Patel and Roger J. Colbran	CALCIUM-CALMODULIN DEPENDENT KINASE II INTERACTS WITH DIACYLGLYCEROL LIPASE AND IS REQUIRED FOR ENDOCANNABINOID- DEPENDENT SYNAPTIC SUPPRESSION IN THE STRIATUM	52
10.00	Ozge Gunduz Cinar, Katie Martin, Resat Cinar, Alexandros Makriyannis, George Kunos and Andrew Holmes	A NOVEL FAAH INHIBITOR FACILITATES EXTINCTION OF FEAR MEMORIES IN MICE	53
10.15	Alex Straiker, Tarun Jain, Jim Wager-Miller and Ken Mackie	BOTH DGL α AND DGL β REGULATE THE PRODUCTION OF 2-ARACHIDONOYL GLYCEROL IN AUTAPTIC HIPPOCAMPAL NEURONS	54

10.30	Jocelijm Meijerink, Michiel Balvers, Pierluigi Plastina, Jean-paul Vincken, Mieke Poland, Mohamed Attya, Kitty Verhoeckx and Renger Witkamp	DHEA, THE ETHANOLAMIDE METABOLITE OF DHA, HAS IMMUNE- MODULATING PROPERTIES	55
10.45 – 12.15	POSTER SESSION 3 COFFEE		P3
12.15	LUNCH		
SYMPOSIUM – “CANNABINOIDS AND HIV PATHOGENICITY” <i>CHAIRS: VISHNUDUTT PUROHIT, DVM, PH.D. AND RAO RAPAKA, PH.D., FAAPS, NIDA</i>			
14.00	Dr. Patricia E. Molina Louisiana State University Health Sciences Center New Orleans, Louisiana, USA	AN UPDATE ON THE ROLE OF CANNABINOIDS IN THE PROGRESSION OF HIV INFECTION: ANIMAL AND HUMAN DATA	
14.30	Dr. Guy A. Cabral Virginia Commonwealth University Richmond, Virginia, USA	CANNABINOID MODULATION OF HIV INFECTION- INDUCED MACROPHAGE/MICROGLIA ACTIVATION IN VITRO SYSTEM	
15.00	Dr. Hava Avraham Harvard Medical School Boston, MA, USA	CANNABINOID MODULATION OF BBB INTEGRITY IN THE PRESENCE OF HIV PROTEINS	
15.30	COMMENTARY AND DISCUSSION DISCUSSANT: <i>MAURO MACCARRONE, PH.D.</i> UNIVERSITY OF TERAMO, ITALY		

15.45	<p style="text-align: center;">SATIVEX: THE CONTINUING SAGA OF THE FIRST PHYTOCANNABINOID MEDICINE</p> <p style="text-align: center;">“SAFETY PROFILE IS IMPROVING OVER TIME”</p> <p style="text-align: center;">ETHAN RUSSO, TILDEN ETGES, COLIN STOTT, STEPHEN WRIGHT, ADO MOHAMMED AND PHILIP ROBSON</p> <p style="text-align: center;">PRESENTED BY <i>ETHAN RUSSO</i> AND <i>COLIN STOTT</i> GW PHARMACEUTICALS</p>	GW1
16.15	ICRS BUSINESS MEETING	
18.00 – 23.00	ICRS BANQUET	

DEPARTURE: SUNDAY, JULY 10TH
TAXIES TO O'HARE OR MIDWAY

FAAH, MAGL, NAAA SYMPOSIUM REGISTRANTS MAY STAY ON

POSTER SESSION 1: TOPICS A & B

DAY 1, WEDNESDAY, JULY 6TH: 15:00 – 17:00

TOPIC A. LEARNING, MEMORY AND PAIN

Daniela Hauer, Gustav Schelling, Benno Roozendaal and Patrizia Campolongo	PROPOFOL ALTERS MEMORY VIA INTERACTION WITH THE ENDOCANNABINOID SYSTEM	P1-1
Adam Steinmetz and John Freeman	CANNABINOID-1 RECEPTOR CONTRIBUTIONS TO CEREBELLAR LEARNING	P1-2
Piray Atsak, Daniela Hauer, Patrizia Campolongo, Gustav Schelling and Benno Roozendaal	ENDOCANNABINOID SIGNALING MEDIATES THE IMPAIRING EFFECTS OF STRESS-LEVEL GLUCOCORTICOIDS ON MEMORY RETRIEVAL	P1-3
Laura Wise, Kelly Long, Jonathan Long, Benjamin Cravatt and Aron Lichtman	DUAL INHIBITION OF FAAH AND MAGL IN MICE IMPAIRS SHORT-TERM SPATIAL MEMORY ASSESSED IN THE WATER MAZE	P1-4
Eli J Shobin, Caroline H Abbott, Richard G Hunter and Joseph A Schroeder	THE CB2 RECEPTOR AGONIST GW405883 ATTENUATES VARIABLE CHRONIC MILD STRESS-INDUCED SPATIAL LEARNING DEFICITS AND NEURONAL CELL LOSS	P1-5
Qing-song Liu, Bin Pan, Wei Wang, Peng Zhong, Jacqueline Blankman and Benjamin Cravatt	ALTERATIONS OF ENDOCANNABINOID SIGNALING, SYNAPTIC PLASTICITY, LEARNING AND MEMORY IN MONOACYLGLYCEROL LIPASE KNOCKOUT MICE	P1-6
Kimberly Tsutsui, Ram Kandasamy and Rebecca Craft	SEX DIFFERENCES IN THC-INDUCED ANTINOCICEPTION AGAINST INFLAMMATORY PAIN	P1-7
Alexa Wakley, Cassidy Kobialka and Rebecca Craft	PROGESTERONE MODULATION OF Δ^9 -TETRAHYDROCANNABINOL-INDUCED ANTINOCICEPTION	P1-8
Sharon Anavi-Goffer, Gemma Baillie, Juerg Gertsch and Ruth Ross	EVALUATION OF THE CANNABINOID CB2 RECEPTOR- SELECTIVE CANNABINOIDS AT GPR55: IMPLICATION FOR DRUG TARGET FOR CHRONIC PAIN	P1-9

Stephen Woodhams, Amy Wong, Stephen Alexander, David Barrett and Victoria Chapman	THE EFFECTS OF SPINAL JZL184 ON NOCICEPTIVE PROCESSING IN THE ANAESTHETISED RAT	P1-10
Barbara Costa, Anita Colombo, Isabella Bettoni, Elena Bresciani, Antonio Torsello and Francesca Comelli	THE ENDOGENOUS LIGAND PALMITOYLETHANOLAMIDE RELIEVES NEUROPATHIC PAIN VIA MAST CELL AND MICROGLIA MODULATION	P1-11
Barbara Costa, Anita Colombo, Isabella Bettoni and Francesca Comelli	PALMITOYLETHANOLAMIDE RELIEVES PAIN IN A MURINE MODEL OF DIABETIC NEUROPATHY	P1-12
Andrew Kwilasz and Steve Negus	EFFECTS OF Δ^9 -TETRAHYDROCANNABINOL ON PAIN-STIMULATED AND PAIN-DEPRESSED BEHAVIOR IN RATS	P1-13
Linda Vaughn, Yao Zhang, Mengwei Liu, Yangmiao Zhang and Raymond Quock	ENDOCANNABINOID MECHANISMS ARE INVOLVED IN NITROUS OXIDE-INDUCED ANTINOCICEPTION	P1-14
Jenny Wilkerson, Ellen Dengler, James Wallace, Audra Kerwin, Ganesh Thakur, Alexandros Makriyannis and Erin Milligan	INTRATHECAL CANNABINOID RECEPTOR 2 (CB2) AGONISTS ALTER SPINAL AND DORSAL ROOT GANGLION INFLAMMATORY FACTORS IN NEUROPATHIC RATS	P1-15
Rukiyah Van Dross and Christian Kuc	J-SERIES PROSTAGLANDINS REGULATE AEA-INDUCED APOPTOSIS	P1-16
TOPIC B. <i>GASTROINTESTINAL AND CANCER</i>		
Cheryl L. Limebeer and Linda A. Parker	CENTRAL, BUT NOT SYSTEMIC, ADMINISTRATION OF A PERIPHERALLY RESTRICTED CB1 AGONIST SUPPRESSES NAUSEA-LIKE BEHAVIOR IN RATS	P1-17
Martin Sticht, Jonathan Long, Erin Rock, Cheryl Limebeer, Raphael Mechoulam, Benjamin Cravatt and Linda Parker	THE MAGL INHIBITOR, JZL184, ATTENUATES LICL-INDUCED VOMITING IN THE SUNCUS MURINUS AND 2AG ATTENUATES LICL-INDUCED NAUSEA-LIKE BEHAVIOR IN RATS	P1-18

Mauro Maccarrone, Natalia Battista, Antonio Di Sabatino, Monia Di Tommaso, Paolo Biancheri, Cinzia Rapino, Francesca Vidali, Cinzia Papadia, Chiara Montana, Alessandra Pasini, Alberto Lanzini, Vincenzo Villanacci and Gino R. Corazza	ALTERATIONS OF ANANDAMIDE METABOLISM IN COELIAC DISEASE	P1-19
Jeffrey Hergenrather and Stacey Kerr	CLINICAL IMPROVEMENT AND REDUCTION OF IMMUNOSUPPRESSIVE DRUG THERAPY IN CANNABIS TREATED PATIENTS WITH CROHN'S DISEASE AND ULCERATIVE COLITIS	P1-20
Clara Andradas, María M. Caffarel, Eduardo Pérez-Gómez, Maria Salazar, Mar Lorente, Guillermo Velasco, Manuel Guzmán and Cristina Sánchez	THE PUTATIVE CANNABINOID RECEPTOR GPR55 PROMOTES CANCER CELL PROLIFERATION VIA ERK	P1-21
Naoki Shinohara, Toru Uyama, Xing-Hua Jin, Kazuhito Tsuboi, Takeharu Tonai, Hitoshi Houchi and Natsuo Ueda	THE TUMOR SUPPRESSOR A-C1 HAS AN N-ACYLTRANSFERASE ACTIVITY INVOLVED IN NAPE FORMATION	P1-22
Marta Solinas, Paola Massi, Valentina Cinquina and Daniela Parolaro	ANTITUMOR EFFECT OF THCV, CBDV AND CBG ON HUMAN GLIOMA CELLS	P1-23
Seok-Woo Park and Myung-Whun Sung	THE RECEPTORS-INDEPENDENT ANTI-CANCER EFFECT OF ANANDAMIDE IN HEAD AND NECK SQUAMOUS CELL CARCINOMA CELLS	P1-24
Sean Emery, David Gewirtz and Aron Lichtman	COMBINING CANNABINOID AGONISTS AND RADIATION THERAPY REDUCES BREAST CANCER GROWTH	P1-25
Somnath Mukhopadhyay, Vladimir Poltoratsky, Shalini Jha, Teresa Collins and Yoseph Saleh	DOES CB2 CANNABINOID RECEPTOR A BETTER TARGET FOR THE REGULATION OF PROSTATE CANCER?	P1-26
Shalini Jha, Vladimir Poltoratsky, Keith Burridge and Somnath Mukhopadhyay	HETEROTRIMERIC G-PROTEIN COUPLED CB2 RECEPTOR-MEDIATED ACTIVATION OF SMALL G PROTEIN REGULATES PROSTATE CANCER CELL MIGRATION	P1-27
Farideh A Javid, Saeed Afshinjavid, Roger M Phillips, Colin Stott and Leo Ayerakwa	THE EFFECT OF CBD (BDS) BOTANICAL CANNABINOID EXTRACT ON MCF-7 HUMAN BREAST CARCINOMA CELLS	P1-28
Stanislav Svetlov, Anatolyi Vakulenko, Mengde Cao and David Nelson	ALKYLOXY-ISOPROPYLAMINO PROPANOLS (AIPS) INDUCE DEATH OF HEPATOCELLULAR CARCINOMA CELLS VIA MULTIPLE GPCRS AND KINASES MODULATION	P1-29

POSTER SESSION 2: TOPICS C, D & E
DAY 2, THURSDAY, JULY 7TH: 15:30 – 17:00

TOPIC C. RECEPTOR SIGNALING & STRUCTURE

Anushka Goonawardena, Andrea Plano, Mathew Elverman, Bettina Platt, Robert Hampson and Gernot Riedel	CHRONIC EFFECTS OF CANNABINOID AGONISTS, ANTAGONISTS AND NEUTRAL ANTAGONISTS ON SLEEP-WAKE CYCLES IN MICE	P2-1
Philip Brownjohn, Ken Mackie and John Ashton	THE USE OF KNOCK OUT TISSUE IS ESSENTIAL TO VALIDATE SPECIFICITY OF CB2 ANTIBODIES	P2-2
Douglas McHugh and Heather Bradshaw	SIRNA KNOCKDOWN OF GPR18 RECEPTORS IN BV-2 MICROGLIA ATTENUATES THE CELL MIGRATION INDUCED BY N-ARACHIDONOYL GLYCINE	P2-3
Dina Navia-Paldanius, Sari Yrjölä, Juha Savinainen, Tarja Kokkola, Hanna Lesch, Seppo Ylä-Herttuala, Jarmo T. Laitinen and Antti Poso	ACTIVATION ASSAYS FOR GPR55 AND ITS NEGATIVE OUTCOME	P2-4
Sari Yrjölä, Tuomo Kalliokoski, Dina Navia-Paldanius, Tuomo Laitinen, Tapio Nevalainen and Antti Poso	LIGAND-BASED VIRTUAL SCREENING FOR CANNABINOID LIGANDS	P2-5
Nicholas Jones, Andrew Hill, Claire Williams, Gary Stephens and Benjamin Whalley	A PRE- AND POST-SYNAPTIC MECHANISTIC INVESTIGATION OF CANNABIDIOL IN ACUTE HIPPOCAMPAL BRAIN SLICES	P2-6
Spyros Nikas, Marion Schimpfen, Demetris Papahadjis and Alexandros Makriyannis	DESIGN AND SYNTHESIS OF NOVEL ENDOCANNABINOID TEMPLATES	P2-7
Amina Bagher, Melanie Kelly and Eileen Denovan-Wright	DIMERIZATION AND FUNCTION OF CB1 RECEPTOR CODING REGION SPLICE VARIANTS	P2-8
Cheryl Habrukowich, John Ladias, Nikolai Zvonok and Alexandros Makriyannis	ENGINEERING CANNABINOID 2 RECEPTOR FOR STRUCTURAL STUDIES	P2-9
Spiro Pavlopoulos, Xiaoyu Tian and Alexandros Makriyannis	MODULATION OF ARRESTIN FUNCTION: SEARCHING FOR A LEAD COMPOUND USING NMR METHODS	P2-10

Nicola Bernabò, Enzo Tettamanti, Marco Chiarini, Mauro Mattioli, Paola Palestini, Laura Botto, Natalia Battista, Mauro Maccarrone and Barbara Barboni	TYPE-1 CANNABINOID RECEPTOR REGULATES MEMBRANE FLUIDITY OF BOAR SPERMATOZOA DURING THE CAPACITATION	P2-11
Julia Kargl, Nariman Balenga, Lene Martini, Jennifer Whistler and Maria Waldhoer	THE GPCR-ASSOCIATED SORTING PROTEIN 1 REGULATES RIMONABANT-INDUCED DOWNREGULATION OF GPR55	P2-12
Chelsea Hutch and Colleen Hegg	IDENTIFICATION OF FUNCTIONAL CANNABINOID RECEPTORS IN THE MOUSE OLFACTORY SYSTEM	P2-13
Mauro Maccarrone, Sergio Oddi, Enrico Dainese, Simone Sandiford, Filomena Fezza, Mirko Lanuti, Valerio Chiurchiù, Antonio Totaro, Giuseppina Catanzaro, Daniela Barcaroli, Vincenzo De Laurenzi, Diego Centonze, Somnath Mukhopadhyay and Allyn C. Howlett	EFFECT OF PALMITOYLATION ON MEMBRANE LOCALISATION AND SIGNALLING OF CB1 CANNABINOID RECEPTOR	P2-14
Gemma Baillie, Sharon Anavi-Goffer, Roger Pertwee and Ruth Ross	SIGNALING TRAFFICKING OF ALLOSTERIC MODULATOR, ORG27569 AT THE CANNABINOID CB1 RECEPTOR	P2-15
Jahan P. Marcu, Ankur Kapur, Megan Trznadel, Patricia H. Reggio, Mary E. Abood, Alex Makriyannis and Ganesh Thakur	AMINO ACIDS D2.63 AND K373 ARE IMPORTANT FOR MAINTAINING THE CB1R BINDING POCKET, WHILE RESIDUES K3.28 AND S1.39 ARE INVOLVED IN SELECTIVE LIGAND RECOGNITION	P2-16
Thomas Gamage, Roger Pertwee, Ruth Ross and Aron Lichtman	INVESTIGATION INTO THE BEHAVIORAL EFFECTS OF THE PURPORTED CB1 RECEPTOR NEGATIVE ALLOSTERIC MODULATOR ORG-27569	P2-17
George Dalton and Allyn Howlett	THE CB1 RECEPTOR TRANSACTIVATES THE EPIDERMAL GROWTH FACTOR RECEPTOR AND THE FLK-1 VASCULAR ENDOTHELIAL GROWTH FACTOR RECEPTOR TO REGULATE ERK, BUT NOT FAK, IN NEURONAL CELLS	P2-18
Carolyn Tanner, Amy Sasman and Cecilia Hillard	BRAIN REGION SPECIFIC CIRCADIAN EFFECTS ON CB1 RECEPTOR BINDING IN MEMBRANES DERIVED FROM ADULT MICE	P2-19
Teniel Ramikie, Salem VanderStel, Megan Deel and Sachin Patel	CHRONIC STRESS EXPOSURE INDUCES SYNAPTIC ADAPTATIONS THROUGH ENDOCANNABINOID SIGNALING MECHANISMS WITHIN THE CENTRAL AMYGDALA	P2-20

Lawrence C. Blume, Caroline E. Bass, Steven R. Childers, George D. Dalton, Jasmine M. Richardson, Dana E. Selley, Ruoyu Xiao and Allyn C. Howlett	CANNABINOID RECEPTOR INTERACTING PROTEIN 1A (CRIP1A) MODULATES STRIATAL NEUROPHARMACOLOGY AND SIGNAL TRANSDUCTION IN CANNABINOID, DOPAMINE AND OPIOID RECEPTOR SYSTEMS	P2-21
Jagjeet Singh, Zhao-Hui Song and Patricia Reggio	THE CANNABINOID CB2 RECEPTOR/GI COMPLEX : CROSSLINKING AND COMPUTATIONAL STUDIES SUGGEST THAT CB2 SIGNALS AS AN ACTIVATED DIMER	P2-22
Pritesh Kumar and Zhao-Hui Song	HOMOGENOUS-TIME-RESOLVED-FLUORESCENCE(HTRF): OPTIMIZATION AND VALIDATION OF A CELL-BASED CAMP SCREENING TECHNOLOGYFOR THE CANNABINOID RECEPTORS CB1 AND CB2	P2-23
Christopher Roberts and Cecilia Hillard	CB1R SIGNALING ENHANCES ADRENAL RESPONSIVENESS TO ACTH TO INCREASE GLUCOCORTICOID SYNTHESIS	P2-24
Herbert Seltzman, Patricia Reggio, Lauren Chun, Zheng-Xiong Xi, Guo-Hua Bi, Eliot Gardner, Frank Navas, III, Ester Fride, Julie Marusich and Jenny Wiley	BEHAVIORAL AND PHARMACOLOGICAL PROPERTIES OF THE CB1 NEUTRAL ANTAGONIST PMSR	P2-25
Rebecca Hartzell, Joseph Meissler, Martin Adler and Toby Eisenstein	CB2-SELECTIVE CANNABINOIDS INHIBIT THE MIXED LYMPHOCYTE REACTION (MLR), AN IN VITRO CORRELATE OF ORGAN GRAFT REJECTION	P2-26
TOPIC D. <i>CARDIOVASCULAR</i>		
Christopher Stanley, Gillian Manning and Saoirse O'Sullivan	CANNABINOID INDUCED VASORELAXATION OF HUMAN MESENTERIC ARTERIES	P2-27
Christopher Stanley, Amanda Wheal, Michael Randall and Saoirse O'Sullivan	CANNABINOIDS IMPROVE ENDOTHELIUM-DEPENDANT VASORELAXATION IN ZUCKER DIABETIC FATTY RAT	P2-28
Carol Paronis, Ganesh Thakur and Alexandros Makriyannis	CANNABINOID AND K-OPIOID MEDIATED DIURESIS IN RATS	P2-29
Chris Schaich, Allyn Howlett and Debra Diz	MODULATION OF BAROREFLEX SENSITIVITY BY CANNABINOID RECEPTOR ACTIVATION IN RAT SOLITARY TRACT NUCLEUS	P2-30

TOPIC E. METABOLISM

Jayendra Patel, Anna Minkkilä, Teija Parkkari, Susanna Saario, Juha Savinainen, Jarmo Laitinen and Tapio Nevalainen	DESIGN, SYNTHESIS AND STRUCTURE ACTIVITY RELATIONSHIPS OF 5-ALKOXY-1,3,4-OXADIAZOL-2-ONES AS POTENT AND SELECTIVE INHIBITORS OF FATTY ACID AMIDE HYDROLASE (FAAH) AND MONOACYLGLYCEROL LIPASE (MGL)	P2-31
Satoshi Yamaori, Yasuka Okamoto, Ikuo Yamamoto and Kazuhito Watanabe	INHIBITORY MECHANISM OF MAJOR PHYTOCANNABINOIDS ON CYTOCHROME P450 2D6 ACTIVITY	P2-32
Rongrong Jiang, Satoshi Yamaori, Shuso Takeda, Ikuo Yamamoto and Kazuhito Watanabe	METABOLISM OF CANNABIDIOL BY CYTOCHROME P450S IN HUMAN LIVER MICROSOMES	P2-33
Mahmoud Nasr, Xiaomeng Shi, Ioannis Karageorgos, Christopher Morgan, Xiaoyu Tian, Nikolai Zvonok, John Engen and Alexandros Makriyannis	STUDIES ON THE DYNAMIC PROPERTIES OF MONOACYLGLYCEROL LIPASE IN NANODISC MODEL MEMBRANE	P2-34
Mauro Maccarrone, Maria Grazia Signorello, Enrica Giacobbe, Alessia Segantin, Luciana Avigliano, Fabiola Sinigaglia and Giuliana Leoncini	ACTIVATION OF HUMAN PLATELETS BY 2-ARACHIDONOYLGLYCEROL: FURTHER INSIGHTS INTO THE UNDERLYING SIGNALING	P2-35
JodiAnne Wood, Holiday Durham, Kyle Whitten, Subramanian Vadivel, Alexandros Makriyannis and Carol Lammi-Keefe	DETECTION OF THE ENDOCANNABINOID METABOLOME IN HUMAN BREAST MILK	P2-36
Bogna Ignatowska-Jankowska, Maciej Jankowski and Artur Swiergiel	BIDIRECTIONAL EFFECTS OF CANNABIDIOL ON BODY WEIGHT GAIN IN RATS	P2-37
Neta Rimmerman	THE NON-PSYCHOACTIVE PLANT CANNABINOID, CANNABIDIOL AFFECTS CHOLESTEROL METABOLISM-RELATED GENES IN MICROGLIAL CELLS	P2-38
Cecilia Hillard and Leyu Shi	CANNABIDIOL ACTIVATES GLYCOGEN SYNTHASE KINASE 3 IN NEURONS VIA INHIBITION OF PKA-MEDIATED PHOSPHORYLATION	P2-39

POSTER SESSION 3: TOPICS F - J
DAY 4, SATURDAY JULY 9TH: 10:45 – 12:15

**TOPIC F. NEUROBIOLOGY &
 NEURODEGENERATIVE DISORDERS**

Mauro Maccarrone, Monica Bari, Natalia Battista, Marta Valenza, Marinella Malaponti, Giuseppina Catanzaro, Chiara Di Pancrazio, Nicolina Mastrangelo, Diego Centonze, Alessandro Finazzi-Agrò and Elena Cattaneo	ALTERATIONS OF THE ENDOCANNABINOID SYSTEM IN <i>IN VITRO</i> AND <i>IN VIVO</i> MODELS OF HUNTINGTON'S DISEASE	P3-1
Concepción García, Valentina Cinquina, Cristina Palomo-Garo, Miguel Moreno-Martet, María Gómez-Cañas, Raphael Mechoulam and Javier Fernández-Ruiz	IDENTIFICATION OF CB2 RECEPTORS IN GLIAL ELEMENTS IN THE SUBSTANTIA NIGRA OF PATIENTS AND EXPERIMENTAL MODELS OF PARKINSON'S DISEASE	P3-2
Carmen Rodríguez-Cueto, Francisco Espejo-Porras, Eva de Lago, Javier Fernández- Ruiz, Mariluz Hernández-Gálvez and María Gómez-Ruiz	CHANGES IN CB1 AND CB2 RECEPTORS AND FAAH ENZYME IN THE CEREBELLUM OF PATIENTS WITH SPINOCEREBELLAR ATAXIAS	P3-3
Onintza Sagredo, Sara Valdeolivas, Valentina Satta, José A. Ramos, Roger G. Pertwee and Javier Fernández-Ruiz	NEUROPROTECTIVE EFFECTS OF THE PHYTOCANNABINOID-BASED MEDICINE SATIVEX® IN EXPERIMENTAL MODELS OF HUNTINGTON'S DISEASE	P3-4
Daniele De Filippis, Mariateresa Cipriano, Annapina Russo, Massimo Vaia, Giuseppe Esposito, Giulia Russo, Luca Steardo, Vincenzo Di Marzo and Teresa Iuvone	EFFECT OF CANNADINOIS IN <i>IN VITRO</i> MODELS OF ALZHEIMER'S DISEASE	P3-5
Stanislav Svetlov, Ronald Hayes and Kevin Wang	BIOMARKER-GUIDED TARGETING ENDOCANNABINOID SYSTEM AFTER TRAUMATIC BRAIN INJURY (TBI)	P3-6
Sabina Adhikary	MODULATION OF INFLAMMATORY RESPONSE BY A CANNABINOID RECEPTOR-2-SELECTIVE AGONIST FOLLOWING SPINAL CORD INJURY	P3-7

Shailendra Devkota and Somnath Mukhopadhyay	CANNABINOID-ETHANOL INTERACTION IN THE REGULATION OF DEVELOPING NEUROGENESIS IN ZEBRAFISH BRAIN	P3-8
Joanna Slusar, Anna-Maria Szczeniak and Melanie Kelly	URB597-MEDIATED ACTIONS IN AN OPTIC NERVE INJURY MODEL	P3-9
TOPIC G. <i>PSYCHIATRIC</i>		
Matthew Hill, Shobha Anil Kumar, Cecilia Hillard, Shona Chattarji and Bruce McEwen	FAAH DEFICIENT MICE ARE RESISTANT TO THE EFFECTS OF CHRONIC STRESS ON ANXIETY AND MICROSTRUCTURE OF THE AMYGDALA	P3-10
Silvain Dang, Tiffany Lee, Matthew Hill and Boris Gorzalka	EFFECTS OF ACUTE CANNABINOID ADMINISTRATION ON ADOLESCENT EMOTIONALITY	P3-11
Tiffany Lee, Matthew Hill, Silvain Dang, Cecilia Hillard and Boris Gorzalka	LACK OF STRESS HABITUATION IN ADOLESCENT RATS IS ASSOCIATED WITH AN ABSENCE OF AMYGDALAR ENDOCANNABINOID SIGNALING RECRUITMENT	P3-12
Ryan McLaughlin, Matthew Hill, Francis Bambico, Gabriella Gobbi and Boris Gorzalka	PREFRONTAL CORTICAL ANANDAMIDE SIGNALING COORDINATES BEHAVIORAL COPING RESPONSES TO STRESS THROUGH A SEROTONERGIC PATHWAY	P3-13
Roger Cachope, Patricio O'Donnell and Joseph Cheer	ENDOCANNABINOID-MEDIATED MODULATION OF MOTIVATION IN SCHIZOPHRENIA	P3-14
Andrea Dlugos	MORE ANXIETY, MORE CONFUSION: FUNCTIONAL FAAH GENE POLYMORPHISM BUT NOT CNR1 GENE POLYMORPHISM INFLUENCES ACUTE STRESS RESPONSE IN HEALTHY HUMANS	P3-15
David Taylor, Anand Gururajan and Daniel Malone	THE EFFECT OF CANNABIDIOL IN A MK-801-INDUCED RAT MODEL OF ASPECTS OF SCHIZOPHRENIA	P3-16
TOPIC H. <i>CANNABIS, HUMAN USE & ADDICTION</i>		
Patrik Roser, Ida S. Haussleiter and Georg Juckel	AGGRAVATION OF KETAMINE-INDUCED DEFICITS IN AUDITORY SENSORY MEMORY BY INHIBITION OF CEREBRAL TYPE 1 CANNABINOID RECEPTORS IN HEALTHY HUMAN SUBJECTS	P3-17

Jeff Howard, Stan Banks, Miro Golinski and Audra Stinchcomb	DEVELOPMENT OF CANNABIDIOL PRODRUGS FOR USE WITH MICRONEEDLES FOR THE TREATMENT OF ALCOHOL USE DISORDERS	P3-18
Sherrica Tai, Torbjörn U.C. Järbe, Spyros P. Nikas and Alexandros Makriyannis	CHARACTERIZATION OF A CB1 RECEPTOR AGONIST (AM2389) WITH A LONG DURATION OF EFFECT TO FACILITATE THE STUDY OF CB1 DEPENDENCE/WITHDRAWAL	P3-19
Amanda Reiman	HUMAN CONSUMPTION OF WHOLE PLANT MEDICINE: EXPLORING DISPENSARY-BASED RESEARCH	P3-20
Torbjörn U.C. Järbe, Subramanian K. Vadivel and Alexandros Makriyannis	CANNABINERGIC AMINOALKYLINDOLES, INCLUDING AM678=JWH018 FOUND IN 'SPICE', EXAMINED USING DRUG (Δ^9 -THC) DISCRIMINATION FOR RATS	P3-21
Divya Ramesh, Robert A. Owens, Steven G. Kinsey, Benjamin F. Cravatt, Laura J. Sim-Selley and Aron H. Lichtman	EFFECTS OF CHRONIC MANIPULATION OF THE ENDOCANNABINOID SYSTEM ON PRECIPITATED OPIOID WITHDRAWAL	P3-22
Lisa Brents, Anna Gallus-Zawada, Anna Radominska-Pandya, William Fantegrossi, Jeffery Moran and Paul Prather	A MONO-HYDROXYLATED METABOLITE OF THE K2 SYNTHETIC CANNABINOID JWH-073 DISPLAYS HIGH AFFINITY NEUTRAL ANTAGONISM AT CB1 RECEPTORS	P3-23
Pretal Muldoon, Aron Lichtman and Imad Damaj	THE ROLE OF 2-AG ENDOCANNABINOID NEUROTRANSMISSION IN NICOTINE REWARD AND WITHDRAWAL	P3-24
Linda Vaughn, Oliver Vranjkovic, Gregory Stroh, Margaret LaCourt, John Mantsch and Cecilia Hillard	CANNABINOID INVOLVEMENT IN STRESS-INDUCED COCAINE RELAPSE THROUGH INTERACTION WITH NORADRENERGIC PATHWAYS	P3-25
D. Matthew Walentiny, Robert E. Vann, Brittany L. Andres, Michelle M. Hodges, Boglarka Vizu, Anu Mahadevan and Jenny L. Wiley	PHARMACOLOGICAL EVALUATION OF 3-SUBSTITUENT RIMONABANT ANALOGS	P3-26
Jason Wiebelhaus, Aron Lichtman, Kristen Tobey, W. Carter Lowery and Robert Vann	ASSESSING REINFORCING EFFECTS OF CANNABINOIDS USING INTRACRANIAL SELF-STIMULATION	P3-27
Girish Chopda, Joseph Anderson, Ganesh Thakur, Alexandros Makriyannis and Carol Paronis	DIURESIS: A SIMPLE AND EFFICIENT MEASURE TO SCREEN CANNABINOIDS	P3-28
William Courtney	PHYSIOLOGIC TO PATHOPHYSIOLOGIC INDIVIDUALLY TAILORED PHYTOGENETICS	P3-29

TOPIC I. ENDOCANNABINOID SIGNALING

Mariateresa Cipriano, Daniele De Filippis, Daniela De Stefano, Annapina Russo, Giulia Russo, Rosa Carnuccio and Teresa Iuvone	PALMITOYLETHANOLAMIDE CONTROLS RMCP-5 ACTIVITY BY THE REGULATION OF MICROPHALMIA-ASSOCIATED TRANSCRIPTION FACTOR	P3-30
Ioannis Karageorgos, Sergiy Tyukhtenko, Nikolai Zvonok, Thomas Wales, John Engen and Alexandros Makriyannis	EXPRESSION, PURIFICATION AND BIOPHYSICAL (NMR,MS) STUDIES OF THE ENDOCANNABINOID ENZYME MONOACYLGLYCEROL LIPASE	P3-31
John Sesay, Anushka Goonawardena, Cheryl Ann Sexton and Robert Hampson	ANANDAMIDE DIFFERENTIALLY MODULATES HIPPOCAMPAL NEURONAL FIRING IN ANESTHETIZED VS. BEHAVING RATS	P3-32
Holiday Durham, JodiAnne Wood, Alexandros Makriyannis and Carol Lammi-Keefe	THE ENDOCANNABINOID METABOLOME-LEPTIN CONNECTION DURING THE SECOND AND THIRD TRIMESTERS OF PREGNANCY	P3-33
Jay West, Kyle Whitten, S.K. Vadivel, Nikolai Zvonok and Alex Makriyannis	MASS SPECTROMETRIC CHARACTERIZATION OF HUMAN NAAA	P3-34
Anna Louise Bowman and Alexandros Makriyannis	DEVELOPING DYNAMIC PHARMACOPHORE MODELS OF MONOACYLGLYCEROL LIPASE AND RELATED ENZYMES	P3-35
Kyle M. Whitten, Subramanian K. Vadivel and Alexandros Makriyannis	NOVEL ENDOCANNABINOID PROBES	P3-36
Elizabeth Sabens Liedhegner, Amy Sasman and Cecilia J. Hillard	BRAIN REGION-SPECIFIC CIRCADIAN RHYTHMS OF N-ACYLETHANOLAMINE CONTENTS AND FATTY ACID AMIDE HYDROLASE	P3-37
S. John Gatley, Richard Duclos, Kun Qian and Sherrye Glaser	BRAIN TRANSPORT AND METABOLISM STUDIES WITH 2-ARACHIDONOYLGLYCEROL (2-AG) AND FATTY ACID ETHANOLAMIDES	P3-38
Noriko Shinjo, Vincenzo Di Marzo, Fabiana Piscitelli, Roberta Verde, Cristoforo Silvestri and Piero Orlando	THE EFFECT OF POLYUNSATURATED FATTY ACIDS ON THE ENDOCANNBINOID SYSTEM IN NEURAL STEM/PROGENITOR CELLS	P3-39

Kara Stuhr, Anyu He and Cecilia Hillard	CHARACTERIZATION OF ANANDAMIDE SYNTHESIS INHIBITORS	P3-40
TOPIC J. <i>IMMUNITY</i>		
Lennard B. Karger, Linda E. Klumpers, Matthijs Moerland and Joop M.A. van Gerven	Δ^9 -TETRAHYDROCANNABINOL DOES NOT REDUCE TNF- α PRODUCTION IN <i>EX VIVO</i> LPS- AND SEB-CHALLENGED WHOLE BLOOD	P3-41
Guy Cabral, Erinn Raborn, Gabriela Ferreira and Daniel Fraga	CANNABINOID INHIBITION OF MACROPHAGE-LIKE CELL MIGRATION TO THE TAT TRANS-ACTIVATING PROTEIN OF HIV-1 IS LINKED TO THE CB2 CANNABINOID RECEPTOR	P3-42
Patricia Molina, Peter Winsauer, Angela Amedee and Nicole LeCapitaine	CHRONIC Δ^9 -THC MODULATION OF SIMIAN IMMUNODEFICIENCY VIRUS INFECTION	P3-43
J. Thomas Toguri, Ryan Moxsom, Juan Zhou, Vladimer Cerny, Sarah Whynot, Melanie Kelly and Christian Lehmann	EFFECTS OF CANNABINOID RECEPTOR MODULATION ON LEUKOCYTE-ENDOTHELIAL INTERACTIONS IN ENDOTOXIN-INDUCED UVEITIS	P3-44
Barbara Romano, Gabriella Aviello, Simona Pace, Francesca Borrelli and Angelo Antonio Izzo	EFFECT OF NON-PSYCHOTROPIC CANNABINOIDS ON NITRITE PRODUCTION IN MOUSE PERITONEAL MACROPHAGES	P3-45
Schuyler Pike, Weimin Chen, Barbara Kaplan and Norbert Kaminski	AN IN VITRO IMMUNE RESPONSE MODEL MIMICKING EARLY-STAGE HIV INFECTION FOR ELUCIDATING MECHANISMS OF CANNABINOID- INDUCED IMMUNE MODULATION	P3-46

NOTES:

FAAH, MAGL, NAAA

**DAY 5 – ICRS SATELLITE SYMPOSIUM
SUNDAY, JULY 10TH**

7.30	BREAKFAST	
THE ICRS PRESENTS A NIDA-SPONSORED SATELLITE SYMPOSIUM ENTITLED “ ENDOCANNABINOID METABOLIC ENZYMES AND DRUG DEVELOPMENT ” A.K.A. FAAH, MAGL, NAAA ORGANIZED BY RAO S. RAPAKA, PH.D., FAAPS, NIDA		
8.40	WELCOME DR. RAO S. RAPAKA	
8.45	DR. DAVID SHURTLEFF NIDA, ACTING DEPUTY DIRECTOR	
TRANSLATIONAL RESEARCH CHAIRPERSON: DR. CRAVATT		
9.00	Dr. Ben Cravatt The Scripps Research Institute	ENDOCANNABINOID HYDROLASES AS TARGETS FOR DRUG DEVELOPMENT
9.15	Dr. Kay Ahn Pfizer	DISCOVERY AND PHARMACOLOGICAL CHARACTERIZATION OF PF-044577845; A HIGHLY POTENT, ORALLY BIOAVAILABLE, AND SELECTIVE FAAH INHIBITOR
9.30	Dr. Dale Boger The Scripps Research Institute	INHIBITORS OF FATTY ACID AMIDE HYDROLASE

9.45	Dr. Alex Makriyannis Northeastern University	FAAH AND MAGL INHIBITORS AS DRUGS
10.00	Dr. Nephi Stella University of Washington	TARGETING ABHD6 TO TREAT NEUROLOGICAL DISEASES
10.15	Dr. Natsuo Ueda Kagawa University	INTRACELLULAR FORMATION OF N-ACYLPHOSPHATIDYLETHANOLAMINE BY CALCIUM-INDEPENDENT N-ACYLTRANSFERASES
10.30	BREAK	
THERAPEUTIC PROFILING CHAIRPERSON: DR. KUNOS		
11.00	Dr. Jack Bergman McLean Hospital, Boston	FAAH INHIBITORS AND SMOKING CESSATION
11.15	Dr. Imad Damaj Virginia Commonwealth University	EFFECTS OF FAAH INHIBITION IN MOUSE MODELS OF NICOTINE DEPENDENCE
11.30	Dr. Aron Lichtman Virginia Commonwealth University	ACUTE AND PROLONGED INHIBITION OF ENDOCANNABINOID CATABOLIC ENZYMES IN PRECLINICAL PAIN MODELS
11.45	Dr. George Kunos NIH/NIAAA	PHARMACOLOGICAL, BEHAVIORAL AND METABOLIC PROFILE OF THE NOVEL FAAH INHIBITOR AM3506
12.00	LUNCH	

13.30	Dr. Andrea Hohmann University of Georgia	INHIBITORS OF FATTY-ACID AMIDE HYDROLASE AND MONOACYLGLYCEROL LIPASE DIFFERENTIALLY SUPPRESS PAIN INITIATION THROUGH PERIPHERAL ENDOCANNABINOID MECHANISMS
13.45	Dr. Loren Parsons The Scripps Research Institute	EVALUATION OF FAAH AND MAGL INFLUENCE ON EXTRACELLULAR eCB LEVELS IN RODENT BRAIN
<p>SPECIAL TOPICS</p> <p>CHAIRPERSONS: DRS. MACCARRONE & RAPAKA</p>		
14.00	Dr. Sudhansu Dey University of Cincinnati	FAAH'S ROLE IN REPRODUCTION
14.15	Dr. Mauro Maccarrone University of Teramo	NEW STRUCTURAL AND FUNCTIONAL ASPECTS OF THE ENDOCANNABINOID DEGRADING ENZYME FAAH
14.30	Dr. Heather Bradshaw Indiana University	MULTIPLE OUTCOMES FOR FAAH INHIBITION: UP AND DOWN
14.45	BREAK	
15.00	Dr. Cecilia Hillard Medical College of Wisconsin	FAAH AS A TARGET OF GENERAL ANESTHETICS
15.15	Dr. Dale Deutsch SUNY Stony Brook	THE FATTY ACID BINDING PROTEINS (FABPS) AND THEIR ROLE IN ENDOCANNABINOID METABOLISM
15.30	BREAK	
15.40	<p>WORKSHOP DISCUSSION</p> <p>CHAIRPERSONS: DRS. CRAVATT, KUNOS, RAPAKA & MACCARRONE</p>	
17.30	ADJOURN	

CANNABINOIDS ALTER NEURAL ENCODING BUT NOT RECALL OF MEMORY

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Systemically administered cannabinoid receptor (CB1) agonists WIN 55,212-2 and delta-9-THC suppress hippocampal neural encoding of task-relevant information in rats performing a delayed-nonmatch-to-sample (DNMS) task (Hampson et al. Behav Pharm 2007). The CB1 antagonist/inverse agonist Rimonabant blocks the effects of exogenously applied agonists, but when applied alone produces enhanced neural encoding and facilitated DNMS behavior at delays > 10 sec. Likewise, modulators which inhibit the metabolism of the endogenous cannabinoids anandamide and 2-AG show the inverse effects, with impairment of neural encoding and behavior. This suggests that a major role of the endocannabinoid system in learning and memory is to modulate the strength of neural encoding.

A key finding of these studies is that when animals are run in a Closed Loop Feedback version of the DNMS task, in which trial delay depends on the strength of hippocampal encoding, animals infused intrahippocampally with a CB1 agonist can still successfully perform the task at delays commensurate with neural encoding strength. When the CB1 antagonist Rimonabant was infused intrahippocampally, neural encoding is facilitated, as is performance at long delay trials. In both cases, it is the neural activity of the Sample (encoding) phase of the task which is altered, and not the Nonmatch (recall) phase, suggesting that the cannabinoids do not alter the mechanisms of memory per se, but rather modulate the strength of encoded information.

Results will be presented from a novel stimulation technique to restore neural activity in animals with impaired information encoding, and hence behavioral performance, by CB1 receptor activation. Such results confirm that CB1 receptors alter encoding without altering specific mnemonic mechanisms, and suggest that endocannabinoids may normally play a role in "tuning" encoding strength and information content within brain areas that process cognitive information.

Supported by NIDA grants DA008549 to R.E.H. and DA007625 to S.A.D.

CANNABINOID AGONISM REDUCES SONG PERCEPTION-INDUCED ARC EXPRESSION IN AN AUDITORY REGION OF ZEBRA FINCH TELEENCEPHALON

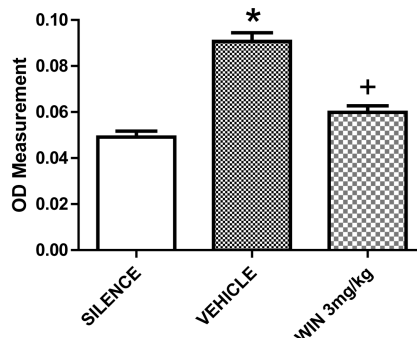
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Currently, we are working to understand mechanisms by which cannabinoid agonism alters dendritic morphology and interferes with both habituation and memory formation in our zebra finch song learning model. Arc, an immediate-early gene product essential for long-term potentiation, is rapidly induced in zebra finch NCM (a secondary auditory region of telencephalon) in response to hearing novel song. Arc expression in NCM gradually habituates after repeated presentations of a novel song stimulus. Arc's mRNA/protein temporal expression parallels other immediate early genes, but is unique in its dendritic localization. Distinct dendritic Arc expression suggests a potential role in cannabinoid-altered dendritic morphology that we have discovered previously; we hypothesize that altered vocal learning may involve a CB₁ activity-related reduction of Arc protein expression.

Methods: Adult male zebra finches were assigned to treatment: (WIN 3mg/kg), vehicle, or silence groups (n = 4 each). Birds were housed singly in visually-isolated soundproof chambers. IM injections were administered into pectoralis 30 min before exposure to novel song. Birds were exposed to 30 min of song, followed by 90 min of silence. Animals were anesthetized with Equithesin, perfused, and brains dissected. 30 μm tissue sections containing NCM, were collected and processed for immunolabeling of Arc protein. Mean optical density (OD) measurements of Arc staining within NCM were taken from 1000 X images.

One-way ANOVA revealed that WIN treatment significantly reduced Arc expression within NCM ($F_{(2,252)} = 48.22, p < 0.05$). Mean OD's were 0.049 in the silence-control group, 0.091 in vehicle and 0.060 in WIN-treated groups. In summary, these results demonstrate that cannabinoid administration disrupts auditory-perception-related expression of Arc, a protein critical to dendritic morphology. This suggests that altered Arc expression may be involved in the mechanism responsible for elevated dendritic spine densities following cannabinoid-altered vocal learning.



**ALLELIC VARIANTS OF AN INTRONIC CB1 ENHANCER SHOW
DIFFERENTIAL RESPONSES IN HIPPOCAMPAL AND HYPOTHALAMIC
NEURONES; IMPLICATIONS FOR CANNABIS USE.**

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Studies have shown that the development of disorders such as memory impairment and psychosis that occur in ~ 10% of young cannabis users has a genetic basis. We explored the hypothesis that gene regulatory variation may underlie these findings. Because no polymorphisms were found in the CNR1 gene promoter we looked for functional non-coding polymorphisms close to the CNR1 transcriptional start site using the ECR browser and the UCSC browser. We found a polymorphism (rs9444584 -C/T) within intron 1 of the CNR1 gene that occurred in a 400bp region (ECR1) of sequence that had been highly conserved for 300 million years. Examination of the population diversity of these alleles demonstrates that in European and Asian populations the T allele was comparatively rare and only found in 13-20% of the population. However, in Sub-Saharan Africa the balance between C and T was roughly equal (47% and 53% respectively). A luciferase reporter construct containing the major allele of ECR1 (ECR1(C)) was magnetofected into hippocampal and hypothalamic neonatal primary cell cultures, where CB1 is highly expressed, that were then treated with different cell signalling agonists. ECR1(C) could activate CNR1 promoter activity within hypothalamus cells through the MEKkinase pathway but was inactive in hippocampal cells. However, ECR1(T) drove high levels of expression and responded strongly to angiotensin in hippocampal cells where activation of CB1 can stimulate MAPkinase pathways. These results are consistent with the possible involvement of ECR1(T) in a hippocampal CB1 driven autocrine loop. Furthermore, ECR1(C) was predicted to have relatively low affinity for the AP-1 transcription factor whereas the ECR1(T) allele would have a significantly higher affinity. This intriguing observation is made more interesting if we consider evidence that the ECR1(T) allele was selected against following the arrival of Eurasian ancestors in the Middle East and Central Asia where Cannabis Sativa was indigenous. Thus the observations described in this study constitute an important step in understanding the involvement of regulatory polymorphisms in the development of cannabis induced pathologies.

CANNABINOID IMPAIRMENT OF SPATIAL WORKING MEMORY VIA HIPPOCAMPAL ASTROCYTE-MEDIATED SYNAPTIC LONG-TERM DEPRESSION

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Introduction: Spatial Working memory (SWM), the ability to transiently store and manipulate spatial and temporal information in order to perform many activities of daily life such as driving a car, is impaired in patients with schizophrenia and Alzheimer disease. Cannabis (marijuana or cannabinoids) can impair SWM through unknown mechanisms. Recent evidence indicates a link of impairment of long-term potentiation (LTP) at excitatory synapses connecting hippocampal CA3 axons and CA1 pyramidal neurons (CA3-CA1PN synapses) with SWM impairment. Cannabinoids may impair SWM as a result of synaptic LTP impairment through cannabinoid depression of excitatory synaptic transmission, because recent studies have consistently shown that cannabinoid application to hippocampal slices depresses glutamate synaptic transmission at CA3-CA1PN synapses. It is entirely unknown, however, whether cannabinoids are able to induce *in vivo* long-term depression (LTD) at CA3-CA1PN synapses. More importantly, whether LTP impairment or LTD induction at these synapses causatively contributes to cannabinoid impairment of SWM has never been addressed. We explored these essential issues with *in vivo* field recordings of the excitatory postsynaptic potentials (EPSP) from CA3-CA1PN synapses.

Results: An *in vivo* exposure of cannabinoids induces *in vivo* LTD at CA3-CA1PN synapses because an i.p. injection of HU210 or Δ^9 -tetrahydrocannabinol decreases EPSP amplitude about 40% of the baseline levels for longer than 24 hours, which was blocked by the CB1 receptor (CB1R) antagonist AM281 10 min before but not 10 min after HU210 injection, and because the later-phase, but not early-phase, expression of the depressed synaptic transmission is protein-synthesis dependent. HU210-induced *in vivo* LTD at CA3-CA1 synapses can be blocked (1) by adenoviral vectors to knock down CA1 expression of CB1R or the astrocyte-specific connexin43 (Cx43), (2) in Cx43^{+/-} mice but not in wild-type mice (we are now working on mutant mice without CB1R expression selectively in glutamatergic neurons or astrocytes), (3) by the NMDA receptor antagonist AP-5 and NR2B-preferring antagonists Ro25,6981 and ifenprodil, but not by the NR2A-preferring antagonist NVP-AAM077, and (4) by the AMPA receptor endocytosis blocker Tat-GluR2 peptide but not its control peptide. HU210 impairment of SWM is abolished in Cx43^{+/-} mice or by pretreatment with Ro25,6981 or Tat-GluR2.

Conclusion: cannabinoids stimulate astrocyte CB1R to release glutamate, which activates postsynaptic NR2B receptor to induce AMPA receptor endocytosis, leading to *in vivo* LTD expression at CA3-CA1PN synapses and subsequent SWM impairment.

CANNABINOID MODULATION OF VISUAL LEARNING AND MEMORY IN RHESUS MACAQUES

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While marijuana is the most commonly abused illicit drug in the United States (Johnston *et al.*, 2010), the cognitive impact of acute marijuana exposure is poorly understood. Some studies suggest that smoking marijuana produces discrete cognitive deficits in humans that may persist for up to 28 days (Bolla *et al.*, 2002), while other studies suggest that marijuana-related cognitive deficits are generally mild (Brown, McKone, & Ward, 2010). In humans and non-human primates, visual learning and memory can be measured with a paired associates learning (PAL) task. In the PAL task, subjects are presented with up to 4 visual stimuli, each occupying a unique position on a touch-screen. To be reinforced, subjects are required to recall the screen position associated with each of the previously-viewed stimuli. A series of experiments were undertaken to measure the degree to which Δ^9 -THC (0 to 0.5 mg/kg, i.m.) alters visual learning and memory in non-human primates. In these experiments, adult male Rhesus macaques ($N=8$) were trained to perform the PAL task using touch-sensitive LCD screens controlled by CANTAB® software (Lafayette Instruments, Lafayette, Indiana, USA). Under these conditions, the degree to which Δ^9 -THC altered visual learning and memory was dependent upon the number of stimuli presented during the trial. A 2-way repeated measures ANOVA confirmed a significant interaction between the dose of Δ^9 -THC and the number of stimuli presented on the overall percentage of correct responses during the PAL task. Subsequent post-hoc analyses confirmed that a 0.5 mg/kg dose of Δ^9 -THC reliably reduced the overall percentage of correct responses when 3 or more stimuli were presented. Additionally, all tested doses of Δ^9 -THC reliably reduced the overall percentage of correct responses when 4 stimuli were presented. Importantly, Δ^9 -THC did not significantly alter choice latency in these trials or performance in a subsequent test of manual dexterity. These data suggest that the cognitive impairment produced by Δ^9 -THC is dependent upon on the difficulty of the task undertaken. Further, the cognitive impairment produced by Δ^9 -THC is dissociable from the psychomotor effects of the drug in these experiments.

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CANNABINOID CB₂ RECEPTORS ACTIVATION SUPPRESSES NEUROPATHIC PAIN EVOKED BY THE CHEMOTHERAPEUTIC AGENT CISPLATIN IN RATS

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Neuropathic pain is observed following administration of cisplatin, a platinum-based chemotherapeutic agent. Few studies have investigated the involvement of CB₂ agonists in suppressing chemotherapy induced neuropathy. The present study was designed to evaluate and compare the efficacy of two CB₂ agonists (AM2301 and AM1710) from the cannabillactone class of cannabinoids.

Rats received once weekly injections of cisplatin for 4 weeks to produce neuropathy. Mechanical allodynia was evaluated using an electronic von Frey stimulator before and during the development of cisplatin-induced allodynia. CB₂ agonists AM1710 and AM2301 were administered to rats receiving either cisplatin (3 mg/kg i.p.) or saline. Separate groups of rats received either vehicle, the CB₂ antagonist AM630 (3 mg/kg i.p.) or either AM1710 (1 or 5 mg/kg) or AM2301 (1 or 5 mg/kg) co-administered with AM630 on day 28 following initial cisplatin dosing.

Mechanical allodynia developed starting at day 4 post-cisplatin injection and lasted for 28 days following initial cisplatin dosing. AM2301 and AM1710 produced similar suppressions of cisplatin-evoked mechanical allodynia, normalizing mechanical withdrawal threshold to pre-cisplatin levels. This anti-allodynic effect was still observed at 2.5 h post-injection. By contrast, both CB₂ agonists failed to produce antinociception in animals receiving saline instead of cisplatin. These effects were mediated by cannabinoid CB₂ receptors, because antiallodynic effects of the cannabillactones were blocked by the CB₂ antagonist AM630.

In conclusion, cannabinoid CB₂ agonists from the cannabillactone class suppress the maintenance of neuropathic pain behavior produced by cisplatin treatment. These anti-allodynic effects are mediated by CB₂-specific mechanisms. CB₂ agonists represent a promising therapeutic target for the treatment of chemotherapy-induced neuropathy produced by different classes of chemotherapeutic agents.

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EXPERIMENTAL OSTEOARTHRITIS IN RATS IS ATTENUATED BY ORAL ADMINISTRATION OF PALMITOYLETHANOLAMIDE.

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Osteoarthritis (OA) is the most common form of arthritis and is characterized by extensive remodelling of subchondral bone and permanent destruction of articular cartilage leading to joint pain. An established animal model of OA pain involves the intra-articular injection of the glycolysis inhibitor sodium mono-iodoacetate (MIA) which disrupts cartilage metabolism, leading to OA like lesions, nerve sensitization and joint pain. Cannabinoids are emerging as pertinent treatment option for the management of chronic pain and, recently, constitutive expression of both CB1 and CB2 receptors have been found on chondrocytes and implicated in a potential disease modifying role in OA. Cannabinoid agonists inhibit pain related behaviour in different models of arthritic pain even if the main drawback of this therapy is the development of centrally mediated side effects. On this basis and in the light of the recent finding showing that the levels of anandamide, 2-AG, PEA, and OEA were elevated in the spinal cords of MIA-treated rats indicative for a functional role of these endogenous compounds in limiting increases in the excitability of spinal neurons (Sagar *et al.* *Arthritis & Rheumatism* 62, 3666-3676, 2010), the aim of the present study was to assess whether the administration of exogenous PEA can be useful in counteracting OA-associated pain. To induce OA, 25 μ l of 2mg sodium MIA in saline was injected into the joint cavity through the patellar ligament of male Wistar rats. In other animals, 25 μ l of saline was injected into the knee joint and this cohort served as a sham control group. Starting from the day after, PEA was orally administered at a dose of 30mg/kg, once a day for two weeks. Knee diameters were measured to determine the amount of tissue swelling as an index of inflammation: a brief period of inflammation was noted after MIA injection, and PEA treatment evoked a significant reduction of knee swelling during the first days of administration. This effect was accompanied by a significant reduction of TNF α content in the synovial fluid of MIA rats treated chronically with PEA. Using the walking track analysis, we estimated the motor recovery of the lower limb by assessing some sciatic nerve functional parameters (Sciatic Functional Index). The reduced performance of MIA rats was significantly recovered following PEA administration suggesting an improvement of peripheral nerve function. As expected, after the MIA injection, rats developed mechanical allodynia to normally innocuous mechanical stimulation with a von Frey filament, as compared to saline injected animals. Treatment of MIA rats with PEA resulted in a partial but significant relief of pain. We assessed the efficacy of PEA in counteracting pain associated with OA also when administered later (two week after MIA), when the pathology, and especially pain symptoms, are well established. The data support the anti-allodynic properties of PEA also in a situation that more closely reproduces the clinical one. Because pain is one of the most physically and psychologically debilitating symptoms of OA and is often resistant to actual treatments the pain reduction by PEA could offer a promising approach to treat this aspect of OA.

Acknowledgments: authors are grateful to Epitech for supporting this study.

CARRAGEENAN-INDUCED PAIN BEHAVIOUR IS ASSOCIATED WITH PPAR α POSITIVE IMMUNE CELL RECRUITMENT, AND IS MODULATED BY PALMITOYLETHANOLAMIDE

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Previous work in our laboratory has shown that intraplantar injection of 2% λ -carrageenan produces a rapid (within 3 hour) increase in the levels of PPAR α protein in rat paw tissue. The aim of the present study was to determine whether this increase is due to the recruitment of PPAR α -expressing inflammatory cells, or due to increased expression of PPAR α in resident skin cells.

Rats were injected (Intraplantar) with either an intervention (PPAR α agonist/antagonist) or vehicle followed by 2% (100 μ l) λ -carrageenan. Changes in hindpaw weight bearing (a measure of hyperalgesia) and hindpaw oedema were recorded for three hours following carrageenan administration. Rats were then overdosed and transcardially perfused, skin tissue was collected, and stored in chilled sucrose solution. Skin was cut into 40 μ M sections and sections were either stained using hematoxylin and eosin, or immunofluorescence was conducted on the skin sections for immune cell quantification.

Dramatic histological differences were noted between skin tissue taken from carrageenan-treated rats. Skin taken from carrageenan-treated rats, showed an extremely high volume of infiltrating cells, which formed dense clusters, and exhibited widespread distribution in the basal membrane. The presence of infiltrating cells was confirmed with immuno-fluorescent experiments, which showed a significant increase, in the number of neutrophils (2 +/- 0.06 in vehicle and 96 +/- 16.12 cells in carrageenan injected skin) and ED1 (cluster of differentiation 68, a glycoprotein that is found on monocytes/macrophages/giant cells), positive cells (1 +/- 0.11 in vehicle and 18 +/- 1.53 giant cell clusters in carrageenan injected skin). We then determined whether these infiltrating cells expressed PPAR α protein. Both immune cell populations in skin tissue expressed PPAR α protein, which was considerably higher in carrageenan-treated skin (1 +/- 0.67 positive cell clusters in vehicle skin vs. 11 +/- 2.27 positive cell clusters in skin taken from carrageenan treated rats). Furthermore, PPAR α expression in immune cells was predominantly expressed in cell nuclei (as confirmed by DAPI, PPAR α , and immune marker triple-staining).

Local administration of 50 μ g of the PPAR α agonist palmitoylethanolamine (PEA) did not alter oedema formation, but did profoundly decrease carrageenan-induced changes in weight bearing. This effect of PEA was abolished by the PPAR α antagonist GW6471 (30 μ g), confirming a role for this receptor.

In summary, carrageenan-induced inflammation induces a rapid recruitment of neutrophils and ED1 positive cells (monocytes/macrophages/giant cells), which express high levels of PPAR α protein. Activation of PPAR α , whether on recruited immune cells, or on resident cells of paw skin tissue, attenuates pain behaviour. Ongoing studies will examine the effects of PEA and GW6471 on immune cell recruitment in this model. Taken in a wider context, it is possible that potential immune modulatory effects of PEA, may have an important contributory role in the anti-inflammatory and analgesic effects induced by inhibiting endocannabinoid catabolism.

PRESYNAPTIC AND POSTSYNAPTIC MECHANISMS MODULATING ENDOCANNABINOID-DEPENDENT NOCICEPTIVE SIGNALING

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Previous studies in our laboratory have shown that long-term depression (LTD) of nociceptive synaptic transmission in the leech CNS is mediated by endocannabinoid activation of a TRPV-like receptor (Yuan & Burrell (2010). *J. Neurophysiology*, Vol 104: 2677-2777). This endocannabinoid-mediated LTD (ecLTD) requires postsynaptic synthesis of the endocannabinoid 2AG which travels in a retrograde manner to activate presynaptic TRPV-like receptors. The ability of TRPV receptors to mediate ecLTD in place of CB1 receptors has only recently been appreciated and the cellular properties and functional role of this form of synaptic plasticity is not well understood. Therefore, we examined pre- versus postsynaptic processes that mediate this endocannabinoid/TRPV-dependent LTD.

In the medicinal leech, both non-nociceptive touch (T) and nociceptive (N) sensory cells have synaptic input onto the longitudinal (L) motor neuron. Low frequency stimulation (LFS) of the T-cell induces ecLTD in the N-to-L synapse. Injection of the Ca^{2+} chelator EGTA into either the N- or L-cell blocked ecLTD, indicating that increased intracellular Ca^{2+} is required in both pre- and postsynaptic neurons. Given that TRPV receptors flux Ca^{2+} and Ca^{2+} sensitive protein phosphatases have been shown to contribute to ecLTD, we examined the role of protein phosphatase 2B/calcineurin during this form of nociceptive synaptic depression. Cyclosporine A, an inhibitor of calcineurin, blocked ecLTD at the N-to-L synapse indicating that this protein phosphatase is necessary for this endocannabinoid/TRPV-mediated synaptic plasticity. Furthermore, intracellular injection of calcineurin auto-inhibitory peptide was found to block LFS-induced depression at the presynaptic, but not postsynaptic neuron. We also examined the role of RNA and protein synthesis during ecLTD. Emetine, a protein synthesis inhibitor, blocked ecLTD. Synaptic depression was also blocked by the two RNA synthesis inhibitors tested, actinomycin D and DRB. Furthermore, postsynaptic injection of DRB blocked ecLTD while presynaptic injection had no effect. This suggests that the transcription-dependent component of ecLTD occurs selectively in the postsynaptic neuron and may involve the synthesis of DAG lipase.

We propose that persistent synaptic activation stimulates postsynaptic transcription- and translation-dependent processes that result in 2AG synthesis in a Ca^{2+} -dependent manner, possibly as a result of increased synthesis of DAG lipase. This 2AG is then transmitted in a retrograde manner, activating presynaptic TRPV-like receptors and the resulting Ca^{2+} influx calcineurin and possibly other translation-dependent processes that result in decreased neurotransmitter release. These results represent a potentially important process involving endocannabinoids and TRPV activation in modulating synapses in general.

CB₁ RECEPTOR FUNCTION IS MAINTAINED FOLLOWING CHRONIC ADMINISTRATION OF LOW DOSES OF THE MONOACYLGLYCEROL LIPASE (MAGL) INHIBITOR JZL184

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JZL184, the selective inhibitor of monoacylglycerol lipase (MAGL), produces antinociceptive effects and reduces the ulcerogenic effects of nonsteroidal anti-inflammatory drugs (NSAIDs). However, chronic administration of a high dose of JZL184 (40 mg/kg) leads to tolerance to its antinociceptive effects, accompanied with cross-tolerance to the pharmacological effects of cannabinoid receptor agonists as well as CB₁ receptor downregulation and desensitization. This functional CB₁ receptor tolerance represents a hurdle in developing MAGL inhibitors for therapeutic uses. In the present study, we tested whether the beneficial effects of JZL184 in reducing pain and NSAID-induced ulcers would be maintained following chronic administration of low doses. Mice were given a daily injection of vehicle or JZL184 (1.6-40 mg/kg) for six days and evaluated in the chronic constrictive injury of the sciatic nerve (CCI) model or NSAID-induced ulcer model. Whereas the antinociceptive and anti-ulcerogenic effects of high doses of JZL184 (≥ 16 mg/kg) underwent tolerance following chronic administration, these effects were maintained following low doses of JZL184 (≤ 8 mg/kg). In additional experiments, mice were treated chronically with JZL184 (1.6-40 mg/kg) and evaluated for cross-tolerance to the antinociceptive, cataleptic, and hypothermic effects of THC. Consistent with the previous study, high dose of chronic JZL184 (≥ 16 mg/kg) resulted in rightward shifts of the THC dose-response curves, while THC retained its potency in mice treated chronically with low doses of JZL184 (≤ 8 mg/kg). In the final set of experiments, we investigated the impact of subchronic JZL184 on whole brain CB₁ receptor desensitization using CP55,940 stimulated [³⁵S]GTP γ S activation assays, as well as downregulation of CB₁ receptor density measured via [³H]rimonabant binding. Whereas chronic administration of high doses of JZL184 (≥ 16 mg/kg) produced CB₁ receptor desensitization and downregulation, CB₁ receptor expression and function were maintained following chronic administration of low doses of JZL184 (≤ 8 mg/kg). These results indicate that prolonged inhibition of partial MAGL inhibition continues to produce potentially beneficial antinociceptive effects as well as reductions in NSAID-induced ulcers, without producing functional CB₁ receptor tolerance.

PRESENCE AND FORMATION OF N-ACYL-SEROTONINS IN THE INTESTINE

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Following the discovery of anandamide (arachidonoyl ethanolamide, AEA) and other N-acyl-ethanolamines, many other compounds have been found in which amino acids or neurotransmitters rather than ethanolamide are linked to fatty acids.

Several studies have shown that the local relative availability of fatty acid precursors, which in turn is modulated by dietary intake of lipids, determines the pattern of conjugates formed. Much less information is available on the question whether the same might be true for the amines or neurotransmitters involved. Remarkably little is known on the presence and formation of possible conjugates with serotonin. More than 10 years ago, Bisogno *et al.* (1) reported the chemical synthesis of arachidonoyl-serotonin (AA-5-HT) and they showed that this compound is able to inhibit the activity of the enzyme fatty acid hydrolase (FAAH). FAAH is responsible for the degradation of NAEs and therefore inhibition of this enzyme may lead to the indirect activation of mechanisms where NAEs are involved in. We hypothesized that AA-5-HT could be a compound that is endogenously present in those tissues that have high contents of serotonin, including the gut, and that analogues of AA-5HT are also likely to exist (e.g. N-acyl-serotonins of stearic acid, oleic acid, palmitic acid, docosanoic acid, and eicosapantanoic acid. For this purpose, an LC-MS/MS method was developed and applied to investigate the endogenous presence of AA-5-HT and its analogues in different parts throughout the gastro-intestinal (GI) tract of pigs and mice. In pigs, we discovered that AA-5-HT, oleoyl-serotonin, palmitoyl-serotonin, and stearoyl serotonin were indeed endogenously present, in particularly in the jejunum and ileum. Their formation *in vitro* was stimulated by the addition of serotonin to intestinal tissue incubations. Further more in a mouse study we showed that the pattern of formation is dependent on the relative amount of fatty acids in the diet. Preliminary data showed that several of the serotonin conjugates are able to inhibit glucagon-like peptide-1 secretion and FAAH activity *in vitro*. Taken together, our data suggest that N-acyl serotoninins are a novel class of lipid mediators present in the gut with highly promising biological properties.

(1) Bisogno, T., Melck, D., De Petrocellis, L., Bobrov, M.Y., Gretskaya, N.M., Bezuglov, V.V., Sitachitta, N., Gerwick, W.H., and Marzo, V.D. (1998). Arachidonoylserotonin and other novel inhibitors of fatty acid amide hydrolase. *Biochemical and Biophysical Research Communications* 248, 515-522

CANNABIDIOL MAY ATTENUATE VOMITING AND NAUSEA BY ACTING AS AN INDIRECT 5-HT_{1A} AGONIST

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Cannabidiol (CBD), a non-intoxicating component of cannabis suppresses cisplatin-induced vomiting in *Suncus murinus* (house musk shrew; Kwiatkowska et al., 2004) and reduces lithium chloride (LiCl)-induced conditioned gaping in rats (a selective measure of conditioned nausea; Parker & Mechoulam, 2003), but the mechanism of action mediating these effects is unknown. We investigated the involvement of somatodendritic 5-hydroxytryptamine 1A (5-HT_{1A}) auto-receptors in CBD's anti-emetic/anti-nausea effects, as evidence suggests its involvement in the neuroprotectant and anxiolytic properties of CBD.

A series of experiments were conducted to examine the action of CBD at the 5-HT_{1A} receptor, using the 5-HT_{1A} antagonist WAY100135, to block the anti-emetic/anti-nausea effects of CBD in shrews and rats respectively.

Cannabidiol (5 mg/kg) suppressed nicotine-, LiCl-, and cisplatin (20 mg/kg)-induced vomiting in the shrew, and LiCl-induced conditioned gaping in rats. The anti-emetic/anti-nausea effects of CBD were reduced by systemic pretreatment with WAY100135. As well, the more selective 5-HT_{1A} receptor antagonist, WAY100635, administered systemically or intracranially into the dorsal raphe nucleus (DRN), a site of somatodendritic 5-HT_{1A} autoreceptors, interfered with the suppressed conditioned gaping produced by CBD in rats. When administered intracranially into the DRN, CBD completely abolished LiCl-induced conditioned gaping in rats. In addition, CBD was found to display significant potency at enhancing the ability of the 5-HT_{1A} receptor-selective agonist, 8-OH-DPAT, to stimulate [³⁵S]GTPγS binding to rat brainstem membranes *in vitro*. Furthermore, in combination CBD (0.5 mg/kg) and 8-OH-DPAT (0.005 mg/kg) synergistically reduced conditioned gaping, but these doses alone were not effective.

These results suggest that the anti-emetic/anti-nausea effects of CBD may be mediated by increasing activation of 5-HT_{1A} somatodendritic auto-receptors in the DRN, perhaps by reducing the release of forebrain 5-HT.

MAGL OR FAAH INHIBITION PROTECTS AGAINST NSAID-INDUCED GASTRIC MUCOSAL DAMAGE

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Non-steroidal anti-inflammatory drugs (NSAIDs) are commonly used analgesics that also cause undesirable side effects, including gastric hemorrhages, erosion, and ulceration. The incidence of these side effects is increased by chronic use, and thus antacids such as proton pump inhibitors (PPIs) are routinely co-prescribed with NSAIDs. However, chronic PPI use may also cause a host of side effects, including vitamin deficiencies, gastric polyp formation, and increased susceptibility to bacterial infection. The endocannabinoid (eCB) system, comprised of cannabinoid receptors (CB₁ and CB₂), endocannabinoid ligands, and eCB catabolic enzymes, offers several targets to treat inflammatory disorders. We hypothesized that elevating the eCB 2-arachidonoylglycerol (2-AG) via the selective MAGL inhibitor JZL184, or elevating the eCB anandamide via the selective FAAH inhibitor PF-3845 protects against NSAID-induced gastropathy.

Methods: Male C57BL/6J mice were fasted for 22 h and then administered JZL184 (1-40 mg/kg, ip), PF-3845 (10 mg/kg, ip), Δ^9 -tetrahydrocannabinol (THC; 10 mg/kg, ip), or the PPI Omeprazole (20 mg/kg, ip). At 24 h, the mice were administered the NSAID diclofenac (100 mg/kg, po) to induce gastric hemorrhages, which were assessed at 30 h. Complementary genetic and pharmacological approaches were used to investigate the contribution of CB₁ and CB₂ receptors. We also evaluated whether the gastroprotective actions of JZL184 or PF-3845 would be retained after repeated administration.

Results: JZL184 blocked hemorrhage development and increased stomach levels of 2-AG. Pharmacological inhibition or genetic deletion of CB₁ or CB₂ receptors revealed that the gastroprotective effects of JZL184 and THC were mediated via the CB₁ receptor. The anti-hemorrhagic effects of JZL184 underwent tolerance after repeated administration of 16 mg/kg or higher doses, but persisted at low doses (e.g. 4 mg/kg). PF-3845 attenuated diclofenac-induced hemorrhages after acute or repeated dosing.

Conclusions: These data indicate that MAGL or FAAH inhibition protects against NSAID-induced gastropathy, even after chronic administration. Previous results demonstrating that eCB catabolic enzyme inhibitors produce antinociceptive actions along with the findings of the present study, suggest that MAGL and FAAH offer promising targets for development of analgesic, gastroprotective therapeutics. Research supported by NIDA.

INCREASING 2-ARACHIDONOYLGLYCEROL LEVELS COUNTERACTS COLITIS AND RELATED SYSTEMIC INFLAMMATION

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Inflammatory bowel diseases (IBD) are chronic inflammatory disorders with an undeniable need for new therapeutic approaches. Genetic and pharmacological evidence points towards a protective role of the activation of CB₁ and CB₂ cannabinoid receptors in IBD and experimental models of colitis.

Here we tested the hypothesis that increasing the endogenous levels of the main full agonist of these receptors – i.e. 2-arachidonoylglycerol (2-AG) – would result in reduced inflammation in a mouse model of Crohn's disease.

We used the TNBS-induced colitis mouse model, and raised 2-AG levels in diseased mice by inhibiting monoacylglycerol lipase (MAGL), the primary enzyme responsible for its hydrolysis, using the selective inhibitor JZL184.

MAGL inhibition in TNBS-induced colitis mice resulted in increased levels of 2-AG as well as in a reduction of the macroscopic and histological colon alterations. The treatment also reduced colonic expression of pro-inflammatory cytokines (IL-12, IL-6, TNF- α , and MCP-1). The restored integrity of the intestinal barrier function following MAGL inhibition resulted in reduced endotoxemia (i.e. lipopolysaccharide plasma levels) and decreased levels of circulating inflammatory cytokines (TNF- α , IL-1 β , IL-6, MCP-1, MIP-1 α and IP-10) as well as reduced liver and brain inflammation.

To test the implication of either or both cannabinoid receptors in mediating 2-AG's beneficial effects, we used CB₁ (SR141716) or CB₂ (AM630) selective antagonists. Coadministration of JZL184 with SR141716 or AM630 completely abolished the protective effect of MAGL inhibition on TNBS-induced colon alterations thus demonstrating the involvement of both cannabinoid receptors.

In conclusion, we demonstrated that increasing 2-AG levels results in a dramatic reduction of colitis, as well as the related systemic and central inflammation mediated by increased levels of circulating lipopolysaccharide. In addition to providing a novel insight into the pathophysiology of the disease, this could offer a novel pharmacological approach for the treatment of IBD based on the new protective role of 2-AG described here.

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ROLE OF THE CB₂ CANNABINOID RECEPTOR IN THE PATHOGENESIS OF BREAST CANCER

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A large body of evidence has demonstrated that plant-derived, endogenously produced and synthetic cannabinoids exert antitumoral actions in different models of cancer, including cell cultures, xenografted animals and genetically engineered mice. However, little is known about the role of the endocannabinoid system in tumor physio-pathology. In particular, although strong evidence point to the CB₂ cannabinoid receptor as target for anti-cancer therapy, there is no information about its role in tumor generation and progression. To shed light on this issue, we generated, animals with two genetic modifications, specifically, ErbB2 overexpression directed to the mammary epithelium, which triggers the spontaneous generation of breast tumors, and genetic ablation of the CB₂ cannabinoid receptor. To transfer the CB₂ knockout allele to the genetic background of the tumor-prone animals, mice were backcrossed for several generations using a marker-assisted selection protocol. We observed that the absence of CB₂ receptors produced a striking delay in tumor appearance, reduced the number of tumors generated per animal, slowed down their growth and diminished the percentage of animals with lung metastasis. Together, these results suggest that CB₂ receptors play an important role in breast tumor generation and progression. Work is currently in progress to elucidate the molecular bases of this action.

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A ROLE FOR GPR55 IN MULTISTAGE MOUSE SKIN CARCINOGENESIS

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It has been recently proposed that the putative cannabinoid receptor GPR55 modulates cancer cell proliferation and migration. To further understand the role of this receptor in cancer we used a classical mouse skin carcinogenesis model. The two-stage chemical protocol involves the treatment of mice with a single dose of a carcinogen; i.e., 7,12-dimethylbenz(a)anthracene (DMBA), followed by repeated applications of the tumor promoter 12-O-tetradecanoylphorbol-13-acetate (TPA). This treatment results in the outgrowth of highly differentiated benign papillomas. A small subset of papillomas eventually progresses to malignant squamous cell carcinomas (SCC), some of which undergo an epithelial–mesenchymal transition to spindle cell carcinomas (SpCC), the aggressive and metastatic stage.

Our results show that GPR55 is weakly expressed in mouse skin but it is upregulated during mouse skin carcinogenesis as well as after short-term TPA treatment. GPR55 deficient mice showed reduced papilloma formation in comparison with wild type mice. Further analysis revealed that GPR55 knockout mice were resistant to TPA–induced epidermal hyperproliferation, and had significantly reduced levels of c-fos expression. In summary, our study suggests that GPR55 is required for tumor promotion in skin carcinomas.

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MECHANISMS OF THE ANTI-CANCER EFFECTS OF CANNABIDIOL AND OTHER NON-PSYCHOTROPIC CANNABINOIDS ON HUMAN PROSTATE CARCINOMA

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Prostate cancer is a major health problem in adult males as long as current therapies are unable to completely eliminate the androgen-independent prostate cancer cells that remain after androgen ablation therapy, thus making necessary to explore novel approaches for the treatment of this type of cancer. Recent studies have focused on the role of non-selective, calcium permeable cation channels of the transient receptor potential (TRP) channels in prostate cancer initiation and progression, suggesting that they might be novel molecular targets useful for the diagnosis and treatment of the disease.

We investigated the effect of several plant cannabinoids (CBD, CBG, CBC, THC, THCv, THCVa, CBN, CBGA, CBGV, CBDA, CBDV and CBN) and relative enriched extracts (BDSs) on human prostate cancer cell (HPCC) viability, with particular regard to possible TRP-mediated mechanism(s) of action in both androgen receptor dependent (LNCaP and 22RV1) and independent (DU-145 and PC3) cells. Generally, among all pure compounds tested, CBD was the most efficacious at reducing cell viability (as assessed by MTT assay), and, in many cases, the BDSs were more potent than pure compounds. Under these conditions, CBD, and to a lesser extent CBG and CBC, were able to induce apoptosis (as assessed by chemoluminescence-based assay of caspase-3/7 activity, DNA fragmentation and FACS analysis) in LNCaP but not in the other HPCCs. The effect of CBD was accompanied by a dramatic up-regulation of the expression of PUMA, a p53-regulated modulator of apoptosis and a pro-apoptotic member of the Bcl-2 protein family.

We previously reported that CBD is potent antagonist at TRPM8 channels (see De Petrocellis et al., Br J Pharmacol, 2010), and therefore we investigated the role of TRPM8 blockade in CBD-induced apoptosis. Among all the cell lines used in the study, TRPM8 receptor was highly expressed only in LNCaP cells (as assessed by quantitative RT-PCR), and its expression was found to be strictly dependent on androgen receptor (AR) activity. In LNCaP cells, CBD significantly down-regulated AR expression and, in a serum/hormone-deprived medium, it blocked the stimulatory effect of dihydrotestosterone on TRPM8 receptor expression. Finally, when LNCaP cells were transformed into a more aggressive, neuroendocrine-like phenotype, identified by the formation of fiber-like processes and over-expression of neuronal enolase (NSE), CBD was even more potent at inducing apoptosis and decreased NSE expression.

We suggest that CBD, and possibly other cannabinoids, strongly reduce HPCC viability through both apoptosis-independent and -dependent mechanisms, the latter being exerted through several concurring molecular mechanisms due not uniquely to a direct TRPM8 antagonism, and including AR down regulation, which in turn can lead to TRPM8 down-regulation. **Supported by GW Pharmaceutical**

CANNABIDIOL INHIBITS GLIOBLASTOMA DISPERSAL AND EXPRESSION OF STEM CELL MARKERS IN PATIENT-DERIVED PRIMARY CULTURES

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In glioblastoma, standard-of-care includes surgical resection, radiation therapy, and adjuvant chemotherapy such as Temozolomide. These treatments are designed to primarily target tumor growth and survival but have done little to increase the overall survival of patients (median of 14 months). In many cases, this is due to postsurgery recurrences arising from tumor cells that have migrated and invaded (dispersed) into distant tissues. It is, therefore, critical to discover master regulators that specifically control tumor dispersal and target them in addition to targeting pathways controlling glioblastoma growth and survival. We determined that the transcriptional regulator, Id-1, plays a critical role in modulation of invasiveness of high-grade glioblastoma. Its expression correlates with enhanced glioblastoma cell invasiveness in culture and higher tumor grade in human biopsies. Moreover, its specific knockdown dramatically reduces cell invasion and induces profound morphological changes as well as down-regulation of cancer stem cells markers in patient-derived primary cultures. We further demonstrate that the non-toxic, and non-psychoactive cannabinoid, cannabidiol, significantly down-regulates Id-1 gene expression and associated cell invasiveness and self-renewal in primary cultures of glioblastoma. In addition, cannabidiol inhibits the proliferation and survival of glioblastoma in culture and *in vivo*. Taken together our data suggests that cannabidiol may be a promising compound for combined therapy with standard-of-care in human patients.

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CARCINOGENIC-INDUCED COLON CANCER IN MICE: ROLE OF THE ENDOCANNABINOID SYSTEM AND CHEMOPREVENTIVE EFFECTS OF CANNABIDIOL

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Introduction (Endo)cannabinoids exert protective effect in several experimental models of cancer, including colon cancer (Izzo and Sharkey, *Pharmacology & Therapeutics*. 126 (2010) 21–38). However, the role of the endocannabinoid system (ECS) in chemically-induced colon is still largely unexplored. This study aimed at 1) providing insights into the role of the ECS in chemically-induced colon cancer *in vivo* and 2) evaluating the potential chemopreventive effect of the non-psychotropic *Cannabis* component cannabidiol (CBD).

Methods Aberrant crypt foci (ACF), polyps and tumours were induced *in vivo* by azoxymethane (AOM); receptors and proteins of the ECS were analyzed by quantitative RT-PCR; endocannabinoid levels by HPLC-MS; phosphoAkt, iNOS, COX-2 and caspase-3 by western blot analysis. Cell proliferation was evaluated in colon adenocarcinoma cell lines using trypan blue staining, MTT assay and ³H-thymidine incorporation.

Results AOM administration was associated with the formation of ACF, polyps, tumours, increased endocannabinoid levels, changes in proteins of the ECS, up-regulation of phosphoAkt, iNOS and COX-2 and down-regulation of caspase-3 expression. The CB₁ agonist ACEA - but not the CB₂ receptor agonist JHW033 - and cannabidiol reduced ACF, polyps and tumours. Cannabidiol counteracted phosphoAkt and caspase-3 (but not COX-2 or iNOS) changes induced by AOM. Cannabidiol reduced cell proliferation in a CB₁ antagonist-, TRPV1- and PPAR- γ -sensitive manner.

Conclusions Chemically-induced tumours are associated to adaptive changes of the colonic ECS. CBD, which reduces tumour formation *in vivo* and cell proliferation through multiple mechanisms, may represent a potential chemopreventive agent for colon carcinogenesis.

RATIONAL SYNTHESIS OF CANNABINOID RECEPTOR 1 ANTAGONISTS FOR PERIPHERAL SELECTIVITY

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Cannabinoid receptor (CB1R) antagonists show potential for the treatment of several diseases such as obesity, liver disease, and diabetes. Recently, several CB1R antagonists were pulled from clinical development due to adverse CNS related side effects observed with rimonabant – the first clinically approved CB1R inverse agonist. However, several recent studies indicate that regulation of peripherally expressed CB1R with CNS-sparing compounds is a viable strategy to treat several important disorders. To date, our efforts aimed at rationally designing peripherally restricted CB1R antagonists have resulted in two classes of compounds that limit blood-brain barrier (BBB) permeability. The first group comprised of permanently charged compounds, such as alkyl pyridinium salts and N-oxides, which do not normally cross the BBB. The second group of compounds was designed to have high topological polar surface areas (TPSA). Compounds with high TPSAs, such as sulfonamides and sulfamides, do not usually cross the BBB passively. Functional activity of compounds at CB1R was determined using a calcium mobilization assay and binding affinity was determined by radioligand (³H-SR141716) displacement. Selectivity for CB1R over CB2R was determined by comparing the radioligand displacement of ³H-CP55940 at cells expressing either CB1R or CB2R. Permanently charged compounds had relatively poor functional activity at CB1R with Ke values ranging from 1 - >10 μM, however, at least one compound demonstrated surprisingly good binding affinity (Ki = 61 nM, ³H-SR141716). Compounds with high TPSAs (TPSA = 101-127) and acceptable functional activity (Ke ranging from 30-300 nM at CB1R) were also identified. In general, compounds showed modest selectivity for CB1R over CB2R (CB1R:CB2R, 1.3-39). Promising compounds are currently undergoing further refinement and testing.

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AM994-BINDING ARCHITECTURE OF HUMAN CANNABINOID RECEPTOR 2 USING LIGAND-ASSISTED PROTEIN STRUCTURE FOLLOWED BY MOLECULAR DYNAMICS

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Human cannabinoid receptor 2 (hCB2) has been shown to play important physiological roles in the immuno system, and is a target for development of therapeutic medications. To gain insight on the ligand binding site(s) and structural features of activation, we applied our multidisciplinary experimental approach named ligand-assisted protein structure (LAPS) to obtain structural information on the ligand-receptor binding motifs. Implementation of LAPS include high affinity covalent cannabinergic probes, site-directed mutagenesis followed by computational molecular dynamics. Additional experimental data can be obtained by carrying out LC/MS based proteomic experiments.

In the present work, we designed and synthesized AM994, a classical cannabinoid affinity label that incorporates an isothiocyanate moiety as an electrophilic reactive group capable of interacting irreversibly with a suitably located and properly oriented cysteine residue, at or near the binding site. To obtain evidence for the site of covalent attachment of AM994, five cysteine residues in transmembrane helices (TMH) were mutated to serine, an amino acid residue that does not react with isothiocyanate groups. Wild-type (WT) and mutant hCB2 receptors were evaluated for their abilities to recognize cannabinergic ligands, i.e., AM994 and ³H-labeled CP55940. It is noteworthy that AM994 was shown to bind irreversibly to WT hCB2 but exhibited no covalent attachment with the mutants of C7.38 suggesting irreversible attachment to hCB2 in its active state. The data were used to identify the docking motif of AM994 at the hCB2 receptor in molecular models. The evidence presented identifies C7.38 as the site of covalent bond formation with AM994, supported by the molecular modeling, which demonstrated the architecture of binding motif for covalent attachment of AM994 to hCB2 TMH7.

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EVIDENCE THAT AM630 IS A HUMAN CB₂ RECEPTOR PROTEAN AGONIST

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Previous research by our group has shown that AM630 behaves as a low-potency neutral antagonist of CP55940 and THCv in human CB₂ receptor-transfected Chinese Hamster Ovary cells (hCB₂ CHO cells) that have been AM630-preincubated, and as a higher-potency inverse agonist/antagonist in unpreincubated hCB₂ CHO cells (Bolognini *et al.*, 2010). We now describe results obtained with another CB₂ receptor inverse agonist/antagonist, SR144528, indicating that AM630 may be a protean agonist.

Interactions between CB₂ receptors and CP55940, AM630 or SR144528 were monitored by measuring the ability of these compounds to affect cyclic AMP production stimulated by 10 μ M forskolin in hCB₂ CHO cells (n=6-9). For all our experiments, these cells were preincubated for 24 hours with 10 μ M AM630, SR144528 or vehicle (DMSO) and then subjected to intense washing.

First, we found that SR144528 by itself enhanced forskolin-induced stimulation of cyclic AMP by hCB₂ CHO cells (EC₅₀= 42nM; E_{max}= -607%). Although this effect was still detectable in cells that had been preincubated with AM630, SR144528 displayed significantly less efficacy, although similar potency in these preincubated cells (EC₅₀= 61.1nM; E_{max}= -67.1%) than in unpreincubated cells, suggesting that it is a stronger inverse agonist than AM630 (Bolognini *et al.*, 2010). Next we preincubated hCB₂ CHO cells with SR144528. We found that in these cells, SR144528 retained a slight but significant ability to enhance forskolin-induced stimulation of cyclic AMP production (E_{max}= -16.1%). We also found that CP55940 displayed similar efficacy but less potency in SR144528-preincubated cells compared to unpreincubated cells. Importantly, when we tested AM630 in SR144528-preincubated cells, we discovered that this compound now inhibited forskolin-induced stimulation of cyclic AMP production, suggesting that AM630 may be a protean agonist. We then went on to investigate the ability of SR144528 to antagonize CP55940 in SR144528-preincubated cells. We found that in these cells, SR144528 (0.1 or 25 μ M) produced neither a downward nor a rightward shift in the log concentration-response curve of CP55940.

In conclusion, we have obtained evidence that AM630 is a hCB₂ receptor protean agonist. Our results also suggest that SR144528 displays significantly greater potency as a CB₂ inverse agonist than as a CB₂ competitive antagonist, at least in our hCB₂ receptor cell-line, that this potency ratio is greater for SR144528 than for AM630, and that the data we have obtained with these two compounds can be explained in terms of the two-state model of receptor activation. Further experiments with cannabidiol and other CB₂ receptor inverse agonists are now underway.

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INHIBITION OF ALCOHOLIC HEPATIC STEATOSIS BY A TYPE 1 CANNABINOID RECEPTOR NEUTRAL ANTAGONIST

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Cannabinoid receptors are attractive targets to treat liver diseases. The two cannabinoid receptors CB1R and CB2R are expressed in the central nervous system (CNS) and peripherally in a number of tissues. Protein levels of both receptors are increased upon liver injury or onset of alcoholic liver disease (ALD). Past reports suggest that CB1R activation is pro-fibrotic and antagonism of this receptor is an emerging strategy to treat both early and late stage liver disease. Unfortunately, first generation inverse agonists of CB1R have serious psychiatric side effects and are no longer in clinical use or development. However, neutral antagonists or CNS-sparing peripherally restricted antagonists of CB1R may be useful as therapeutics. These compounds should bypass the adverse effects associated with inhibition of endogenous endocannabinoid activity in the CNS. We have recently developed 5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-3-[(E)-piperidinoiminomethyl]-1H-pyrazole (PIMSR1), a potent and selective neutral antagonist of human CB1R (Ki~17 nM). This compound was tested in a refined mouse model of alcoholic steatosis (AS) induced by a single acute challenge with ethanol. Accumulation of lipids in liver, levels of liver enzymes aspartate aminotransferase (AST), alanine aminotransferase (ALT) and intracellular lactate dehydrogenase (LDH), a marker of cytotoxicity were increased upon (6g/kg) alcohol challenge. However, while accumulation of fat was visible at lower doses of alcohol (3 g/kg and beyond), liver enzyme levels were only elevated at 6 g/kg but not at lower alcohol doses. Gross necropsy revealed visible liver lesions at doses of 3 g/kg and beyond (pale liver, focal deposition of fat) in some animals – a finding that was consistent at 6 g/kg alcohol. Subsequently, efficacy of PIMSR1, a neutral antagonist based on the SR141716 core was tested for prevention of AS. At a 30 mg/kg dose, PIMSR1 was able to significantly inhibit AS in mice induced by 6g/kg alcohol. Pre-treatment of mice with PIMSR1 significantly reduced markers of liver injury (AST, ALT), cytotoxicity (LDH) and steatosis. Microarray analyses of gene expression indicated that PIMSR1 normalized alcohol-induced changes in expression of genes associated with cytoskeletal remodeling, cell adhesion, immune response and chemotaxis. In conclusion, further development of neutral or peripherally selective CB1R antagonists represents an exciting strategy to treat ALD.

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**REGULATION OF CANNABINOID RECEPTOR SIGNALING BY RGS
(REGULATORS OF G-PROTEIN SIGNALING) PROTEINS: A NEW PARADIGM FOR
MODULATING CB1 AND CB2 RECEPTOR-MEDIATED SIGNALOSOME**

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CB1 and CB2 cannabinoid receptors belong to Gi protein-coupled receptor superfamily. RGS proteins are classically known for turning off Gi and Gq-coupled receptor signaling. CB1 and CB2 cannabinoid receptors are known to elicit a plethora of cellular responses acting through different G-proteins. However, till date almost nothing is known about RGS-mediated regulation of cannabinoid receptor signal transduction. In the current study we attempt to define the role of RGS-proteins in the regulation of CB1 and CB2 receptor signaling in neuronal and endothelial cells respectively.

Previous results from our laboratory and others have showed that stimulation of CB1 and CB2 receptors produce nitric oxide via the activation of nitric oxide synthase (nNOS or eNOS depending on the cell type) in a pertussis-toxin sensitive manner. In the current study using real-time PCR we first screened for native expression of individual RGS proteins in CB1 and CB2 receptor expressing neuronal and endothelial cells lines respectively. Once we found the expression profile of RGS proteins in the specific cell lines we selectively knock down (using SiRNA) individual RGS proteins in these cell lines. We then measured nitric oxide production following CB1 or CB2 receptor activation in these RGS-knock down cell lines to determine the specificity of RGS proteins for intercepting CB1 vs. CB2 receptor signaling. Our initial findings showed that a) different RGS proteins are coupled to CB1 and CB2 receptor signaling and b) RGS proteins can differentially regulate (inhibit or activate) CB1 and/or CB2 receptor-mediated nitric oxide production. These results suggest that RGS proteins play a pivotal role in the regulation of cannabinoid receptor signalosomes.

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FUNCTIONAL IDENTIFICATION OF NEW AGONISTS AND ANTAGONISTS AT GPR55

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GPR55 is a rhodopsin-like (Class A) G protein-coupled receptor (GPCR) that was originally de-orphanized as a cannabinoid receptor. GPR55 is widely expressed in the body, especially in regions of the CNS, spleen, gut, and adrenal glands thus implying a role in diverse physiological processes. Indeed recent studies suggest a role for GPR55 in inflammatory and neuropathic pain, bone development and cancer. However, although some CB1/CB2 agonists have been reported to activate GPR55, the designation of GPR55 as a “third” cannabinoid receptor remains controversial as lysophosphatidyl-inositol, (LPI) has consistently been reported to be the most efficacious agonist at GPR55 so far. Moreover, only CP55940 and Cannabidiol were reported to antagonize GPR55. To better understand GPR55’s specific role, a search for selective agonists and antagonists was employed. To identify GPR55 active compounds, we used high throughput assays that were based upon the ability to monitor the activation states of ligand-bound GPR receptors using high content imaging employing beta-arrestin green fluorescent protein biosensors (PubChem AID1965, AID2026). We have identified potent GPR55 selective agonists ML184, ML185 and ML186 (EC₅₀ values 263 nM, 658 nM and 305 nM respectively) and GPR55 selective antagonists ML191, ML192 and ML193 (IC₅₀ values 1076 nM, 702 nM and 221 nM respectively). Two secondary assays were utilized to validate the new identified compounds: Recruitment of PKC β II GFP to the plasma membrane, and ERK1/2 phosphorylation. All compounds showed the appropriate membrane recruitment indicating activation or inhibition of activity in this signaling pathway. The agonists (1 μ M) elicited ERK1/2 phosphorylation in U2OS cells expressing GPR55E. Notably, at 1 μ M each of the agonists resulted in ERK1/2 phosphorylation that was at least as high as compared to treatment with 10 μ M LPI. Moreover, the response mediated by 10 μ M LPI was markedly decreased following pre-incubation with each of the antagonists yielding IC₅₀ values of 328 nM, 1827 nM, and 65 nM for ML191, ML192 and ML193 respectively. Further, our modeling data indicate that the similarity between the new compounds and LPI enables them all to be recognized by a single GPR55 binding pocket, the primary interaction of which occurs on TMH2 between K2.60 and an exposed highly electronegative region on each ligand. Our study demonstrates that substitution of lysine in position 2.60 to alanine, results in loss of responsiveness to the various compounds, while the receptor expression is not affected.

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NOVEL AGONISTS AND ANTAGONISTS OF THE GPR55 RECEPTOR

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GPR55 is a rhodopsin-like (Class A) G protein-coupled receptor (GPCR), highly expressed in human striatum. Characterization of GPR55^{-/-} knock-out mice reveal a role for GPR55 in inflammatory pain, neuropathic pain (Staton et al, *Pain*, 2008), and bone development (Whyte et al. *PNAS* 2009); while other studies indicate that GPR55 activation is pro-carcinogenic (Andradas et al. *Oncogene* 2010, Ford et al. *Br J Pharmacol* 2010; Pineiro et al. *Oncogene* 2010). The orphan receptor GPR55 binds a subset of CB1/CB2 ligands and has been proposed as a cannabinoid receptor (Ryberg et al *Br. J. Pharmacol* 2007). However, lysophosphatidylinositol (LPI) was also identified as a GPR55 agonist (Oka et al. *Biochem Biophys Res Commun* 2007). None of these reported ligands, however, are very potent or very selective. From a β -arrestin, high-throughput, high-content screen of 300,000 compounds run in collaboration with the Molecular Libraries Probe Production Centers Network initiative (PubChem AID1965), we identified potent GPR55 selective agonists and antagonists that belong to novel, unreported GPR55 chemotypes. Here we report the docking of a number of these agonists and antagonists in a computational model of the GPR55 receptor.

Three of the identified agonists (CID1792197, CID1172084 and CID2440433) were docked in a model of GPR55 R* receptor that had been pre-equilibrated for 50 ns in a POPC bilayer. Additionally, four antagonists belonging to four different chemotypes (CID1077538, CID1261290, CID3193014 and CID23612567) were docked in a model of the GPR55 R receptor. The GPR55 R* state model binding site accommodates ligands that are inverted-L or T shapes with long, thin profiles that can fit vertically deep in the receptor binding pocket while their broad head regions occupy the horizontal binding pocket opening near the EC loops. The GPR55 R state model binding pocket accommodates ligands that possess a central core that is relatively flat and with substituents of sufficient length to reach F6.48 in the SFLP hinge region. The agonists as well as the antagonists bind GPR55 in the TMH 2, 3, 5, 6 and 7 region. The primary interaction for these ligands with GPR55 occurs between K2.60 and an exposed highly electronegative region on each ligand. The antagonists have their main bulk higher than the agonists but are able to reach the residues M3.36/F6.48 and prevent flexing of the TMH6 CWXP hinge motif keeping GPR55 in the inactive state. [Support: NIH DA DA023204 (MEA) and DA021358 (PHR)].

CHARACTERISATION OF 2-ARACHIDONOYLGLYCEROL-INDUCED VASORELAXATION IN HUMAN MESENTERIC ARTERIES

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In human mesenteric arteries, we have shown that the endocannabinoid 2-arachidonoylglycerol (2-AG) causes significantly greater vasorelaxation than anandamide (Stanley *et al.*, this conference). Therefore, the aim of the present study was to explore the mechanisms of 2-AG-induced vasorelaxation in human mesenteric arteries.

With ethical approval and written informed consent, human mesenteric arteries were taken from patients receiving colorectal surgery. Arteries were dissected and mounted on a Mulvany-Halpern myograph and bathed in oxygenated physiological salt solution at 37°C under a set pressure of 90% of 100 mmHg. U46619 and endothelin-1 were added to increase tension by a minimum of 5 mN. Once a stable contraction had been achieved, concentration-responses curves were carried out to 2-AG. Mechanisms underlying 2-AG induced vasorelaxation were investigated using the following; CB₁ and CB₂ receptor antagonism (100 nM AM251 and AM630), TRPV1 desensitisation (10 µM capsaicin), endothelium denudation, nitric oxide production (300 µM L-NAME) and metabolism of 2-AG via cyclooxygenase (COX) (10 µM indomethacin or 10 µM Nimesulide) monoacylglycerol lipase (MAGL) (1 µM JZL184) or fatty acid amide hydrolase (FAAH) (1 µM URB597).

2-AG causes vasorelaxation significantly different to vehicle control ($P < 0.001$ Student's unpaired *t*-test) of pre-constricted human mesenteric arteries (pEC₅₀ = 5.4±0.2 s.e.m, R_{max} = 53.2±2.8% relaxation, $n = 29$). Pre-treatment with AM251, AM630, capsaicin, L-NAME, JZL184, URB579 or endothelial denudation did not alter the vasorelaxant responses to 2-AG. However, in the presence of indomethacin, 2-AG responses were partially inhibited (control R_{max} = 57.9±4.7% relaxation, indomethacin R_{max} = 37.4±5.8% relaxation, $P < 0.05$ Student's paired *t*-test, $n = 8$). The COX-2 selective inhibitor nimesulide did not inhibit 2-AG-induced vasorelaxation.

We have shown that 2-AG causes vasorelaxation in human mesenteric arteries. This is not mediated by CB₁, CB₂ or TRPV1 receptors, and is also not dependant on the production of nitric oxide, the endothelium or FAAH and MAGL activity. 2-AG induced vasorelaxation was sensitive to non-selective COX inhibition, but not COX-2 inhibition, suggesting that 2-AG is metabolised by COX-1 into vasoactive prostanoids in human mesenteric arteries.

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DEMONSTRATING PERIPHERAL RESTRICTION OF CB₁ ANTAGONIST TM38837 IN HUMANS

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Cannabinoid receptor type 1 (CB₁) antagonists, such as rimonabant, show beneficial effects in reducing body weight in obese subjects and reversing associated metabolic disorders. However, by adverse psychiatric effects of first generation, brain penetrating compounds have led to withdrawal of rimonabant from the market and cancellation of several development programs. These unfavourable side effects are attributed to central CB₁ receptor engagement. There are however, indications that the beneficial effects are peripherally mediated. TM38837 is a novel CB₁ antagonist that pre-clinically showed negligible penetration into the central nervous system, whereas clear weight loss and metabolic effects comparable to rimonabant were observed. In the current study, TM38837 was studied in healthy male subjects, using a 9-delta tetrahydrocannabinol (THC) challenge test with rimonabant as a positive control. Different tests were used to probe the reversal of peripheral and central effects of THC, and which have previously shown inhibition by various CB₁-antagonists.

This was a double-blind, double dummy, randomized, placebo-controlled, cross-over, partial parallel study. During occasions 1 to 4, 24 subjects were treated with TM38837 100 mg, 500 mg, placebo TM38837 or placebos only. During occasion 5, subjects received either rimonabant 60 mg or placebo rimonabant, both combined with placebo TM38837. Five THC 4 mg inhalation challenge tests over two days were performed during each occasion. Blood samples were drawn for pharmacokinetic (PK) analyses of TM38837, rimonabant and THC. Body sway, visual analogue scales (VAS) by Bond & Lader (mood, alertness and calmness) and Bowdle (psychedelic effects), and heart rate were assessed frequently as pharmacodynamic (PD) measures. The PK of THC, TM38837 and rimonabant, and the effects of these compounds on the THC challenge test were also quantified by population modelling.

THC particularly affected VAS feeling high, body sway, and heart rate. These effects were partly antagonized by Rimonabant 60 mg and TM38837 500 mg, whereas the 100 mg dose of TM38837 had no measurable impact on THC-induced VAS feeling high and body sway and only limited effect on heart rate. TM38837 was well tolerated, and most adverse events were ascribed concomitant administration of THC.

Rimonabant 60 mg showed a larger antagonizing potential on all THC-induced effects than TM38837 500mg, except for heart rate where the antagonizing effect was considered to be similar. The 100 mg dose of TM38837 had no measurable impact on THC-induced CNS-effects, suggesting that this dose does not penetrate the brain. TM38837 has been shown to be equipotent to rimonabant with regard to weight loss and other metabolic effects in rodent obesity models; hence these results are very encouraging for further development of TM38837 as a peripherally restricted CB₁ receptor antagonist for indications such as obesity and metabolic disorders without inducing psychiatric side effects.

THE EFFECTS OF ENDOCANNABINOIDS ON HUMAN OSTEOBLAST GROWTH *IN VITRO*

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Bone is a dynamic tissue consisting of osteoblasts, responsible for bone formation, and osteoclasts, their bone resorbing counterpart. Overall bone turnover is controlled by multiple pathways and the resulting balance maintains constant bone mass. Recent studies have shown that endocannabinoids (ECs) have an effect on bone cells (osteoblasts and osteoclasts) and their precursors, *in vitro* and on bone growth *in vivo* (Idris and Ralston, 2010). However, much is still unknown about the actions of the endocannabinoid system on bone growth. This study assesses the effect of 2 endogenous endocannabinoids, anandamide (AEA) and 2-arachidonoylglycerol (2-AG) on the proliferation and differentiation of human osteoblasts (HOBs) *in vitro*. HOBs (previously isolated from femoral head trabecular bone) were grown *in vitro* for 24 hours or 5 days in an osteogenic medium (containing 150µg/ml L ascorbic acid, 10nM dexamethasone and 10mM sodium beta glycerophosphate) and supplemented with a range of concentrations (1nM-10µM) of AEA or 2-AG. The range of concentrations represented physiological and pathophysiological concentrations in human trabecular bone. At each time-point both DNA concentration (using Hoechst 33258) and relative alkaline phosphatase activity (using p-nitrophenyl phosphate) were measured. The results were analysed and compared against the vehicle to show the effect of ECs on osteoblast proliferation and differentiation respectively. Additionally, the HOB cell cultures (without EC supplementation) were assayed, using western blotting for the presence of the CB1 and CB2 receptors at 1 and 5 days.

Supplementation with ECs was shown to affect both proliferation and differentiation of HOBs. Differentiation was significantly increased ($p < 0.001$) across the micromolar range of concentrations of both AEA and 2-AG tested after 5 days and proliferation was reduced after 5 days ($p < 0.001$). Neither differentiation or proliferation or differentiation was affected by endocannabinoid supplementation after 24 hours. The receptors for both CB1 and CB2 appeared to be present in 5 day cultures of HOBs using western blot analysis.

The findings of this study suggest that HOBs in culture possess receptors for both CB1 and CB2. Both of the endogenous ECs tested have a significant effect on osteoblast proliferation and differentiation and further studies will establish whether other markers of differentiation are affected over longer time periods.

PERIPHERAL CANNABINOID CB₁ RECEPTOR BLOCKADE REDUCES DEVELOPMENT OF GLUCOCORTICOID-MEDIATED METABOLIC SYNDROME

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Pervasive exposure to stress, and the associated increase in circulating glucocorticoids, is accepted as one of the mediators of the obesity and metabolic syndrome epidemic plaguing our nation. Glucocorticoids are powerful regulators of metabolism, endocrine function, and adipocyte differentiation and promote the development of upper body obesity, especially visceral fat stores. Recent studies have demonstrated that glucocorticoids possess the ability to increase the production and release of endocannabinoid molecules. We have previously reported that the ability of glucocorticoids to induce obesity and the metabolic syndrome is absent in CB₁ receptor deficient mice, indicating that endocannabinoid signaling is a mediator of glucocorticoid-induced obesity. Endocannabinoids, however, are potent regulators of appetite, energy balance and metabolic processes through both central and peripheral regulation of feeding and metabolism, and it is not known whether glucocorticoids recruit central or peripheral endocannabinoid signaling to promote obesity. The aim of the current study was to extend our previous findings obtained in CB₁ receptor deficient mice to a pharmacological approach, and further to use pharmacological tools to dissect if central or peripheral endocannabinoid signaling was a driving force in mediation of obesity from glucocorticoids. To this extent, we employed our previously validated non-invasive model of glucocorticoid administration through the drinking water of mice which results in rapid and dramatic increases in weight gain, increased adiposity, elevated plasma leptin, insulin and triglyceride levels, and hyperphagia. In tandem with this, we performed daily administration of either a global CB₁ receptor antagonist AM251 (2 mg/kg) or the recently characterized, peripherally restricted CB₁ receptor antagonist AM6545 (10 mg/kg) for 4 weeks. Administration of both AM251 and AM6545 significantly prevented the development of obesity and attenuated changes in metabolic markers, such as liver steatosis and adipocyte hypertrophy. Interestingly, glucocorticoid-induced hyperphagia, which was absent in CB₁ receptor deficient mice, was also blocked by the peripherally acting antagonist AM6545, further supporting the argument that cannabinoids regulate feeding through a peripheral mechanism. However, additional studies by our group demonstrated that food restriction did not prevent glucocorticoid-induced obesity, suggesting that while hyperphagia may accompany this phenotype it is not the driving force. Accordingly, our current data argue that persistent elevations in circulating glucocorticoid produce a state of obesity and the metabolic syndrome, which is dependent upon endocannabinoid signaling in the periphery. Preliminary data from gene arrays obtained in adipose tissue indicate that this process may involve a breakdown in insulin receptor signaling, which is preserved by peripheral blockade of CB₁ receptors.

CANNABINOID REGULATION OF BODY GROWTH

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It is now established that the endocannabinoid (EC) system is involved in the regulation of skeletal remodeling and bone mass. Another critical skeletal activity is driving body growth. In vertebrates, skeletal elongation occurs mainly by enchondral ossification, an intricate process controlled by multiple hormones and growth factors intrinsic to the epiphyseal growth plate (EGP) whose action is concerted to ensure the precise rate of skeletal formation and to confer unique growth properties to the organism. Because the use of marijuana/hashish during pregnancy leads to babies shorter than those born to nonusers, we set out a project to examine the role of EC system in skeletal growth. We show here in femoral and vertebral cartilaginous growth plates that CB1 and CB2 are specifically expressed in hypertrophic, but not other chondrocytes. In addition, these cells express the 2-AG biosynthetic enzymes diacylglycerol lipase (DAGL) α and DAGL β . We could also isolate 2-AG from growth plates. Femora of CB1 and/or CB2 deficient mice at the end of the accelerated growth phase are approximately 5 percent longer than those of wild type controls. Ovariectomized (OVX) mice show enhanced growth rate due to the alleviation of estrogen growth inhibitory effects. Four-week treatment of the OVX mice with the CB2 specific agonist HU-308, at 10 mg/Kg/day, reverses this growth acceleration. Administration of THC, at 5 mg/Kg/day to normal mice during their rapid growth phase (5-11 weeks of age) slowed down their skeletal growth, which in turn resulted in lower weight but unaltered fat content. Ex vivo THC challenging of growth plate chondrocytes dose dependently (10^{-11} - 10^{-7} M) inhibited hypertrophic-cell nodule formation. Collectively, these findings demonstrate a local EC system in the cartilaginous growth plate with a growth inhibitory role for CB1 and CB2. Physiologically, CB activation downregulates longitudinal skeletal elongation. Defining the involvement of ECs and their receptors in body growth may aid the development of therapies to prevent short stature. Perhaps more importantly, our findings may provide an argument against the use of cannabis among teenagers. Also, growth retardation/stimulation becomes a parameter to be considered in initiation of cannabinoid based therapies for patients at an age prior to growth cessation.

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ENDOCANNABINOID MODULATION ATTENUATES ETHANOL-INDUCED NEURODEGENERATION DURING WITHDRAWAL

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It is widely accepted that endocannabinoids protect the CNS from a variety of insults and neurodegenerative diseases including traumatic brain injury, stroke and multiple sclerosis. Literature suggests that this protection could be the result of an attenuation of excitotoxicity and inflammation. Interestingly, binge alcohol consumption, a pattern of intake characteristic of alcohol use disorders, increases susceptibility neurodegeneration and studies have shown that excitotoxicity and inflammation are key contributing mechanisms. It has been hypothesized that alcohol-induced brain damage and the associated cognitive deficits play a critical role in the transition from casual consumption to compulsive alcohol intake. Therefore, it is reasonable to speculate that targeting the endocannabinoid system will be efficacious in preventing alcohol-induced brain damage and aid in the recovery from alcoholism. We hypothesized that targeting the endocannabinoid system in a model of alcohol-induced excitotoxicity would attenuate neuronal cell death. To test this hypothesis, organotypic hippocampal slice cultures (OHSC) were used. Bilateral hippocampi were dissected from 8 day old Spague-Dawley rat pups. Hippocampi were then sectioned on the coronal plane at 200 μm and plated on biopore membrane inserts in culture medium. The resulting cultures were allowed to mature *in vitro* for 5 days before being exposed to either control media or 50 mM ethanol (EtOH) media for 10 days. Following exposure, cultures were withdrawn for 24 hours in the presence of 5 μM NMDA, 2.5 $\mu\text{g/mL}$ Propidium Iodide (to assess cellular damage), and respective drug treatment. As expected, we found that 10 days of EtOH exposure significantly potentiated NMDA toxicity ($p = < 0.0001$) after 24 hours of withdrawal. Interestingly, application of 50.0 nM URB597, a fatty acid amide hydrolase (FAAH) inhibitor, was able to completely reverse the ethanol withdrawal-induced (EWD) potentiation of NMDA toxicity ($p = < 0.0001$), while having no effect on NMDA treatment alone. In order to determine whether the neuroprotective effects of FAAH inhibition were mediated through CB1 receptor activation, we applied the CB1 antagonist SR141716 (0.01 to 10.0 μM) to both control cultures and EWD cultures. Surprisingly, we found that SR141716 also attenuated EWD potentiation of NMDA neuronal damage in a concentration-dependent manner with statistical significance reached at the 10 μM dose of SR141716 ($p = < 0.05$). Taken together, our data suggests that the endocannabinoid system is a valid target for preventing alcohol-induced neurotoxicity. Additionally, it appears that the observed neuroprotection is dependent upon EtOH-induced neuroadaptations in the hippocampus as neuroprotection was only observed in EtOH treated cultures. It is interesting that we observed neuroprotection by indirectly activating cannabinoid activity by FAAH inhibition and by directly antagonizing CB1 receptors and leads us to speculate on the differential mechanisms underlying these different treatment strategies.

EXPRESSION AND CELLULAR DISTRIBUTIONS OF CANNABINOID CB₂ RECEPTORS IN MOUSE BRAIN

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The presence of CB₂ receptors in the brain has been subject to debate. Previous studies using *in situ* hybridization failed to detect CB₂ receptor mRNA in brain. However, more recent studies have claimed to find CB₂ expression on both neurons and glial cells in brain and two CB₂ receptor isoforms (CB_{2A}, CB_{2B}) in the brain using highly sensitive Taqman probes. To further address this issue, we used quantitative RT-PCR, immunoblot and double immunohistochemistry assays to detect mouse brain mCB₂ mRNA and proteins in wild-type (WT) mice and a strain of CB₂-knockout (CB₂^{-/-}) mice in which the C-terminal-coding mRNA sequence is deleted (Buckley, et al., 2000). Using the specific Taqman mCB_{2A} and mCB_{2B} probes that target the N-terminal-coding sequence of the gene, we detected both mCB_{2A} and mCB_{2B} isoforms with brain mCB_{2A} 5-10-fold higher than mCB_{2B} in WT and CB₂^{-/-} mice. However, when using a specific Taqman probe (mCB_{2A}-ko) that target the deleted C-terminal-coding gene sequence, we detected mCB₂-mRNA in spleen and striatum in WT and CB₁^{-/-}, but not CB₂^{-/-} mice. These data suggest that specific mCB₂-mRNAs are expressed in mouse brain. Next, we examined whether mCB₂ receptors are expressed in mouse brain by Western immunoblot assay. We initially tried two CB₂ antibodies purchased from Santa Cruz and α -Diagnosis, which recognize the C-terminal of mCB₂ receptors, but failed due to very low immunoreactivity of the antibodies (no band by 1:50). However, with CB₂ antibody purchased from Alomone (ACR-002) that recognizes the 3rd intracellular loop (epitope: 228-242 aa) or another antibody custom designed and generated at NIDA (NIDA-5633) that recognizes the C-terminus (epitope: 326-340 aa) of mCB₂ receptors, we detected a high density of CB₂-immunoblotting band (at a MW ~38kD) in spleen and a low-to-moderate density in striatum in WT and CB₁^{-/-} mice, which was substantially diminished in the present strain of CB₂^{-/-} mice. Further, double immunofluorescent labeling demonstrated a moderate density of mCB₂-staining in multiple types of neurons, including dopaminergic neurons (labeled by TH-Ab) in the ventral tegmental area and substantia nigra, GABAergic neurons (by parvalbumin-Ab) in the cerebral cortex, striatum and cerebellum, and glutamergic neurons (by vGluT2-Ab) and astrocytes (by GFAP-Ab) in the cortex and cerebellum. These findings, combined with our recent behavioral, neurochemical and electrophysiological findings, suggest that CB₂ receptors may be expressed on neuronal cells and functionally modulate brain function.

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ENDOCANNABINOID 2-ARACHIDONOYLGLYCEROL SUPPRESSION OF COX-2 EXPRESSION IS MEDIATED VIA PPAR- γ

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Endocannabinoids display anti-inflammatory and neuroprotective properties. However, the mechanisms responsible for these beneficial effects are largely unknown. We demonstrated previously that endocannabinoid 2-arachidonoylglycerol (2-AG) protects hippocampal neurons by limiting COX-2 expression via a CB₁ receptor-dependent MAPK/NF- κ B signaling pathway (Zhang & Chen, JBC 283: 22601-22611, 2008). The purpose of the present study was to determine whether peroxisome proliferator-activated receptor- γ (PPAR γ), an important nuclear receptor, mediates 2-AG-induced inhibition of NF- κ B phosphorylation and COX-2 expression and COX-2-enhanced miniature spontaneous excitatory postsynaptic currents (mEPSCs) in hippocampal neurons in culture. We observed that both exogenous and endogenous 2-AG-produced suppressions of NF- κ B-p65 phosphorylation, COX-2 expression and excitatory synaptic transmission in response to pro-inflammatory interleukin-1 β (IL-1 β) and lipopolysaccharide (LPS) were inhibited by GW9662, a selective PPAR γ antagonist. PPAR γ agonists 15-deoxy- Δ 12,14-prostaglandin J₂ (15d-PGJ₂) and rosiglitazone mimicked the effects of 2-AG on NF- κ B-p65 phosphorylation, COX-2 expression and mEPSCs, and these effects were eliminated by antagonism of PPAR γ . Moreover, exogenous application of 2-AG or elevation of endogenous 2-AG by inhibiting its hydrolysis with URB602 or JZL184, selective inhibitors of monoacylglycerol lipase (MAGL), rescued the IL-1 β - and LPS-induced reduction of PPAR γ expression. However, the 2-AG-produced restoration of the reduced PPAR γ expression by LPS was blocked or attenuated by pharmacological or genetic inhibition of the CB₁ receptor. Our results suggest that 2-AG suppression of COX-2 expression is through CB₁ receptor-dependent PPAR γ expression, which is an important and novel signaling pathway underlying endocannabinoid 2-AG-produced resolution of neuroinflammation in response to pro-inflammatory insults.

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2-ARACHIDONOYLGLYCEROL MODULATES CUE-EVOKED DOPAMINE RELEASE DURING REWARD-SEEKING

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Endocannabinoids are theorized to modulate brain reward processes. When an animal experiences a rewarding event, situational environmental stimuli (i.e., cues) predicting reward availability develop incentive properties which then motivate the seeking of future rewards. Our working hypothesis is that endocannabinoids in the ventral tegmental area (VTA) specifically affect reward-seeking by modulating cue-evoked subsecond dopamine release events, which are thought to carry incentive salience. When an animal is presented with a motivationally salient cue, VTA dopamine neurons fire in brief high frequency bursts. As a result, subsecond dopamine concentrations are released into terminal fields such as the nucleus accumbens while endocannabinoids are released from cell bodies in the VTA. We investigate how endocannabinoids affect subsecond dopamine release during reward-seeking by measuring accumbal dopamine concentrations in near real-time using fast-scan cyclic voltammetry while pharmacologically manipulating endocannabinoid signaling. Using this approach, we previously demonstrated that disrupting endocannabinoid signaling using rimonabant (0.3 mg/kg IV) decreased cue-evoked dopamine concentrations and reward-seeking (assessed as the latency to respond following lever presentation) in Sprague-Dawley rats. These findings led us to further hypothesize that increasing endocannabinoid levels will facilitate reward-seeking and cue-evoked dopamine release.

In the present study we tested the effects of multiple drugs that have been shown to raise endocannabinoid tissue content on cue-evoked dopamine concentrations during reward-seeking. Reward-seeking was maintained in a cued-intracranial self-stimulation task. In this task, responding for electrical currents delivered directly into the VTA was maintained under a fixed ratio 1 schedule with variable inter-trial intervals while dopamine concentrations were simultaneously assessed in the nucleus accumbens. We first tested the effects of the putative endocannabinoid uptake inhibitor VDM11. Contrary to our expectations, VDM11 (3-5.6 mg/kg IV) failed to facilitate reward-seeking or dopamine signaling. Rather, VDM11 produced a dose-dependent decrease in reward-seeking and cue-evoked dopamine concentrations, a finding that is more consistent with VDM11 decreasing presynaptic CB1 receptor activation. We next tested the effects of URB597 (0.1-0.56 mg/kg IV), a fatty acid amide hydrolase inhibitor, which failed to alter reward-seeking. Finally we tested the effects of JZL184, a monoacylglycerol lipase inhibitor. JZL184 (3-10 mg/kg IV) produced a dose-dependent increase in reward-seeking and cue-evoked dopamine concentrations. Together, these data suggest: a) the endocannabinoid system modulates reward-seeking by sculpting supranormal patterns of dopamine release b) during reward-seeking endocannabinoids are released on demand through an endocannabinoid transporter that is involved in the bidirectional support of these molecules c) 2-arachidonoylglycerol but not anandamide is the primary endocannabinoid involved in modulating cue-evoked dopamine signaling during reward-seeking.

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DEPRESSION IN CARDIAC SURGERY PATIENTS IS ASSOCIATED WITH REDUCED PERIOPERATIVE PLASMA ENDOCANNABINOID LEVELS

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Introduction: Patients after cardiac surgery (CS) are at risk for the development of stress-related disorders including posttraumatic disorder, anxiety and depression resulting in an impaired long-term outcome. Depression is one of the most common complications after heart surgery. There is evidence that the endocannabinoid system (ECS) plays a role in depression and other stress-related disorders¹ as activated endocannabinoid signalling in the brain controls adaptation processes to aversive situations and limits the stress reaction². It has already been shown that impairment of endocannabinoid signalling by CB1 receptor blockade in patients with cardiometabolic disorders led to a significant increase in the incidence of depression, particularly in patients exposed to stressful situations or experiences³. We therefore measured plasma levels of the endocannabinoids ANA and 2-AG during the perioperative phase of CS and showed that patients with an impaired EC response were at an increased risk for postoperative depression.

Methods: ANA and 2-AG were measured in 106 CS patients pre- and postoperatively, at post-operative days 1-3 (POD 1-3), at ICU discharge (97 patients) and at 6 months after CS (68 patients). Depression was diagnosed by using the depression subscale of the PTSS-10 stress symptom questionnaire⁴, the Hamilton Depression Rating Scale (HDRS) and a standardized psychological interview.

Results: In 16 out of the 68 interviewed patients (23.5%) depression could be diagnosed at 6 months after CS. These patients had significantly higher PTSS-10 depression subscores at that timepoint ($p < 0.01$) with no significant differences at the preoperative time point and at discharge from the ICU ($p = 0.22$). Patients developing depression had significantly *lower* anandamide plasma levels during the immediate post-operative period followed by an ANA increase at discharge from the ICU. Likewise, the perioperative 2-AG response in patients with depression was significantly *lower* when compared to mentally healthy individuals who demonstrated a statistically significant endocannabinoid *increase* throughout the post-operative period (Figure 2). HDRS scores correlated negatively with higher anandamide ($r = -0.32$, $p = 0.04$, $n = 58$) and 2-AG levels ($r = -0.44$, $p < 0.01$, $n = 58$) during ICU treatment.

Conclusions: These data suggest that cardiac surgery patients with reduced perioperative plasma endocannabinoid levels have an increased risk developing postoperative depression.

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- (1) Neurosci.Biobehav.Rev. 2008; 32: 1152-60
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- (3) Journal of the American Medical Association 2008; 299: 1547-60\
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CANNABIDIOL REDUCED ANXIETY IN TREATMENT-NAÏVE SOCIAL PHOBIC PARTICIPANTS

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Generalized Social Anxiety Disorder (SAD) is one of the most common anxiety conditions, with lifetime prevalence estimates of 7–12%. SAD is characterized by excessive and persistent fear and avoidance of a wide variety of social situations. Cannabidiol (CBD), one major non-psychotomimetic compound from *Cannabis sativa*, showed anxiolytic effects in animal and human studies. In the present study, we investigated the anxiolytic effects of CBD in participants with SAD taking part in the simulation of public speaking test (SPST). Twenty-four subjects with generalized SAD and 12 healthy controls were selected. All participants were undergraduate students, treatment-naïve (either by pharmacotherapy or psychotherapy) and without any other concomitant psychiatric disorder. Subjects were randomly allocated into three experimental groups: 1) 12 SAD subjects received CBD 600 mg (SAD-CBD); 2) 12 SAD subjects received placebo (SAD-P) and 3) 12 healthy controls (CONTROL) received no medication. Groups were matched according to gender, age, years of education and socioeconomic level. Each volunteer participated in only one experimental session. The Visual Analogue Mood Scale (VAMS) and Negative Self-Statement scale (SSPS-N) were administered at six different time points during SPST. Baseline measurements (B) were collected after a 15min adaptation, followed by a double blind single oral CBD or placebo dose, and a pre-stress measurement (P) 80min after drug ingestion. Immediately thereafter, subjects received instructions and had 2 minutes to prepare a 4-minute speech about “the public transportation system of your city.” Anticipatory speech measurements (A) occurred before the start of speaking, and the Speech was interrupted in the middle for speech measurements (S). Post stress measurements (F1 and F2) were made 15 and 35 minutes after the end of the speech, respectively. VAMS anxiety factor scores were significantly higher for SAD-P in relation to CONTROL in the A, S, and F1 measures. SAD-CBD had an intermediate score that differed significantly from SAD-P and CONTROL during the S phase. Scores of the SSPS-N evidenced significant differences between SAD-PLAC and SAD-CBD at the A and S phases and between SAD-PLAC and CONTROL at the S phase. No significant differences were observed between SAD-CBD and CONTROL. These results provide further evidence that CBD has anxiolytic properties, although additional double blind, placebo controlled studies are needed.

POSTNATAL NMDA-INHIBITION INDUCES ALTERATIONS IN CANNABINOID CB₂ RECEPTOR EXPRESSION

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Inhibition of the glutamate NMDA receptor in mice induces impairment of emotional and cognitive functions, and alterations in the sensorimotor gating which resembles human schizophrenic behaviour. We have previously shown that postnatal administration of phencyclidine (PCP), an NMDA antagonist, leads to behaviour paradigms relevant to anxiety and symptoms associated with schizophrenia when animals reach adulthood. In this study, we have characterised the biochemical expression of CB₂ receptors in different brain regions. PCP was administrated at 20 mg/kg every other day between postnatal days 3 and 15. The CB₂ receptor expression was evaluated by Western blot analysis and real-time PCR. Fluorescent labelling of the CB₂ receptor antibody revealed a major band at 45 kDa. In the brain stem, CB₂ receptor expression was significantly reduced from $100.0 \pm 7.7\%$ (n=11) in vehicle-injected mice to $77.6 \pm 6.0\%$ ($p < 0.05$; n=10) and in the basal ganglia to $86.5 \pm 10.7\%$. A comparison between the left and the right cortices revealed that CB₂ receptor expression was reduced to $83.7 \pm 8.4\%$ in the left cortex while no change was detected in the right cortex. The reduction in CB₂ receptor expression in the brain stem of PCP-treated mice led to a significant inhibition in receptor activation as measured by radioligand [³⁵S]GTP_γS binding. Further characterization of mRNA expression level of the CB₂ receptor revealed a significant increase of $37.6 \pm 0.08\%$ ($p = 0.005$) in the brain stem. In the left cortex there was an increase of $16.1 \pm 0.06\%$ while no change has been detected in the right cortex. These results suggest that inhibition of glutamate transmission induces cerebral lateralization in CB₂ receptor expression. Alterations seen in the left but not in the right cortex are in line with human studies which have detected cerebral lateralization in schizophrenics using MRI imaging. These results suggest a role for cannabinoid CB₂ receptor in schizophrenia.

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STRESS-INDUCED BEHAVIORAL ADAPTATION IS ASSOCIATED WITH ENHANCED 2-ARACHIDONOYLGLYCEROL SIGNALING IN THE AMYGDALA AND PREVENTED BY CHRONIC ENDOCANNABINOID AUGMENTATION

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Chronic stress is the most important environmental risk factor the development and exacerbation of mental illnesses ranging from depression to schizophrenia. Chronic homotypic stressors exert profound effects on the endocannabinoid (eCB) system. Accumulating evidence indicate that chronic stress down-regulates CB₁ receptor function in several brain regions, but paradoxically increases levels of 2-arachidonoylglycerol (2-AG). However, the effects of these divergent adaptations on eCB-mediated synaptic plasticity and stress-related behavioral changes are not well understood.

We utilized whole-cell patch clamp electrophysiology to determine the effects of chronic restraint stress on eCB-mediated synaptic plasticity at inhibitory synapses in the basolateral amygdala (BLA) of male ICR mice. We also determined the effects of chronic restraint stress on behavioral measures of anxiety and depression, and the effects of pharmacological augmentation of 2-AG levels on these behavioral changes. The behavioral effects 2-AG augmentation in control mice was also determined.

Recordings of evoked inhibitory postsynaptic currents (IPSCs) from BLA pyramidal neurons were conducted in control mice, and mice exposed to 10 consecutive days of restraint stress. In control animals, low-frequency afferent stimulation (LFS) had no effect on IPSC amplitude, however, in stressed mice LFS produced a robust presynaptic long-term depression (LTD_{GABA}). This LTD_{GABA} was blocked by pre-incubation with SR141716, and attenuated by the DAGL inhibitor THL, and a combination of group I metabotropic glutamate receptor antagonists. The ability to induce LTD_{GABA} was lost after 7 days of recovery from stress. LTD_{GABA} could be induced in control mice in the presence of the MGL inhibitor JZL184, which alone produced a small depression in IPSC amplitude in control mice. JZL184 administered acutely increased brain 2-AG levels and reduced anandamide levels, but had no behavioral effect on the elevated plus-maze, novelty-induced suppression of feeding test, and did not affect general locomotor activity. However, chronic administration of JZL184 prior to each restraint exposure prevented the development of stress-induced anxiety.

These data highlight a role for 2-AG in the synaptic and behavioral adaptations to chronic stress. In the BLA, chronic stress gates the induction of LTD_{GABA}, indicating a hyperactive eCB system in this region after chronic stress. Our behavioral data suggest augmentation of 2-AG signaling can prevent certain aspects of stress-induced pathology, and that MGL inhibition could represent a novel potential target for the treatment of stress-related neuropsychiatric disorders.

ACUTE STRESS INCREASES CIRCULATING N-ACYLETHANOLAMINES, INCLUDING ANANDAMIDE, IN HEALTHY HUMANS

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Stress plays an important role in psychiatric disorders, and recent preclinical evidence indicates that the endocannabinoid system is involved in responses to stress. Stress activates the central endocannabinoid system, which, in turn, is thought to negatively modulate HPA-axis activation during stress-recovery. This study aimed to investigate the effect of acute psychological stress on circulating levels of endocannabinoids (eCB) and their structural analogues in healthy human volunteers.

71 young adults participated in two sessions in which they were exposed to either a standardized psychosocial stress procedure (Trier Social Stress Test) or a control task. Blood samples for eCB and cortisol assays, cardiovascular and subjective measures were obtained before and at regular intervals after the stress or the control task. Serum levels of the endocannabinoids arachidonylethanolamide (anandamide, AEA) and 2-arachidonoylglycerol (2-AG), as well as of the N-acylethanolamides (NAEs) N-palmitoylethanolamine (PEA) and N-oleoylethanolamine (OEA) were determined using isotope-dilution liquid chromatography/mass spectrometry.

Compared to the control condition, stress increased serum concentrations of the NAEs including AEA. Increases in PEA after stress were positively correlated with increases in serum cortisol after stress. Baseline (pretask) levels of Anxiety were negatively correlated with baseline levels of AEA and OEA.

These results represent the first data in healthy humans indicating that stress increases circulating levels of NAEs including AEA in serum. This finding is consistent with preclinical findings supporting a role for eCBs in stress. Further research is needed to elucidate the function of these lipid mediators, and to explore their potential as therapeutic agents.

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CHRONIC ANTIPSYCHOTIC ADMINISTRATION DURING ADOLESCENCE ALTERS LATER THC EFFECTS ON COGNITION

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Marijuana abuse and psychiatric disorders are highly co-morbid. Although the direction and nature of this association have not been fully elucidated, one consequence is that co-administration of marijuana and psychiatric drugs is not uncommon and may occur as early as during adolescence. In an earlier study, we reported that antipsychotic administration affected endocannabinoid system functioning [Wiley et al., 2008, *Neuropharmacology*, 55: 1183-1190]. In this study, sub-chronic treatment with the antipsychotics haloperidol and clozapine attenuated maximal stimulation of CB₁ receptor-mediated G-protein activity in the striatum (clozapine) and prefrontal cortex (both antipsychotics) without changes in CB₁ receptor number in adult female rats whereas it did not alter CB₁ receptor-mediated G-protein activation in any brain region in adult male rats and in adolescents of either sex. These findings suggest that sex is an important determinant of antipsychotic modulation of the endocannabinoid system.

The purpose of this study was to determine the effects of chronic administration of antipsychotic medication during adolescence on later response to the effects of Δ^9 -tetrahydrocannabinol (THC) on cognitive functioning. Specifically, adolescent male and female rats were exposed to saline, haloperidol (0.3 mg/kg) or clozapine (10 mg/kg) twice daily from postnatal day (PN) 27 to PN80. Subsequently, they were trained to perform a sustained attention task with multiple trials, in which one response was required following presentation of a light and another response was required when the light did not appear. All rats acquired the task. In rats that received saline during adolescence, THC dose-dependently decreased accuracy primarily in the signaled trials (i.e., increased miss rate). While both sexes showed increased omissions, females showed this effect at lower THC doses. Haloperidol-treated rats exhibited a similar pattern of effects across sexes. In contrast, clozapine-treated male rats showed greater deficits in sustained attention at THC doses that did not alter omission rates than did clozapine-treated female rats. These results suggest that chronic dosing with the atypical antipsychotic, clozapine, during adolescence may enhance the detrimental effects of later administration of THC on attention in males, but not in females.

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CANNABINOID PHARMACOKINETICS IN EXPECTORATED ORAL FLUID FOLLOWING CONTROLLED SMOKED CANNABIS

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Introduction: Oral fluid (OF) is increasingly accepted as an alternative matrix for drug testing due to its non-invasive collection under direct observation by gender-neutral staff. However, questions remain about its effectiveness for monitoring cannabinoid intake. Novel analytical approaches achieving simultaneous cannabinoid quantification in OF suggest that recent cannabis smoking can be identified. Controlled cannabis administration studies are needed to establish which cannabinoid analytes to monitor, appropriate cutoff criteria, and OF detection windows, all key parameters for improving interpretation of OF tests. **Methods:** Healthy cannabis smokers provided written informed consent to participate in this IRB-approved study. Participants smoked 1 marijuana cigarette (6.8% THC) *ad libitum*. OF specimens were collected by expectoration once prior to and periodically after smoking for up to 22h. Specimens were analyzed by 2-dimensional GCMS with limits of quantification of 0.25 ng/mL for Δ^9 -tetrahydrocannabinol (THC), cannabidiol (CBD), and 11-hydroxy-THC (11-OH-THC), and 1 ng/mL for cannabinol (CBN), all with electron impact, and 5 pg/mL for 11-nor-9-carboxy-THC (THCCOOH) with negative chemical ionization. **Results:** 10 cannabis smokers (9M, 1F, 18-46 years) self-reported smoking a mean \pm SD 11.4 \pm 2.2 days in the 14 days prior to admission. OF specimens were collected -0.5h before and 0.25, 0.5, 1, 2, 3, 4, 6, and (optionally, $n=6$) 22h after the start of smoking. Only 5 THC (median 0.2 ng/mL; range 0.0-43.6) and 10 THCCOOH (33.6 pg/mL; 8.4-581.3) positive specimens were obtained at baseline. Six specimens from 4 individuals could not be collected between 0.25-1h due to reduced salivary excretion after cannabis smoking. Maximum THC, CBD, and CBN concentrations occurred 0.25h after smoking, while THCCOOH concentrations generally peaked within 2h. THC was quantifiable in 72 specimens (90.0%) at concentrations up to 22,370 ng/mL. Median (range) THC concentration was 2634 ng/mL (264.5-22370) at 0.25h, decreasing 10-fold within 1h to 283.5 ng/mL (35.4-1030) and 100-fold over 3h to 23.5 ng/mL (1.5-206.0). Four of 6 specimens were still THC-positive (median 0.7 ng/mL; range 0.0-10.3) 22h after dosing. Four specimens in 3 participants were 11-OH-THC-positive (range 0.3-1.3 ng/mL) within 2h after smoking. THCCOOH was identified in 71 specimens (88.8%) at concentrations up to 3519 pg/mL. Five of 6 specimens were THCCOOH-positive (median 10.9 pg/mL; range 0.0-24.0) at 22h. CBD in 50 (62.5%) and CBN in 46 (57.5%) OF specimens were always with concurrent THC. CBD duration of detection was >22h and <22h for CBN. THC duration of detection was >22h in 3 of 6 participants at the recommended Driving under the Influence of Drugs, Alcohol and Medicines (DRUID) confirmation cutoff of 1 ng/mL, and in 2 subjects at the US Substance Abuse and Mental Health Services Administration (SAMHSA) 2 ng/mL cutoff. A suggested criterion of THC \geq 2 ng/mL and THCCOOH \geq 20 pg/mL reduced detection windows to <22h with 50 of 72 THC-positive specimens. **Conclusions:** Quantification of multiple cannabinoids in OF improved interpretation of OF tests by identifying recent cannabis smoking, extending the cannabinoid detection window and reducing the potential for detection of passive cannabis smoke exposure. Supported by the IRP, National Institute on Drug Abuse (NIDA), NIH.

INHIBITORY CONTROL IN YOUNG CANNABIS USERS: RELATIONSHIPS WITH CANNABIS USE AND SYMPTOMS OF CANNABIS ADDICTION

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Cannabis use is associated with deficits in learning and memory that appear to dissipate approximately one month after abstinence. Recent studies show deficits in inhibitory control among heavy cannabis users that may persist for longer. It remains unclear if such deficits predate the onset of a cannabis use disorder or if they are a consequence of cannabis use. Indeed, the role of inhibitory control in symptoms of cannabis addiction is not well understood. In this study, we compared 17 to 24 year-old current cannabis users (CU, $n = 65$) and matched non-using controls (NU, $n = 65$) on a measure of episodic memory (the Hopkins Verbal Memory Test – Revised; HVLTR) and several measures of inhibitory control: the Iowa Gambling Task (IGT), Go-Stop Task, and Monetary Choice Questionnaire. We also examined relationships between neurocognitive performance and cannabis addiction severity using the Marijuana Screening Inventory (MSI-X) and the total number of current DSM-IV symptoms for cannabis abuse and dependence (DSM-SX). Groups were well matched on numerous demographic, mental health, and substance use parameters. We used a fairly stringent exclusion criteria, which included history of significant neurological or mental health problems; developmental or learning disorders; current use of psychotropic medications; positive urine toxicology for substances other than cannabis; significant or frequent, recent alcohol consumption; significant or recent use of other drugs; or abuse or dependence for any other drugs. Participants in the CU group were regular cannabis users, all endorsed cannabis as their drug of choice, and used cannabis within the 30 days prior to their study visit. One-way ANOVAs revealed significant differences between the CU and NU groups on HVLTR Total Immediate Recall ($p = .01$) and Delayed Recall ($p = .03$). No significant differences were observed for any of the inhibitory control tasks (all p -values $> .43$). Among CU, multiple regressions were conducted with performance on each inhibitory control task and HVLTR Immediate Recall as independent variables and MSI-X or DSM-SX as dependent variables. Only IGT scores predicted greater severity of DSM-SX ($p < .01$), even when controlling for amount of cannabis use. No significant associations were evident with the MSI-X. In summary, we found that young cannabis users with minimal comorbidities showed deficits in memory, but not in inhibitory control relative to healthy matched controls. However, poorer decision-making in this sample was associated with more symptoms of cannabis addiction, regardless of their amount of cannabis use. Our results suggest that, unlike deficits in memory, deficits in inhibitory control may not be a consequence of cannabis use; rather, they may be more relevant to the development of cannabis addiction among young cannabis users.

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THE EFFECT OF ADOLESCENT THC TREATMENT ON THE ENDOCANNABINOID SYSTEM MATURATION IN SPECIFIC BRAIN AREAS AND NEURAL REFINEMENT

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Adolescence is increasingly viewed as an important developmental window, where ongoing neuroplastic modifications occur in the central nervous system. These include changes in dendritic spine density, synaptic rearrangements, myelination of nerve fibers, changes in neurotransmitter concentrations and their receptor levels. The endocannabinoid system too seems to undergo maturation during the adolescent transition period, therefore, exposure to cannabinoids during adolescence may conceivably alter the physiology of the endocannabinoid system. Moreover, as this system seems to be critically involved also in the late brain developmental phase, adolescent exposure to cannabinoids might as well disrupt processes involved in brain maturation and neural refinement specific of this developmental window.

On these bases, the first aim of the present work was to study the maturation of the endocannabinoid system from adolescence into adulthood, in term of receptors and endogenous ligands, in the brain areas more involved in the modulation of emotional behaviour (i.e. the prefrontal cortex, nucleus accumbens, amygdala and hippocampus) and how adolescent exposure to THC may affect it. To this aim, CB1 receptor binding studies and evaluation of anandamide (AEA) and 2-arachidonoylglycerol (2-AG) levels were performed at 46 post natal day (PND), 60 PND and 75 PND, representing the middle and late adolescence, and adulthood, respectively. The same study was then performed on the brains from animals exposed to THC during adolescence (35-45 PND). Our results indicate that both CB1 receptor and AEA increased during adolescence and then decreased with different intensity when animals reached adult age. 2-AG levels showed a mirrored pattern, decreasing through adolescence and then increasing at adulthood. Adolescent THC treatment altered this course, with lower CB1 receptor levels during adolescence and, in some brain regions, also at adulthood; lower AEA in the prefrontal cortex and nucleus accumbens and increased 2-AG in the hippocampus and amygdala.

The second step of this work was to investigate the occurrence of neuronal refinement in this specific time window and the impact of adolescent THC treatment on it. To this aim we monitored synaptophysin and PSD95 levels at 46, 60 and 75 PND in control and THC-treated rats. PSD95 progression appears to be the most affected by adolescent exposure to THC. In fact, while control animals exhibited a decrease in this protein levels at 60 PND and then an increase at 75 PND, this trend was flattened in the nucleus accumbens and significantly altered in the other brain areas.

Taken together these results suggest that adolescent THC treatment disrupts the physiological course of the endocannabinoid system maturation into adulthood and the processes of synaptic remodelling proper of the adolescent period.

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NICOTINE REWARD: A ROLE FOR CB2 RECEPTORS?

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Nicotine is the main addictive component of tobacco that plays a major role in dependence. Nicotine acts on the brain to produce both dependence and withdrawal upon tobacco smoking or cessation. Emerging evidence suggest that the endogenous endocannabinoid system play an important role in nicotine reward and reinforcement. Our lab and others has previously reported that CB1 receptors mediate nicotine reward in rodents (Merritt *et. al.* 2008). Rimonabant, a CB1 antagonist, blocked nicotine effects in the conditioned place preference (CPP) and i.v. self-administration in mice and rats. However, the involvement of CB2 receptors has yet to be studied in nicotine's effect.

We therefore investigated the role of CB2 receptor on nicotine reward using SR144528, a selective CB2 antagonist. Nicotine reward in the mouse was evaluated in an unbiased conditioned place preference paradigm (CPP) in induction. Mice were pretreated with either SR144528 (1 and 3 mg/kg i.p.) or Vehicle (i.p.) 15 min before conditioning in the morning for days 2-4. On test day the mice were tested in a drug free state. Our results showed that CB2 antagonist dose-dependently decreased nicotine preference compared to nicotine control in our CPP paradigm. Similarly, nicotine preference at the dose of 0.5 mg/kg was significantly decreased in CB2 knockout mice. Furthermore, pretreatment with O-1966, a selective and potent CB 2 agonist, dose-dependently potentiated the effects of a low dose of nicotine (0.1 mg/kg) in the CPP test. Finally, nicotine-induced antinociception and hypothermia after acute injection was not reduced by SR144528 or changed in CB2 CB2 knockout mice.

Our results suggest that CB2 receptors may play an important role in nicotine reward.

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PRECLINICAL EVALUATION OF MARIJUANA'S APPETITIVE, REWARDING AND PSYCHOACTIVE PROPERTIES

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Marijuana use produces a wide array of physiological effects in humans, including increases in appetite, facilitation of reward and psychoactive intoxication. These and other effects of marijuana have been successfully modeled in several preclinical paradigms using THC and other cannabinoids. These studies have provided a great deal of information regarding the behavioral effects associated with marijuana use, their underlying mechanisms, and have assisted in evaluating therapeutic utility of the endocannabinoid system. Unfortunately, few, if any, studies have validated the effects of inhaled marijuana in preclinical models. Thus, the present study sought to evaluate the effects of inhaled marijuana in established mouse models of feeding, intracranial self-stimulation (ICSS) and drug discrimination.

Mice were exposed to marijuana smoke using an apparatus that drew smoke from burning plant material (marijuana or placebo) through Tygon tubing to a manifold under vacuum pump and flow regulation. A solenoid alternated the flow of smoke and fresh air to mice every 8 s to mimic puffing. Mice were placed into individual holding tubes that connected to the manifold and actively inhaled smoke during the exposure period.

First, the appetitive effects of marijuana were examined in male ICR mice that were food-deprived 24 h prior to marijuana exposure, then were allowed access to standard rodent chow for 2 h. Compared to placebo, marijuana increased food consumption, as did THC. Next, marijuana and THC's effects were evaluated in C57BL/6 mice trained to respond for ICSS under a progressive schedule of reinforcement. Under this schedule, the response requirement for a subject to obtain a reinforcer increases progressively (FR1, FR2, FR4, etc.). Once a subject ceases to respond for reinforcement this is referred to as the "breakpoint", a dependent measure of reward strength. Marijuana and THC both increased breakpoints, and this was reversed by rimonabant, demonstrating a CB1-mediated facilitation of responding for ICSS. Finally, male C57BL/6 mice were trained to discriminate 5.6 mg/kg THC from vehicle in a two-choice drug discrimination task and marijuana's effects were evaluated 10 and 30 min following inhalation exposure. Marijuana dose-dependently substituted for THC 10 min following exposure, which was attenuated by rimonabant. However, marijuana failed to substitute for THC 30 min post-exposure.

These findings demonstrate marijuana's ability to alter feeding behavior, facilitate reward and produce THC discriminative stimulus effects in a manner comparable to other cannabinoids. Taken together, these results provide evidence of the predictive validity of these animal models as relates to the potential of cannabinoids to model relevant physiological changes in humans following marijuana use.

ADVANCED ANALYTICAL METHODS FOR THE SURVEILLANCE AND DETECTION OF SYNTHETIC CANNABINOIDS IN HERBAL FORMULATIONS AND BIOLOGICAL MATRICES

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The appearance of synthetic cannabinoids in herbal products marketed as “spice” or “incense” marks one of the latest stages in the distribution and abuse of designer drugs or controlled substance analogs, the history of which includes the design of fentanyl in the 1980s; the development of the ring-substituted phenethylamines in the late 1980s and the tryptamines in the 1990s; and the piperazines and cathinone derivatives in the 2000s. Of particular concern is the recognition that relatively little is known about the prevalence and long term health consequences of their use, as methods for the detection and confirmation of synthetic cannabinoids in bulk, drug formulations, and biological fluids are currently being developed. Furthermore, there are hundreds of published compounds with cannabinoid receptor affinity and activity, in several chemical classes, and it might be anticipated that new substances will continue to appear in various formulations for their illicit use. This presents a constant challenge for the forensic and toxicological identification of new substances for the prompt assessment of risk and, where necessary, implementation of control measures by both public health and law enforcement agencies.

In an effort to develop rapid methods to analyze herbal formulations sold as incense, but suspected to contain synthetic cannabinoids, an automated solid-phase micro-extraction and gas chromatography/mass spectrometry (SPME-GC/MS) method was developed. The analytical approach allows for the detection of JWH-018, JWH-073 and other synthetic cannabinoid analogs, from small quantities of materials (~50 mg), without requiring extraction, concentration or derivatization. Analysis of over 20 products, obtained from retail stores in North Carolina, revealed the presence of at least one synthetic cannabinoid in each product. The most frequently encountered compounds in these products were JWH-073 and JWH-018. Several products had two or more analogs, and one product appeared to have a novel, previously unreported JWH analog present. RTI scientists are also developing high resolution MS approaches to enhance selectivity and sensitivity, as well as enable data interrogation approaches that provide better surveillance across entire classes of compounds. The analytical information (e.g., MS, NMR, FTIR, etc.) obtained from the analysis of herbal preparations containing synthetic cannabinoids, as well as synthetic standards of cannabinoid analogs and metabolites, are currently being incorporated into 'Forensic DB' (www.forensicdb.org), a web-based cheminformatic database for the retention, review and ongoing collection of spectral data pertaining to toxins, drugs and other compounds of interest to the forensic community.

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THE GENOME OF *CANNABIS SATIVA* L.

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Cannabis sativa L. (marijuana, hemp; Cannabaceae) has a long history of cultivation as a source of fibre and oil-rich seeds, and as a medicinal and psychoactive herbal drug. Its unique effects are due to the presence of cannabinoids, which include Δ^9 -tetrahydrocannabinol (THC) and more than 70 related prenylated polyketide natural products. Despite the widespread cultivation and use of cannabis, our understanding of cannabis biology and cannabinoid biosynthesis has been hampered by the lack of genomic information.

Here we report the draft genome of a medical marijuana variety of *Cannabis sativa* ('Purple Kush') and its comparison to two hemp cultivars ('Finola' and 'USO-31'). We used the Illumina and Roche/454 platforms to generate an approximate $\times 100$ combined average coverage of the ~ 900 Mb diploid cannabis genome. Both cultivars were sequenced to $\times 50$ coverage using Illumina technology. We also generated RNA-Seq data of a normalized whole-plant 'Purple Kush' cDNA library, as well as from individual 'Purple Kush', 'Finola' and 'USO-31' tissues (root, shoot, stem, leaf and flower). Our preliminary genome assembly encompasses 350 Mb of the non-repetitive genome, covering an estimated 90% of gene sequences. Assembly of the RNA-Seq reads gave 33,167 contigs with an N50 of 1.64 kb.

Cannabinoids accumulate in high concentrations in glandular trichomes on the floral tissues of female cannabis plants. In addition to the genome and whole plant transcriptome approaches described above, we have used targeted analysis of the transcriptome of glandular trichomes for discovery of cannabinoid biosynthetic enzymes. These analyses have yielded several candidate enzymes involved in cannabinoid pathway and precursor supply, including polyketide synthases and aromatic prenyltransferases. Our genome analysis provides sequence information useful in determining the genetic basis for the metabolic divergence of marijuana and hemp, and for breeding and biotechnological improvement of this important medicinal and agricultural plant.

ENDOCANNABINOID SIGNALING ORIGINATING IN THE LUMINAL EPITHELIUM IS CRITICAL FOR STROMAL DECIDUALIZATION

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Although the predecidual response in mice is initiated on day 4 of pregnancy prior to the attachment of the blastocyst with the luminal epithelium (LE), the full-fledged stromal decidualization begins with blastocyst implantation on day 5. Initially, LE cells are in direct contact with the blastocyst. Previous studies have shown that the LE is indispensable for triggering the decidualization response. However, the underpinning mechanism by which the LE influences the decidualization process in the stroma remains elusive. It is also not clearly understood as to how the blastocyst attachment signaling is transmitted to the stromal cells by the LE. In this study, we found that cannabinoid receptor 1 (CB1) is expressed in the LE, and its ligand anandamide is also detected in the uterus around the time of implantation. These results suggest that endocannabinoid signaling plays an important role in early pregnancy events. In fact, we found that decidual response is remarkably dampened in mice missing CB1. In contrast, the response was more robust in *Faah* null females. The fact that CB1 is primarily detected in the LE supports our hypothesis that CB1 plays a role in regulating the epithelium-mediated signaling in stromal decidualization. COX2 is induced in the epithelial cells at the initial stages of decidualization. We found that COX2 expression is appreciably lower in CB1 deficient uteri at the time decidualization. These findings provide evidence that the LE lacking CB1 transmits sub-threshold signals to the underlying stroma, compromising decidualization and thereby pregnancy outcome.

Acknowledgements: This work was supported by an NIH Grant (Grant No. DA06668).

FATTY ACIDS, *N*-ACYL ETHANOLAMINES AND INFLAMMATION: TISSUE, TIME AND COMPOUND SPECIFIC EFFECTS

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N-acylethanolamines (NAEs) are fatty acid (FA) derived compounds and involved in a variety of biological processes, including inflammation. The best studied NAE so far is arachidonoyl ethanolamide (AEA), but combinations with other fatty acids also exist, such as oleoyl ethanolamide (OEA), stearoyl ethanolamide (SEA), palmitoyl ethanolamide (PEA), docosahexaenoyl ethanolamide (DHEA) and eicosapentaenoyl ethanolamide (EPEA). The profile of NAEs is a reflection of long-term dietary FA intake. FAs are known to affect inflammatory processes, and this could in part be mediated through conversion to their NAEs since AEA, PEA, DHEA and EPEA display anti-inflammatory properties. At the same time, LPS triggers the synthesis and release of NAEs, suggesting that there is a link between dietary fatty acids, their related NAEs, and inflammation. However, this has not been thoroughly investigated yet.

We examined the effect of LPS on NAE levels in liver, ileum and adipose tissue in adult male C57/bl6 mice. In the first study, mice were on a n-3 FA rich diet for six weeks. LPS or saline was administered i.p. and mice were euthanized at t=0, 2, 4, 8, and 24 hr (4/group). In the second study, mice were on either a control diet, or a 1% (w/w) or 3% (w/w) fish oil (FO) diet for six weeks to investigate the effect of dietary FAs on NAE levels. Mice were euthanized 24 hrs post injection with LPS or saline (8/group).

Time- and tissue-dependent effects of LPS on NAE levels were observed in the first study. Interestingly, after an increase at 2 hr LPS, ileal levels of AEA, DHEA, EPEA, and OEA decreased gradually, whereas PEA remained high until 24 hr, and SEA levels continued to increase from 4 hr after LPS. In liver, levels of most NAEs increased 4 hrs after LPS and decreased subsequently, but PEA levels decreased at 24 hr and SEA remained unaffected by LPS. In adipose tissue, most compounds were increased by LPS from 4 hr and further, whereas SEA was decreased by LPS after 24 hr. In the second study, the FO diets dose-dependently increased DHEA and EPEA levels, and were further increased by LPS in liver and adipose tissue. The FO diets had different effects on other NAE levels and their modification by LPS. For example, ileal AEA was reduced in the 1% FO diet, but unaffected in the 3% FO group compared to the control diet. The increase in AEA levels after LPS was less in the FO groups compared to the control diet group.

In conclusion, i.p. LPS differentially affects *in vivo* NAE levels in liver, ileum and adipose tissue. The FO diets result in different NAE patterns, and also modulate the effect of LPS on tissue NAE levels.

ROLE OF CALCIUM RELEASE FROM INTRACELLULAR STORES IN ENDOCANNABINOID PRODUCTION AND RETROGRADE SYNAPTIC SIGNALING IN THE CEREBELLAR CORTEX

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Introduction. Presynaptic CB₁ cannabinoid receptors can be activated by endogenous cannabinoids (endocannabinoids) synthesized by postsynaptic neurons. This phenomenon is termed endocannabinoid-mediated retrograde synaptic signaling (for review see Kano et al., *Physiol Rev* 89: 309–380, 2009). The production of endocannabinoids in postsynaptic neurons can be triggered by an increase in intracellular Ca²⁺ concentration and by activation of G $\alpha_{q/11}$ protein-coupled receptors. The aim of the present study was to clarify the role of Ca²⁺ released from the endoplasmic reticulum (ER) in endocannabinoid production triggered by activation of G $\alpha_{q/11}$ protein-coupled receptors.

Methods. The experiments were performed on mouse cerebellar slices. Glutamatergic excitatory (eEPSCs) and GABAergic inhibitory (eIPSCs) postsynaptic currents were elicited by electrical stimulation in the molecular layer of the cerebellar cortex and were recorded in Purkinje cells with patch-clamp techniques.

Results. Superfusion of DHPG (5 x 10⁻⁵ M), an agonist of the G $\alpha_{q/11}$ protein-coupled mGluR1 receptor, suppressed eEPSCs recorded in Purkinje cells. The CB₁ antagonist rimonabant prevented this suppression, pointing to the involvement of endocannabinoids and CB₁ receptors. The DHPG-induced suppression of eEPSCs was delayed and strongly attenuated after depletion of the ER calcium stores by the SERCA pump inhibitors thapsigargin (5 x 10⁻⁵ M) and cyclopiazonic acid (3 x 10⁻⁵ M). Similarly, the suppression of GABAergic eIPSCs by DHPG was also attenuated after depletion of ER calcium stores. In a further series of experiments, mGluR1 receptors in the postsynaptic Purkinje cells were activated by endogenous glutamate released by burst stimulation (10 pulses / 100 Hz) of the presynaptic parallel fibres. The burst stimulation suppressed eEPSCs, and abolishment of this suppression by rimonabant verified the role of endocannabinoids and CB₁ receptors. Calcium store depletion by thapsigargin strongly attenuated the burst-induced suppression of eEPSCs.

Conclusions. The suppression of the glutamatergic and GABAergic synaptic transmission by DHPG and endogenous glutamate was delayed and strongly attenuated in experiments in which intracellular Ca²⁺ stores were depleted by SERCA pump inhibitors. This observation points to the important role of Ca²⁺ release from intracellular Ca²⁺ stores in endocannabinoid production when this production is triggered by activation of G $\alpha_{q/11}$ protein-coupled receptors. Probably, stimulation of PLC β by G $\alpha_{q/11}$ proteins alone does not maximally activate the synthetic pathway of the endocannabinoid 2-arachidonoylglycerol.

CALCIUM-CALMODULIN DEPENDENT KINASE II INTERACTS WITH DIACYLGLYCEROL LIPASE AND IS REQUIRED FOR ENDOCANNABINOID-DEPENDENT SYNAPTIC SUPPRESSION IN THE STRIATUM

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The striatum is the input nucleus of the basal ganglia, integrating cortical and thalamic excitatory afferents to modulate action selection and motor coordination. Endocannabinoids (eCBs) mediate short- and long-term depression (LTD) of excitatory transmission in the dorsal striatum, and alterations in eCB signaling have been observed in rodent and primate models of Parkinson's disease. The most abundant eCB in the central nervous system is 2-arachidonoyl glycerol (2-AG), which is produced on demand through the hydrolysis of 1-stereoyl-2-arachidonoyl glycerol (SAG) by diacylglycerol lipase α (DGL α) in response to mGluR and L-type calcium channel activation. However, the molecular basis for Ca²⁺-dependent regulation of DGL activity is unclear. In order to provide insight into these mechanisms, we isolated DGL α immune complexes from solubilized mouse striatum and analyzed them using a shotgun proteomics approach. Calcium/calmodulin-dependent protein kinase II (CaMKII α) was identified in striatal DGL α immune complexes, and we confirmed that CaMKII α interacts with and phosphorylates DGL α in co-transfected HEK293T cells. Moreover, the interaction is stabilized by CaMKII activation and autophosphorylation at Thr286.

To determine the synaptic consequence of the interaction between DGL α and CaMKII α , we used whole cell patch clamp recordings from T286A-KI mice (with a knock-in mutation of Thr286 to Ala in CaMKII α to prevent autophosphorylation), and found that these mice have a strong deficit in striatal eCB-LTD. In whole cell patch experiments in WT mice, the intracellular loading of the DGL α substrate SAG caused significant CB1 receptor-dependent depression of evoked EPSCs. This effect was blocked by the DGL inhibitor tetrahydrolipstatin (THL) and was absent in both CB1KO and T286A-KI mice. These data indicate a strong deficit in striatal 2-AG signaling in T286A-KI mice, which could contribute to the hyperactive behavioral phenotype they display. Consistent with this hypothesis, hyperactivity of T286A-KI mice in open field arenas can be rescued by pharmacological augmentation of 2-AG levels by JZL-184, which has no significant effect on locomotion in WT mice. In combination, these data demonstrate that CaMKII is a critical regulator of striatal 2-AG signaling and synaptic plasticity through its interaction with DGL α . Deficits in this pathway may contribute to movement disorders and other neurological disorders involving the striatum.

A NOVEL FAAH INHIBITOR FACILITATES EXTINCTION OF FEAR MEMORIES IN MICE

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Fear extinction is a form of learning in which a conditioned fear response is reduced by repeated (non-reinforced) exposure to a learned fear stimulus. Previous work has shown that fear extinction is impaired in mice lacking the CB1 receptor. Here we tested the hypothesis that administration of a novel fatty acid amide hydrolase (FAAH) inhibitor (AM3506) would boost brain endocannabinoid levels and facilitate fear extinction in mice. We found that systemic administration of AM3506 (0.25-1.0 mg/kg i.p.) preferentially inhibited FAAH activity in the brain and increased anandamide induced stimulation on CB1 receptor *ex vivo* by [³⁵S]GTPγS binding assays. Endogenous anandamide levels in the basolateral amygdala and prefrontal cortex were elevated after fear extinction training, and augmented by pre-training systemic administration of AM3506 (1 mg/kg, i.p.). In a mouse model of impaired extinction (the S1 inbred strain), systemic administration of AM3506 significantly facilitated fear extinction. This effect was blocked by systemic co-administration of the CB1 receptor antagonist rimonabant (10 mg/kg, i.p.). The fear-extinction facilitating effect of systemic AM3506 was also blocked by bilateral microinfusion of rimonabant (2 μg) into the basolateral amygdala. These data demonstrate that AM3506 is a potent enhancer of fear extinction, and likely acts via stimulation of CB1 receptors in the amygdala. Our study identifies FAAH as a novel target for the treatment of neuropsychiatric disease characterized by impaired fear extinction, such as posttraumatic stress disorder.

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BOTH DGL α AND DGL β REGULATE THE PRODUCTION OF 2-ARACHIDONOYL GLYCEROL IN AUTAPTIC HIPPOCAMPAL NEURONS

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Cannabinoids are part of an endogenous signaling system consisting of cannabinoid receptors and endogenous cannabinoids (eCBs) as well as the enzymatic machinery to produce and break down these eCBs. Depolarization-induced suppression of excitation (DSE) is a form of cannabinoid CB1 receptor-mediated inhibition of synaptic transmission that involves the production of the eCB 2-arachidonoyl glycerol (2-AG). The role of diacylglycerol lipase (DGL) in the formation of 2-AG during depolarization is controversial: hippocampal DSI is absent in DGL α knockout mice (Tanimura et al., 2010, Gao et al., 2010), yet DGL inhibitors do not always block DSI (Min et al., 2010). Furthermore, the two isoforms of DGL—DGL α and DGL β —are impossible to distinguish by available pharmacological tools. Of these DGL α has received considerable attention in 2-AG production. What role, if any, might be played by DGL β remains largely unexplored. Autaptic hippocampal neurons are well-suited to a comparative study of the roles of these enzymes in mediating the robust endogenous DSE present in these cultured neurons under controlled conditions (Straiker & Mackie, 2005).

To investigate the importance of DGL α and DGL β for 2-AG production in DSE we developed siRNA constructs for each enzyme. We found that our constructs reduced expression of these proteins by 70% and 72% in DGL α - and DGL β -expressing HEK293 cells, respectively. We then used these constructs to knockdown DGL expression in autaptic hippocampal neurons.

We find that knockdown of DGL α results in a substantial reduction of DSE, shifting the ‘depolarization response curve’ from an EC50 value of 1.6 sec to 6.4 sec (non-overlapping 95% confidence intervals). Interestingly, DGL β diminishes DSE as much or more (EC50 54.8 sec, non-overlapping CI), suggesting that DGL β is responsible for a portion of 2-AG production in autaptic neurons. In separate experiments we confirmed that siRNA for each DGL did not reduce the expression of the other DGL in HEK293 cells.

We conclude that both DGL α and DGL β play a role in endogenous cannabinoid signaling. Our results identify DGL β as a new potential target for modulation of the cannabinoid signaling system.

DHEA, THE ETHANOLAMIDE METABOLITE OF DHA, HAS IMMUNE-MODULATING PROPERTIES

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Dietary consumption of long chain omega-3 poly-unsaturated fatty acids (*n*-3 LCPUFAs), in particular DHA (22 : 6*n*-3) and EPA (20 : 5*n*-3), has been associated with several positive health effects, including a reduction of risk factors for cardiovascular diseases, diabetes, obesity/metabolic syndrome and inflammatory bowel disease. A low-grade inflammatory process is thought to play a pivotal role in the sustainment and possibly the induction of many of these disorders. After dietary intake, LC-PUFAs are incorporated in membranes and can be converted to their corresponding *N*-acyl ethanolamines (NAEs). Members of the group of NAEs, e.g. AEA, PEA and OEA, are well-known to possess biological activity via cannabinoid or PPAR receptors. However, little is known on the biological role of the 'fish-oil-derived' NAE metabolites.

Studies were conducted in mice RAW264.7 and thioglycollate-induced peritoneal macrophages, stimulated by LPS (0.1-1 µM). Nitric oxide, MCP-1 and IL-6 release were assessed by Griess assay and ELISA, respectively. Expression levels of MCP-1 and iNOS were established by RT-qPCR. Levels of DHEA and EPEA were determined using LC-MS/MS.

We found that DHEA (docosahexaenoyl ethanolamine), the ethanolamide of DHA has anti-inflammatory properties. Among a series of fatty acid-derived unsaturated NAEs (including AEA), DHEA was found to be the most potent inhibitor of LPS-induced nitric oxide release in RAW264.7 macrophages. In LPS-stimulated peritoneal mice macrophages, DHEA significantly suppressed production of monocyte chemoattractant protein-1 (MCP-1), IL-6 and nitric oxide, all key inflammatory mediators in diseases associated with the metabolic syndrome. Inhibitory effects of DHEA were found to take place at the transcriptional level as DHEA reduced gene expression of MCP-1 and inducible nitric oxide synthase (iNOS). Additionally, studies were undertaken to reveal the molecular pathways via which DHEA exerts its action. Moreover, it was confirmed that 3T3-L1 preadipocytes can form DHEA and eicosapentaenoyl ethanolamine (EPEA) out of their fatty acid precursors DHA and EPA, respectively, and we demonstrated the presence of DHEA in human plasma. These results imply a potential modulatory role for DHEA as DHA-derived mediator in inflammatory processes. Further studies should elucidate the relevance of this 'fish oil mediator' *in vivo* and the relation to other inflammatory pathways in which DHA is involved.

PROPOFOL ALTERS MEMORY VIA INTERACTION WITH THE ENDOCANNABINOID SYSTEM

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Introduction: Propofol is a popular and very commonly used anesthetic that is known to reduce postoperative nausea and vomiting (PONV), shows postoperative mood alterations and is associated with a higher incidence of dreaming compared to other general anesthetics. It is also known to inhibit fatty acid amide hydrolase (FAAH) degrades the endocannabinoid anandamide. It already has been shown that i.p. administration of propofol leads to increased brain anandamide contents in mice¹. In patients, our group has shown that propofol anesthesia results in moderate increases or unchanged plasma endocannabinoid activity while in those patients undergoing anesthesia with volatile agents had decreased endocannabinoid plasma levels.

As the endocannabinoid system (ECS) plays a crucial role in regulating emotional arousal and stress hormone effects that strengthen memory² we are now able to show that propofol alters memory consolidation specifically while midazolam and pentobarbital does not affect memory. In a second set of experiment we administered propofol together with cannabinoid receptor 1 blocker rimonabant to investigate the interaction of propofol and ECS.

Methods: Male Sprague-Dawley rats were trained and tested in an inhibitory avoidance apparatus and received inescapable footshock after entering the dark compartment. On retention test 48 hours later the latency to enter the dark compartment completely was recorded and taken as a measure of retention. Drug administration was performed either immediately, 30 or 180 minutes after training to clarify what part of memory consolidation is effected.

We administered propofol (200 and 300 mg/kg) together or alone with rimonabant (1mg/kg), midazolam (30 and 50 mg/kg) or vehicle i.p.

Results: Propofol in anesthetic dose (300 mg/kg) leads to significant longer retention latency that represents a stronger memory consolidation when administered immediately or 30 minutes after training session. This effect does not occur when infusing propofol 180 minutes after training or when rimonabant was administered concomitantly.

Midazolam do not affect retention latencies, no matter of dose or timepoint of administration.

Conclusion: We showed in rats that propofol induces memory consolidation via an interaction with the endocannabinoid system while midazolam does not affect consolidation.

Like it is also known for cannabinoids³ and glucocorticoids⁴ this memory modulation is time-dependent. Concomitant administration of rimonabant blocked memory consolidation induced by propofol what leads to the conclusion that this memory effect of propofol needs endocannabinoid activity. Therefore propofol, in contrast to the benzodiazepine midazolam, enhances emotional memory consolidation when administered immediately after a stressful event by augmenting endocannabinoid signaling.

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CANNABINOID-1 RECEPTOR CONTRIBUTIONS TO CEREBELLAR LEARNING

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The cerebellar cortex, particularly the molecular layer, contains the highest density of cannabinoid receptors (CBR1) in the mammalian brain. The CBR1s are located on the axon terminals of parallel fibers, climbing fibers, stellate cells, and basket cells where they inhibit neurotransmitter release. One way to probe the function of these cerebellar receptors is through eyeblink conditioning. Eyeblink conditioning is a type of learning established by paired presentations of a conditioned stimulus (CS) such as a tone or light and an unconditioned stimulus (US) that elicits the blink reflex. Conditioned stimulus information is projected from the basilar pontine nuclei to the cerebellar interpositus nucleus and cortex. The present study examined the effects of a CBR1 agonist, WIN55,212-2, on acquisition, retention, and extinction of eyeblink conditioning in rats. In Experiment 1, rats were given subcutaneous administration of 1, 2, or 3 mg/kg of WIN55,212-2 during 10 days of acquisition training. There was a dose-dependent impairment in acquisition, with no effect on spontaneous or non-associative blinking. In Experiment 2, 3 mg/kg of WIN55, 212-2 was given after 5 days of training with no infusions, followed by a day of training with an injection of the vehicle. Extinction (a type of inhibitory learning) training with Win 55,212-2 or vehicle injections was then administered for 2 days. Administration of WIN55,212-2 after learning produced a mild impairment in retention. Extinction of eyeblink conditioning was slower in the rats that were given WIN55,212-2. The findings support the hypothesis that CBR1s in the cerebellar cortex play important roles in cerebellar learning and extinction.

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ENDOCANNABINOID SIGNALING MEDIATES THE IMPAIRING EFFECTS OF STRESS-LEVEL GLUCOCORTICOIDS ON MEMORY RETRIEVAL

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It is well known that glucocorticoids (GCs) administered to rats or humans interact with the noradrenergic system to impair the retrieval of memory of emotionally arousing experiences (de Quervain et al., *Nature*, 394 (1998) 787-780; de Quervain et al. *Nat. Neurosci.*, 3 (2000) 313-314; Roozendaal et al., *J. Neurosci.*, 24 (2004) 8161-8169). These findings have important implications for understanding the neurobiological mechanisms underlying traumatic memories and anxiety disorders, e.g. post-traumatic stress disorder (PTSD). Clinical studies indicated that GC treatment results in a long-lasting reduction of re-experiencing (=retrieval) of traumatic information and other stress symptoms in PTSD patients (Schelling et al., *Biol. Psychiatry*, 55 (2004) 627-633). However, the mechanism of how GCs impair such memory retrieval mechanisms is poorly understood. Here, we investigated the hypothesis that the endocannabinoid (eCB) system, a fast-acting lipid-based stress-response system, might play an important role in regulating the impairing effects of GCs effects on memory retrieval.

We tested this hypothesis with four sets of experiments. For all experiments, rats were trained on a hippocampus-dependent contextual fear-conditioning (CFC) task and retention was tested 24 h later, all drug treatments were given 60 min before retention testing. First, we showed that systemic injections of corticosterone (0.3-3.0 mg/kg, sc), the major GC in rats, dose-dependently impaired the retrieval of CFC memory. Second, we found that a blockade of the eCB system in the hippocampus with the cannabinoid receptor type 1 (CB1) antagonist AM251 (0.35 ng) prevented the memory retrieval impairment induced by co-administered systemic corticosterone. Third, we found that a CB1 receptor agonist WIN 55,212-2 (10-30 ng) administered into the hippocampus is sufficient to induce CFC memory retrieval impairment. Fourth, we investigated a possible interaction between eCB and noradrenergic signaling in the hippocampus. We found that intrahippocampal infusions of the beta-adrenoceptor antagonist propranolol (1.25 µg) blocked the memory retrieval impairment induced by the CB1 receptor agonist WIN 55,212-2. Collectively, these findings indicate that memory retrieval impairment induced by GCs depends on both eCB and noradrenergic transmission.

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**DUAL INHIBITION OF FAAH AND MAGL
IN MICE IMPAIRS SHORT-TERM SPATIAL MEMORY
ASSESSED IN THE WATER MAZE**

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Acute administration of marijuana and THC impair short-term spatial memory in water maze tasks through a CB1 receptor mechanism of action. N-arachidonoyl ethanolamine (anandamide; AEA) and 2-arachidonoylglycerol (2-AG) are endogenous ligands for the CB1 receptor. Pharmacological inhibition or genetic deletion of either fatty acid amide hydrolase (FAAH) or monoacylglycerol lipase (MAGL), enzymes that regulate AEA and 2-AG, respectively, produce distinct pharmacological profiles of direct CB1 agonists, such as THC. However, dual inhibition of FAAH and MAGL elicits THC-like behaviors, such as THC generalization in drug discrimination. In the following studies we tested the hypothesis that dual inhibition of FAAH and MAGL would produce THC-like short-term memory impairment in the Morris water maze. FAAH $-/-$ and wild-type mice were trained in a repeated acquisition memory task mice. Doses (0, 20 and 40 mg/kg) of the selective MAGL inhibitor, JZL184, were then tested in each genotype. We then evaluated whether the memory impairing effects of JZL184 were mediated by a CB1 receptor mechanism of action. Finally, for comparison, the dual FAAH/ MAGL inhibitor JZL195 (20 mg/kg) and THC (10 mg/kg) were evaluated in the repeated acquisition memory task mice. Although the high dose of JZL184 disrupted memory performance in both genotypes, the low dose of JZL184 only disrupted memory in FAAH $-/-$ mice, indicating that spatial short-term memory impairment is particularly sensitive to dual inhibition of FAAH and MAGL. The CB1 receptor antagonist rimonabant blocked JZL184-induced memory impairment in both genotypes indicating that the memory impairing effect of MAGL inhibition, as well as the memory impairing effect of dual FAAH/ MAGL inhibition, was mediated by a CB1 receptor mechanism of action. Both JZL195 and THC also disrupted memory performance in the water maze providing complementary findings that dual inhibition of FAAH and MAGL can produce THC-like spatial short-term memory impairment. The results of this study reveal short-term memory impairment in mice treated with a high dose of the MAGL inhibitor, JZL184. Moreover, given that the low dose of JZL184 disrupted memory in FAAH $-/-$ mice and that the dual FAAH/ MAGL inhibitor JZL195 disrupted memory performance, these findings also indicate that dual inhibition of FAAH and MAGL can also impair short-term memory.

**THE CB2 RECEPTOR AGONIST GW405883 ATTENUATES
VARIABLE CHRONIC MILD STRESS-INDUCED SPATIAL
LEARNING DEFICITS AND NEURONAL CELL LOSS**

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Prolonged exposure to stress may result in a depressive state that is associated with hippocampal damage mediated by inflammatory cytokines produced by activated microglia. Recent evidence suggests that type 2 cannabinoid receptors (CB2) are expressed by central nervous system microglial cells. CB2 receptor activation inhibits activated microglia inflammation and promotes neuron survival and proliferation. In this study, rats were exposed to a 3-week variable chronic mild stress (CMS) paradigm followed by behavioral assessment of spatial navigation ability. Compared to untreated stress-exposed controls, rats that received daily injections of the CB2 agonist GW-405883 during CMS performed significantly better in Morris water maze evaluation of spatial learning and memory. Immunohistochemical evaluation NeuN cell counts demonstrated a greater number of neurons in CMS animals treated with GW-405833. Results suggest that the CB2 receptor may play a role in controlling stress-induced spatial learning and memory deficits associated with activated microglial-mediated hippocampal damage.

ALTERATIONS OF ENDOCANNABINOID SIGNALING, SYNAPTIC PLASTICITY, LEARNING AND MEMORY IN MONOACYLGLYCEROL LIPASE KNOCKOUT MICE

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Endocannabinoids (eCBs) are key signaling molecules that regulate synaptic transmission and a variety of physiological functions. eCB signaling is tightly regulated by eCB biosynthetic and degradative enzymes. The CB 2-arachidonoylglycerol (2-AG) is hydrolyzed primarily by monoacylglycerol lipase (MAGL). Here, we investigated whether eCB signaling, synaptic function and behavior were altered in MAGL knockout mice. MAGL^{-/-} mice exhibited prolonged depolarization-induced suppression of excitation (DSE) in cerebellar slices and inhibition (DSI) in hippocampal slices, providing genetic evidence that the inactivation of 2-AG by MAGL determines the time course of DSE and DSI. On the other hand, the magnitude of DSE or DSI was decreased, and a CB1 receptor agonist induced significantly less synaptic depression in MAGL^{-/-} mice, suggesting that sustained elevations of 2-AG in MAGL^{-/-} mice cause CB1 receptor desensitization. Genetic deletion of MAGL selectively enhanced theta burst stimulation (TBS)-induced long-term potentiation (LTP) in CA1 region of hippocampal slices but had no significant effect on LTP induced by high-frequency stimulation or long-term depression induced by low-frequency stimulation. The enhancement of TBS-LTP in MAGL^{-/-} mice appears to be mediated by 2-AG-induced suppression of GABA_A receptor-mediated inhibition. MAGL^{-/-} mice exhibited enhanced learning as shown by improved performance in novel object recognition and Morris water maze. These results indicate that genetic deletion of MAGL causes profound changes in eCB signaling, long-term synaptic plasticity and learning behavior.

SEX DIFFERENCES IN THC-INDUCED ANTINOCICEPTION AGAINST INFLAMMATORY PAIN

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Cannabinoids are antinociceptive in both acute and inflammatory pain models in male rodents. Additionally, cannabinoids are more potent and in some cases more efficacious in female compared to male rats, using acute pain models; however it is not known whether males and females respond differently to Δ^9 -tetrahydrocannabinol (THC) under conditions of chronic pain. As such, the aim of this study was to determine whether there are sex differences in THC's anti-hyperalgesic, anti-allodynic, and anti-inflammatory effects using the complete Freund's adjuvant (CFA) model. The behavioral and anti-inflammatory effects of vehicle vs. THC (0.32 or 3.2 mg/kg i.p.) were compared between gonadally intact male and female Sprague-Dawley rats at 1, 3, and 7 days after intra-plantar CFA injection; vehicle or THC was given each day, 30 min before testing began. THC attenuated both mechanical allodynia and thermal hyperalgesia as assessed by the von Frey and Hargreaves assays, respectively. Females showed less mechanical allodynia than males on day 3, whereas there was no sex difference in anti-hyperalgesia. Overall, THC decreased locomotor activity more in females than males. THC also decreased paw inflammation more in females than males. These results agree with previous studies demonstrating sex differences in cannabinoid-induced antinociception using acute pain models, and also suggest possible sex differences in the anti-inflammatory properties of THC. However, it is possible that THC's greater anti-allodynic effect in females is secondary to THC's greater sedative effect in females. Future studies comparing the effects of peripherally administered cannabinoids in females vs. males will test this hypothesis.

PROGESTERONE MODULATION OF Δ^9 – TETRAHYDROCANNABINOL-INDUCED ANTINOCICEPTION

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Systemically administered cannabinoids produce greater antinociceptive effects in female compared to male rats (Romero et al., 2002; Tseng & Craft, 2001). We have also demonstrated estrous cycle-related fluctuations in THC-induced antinociception (Craft & Leidl, 2008; Wakley & Craft, 2011). Whether these fluctuations are due to changes in estradiol, progesterone, or both is not known; there is limited evidence that each hormone can modulate cannabinoid-induced antinociception (progesterone in mice: Kalbasi Anaraki et al., 2008; estradiol in rats: Craft & Leidl, 2008). Therefore, the aim of this study was to examine how progesterone and estradiol alone and in combination modulate the effects of THC on measures of antinociception and locomotion in the rat.

Female Sprague-Dawley rats (N=6-10/hormone group) were ovariectomized (OVX) and a blank or estradiol-filled capsule was implanted *s.c.* On post-surgery day 8, a *s.c.* injection of progesterone or oil was administered; 4 hours later, Δ^9 -tetrahydrocannabinol (THC, 5 mg/kg, *i.p.*) or vehicle (1:1:18 ethanol:cremophor:saline) was administered. Antinociception was measured using warm water tail withdrawal and paw pressure tests at 15-240 min post-THC injection. Horizontal locomotion was examined in 5-min periods at 30-240 min post-injection, and catalepsy was measured at 30 and 60 min post-injection. THC produced time-dependent antinociception on the paw pressure test (time x THC: $F(3.5, 194.3) = 3.72, p < 0.01$). Peak paw pressure antinociception was observed between 15 & 30 min post-injection in all hormone groups, except females given progesterone alone (peak occurred at 60 min post-injection; time x THC x P4: $F(3.5, 194.3) = 2.85, p < 0.05$). No significant hormone group differences were observed in THC-induced antinociception on the tail withdrawal test. THC significantly suppressed locomotor activity 30 – 240 min post-injection (THC x time: $F(2.23, 121.4) = 13.35, p < 0.001$), but neither estradiol nor progesterone significantly altered THC-induced locomotor suppression. Similarly, neither hormone significantly altered THC-induced catalepsy. Estradiol significantly increased uterine weight (estradiol: $F(1, 53) = 271.27, p < 0.001$), however, THC slightly blunted the estradiol-induced increase in uterine weight (THC x estradiol: $F(1, 53) = 4.31, p < 0.05$).

These results suggest that while progesterone alone can modulate THC-induced antinociception in OVX females, its effects are assay-dependent. Neither ovarian hormone appears to modulate the motoric effects of THC. However, in both of our studies estradiol was administered chronically rather than cyclically; thus its effect (or lack thereof) may not reflect estradiol's actual modulation of cannabinoid action in a cycling female. We are currently employing an intermittent hormone injection approach to more accurately model the estrous cycle of female rats.

EVALUATION OF THE CANNABINOID CB₂ RECEPTOR-SELECTIVE COMPOUNDS AT GPR55: IMPLICATIONS FOR DRUG TARGETS IN PAIN

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The antinociceptive effect of CB₂ receptor selective cannabinoid agonists has been extensively investigated. Subsequently, selective CB₂ receptor agonists have been developed by scientists and pharmaceutical companies as alternative treatments aimed at alleviation of neuropathic pain. However, a pool of cannabinoid ligands also modulates GPR55 activity. Interestingly, a comparison of the behavioural responses of wild type mice with GPR55 knockout mice in pain models revealed the involvement of GPR55 in the conduction of neuropathic pain. These results suggest that the analgesic effects of certain CB₂ receptor selective ligands may be mediated by GPR55. In this study we evaluated the pharmacological actions of several CB₂ receptor selective ligands on the phosphorylation of ERK1/2 in human GPR55 transfected cells using the AlphaScreen® Surefire® assay. The compounds were evaluated in the presence or absence of LPI (L- α -lysophosphatidylinositol), an endogenous GPR55 agonist. JWH-133 was an inverse-agonist of GPR55-mediated ERK1/2 phosphorylation with an EC₅₀ of 164 nM and inverse of maximal stimulation of (-)28.9% \pm 6.40. Of the tested compounds, HU-308, BCP ((E)-beta-caryophyllene) and AM1241 had little/no effect up to a concentration of 10 μ M in which ERK1/2 phosphorylation above basal was 20.1% \pm 6.25 (n=3, p<0.02) for HU-308, 22.4% \pm 8.39 (n=3, p<0.05) for BCP, and 20.7% \pm 9.5 (not significant) for AM1241. LPI induced the stimulation of ERK1/2 phosphorylation between 146-250%. At 3 μ M, BCP and HU-308 had no effect on the maximal stimulation of LPI-induced ERK1/2 phosphorylation. But JWH-133 at this concentration significantly inhibited LPI-induced response (n=3, p=0.03). These results suggest that HU-308 and BCP are indeed CB₂ receptor selective ligands while some of the actions of JWH-133 may be mediated by GPR55.

THE EFFECTS OF SPINAL JZL184 ON NOCICEPTIVE PROCESSING IN THE ANAESTHETISED RAT

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The analgesic effects of the endocannabinoids 2-AG and AEA are established, but limited by their rapid enzymatic degradation by FAAH and MAGL, respectively. Focus has, therefore, shifted to preventing endocannabinoid catabolism via specific enzyme inhibitors. Recent reports identify JZL184 as a selective and potent MAGL inhibitor, with analgesic properties in the mouse. Here, we investigate the effects of spinal JZL184 on nociceptive processing in the rat.

In vivo spinal electrophysiology was utilised to determine the effects of cumulative spinal doses of JZL184 (25, 50 & 100µg) or vehicle (50µl) on mechanically-evoked responses of wide dynamic range (WDR) neurones in the dorsal horn of naive, anaesthetised, male rats. The role of the CB₁ receptor in these effects was also determined via spinal pre-administration of AM251 (1µg). In a separate group of rats, the ability of spinal application of JZL184 to alter carrageenan-induced WDR receptive field expansion, a marker of central sensitisation, was also investigated. To ascertain the mechanism of action, effects of spinal JZL184 on levels of endocannabinoids were assessed via LC-MS-MS, and MAGL activity was determined via an *ex vivo* tritiated 2-OG hydrolysis assay.

Spinal JZL184 dose-dependently inhibited both innocuous and noxious mechanically evoked responses of WDR neurones, with significant inhibition (>50%, p<0.001) seen with the highest dose. This effect was partially, but significantly, attenuated by CB₁ blockade via a single dose of AM251. Spinal JZL184 also blocked carrageenan-induced expansion of receptive fields of WDR neurones. Despite these physiological effects, we were unable to demonstrate significant elevation of 2-AG levels, nor blockade of MAGL activity, in the spinal cords of JZL184-treated rats.

Data reported here represent the first direct evidence of efficacy of JZL184 on nociceptive neuronal activity in the rat, and indicate the spinal cord as a possible site of action. These data are in line with previous reports of the anti-nociceptive properties of JZL184 in the mouse and rat. However, our inability to demonstrate a concomitant elevation of spinal cord 2-AG, or blockade of MAGL activity, following JZL184 treatment raises questions as to its mechanism of action in the rat.

THE ENDOGENOUS LIGAND PALMITOYLETHANOLAMIDE RELIEVES NEUROPATHIC PAIN VIA MAST CELL AND MICROGLIA MODULATION.

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We have recently shown that PEA induces relief of neuropathic pain partially through an action upon receptors located on the nociceptive pathway (Costa et al., Pain, 2008). The same receptors are present also on non-neuronal cells strongly involved in the maintenance of neuropathic pain; thus the aim of the present work was to ascertain whether part of the antinociceptive properties of PEA might be ascribed to a downregulation of non-neuronal mediators of pain response, such as mast cells in the periphery and microglia in the spinal cord. PEA (10 mg/kg) was administered i.p. to mice with chronic constriction injury of sciatic nerve (CCI) once a day for one week starting the day after the lesion. This therapeutic regimen evoked a relief of both thermal hyperalgesia and mechanical allodynia in neuropathic mice. After assessing PEA-induced relief of neuropathic pain, mice were sacrificed and sciatic nerves were submitted to different preparations and inclusions in order to evaluate the axon morphology (indicative of Wallerian degeneration) and the number of intact or degranulated mast cells, through both toluidine staining and immunostaining employing the polyclonal antibody anti-mouse mast cell protease I (MCP-I). Lumbar spinal cords (L3–L5) were excised, post-fixed over night in the paraformaldehyde solution and microglial cells were identified by immunoreactivity for Iba-1. The histological analysis of sciatic nerve sections showed a marked degeneration of myelinated fibers in CCI mice, which was substantially reduced after repeated administration of PEA, suggesting that the compound may favour myelin repair. The staining revealed that nerve injury caused a significant recruitment of mast cells that were confined in the side region of the sciatic nerve at 3 days after the injury, with a subsequent infiltration towards the inner part of the nerve at 8 day time point, when also mast cell morphology was clearly changed with many degranulated cells. Treatment of mice with PEA appeared to delay mast cell recruitment and protect mast cell against degranulation. Furthermore, there is microglial activation in the dorsal horn of the spinal cord following CCI-induced neuropathy that is modulated after PEA administration. In vitro studies performed on N9 microglial cells were conducted to ascertain a direct effect of PEA on activated microglia. These findings, in addition to prove the beneficial effects of PEA in chronic pain, suggest that the modulation of non-neuronal cells might represent an important tool to counteract the development of hyperalgesia in neuropathic pain states thus identifying new potential targets for analgesic medicine.

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PALMITOYLETHANOLAMIDE RELIEVES PAIN IN A MURINE MODEL OF DIABETIC NEUROPATHY

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The endogenous lipid palmitoylethanolamide (PEA) was shown to exert potent anti-inflammatory and analgesic effects in experimental models of inflammatory, visceral and neuropathic pain by acting via several possible mechanisms. On these bases, the present study wanted to expand the knowledge about PEA activity by exploring its antinociceptive properties in a mouse model of diabetic neuropathy, one of the most common peripheral neuropathies that occurs widely as a long-term complication of diabetes. Type I diabetes was induced in mice through chemical pancreatectomy by a single intraperitoneal (i.p.) injection of streptozotocin (STZ) at 120 mg/kg. Two weeks after STZ administration, when a significant mechanical allodynia was evident, diabetic mice received PEA (10 mg/kg i.p.) or its vehicle, once a day, for 7 days. The repeated treatment with PEA completely relieved mechanical allodynia already after 4 administrations, suggesting a strong efficacy of this drug in counteracting allodynic pain associated with diabetes. The relief of diabetic neuropathy induced by PEA was not due to a decrease in blood glucose levels: in fact, PEA-treated mice showed a marked hyperglycaemia like control ones. However, PEA treatment partially restored insulin serum levels to normal ones in diabetic mice, indicative of a first step of glycaemic control. Histological evaluation of pancreas from control and PEA-treated animals were performed to evaluate an eventual protective effect exerted by PEA. Since one of the important consequences of chronic hyperglycaemia is the enhanced oxidative stress, further studies were conducted investigating whether PEA can attenuate the diabetes-associated free radical injury; the repeated treatment with PEA evoked a significant reduction of the hepatic MDA levels, indicating that this drug was partially efficacy to protect against diabetes oxidative damage. In the light of the pivotal role of the growth factor NGF in nerve regeneration and that the diabetic neuropathy was associated with reduced levels or activity of NGF, we demonstrated that PEA treatment was able to increase the reduced levels of NGF at the level of the sciatic nerve, so promoting nerve regeneration and consequently suggesting an involvement of this neurotrophic factor in PEA-induced anti-allodynic effect. In conclusion, this study demonstrated PEA efficacy in relieving neuropathic pain associated with diabetes, and contributed to enlarge the therapeutic potential of this lipid molecule.

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EFFECTS OF Δ^9 -TETRAHYDROCANNABINOL ON PAIN-STIMULATED AND PAIN-DEPRESSED BEHAVIOR IN RATS

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Δ^9 -Tetrahydrocannabinol (THC), the primary active constituent of marijuana, produces robust antinociception in preclinical assays of pain-stimulated behavior; however, clinical studies have reported only small and inconsistent analgesic effects of THC in humans. This disparity suggests that preclinical antinociception may result from non-selective behavioral suppression rather than from a selective decrease in sensitivity to pain. To evaluate this possibility, the present study compared THC's effects in assays of pain-stimulated and pain-depressed behavior. Intraperitoneal (IP) injection of dilute lactic acid (1.8% in 1 ml/kg) stimulated a stretching response or depressed intracranial self-stimulation (ICSS) in separate groups of male Sprague-Dawley rats. THC (1-10 mg/kg, IP) dose-dependently blocked acid-stimulated stretching but only exacerbated acid-induced depression of ICSS. THC doses that decreased stretching and exacerbated acid-induced depression of ICSS also decreased control ICSS in the absence of a noxious stimulus. These findings suggest that preclinical antinociceptive effects of acute THC in assays of pain-stimulated behavior can be attributed to non-selective behavioral suppression rather than to a selective decrease in sensitivity to pain. Moreover, these results suggest that assays of pain-depressed behavior may be especially useful in translational research on development of cannabinoids as candidate analgesics. Finally, these results may have implications for the deployment of medical marijuana. "Pain" is cited as one indication for the use of medical marijuana in 14 of the 15 states that have approved its use, and large proportions of medical marijuana users within those states endorse "pain" as their primary diagnosis. However, the present results agree with clinical data in challenging the notion that THC is effective as an analgesic.

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ENDOCANNABINOID MECHANISMS ARE INVOLVED IN NITROUS OXIDE-INDUCED ANTINOCICEPTION

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Endocannabinoids and nitrous oxide (N₂O) are both involved in the control of pain and anxiety. They both produce their spectrum of behavioral effects through a variety of mechanisms, including interacting with the endogenous opioid system, GABA and glutamate synaptic activity, nitric oxide, and K⁺ “leak” channels. This study was conducted in order to determine whether N₂O might interact with the endocannabinoid system to produce its antinociceptive effects.

The antinociceptive responsiveness of male NIH Swiss mice to N₂O was assessed using the acetic acid abdominal constriction test, a test which is significantly more sensitive to detection of κ opioid antinociceptive activity than are thermal tests. Our previous studies have implicated κ opioid mechanisms in mediation of N₂O-induced antinociception. Different groups of mice were pretreated with the CB1 receptor antagonist/inverse agonist AM 251 (1 or 3 mg/kg); CB2 antagonist/inverse agonist AM 630 (3 or 10 mg/kg); anandamide transport-inhibitor AM 404 (1 mg/kg); or their respective vehicles. The mice were then exposed to either 25, 50 or 70% N₂O, and their response to in the abdominal constriction test was measured.

We found that: 1) Exposure to increasing concentrations of N₂O produced a concentration-related antinociceptive response; 2) Pretreatment with the CB1 receptor antagonist AM 251 but not the CB2 antagonist AM 630 caused a reduction in the magnitude of the N₂O-induced antinociceptive response; and 3) Pretreatment with AM 404 enhanced the antinociceptive response to N₂O.

These findings are consistent with the hypothesis that the endocannabinoid system contributes to N₂O-induced antinociception.

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INTRATHECAL CANNABINOID RECEPTOR 2 (CB2) AGONISTS ALTER SPINAL AND DORSAL ROOT GANGLION INFLAMMATORY FACTORS IN NEUROPATHIC RATS

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Chronic neuropathic pain is mediated by activated glial cells in the spinal cord and dorsal root ganglia (DRG). Chronic constriction injury (CCI) of the rodent sciatic nerve leads to light touch mechanical sensitivity (allodynia), and produces spinal (astrocytes and microglia) and DRG (Schwann cell) glial activation. Glial cell activation is well characterized to increase proinflammatory cytokine IL-1 β mRNA through the p38 MAPK and JNK signaling pathways. The cannabinoid receptor type 2 (CB2), identified in the central nervous system, is found predominantly on immune cells. Prior reports support that peri-spinal (intrathecal; i.t.) injections control pathological pain. Here we examined if pathological pain (allodynia) induced by CCI, as well as corresponding changes in spinal and DRG protein expression could be altered by i.t. CB2 agonists. We examined two structurally distinct CB2 agonists, AM1241 and AM1710, to determine maximal efficacy during neuropathy induced by CCI. Following i.t. AM1241 or AM1710, L4-L6 spinal cord with intact DRG were collected and fluorescent immunohistochemical (IHC) detection of markers for glial activation, phosphorylated p38MAPK (p-p38MAPK), inflammatory cytokines, as well as endocannabinoid degradative enzymes were examined. IHC fluorescent levels were quantitatively analyzed using fluorescent spectral analysis, which converts fluorescent wavelength intensity to a numerical value and subtracts background and autofluorescence, yielding a true fluorescent signal.

Results revealed neither AM1241 nor AM1710 altered normal threshold responses in sham-treated rats, but dose-dependently reversed CCI-induced allodynia for 1.5 and 3 hr, respectively, compared to vehicle injection. CCI-treated rats displayed increased spinal glial cell activation, p-p38 MAPK, as well as endocannabinoid degradative enzyme and cytokine levels compared to basal levels observed in sham-treated rats. While both CB2 agonists reset cytokine makers back to basal levels, only AM1241 decreased spinal p-p38MAPK expression. Similar effects were observed in DRG. These data support that i.t. AM1241 and AM1710 alter the expression of factors responsible for neuropathic pain.

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J-SERIES PROSTAGLANDINS REGULATE AEA-INDUCED APOPTOSIS

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Arachidonoyl ethanolamide (AEA) is a potent inducer of tumor cell apoptosis however its mechanism of cytotoxicity is unclear. We reported previously that AEA induces cell death in a COX-2-dependent manner. Our data now show that AEA-induced apoptosis is regulated by COX-2 metabolic products of the J-series. In tumorigenic keratinocytes which overexpress COX-2, AEA caused a concentration-regulated increase in J-series prostaglandin (PG) production and apoptosis. AEA-induced apoptosis was inhibited by the antioxidant, N-acetyl cysteine, indicating that reactive oxygen species generation was required. Antagonists of cannabinoid receptor 1, cannabinoid receptor 2, or TRPV1 did not block AEA-mediated cell death. In contrast, fatty acid amide hydrolase (FAAH) inhibition potentiated AEA-induced J-series PG synthesis and apoptosis. These results suggest that the metabolism of AEA to J-series PGs in cells that overexpress COX-2 activates the apoptotic pathway. Our data further indicate that the pro-apoptotic activity of AEA can be enhanced inhibiting FAAH. Since epithelial tumors commonly overexpress COX-2, AEA may be an effective agent to eliminate these cells.

**CENTRAL, BUT NOT SYSTEMIC, ADMINISTRATION OF A
PERIPHERALLY RESTRICTED CB₁ AGONIST SUPPRESSES
NAUSEA-LIKE BEHAVIOR IN RATS**

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Although rats do not vomit in response to a toxin challenge, they express conditioned gaping reactions when exposed to a flavor previously paired with an emetic agent. This conditioned gaping reaction serves as a rodent model of nausea-like behavior. CB₁ agonists modify conditioned gaping in rats in a manner that suggests they reduce nausea as well as vomiting. Systemic administration of the potent CB₁ agonist, HU-210, interferes with the establishment of LiCl-induced conditioned gaping reactions. There is evidence that both central and peripheral CB₁ receptors may be involved in the control of vomiting responses in emetic species but it is unclear whether the potential of CB₁ agonists to interfere with LiCl-induced nausea-like behavior in rats is centrally or peripherally mediated. We evaluated the ability of the peripherally restricted CB₁ agonist, CB13, delivered systemically and centrally to the lateral ventricles to modify the establishment of LiCl-induced conditioned gaping reactions in rats.

All rats had intra-oral cannulas surgically implanted prior to beginning experimental manipulations. Rats in the centrally administered groups were also implanted with a unilateral guide cannula directed at the lateral ventricle. For systemic administration, CB13 was prepared in a vehicle of 45% 2-hydroxypropyl- β -cyclodextrin at 0.3, 1.0 and 3.0 mg/2 ml. For central administration, CB13 was prepared in 100% DMSO in concentrations of 10 and 30 μ g/ μ l and 1 μ l was delivered at the rate of 1 μ l/min. Rats were pretreated with the appropriate CB13 dose and infused with 0.1% saccharin solution for 2 min followed immediately by an ip injection of LiCl (20 ml/kg of 0.15M). Forty eight hr later, the rats received a drug-free test trial and their orofacial reactions were video recorded and later scored.

When centrally administered, CB13 interfered with LiCl-induced conditioned gaping at 30 μ g, but not at 10 μ g. However, when peripherally administered CB13 did not attenuate LiCl-induced conditioned gaping at any dose. These results suggest that the anti-nausea-like effects of CB₁ agonists are centrally mediated.

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THE MAGL INHIBITOR, JZL184, ATTENUATES LiCl-INDUCED VOMITING IN THE *SUNCUS MURINUS* AND 2AG ATTENUATES LiCl-INDUCED NAUSEA-LIKE BEHAVIOR IN RATS

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The endogenous cannabinoid, anandamide, has been shown to suppress toxin-induced vomiting in a number of emetic species (Darmani, 2002; Van Sickle *et al.*, 2001; Parker *et al.*, 2009), as well as reduce lithium chloride (LiCl)-induced conditioned gaping in rats (a selective measure of conditioned nausea; Cross-Mellor *et al.*, 2007). However at present, only a few studies have evaluated the effects of the endogenous cannabinoid, 2-arachidonoylglycerol (2AG), on emesis, while its effect on nausea-like behaviors in rats remains unknown. We investigated the role of 2AG, as well as inhibition of its inactivating enzyme, monoacylglycerol lipase (MAGL), in the regulation of nausea and vomiting using a shrew (*Suncus murinus*) model of emesis and LiCl-induced conditioned gaping in rats.

A series of experiments evaluated the effects of pretreatment with the selective MAGL inhibitor, JZL184, as well as exogenous 2AG prior to administration of the emetogenic drug, LiCl. JZL184 (0, 16, 40 mg/kg), dose-dependently suppressed vomiting in the shrew in response to LiCl, and this effect was reversed by pretreatment with the CB1 antagonist/inverse agonist, AM-251. The ability of JZL184 to selectively inhibit MAGL in shrew brain tissue was observed using competitive activity based protein profiling (ABPP). Pretreatment with exogenous 2AG (0, 0.5, 1.25, 2.0 mg/kg) dose-dependently suppressed LiCl-induced nausea-like behavior (conditioned gaping) in rats, however, the anti-nausea-like effects of 2-AG were not reversed by pretreatment with AM-251 or the CB2 antagonist, AM-630. Instead, pretreatment with the cyclooxygenase (COX) inhibitor, indomethacin, reversed the ability of 2-AG, as well as its downstream metabolite, arachidonic acid (AA), to suppress LiCl-induced conditioned gaping. On the other hand, when rats were pretreated with a high dose of JZL184 (40 mg/kg), the suppression of conditioned gaping by 2AG was partially reversed by AM251. The suppression of conditioned gaping was not due to interference with learning because the same dose of 2AG did not modify the strength of conditioned freezing to a shock-paired tone.

These findings suggest that manipulations resulting in an elevation of 2AG may have anti-emetic/anti-nausea potential, and that downstream metabolites of 2AG and AA may be partially responsible for mediating the anti-nausea effects of exogenous administration.

ALTERATIONS OF ANANDAMIDE METABOLISM IN COELIAC DISEASE

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The endocannabinoid system has been extensively investigated in experimental colitis and inflammatory bowel disease, but not in coeliac disease where only a single study showed increased levels of the major endocannabinoid anandamide in the atrophic mucosa.

Here, we aimed at investigating anandamide metabolism in coeliac disease by analysing transcript levels (through quantitative real time reverse transcriptase-polymerase chain reaction), protein concentration (through immunoblotting) and activity (through radioassays) of enzymes responsible for anandamide synthesis (*N*-acylphosphatidylethanolamine specific phospholipase D, NAPE-PLD) and degradation (fatty acid amide hydrolase, FAAH) in the duodenal mucosa of untreated coeliac patients, coeliac patients on a gluten-free diet for at least 12 months, and control subjects. Also treated coeliac biopsies cultured *ex vivo* with peptic-tryptic digest of gliadin were investigated.

Our *in vivo* experiments showed that mucosal NAPE-PLD expression and activity are higher in untreated coeliac patients than treated coeliac patients and controls, with no significant difference between the latter two groups. In keeping with the *in vivo* data, the *ex vivo* activity of NAPE-PLD was significantly enhanced by incubation of peptic-tryptic digest of gliadin with treated coeliac biopsies. On the contrary, *in vivo* mucosal FAAH expression and activity did not change in the three groups of patients and, accordingly, mucosal FAAH activity was not influenced by treatment with peptic-tryptic digest of gliadin.

In conclusion, our findings provide a possible pathophysiological explanation for the increased anandamide concentration previously shown in active coeliac mucosa.

CLINICAL IMPROVEMENT AND REDUCTION OF IMMUNOSUPPRESSIVE DRUG THERAPY IN CANNABIS TREATED PATIENTS WITH CROHN'S DISEASE AND ULCERATIVE COLITIS

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California physicians involved in the practice of cannabis consultations regularly encounter patients with autoimmune and idiopathic inflammatory conditions; a large proportion of these have inflammatory bowel disease. SCC physicians performed a retrospective evaluation of 38 patients with an independent diagnosis of Crohn's disease (ICD-9 555.9) and ulcerative colitis (ICD-9 556.9). The primary aim of the study was to evaluate the efficacy of ad lib use of cannabis in alleviating symptoms of active inflammatory bowel disease, both with and without concomitant use of conventional medications. A secondary aim was to determine if cannabis alone or in combination with conventional medications leads to better response, longer periods of disease quiescence, and reduction in the use of conventional medications. Conventional medications included immunosuppressive drugs, anti-inflammatory drugs, steroids, antimicrobials, and TNF-alpha binding medications.

All study patients were self-referred and approved to use cannabis for relief of the symptoms of inflammatory bowel disease. Study inclusion criteria included the completion of a questionnaire designed to elicit details of the clinical course and use of all medications, including cannabis. The patients utilized their own supply of cannabis with methods of administration of their choice. The quantity of cannabis used was self-adjusted in accordance with symptoms, concomitant use of other medications, side effects, and employment considerations.

Patients report statistically significant improvement in their signs and symptoms when using cannabis. Stools per day, flare-up frequency, and flare-up severity were significantly reduced when patients used cannabis (all $p < .001$). Patients' appetite, activity, and average weight all increased significantly with the use of cannabis (all $p < .01$). A scale of symptoms including pain, nausea, vomiting, fatigue, and depression showed an average reduction by almost 50% when patients used cannabis (mean score using cannabis = 12.87, mean score without cannabis = 25.67, $p < .001$). Patients report a marked reduction of conventional pharmacotherapy associated with the regular use of cannabis.

Cannabis is preferred over conventional medications with nearly half of the study patients using cannabis only in their daily management of inflammatory bowel disease. Cannabis is believed to function as an effective immunomodulator, appetite stimulant, antispasmodic, and pain relieving medication with a wide margin of safety.

THE PUTATIVE CANNABINOID RECEPTOR GPR55 PROMOTES CANCER CELL PROLIFERATION VIA ERK

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GPR55 is an orphan G protein-coupled receptor that may be engaged by some lipid ligands such as lysophosphatidylinositol and cannabinoid-type compounds. Very little is known about its expression pattern and physio-pathological relevance, and its pharmacology and signaling are still rather controversial. Research carried out during the past decade has shown that cannabinoids have anti-tumor properties. Specifically, they generally induce a biphasic effect: while “low” concentrations of cannabinoids increase cancer cell proliferation rates, “high” concentrations exert the well known antiproliferative action. Thus, we decided to investigate the role of GPR55 in cancer physiopathology, focusing on its involvement on the regulation of cancer cell proliferation.

Our data show that GPR55 expression in human tumors from different origins correlates with their aggressiveness. Moreover, GPR55 promotes cancer cell proliferation, both in cell cultures and in xenografted mice, through the overactivation of the extracellular signal-regulated kinase cascade (ERK cascade). We also observed that GPR55 mediates the pro-proliferative effect elicited by low doses of cannabinoids, an effect that is abolished by GPR55 specific silencing and enhanced by GPR55 overexpression.

These findings suggest that GPR55 plays an important role in human cancer and that this receptor could constitute a new biomarker of tumor aggressiveness and a new potential target in oncology.

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THE TUMOR SUPPRESSOR A-C1 HAS AN *N*-ACYLTRANSFERASE ACTIVITY INVOLVED IN NAPE FORMATION

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N-Acylphosphatidylethanolamines (NAPEs) are the precursors of bioactive *N*-acylethanolamines, including anandamide, and are formed in animal tissues by transfer of an acyl chain from the *sn*-1 position of glycerophospholipid to the amino group of phosphatidylethanolamine. This reaction is catalyzed by membrane-associated, Ca²⁺-dependent *N*-acyltransferase. However, its molecular properties are largely unknown. Recently, we found that four human proteins of the tumor suppressor HRASLS family (iNAT, H-rev107, HRASLS2, and TIG3) have a NAPE-forming *N*-acyltransferase activity together with phospholipase (PL) A_{1/2} and lysophospholipid *O*-acyltransferase activities. A-C1 protein is another member of this family and regulates negatively the oncogene Ras. Here, we characterized A-C1 as an enzyme. We cloned cDNAs of A-C1 from rat, mouse and human, and expressed them in COS-7 cells. The cell homogenates exhibited PLA_{1/2} activity for phosphatidylcholine. Further examination with the purified recombinant A-C1 revealed that the protein also possesses *N*-acyltransferase and lysophospholipid *O*-acyltransferase activities. Ca²⁺ was not required for the full activity. In human, mouse and rat, testis, skeletal muscle, brain and heart abundantly expressed A-C1 mRNA. Taken together, these results show that all five members of the HRASLS family are phospholipid-metabolizing enzymes with *N*-acyltransferase activity.

ANTITUMOR EFFECT OF THCV, CBDV AND CBG ON HUMAN GLIOMA CELLS

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In the last ten years different research groups, including our own, showed that the non psychoactive component of *Cannabis sativa* extract, Cannabidiol, possesses antiproliferative effect against different kinds of tumor, such as glioma. More specifically, CBD reduced U87/U373 glioma cells proliferation both *in vitro* and *in vivo* and impaired glioma cells migration and invasion. These effects are due to a mechanism independent of cannabinoid- or vanilloid-receptors activation, but based on the alteration of several intracellular pathways involved in tumor cells proliferation, such as ERK, Akt and HIF1. Moreover CBD showed antiangiogenic action on HUVE cells (endothelial spheroids test) and reduced the haemoglobin content in matrigel sponges implanted subcutaneously in mice. Based on these evidences our aim was to explore the potentiality of other phytocannabinoids as tools for reducing glioma cells proliferation. To this purpose we examined the effect of THCV, CBDV and CBG on the viability of U87 MG glioma cells exposed to the different drugs for 24h. We also evaluated U87 MG cells migration and invasion through Boyden chamber assay in absence or presence, respectively, of a thin layer of Matrigel, to mimic extracellular matrix. Finally for each compound a time course study of the cell viability (Trypan blue counting), apoptosis (propidium iodide test) and orange acridine staining (FACS analysis) was performed.

THCV induced a dose-dependent reduction of U87 MG cells viability, as assessed both by the Trypan blue counting and by the MTT assay, with an IC₅₀ of 14 μ M. This effect correlated to the induction of apoptosis and with a positive staining to acridine orange. Neither AM251 (CB1 antagonist), nor AM630 (CB2 antagonist), nor capsazepine (TRPV1 antagonist) antagonized the antiproliferative effect of THCV. Moreover, THCV significantly reduced glioma cells migration and invasion. This effect is evident at a lower dose range (between 0.25 and 5 μ M) than the one evaluated in viability experiments. As for CBDV, its pharmacological profile resembles that of THCV for its ability to reduce U87 MG cells viability and for its insensitivity to cannabinoid- and vanilloid-receptor antagonists. The antiproliferative effect correlated with induction of apoptosis and positive staining to acridine orange. With respect to U87 MG cells migration and invasion, they were both significantly reduced, although no clear dose-response pattern was observed. CBG pharmacological profile and potency in reducing cell viability were the same as for THCV and CBDV. In contrast CBG induced a lower reduction of cells migration and had no significant effect on their invasion.

In the whole, the present work promotes further studies on these compounds to sustain the hypothesis of their possible use as effective tools in glioma therapy.

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**THE RECEPTORS-INDEPENDENT ANTI-CANCER EFFECT OF
ANANDAMIDE IN HEAD AND NECK SQUAMOUS CELL
CARCINOMA CELLS**

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It has been suggested that endocannabinoids, anandamide (AEA) and 2-arachidonoyl glycerol (2-AG), might be the promising anti-cancer agents in clinical fields of cancer treatment. In this study, we tried to check the anti-cancer effects of AEA and 2-AG in head and neck squamous cell carcinoma (HNSCC) cell lines. AEA inhibited effectively cell proliferation of three HNSCC cells (SNU-1041, 1066 and 1076) but 2-AG did not. CB1 was expressed only in SNU-1066 and expression of VR1 was observed in all tested cells (no CB2 was detected). Anti-cancer effect of AEA seemed to be mediated by their receptors-independent action since antagonist of CB1 and VR1 (AM251, cay10448 and capsazepine) did not reverse AEA-inhibited cell proliferation. From references on cancer cell killing effect of COX-2 metabolites of AEA in some types of cancer such as colon cancer, we checked the possibility of COX-2-mediated anti-cancer effect of AEA in HNSCC cells but COX-2 inhibition (by inhibitors and siRNA) had no effect on it. Instead, we observed increase of reactive oxygen species (ROS) and 8-isoprostane production by AEA and antioxidants (NAC and ebselen) reversed AEA-inhibited cell proliferation partially. In addition, AM404 and cay10412 (inhibitors of AEA transporter) reversed perfectly AEA-inhibited cell proliferation. These findings suggest that AEA might have anti-cancer effect by their receptors-independent action in intracellular localization of HNSCC cells (such as increase of ROS). The investigation on concrete mechanism of anti-cancer action of AEA in HNSCC cells is ongoing.

COMBINING CANNABINOID AGONISTS AND RADIATION THERAPY REDUCES BREAST CANCER GROWTH

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Reports from several preclinical models indicate that cannabinoids have the potential to improve the effectiveness of cancer treatment via several mechanisms including antiproliferative, anti-angiogenic, and anti-invasive effects. These actions of cannabinoids are considered to be mediated through the traditional cannabinoid G-protein coupled receptors (GPCR) CB1 and CB2. Both CB1 and CB2 are commonly accepted to signal through a Gi/o mechanism, with some evidence for Gs signaling as well. Given that cannabinoids are already used as a palliative treatment in cancer treatment, we investigated whether cannabinoid agonists enhance the effectiveness of radiation therapy, as used in treatment of breast cancer. We assessed the impact of WIN55, 212-2 (WIN2), a full efficacy agonist for CB1 and CB2, in combination with radiation. Changes in cell population were quantified by cell count or the Crystal Violet assay. Cannabinoid agonists, including Cannabidiol (CBD) and Δ^9 -Tetrahydrocannabinol (THC) were assessed in combination with radiation. In addition, Nabilone (NAB) was used to determine possible signaling pathways. All experiments were conducting in MCF-7, MDA-MB-231 and 4T1 breast tumor cell lines, unless otherwise noted. There was a significant enhancement of the antiproliferative actions of radiation at a dose of 1x2gy when combined with WIN2. Curiously CBD and THC did not show an enhancement similar to WIN2 in the MCF-7 cells, suggesting a possible difference between the aminoalkylindole and phytocannabinoid classes. A comparison of WIN2 to its enantiomer WIN55,212-3 (WIN3), which possesses poor binding affinity to CB1 and CB2 receptors, demonstrated clear stereoselectivity in inhibiting the proliferation of all three cell lines. Moreover, WIN3 did not enhance the antiproliferative effects of radiation in MCF-7 cells. The CB1 and CB2 antagonists, rimonabant and SR144528, inhibited the antiproliferative actions of WIN2, THC, CBD, and NAB in all of the breast tumor cell lines. Unexpectedly, neither pertussis toxin, which inhibits Gi/o, nor cholera toxin, which inhibits Gs, interfered with the actions of any of the agonists. The stereoselectivity of WIN2 and our ability to antagonize the actions of all four cannabinoids suggest that these cannabinoids are acting in breast cancer cells via CB1 and CB2 receptors. However, the indications that Gi/o and Gs are apparently not involved in the antiproliferative actions of the cannabinoids leaves it unclear for now as to how these receptors are signaling to induce their actions. Regardless of the signaling pathway of the receptor through which cannabinoid agonists act to reduce cancer, our data indicate that WIN55,212-2 has the potential to enhance current radiation therapy used against breast cancer. Based on these data, the synthetic cannabinoid agonist WIN55,212-2 may be more effective as an adjuvant breast cancer treatment than the current clinically approved cannabinoids for chemotherapy (e.g., THC and Nabilone) in combination with radiation.

DOES CB2 CANNABINOID RECEPTOR A BETTER TARGET FOR THE REGULATION OF PROSTATE CANCER?

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In past decade cannabinoid receptors have been described as potential drug targets for several types of cancer. The anti-cancer efficacy of CB1 cannabinoid receptor is well documented in gastric cancer, skin cancer, prostate cancer and glioma. However, the role of CB2 cannabinoid receptor in the regulation of tumor growth is not well studied. CB2 receptors expression in brain is much lower than CB1 receptor expression and is limited to brain stem neurons. Thus, in contrast to CB1 receptor-mediated effects, CB2 receptor activation is expected to have much lesser neurobehavioral side effects. Further, CB2 receptors are expression is higher in many cancer tissues including prostate cancer. These facts make CB2 receptor a better target for anti-cancer drug development. In the current study we have investigated the mechanism of CB2 receptor-mediated regulation of androgen-sensitive and androgen-insensitive prostate cancer growth, viability and metastasis.

We have recently showed that activation of CB2 receptor inhibits human prostate cancer cell (LNCaP and DU145) proliferation, viability and chemotactic motility without affecting that of non-malignant prostate epithelial cells (PrEc). Here, we have extended these studies with analysis of transcriptome for the genes that control cell proliferation, viability and anchorage independent growth. We have found that activation of the CB2 receptor significantly decreased transcriptional factor E2F and E2F-dependent gene (like cdc2, cyclinD and cyclinE) transcription. These responses were blocked by CB2 receptor antagonist SR144528. We then focused on the role of endocannabinoids in the regulation of cell cycle and tumorigenicity. For this purpose we have developed an inducible system to regulate the endocannabinoid (anandamide and 2AG) level in normal and prostate cancer cells. Using this system and pharmacological tools we are currently investigating role of endogenous cannabinoids in the regulation of cell cycle in relation to tumorigenicity and metastasis. Results from these studies will shed new light on the role of CB2 cannabinoid receptor in the regulation of cell cycle switch for the development of tumor.

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**HETEROTRIMERIC G-PROTEIN COUPLED CB2
RECEPTOR-MEDIATED ACTIVATION OF SMALL G PROTEIN
REGULATES PROSTATE CANCER CELL MIGRATION**

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One of the earliest responses of cancer cells to extracellular factors is a rapid reorganization of their actin cytoskeleton. Two distinct types of reorganization have been observed on addition of growth factors: changes in the number of actin stress fibers, and accumulation of actin filaments at the plasma membrane forming membrane ruffles. These processes of cell migration and the actin cytoskeleton reorganization are regulated by small g proteins known as Rho family of GTPases. Rho family GTPases serve as molecular switches that transduce signals from the extracellular environment via transmembrane receptors to elicit a plethora of cellular responses including cell migration.

We have recently showed that activation of CB2 receptor inhibits human prostate cancer cell (LNCaP and DU145) proliferation and viability. Further, we also showed CB2 receptor stimulation inhibits chemotactic motility in human prostate cancer cells (LNCaP and DU145). In the current investigation, we have extended these studies to characterize the molecular mechanism of CB2 receptor-mediated cell migration. We have found that in androgen sensitive human prostate cancer cell, CB2 receptor activation produced RhoA activation and stress-fiber formation in a dose and time-dependent manner. This response was blocked by CB2 receptor antagonist SR144528 as well as by knock down of CB2 receptors. Further, we also found that blockade of RhoA activation attenuated CB2 receptor-mediated inhibition of cell migration in prostate cancer cells. Collectively these results suggest that CB2 receptor-mediated signaling cross-talks with RhoA activation pathway to regulate prostate cancer cell migration. We are currently investigating the signaling mechanism of this cross-talk between Gi-coupled Cb2 receptor with RhoA family of g proteins cancer cells.

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THE EFFECT OF CBD (BDS) BOTANICAL CANNABINOID EXTRACT ON MCF-7 HUMAN BREAST CARCINOMA CELLS

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The use of cannabinoids in cancer treatment has recently attracted attention (Alexander et al., 2009). A number of studies have shown that the apoptosis or reduced growth induced in tumour cells by cannabinoids involves an increase in the expression of CB2 receptors (Alexander et al., 2009; Pisanti et al., 2009). The aim of the present study was to investigate the potential anti-tumour activity of CBD (BDS), a botanical cannabinoid extract on breast tumour cells. MCF-7 cells (American Type Culture Collection) were grown and maintained in RPMI 1640 medium supplemented with 10% fetal bovine serum at 37°C, 5% CO₂. The cells were plated in 96-well culture plates at a density of 1x10⁴ cells/well and allowed to adhere at 37°C for 24 hours. The following day, various doses of extract in the absence and presence of AM251, SR144528 and capsazepine, were added to the cells and further incubated for 4 days. Then the supernatant was removed and MTT (3-(4,5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide) was added for 4 hours. The ability of cells to form formazan crystals by active mitochondrial respiration was determined by using a Microplate reader after dissolving the crystals in DMSO. Cytotoxicity was expressed as a relative percentage of the absorbance measured at 540 nm in the control and extract-treated cells.

CBD (BDS) extract induced dose-dependent cytotoxic effects on MCF-7 cells with an IC₅₀ of 0.046 mg/ml. Pre-treatment with AM251, SR144528 and Capsazepine, CB1, CB2 and TRPV1 receptor antagonists, respectively, did not reverse the cytotoxicity afforded by CBD (BDS). Single application of antagonists alone or vehicle did not affect the survival rate of the MCF7 cells. The data suggest the unlikely involvement of CB1, CB2 and TRPV1 receptors in mediating CBD (BDS)-induced apoptosis in MCF-7 tumour cells. Further experiments are required to investigate the receptor type/subtypes involvement and the mechanism of cell death.

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ALKYLOXY-ISOPROPYLAMINO PROPANOLS (AIPs) INDUCE DEATH OF HEPATOCELLULAR CARCINOMA CELLS VIA MULTIPLE GPCRS AND KINASES MODULATION

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We developed and characterized a novel anti-cancer modality, fatty acid derivatives of isopropylamino propanol, (AIPs) and examined drug potency against human and mouse HCC *in vitro* and *in vivo*. Here, we show that C16-hexadecyl- (AIP-1) and C18-octyl- (AIP-2) analogs inhibited proliferation and caused cell death in cultured bulk HUH-7 and 1MEA.7R.1 (1MEA) cells.

Important, AIP-1 and AIP-2 blocked activation of putative liver cancer stem cells. In contrast, AIPs analogs exhibited strikingly lower toxicity against normal human or rat hepatocytes in primary cultures. AIP-1 treatment induced both autophagy and apoptosis of HCC cells as indicated by up-regulation of Becline-1, caspase-3 and down regulation of anti-apoptotic Mcl-1. AIPs utilize multifaceted mechanisms mediating HCC death, including inhibition of sphingosine phosphate kinase 2 (SPHK2), Akt1 and Akt2, as well as modulation of GPCR endocannabinoid receptors CB1/CB2 and GPR40. AIP-1 has shown a significant CB1 antagonistic activity with IC₅₀ of 3.5 μ M, a nearly 2-fold lower than IC₅₀ toward HCC cell death, and a mild CB2 receptor blockade. In contrast, AIP-2 did not affect significantly CB1/CB2 signaling. In kinase activity assay, AIP-1 strongly suppressed CAMK-I, while modestly inhibited sphingosine kinase 2 (SPHK2) and Akt2 activities. AIP-2 substantially inhibited SPHK2, but not SPHK-1, suppressed PDK1 and Akt2, and slightly affected CAMK-I. Collectively, these data demonstrate AIPs as a novel class of simple endogenous lipid-like anti-cancer compounds against HCC *in vitro* with diverse mechanisms of action. Extensive studies of AIPs efficacy in mouse models of HCC-driven tumors are under way.

Materials and Methods

Synthesis and purification of AIPs. *Synthesis of 3-alkyloxy(3-alkenyloxy)-1,2-epoxypropanes (2a–c, Fig. 1).* **1a–c** (10 mmol) in 20 mL of dry THF was added under N₂ to a stirred suspension of NaH (10 mmol) at 5–10 °C and the mixture was stirred at 22 °C for 0.5 h. Epichlorohydrin (20 mmol) was added and the mixture was heated at reflux for 3–9 h. After evaporation and extraction with EtOAc (3 x 35 mL) the product was purified by chromatography to give **2a–c** with 64, 47 and 54% yields (**Fig. 1**). *Synthesis of 1-alkyloxy(1-alkenyloxy)-3-isopropylamino-propan-2-oles (3a–c).* Oxirane (**2a–c**) (4 mmol) and isopropylamine (32 mmol) in 2-propanol (40 mL) was stirred at 45–55 °C for 7–15 h. After solvent evaporation the residue was purified by recrystallization or chromatography to obtain pure hexadecyl-AIP-1 (**3a**, mp 55–57 °C), octadecenyl-AIP-2 (**3b**, colorless oil) and octyl-AIP-3 (**3c**, colorless oil) in 83, 86 and 89% yields, respectively (**Fig. 1**). Chemical structure of AIP-bases **3** was confirmed by NMR and mass spectrometry (data not shown). *Preparation of 1-alkyloxy(1-alkenyloxy)-3-isopropylamino-propan-2-ol hydrochlorides (4a–c).* 1-Alkyloxy(1-alkenyloxy)-AIP (**3a–c**) (2 mmol) was dissolved in ether (35 mL) and HCl (4 mmol, 2M in ether) was added dropwise at 5–10 °C with stirring. The solid was filtered after 0.5 h and washed with ether for (**4a**), or the solvent was evaporated for (**4b,c**) to yield pure C16-AIP-1-HCl (**4a**) (91%, white microcrystals, mp 75–77 °C), C18-AIP-2-HCl (**4b**) (97%, yellowish oil) or C8-octyl-AIP-3 (**4c**) (96%, brownish microcrystals, mp 37–38 °C).

Human and mouse HCC cell culture and treatment.

Multifaceted drugs are required to overcome the molecular diversity of HCC and resistance of putative stem/progenitor cells to chemotherapy, and addition of AIPs may provide a new angle in prevention and treatment strategies. The active drugs are lipid-fatty acid derivatives, thus capable of forming liposomes, which enhances self bioavailability and delivery via either oral or locoregional transarterial hepatic tumor chemoembolization. Mechanisms of AIP action, effectiveness, and safety of nano-liposomal formulation of the drugs and potential clinical implication will be discussed.

CHRONIC EFFECTS OF CANNABINOID AGONISTS, ANTAGONISTS AND NEUTRAL ANTAGONISTS ON SLEEP-WAKE CYCLES IN MICE

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Endocannabinoids such as anandamide (AEA) and 2-arachidonylglycerol (2-AG) are fatty acid derivatives that have a variety of biological actions, most notably via the activation of cannabinoid (CB1) receptors. These compounds are known to modulate diverse neurobiological functions including learning and memory, pain, feeding, and sleep generation. Previous studies have shown that administration of cannabinoid receptor agonists such as anandamide and Δ^9 -THC promote sleep via the activation of CB1 receptors, since this effect is blocked following pre-treatment of the CB1 receptor antagonist, rimonabant. Furthermore, rimonabant on its own dose-dependently increased the time spent in wakefulness at the expense of slow-wave sleep (SWS) and REM sleep in rats. We have recently demonstrated that acute infusions of the potent CB1 agonist, WIN55,212-2 (WIN-2), induced an increase in non-rapid eye movement (NREM) sleep and a reduction in rapid eye movement (REM) sleep, while the CB1 antagonist/inverse agonist AM251 increased wakefulness in mice. However, the chronic effects of these compounds on the sleep-wake cycle have not been investigated. Here we assessed the long-term effects of WIN-2, AM251, and the novel CB1 neutral antagonist, ABD459, on circadian activity in freely moving mice using time scoring and power spectral analysis of the quantitative electroencephalogram (EEG). Adult, C57/BL6 mice were surgically implanted with epidural gold screws anchored to the skull directly over the medial prefrontal cortex, and left and right dorsal hippocampus. EEG was recorded using wireless Neurologger microchips attached directly to their heads in home cages. Animals were treated (i.p.) with WIN-2 (1mg/kg/day) or AM251 (1mg/kg/day) or ABD459 (1mg/kg/day) or vehicle for 2 weeks and alterations in their sleep patterns over a 6h-recording period (1:00-7:00pm: *light phase*) were assessed after the final day of treatment (day 14). Results demonstrate the long-term effects of these cannabinoids on (i) vigilant states (Wakefulness, NREM and REM sleep) and (ii) EEG spectral power across different frequency bands (delta, theta, alpha and beta). The outcome of these results increase the understanding of how the endocannabinoid system may affect the sleep-wake cycle following chronic cannabinoid treatment and help in the development of therapeutics for sleep disorders.

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THE USE OF KNOCK OUT TISSUE IS ESSENTIAL TO VALIDATE SPECIFICITY OF CB2 ANTIBODIES

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The CB2 receptor has traditionally been viewed as a peripheral receptor. Recent reports, however, have suggested that the CB2 receptor may be present in the central nervous system, in both resting and induced states. These reports rely mainly on immunohistochemical (IHC) evidence to prove existence, using a variety of positive and negative controls to validate specificity. In this study, we aimed to validate the specificity of a commercially available antibody that has been used in several published reports of CB2 expression. N terminus CB2 knockout (KO) mice (The Jackson Laboratory, ME, USA) were compared to wild type (WT) mice of the same C57BL/6 strain. Briefly, mice were anaesthetised and trans-cardially perfused with saline followed by 4% paraformaldehyde (PFA), then spleen and lumbar enlargements excised, soaked overnight in PFA, and sixteen micron sections cut at -20°C on a cryostat. Sections were quenched with hydrogen peroxide, blocked with goat serum, and incubated overnight with a primary antibody raised against amino acids 20-33 of the human CB2 receptor (Cayman Chemicals, MI, USA). Sections were incubated with a secondary antibody, and the signal amplified with the ABC system, before visualisation with DAB nickel. Adjacent sections were incubated with the primary antibody preadsorbed with its immunising peptide, or in the absence of primary antibody.

Immunolabeling was observed in cellular membranes in the dorsal horn of lumbar spinal sections from both WT and KO mice. Scatterings of labelled cells were also observed in the spleen of WT and KO mice. Omission of the primary antibody, or preadsorption with its immunising peptide, abolished or suppressed, respectively, this observed immunolabelling. The results of this study indicate that the tested antibody is not specific in its labelling of CB2 receptors in either spinal tissue or spleen. Furthermore, it can be concluded that preadsorption of the primary antibody with its immunising peptide is not a sufficient negative control, and instead, KO tissue should serve as the gold standard for testing antibody specificity. Finally, this study highlights the inherent difficulties in using polyclonal antibodies to detect complex, membrane bound GPCRs such as the cannabinoid receptors.

**siRNA KNOCKDOWN OF GPR18 RECEPTORS IN BV-2 MICROGLIA
ATTENUATES THE CELL MIGRATION INDUCED BY
N-ARACHIDONOYL GLYCINE**

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N-arachidonoyl glycine (NAGly) is an endogenous enzymatically oxygenated metabolite of the endocannabinoid AEA that acts as a high affinity ligand for the G_{i/o}-coupled GPCR GPR18 and a partial agonist of G_{q/11}-coupled GPR92 receptors. We have previously shown that BV-2 mouse microglial cells natively express high levels of GPR18 mRNA and exhibit a novel pharmacology for the stimulation of cell migration by NAGly, abnormal cannabidiol (Abn-CBD) and O-1602. NAGly is the most potent pro-migratory ligand with regard to BV-2 cells reported to date, able to elicit a migratory response double that of 1 μM *N*-formyl-methionine-leucine-phenylalanine (fMLP), an established chemotactic peptide, at a concentration of 170 pM. The migration produced is predominantly chemotaxis over chemokinesis, abolished by pre-treatment with pertussis toxin, and attenuated in the presence of 1 μM O-1918, 1 μM *N*-arachidonoyl serine or 1 μM cannabidiol (CBD) (McHugh *et al.*, 2010). Our first hypothesis is that NAGly activates GPR18 receptors in BV-2 microglial cells to trigger a signalling cascade that results in effective cell migration. A second hypothesis is that GPR18 is the unidentified Abn-CBD receptor. Here we present siRNA knockdown data in support of both of these.

The pSUPER vector system is designed specifically for the expression of short interfering RNA (siRNA). To effect the silencing of murine GPR18, a custom oligonucleotide corresponding to a unique 19-nt sequence within murine GPR18 mRNA was cloned into the pSUPER GFP vector, thereby creating a plasmid that would simultaneously produce GPR18 siRNA and GFP when successfully transfected into BV-2 microglial cells. GFP⁺/siRNA⁺ transfected BV-2 cells were then selected via flowcytometry. Using a 96-well Boyden chamber migration assay, the migratory response evoked by 1 μM NAGly, 1 μM Abn-CBD and 1 μM O-1602 in GFP⁺/siRNA⁺ transfected BV-2 cells is significantly attenuated compared to wildtype BV-2 microglia.

Microglia fulfil a broad variety of tasks within the central nervous system related to both developing an effective immune response and maintaining homeostasis. Under neurodegenerative or neuroinflammatory conditions, microglia proliferate and migrate towards sites of injury where their responses contribute to and expand neurodestructive effects, worsening the disease process; therefore, a sound understanding of the mechanisms that control their motile and proliferative capacity is of crucial therapeutic importance.

Reference: McHugh, D., Hu, S. S-J., Rimmerman, N., Juknat. A., Vogel, Z., Walker, J.M., and Bradshaw, H. B. (2010). “*N*-arachidonoyl glycine, an abundant endogenous lipid, potently drives directed cellular migration through GPR18, the putative abnormal cannabidiol receptor.” *BMC Neuroscience*, 11:44.

ACTIVATION ASSAYS FOR GPR55 AND ITS NEGATIVE OUTCOME

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GPR55 is a recently orphanized G protein-coupled receptor that is under a great scientific interest since it has been suggested to have role as a novel cannabinoid receptor. Even though its physiological functions are not yet clear, it has been proposed that GPR55 has a role in neuropathic pain, bone cell function and invasive capability of several breast cancer cell lines. The field of GPR55 research has provided inconsistent results. There are not many publications that would support each other and the outcome is often different, depending on what functional aspects have been studied.

In our experiments we have used N-terminally HA-tagged human GPR55 expressing HEK293 cell lines that we have generated by transducing cells with a third-generation self-inactivating lentivirus transfer plasmid. 3xHA-HEK-hGPR55 positive cells were isolated and receptor expression level was verified by Western Blotting as well as receptor ELISA.

After we had verified 3xHA-HEK-hGPR55 expressing cell lines, we began to optimize different assays in order to assess activity of GPR55 receptor in at least three cell lines with different expression level of the receptor. ELISA assay was used to obtain quantitative data on GPR55 internalization as changes in total and cell-surface receptor number can be monitored in response to agonist stimulation. For comparative purposes, GPR55 internalization was examined also with immunofluorescence staining and visualized by confocal microscopy. GPR55 function was also studied by monitoring agonist-induced ERK phosphorylation (pERK). We used both traditional Western Blotting and a novel In-Cell Western approach to detect pERK levels. In addition, G-protein activity in response to GPR55 stimulation was assessed with traditional [³⁵S]GTPγS membrane binding assays. In each of the functional assays, HEK and HEK hGPR55 cells (or membranes) were treated with the GPR55 agonists LPI and AM251. However, although control compounds produced the expected responses in the functional assays, we observed no GPR55-dependent signal using these assays.

LIGAND-BASED VIRTUAL SCREENING FOR CANNABINOID LIGANDS

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The cannabinoid receptors CB1 and CB2 are the primary targets of endocannabinoids and cannabinergic ligands possessing therapeutic potential. Recently found orphan G protein-coupled receptor GPR55 has been suggested to be the third cannabinoid receptor because it has been reported to be activated by some exogenous and endogenous cannabinoids.

In our study, we used *in silico* screening methods to find new lead compounds for cannabinoid receptor ligand development. Promising compounds were screened for CB1/CB2 agonist and antagonist activity. Our intention is also to screen compounds for agonist and antagonist activity at the GPR55.

We used ligand-based virtual screening utilizing the properties of SR141716A (rimonabant) which is known as CB1 antagonist and GPR55 agonist. First, several databases were confined by general descriptors such as molecular weight, log P and the number of hydrogen bond donors and acceptors. After that, we used Brutus virtual screening software to search compounds with steric and electrostatic fields similar to those of SR141716A. As a result, we ordered 118 compounds, of which CB1/CB2 receptor activities were determined using the [³⁵S]GTPγS binding assay. Of the ordered compounds, 12 showed CB1 receptor antagonist activity, of which 4 showed also CB2 antagonist activity. Three compounds were both CB1/CB2 agonists, seven compounds behaved as CB2 agonists and three as CB2 antagonists.

We also utilized the results of PubChem bioassay AID 2013 (Image-Based HTS for Selective Antagonists for GPR55) which reported 370 active GPR55 antagonists. Those antagonists were docked into several conformers of the GPR55 homology model. Based on the docking score, activity and synthetic feasibility, the compound CID2937915 was chosen. In addition, ten commercially available compounds similar to CID2937915 were screened, which all showed CB1 antagonist activity.

From PubChem bioassay AID 1961 (Image-based HTS for Selective Agonists of GPR55) we searched GPR55 agonists structurally similar to CID2937915. The two compounds, CID1792579 and CID1252842, were selected and screened together with 10 structurally similar commercially available compounds. As a result, one of them showed CB1 agonist activity.

The found active compounds can be used as starting points when designing new potent and selective cannabinoid receptor ligands.

A PRE- AND POST-SYNAPTIC MECHANISTIC INVESTIGATION OF CANNABIDIOL IN ACUTE HIPPOCAMPAL BRAIN SLICES

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Introduction: Cannabidiol (CBD) reliably inhibits epileptiform activity *in vitro* and consistently reduces seizure severity and lethality *in vivo*, strongly supporting reconsideration of its use for the treatment of human epilepsies. However, CBD has only very low affinity for cannabinoid CB₁ and CB₂ receptors and effects of CB receptor ligands upon seizures are highly varied and unpredictable. Consequently, we have begun to investigate CBD effects upon receptors and ion channels in hippocampal neurons using *in vitro* electrophysiological methods to elucidate mechanisms underlying CBD's anti-convulsant effects.

Methods: The effects of pure CBD (10 µM) in adult rat hippocampal acute brain slices (300 µm thickness) were assessed. Whole-cell patch clamp recordings in current clamp mode were undertaken to assess the effects of CBD upon synaptic and non-synaptic responsiveness of visually identified CA1 pyramidal neurons. Synaptic responses were evoked via a bipolar stimulating electrode (0.1-9 V amplitude, 0.5 ms duration) positioned in the Schaffer collaterals. Non-synaptic responses were assessed by application of depolarising and hyperpolarising current via the recording micropipette (-350-350 pA; 150 ms). In all cases, $P \leq 0.05$ was considered significant.

Results: CBD significantly decreased the amplitude of evoked sub-threshold synaptic responses. Moreover, in response to injected current, CBD significantly reduced mean spike count, instantaneous firing frequency and resting membrane resistance, increased second spike duration but did not affect spike amplitude or activation threshold.

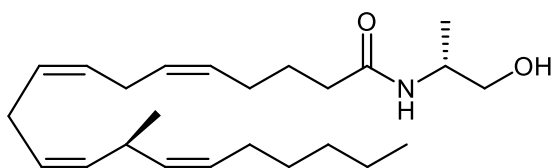
Conclusion: Results from direct current injection (non-synaptic) demonstrate that CBD exerts significant effects on voltage-dependent conductances (e.g. Na⁺ and/or Ca²⁺) that are consistent with its anti-convulsant profile. Furthermore, synaptic stimulation results support additional CBD effects at a number of pre- and/or post-synaptic ion channels and/or receptors. Taken together, these findings suggest a number of specific targets, the elucidation of which and their relative contributions to CBD's anti-convulsant profile require the subsequent use of selective pharmacology. In addition to these results further encouraging the clinical development of CBD as an anti-convulsant (particularly given its excellent side-effect profile *in vivo*), the diverse range of effects presented here support the assertion that CBD could represent a 'new in class' anti-convulsant.

DESIGN AND SYNTHESIS OF NOVEL ENDOCANNABINOID TEMPLATES

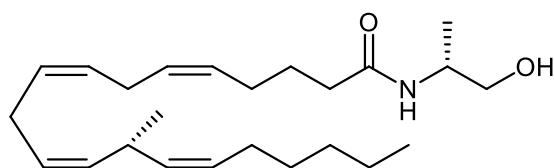
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The endogenous *N*-arachidonylethanolamine (anandamide, AEA) and 2-arachidonoyl glycerol (2-AG) act at the CB1 and CB2 receptors, two G_{i/o}-protein-coupled cannabinoid receptors (CBs) known to modulate a number of physiological and pathological processes. The biological actions of AEA and 2-AG are terminated by a transport mechanism and enzymatic deactivation. In most tissues, AEA is metabolized hydrolytically by fatty acid amide hydrolase (FAAH), and 2-AG is metabolized by monoacylglycerol lipase (MAGL). However, recent investigations have demonstrated that oxidative enzymes of the arachidonate cascade, including lipoxygenases (LOX), cytochrome P450, and cyclooxygenase-2 (COX-2), can transform AEA and 2-AG into eicosanoid related bioactive products. As a part of our ongoing program in cannabinoid medicinal chemistry, we focus on the development of novel endocannabinoid templates that possess high CB receptor binding affinity as well as metabolic stability to the action of the COX-2, LOX, FAAH and MGL enzymes. We report herein two novel arachidonic acid derivatives referred to as (13*S*)-methyl-arachidonic acid *N*-[(*R*)-1-hydroxypropan-2-yl]amide (AMG315), and (13*R*)-methyl-arachidonic acid *N*-[(*R*)-1-hydroxypropan-2-yl]amide (AMG317) which were synthesized *de novo* using a nineteen step chiroselective synthesis. *In vitro* evaluation of the novel compounds demonstrated that the 13*S*-analog is an endocannabinoid with remarkably high affinity for CB1, while the 13*R*-isomer is not recognized by the CB receptors. A detailed SAR study as well as a full biological evaluation of the novel endocannabinoid analogs reported here is underway.



AMG 315



AMG 317

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DIMERIZATION AND FUNCTION OF CB1 RECEPTOR CODING REGION SPLICE VARIANTS

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The pharmacological functions of the type 1 human cannabinoid receptor (hCB1) is thought to be modulated through the isoform encoded by the most abundant hCB1 mRNA. Two other mRNA variants of the coding region of this receptor have been described. These less abundant mRNAs encode receptors with a small deletion in the amino terminus of the coding region (hCB1b) or a different amino terminus (hCB1a). The transcripts of the three coding region splice variants are heterogeneously distributed through human brain and peripheral tissues. The distribution profiles of these transcripts have been reported to change during development or in pathophysiological conditions (Shire *et al.*, 1995, Ryberg *et al.*, 2004, Sarzani *et al.*, 2006). The contribution of these variants to endocannabinoid physiology and pharmacology remains unclear.

Similar to other members of the family of type A GPCRs, the hCB1 receptor is able to physically interact and form heterodimers with other type A GPCRs such as the D2 dopamine (Kearn *et al.*, 2005), A2a adrenergic (Carriba *et al.*, 2007), B2 adrenergic (Hudson, 2010), orexin-1 (Ellis *et al.*, 2006) and opioid receptors (Rios *et al.*, 2002). These interactions have profound impact on hCB1 receptor trafficking, ligand binding and G protein coupling. Homo- and heterodimerization between and among hCB1 variants has not been examined. The overlapping patterns of distribution of the mRNAs of the three coding region variants in addition to alterations in the relative abundance of the mRNAs of the three variants raises the possibility that dimerization may occur and influence the function of hCB1 receptor complexes. Therefore, the present study aimed to understand the relative abundance of the variants and CNS distribution, as well as interactions between receptors and effect of variants on the trafficking and signaling of hCB1.

We have examined the expression of hCB1 mRNA variants in RNA isolated from total brain, the frontal cortex, parietal cortex, caudate and putamen and cerebellum. It appears that hCB1 is the major isoform in all brain regions examined. hCB1, hCB1a and hCB1b can each form homodimers as demonstrated by BRET saturation curves of homogenates isolated from transiently transfected HEK293 cells. Heterodimers between hCB1 and either hCB1a or hCB1b have also been demonstrated. We are currently examining whether these proteins can be isolated as complexes using co-immunoprecipitation. We are also examining the cell surface expression and trafficking in the presence and absence of CB1 receptor agonists and antagonists.

In conclusion, the results of the present study demonstrate that the mRNAs of the three coding region variants are heterogeneously distributed through human brain. The two splice variants are capable of dimerization with the full-length hCB1; however, further studies are required to understand the influence of such interactions.

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ENGINEERING CANNABINOID 2 RECEPTOR FOR STRUCTURAL STUDIES

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Structural characterization of G protein coupled receptors (GPCRs) has been very challenging due to the inherent difficulties in overexpressing, solubilizing, purifying, maintaining and manipulating of these hydrophobic membrane proteins. Inactive and active state crystal structures of the β 2AR and β 1AR receptors have been solved only after protein stabilizing modification using covalent ligand attachment, antibody, “nanobody”, and multiple single point mutations. We have undertaken the first steps to obtain the human cannabinoid 2 receptor (hCB2) suitable for NMR studies. We developed binding assays to evaluate the thermal stability of the hCB2- 3 H]-CP55940 complex using the receptor overexpressed in mammalian HEK293 cell lines. To obtain essential quantity of protein, several constructs including wild type (WT) and non-essential domain truncated hCB2 receptor variants containing N-terminal maltose binding protein followed by FLAG and C-terminal hexa-histidine tags were created and expressed in *E.coli*. The functional properties of the modified proteins were evaluated for their ability to interact with CP55940, a CB2 receptor agonist. The hCB2 protein was partially purified using metal affinity chromatography and characterized by mass spectrometry. We are performing receptor stabilization using single point mutations and covalent ligands attachment. Currently, we are optimizing the bacterial expression and purification conditions of the hCB2 receptor.

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MODULATION OF ARRESTIN FUNCTION: SEARCHING FOR A LEAD COMPOUND USING NMR METHODS

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Arrestins are cytosolic proteins that mediate the desensitization and internalization of G-Protein Coupled Receptors (GPCRs); events that have been consistently implicated in the onset of tolerance in many systems. Following activation, the phosphorylation of the GPCR C-terminus by G-protein coupled Receptor Kinases (GRK) leads to binding with arrestin. This results in a decrease in the interaction with the G-protein, and the arrestin molecule serves as a scaffold for proteins necessary for internalization. Furthermore, arrestin mediates the coupling of internalized GPCRs to alternate signaling pathways. There is evidence that there may be structurally differing arrestin-GPCR complexes that result in different biological outcomes. Thus, arrestin represents a novel protein target. The identification of ligands that modulate arrestin function may serve as useful pharmacological tools for biological discovery, and may lead to new therapeutic paradigms.

In order to model the interaction between GPCRs and arrestin, we synthesized phosphorylated peptides that mimic key segments of the cannabinoid receptor, CB1. We present studies of complexes between these fragments of CB1 and arrestin-2 that yield insights as to the location of the arrestin site responsible for interaction with the GPCR. HSQC and transferred NOE data show these peptides interacting in a manner that is consistent with the model of arrestin action. We further outline how we will utilize this information to select likely ligand candidates from a library of compounds and describe NMR methods by which we intend to screen these selected small molecule ligands.

TYPE-1 CANNABINOID RECEPTOR REGULATES MEMBRANE FLUIDITY OF BOAR SPERMATOZOA DURING THE CAPACITATION

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Mammalian spermatozoa acquire the ability to fertilize within the female genital tract where they are exposed to inverse gradients of inhibiting (endocannabinoids) and stimulating (bicarbonate) molecules. In the sperm activation, the capacitation, membrane lipid remodeling have a crucial role, allowing the sperm–egg recognition and increasing plasma and outer acrosome membranes instability required for acrosome reaction. The present research has been designed to study the role exerted by anandamide (AEA) on the sperm membrane remodeling. To this aim, boar sperm were *in vitro* incubated under capacitating condition (plus bicarbonate) with or without the non-hydrolysable analogue of AEA, the methanandamide (Met-AEA). Moreover, in order to verify whether Met-AEA influences are mediated by CB1R, the cultures were performed by adding the specific receptor antagonist (SR141716, SR1) and/or a permeable cAMP analogue (8Br-cAMP). The functional effects of the different cultural conditions were then evaluated by analyzing: CB1R sperm distribution, phospholipid scrambling, membrane cholesterol content, membrane fluidity and anisotropy. Immunocytochemistry revealed that Met-AEA blocked CB1R translocation from the post-equatorial to equatorial region of sperm head induced in capacitated cells by bicarbonate. In addition, Met-AEA totally inhibited the bicarbonate-induced phospholipid scrambling (MC450 staining) thus preventing the redistribution of cholesterol to the apical head region (filipin staining) and sterol extraction induced in capacitated cells by BSA (C/P content on membrane enriched fraction). As a consequence, Met-AEA treated spermatozoa did not increase membrane fluidity and anisotropy either during the incubation with bicarbonate or after BSA exposure, as documented by EPR spectroscopy and biochemical analysis. All these inhibitory effects of Met-AEA were mediated by CB1R, since they are totally prevented by SR1 or cAMP addition. These results suggested that AEA-CB1R activation operated as a membrane stabilizing pathway able to inhibit membrane the protein redistribution (CB1R itself) and the increase in membrane fluidity required for capacitation.

THE GPCR-ASSOCIATED SORTING PROTEIN 1 REGULATES RIMONABANT-INDUCED DOWNREGULATION OF GPR55

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The G protein-coupled receptor 55 (GPR55) has recently been suggested to be responsible for those cannabinoid responses that could not be attributed to either the cannabinoid 1 (CB1) or cannabinoid 2 (CB2) receptor. Potent GPR55 agonists were identified, such as lysophosphatidylinositol (LPI) and several synthetic cannabinoids: One of these is Rimonabant (SR141716A), an antagonist at the CB1 receptor, which showed clinical promise, but approval was revoked due to adverse events. Generally, the activity of GPCRs is coordinated by receptor signaling, receptor desensitization and receptor resensitization. One regulatory mechanism to guarantee appropriate GPCR expression levels in physiological conditions is that of downregulating GPCRs via the G protein-coupled receptor-associated sorting protein 1 (GASP-1), thus leading to an attenuation of cellular signaling events. GASP-1 was originally found to target δ opioid receptors to lysosomes and, hence, to the degradative pathway. It was shown that GASP-1 is a key determinant in the development of analgesic tolerance to cannabinoids via its role in facilitating downregulation of the CB1 receptor.

We evaluated the direct protein-protein interaction of GPR55 with GASP-1 using GST-binding assays and co-immunoprecipitation assays in GPR55-HEK293 cells with endogenous GASP-1 levels. We further tested the internalization, recycling and degradation of GPR55 using confocal fluorescence microscopy and biotinylation assays in the presence and absence of GASP-1.

We demonstrated that Rimonabant promotes downregulation of GPR55 via the GPCR-associated-sorting-protein-1 (GASP-1) *in vitro* and *in vivo*. We show that GPR55 interacts with GASP-1 *in vitro* and that disrupting the GPR55-GASP-1 interaction prevents post-endocytic receptor degradation, and thereby allows receptor recycling. Together, these data implicate GASP-1 as an important regulator of Rimonabant-mediated downregulation of GPR55.

This work provides tangible evidence that GPR55 is degraded after prolonged agonist stimulation and this mechanism is regulated by the G protein-coupled receptor-associated sorting protein 1.

IDENTIFICATION OF FUNCTIONAL CANNABINOID RECEPTORS IN THE MOUSE OLFACTORY SYSTEM

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The peripheral olfactory epithelium exhibits continual natural regeneration throughout adulthood. The olfactory epithelium is able to regenerate its various cell types through the resident stem cell population. However, the signaling cues received by stem cells that trigger regeneration are not fully known. The endocannabinoid system has been shown to stimulate adult neurogenesis in the central nervous system. We tested the hypothesis that cannabinoid receptors are located in the mouse olfactory epithelium and assist in proliferation.

First, the presence of cannabinoid receptor 1 (CB1) and cannabinoid receptor 2 mRNA in the mouse olfactory epithelium was confirmed using RT-PCR. Next, CB1 protein was detected using western blot. Third, using a neonatal mouse olfactory epithelium slice model and calcium imaging, we found that cannabinoid receptors are physiologically functional, responding to the pan cannabinoid receptor agonist WIN55,212-2. Transient increases in calcium evoked by either 100 nM or 1 μ M WIN55,212-2 were observed in 70% (30 out of 43) of ATP-responsive non-neuronal cells, and in 23% (10 of 43) of odorant-responsive neuronal cells. These data suggest that cannabinoid receptors are present on both olfactory sensory neurons and non-neuronal sustentacular cells.

We then tested if cannabinoid receptors effect proliferation in the mouse olfactory epithelium. Adult Swiss Webster male mice were intranasally administered either saline vehicle or 10 μ M WIN55,212-2 to activate cannabinoid receptors followed by bromodeoxyuridine (BrdU) intraperitoneal injection 44 hours later to measure proliferation. There was a significant increase in BrdU+ cells in WIN55,212-2 treated animals over vehicle control animals (26.9 ± 2.2 vs. 20.1 ± 1.0 BrdU+ cells/mm, $p < 0.05$). Administration of the specific CB1 antagonist, AM-251 inhibited the WIN55,212-2 induced increase to control levels (15.7 ± 0.8 vs. 20.1 ± 1.0 BrdU+ cells/mm, $p < 0.05$). Our data identifies expression of functional cannabinoid receptors in the mouse olfactory epithelium and suggests endocannabinoids may play a role in proliferation in the olfactory system.

EFFECT OF PALMITOYLATION ON MEMBRANE LOCALISATION AND SIGNALLING OF CB₁ CANNABINOID RECEPTOR

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The CB₁ cannabinoid receptor is regulated by its association with membrane microdomains like the lipid rafts. The purpose of this study was to investigate the role of CB₁ palmitoylation by analyzing the functional consequences of site-specific mutation of cysteine 415, the likely palmitoylated residue at the end of helix 8, in terms of membrane association, raft targeting and signalling of the receptor.

The palmitoylation state of the CB₁ receptor was assessed in rat forebrain by depalmitoylation/repalmitoylation experiments. Cysteine 415 was replaced with alanine by site directed mutagenesis. Green fluorescence protein chimeras of both wild-type and mutant receptors were transiently expressed and functionally characterised in human neuronal cells by means of confocal microscopy, cytofluorimetry and competitive binding assays. Confocal fluorescence recovery after photobleaching was used to assess receptor membrane dynamics, whereas cAMP and co-immunoprecipitation assays were employed to assess signalling activity.

Endogenous CB₁ receptors were found to be palmitoylated in rat brain. Mutation of cysteine 415 significantly reduced receptor recruitment at both the plasma membrane and the lipid rafts; it also increased protein diffusional mobility, whereas the immobile fraction remained unaltered. The same mutation markedly reduced the functional coupling of CB₁ receptor with adenylyl cyclase, whereas depalmitoylation abrogated receptor association with G proteins.

Taken together, we found that CB₁ receptor is post-translationally modified by palmitoylation and that mutation of cysteine 415 gives rise to a form of receptor that is functionally impaired in terms of membrane targeting and signalling.

SIGNALLING TRAFFICKING OF ALLOSTERIC MODULATOR, ORG27569 AT THE CANNABINOID CB₁ RECEPTOR

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ORG27569, an allosteric modulator of the cannabinoid CB₁ receptor (Price et al, 2005), increases the binding affinity of agonists for CB₁ receptors but reduces the signalling efficacy of these receptors. It has been suggested that allosteric modulators can have differential effects on various signalling pathways (Zhang et al, 2005). The aim of the present study was to investigate how the allosteric modulator of CB₁, ORG27569, affects different signalling pathways that are linked to the CB₁ receptor. The effect of ORG27569 on agonist-induced CB₁ receptor signalling was investigated using [³⁵S]GTPγS binding, cyclic AMP, beta arrestin and pERK assays.

ORG27569 displayed different effects on CP55940 signalling. Table 1 summarises the effect of 100nM or 1μM ORG27569 on CP55940 signalling in all assays investigated.

Table 1: E_{max} values with 95% confidence limits and pEC₅₀ values for the effect of ORG27569 on the CB₁ receptor agonist CP55940 in each assay type.

Assay used	Vehicle/modulator	E_{max} (95% C.L)	pEC ₅₀ ± S.E.M
GTPγS binding	DMSO	61 % (47-75)	8.14 ± 0.25
	100nM ORG27569	18 % (14-22)	8.60 ± 0.37
Beta-Arrestin	DMSO	99 % (95-103)	7.80 ± 0.06
	100nM ORG27569	-	-
Cyclic AMP	DMSO	87 % (75-97)	8.09 ± 0.19
	100nM ORG27569	-	-
pERK	DMSO	221.5 % (201-241)	7.80 ± 0.12
	1μM ORG27569	175.5 % (149-202)	7.72 ± 0.21

100nM ORG27569 completely abolished the effect of CP55940 in both the beta arrestin assay and cyclic AMP assay compared to the [³⁵S]GTPγS binding assay in which 100nM ORG27569 attenuated the effect of CP55940 and the pERK assay in which 1μM ORG27569 had no significant effect on CP55940 functional efficacy.

These data provide evidence that allosteric modulators binding to the cannabinoid CB₁ receptor can preferentially impact signalling through some pathways and not others. This indicates that allosteric modulators could be used to selectively alter a desired or undesired response to receptor activation without affecting others.

Price, M.R et al. (2005) *Mol Pharmacol* 68, 1484-1495
Zhang, Y et al. (2005) *J. Pharmacol Exp Ther* 315 (3), 1212-1219

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ALLOSTERIC BINDING TO CANNABINOID RECEPTORS

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Allosteric modulation provides a potential opportunity to produce clinically relevant drugs that target G protein-coupled receptors in a manner that offers advantages over compounds that bind to orthosteric binding sites. Thus, allosteric modulators bind to a site on the receptor which is less conserved than the orthosteric site, and hence display greater selectivity than orthosteric ligands. In addition, allosteric modulators display both a 'ceiling effect' that lessens the risk of toxicity, and the potential to maintain a more physiological profile than an orthosteric ligand, by up- or down-regulating the response to an endogenously released agonist.

This study investigated Org27569, an allosteric modulator of cannabinoid CB₁ receptors. The actions of allosteric modulators of CB₁ receptors are not fully understood. To obtain a clearer understanding of these actions we attempted to address three questions. First, does Org27569 display agonist-dependence? Second, where does Org27569 bind to the CB₁ receptor? Finally, are there any explanations for why Org27569 differentially affects the binding of cannabinoid compounds?

To investigate these questions, two kinds of radioligand binding assay were used: [³⁵S]GTPγS binding and equilibrium binding assays. Results obtained in the [³⁵S]GTPγS binding assays show that Org27569 displays agonist-dependent potency as an apparent allosteric antagonist of CP55940, WIN55212-2 and anandamide. Its IC₅₀ values are presented in Table 1.

Table 1: IC₅₀ values for the effect of Org27569 on various CB₁ receptor agonists

Agonist	Modulator	IC ₅₀ (nM)
CP55940	Org27569	2.3
WIN55212-2	Org27569	555.4
Anandamide	Org27569	38.6

As well as the agonist dependence it displays in the [³⁵S]GTPγS binding assay, Org27569 differentially affects the binding of radiolabelled CP55940, SR141716A and WIN55212-2 in equilibrium binding assays. It has previously been found that SR141716A and WIN55212-2 bind to the same binding pocket on the CB₁ receptor which is distinct yet overlapping with the binding pocket to which CP55940 binds. Using a mutated receptor cell line with the amino acid residue W5.43 changed to alanine, we discovered that Org27569 no longer exerts its allosteric effect on CP55940 in the [³⁵S]GTPγS binding assay. The same mutation is also known to affect binding of both SR141716A and WIN55212-2 but not of CP55940 to the CB₁ receptor.

We conclude that Org27569 displays agonist-dependence as a CB₁ allosteric inhibitor and, also, that it binds to a site on the CB₁ receptor that overlaps with the binding pockets of SR141716A and WIN55212-2 which contain the amino acid residue W5.43.

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AMINO ACIDS D2.63 AND K373 ARE IMPORTANT FOR MAINTAINING THE CB₁R BINDING POCKET, WHILE RESIDUES K3.28 AND S1.39 ARE INVOLVED IN SELECTIVE LIGAND RECOGNITION

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Previous work has demonstrated that specific amino acid residues in G-protein coupled receptors (GPCRs) are important for receptor stimulation and ligand recognition. In the absence of crystal structures for the CB₁ or CB₂ proteins, much of the structural information on these proteins has been gained from biochemical, mutational, and modeling studies. D2.63 is a unique aspartate that is at the extracellular end of TMH2, facing into the binding pocket of CB₁. We have previously demonstrated that mutation of D2.63 to N does not affect ligand binding but does play a crucial role in modulating signal transduction (Kapur et al, 2008). K373 is located on the 3rd EC loop of the CB₁ receptor. Together, D2.63 and K373 are thought to form a salt bridge under normal receptor activation. The 3rd transmembrane domain contains a highly conserved lysine residue at position K3.28 (K192). Previously, an alanine substitution of K3.28 (K3.28A) showed that only WIN55,212 (WIN) could maintain CB₁ receptor binding (Song and Bonner, 1996; Chin et al, 1998). CP55,940 (CP) binding was eliminated, as was that for HU210 and anandamide. These and subsequent studies suggest that specific residues are important for recognizing different ligands, while other residues may be important for maintaining the binding pocket. We have also created a novel mutation in the first transmembrane domain of CB₁ at S1.39. We generated alanine substitutions in the CB₁ receptor by site directed mutagenesis, with stable expression in HEK293 cells. We used [³⁵S]GTPγS binding to measure the stimulation of WT and mutant cannabinoid receptors with distinct ligands. In the D2.63A mutant, each cannabinoid that was tested generated a lower E_{max} and increased EC₅₀ compared to WT. The values became increasingly significant when the ‘salt-bridge’ was completely disrupted at the D2.63A-K373A mutant. The EC₅₀ increased greatly for JWH-018 (43 fold) in the D2.63A-K373A mutant receptor, while a 2 fold increase was seen at the D2.63A. Likewise, the E_{max}S for D2.63A and D2.63A-K373A mutants for JWH-018 were 88.7% and 59.2%. E_{max}S for CP stimulation at the D2.63A and D2.63A-K373A mutants were 73.4% and 28.9% and the EC₅₀S were 5.7 and 3.1 fold higher, respectively. The D2.63A-K373A mutant showed a severe impairment of receptor stimulation, representing a disruption of the ‘salt-bridge’ that is involved with maintaining the structure and functionality of the CB₁ receptor.

K3.28A and S1.39A may be residues which are important for selective ligand recognition. While the values generated for the K3.28A and S1.39A mutants by WIN stimulation were not significantly different from WT. At the K3.28A mutant but not the S1.39A, the indole JWH-018 was able to stimulate GTPγS binding similar to WT. The existence of amino acid residues in the CB₁ receptor involved in selective ligand recognition is supported by our findings.

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INVESTIGATION INTO THE BEHAVIORAL EFFECTS OF THE PURPORTED CB₁ RECEPTOR NEGATIVE ALLOSTERIC MODULATOR ORG-27569

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Recent efforts have led to the discovery of small molecules that behave as allosteric modulators of the cannabinoid type-1 (CB₁) receptor. Allosteric modulators bind to an area topographically distinct from a receptor's orthosteric site and impart changes in protein conformation, thereby altering affinity and/or efficacy of the receptor's ligands. Thus, a CB₁ allosteric site represents a novel and promising addition to the current targets in cannabinoid drug development as it could allow for the selective fine tuning of receptor pharmacology. Negative allosteric modulators exhibit positive cooperativity with known agonists (i.e. increasing their specific binding), yet abate their responses in GTPγS binding and mouse vas deferens functional assays. One of these compounds, Org-27569, increases specific binding of the CB₁ receptor agonist [³H]CP55,940 but antagonizes CB₁ receptor activation *in vitro*, suggesting it is acting at an allosteric site. The purpose of the present study was to test the hypothesis that Org-27569 behaves as a CB₁ negative allosteric modulator in whole animals. To this end, we tested if it would reduce food intake, a phenotypic response in CB₁^{-/-} mice or wild-type mice treated with CB₁ receptor antagonists. In addition, we tested whether Org-27569 attenuates the pharmacological effects of the primary active constituent of cannabis, Δ⁹-tetrahydrocannabinol (THC). For the first series of experiments, male C57BL/6 mice were food restricted for 24 h and then allowed 2 h access to pre-measured rodent chow. Org-27569 (10 mg/kg) significantly reduced food consumption in C57BL/6 mice and attenuated body weight recovery at 24 h. In order to evaluate whether this anorectic effect of Org-27569 was CB₁ receptor mediated, we evaluated Org-27569 in male and female CB₁^{+/+} and CB₁^{-/-} mice. Surprisingly there was a significant decrease of food intake in CB₁^{-/-} mice. In the final set of experiments, we evaluated whether Org-27569 attenuates the cannabimimetic effects of THC on tetrad effects, i.e., hypomotility, catalepsy, antinociception, and hypothermia. THC (50 mg/kg) produced robust effects in the cannabinoid tetrad; however, Org-27569 (30 mg/kg) failed to attenuate any of these effects. We next tested whether Org-27569 would cause a rightward shift in the dose-response relationship of THC (3, 10, 30, 100 mg/kg). Org-27569 did not alter THC's tetrad effects. We then tested whether lower doses of Org-27569 (3, 10, 30 mg/kg) would antagonize the *in vivo* effects of THC (30 mg/kg). Again, Org-27569 failed to alter the cannabimimetic effects of THC. These studies indicate that Org-27569's anorectic effects on feeding are CB₁ receptor independent and that systemic administration of this compound does not antagonize the behavioral effects of THC. Although these findings do not support the assertion that Org-27569 acts as a CB₁ receptor negative allosteric modulator *in vivo*, it is possible that its lack of effects in the tetrad were due to pharmacokinetic factors (e.g., metabolism, poor CNS penetration). We are in the process of examining the *in vivo* actions of other compounds that act as CB₁ receptor negative allosteric modulators *in vitro*.

THE CB₁ RECEPTOR TRANSACTIVATES THE EPIDERMAL GROWTH FACTOR RECEPTOR AND THE FLK-1 VASCULAR ENDOTHELIAL GROWTH FACTOR RECEPTOR TO REGULATE ERK, BUT NOT FAK, IN NEURONAL CELLS

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Signaling networks that regulate the time-course of CB₁ receptor-mediated extracellular signal-regulated kinase (ERK) and focal adhesion kinase (FAK) activation in neurons are poorly understood. In the present study, murine N18TG2 neuronal cells that express endogenous CB₁ receptors, epidermal growth factor receptors (EGFR), and Flk-1 vascular endothelial growth factor receptors (Flk-1 VEGFR) were used to analyze the role that EGFR/Flk-1 VEGFR transactivation plays in CB₁ receptor regulation of ERK and FAK tyrosine phosphorylation. The LI-COR In Cell Western assay and immunoblotting experiments revealed that CB₁ receptor-mediated ERK and FAK activation can be divided into three phases in N18TG2 cells: phase I (0-5 min) involves maximal ERK activation, phase II involves a decline in ERK activation (5-10 min), and Phase III involves a plateau in ERK activation (> 10 min). Phases I and III ERK phosphorylation are mediated by CB₁ receptor-stimulated ligand-independent transactivation of the EGFR and Flk-1 VEGFR and require activation of Src kinase and the tyrosine phosphatase PTP1B. PTP1B functions as a key positive regulator of Src kinase activity by catalyzing the dephosphorylation(Tyrosine 527)/activation of Src kinase. In contrast to ERK activation, CB₁ receptor-mediated FAK phosphorylation is mediated by activation of Src kinase and PTP1B in N18TG2 cells, but does not involve EGFR/Flk-1 VEGFR transactivation. Protein kinases, protein phosphatases, and RTK transactivation play a central role in the endocannabinoid-mediated signaling networks that regulate cellular behavior and also represent potential drug targets in disease. These studies will contribute to the growing body of evidence that CB₁ receptor agonists and antagonists have therapeutic benefits in the treatment of substance abuse and neurodegenerative diseases.

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BRAIN REGION SPECIFIC CIRCADIAN EFFECTS ON CB₁ RECEPTOR BINDING IN MEMBRANES DERIVED FROM ADULT MICE

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Many biochemical and behavioral processes in mammals are regulated by circadian rhythms. Disruption of circadian cycles, either genetically or environmentally can contribute towards a number of pathophysiological processes (Hastings et al, 2008). The endocannabinoid system, and its manipulation by exogenous cannabinoids has been demonstrated to be implicated in the regulation of a number of processes that are controlled by circadian cycles. These include feeding behavior, body temperature regulation, the sleep/wake cycle, food consumption, CNS regulation of autonomic and endocrine functions and mood (reviewed in Vaughn et al, 2010). Thus, it is possible that the CB₁ receptor, expressed primarily in the central nervous system, may be implicated in these circadian cycles. To study this hypothesis, we examined the relationships among CB₁ receptor affinity for agonist (K_d) and binding site density in membranes harvested from adult male ICR mice sacrificed at 7am, 11am, 3pm, 7pm, 11pm and 3am, after maintenance on a 12 hour light/dark cycle, where the light phase began at 6am. CB₁ receptor saturation binding experiments were performed with brain region membranes harvested from prefrontal cortex, striatum, hippocampus, hypothalamus, amygdala and cerebellum.

In membranes prepared from hippocampus and hypothalamus, differences in only the CB₁ receptor K_d were observed, and B_{max} remained unaltered. In the prefrontal cortex, both CB₁ receptor K_d and B_{max} were lowest at 7am, significantly increased at 11am, and then decreased over the remainder of the light cycle period, with a significant decrease observed between 3pm and 7pm. In the striatum, the CB₁ receptor K_d was highest at 7am, and lowest at 7pm. The K_d was significantly lower at 3pm, 7pm and 3am, compared to 7am. The CB₁ receptor B_{max} in the striatum was highest at 11pm and lowest at 3pm. In the amygdala, CB₁ K_d was lowest at 3pm and increased sharply at 7pm. The K_d was significantly lower at 11am and 3pm compared to 7pm. In the cerebellum, both the CB₁ receptor K_d and B_{max} were lowest at 7am, highest at 3am, then decreased sharply and remained low throughout the dark cycle. The K_d was significantly higher at 3pm than any other time point, whereas the B_{max} was significantly lower at 7pm, 3am and 7am compared to 3pm. In both the hippocampus and hypothalamus, the CB₁ receptor K_d remained high during most of the light cycle, greatly decreasing at 7pm. K_d was significantly higher at 7am, 11am and 3pm compared to 7pm. These data suggest that both the affinity and the density of the CB₁ receptor are altered in a circadian manner, further confirming a role for the endocannabinoid system in circadian cycles. Furthermore, the data suggest brain region specific circadian effects of the CB₁ receptor. However, the mechanisms mediating these alterations are currently unknown. It is possible that alterations in the affinity of CB₁ could be attributed to changes in the activation state of the receptor, receptor desensitization or downregulation. Alterations in CB₁ receptor density could be due to posttranslational modifications of the receptor. Further experiments are required to further investigate the mechanisms underlying the role of the CB₁ receptor in circadian rhythm.

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Hastings et al, (2008). *Journal of Neuroendocrinology*, **20**, 812 – 819; Vaughn et al, (2010). *British Journal of Pharmacology*, **160**, 530 - 543

CHRONIC STRESS EXPOSURE INDUCES SYNAPTIC ADAPTATIONS THROUGH ENDOCANNABINOID SIGNALING MECHANISMS WITHIN THE CENTRAL AMYGDALA

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Numerous studies have revealed the considerable role of endocannabinoid (eCB) signaling in both the neural adaptations to chronic stress (CS) and the pathophysiology of stress-related psychiatric illnesses. Though recent studies within the basolateral amygdala (BLA) demonstrated synaptic correlates of stress-induced neuroadaptations, the role of the main ‘output’ station of the amygdala, the central amygdala (CeA), remains less clear. As such, this study aims to further elucidate eCB-mediated synaptic adaptations within the CeA in response to chronic stress (CS) exposure.

Evoked field excitatory postsynaptic potentials (fEPSPs) were used to determine synaptic adaptations of CeA neurons in response to CS exposure. Male ICR mice were exposed to either 0 days (control mice) or 10 consecutive days of restraint stress (CS mice) for 1 hour per day. Thereafter, evoked fEPSPs were recorded in the lateral division of the CeA in the presence of Picrotoxin. Furthermore, low-frequency stimulations (LFS) were used to induce long-term depression (LTD) at brainstem glutamatergic inputs to the CeA.

Our results show that the CB1 receptor agonist, CP55940, decreased fEPSP amplitude by ~40% in wildtype mice, an effect absent in CB1 knock-out mice. CP55940 also decreased fEPSPs significantly more in CS mice as compared to controls. Furthermore, the selective inhibitor of monoacylglycerol lipase (MAGL), JZL184, revealed a significantly greater decrease of fEPSPs in CS mice. Moreover, the FAAH inhibitor, URB597, produced a small depression of fEPSP amplitude, which was similar in magnitude in both stressed and control mice. Lastly, LFS induced a greater CB1 receptor-dependent LTD at glutamatergic synapses in the CeA of CS mice as compared to controls, and this effect was reversed by the selective CB1 receptor antagonist, Rimonabant.

These data demonstrate that eCB signaling plays a significant role in chronic-stress-induced synaptic adaptations within the CeA. Given the CeA’s essential role in amygdaloidal functionality, further understanding the effects of chronic stress on CeA synaptic efficacy may allow for improved targeting of pharmacological agents used in the treatment of stress-related psychiatric illnesses.

**CANNABINOID RECEPTOR INTERACTING PROTEIN 1A (CRIP1A)
MODULATES STRIATAL NEUROPHARMACOLOGY AND
SIGNAL TRANSDUCTION IN CANNABINOID, DOPAMINE
AND OPIOID RECEPTOR SYSTEMS**

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CB1 cannabinoid receptors, D2 dopamine receptors and DOR1 delta opioid receptors are both members of the GPCR superfamily of transmembrane receptors, are predominately expressed on presynaptic terminals, couple to Gi/o proteins, and initiate similar signal transduction pathways. Together these receptor systems, interact to varying degrees, and have been implemented in neuropathologies and the addiction process for drugs of abuse (Saxon et al., 2005). Therefore, this study utilized shRNA-mediated knock-down of receptors in a circumscribed brain region of adult rats to investigate the interactions of CB1R and CRIP1a, as well as D2R in neurotransmitter regulation, and the potential for alterations in G protein activation and signal transduction in response to manipulating the expression of these proteins.

AAV that contain transgenes to over-express CRIP1a or shRNA designed to knock-down CB1R and D2R were created and injected via unilateral stereotaxic injections into the dorsal striatum of anesthetized adult male Sprague-Dawley rats. Animals were sacrificed at days 3, 5, 10, 17, 28, or 56, and 2mm coronal brain slices were taken at the site of injection. RNA was isolated and purified, converted to cDNA, and quantitated by real-time qPCR. Striata from AAV-CRIP1a over-expressing rats displayed a significant decrease in mRNA levels of prodynorphin (PDYN) and preproenkephalin (PENK) which was followed in time by increased DOR1 levels. Interestingly, knock-down of either CB1R or D2R resulted in significant reductions in the transcript levels of both receptors. Furthermore, shRNA-mediated knock-down of CB1R or D2R parallel the effects seen during CRIP1a over-expression; where CRIP1a was up-regulated and DOR1 mRNA levels increased following reductions in PENK and PDYN.

To determine how alterations in cannabinoid, dopamine and opioid receptor systems observed during CRIP1a over-expression, CB1R and D2R knock-down experiments affect G protein activity and signal transduction, rats received unilateral stereotaxic injections with AAV-transcripts. Animals were sacrificed at 17 days, and either 20 or 40 micrometer coronal brain slices were collected for [³⁵S]GTP gamma S binding studies and in-cell-western techniques to identify changes in receptor activity and ERK and CREB phosphorylation, respectively. Both CRIP1a over-expression and knock-down of CB1 significantly reduced phosphoERK levels in the dorsal striatum. Effects of CREB phosphorylation were not observed during CRIP1a over-expression, CB1 or D2R knock-down.

Interplay between CRIP1a, CB1R, D2R and the opioid receptor systems are revealed phenotypically through the changes observed in this study. These data indicate that CRIP1a may dependently or independently regulate these receptor systems in vivo.

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**THE CANNABINOID CB2 RECEPTOR/Gi COMPLEX :
CROSSLINKING AND COMPUTATIONAL STUDIES SUGGEST
THAT CB2 SIGNALS AS AN ACTIVATED DIMER**

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Recent developments in endocannabinoids indicate that Cannabinoid receptor subtype 2 (CB2), has therapeutic potential in the areas of inflammation and neuropathic pain (Fernandez-Ruiz et al., Mol and Cell Endo. 286 (2008) 91-96). The human CB2 receptor is a Class A GPCR that interacts with Gi/o heterotrimers (Glass and Northup, Mol Pharm. 56 (1999) 1362-1369). In order to determine the structure of the complex that CB2 forms with G protein, we undertook a series of cross-linking experiments that were analyzed using mass spectroscopy. Using a homobifunctional Lys-Lys linker, disuccinimidyl suberate (DSS; space arm length = 11.4 Å, <http://www.thermo.com>) combined with MS/MS analysis, we identified the following crosslinks between CB2 and Gi protein: (1) Gi: K349 (i-5 C term G-alpha / CB2: K6.35(245) and (2) Gi: K317 ($\alpha4\beta6$ loop region G-alpha) / CB2: K5.64 (215). Using HgCl₂ we were also able to establish a disulfide bridge between Gi: C351 (i-3 C term G-alpha) / CB2: C3.53(134) and verify via MS/MS analysis. This data was then used in the modeling study of the CB2/ Gai1 β 1 γ 2 complex.

Our recently published models of the CB2 inactive (R) and active (R*) states (Hurst et al., J Biol Chem. 285 (2010) 17954-17964) along with the crystal structure of Gai1 β 1 γ 2 (Wall et al., Cell. 83 (1995) 1047-1058) were employed in this study. Because the C terminus of Gai is missing in this structure, the NMR structure of the C-terminal undecapeptide of G α T (Kisselev et al., PNAS. 95 (1998) 4270-4275) was used to complete the C terminus of Gai.

Initial docking studies of the CB2 R* state with Gai1 β 1 γ 2 using the experimental constraints above, proved that the links could not occur simultaneously with a monomeric receptor. We therefore explored the hypothesis proposed by Javitch and Weinstein (Han et al., Nat Chem Biol. 5(9) (2009) 688-695), that the minimal GPCR signaling unit is two receptors and a single G protein and that this complex is maximally activated by agonist binding to a single protomer. When the CB2 R/R* dimer complex with Gai1 β 1 γ 2 was modeled with Rhodopsin TMH4-TMH5 dimer interface (Guo et al., PNAS 102 (2005) 17495-19500), the crosslink between K317 (Gi $\alpha4\beta6$ loop) and K5.64(215) was inaccessible. However, using a TMH5/TMH5 dimer interface as seen recently in the x-ray crystal structure of the chemokine CXCR4 receptor (Wu et al., Science 330 (2010) 1066-1071), all three experimental crosslinks were possible simultaneously. [Support: DA011551 (ZHS) and DA003934 and DA021358 (PHR)]

HOMOGENOUS-TIME-RESOLVED-FLUORESCENCE(HTRF): OPTIMIZATION AND VALIDATION OF A CELL-BASED cAMP SCREENING TECHNOLOGYFOR THE CANNABINOID RECEPTORS CB1 AND CB2

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Intro

The cannabinoid receptors hCB1 and hCB2 belong to a family of receptors known as GPCRs (G-Protein Coupled Receptors). Specifically, hCB1 and hCB2 receptors are classified as G_i since activation results in the inhibition of adenylate cyclase. Here, we report the pharmacological evaluation and validation of a novel cell-based cAMP measuring assay known as Homogenous-Time-Resolved-Fluorescence (HTRF) for screening purposes.

Methods:

In the present study, we investigated the effects of the cannabinoid agonist CP55-940 on hCB1 and hCB2 activation in HEK293 cells by measuring cAMP levels. Forskolin-induced release of cAMP was measured via the HiRange cAMP kit from CisBio International.

Results

The hCB1 and hCB2 receptors are negatively coupled to adenylate cyclase (G_i) and activation results in the inhibition of forskolin-stimulated cAMP production. Sigmoidal dose-response curves were created and analyzed via Prism. CP-55,940 inhibited forskolin-stimulated cAMP production concentration-dependently at hCB1 and hCB2. In the forskolin dose-response curve, the EC₅₀ values for hCB1 and hCB2 were 2.670 μ M and 1.4396 μ M, respectively. The 95 % confidence interval for hCB1 and hCB2 for forskolin was 2.6070 μ M to 3.8287 μ M and 1.4396 μ M to 2.3581 μ M, respectively. The overall assay performance was robust due to calculated Z' values > 0.75 and showed good S/B (signal to background) values. Specifically, the signal to background (S/B) values for CB1 and CB2 were 1.37 and 1.77, respectively.

Conclusions

We have validated and optimized the HTRF HiRange cell-based assay for screening purposes for the G_i cannabinoid receptors CB1 and CB2. Overall, this cell-based assay can be used as a primary screening tool to search for novel ligands for these receptors.

CB1R SIGNALING ENHANCES ADRENAL RESPONSIVENESS TO ACTH TO INCREASE GLUCOCORTICOID SYNTHESIS

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INTRODUCTION: The endocannabinoids (eCBs) modulate circadian and stress-induced regulation of the hypothalamic-pituitary-adrenocortical (HPA) axis through CB1 receptor interactions in the paraventricular nucleus of the hypothalamus [Patel, S. *Endocrinology*, 2004. **145**: 5431.; Di, S. *J Neurosci*, 2003. **23**: 4850.] and the anterior pituitary [Cota, D. *Endocrinology*, 2007. **148**: 574.]. However, the ability of CB1R signaling to alter the HPA axis outside of the CNS has been minimally investigated. Therefore, we investigated using *in vivo* and *in vitro* approaches the ability of the CB1R to alter corticosterone (CORT) concentrations via direct actions at the adrenal or via changes in the clearance of CORT from the blood.

METHODS: ICR mice (>8 weeks of age) were used in these studies. In experiment one, whole adrenals were harvested and CORT release from freshly dissociated adrenal cells was examined. Adrenal cells stimulated with either ACTH (2-10 $\mu\text{g}/\text{mL}$) or dibutyryl-cAMP (0.75-1.0 mM) in the presence and absence of the CB1R agonist, WIN55,212-2 (100 nM). In experiment two, WT and CB1R KO males were treated with dexamethasone (2.5 mg/kg) to shut down endogenous ACTH production 40, 22, 16 and 3 hours prior to administration of porcine 1-39 ACTH (40 $\mu\text{g}/\text{kg}$). Blood was sampled at 15 min intervals for an hour after ACTH administration to measure adrenal production of CORT. In experiment three, CORT clearance was measured in female and male mice by collecting blood via tail vein. These mice were treated with dexamethasone (2.5 mg/kg) for 35, 21, 14 and 2.5 hours before the injection of vehicle or SR141716 (5 mg/kg, i.p.). Thirty minutes later a basal blood sample was collected and a bolus of exogenous CORT (160-320 $\mu\text{g}/\text{kg}$) was administered immediately afterward. Serum CORT was determined after 15 to 60 minute intervals.

RESULTS: In experiment one, WIN55212-2 treatment of the adrenal cells did not affect basal CORT release but significantly potentiated CORT release induced by both ACTH and dibutyryl-cAMP. This suggests that activation of CB1 receptors in the adrenal potentiates the ability of cAMP to stimulate CORT synthesis. This conclusion was also reached in experiment 2 as ACTH stimulation of CORT release *in vivo* was lower in CB1R KO mice than WT. In experiment 3, there was no statistically significant difference in CORT clearance, suggesting that the effects in experiment 2 were due to changes in release rather than removal of CORT.

CONCLUSIONS: These data suggest that CB1R signaling could have effects outside of the CNS that also modulate systemic glucocorticoid concentrations. Specifically, the *in vitro* and *in vivo* ACTH stimulation data suggest that CB1R signaling increase CORT release at the level of the adrenal. This effect is the opposite of the effects of inhibitory effects of CB1R activation at higher points in the HPA axis.

Supported by Advancing a Healthier Wisconsin.

BEHAVIORAL AND PHARMACOLOGICAL PROPERTIES OF THE CB1 NEUTRAL ANTAGONIST PIMSR

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Moderating the activity of the cannabinoid CB1 receptor (CB1R) with a neutral antagonist is envisioned to maintain the basal state of this constitutively active receptor. This should re-establish a corresponding endocannabinoid tone of the natural state in circumstances when dysregulation produces behavioral and pharmacological imbalances. One example of dysregulation would be the over activation of the receptor leading to appetite stimulation and lipogenesis. Attempts at blocking the active state of the CB1R with inverse agonists such as rimonabant have led to overshooting the basal state and inducing consequences of an inactive CB1R, such as adverse CNS behavioral effects.

We will present pharmacological and behavioral studies of PIMSR that demonstrate neutral antagonism of CB1R and behavioral properties that include its effects on anxiety, depression, and appetitive conditions such as cocaine enhanced electrical brain stimulation and food intake. Of special note is the electrical brain stimulation reward (eBSR) study that demonstrates the absence of the dysphoria that prevented rimonabant from continued medical use. The eBSR study has been conducted over a wider range of doses than previously reported and continued to show the absence of dysphoric effects and the ability to reduce the cocaine enhancing effects on eBSR, demonstrating a good therapeutic index versus rimonabant. A one-day food intake study showed no change in food consumption, which informs the effect of neutral antagonism on normal appetite and stands in contrast to its effect on suckling in newborn mice in Failure to Thrive Syndrome.

These studies point to a neutral antagonist of CB1R as affording key behavioral effects that distinguish it from inverse agonists and suggest potential utility in moderating the activity of the CB1R to the benefit of the treatment of appetitive disorders and possibly to wider physiological conditions where CB1 inverse agonists have shown efficacy.

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**CB₂-SELECTIVE CANNABINOIDS INHIBIT THE MIXED
LYMPHOCYTE REACTION (MLR), AN IN VITRO CORRELATE
OF ORGAN GRAFT REJECTION**

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Cannabinoids with selectivity for the CB₂ receptor have been shown to have anti-inflammatory and immunosuppressive properties in several different animal models including experimental allergic encephalitis (a mouse model of multiple sclerosis), induced ischemic stroke, and spinal cord injury. Two CB₂-selective agonists, JWH-015 and O-1966, were tested for their potential utility in blocking organ graft rejection. Current medications to prevent graft rejection have undesirable side effects including kidney toxicity and destruction of islet cells of the pancreas with resultant type I diabetes. The Mixed Lymphocyte Reaction (MLR) is considered a reliable in vitro correlate of graft rejection. It was found that both CB₂ agonists inhibited the MLR in a dose dependent manner using cells from wild-type mice, but not when cells from CB₂ receptor deficient mice (CB₂^{-/-}) were used. The CB₂ agonists primarily affected T-cells, as shown by experiments in which murine spleen cells were sorted using flow cytometry into subpopulations of T-cells (CD3⁺) and accessory cells including monocytes, macrophages, and dendritic cells (CD11b⁺). Treatment of a sorted population with a CB₂ agonist before adding it back to the remainder of the untreated cells demonstrated that inhibition of the MLR occurred when the T-cell population was exposed to the cannabinoid. Treatment of the accessory cell population was less effective. These experiments provide another system in which CB₂ selective compounds may have useful application, and also provide a rationale for testing them in vivo for their efficacy in blocking organ or skin graft rejection. This work was supported by NIDA grants DA13429, DA06650, and T32-DA07237.

CANNABINOID INDUCED VASORELAXATION OF HUMAN MESENTERIC ARTERIES

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Cannabinoid induced vasorelaxation has been well studied in a range of animal models. To date the vascular effects of cannabinoids in human mesenteric arteries has not been investigated. Therefore, the aim of the present study was to investigate the vasorelaxant effects of a range of cannabinoids in human mesenteric arteries.

With ethical approval and following written informed consent, human mesenteric arteries were taken from patients receiving colorectal surgery and either used fresh or stored overnight at 4°C. Arteries were dissected and mounted on a Mulvany-Halpern myograph and bathed in oxygenated physiological salt solution at 37°C under a set pressure of 90% of 100 mmHg. U46619 and endothelin-1 were added to increase active tension by a minimum of 5 mN. Once a stable contraction had been achieved the vasorelaxant effects of anandamide, 2-arachidonoylglycerol (2-AG), Δ^9 tetrahydrocannabinol (THC), cannabidiol (CBD) and CP55,940 were assessed as cumulative concentration-response curves.

CP55,940 was the most efficacious cannabinoid tested producing vasorelaxation that was significantly greater than CBD ($n=12$, $P<0.05$, ANOVA), THC and AEA ($n=12$, $P<0.001$, ANOVA). 2-AG ($n=24$) was the most efficacious endocannabinoid tested producing vasorelaxation that was significantly greater than AEA ($n=12$, $P<0.001$, ANOVA). 2-AG also produced vasorelaxation that was significantly greater than THC ($n=12$, $P<0.01$, ANOVA). CBD induced modest vasorelaxation at high concentrations when compared to vehicle control ($n=12$, $P<0.05$ Student's unpaired t -test). THC and AEA caused modest vasorelaxation at low concentrations compared to vehicle control ($n=12$, $P<0.05$ Student's unpaired t -test).

In conclusion, CP55,940, 2-AG, CBD, THC and AEA cause vasorelaxation of human mesenteric arteries. However, cannabinoids demonstrated less potency and efficacy in human arteries than in animal vasculature. Also, in contrast to animal studies, we found that 2-AG has greater efficacy in human mesenteric arteries than that of AEA.

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CANNABINOIDS IMPROVE ENDOTHELIUM-DEPENDANT VASORELAXATION IN ZUCKER DIABETIC FATTY RAT

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Incubation with Δ^9 tetrahydrocannabinol (THC) for 2 h improves endothelium-dependant vasorelaxation in Wistar rats (O'Sullivan *et al.*, 2006). Since diabetes is associated with endothelial dysfunction, the aim of the present study was to assess the ability of cannabinoids to similarly improve endothelial-dependant vasorelaxation in a model of type 2 diabetes, the Zucker Diabetic fatty (ZDF) rat.

Age-matched ZDF rats (weight 370 – 395 g; blood glucose 18 -25 mmol/L) and Zucker lean controls (weight 320-335 g; blood glucose 3-9 mmol/L) were killed by cervical dislocation, and the thoracic aortae removed, cleaned and cut into ring segments. Aortic segments were mounted onto fixed support pins in a myograph (model 610M; Danish Myo Technology). Vessels were bathed in oxygenated Krebs' at 37°C at 1 g tension. Vessels were incubated for 2 h with cannabidiol (CBD), anandamide (AEA), 2-arachidonoylglycerol (2-AG) or vehicle (1 μ M or 10 μ M), after which time they were contracted using methoxamine or U46619. Once a stable contraction was achieved, cumulative concentration-response curves were carried out to acetylcholine (ACh, 1nM-100 μ M).

In the ZDF rats, CBD caused a concentration-dependent increase in the maximal vasorelaxant response to ACh (1 μ M, $P < 0.01$, $n = 6$; 10 μ M, $P < 0.001$, $n = 6$). In the lean Zucker control, CBD (1 or 10 μ M) also significantly enhanced the maximal response to ACh, albeit to a lesser extent ($P < 0.05$, $n = 6$). In the ZDF rats, incubation with AEA significantly increased the maximal vasorelaxant response to ACh at 1 μ M ($P < 0.001$, $n = 6$) and 10 μ M ($P < 0.01$, $n = 6$). By contrast, in the lean Zucker control, incubation with AEA had no effect on vasorelaxation to ACh. In the ZDF rats, incubation with 2-AG caused an increase in the maximal vasorelaxant response to ACh at 1 μ M ($P < 0.01$, $n = 6$) only. By contrast, in the lean Zucker control, 10 μ M 2-AG significantly inhibited the maximal response to ACh ($P < 0.001$, $n = 6$).

In conclusion, CBD improves endothelium-dependent vasorelaxation, and could be beneficial in the treatment of disorders associated with endothelial dysfunction such as diabetes. Similar results were found in the ZDF rats after exposure to exogenously applied endocannabinoids.

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CANNABINOID AND κ -OPIOID MEDIATED DIURESIS IN RATS

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Interactions between opioid and cannabinoid systems have been proposed to modulate some effects of both opioid and cannabinoid drugs. The present studies examined whether these modulatory effects extend to the diuretic actions of cannabinoid and κ -opioid agonists. The diuretic effects of a novel cannabinoid agonist AM4054 (0.01-1.0 mg/kg), the prototypical cannabinoid agonist Δ^9 -THC (0.3-10.0 mg/kg), the cannabinoid antagonist rimonabant (0.1-3.0 mg/kg), the κ -opioid agonist U50,488 (0.1-10.0 mg/kg), and the nonselective opioid antagonist naltrexone (0.3-10.0 mg/kg) given alone or in combination, were determined in female SD rats, n=6/group. For comparison, full dose-effect functions were also obtained for the hypothermic and analgesic effects of AM4054 and Δ^9 -THC. Diuresis was measured by placing the rats in customized restraint devices lined with absorbent pads; differences in pad weight before and after 2-6h experimental sessions were taken as a measure of voided urine. Colonic temperature was measured over 6 hr in restrained animals with thermal probes inserted 6cm; antinociceptive responses were measured using a radiant heat tailflick device (baseline = 1-2 sec, cut-off = 6 sec). Results show that AM4054, Δ^9 -THC, and U50,488 all increased diuresis and rimonabant and naltrexone had neither diuretic nor antidiuretic effects. Peak effects of AM4054, Δ^9 -THC and U50, 488 yielded, respectively, 32±6, 26±4 and 27±3 g/kg urine; following vehicle injection average urine output was approximately 2-4 g/kg urine. Over the same dose ranges as had diuretic effects, AM4054 produced 92±7% of the maximum possible analgesic effect (%MPE) and decreased temperature by 6.0±0.4°C and Δ^9 -THC produced 74±15 %MPE and decreased temperature by 3.4±0.3°C. The diuretic effects of AM4054 were antagonized by 0.1-3.0 mg/kg rimonabant, and the effects of U50, 488 were antagonized by 0.3-3.0 mg/kg naltrexone. However, 3.0-10.0 mg/kg naltrexone did not antagonize the effects of AM4054 or Δ^9 -THC and co-administration of 3.0 mg/kg Δ^9 -THC with 0.3-1.0 mg/kg U50,488 had only additive or less than additive effects. These results demonstrate that cannabinoid agonists dose-dependently increase diuresis at the same doses as produce other characteristic cannabinoid effects. The diuretic effects of cannabinoid agonists are equivalent to those produced by the κ -opioid agonist U50,488. The inability of naltrexone to block the diuretic effects of AM4054 and Δ^9 -THC indicates the different mechanisms mediate the diuretic effects of cannabinoid and κ -opioid agonists, and the lack of supra-additive effects of co-administered U50,488 and Δ^9 -THC suggests that these mechanisms do not interact in a synergistic manner.

MODULATION OF BAROREFLEX SENSITIVITY BY CANNABINOID RECEPTOR ACTIVATION IN RAT SOLITARY TRACT NUCLEUS.

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The endocannabinoid system (ECS) is a widespread central and peripheral cellular signaling system comprised of the biologically active endocannabinoids and the G protein-coupled receptors that mediate their effects. Presynaptic CB1 cannabinoid receptors are the primary neuronal target of the endocannabinoids, and are densely expressed in the solitary tract nucleus (NTS), the primary site for termination of baroreceptor afferent nerve fibers, as well as in vagal afferent neurons of the nodose ganglion. NTS neurons are sensitive to cannabinoid ligands, implicating the potential of the ECS to exert a modulatory influence over the arterial baroreceptor reflex. Our data suggest that activation of cannabinoid receptors within the NTS via direct bilateral microinjection of the CB1 agonist CP55,940 attenuates baroreflex sensitivity (BRS) for control of heart rate (HR) in anesthetized Sprague-Dawley. Resting BRS was 1.06 ± 0.08 ms/mmHg and MAP was 96 ± 3 mm Hg. Acute bilateral NTS microinjection of CP55,940 ($n = 5$) reduced BRS for control of HR to a new baseline of 0.35 ± 0.06 ms/mmHg ($p < 0.01$), a decrease of approximately 65%, while microinjection of vehicle (10% DMSO in 120 nl aCSF, $n = 5$) had no effect on BRS. The effect of CP55,940 on BRS was completely blocked by prior microinjection of the CB1 antagonist SR141716 into the NTS, confirming the effect of CP55,940 was due to activation of NTS CB1 receptors. There was no significant effect of the antagonist alone in these normotensive animals, but it is not clear how chronic administration of CB1 antagonists will affect cardiovagal reflex control of the circulation in the face of hypertension or metabolic syndrome.

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DESIGN, SYNTHESIS AND STRUCTURE ACTIVITY RELATIONSHIPS OF 5-ALKOXY-1,3,4-OXADIAZOL-2-ONES AS POTENT AND SELECTIVE INHIBITORS OF FATTY ACID AMIDE HYDROLASE (FAAH) AND MONOACYLGLYCEROL LIPASE (MGL)

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N-arachidonylethanolamine (AEA) and 2-arachidonoylglycerol (2-AG) are the two major endocannabinoid of the endocannabinoid system. They activate the cannabinoid receptors CB1 and CB2 inducing several biological effects such as appetite stimulation and relief of pain and anxiety. However, these effects remain weak owing to their rapid inactivation by the hydrolytic enzymes fatty acid amide hydrolase (FAAH) and monoacylglycerol lipase (MGL). Hence, the inactivation of FAAH and MGL by specific enzyme inhibitors increases the levels of AEA and 2-AG producing aforementioned therapeutic effects.

In continuation of our research for the development of potent and selective inhibitors of FAAH and MGL¹, we describe extension of structure activity relationships (SAR) of 5-alkoxy-1,3,4-oxadiazol-2-ones (Fig.1). The FAAH and MGL inhibitory activity of these compounds were measured by the procedures described previously.²⁻³

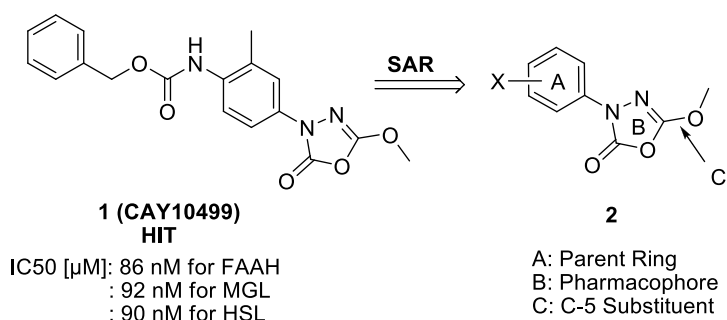


Fig. 1. Oxadiazolone derivatives

The *in-vitro* results give us initial clue for the development of potent and selective FAAH & MGL inhibitors. The further structural modification optimization of 5-alkoxy-1,3,4-oxadiazol-2(3*H*)-ones is under progress for the development of MGL inhibitors.

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INHIBITORY MECHANISM OF MAJOR PHYTOCANNABINOIDS ON CYTOCHROME P450 2D6 ACTIVITY

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Epidemiological studies indicate that many abusers of marijuana concurrently use other recreational drugs, such as amphetamine and 3,4-methylenedioxymethamphetamine, which have been reported to be mainly metabolized by cytochrome P450 (CYP) 2D6. Marijuana leaves contain at least 70 cannabinoids, devoid of nitrogen in their structures. Δ^9 -Tetrahydrocannabinol (Δ^9 -THC), cannabidiol (CBD) and cannabinol (CBN), the three major cannabinoids, have been shown to inhibit CYP-mediated drug oxidations. However, inhibitory effects of cannabinoids on CYP2D6 activity remain to be elucidated. In this study, we examined the potency and mechanism of CYP2D6 inhibition by these major cannabinoids. Δ^9 -THC, CBD and CBN inhibited 3-[2-(*N,N*-diethyl-*N*-methylammonium) ethyl]-7-methoxy-4-methylcoumarin (AMMC) and dextromethorphan *O*-demethylase activities of recombinant CYP2D6 and pooled human liver microsomes ($IC_{50} = 3.0$ - $25 \mu\text{M}$). Among the cannabinoids tested, CBD showed the strongest inhibition against CYP2D6. CBD competitively inhibited the CYP2D6 activities with the apparent K_i values of 1.1- $2.2 \mu\text{M}$. To clarify structural requirements for the CYP2D6 inhibition by CBD, effects of CBD-related compounds on the AMMC *O*-demethylase activity of recombinant CYP2D6 were next investigated. Olivetol ($IC_{50} = 7.21 \mu\text{M}$) inhibited the CYP2D6 activity as potently as CBD did ($IC_{50} = 6.52 \mu\text{M}$), although *d*-limonene and pentylbenzene failed to inhibit the activity. In addition, the methylation of either phenolic hydroxyl group in CBD caused a loss of the inhibitory effect. Cannabidivarin having a propyl side chain inhibited the CYP2D6 activity; its inhibitory effect ($IC_{50} = 10.2 \mu\text{M}$) was less potent than that of CBD. On the other hand, orcinol and resorcinol showed lack of inhibition. The inhibitory effect of CBD on CYP2D6 activity was stronger than those of 16 compounds without nitrogen atom tested, such as progesterone. These results reveal that CBD exhibits potent CYP2D6 inhibition, in which two phenolic hydroxyl groups and the pentyl side chain of CBD may play important roles.

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METABOLISM OF CANNABIDIOL BY CYTOCHROME P450S IN HUMAN LIVER MICROSOMES

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Cannabidiol (CBD), one of the major constituents in marijuana, is nonpsychoactive but has several pharmacological effects such as drug-induced sleep prolongation, antiepileptic, anxiolytic and antiemetic actions. Some of these pharmacological effects may be of therapeutic importance. CBD has been shown to be extensively metabolized by experimental animals and humans. However, human liver enzymes involved in the CBD metabolism remain unclear. In the present study, we investigated *in vitro* metabolism of CBD with human liver microsomes (HLMs) to identify cytochrome P450 (CYP) isoforms responsible for the CBD oxidations. CBD metabolites transformed by HLMs and recombinant human CYP isoforms were analyzed by gas chromatography/mass spectrometry after trimethylsilylation. CBD was metabolized by HLMs to produce eight monohydroxylated metabolites (6 α -OH-, 6 β -OH-, 7-OH-, 1"-OH-, 2"-OH-, 3"-OH-, 4"-OH- and 5"-OH-CBDs), which were identified based on the retention times and typical diagnostic ions. Among these metabolites, 6 α -OH-, 6 β -OH-, 7-OH- and 4"-OH-CBDs were the major metabolites as estimated from the relative abundance of m/z 478, which was a predominant fragment ion of trimethylsilyl derivatives of the metabolites. Seven of 14 recombinant human CYP enzymes examined (CYP1A1, CYP1A2, CYP2C9, CYP2C19, CYP2D6, CYP3A4 and CYP3A5) were capable of metabolizing CBD. The correlations between CYP isoform-specific activities and CBD oxidative activities in 16 individual HLMs showed that 6 β -OH- and 4"-OH-CBDs were mainly produced by CYP3A4, which was supported by inhibition studies with ketoconazole and an anti-CYP3A4 antibody. The correlation and inhibition studies also indicated that 6 α -OH-CBD was primarily formed by CYP3A4 and CYP2C19, while 7-OH-CBD was mainly produced by CYP2C19. These results suggest that CYP3A4 and CYP2C19 may play important roles in the formation of 6 α -OH-, 6 β -OH-, 7-OH- and 4"-OH-CBDs in HLMs.

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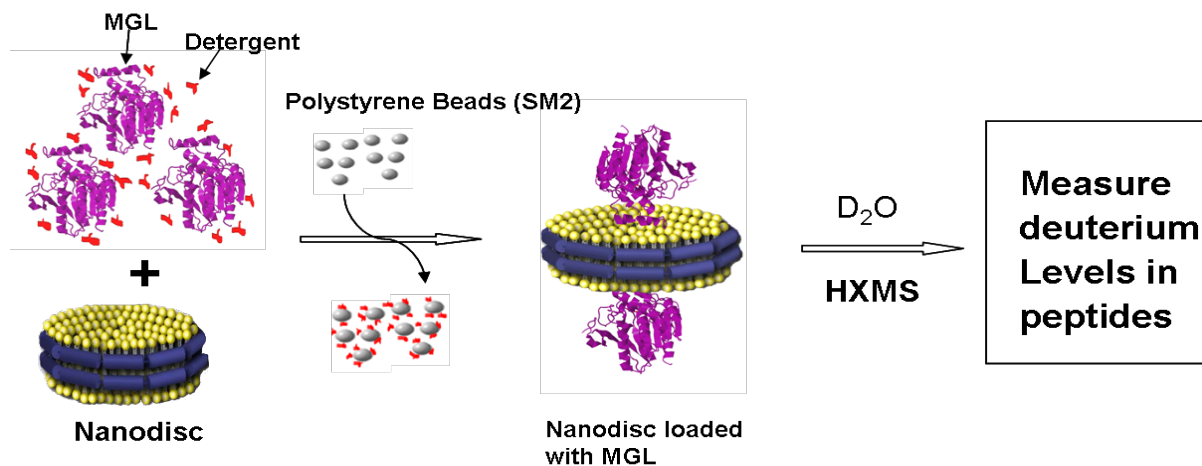
STUDIES ON THE DYNAMIC PROPERTIES OF MONOACYLGLYCEROL LIPASE IN NANODISC MODEL MEMBRANE

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Monoacylglycerol lipase (MGL) is a key serine esterase enzyme of endocannabinoid signaling and is considered an important target for therapeutic intervention. It is known that substrate hydrolysis by a lipase is activated at the lipid-water interface and this triggers a conformational change in the enzyme. This is accompanied by opening of an α -helical lid covering the active site in aqueous solution. The structural details involving the MGL-membrane interaction have not been studied and such information will be of value in understanding the mechanism of action of the enzyme. Nanodiscs have recently been shown to be of great use for studying membrane proteins since they offer a stable, controllable and monodisperse model membrane with a native-like lipid bilayer. We have developed a protocol to prepare negatively charged phospholipid nanodiscs (NCPN) using a membrane scaffold protein (MSP1D1) and POPC-POPG lipids. MGL was attached to NCPN and the biochemical properties of this complex were studied. To obtain information on the dynamic changes accompanying the catalytic action of the free and bound enzyme, we use hydrogen deuterium exchange mass spectrometry (HXMS). Our results will be presented and discussed.



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ACTIVATION OF HUMAN PLATELETS BY 2-ARACHIDONOYLGLYCEROL: FURTHER INSIGHTS INTO THE UNDERLYING SIGNALING

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Human platelets have the biochemical tools to metabolize endocannabinoids, and are activated by micromolar concentrations of 2-arachidonoylglycerol (2-AG) through a CB₁/CB₂-dependent mechanism. However, the question of what molecular pathways underlie the effect of 2-AG on platelet aggregation remains open.

Here, we have measured intracellular calcium, nitric oxide (NO) and cGMP levels, as well as protein kinase C (PKC) and endothelial nitric oxide synthase (eNOS) activities. Moreover, we checked the phosphorylation status of eNOS upon 2-AG treatment and the role of PKC and/or PI3K/AKT on these mechanisms was investigated.

We demonstrated that 2-AG activates dose-dependently washed human platelets and increases intracellular calcium levels. Moreover, 2-AG activated PKC measured as p47 pleckstrin phosphorylation. These parameters were prevented by the thromboxane A₂ receptor antagonist SQ29548, by PLC pathway U73122 and PKC GF109203X inhibitors. No effect on 2-AG-induced platelet activation and calcium elevation in the presence of inhibitors of fatty acid amide hydrolase or monoacylglycerol lipase was observed. In addition, we have shown that 2-AG dose-dependently increased NO and cGMP levels. These effects were abolished by U73122, GF109203X, EGTA and the intracellular calcium chelator BAPTA/AM. Moreover, 2-AG enhanced eNOS activity through the phosphorylation of its positive regulatory residue ser1177 and by dephosphorylation of thr495. The eNOS ser1177 phosphorylation was inhibited by U73122 and GF109203X but it was unaffected by the PI3K/AKT pathway inhibitors LY294002 and MK2206. The dephosphorylation of thr495 was reversed by low concentrations of calyculin A.

Taken together, these data suggest that 2-AG behaves as a true platelet agonist by stimulating PKC activation and Ca²⁺ elevation. Likely 2-AG can modulate platelet activation by increasing NO levels through eNOS activation.

DETECTION OF THE ENDOCANNABINOID METABOLOME IN HUMAN BREAST MILK

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The endocannabinoid system plays a diverse role in reproductive health and CNS development, function and protection. Specific endocannabinoids have been detected in human breast milk, where they stimulate the suckle reflex in the baby's brain to promote ingestion. Our recent report on the endocannabinoid metabolome underlies the role of diet in modulating the response of the endocannabinoid metabolome (Wood et al. *J Lipid Res.*, 2010). Breast milk composition varies with diet; specifically, fatty acid content reflects dietary fatty acids. Our motivation for this work is to characterize the endocannabinoid metabolome of human milk and explore how maternal diet modulates the metabolome constituents. We have developed a targeted metabolomics LC-MS/MS approach for studying the effects of external factors on 15 endocannabinoid metabolites in biological matrices (Williams et al. *Anal Chem.*, 2007). This method is now being applied to human breast milk to determine which members of the endocannabinoid metabolome can be detected and to what extent they can be quantified. Glycerols containing arachidonoyl, palmitoyl, oleoyl, docosahexaenoyl and eicosapentaenoyl groups and acids containing arachidonoyl, docosahexaenoyl and eicosapentaenoyl groups are easily detected in 50 μ L of pooled human milk. Much lower concentrations of ethanolamides containing the arachidonoyl, palmitoyl, oleoyl, docosahexaenoyl groups and eicosapentaenoylglycerol are detected in 3 mL of pooled human milk. We will use this method to show the effects of maternal diet and physiological status on the endocannabinoid profile. This provides an opportunity for exploring and understanding the mechanism by which endocannabinoids are regulated in human milk.

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BIDIRECTIONAL EFFECTS OF CANNABIDIOL ON BODY WEIGHT GAIN IN RATS

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Cannabidiol (CBD) is a major non-psychotropic compound of Cannabis that has wide therapeutic potential. Our previous studies revealed decreased body weight gain following CBD administration in rapidly growing rats (8-10 weeks of age at start of experiment). The present study evaluated the effects of repeated CBD administration on body weight gain, food intake and intra-abdominal adipose tissue accumulation in rats at age of 14-16 weeks, under standard (SD) and high fat (HFD) diet.

Adult male Wistar rats (n=36) weighing approximately 400 g at the beginning of the experiment, fed with SD only, or having access to free choice HFD (60% kcal from fat, 10% kcal from sucrose) received CBD for 14 consecutive days (5 mg/kg/day, intraperitoneal injections). Body weight gain and intake of food and water were measured daily. Total intra-abdominal fat was assessed by a dissection method. Leptin concentration in plasma was assessed by RIA method.

In contrast to previous findings, CBD treatment produced significant increase in body weight gain in rats fed SD, but no significant change in food or water intake was observed. CBD produced tendency for decreased body weight gain in rats fed with HFD, but it did not reach level of significance. CBD did not affect total energy intake in rats fed HFD, but slightly lowered preference for HFD. CBD administration reduced intra-abdominal adipose tissue accumulation, which was accompanied by the decrease in plasma leptin concentration.

The results suggest that CBD may produce bidirectional effects on body weight gain depending on age or the metabolic state of the animal. Moreover, the results indicate that CBD may affect accumulation of intra-abdominal adipose tissue.

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THE NON-PSYCHOACTIVE PLANT CANNABINOID, CANNABIDIOL AFFECTS CHOLESTEROL METABOLISM-RELATED GENES IN MICROGLIAL CELLS.

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Cannabidiol (CBD) is a non psychoactive plant cannabinoid that is clinically used in a 1:1 mixture with the psychoactive cannabinoid Δ^9 - tetrahydrocannabinol (THC) for the treatment of neuropathic pain and spasticity in multiple sclerosis. We previously reported that CBD exerts anti-inflammatory effects on microglial cells. In addition, we found that CBD treatment increases the accumulation of the endocannabinoid *N*-arachidonoyl ethanolamine (AEA), thus enhancing endocannabinoid signaling. Here we proceeded to investigate the effects of CBD on the modulation of lipid related genes in microglial cells. Cell viability was tested using FACS analysis, AEA levels were measured using LC/MS/MS, gene array analysis was validated with real time qPCR, and cytokine release was measured using *ELISA*. We report that CBD significantly upregulated the mRNAs of the enzymes sterol-o-acyl transferase (*Soat2*), which synthesizes cholesteryl esters, and of sterol 27-hydroxylase (*Cyp27a1*). In addition, CBD increased the mRNA of the lipid droplet associated protein, perilipin2 (*Plin2*). Moreover, we found that pretreatment of the cells with the cholesterol-chelating agent, methyl- β -cyclodextrin (MBCD) reversed the CBD-induced increase in *Soat2* mRNA, but not in *Plin2* mRNA. Incubation with AEA increased the level of *Plin2*, but not of *Soat2* mRNA. Furthermore, MBCD treatment did not affect the reduction by CBD of the LPS-induced release of the proinflammatory cytokine IL-1 β . To conclude, CBD treatment modulates cholesterol homeostasis in microglial cells, and pretreatment with MBCD reverses this effect without interfering with CBD's anti-inflammatory effects. The effects of the CBD-induced increase in AEA accumulation on lipid-gene expression are discussed.

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CANNABIDIOL ACTIVATES GLYCOGEN SYNTHASE KINASE 3 IN NEURONS VIA INHIBITION OF PKA-MEDIATED PHOSPHORYLATION

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Glycogen synthase kinase 3 β (GSK-3 β) is a ubiquitously expressed kinase whose activity is critical for the regulation of cell survival and cell death programs. GSK-3 β is inhibited by lithium evidence that hyperactivation of GSK-3 β contributes to the symptoms of bipolar disorder (BPD). Epidemiological studies demonstrate a significant co-morbidity between cannabis use and BPD which suggests that chemicals in cannabis could increase GSK-3 β activity and thereby predispose the development of BPD. Cannabidiol (CBD) is present at high amounts in cannabis. We have tested the hypothesis that CBD affects GSK-3 β activity.

These studies were carried out using cerebellar granule neurons (CGN) from neonatal rats and mice. CGN were maintained for 6-7 days after isolation in depolarizing media, which maintains both cell viability and GSK-3 β in a largely inactive state. The activation state of GSK-3 β is regulated by phosphorylation at serine 9; we used western blotting to measure the ratio of the phosphorylated GSK-3 β (pGSK-3 β) to total GSK-3 β .

CGN exposed to CBD for 6 hours exhibited a concentration-related decrease in pGSK-3 β /GSK-3 β ; significant effects were seen at 1, 3 and 10 μ M CBD. The effect of 1 μ M CBD was time dependent, with significant effects at incubation times of 40 min and longer. The changes in GSK-3 β phosphorylation were accompanied by an increase in enzymatic activity, measured using phosphoglycan synthase peptide-2 as a substrate, indicating that CBD increases GSK-3 β activity via a decrease in serine 9 phosphorylation. Several kinases can phosphorylate GSK-3 β at serine 9, including PKA. We tested the hypothesis that CBD reduces pGSK-3 β /GSK-3 β via inhibition of PKA activity. In support of this hypothesis, incubation of CGN with 1 μ M CBD for 2 hours resulted in complete loss of the phosphorylation at serine 133 of cAMP response element binding protein. We also demonstrated that CBD inhibits forskolin-induced increases in pGSK-3 β /GSK-3 β . The site of action of CBD appears to involve a G protein coupled receptor since pertussis toxin inhibited the effect of CBD to reduce GSK-3 β phosphorylation. We have ruled out known GPCR targets of CBD; the receptor target is currently unknown.

These studies demonstrate that CBD has potent, inhibitory effects on a GPCR/Gi/PKA/GSK-3 β pathway that can result in enhancement of GSK-3 β activity through inhibition of PKA-dependent phosphorylation. These results could have important implications for the exposure of the brain to CBD during critical developmental periods and could contribute to the mechanism by which cannabis use predisposes individuals with other risk factors to develop BPD.

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**ALTERATIONS OF THE ENDOCANNABINOID SYSTEM
IN *IN VITRO* AND *IN VIVO* MODELS OF HUNTINGTON'S DISEASE**

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In this investigation we analyzed the main components of the so-called “endocannabinoid system” (ECS) in R6/2 mice, a widely used model of Huntington’s disease (HD).

We measured the endogenous content of anandamide (AEA) and 2-arachidonoylglycerol (2-AG), of their biosynthetic (NAPE-PLD and DAGL, respectively) and hydrolytic enzymes (FAAH and MAGL, respectively), and of their target receptors (CB₁, CB₂ and TRPV1) in the brain of wild-type and R6/2 mice of different ages. In addition, we measured FAAH activity in lymphocytes of R6/2 mice, in order to evaluate whether central ECS alterations were mirrored by peripheral cells.

In 12-week-old R6/2 mice we found a reduction of NAPE-PLD and DAGL activity, and of CB binding, as well as an increase in 2-AG content when compared to wild-type littermates, without any other change in ECS elements. Our analysis was extended to HD43 cells, an inducible cellular model of HD derived from rat ST14A cells. In both induced and uninduced conditions we demonstrated a fully functional ECS, and we showed that HD43 cells replicate the decrease in FAAH activity (half of that measured in ST14A cells) already observed in human brain and lymphocytes of HD patients.

Overall, our data suggest that ECS is differently affected in mouse and human HD, and that HD43 cells are suitable for high throughput screening of FAAH-oriented drugs affecting HD progression.

IDENTIFICATION OF CB₂ RECEPTORS IN GLIAL ELEMENTS IN THE SUBSTANTIA NIGRA OF PATIENTS AND EXPERIMENTAL MODELS OF PARKINSON'S DISEASE

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Cannabinoid CB₂ receptors have been identified in glial cells recruited at lesioned sites in various neurodegenerative disorders, including Alzheimer's disease, Huntington's chorea, amyotrophic lateral sclerosis, multiple sclerosis, and others, where this receptor seems to play a neuroprotective role (Fernández-Ruiz et al., Trends Pharmacol. Sci. 28, 39-45, 2007). By contrast, the issue has remained controversial in Parkinson's disease (PD) with only a recent study describing CB₂ receptor up-regulation in glial elements and beneficial effects of CB₂ agonists in MPTP-lesioned mice (Price et al., Eur. J. Neurosci. 29, 2177-2186, 2009). By contrast, CB₂ receptor up-regulation was very poor in 6-hydroxydopamine-lesioned rats (García et al., Brit. J. Pharmacol. Brit. J. Pharmacol., in press, 2011) whereas their activation with compounds targeting selectively this receptor did not reduce nigrostriatal damage (García-Arencibia et al., Brain Res. 1134, 162-170, 2007). In this work, we wanted to re-examine the issue by looking at the striatum and the substantia nigra in the case of patients affected by PD (post-mortem samples provided by different biobanks) and also using samples coming from different experimental models of parkinsonism (i.e. rats and mice lesioned with 6-hydroxydopamine, MPTP-lesioned mice, LPS-lesioned mice, and lactacystin-lesioned mice). Our data confirmed that CB₂ receptors are up-regulated in the substantia nigra and/or striatum in experimental parkinsonism, in particular in those cases in which the lesion is provoked by a pro-inflammatory neurotoxin: i.e. LPS. Lower responses were found in those cases, such as 6-hydroxydopamine, in which inflammatory responses are modest. Interestingly, all post-mortem samples from patients that were analysed showed up-regulation of CB₂ receptors. Using double-staining analyses we could identify the cellular substrates where CB₂ receptors are located in lesioned structures, that frequently corresponded to glial elements, in particular microglial cells and also astrocytes. We also obtained solid evidence that the activation of these receptors with selective agonists (i.e. HU-308) in LPS-lesioned mice preserved nigrostriatal dopaminergic neurons, as well as that CB₂ receptor-deficient mice resulted more vulnerable to the effect of LPS. Therefore, these data show for the first time an up-regulatory response of CB₂ receptors in lesioned structures of PD patients which has been also confirmed in various models of experimental parkinsonism, in particular in those models showing greater inflammation and glial activation. In addition, the location of these receptors in glial elements suggests that they should be involved, as in other disorders, in the regulation of glial influence to neurons.

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CHANGES IN CB₁ AND CB₂ RECEPTORS AND FAAH ENZYME IN THE CEREBELLUM OF PATIENTS WITH SPINOCEREBELLAR ATAXIAS

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Spinocerebellar ataxias (SCAs) are a group of neurodegenerative diseases, clinically and genetically heterogeneous, characterized by loss of balance and motor incoordination due to dysfunction/degeneration of the cerebellum and its afferent and efferent connections. SCAs belong to the family of disorders caused by expansion of a polyQ tract, so-called polyglutaminopathies. Whereas in other diseases, cannabinoids improve the motor symptoms and attenuate the progression of brain damage, SCAs remain to be studied in relation with a possible therapy with cannabinoids. With this idea in mind, we wanted to determine the status of the endocannabinoid system in this disease using postmortem human brains from patients with different types of ataxias, obtained from two brain banks (Hospital Clinic, Barcelona, Spain, and Netherlands Brain Bank, Amsterdam, The Netherlands). These samples were used for immunohistochemical analysis of CB₁ and CB₂ receptors and the endocannabinoid-degrading enzyme, FAAH. These analyses proven the existence of differences in CB₁ receptor immunostaining in the cerebellum of patients compared with controls, in particular, we found an increase in immunoreactivity for this receptor that was evident in granular and Purkinje layers but also in the dentate nucleus and areas of white matter. CB₂ receptor immunostaining was also increased in the cerebellum of patients although these changes were evident in Purkinje and granular layers and areas of white matter. Lastly, FAAH immunostaining was found in different parts of the cerebellum, but only in the Purkinje layer and areas of white matter, levels found in patients were higher than in control subjects. In summary, our study demonstrates that the endocannabinoid system, in particular the CB₁ and CB₂ receptors and the FAAH enzyme, is significantly altered in the cerebellum of SCA subjects, which supports the idea that the pharmacological management of this system may have therapeutic value in this disease as happens in other neurodegenerative disorders.

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NEUROPROTECTIVE EFFECTS OF THE PHYTOCANNABINOID-BASED MEDICINE SATIVEX® IN EXPERIMENTAL MODELS OF HUNTINGTON'S DISEASE

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Several cannabinoid agonists afford neuroprotection in different experimental models of Huntington's disease (HD), although the type of cannabinoid compound (i.e. CB₁ agonist, CB₂ agonist, antioxidant cannabinoid) most effective depends on the pathological characteristic of each model used. This observation supports the idea that the type of compound(s) that may be useful in HD patients should be a multi-targeting cannabinoid or a combination of different selective compounds. In this study, we examined whether the combination of botanical extracts enriched with either Δ^9 -tetrahydrocannabinol or cannabidiol, which are the main constituents of the cannabis-based medicine Sativex®, serves as a neuroprotective agent in three different models of HD in which striatal damage depends predominantly on different cytotoxic mechanisms: (i) oxidative injury in 3-nitropropionate-administered rats, (ii) excitotoxicity in quinolinate-lesioned mice, and (iii) inflammation in rats treated with malonate. We obtained solid evidence indicating that Sativex® preserves striatal neurons and attenuates cytotoxic events in these three different models. More specifically, Sativex® removed the deficiency in endogenous antioxidant defenses and attenuated the up-regulation of calpain that occurs in 3-nitropropionate-lesioned rats. It reduced edema and normalized glutamate anomalies typical of quinolinate-lesioned mice, and also reduced edema and inflammatory events predominantly associated with malonate toxicity in rats. In summary, this study provides preclinical evidence in support of a beneficial effect of the cannabis-based medicine Sativex® as a neuroprotective agent capable of delaying disease progression in HD, a disorder that is currently poorly managed in the clinic, prompting an urgent need for clinical trials with cannabinoids that produce positive effects in preclinical studies.

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EFFECT OF CANNABINOIDS IN *IN VITRO* MODELS OF ALZHEIMER'S DISEASE

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Introduction. Investigations aimed at exploring cannabidiol (CBD) effects on A β -induced neurotoxicity demonstrated that this cannabinoid was able to protect differentiated PC12 neuronal cells from the detrimental insult induced by A β peptide exposure, through a combination of antioxidant, anti-inflammatory, and anti-apoptotic properties. The beneficial effects of CBD were also confirmed in an "in vivo" model of AD-related neuro-inflammation induced by the intra-hippocampal injection of the human A β (1-42) fragment in mice, where CBD, dose-dependently, inhibited reactive gliosis by attenuating glial cell activation and pro-inflammatory mediators release.

The considerable and well-documented neuro-protective, anti-inflammatory, and antioxidant properties of CBD encouraged to test other non-THC cannabinoids, such as cannabichromene (CBC), cannabigerol (CBG) and cannabidivarin (CBDV), in the A β -induced toxicity.

Materials and Methods. Glial cells (C6) were cultured in 10% FBS supplemented DMEM, PC12 neuronal cell were cultured in 10% FBS plus 5% HS supplemented DMEM and SHY-5SY were cultured in 10% FBS supplemented RPMI. Neuronal cells were differentiated with retinoic acid. After 24h of starvation glial cells and differentiated PC12 and SHY-5SY were treated with 1 μ g/mL of A β (1-42) in the presence of CBC, CBG and CBDV at concentration of 10⁻⁹ to 10⁻⁶ M. Cell viability was assessed by MTT assay; nitrite production was measured by Griess reaction. In the same experimental conditions RT-PCR was used to evaluate iNOS, transcription.

Results. The obtained results indicate that both CBC and CBDV, but not CBG, reduce the reactive gliosis resulting from A β -treatment of glial cells. In fact, CBC and CBDV treatments decreased both the nitrite production and glial proliferation in A β -stimulated C6 cells. Moreover the administration of CBDV prevented the transcription of iNOS protein.

In parallel experiments with A β -treated PC12 cells, CBC, CBG and CBDV showed no significant effect on A β -neurotoxicity. Finally, only CBDV was able to prevent, in a concentration dependent manner, A β -induced neuronal death in differentiated SHSY-5SY cells.

Conclusions. These data indicate that both CBC and CBDV are able to exert *in vitro* a significant effect on the control of A β -induced reactive gliosis and neurotoxicity. Nevertheless, further investigations are necessary to clarify the anti-inflammatory and neuroprotective effects exhibited by these compounds.

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BIOMARKER-GUIDED TARGETING ENDOCANNABINOID SYSTEM AFTER TRAUMATIC BRAIN INJURY (TBI)

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The endocannabinoid (eCB) system plays an important role in driving neural progenitors in normal development and after injury. A strong expression of CB1 receptors was detected in CD133-positive immature progenitors inside the core of developing clonal neurospheres. In neurospheres differentiating on an adherent matrix, CB1 expression was found in cells of neuronal lineage, in residual CD133 positive cell clusters, and, partially, GFAP-positive cells, suggesting a possible role of CB1 in astrocyte differentiation. Synthetic endocannabinoids sustained growth of neurospheres from stem/progenitor cells of mouse and rat postnatal forebrain, while AM251 abolished the generation of clonal neurospheres.

Given the significance of stem/progenitor cells in tissue repair, we propose that the eCB system is a major player in tune-up responses following brain damage, particularly traumatic brain injury (TBI). It has been suggested that modulation of CB receptors can be exploited for pharmacological intervention of TBI including the use of endocannabinoid-like molecules. However, 2005 clinical trials of synthetic cannabinoid Dexamabinol failed to show benefits in patients with severe TBI. The reason for this has been apparently a lack of therapy criteria. We systematically examined whether CB1 and CB2 receptors can serve as biochemical markers to guide CB1 receptor targeting therapy in the course of TBI in rat models of controlled cortical impact (CCI). While CB1 receptors are abundantly expressed in CNS and undergo down-regulation and remodeling after trauma, CB2 receptors are barely detectable in the normal brain and were up-regulated after TBI, suggesting involvement of inflammatory cells presenting the CB2 receptor.

The time-dependent redistribution of CB1 and CB2 in the injured brain provides a rationale for correct timing of CB1/CB2-directed pharmacological intervention of TBI when the target is present and may be responsive to therapy. Appropriate monitoring of CB1 status by measuring its level in circulation may provide a useful method for early diagnostics of TBI, as well as control of therapy.

MODULATION OF INFLAMMATORY RESPONSE BY A CANNABINOID RECEPTOR–2–SELECTIVE AGONIST FOLLOWING SPINAL CORD INJURY

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Previous studies from our laboratory showed that treatment with the CB2R specific agonist O-1966 improved recovery of motor and bladder function in mouse models of acute spinal cord injury. In SCI, increased infiltration and activation of inflammatory immune cells that follow the initial injury exacerbate neuronal damage. In this study, we assessed the inflammatory response following SCI in animals treated with CB2R agonist.

An acute SCI was caused in mice by generating a contusion in the thoracic spinal cord via an impactor device. Animals were treated with CB2R agonist O-1966 (5 mg/kg) or vehicle at 1 and 24 hr post injury. Spinal cord was harvested at various time points. RNA was extracted for analysis of expression of inflammatory cytokines and chemokines via real-time PCR array. Flow cytometric analysis was performed to determine the extent of peripheral immune cell infiltration into the injured site in the cord. Activation of resident microglia was assessed by immunohistochemistry.

There were no differences in the expression of inflammatory molecules or in the number of infiltrating peripheral immune cells between vehicle treated controls and mice treated with O-1966 at an early time-point. However, at later time-points, we observed a significant decrease in the expression of CXCL family chemokine members CXCL9, 10, and 11, the CC family chemokine receptor members CCR1, 2, 3, the cytokine IL-23 and its receptor, and the Toll like receptors TLR1, 4, 6, and 7 in spinal cords from mice treated with O-1966. This decrease correlated with the reduction in number of infiltrating myeloid cells. We also observed reduced microglia activation as determined by immunohistochemical staining for Iba1. Based on these results, we postulate that the beneficial effect of CB2R agonists in acute SCI is mediated, at least partially, through the inhibition of CNS resident microglia/astrocyte activation, leading to reduced levels of chemokines and pro-inflammatory cytokines, and reduced infiltration of peripheral immune cells.

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CANNABINOID-ETHANOL INTERACTION IN THE REGULATION OF DEVELOPING NEUROGENESIS IN ZEBRAFISH BRAIN

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CB1 cannabinoid receptor (CB1R) activation is known to stimulate neurogenesis in developing and adult rat brain. Chronic ethanol treatment has been shown to inhibit adult brain neurogenesis. We have recently showed that zCB1R mRNA transcript is expressed in the different regions of zebrafish brain at 48 and 72 hpf (hour post-fertilization). We have also showed that activation of CB1R increased zCB1R expression and neurogenesis in zebrafish brain. The objective of the present study is to determine if CB1R regulates ethanol-mediated inhibition of developmental neurogenesis in zebrafish. We have used green fluorescence protein (GFP)-expressing transgenic zebrafish (ISLET-1) embryos to study neurogenesis using confocal microscopy. We showed that knocking down CB1R using morpholino or antagonist-mediated blockade of CB1R attenuated CB1R-mediated increase in neurogenesis. Further we have found that chronic ethanol (0.5%-1.5%)-treatment during embryonic development (6hpf-36 hpf) dose-dependently reduced zCB1R expression and motor neurogenesis. Co-treatment with CB1R agonist CP5540 or FAAH-inhibitor URB597 attenuated ethanol-mediated inhibition of developmental neurogenesis. We also found that CB1R activation stimulates *sox-7* and BDNF gene expression and chronic ethanol (0.5%-1.5%)-treatment reduced BDNF gene expression in developing zebrafish brain. We are currently extending these studies to determine a) if direct and/or indirect activation of BDNF signaling can reverse ethanol-mediated neurogenesis and b) signaling mechanism that regulates CB1R-mediated activation of BDNF and Sox-7 in relation to neurogenesis.

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URB597-MEDIATED ACTIONS IN AN OPTIC NERVE INJURY MODEL

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Increasing levels of the endocannabinoid anandamide (AEA), using the fatty acid amide hydrolase (FAAH) inhibitor, URB597, has been shown to have cell survival effects in the CNS, although the mechanisms are not clear. This study examined the contribution of the CB₁ receptor (CB₁R) to the action of URB597, in a rat optic nerve axotomy model of retinal ganglion cell (RGC) neurodegeneration.

RGCs were retrogradely labelled using fluorogold (FG) 7 days prior to transection of the optic nerve 1 mm behind the eye globe. Vehicle, URB597 (0.3 mg/kg i.p.), the CB₁R antagonist, AM281 (2.5 mg/kg i.p.), URB597 (0.3mg/kg i.p.) + AM281 (2.5mg/kg i.p.) were administered daily until sacrifice. Eucleated eyes were then processed for histology and quantitative analysis. FG+ RGCs and phagocytotic microglia (MG) were quantified in retinal whole-mounts across four retinal quadrants.

URB597 increased RGC survival after 1 and 2 weeks of axotomy ($p < 0.05$) compared to vehicle-treated animals. URB597-treated retinas also had a significant reduction in phagocytotic MG after 2 weeks of axotomy ($p < 0.05$), but not after 1 week. An increase in RGCs after 1 week axotomy was not seen in animals treated with URB597 + the CB₁R antagonist, AM281, but as with URB597 alone, no significant effect on phagocytotic MG were seen at 1 week compared to vehicle-treated retinas. AM281 treatment alone did not result in increased RGC survival after 1 week of axotomy or changes in phagocytotic MG compared to vehicle-treated animals.

These initial results suggest that the observed RGC neuroprotection seen with URB597 treatment following axotomy may involve CB₁R. URB597 effects on MG are currently under investigation.

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FAAH DEFICIENT MICE ARE RESISTANT TO THE EFFECTS OF CHRONIC STRESS ON ANXIETY AND MICROSTRUCTURE OF THE AMYGDALA

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Endocannabinoid signalling is known to modulate anxiety-like behaviours. In particular, inhibition of fatty acid amide hydrolase (FAAH), the enzyme responsible for the metabolism of the endocannabinoid ligand anandamide (AEA), produces anxiolytic effects in an array of preclinical models. Both FAAH activity and AEA content are known to be modulated by stressful stimuli in brain regions, such as the amygdala, which are critical for the generation of anxiety and fear. The current study aimed to examine the role of endocannabinoid signalling in the development of anxiety and morphological changes within the amygdala following chronic stress. Exposure of mice to 21 days of 6 h/day restraint stress resulted in a significant reduction in AEA content within the amygdala, but did not modulate amygdalar levels of the other endocannabinoid ligand 2-arachidonylglycerol, or the binding site density of the cannabinoid CB₁ receptor. This reduction in AEA content was met by a significant increase in the constitutive hydrolytic activity of FAAH within the amygdala, indicating that chronic stress results in an increase in basal FAAH-mediated AEA hydrolysis. To determine if this increase in FAAH and loss of AEA signaling was involved in changes in amygdalar morphology and emotional behaviour we examined the effects of chronic stress in wild type and FAAH deficient mice on anxiety-like behaviour and amygdalar morphology. Wildtype mice exhibited an increase in anxiety-like behaviour in the elevated plus maze and an increase in dendritic spine formation and expansion of the dendritic tree of pyramidal neurons in the basolateral amygdala. Mice lacking FAAH were resistant to both changes in anxiety-like behaviour and architectural changes in pyramidal neurons of the basolateral amygdala. These data suggest that chronic stress results in an activation of FAAH-mediated AEA hydrolysis within the amygdala, which in turn contributes to the development of anxiety-like behaviours and changes in amygdalar morphology. Collectively, these data also support the hypothesis that inhibition of FAAH may be a suitable target for the development of anxiolytic agents.

EFFECTS OF ACUTE CANNABINOID ADMINISTRATION ON ADOLESCENT EMOTIONALITY

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Adolescence is a phase of life in which use and abuse of drugs, including cannabis, often begins. The endocannabinoid system has been shown to play a critical role in neurodevelopment and during adolescence, its content and activity is region specific and varies from that observed in adulthood. Thus, with differential basal levels between adolescence and adulthood, it is reasonable to suggest that the effects of acute cannabinoid administration will result in age-related behavioural differences. Previous work has demonstrated that acute exposure to exogenous cannabinoids in adolescence has effects on locomotion, social behavior, and nociception, with adolescents often showing similar directionality of cannabinoid effects as adults but differential sensitivities. On the other hand, studies examining the expression of adult anxiety and depression-like behaviour following chronic adolescent cannabinoid administration have yielded mixed results. However, no studies have investigated the acute effects of cannabinoids on adolescent emotionality. The current study seeks to investigate whether inhibition or activation of this system has acute effects on anxiety and depression-like behavior in adolescence, and how these compare to adults. In this study, adolescent (post natal day 40) and adult (post natal day 75) male Sprague-Dawley rats were administered either a single IP injection of the CB₁ receptor agonist HU-210 or vehicle. The animals were tested on the elevated plus maze as a measure of anxiety, forced swim test and the sucrose preference test for depressive-like behaviour. Preliminary results suggest that HU-210 reduced anxiety in the elevated plus maze in adolescents. Effects of the drug treatment on the forced swim test and sucrose preference test will also be discussed.

**LACK OF STRESS HABITUATION IN ADOLESCENT RATS
IS ASSOCIATED WITH AN ABSENCE OF AMYGDALAR
ENDOCANNABINOID SIGNALING RECRUITMENT**

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The endocannabinoid (eCB) system is a critical regulator of the neuroendocrine response to physiological and psychological stressors. The eCB system has also been shown to be involved in the regulation of several neurodevelopmental processes throughout early life to maximize cognitive efficiency and flexibility. However, adolescence is also a period in which an individual may be especially susceptible to perturbations (e.g. stress, drug abuse, etc.) that may lead to long lasting or even permanent emotional, cognitive and neural dysfunction in adulthood. Previous work has shown that basal levels of the stress hormones under the control of the hypothalamic-pituitary-adrenal axis (e.g. corticosterone, ACTH) remain relatively constant through to adulthood. However, when subjected to chronic restraint stress, adolescent rats exhibit a distinct stress hormone response from that of adult rats. Presently, the mechanism(s) underlying these differential responses to chronic restraint stress remain unclear. Considering the endocannabinoid system's regulatory role in glucocorticoid feedback and stress habituation, we hypothesized that the eCB system would also exhibit corresponding changes associated with those observed in the adolescent stress response to chronic restraint stress. In this study, adolescent (post-natal day 25) and adult (post natal day 75) male Sprague-Dawley rats were assigned to one of three conditions: 1.) chronic stress (9 consecutive days of 30 min restraint), 2.) acute stress (1 day of 30 min restraint), or 3.) no stress. Immediately following the single or final restraint session (post-natal 40), brain tissue and trunk blood was collected for analyses of hypothalamic and amygdalar eCB content. Our results replicate previous findings that adult male rats exposed to chronic restraint had significantly decreased levels of anandamide in the amygdala, coupled to a spike in 2-AG content within the amygdala, relative to non-stressed adult rats. Adolescent rats, however, failed to exhibit a similar pattern of stress-induced endocannabinoid regulation in the amygdala, in response to chronic and acute restraint stress relative to non-stressed adolescents. Given that our previous work has demonstrated that the recruitment of 2-AG signaling within the amygdala is a contributing factor to the development of stress habituation, these data would suggest that the lack of 2-AG recruitment in the amygdala following repeated stress may be one of the factors impeding habituation in adolescence.

PREFRONTAL CORTICAL ANANDAMIDE SIGNALING COORDINATES BEHAVIORAL COPING RESPONSES TO STRESS THROUGH A SEROTONERGIC PATHWAY

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The endocannabinoid system has recently emerged as a vital component of the stress response in the brain and represents an appealing target for treatment of mood and anxiety disorders. Moreover, recent studies from our laboratory and others have revealed that endocannabinoid signaling within the medial prefrontal cortex (mPFC) is important for stress-induced regulation of emotional behavior. We have previously demonstrated that exposure to forced swim stress induces a rapid and robust decline in anandamide content within this prefrontal subregion, while local mPFC inhibition of FAAH activity elicits an antidepressant-like active coping response in this preclinical test. Additionally, Gobbi and colleagues have revealed that intra-mPFC administration of a CB₁ receptor agonist promotes antidepressant-like responding via a serotonergic mechanism. Therefore, the current study sought to examine whether serotonergic neurotransmission is also involved in the antidepressant-like response elicited by local inhibition of FAAH within the mPFC. Sprague Dawley rats were bilaterally implanted with cannula aimed at the mPFC, given a week to recover, and then pre-treated with the serotonin depletor p-chlorophenylalanine (PCPA; 150 mg/kg i.p.) or vehicle 72 and 48 hours prior to forced swim exposure. An intra-mPFC microinjection of the FAAH inhibitor URB-597 (0.01 µg/side) also preceded forced swim exposure. Results revealed that serotonin depletion prevented the ability of local mPFC FAAH inhibition to promote antidepressant-like active coping responses in the forced swim test. Next, separate cohorts of rats received an intra-mPFC infusion of URB-597 and the firing activity of serotonin neurons emanating from the dorsal raphe was recorded. In support of the above findings, local inhibition of FAAH within the medial PFC significantly augmented the firing rate of dorsal raphe serotonin neurons, an effect that was occluded by global CB₁ receptor antagonism. Collectively, these data suggest that stress-induced changes in endocannabinoid signaling within the mPFC modulate stress coping behaviors through a regulation of serotonin neurotransmission and provide a neuroanatomical framework by which we may understand the mechanism subserving the antidepressant potential of endocannabinoid facilitation.

ENDOCANNABINOID-MEDIATED MODULATION OF MOTIVATION IN SCHIZOPHRENIA

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Since pharmacological antagonism of the cannabinoid CB1 receptor has been linked to decreases in motivation, we hypothesized that increasing endocannabinoid tissue levels could promote motivation. In order to study this possibility, we blocked the activity of 2-arachidonoylglycerol (2-AG)'s hydrolyzing enzyme monoacylglycerol lipase (MAGL) in mice while performing a food-maintained progressive ratio (PR) schedule. Intra-peritoneal injection of the MAGL blocker JZL184 (40 mg/kg) evoked an increase in the breakpoint score performed by wild type mice. This effect was prevented by pretreatment with the CB1 receptor antagonist AM-251 (0.75 mg/kg), at a dose devoid of effects on its own, confirming that the effect of JZL184 was mediated by CB1 receptor activation.

Considered a critical component of the neural circuitry responsible for motivation and reward processing, encoding at the nucleus accumbens (NAc) has also been shown to be a target of cannabinoid receptor modulation. To determine if accumbal encoding is related to the motivational effects of blocking 2-AG hydrolysis, we also performed multiple single-unit recordings from the NAc shell and core of freely moving animals while performing the PR schedule. We found that JZL184 modulates power of frequencies from local field potentials, suggesting that 2-AG promotes neural encoding that accompanies enhanced motivation.

Lack of motivation (avolition) constitutes one of the most striking behavioral features of schizophrenia. In order to test if raising 2-AG tissue levels would influence motivational responses in a model of this disease, we used disrupted-in-schizophrenia 1 (DISC-1) transgenic mice. Intra-peritoneal injection of JZL184 (40 mg/kg) evoked an increase in breakpoints in DISC-1 mice. Confirming the dependence on CB1 activation, this effect was also prevented by pretreatment with AM-251 (0.75 mg/kg). Altogether, our results show that blocking the hydrolysis of the endocannabinoid 2-AG results in enhanced motivation. Moreover, this endocannabinoid-dependent modulation of motivation is intact in the DISC-1 model of schizophrenia. As such, these preliminary results suggest that blocking the hydrolysis of endocannabinoids could constitute a pharmacological tool in the treatment of negative symptoms in schizophrenia.

**MORE ANXIETY, MORE CONFUSION: FUNCTIONAL FAAH GENE
POLYMORPHISM BUT NOT CNR1 GENE POLYMORPHISM INFLUENCES
ACUTE STRESS RESPONSE IN HEALTHY HUMANS**

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Recent evidence has shown that the effects of stress are modulated by endogenous cannabinoids, which act as agonists at the cannabinoid receptor 1 (CNR1) and are degraded by the enzyme fatty acid amide hydrolase (FAAH). In this study, we investigated whether individual differences in mood response to an acute social stressor were related to the rs1049353 CNR1 gene variant or the functional rs324420 (Pro129Thr) FAAH gene polymorphism, that was previously found to be associated with street drug use, addictive traits, amphetamine response, anorexia nervosa and depression.

Caucasian healthy adults (N=73) participated in two sessions involving either a standardized psychosocial stress procedure (Trier Social Stress Test) or a control task. Subjective (Profile of Mood States, POMS) and physiological measures were obtained before and at regular intervals after the tasks. Associations between individual genotypes and levels of self-reported Anxiety and Confusion (POMS) after stress exposure were investigated using two-way ANOVAs/ANCOVAs.

The functional rs324420 (Pro129Thr) FAAH gene variant was significantly associated with increases in Anxiety ($p=0.017$) and Confusion ($p=0.00019$) after stress. The association between rs324420 and increases in Confusion remained significant after adjustment for multiple testing. There was no association between the rs1049353 CNR1 variant and mood response after stress.

These results support the idea that the human endogenous cannabinoid system is significantly involved in stress response. Genetic variability in the FAAH gene may modulate perception of stress and thus modulate the individual's risk for developing a stress-related psychiatric disorder. This finding suggests that manipulations of the cannabinoid system may offer strategies for prevention and treatment of mental illness.

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THE EFFECT OF CANNABIDIOL IN A MK-801-INDUCED RAT MODEL OF ASPECTS OF SCHIZOPHRENIA

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There have been several preclinical and clinical reports that suggest that cannabidiol, the non-psychoactive phytocannabinoid, has antipsychotic effects. The purpose of this study was to further investigate if these effects would be seen using a MK-801-induced rat model of aspects of schizophrenia. MK-801 is an NMDA receptor-antagonist known to produce hyperactivity, deficits in prepulse inhibition (PPI) and social withdrawal, behaviours which correlate well with some of the positive, cognitive and negative symptoms of schizophrenia. We have previously shown this model of aspects of psychosis to possess high face validity and it has been used extensively by others for its predictive antipsychotic validity. We therefore also tested the effect of the atypical antipsychotic clozapine as a comparator.

Following 4 days of acclimatisation to the holding room, rats were familiarised to startle chambers on day 5 and their prepulse inhibition (PPI) was determined on day 6 following intraperitoneal injection with cannabidiol (3–30 mg/kg) or vehicle and MK-801 (0.3–0.6 mg/kg) or vehicle. On day 9, rats were familiarised to the social interaction testing arena and on day 10, following the same treatments as administered on day 6, the rats' levels of social interaction and locomotor activity were determined.

MK-801 induced disruption of PPI, hyperactivity and social withdrawal. Cannabidiol by itself at 10 mg/kg disrupted PPI although this was accompanied by a significant reduction in startle response. Cannabidiol also caused hyperactivity but had no effect on social behaviour. Cannabidiol was unable to inhibit the MK-801-induced disruption of PPI or hyperactivity however it appeared to partially normalise MK-801-induced social withdrawal. Clozapine only partially inhibited MK-801-induced disruption of PPI but was able to normalise MK-801-induced hyperactivity and social withdrawal. In conclusion, cannabidiol showed both propsychotic activity and partial antipsychotic activity in an MK-801-induced model of aspects of schizophrenia. Additional behavioural studies using a range of species, strains, animal models and testing paradigms would be required to conclusively establish the antipsychotic potential of cannabidiol.

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AGGRAVATION OF KETAMINE-INDUCED DEFICITS IN AUDITORY SENSORY MEMORY BY INHIBITION OF CEREBRAL TYPE 1 CANNABINOID RECEPTORS IN HEALTHY HUMAN SUBJECTS

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Preclinical and clinical research suggests that cognitive impairment associated with schizophrenia is due to dysregulation of the endogenous cannabinoid system. In particular, deficient generation of mismatch negativity (MMN) indicating auditory sensory memory is a characteristic finding in schizophrenic patients. Experimental studies implicate a deficient N-methyl-D-aspartate (NMDA) receptor function in such abnormalities.

The primary aim of this study was to investigate the effects of the cannabinoid CB₁ receptor antagonist rimonabant on MMN deficits in the NMDA receptor antagonist model of schizophrenia using ketamine. Thirty healthy male subjects participated in a randomized, double-blind, placebo-controlled cross-over study with subanesthetic doses of intravenous ketamine. The MMNs to frequency and duration deviants were elicited within an auditory oddball paradigm and recorded by 32 channel EEG.

Twenty subjects completed both experimental sessions. Overall, ketamine infusion produced a significant reduction of MMN amplitudes in both deviance conditions. In contrast to placebo, rimonabant significantly enhanced the ketamine-induced MMN deficits at frontal electrodes. The results suggest an involvement of the endogenous cannabinoid system in ketamine-induced impairments of auditory sensory memory as a cognitive key feature in schizophrenia. The CB₁ receptor antagonism seems to aggravate the cognitive performance by disturbing the interaction between endocannabinergic activity and glutamatergic neurotransmission.

DEVELOPMENT OF CANNABIDIOL PRODRUGS FOR USE WITH MICRONEEDLES FOR THE TREATMENT OF ALCOHOL USE DISORDERS

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With over 8.5% of the U.S. population and 75 million people worldwide meeting the diagnostic criteria for alcohol use disorders (AUDs), alcohol abuse is among the top three preventable public health problems in the U.S. and the world. AUDs have a complex etiology where chronic alcohol associated neurodegeneration, withdrawal-related anxiety and persistent alcohol craving are predictive of high relapse and poor clinical outcomes. However, of the three drugs currently FDA-approved for the treatment of excessive alcohol consumption, none address neurodegeneration, withdrawal or anxiety. Cannabidiol (CBD) is a drug that could improve these three syndromes. We hypothesize that a CBD transdermal dosage form can be developed, and that this dosage form could provide therapeutic levels of drug in a future clinical study with alcoholic patients. For this project, CBD prodrug delivery via transdermal microneedles (MN) was conducted. Prodrugs are chemically modified parent drugs that are more skin permeable than the parent, and once they cross the stratum corneum quickly separate back into the parent drug and prodrug moiety.

Full thickness Yucatan pig skin (YPS), stored at -20°C, was used for the experiments. A PermeGear flow-through (In-Line, Hellertown, PA) diffusion cell system was used for the skin permeation studies. Diffusion cells were kept at 32°C with a circulating water bath. Permeation area (area exposed to receiver fluid) of the skin was 0.95 cm². The MN pores were created by applying a 5 MN solid stainless steel array 20x followed by the CBD prodrug in solution. Data was collected from 3-4 diffusion cells per treatment. Ethanol (10%) in ddH₂O was used for the receiver fluid for the studies set at a flow rate of 1.1 mL/h. Samples were collected in 6 h increments for 48 h. Diffusion samples were diluted 1:1 acetonitrile:sample and analyzed by HPLC. At the end of the experiment, skin tissue was extracted for drug content and analyzed by HPLC.

In vitro human skin diffusion studies have shown that sustainable skin permeation can be achieved with topical CBD gel applied twice daily (flux= 6.3 ± 1.4 nmol/cm²/h). However, when prodrug ALL00179 was applied to MN treated full thickness YPS, a flux of 63.2 ± 11.4 nmol/cm²/h was obtained. Preliminary data from CBD prodrugs alone did not improve skin permeation, unless the prodrugs were specifically designed for use with microneedles.

CBD alone did not penetrate through MN created aqueous channels, suggesting that hydrophilic prodrugs of CBD could provide long-term systemic delivery. High permeation rates coupled with ALL00179's complete bio-conversion to CBD within 2 h in human plasma showed that water soluble prodrugs of CBD with the aid of microneedles could be a viable alternative to effective transdermal delivery of CBD.

CHARACTERIZATION OF A CB1 RECEPTOR AGONIST (AM2389) WITH A LONG DURATION OF EFFECT TO FACILITATE THE STUDY OF CB1 DEPENDENCE/WITHDRAWAL

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Δ^9 -THC, the major psychoactive constituent in marijuana, is used to induce dependence in animal models (prior to precipitated withdrawal); however, a CB1 receptor agonist that is capable of producing sustained CB1 receptor activation, such as AM2389 [9-Nor-9 β -hydroxy-3-(1-hexyl-cyclobut-1-yl)-hexahydrocannabinol] may be more efficient in this regard than Δ^9 -THC, a relatively short-acting ligand. Previously, AM2389 was shown to have remarkably high affinity in addition to displaying selectivity (26-fold) for the CB1 ($k_i = 0.16$ nM) compared to the CB2 ($k_i = 4.21$ nM) receptor. In a drug discrimination assay for rats, AM2389 was 105 to 125 times more potent than Δ^9 -THC *in vivo* and its functional half-life was estimated to be ≈ 17 hrs. More recently AM2389, was found to be a useful pharmacological tool for inducing dependence to study antagonist precipitated withdrawal. Evaluating this ligand *in vivo* used the following behavioral assays; body temperature, locomotor activity and scoring somatic signs of withdrawal. Male mice (n=6-8 per group) were used for all assays. Temperature recordings over-time showed that AM2389 had a slower onset, peaked later and had a considerably longer duration of effect compared to Δ^9 -THC. Additionally, AM2389 induced hypothermia appears to be CB1 receptor mediated, since these effects were antagonized by a CB1 receptor selective antagonist. Chronic treatment with AM2389 leads to tolerance to CB1 receptor mediated hypothermia and hypo-locomotion in mice. Abrupt discontinuation of AM2389 after chronic administration does not induce somatic signs of spontaneous withdrawal. Furthermore, an increase in hyper-activity was induced in AM2389-tolerant mice administered rimonabant and not in mice treated with vehicle. Overall, a CB1 receptor agonist (AM2389) produced a long duration of effect that could facilitate the study of CB1 dependence/withdrawal, as sustained exposure likely contributes to tolerance development.

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HUMAN CONSUMPTION OF WHOLE PLANT MEDICINE: EXPLORING DISPENSARY-BASED RESEARCH

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The Schedule I status of cannabis has relegated most cannabis-related research to pre-clinical trials using synthetic cannabinoids. While these models can investigate the effects of specific cannabinoids on micro-level functioning, these models do not have the ability to generate answers to questions about the human experience related to using whole plant medicine (WPM). Medical cannabis dispensaries in states such as California serve hundreds of thousands of individuals who use a variety of cannabis strains on a regular basis, through multiple methods, for a specific therapeutic reason, have a heightened awareness of their use patterns, and are using a product that can be tested to determine its cannabinoid profile. This situation provides a unique opportunity to facilitate the generation of knowledge regarding human consumption of cannabis in the form of WPM. Dispensary-based research can be explored through various models, including recruitment from a dispensary for a study occurring at another location, and partnerships and subcontracts between researchers and dispensaries to conduct research at the dispensary itself.

Dispensary-based research is appealing for several reasons. First, there is a crucial gap in the research on cannabis use in human subjects. Currently, this research is generally limited to general population surveys, and adolescent and treatment seeking samples. Medical cannabis patients possess a heightened awareness of their cannabis use patterns, such as quantity, frequency, strain consumed and method of consumption. Furthermore, product testing employed by many dispensaries can analyze products consumed to provide a cannabinoid profile, including THC, CBD, CBN and terpine levels. Medical cannabis patients also have high levels of cannabis use. General population surveys contain small samples of high level users, and adolescent and treatment seeking samples are not generalizable. Medical cannabis patients represent a wide range of ages, ethnicities, income and education levels. Finally, the medical cannabis patient population can provide samples related to a specific illness (MS, HIV, etc.) or, in states like California, samples related to a wide variety of ailments, more representative of the general population, with chronic pain, anxiety and insomnia most often reported as the condition for which cannabis is used.

Dispensary-based research can reveal patterns of use that are novel, such as the use of cannabis as a substitute for alcohol. Through dispensary-based research, 750 medical cannabis patients have now participated in investigating this behavior, and it is gaining legitimacy as a harm reduction technique for treating alcoholism. Fifteen states, plus Washington D.C. allow for the use of medical cannabis, with laws pending in several others. The growing number of individuals across the country and the world using cannabis as a medicine, coupled with the restrictions on cannabis research, makes dispensary-based research a viable path to increasing our knowledge of the human consumption of whole plant medicine.

CANNABINERGIC AMINOALKYLINDOLES, INCLUDING AM678=JWH018 FOUND IN ‘SPICE’, EXAMINED USING DRUG (Δ^9 -THC) DISCRIMINATION FOR RATS

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Objective To examine four different cannabinergic aminoalkylindole ligands, including one drug (AM678=JWH018) found in herbal ‘Spice’ concoctions, for their ability to substitute for Δ^9 -tetrahydrocannabinol (THC), and the ability of the cannabinoid receptor 1 (CB₁R) antagonist/inverse agonist rimonabant to block the substitution 30 and 90 min after i.p. injection. **Methods** Rats were trained to discriminate between vehicle and 3 mg/kg THC, and then the aminoalkylindoles were examined for their THC-like effects and potential antagonism by rimonabant. **Results** The rank order of potency was: AM5983 \geq AM678 > AM2233 > WIN55,212-2. AM5983 and AM678 appeared 8 times more potent than THC, followed by AM2233 (about twice as potent as THC), and WIN55,212-2 \leq THC at the 30 min-test interval. The aminoalkylindoles showed reduced potency (i.e., an increased ED₅₀ value) at the longer injection-to-test interval of 90 min compared to testing at 30 min. The right-ward shifts by rimonabant were approximately 8 to 12-fold for AM5983 and AM678 compared to an approximately 3-fold right-ward shift for the WIN55,212-2 curve. AM2233 substitution was also blocked by rimonabant. **Conclusion** AM5983 and AM678=JWH018 are potent cannabimimetics derived from an aminoalkylindole template. WIN55,212-2 seemed to interact differently with rimonabant compared to either AM5983 or AM678, indicating differences in the mechanism(s) of action between cannabinergic aminoalkylindoles. The binding affinity of JWH018/AM678 for CB₁R was previously reported to be 9 nM, i.e., a 4-to 5-fold difference in comparison to THC (Aung et al. 2000), and it’s *in vivo* potency *viz-a-vie* THC in mice depended upon which sub-test of the “tetrad” assay was being used for comparison (Wiley et al. 1998). Our binding affinity estimate of 4.5 nM, coupled with current and previous *in vivo* drug discrimination data (Järbe et al. 2010), would suggest that the potency difference could be as high as 8-fold. Regarding time course, the decline in activity at the 90 min post-injection drug tests suggested that the THC-like effects of these aminoalkylindoles were waning.

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EFFECTS OF CHRONIC MANIPULATION OF THE ENDOCANNABINOID SYSTEM ON PRECIPITATED OPIOID WITHDRAWAL

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We have previously shown that acute inhibition of the endocannabinoid catabolic enzymes monoacylglycerol lipase (MAGL) or fatty acid amide hydrolase (FAAH) attenuated the expression of precipitated opioid withdrawal signs in mice. While the MAGL inhibitor, JZL184, attenuated all the signs of withdrawal, the FAAH inhibitor, PF-3845, attenuated only some signs of precipitated withdrawal. Although chronic treatment with high doses of JZL184 produces CB1 receptor downregulation/desensitization and tolerance in pharmacological assays, emerging data show that chronic administration of low doses of JZL184, which cause only partial blockade of MAGL function, does not lead to functional tolerance in either *in vitro* or *in vivo* pharmacological assays. In the present study, we investigated the effects of sub-chronic treatment with MAGL and FAAH inhibitors on precipitated withdrawal in morphine dependent mice. Male ICR mice were treated with either JZL184 or PF-3845 for 6 days. On day 3, the mice were implanted with a 75 mg morphine pellet and challenged with naloxone on day 6 to precipitate withdrawal. The withdrawal signs quantified were incidences of jumps and paw flutters, occurrence of diarrhea, as well as total body weight loss during the 30 min observation period. To our surprise, sub-chronic administration of a high dose of JZL184 (40 mg/kg) retained full efficacy in attenuating the intensity of jumps ($p < 0.05$) and weight loss ($p < 0.05$) as well as blocking the expression of diarrhea. These findings indicate that the anti-dependence effects of MAGL inhibition do not undergo tolerance following repeated administration. These data further support the idea that MAGL is a promising target for the treatment of opioid dependence.

A MONO-HYDROXYLATED METABOLITE OF THE K2 SYNTHETIC CANNABINOID JWH-073 DISPLAYS HIGH AFFINITY NEUTRAL ANTAGONISM AT CB1 RECEPTORS

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K2 and several similar synthetic cannabinoid-spiked incense products that are sold in “headshops” have been marketed and abused as “legal marijuana”. K2 use has a high incidence of dangerous adverse effects that are atypical of marijuana use. One prevalent component of K2 is the aminoalkylindole JWH-073, which is structurally dissimilar to Δ^9 -THC and could conceivably have a different profile of metabolites active at cannabinoid 1 receptors (CB1Rs). Therefore, we hypothesized that four mono-hydroxylated (M1, M3-M5) and one mono-carboxylated (M6) metabolites of JWH-073 retain high affinity and activity at CB1Rs, and thus may contribute to the severe adverse effect profile of K2. Competition receptor binding experiments using [³H]CP-55,940 and G-protein activation assays employing [³⁵S]GTP γ S in mouse brain homogenates were used to determine the affinity and intrinsic activity of ligands for CB1Rs, respectively.

The present study first determined that JWH-073, M1 and M3-M5 completely displace [³H]CP-55,940 in mouse brain homogenates, exhibiting nanomolar (M1, M4, M5) or micromolar (M3) affinities. In contrast, concentrations up to 10 μ M of M6 produce less than 5% displacement, indicating negligible affinity for CB1Rs. Next, 10 μ M concentrations of M1 and M5 produced G-protein activation in mouse brain homogenates with equal efficacy relative to JWH-073. Activation by M1 was also concentration dependent, and 1 μ M of M1 and M5 was blocked by 1 μ M of O-2050, suggesting a CB1R-mediated event. M3, M4 and M6 do not activate G-proteins. M4 was further examined as a potential CB1R neutral antagonist due to its high CB1R affinity and lack of intrinsic activity. M4 concentration-dependently attenuated G-protein activation produced by 100 nM of the CB1R agonist CP-55,940. Because JWH-073 is often added in combination with JWH-018 in K2 preparations, the ability of JWH-073-M4 to shift the concentration-effect curve for G-protein activation produced by JWH-018 was examined. Co-administration with JWH-073-M4 produced a concentration-dependent, rightward shift in the ED₅₀ of JWH-018. Future studies will examine the CB1R antagonist properties of JWH-073-M4 when coadministered with JWH-018 in mice.

Evidence presented indicates that in addition to retaining high CB1R affinity, two JWH-073 metabolites exhibit partial agonist activity and one metabolite acts as a neutral antagonist. This complex mix of metabolically produced CB1R ligands with poor efficacy may lead to abuse of higher doses of co-administered CB1R agonists, contributing to the dangerous adverse effect profile of K2 products containing JWH-073.

THE ROLE OF 2-AG ENDOCANNABINOID NEUROTRANSMISSION IN NICOTINE REWARD AND WITHDRAWAL

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Nicotine is the main addictive component of tobacco that plays a major role in dependence. Nicotine acts on the brain to produce both dependence and withdrawal upon tobacco smoking or cessation. Emerging evidence suggest that the endogenous endocannabinoid system may modulate these effects. Our lab has previously reported that enhancement of endocannabinoids; in particular AEA, enhanced nicotine withdrawal and reward of nicotine and was CB1 mediated (Merritt *et. al.* 2008). However, another EC has yet to be studied in nicotine's effect. 2-AG is the most abundant endocannabinoid in the brain, required for retrograde transmission and exerts its action via CB1 receptors.

We measured the effect of enhanced 2-AG via MAGL inhibition by JZL184, JZL184 is a potent and selective inhibitor of MAGL (Long et al 2009), on nicotine reward and withdrawal. Nicotine reward in the mouse was evaluated in an unbiased conditioned place preference paradigm (CPP) in induction. Mice were pretreated with either JZL184 (40 or 16 mg/kg i.p.) or Vehicle (i.p.) two hours before conditioning in the morning for days 2-4. On test day the mice were tested in a drug free state. Our results showed that degradation of MAGL dose-dependently decreased nicotine preference compared to nicotine control in our CPP paradigm. Next, we than confirmed that we had a true blockade of nicotine CPP and not enhancement because JZL184 (40mg/kg) administered with an inactive dose of nicotine (0.1mg/kg) caused blockade of nicotine reward.

We then assessed JZL184's effect on another important aspect of nicotine dependence, nicotine withdrawal. Mice were implanted with 14-day osmotic minipumps. On Day 15, they were pretreated either with JZL184 (4mg/kg, 8, and 40mg/kg i.p.) or Vehicle (i.p.) two hours before withdrawal. During withdrawal testing they were given either Mecamylamine (2mg/kg s.c.) or Vehicle (s.c.) ten minutes before assessing somatic (paw tremors, body shakes, backing and other signs). JZL184 dose-dependently decreased somatic signs.

Our results suggest that AEA and 2-AG may have differential mechanisms in nicotine withdrawal and reward.

CANNABINOID INVOLVEMENT IN STRESS-INDUCED COCAINE RELAPSE THROUGH INTERACTION WITH NORADRENERGIC PATHWAYS.

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There is strong evidence that the endocannabinoid system (ECS), via the CB1 cannabinoid receptor, is activated in multiple brain regions by stress exposure. Another critical function of ECS is to maintain hedonia during stress. Activation of brain noradrenergic systems also plays an important role in the physiological responses to stress and it has been established that norepinephrine release is critical for stress-induced drug reinstatement. There have been several recent studies that have implicated the ECS in various aspects of cocaine dependency. Therefore, we tested whether the ECS is involved in stress-induced reinstatement of cocaine seeking in extinguished animals and whether this reinstatement involves an interaction between the noradrenergic and endocannabinoid systems.

We used a well-validated preclinical model for human relapse, the rodent conditioned place preference (CPP) assay. In the present study, cocaine-induced place preference was established in C57BL/6 mice using injections of 15 mg/kg i.p. cocaine. To test the role of the ECS in stress- and cocaine-induced reinstatement of cocaine seeking behavior, mice whose preference for the cocaine environment had been extinguished were tested for reinstatement following 6 min of swim stress, with or without the cannabinoid antagonist AM-251 (3 mg/kg, i.p.), and cocaine (15 mg/kg, i.p.) with or without AM-251. Another cohort of mice were tested for reinstatement following administration of cannabinoid agonist CP 55,940 (10, 20 or 40 µg/kg, i.p.). A third group of mice were tested with the alpha-2 adrenergic antagonist BRL 44408 (5 mg/kg, i.p.) with or without CP 55,940 (20 µg/kg).

We found that 1) The cannabinoid antagonist AM-251 can block forced swim-induced cocaine reinstatement; 2) AM-251 does not block cocaine-induced cocaine reinstatement; 3) the cannabinoid agonist CP55,940 does not cause reinstatement of cocaine-seeking behavior; 4) non-reinstating doses of CP55,940 can synergize with non-reinstating doses of the alpha-2 adrenergic antagonist BRL 44408 to cause reinstatement of cocaine seeking behavior.

These results are consistent with the hypothesis that stress exposure but not cocaine triggers the endogenous activation of CB1 receptors and that activation of ECS is required for the stress-induced relapse of the mice to cocaine seeking. Further, the data suggests that the endocannabinoid system interacts with noradrenergic mechanisms to influence stress-induced reinstatement of cocaine-seeking behavior.

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PHARMACOLOGICAL EVALUATION OF 3-SUBSTITUENT RIMONABANT ANALOGS

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A structure activity relationship of rimonabant analogs retaining the pyrazole core of the parent compound revealed that certain alterations in the 3-substituent conferred cannabimimetic activity (i.e., hypomotility, hypothermia, antinociception, catalepsy) in the tetrad battery (Wiley et al., *J Pharmacol Exp Ther* 296 (2001) 1013–22). This was not reversed by rimonabant, nor did these 3-substituent rimonabant analogs reverse THC's tetrad effects. Given the interesting pharmacological profile of this series of analogs, we evaluated two novel 3-substituent rimonabant analogs, O-6629 and O-6658, in C57BL/6 mice using two behavioral assays with differing levels of pharmacological specificity: the tetrad and drug discrimination.

Both O-6629 and O-6658 produced catalepsy, as well as decreases in locomotor activity and body temperature in a dose-dependent manner. O-6629, but not O-6658, significantly increased tail flick latencies. The maximal effects elicited by these compounds differed from those produced by cannabinoids. For instance, cannabinoids maximally decrease body temperature by approximately 6° C, whereas O-6629 and O-6658 produced a 12° C decrease. In mice discriminating 5.6 mg/kg THC from vehicle, O-6629 and O-6658 did not elicit THC-like responding, nor did they appreciably alter the discriminative stimulus of THC. Finally, 5.6 mg/kg O-6629 was trained as a discriminative stimulus in mice and evaluated a variety of compounds to test for potential mechanism/s of its action. O-6629 successfully served as a discriminative stimulus and generalization occurred in a dose-dependent manner. O-6658 fully substituted for O-6629. Rimonabant, THC, anandamide, morphine and cocaine all failed to substitute for O-6629. Interestingly, nicotine produced a moderate amount of O-6629-appropriate responding; however, increasing the sample size and testing higher nicotine doses is necessary before further mechanistic testing (e.g., mecamylamine challenge test) would be prudent.

Collectively, these results provide strong behavioral evidence that 3-substituent rimonabant analogs with cannabimimetic activity in the tetrad are acting through a non-CB1 mechanism. Although the lack of available information about these 3-substituent rimonabant analogs limits the interpretability of these data, the pharmacological specificity of O-6629's discriminative stimulus was evidenced by O-6658's substitution for O-6629. Future directions for investigating the discriminative stimulus properties of O-6629 include testing diazepam, which has been shown to partially substitute for THC-trained animals through a non-CB1 mechanism, as well as chlorpromazine, an antipsychotic agent that is a false positive in the tetrad screen.

ASSESSING REINFORCING EFFECTS OF CANNABINOIDS USING INTRACRANIAL SELF-STIMULATION

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Marijuana (*cannabis sativa*) is the most widely used illicit substance in the U.S., which is due, in part, to its reinforcing effects. On the other hand, appetitive effects of cannabinoids have been inexplicably difficult to observe in animal models of drug reinforcement. Intracranial self-stimulation (ICSS) is a method whereby rodents are trained to press a lever in order to obtain electrical stimulation of a brain area (medial forebrain bundle, MFB) associated with reward. A limited number of cannabinoids have been tested in ICSS and results have been mixed. The objective of the present study was to evaluate the effects of THC compared with cocaine (positive control) on ICSS thresholds in mice. We hypothesized that THC would produce leftward shifts of rate-frequency curves indicating a facilitation of reward, i.e., reduction in reward thresholds. Male C57BL/6J mice were surgically implanted with a bipolar electrode aimed at the MFB. Initially mice were trained under a fixed ratio one (FR-1) schedule of ICSS reinforcement. Then mice were trained to respond for ICSS under a FR-1 schedule of reinforcement using a rate frequency procedure, whereby mice responded during ten 1 min periods of descending half-log frequencies (141-18 Hz). These 12 min components were repeated six times, three for baseline determinations and three for determination of effect (i.e, pre vs. post).

To demonstrate that ICSS responding was facilitated following administration of a drug that produces reinforcing effects, we first evaluated the effects of cocaine (0, 1, 3, 10, and 30 mg/kg, i.p.) administered ten min prior to test sessions. Repeated measures ANOVA revealed a significant effect of cocaine treatment $F(7, 39) = 18.60, p < 0.05$. Post hoc tests revealed that 10 and 30 mg/kg cocaine significantly decreased ICSS thresholds compared to saline. Our initial tests with THC have revealed robust rate decreasing effects at high doses tested (e.g., 10 mg/kg). We are currently testing assesses lower doses of THC to assess its ability to alter ICSS responding.

DIURESIS: A SIMPLE AND EFFICIENT MEASURE TO SCREEN CANNABINOIDS

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There is a sparse literature on diuretic effects of Δ^9 THC in humans and rats; however no study to date has characterized cannabinoid (CB) mediated diuresis in mice. We established that cannabinoids produce diuresis in mice and, further, characterized these effects as CB receptor mediated effects. In a separate series of studies we similarly evaluated cannabinoid diuresis in rats. Rats and mice were injected with vehicle or cannabinoid agonists (Δ^9 THC, AM4054 and WIN55212-22). Voided urine was measured over 2hr and 6hr, in rats and mice respectively using single dosing procedure. As a comparative effect, analgesia was measured using cumulative dosing procedures in a hot water (52°C) tail-withdrawal assay (8-sec cut-off, baseline latency = 2.2±0.1 sec) in mice; in rats a radiant heat tail flick assay (cut-off 6s, baseline latency = 2.0±0.1 sec) with a single dosing procedure was used. In antagonism studies, 0.3-10.0 mg/kg rimonabant was administered as a 30min pretreatment. All cannabinoid agonists increased diuresis with maximum voided urine ranging from 28-35 g/kg; urine output after vehicle injection ranged from 5-12 g/kg (mice) and 1-4 g/kg (rats). Rimonabant dose dependently shifted the diuretic and analgesic dose response curve of THC or AM4054 to the right, and was marginally more potent in the diuresis assays. Our results indicate that cannabinoid agonists produce diuresis in both species by acting on CB1 receptors. Peak diuretic effects occurred at doses lower than those that produced maximum analgesic effects. This along with the simplicity of the assay makes diuresis a more cost efficient and an objective measure to screen cannabinoids *in vivo*.

PHYSIOLOGIC TO PATHOPHYSIOLOGIC INDIVIDUALLY TAILORED PHYTOGENETICS

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Introduction: 10,000 years of selective hybridization has increased THCA concentration to over 30.0 Wt% with concomitant reduction to elimination of the other 79 and counting cannabinoids. 99.9% of cannabis usage in California is in 10 mg doses of decarboxylated THCA whether vaporized, steeped, in confections or smoked. By comparison FDA has approved of 600 mg of CBD/day as an IND. Recently Dr. Ross identified that CBDA/CBGA have a 2-4 fold greater antagonistic activity at GPR55/CB3/Phyto-Cannabinoid Receptor than CBD/CBG. CBD/CBG are only present in negligible amounts in dietary raw cannabis.

Methods: The initial plant was assessed by GC/MS at CBD 6.9 Wt% and THC 6.2% Wt%. A clone of which, was stressed to convert to staminate pollen production, which was used to fertilize a second female clone. While it was suggested that the cannabinoid profile of feminized seeds would be similar to the parent plant, our findings differed. A Shimadzu LC-20XR Ultra-Fast Liquid Chromatograph equipped with an SPD-M20A Photodiode Array Detector analyzed 17 feminized seeds grown to maturity.

Results: CBDA range 0.08 Wt% to 22.0 Wt%, THCA range 1.12 Wt% to 30.1 Wt%. The extreme range of chemotype and phenotype variation would improve offspring survival in a stressed environment. CBDA expression separates into two distinct groups:

0.08|0.09|0.10|0.10|0.10|0.13|0.17|0.61}12.1|12.6|13.2|14.5|15.3|16.4|16.5|16.5|22.0

By comparison THCA expression varies in a continuous incremental increase:

CBDA: 22.0|12.1|12.6|15.3|13.2|16.4|14.5|16.5|16.5|0.08|0.15|0.10|0.61|0.10|0.10|0.09|0.17

THCA: 1.12|4.76|5.37|5.65|6.34|7.07|7.72|8.40|9.29|13.9|16.8|17.3|23.1|26.6|26.7|28.9|30.1

Conclusions: Our understanding of constituents and genetics of chemotype production is incomplete but CBDA:THCA ratios in Alternative Cannabinoid Dietary Cannabis such as ACDC 22:1 and ACDC 0:30 now provide compounding stock. The disclosed methodology combines compounding stocks in published ratios associated with particular medical conditions, which are then titrated to accommodate individual bioavailability; digestive, absorptive, first pass liver effects, patho-physiologic overlays. Serum levels assess adjustments for bioavailability. An algorithm converts the imperfect compounding stock profiles into a specific cannabinoid ratio. Next a complementary terpene profile is determined. The individualized cannabinoid-terpene clone is then grown in succession to provide 85-day leaf and or mature flower for unheated, divided dose, dietary use. Complementing this Cloned Mono-Therapy/CMT profile are plants grown from seed that deliver a randomly varying profile that provides diverse ECS support for autocrine and paracrine maintenance and prevention of disease across diverse cell types. The severity of the pathophysiological state determines the ratio of Preventative Random Seed/PRS to CMT consumed. Life Threatening Patho-physiology 0PRS:5CMT/ Severe Pathology 1PRS:4CMT / Moderate Pathology 2-3PRS:3-2CMT / Familial Predisposition 3-4PRS:2-1CMT /Healthy, Prevention only 5PRS:0CMT. The ratio of physiologic based prevention to patho-physiologic determined therapeutic dosing can be varied across the day if motivated, otherwise across the week. If fresh juice is unavailable, blister packed, frozen cubes of juiced leaf/bud are diluted for palatability / compliance / convenience.

PALMITOYLETHANOLAMIDE CONTROLS RMCP-5 ACTIVITY BY THE REGULATION OF MICROPHALMIA-ASSOCIATED TRANSCRIPTION FACTOR

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Introduction. We have previously demonstrated that the chymase, rat mast cell protease-5 (rMCP-5), exhibits pro-inflammatory and pro-angiogenic properties during λ -carrageenin-induced granuloma formation in rats. Several evidences show that rMCP-5 transcription is under the control of a basic helix–loop–helix leucine zipper (bHLH-Zip) DNA-binding protein, the Microphthalmia-associated Transcription factor (MITF). MITF is predominantly expressed in mast cells (MCs), where it controls specific cellular functions. Animals with a mutation in MITF gene exhibit a deficit in MCs number and develop several diseases, thus evidencing a close correlation between MITF, chymase and MCs.

Palmitoylethanolamide (PEA), an endogenous compound, belongs to a class of lipid mediators showing anti-nociceptive, immuno-modulating and anti-inflammatory properties. Different mechanisms of action are responsible of PEA effects; the most known is the one related to the *Autacoid Local Injury Antagonism*, according to which PEA modulates MCs activation. We have already demonstrated that PEA inhibited granuloma formation, a model of chronic inflammation sustained by MCs activation. Therefore in the present study we investigate the effect of PEA on MITF activity in λ -carrageenin-induced granuloma formation in rats.

Methods. MITF activity was evaluated in a *in vivo* model of granuloma, a feature of chronic inflammation, that was induced by subcutaneous λ -carrageenin-soaked sponge implants, on the back of male Wistar rats. PEA was injected into each sponge at the concentration of 200, 400, 800 μ g/mL. After 96 hours, granulomas were detached and tissues were processed to evaluate various parameters: the effect of PEA on chymase and MITF expressions was determined using Western Blot analysis, while semi-quantitative RT-PCR was used to evaluate mRNA levels of rMCP-5. EMSA essay was performed to evaluate PEA action on MITF binding to the DNA. Furthermore, in order to evaluate the effect of phosphorylation of MITF serine sites on its transcriptional activity, immuno-precipitation assay was performed.

Results and Conclusions. Local administration of PEA caused a concentration-dependent reduction in rMCP-5 mRNA levels and in chymase expression, in granulomatous tissue, in confront to animals treated only with λ -carrageenin. Moreover, MITF protein expression was reduced in λ -carrageenin tissues, while the amounts of phosphorylated were increased. PEA treatment restored MITF protein expression and, in parallel, reduced the amounts of phosphorylated MITF. Finally, PEA reduced the MITF/DNA binding, that was increased in tissues treated with λ -carrageenin alone.

In conclusions, our results show that PEA is able to modulate both the pro-inflammatory and angiogenic rMCP-5 levels and the transcriptional activity and stability of MITF in λ -carrageenin-induced granuloma in rats.

Therefore, PEA may be a considerable modulator of chronic inflammatory diseases sustained by MC activation.

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EXPRESSION, PURIFICATION AND BIOPHYSICAL (NMR/MS) STUDIES OF THE ENDOCANNABINOID ENZYME MONOACYLGLYCEROL LIPASE

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Monoacylglycerol lipase (MGL) is a major degrading enzyme of the endocannabinoid system and is involved in the hydrolytic deactivation of 2-arachidonoyl glycerol (2-AG). MGL belongs to the family of serine esterases in which the catalytic domain is comprised of Ser-Asp-His triad. We have used MS and NMR methods to obtain structural and functional information of MGL. Human and rat MGL were expressed in *E.coli* as N-terminal hexa-histidine tagged proteins. The enzymes were first purified using IMAC chromatography, characterized biochemically and used for NMR structural studies. To improve its solubility and enhance its stability for our NMR work we have carried out suitable modifications of this enzyme while striving to maintain its catalytic activity. We showed that deletion of the highly hydrophobic region (T165-L191) comprising 17 hydrophobic residues from the lid domain or replacement of specific residues in the lid can provide suitable samples for the NMR work. We used 2D ^1H - ^{15}N HSQC NMR spectroscopy as well as hydrogen-deuterium exchange mass spectrometry (HXMS) to probe the conformational changes in MGL upon ligand binding. The advantages of using these constructs for drug discovery studies will be discussed and results comparing the hMGL and rMGL will be presented.

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ANANDAMIDE DIFFERENTIALLY MODULATES HIPPOCAMPAL NEURONAL FIRING IN ANESTHETIZED VS. BEHAVING RATS

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The natural cannabinoid Δ^9 -THC and the synthetic compound WIN 55,212-2 have been shown to alter performance of a spatial memory task through the modulation of neuronal firing of hippocampal pyramidal neurons (Hampson and Deadwyler, 2003). However, the role of anandamide on the firing rates and burst characteristics of hippocampal principal neurons is not fully understood. Many studies have demonstrated that inhibition of fatty acid amide hydrolase (FAAH) and inhibition of anandamide transport enhance anandamide levels in the brain (Piomelli, 2003). Therefore, this study examined the neurophysiological changes that occur in hippocampal spike train activity when anandamide levels are enhanced. Extracellular action potentials were recorded using multi-neuron wires electrodes implanted in the CA1 and CA3 subfields of the hippocampus in anaesthetized animals and in animals performing a Delayed-Non-Match-to-Sample (DNMS) task. The acute effects of *R*-Methanandamide, the stable analog of anandamide, URB597, a FAAH inhibitor, and VDM11, an anandamide membrane transport inhibitor, were investigated by examining firing rates and bursting characteristics of hippocampal cells.

In both awake behaving animals and anesthetized animals, *R*-methanandamide and URB597 produced a consistent decrease in firing rates, and no significant increase in number of “bursts”(spontaneous spike train firing >10 Hz) activity of pyramidal neurons; as well as a significant increase in mean burst duration and interburst intervals, when compared with effects of the corresponding vehicle. However, VDM11 increased firing rate, number of bursts, burst duration and decreased interburst interval in anesthetized animals when compared with the corresponding vehicle. In awake animals, VDM11 decreased firing rates and increased the burst duration, number of bursts, and interburst intervals. All drugs tested revealed that the substantial change in firing characteristics and bursting was more significant in awake than in anesthetized animals, Therefore the neurophysiological consequences of elevating anandamide are more pronounced when the animal is awake and performing a behavioral task. These results suggest that, in rats, endocannabinoid modulation of hippocampal bursting may be related to alteration of short term spatial memory processing.

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THE ENDOCANNABINOID METABOLOME-LEPTIN CONNECTION DURING THE SECOND AND THIRD TRIMESTERS OF PREGNANCY

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Introduction: During pregnancy the endocannabinoid-cytokine relationship underlies important regulatory events by which endocannabinoids, their congeners, cytokines and hormones may interact to alter the course of pregnancy. Endocannabinoids are emerging as important regulators of physiological processes, including functions in reproduction, inflammation and weight regulation. Leptin is a cytokine produced by white and brown adipose tissue, syncytiotrophoblasts, ovary, and mammary epithelial cells. Like anandamide and 2-arachidonolglycerol, leptin plays a tight regulatory role in implantation and pregnancy maintenance. Little is known about regulation of these endocannabinoids in conjunction with cytokines, such as, leptin during the course of pregnancy, especially among women with different body mass indexes (kg/m²; BMI).

Objective: At 20-22, 23-26, 32 and 38-40 weeks, evaluate the relationship between plasma endocannabinoids and leptin of pregnant women who were underweight, normal, overweight or obese prior to pregnancy.

Methods: Women were categorized into BMI categories based on self-reported pre-pregnancy body weights (N=35). We quantified plasma endocannabinoids (anandamide, palmitoylethanolamine, oleoylethanolamine (OEA), docosahexaenoyl, ethanolamine, 2-arachidonolglycerol (2-AG), 2-palmitoylglycerol, 2-oleoyglycerol) using liquid chromatography-mass spectrometry. Leptin was measured using the Enzyme-Linked ImmunoSorbent Assay. Canonical correlations were used to evaluate relationships between endocannabinoids and leptin after adjusting for weeks of pregnancy and BMI categories.

Results: During the second and third trimesters of pregnancy, leptin was positively related to AEA and OEA (p<0.01). Leptin was not related to the other measured endocannabinoids in the significant canonical variate.

Conclusions: These exploratory findings point to a positive relationship between leptin and AEA and OEA during the second and third trimesters of pregnancy. These findings, among women with term pregnancies, suggest that with placental formation and increased adiposity during the later stages of pregnancy, leptin production increases which in turn mirrors the tight regulation of AEA and its congener, OEA. AEA and OEA plasma levels have implications for maintaining a successful pregnancy. There is a need in future work to: i.) investigate the role of the endocannabinoid metabolome longitudinally from pre-pregnancy and throughout pregnancy in relation to leptin regulation. ii.) examine the longitudinal relationship between the endocannabinoid metabolome and other cytokines in underweight, normal, overweight and obese pregnant women. To our knowledge, this is the first report of the relationship between the endocannabinoid metabolome and leptin during later pregnancy. Funded in part by: Nestec, Ltd Switzerland, LSU AgCenter, and Agriculture and Food Research Initiative Grant 2009-65200-05991 from the USDA National Institute for Food and Agriculture.

MASS SPECTROMETRIC CHARACTERIZATION OF HUMAN NAAA

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We performed mass spectrometry based proteomic and ligand-assisted active site characterization studies of the human lysosomal enzyme NAAA, purified from the media of HEK293 cells stably expressing the enzyme. A hexa-histidine C-terminal affinity tag was incorporated into the protein, allowing an efficient single step affinity purification of the enzyme. We developed a high throughput fluorescent reporter assay, using a fluorogenic synthetic analog of PEA, for rapid compound screening. Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) and SDS-PAGE demonstrated the enzyme to be mainly in the precursor (inactive) form with an approximate molecular weight of 47.7 kDa. At pH 4.5 the precursor was converted into the active form of two subunits with approximate molecular weights of 14.6 kDa (α -subunit) and 33.3 kDa (β -subunit). The molecular mass of the active form of the enzyme was estimated by size exclusion chromatography to be 45 ± 3 kDa, suggesting it is a heterodimer in its native form. MALDI-TOF MS analysis of the purified protein after deglycosylation by PNGaseF showed the molecular weights of the precursor and α - and β -subunits to be 40.6 kDa, 10.9 kDa, and 29.7 kDa respectively. Analysis of the trypsin digest by MALDI-TOF MS/MS revealed the N-terminal sequences of the α - and β -subunits.

The mechanism of NAAA inhibition was determined for two compounds from different classes that are each believed to inhibit via covalent modification: AM6701 and serine β -lactone analog (SBL). AM6701 is a potent inhibitor of NAAA (IC_{50} in the low nanomolar range), and it inhibits in a competitive and time-dependent manner, whereas SBL inhibits in a non-competitive and time dependent manner and has an IC_{50} in the low micromolar range. MALDI-TOF MS/MS analysis of the trypsin digests of both AM6701 and SBL treated NAAA identified modifications exclusively at the N-terminal cysteine of the β -subunit. These experiments clearly suggest that NAAA belongs to the N-terminal nucleophile class of enzymes, with the N-terminal cysteine of the β -subunit as the critical catalytic active site residue and which is the target of these two structurally distinct inhibitors.

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DEVELOPING DYNAMIC PHARMACOPHORE MODELS OF MONOACYLGLYCEROL LIPASE AND RELATED ENZYMES

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Monoacylglycerol lipase (MGL) is primarily responsible for the inactivation of 2-arachidonoylglycerol (2-AG), an endocannabinoid signaling lipid with full agonist activity at both cannabinoid receptors. The enhanced tissue 2-AG levels consequent to MGL inhibition are considered therapeutic against pain, inflammation, and neurodegenerative/neuroinflammatory disorders including Alzheimer's and Parkinson's diseases. The most common "off-target" for MGL inhibitors is considered to be fatty acid amide hydrolase (FAAH) the enzyme mainly responsible for degrading anandamide, although FAAH can also inactivate 2-AG in cellular systems.

Here we describe the development of a structure based pharmacophore model of MGL. The method utilizes an ensemble of conformations of the MGL active site identifying regions of most favorable interaction termed "hot-spots". Hot-spots which are consistently present in the majority of the protein conformations despite the inherent motion of the active site create a consensus of hot-spot regions, which are translated into a pharmacophore model. Comparison of pharmacophore models from MGL and FAAH allows insight into the requirements for selective inhibition and will inform the rational design of targeted inhibitors as pharamcotherapeutics.

NOVEL ENDOCANNABINOID PROBES

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The structural characterization of G-protein coupled receptors is important for the design of novel selective ligands. For this reason we have sought to study the interactions of selective cannabinergic ligands with the CB1 and CB2 cannabinoid receptors to obtain information on the pharmacophoric requirements for ligand/drug activities.

Towards this goal we have based an approach developed in our laboratory entitled ligand-assisted protein structure (LAPS). This approach involves the combined use of covalent ligands, CB1 and CB2 mutants, as well as LC/MS based proteomic methods. The covalent ligands reported here are based on the endocannabinoid anandamide structure. We developed a convergent synthetic route to generate bi-functional, high affinity CB1 and CB2 compounds carrying reactive groups in strategic sites within each ligand. The functional groups selected for covalent linkage are either the azido or isothiocyanato groups. The azido group, when activated by UV radiation, forms a nitrene that can after rearrangement covalently attach to nearby amino acid residues. On the other hand, the electrophilic isothiocyanate group reacts selectively with cysteine residues at or near the binding domain.

Our effort has led to the synthesis of a number of high affinity ligands ($K_i \sim 3\text{nM}$ CB1, 15nM CB2). We have also developed novel covalent ligands that are stable in the presence of fatty acid amide hydrolase (FAAH) while retaining receptor affinity. These ligands can be used for covalent binding experiments to form ligand-receptor complexes. Analysis of these complexes provides information on the binding domains of the CB1 and CB2 receptors.

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**BRAIN REGION-SPECIFIC CIRCADIAN RHYTHMS OF
N-ACYLETHANOLAMINE CONTENTS
AND FATTY ACID AMIDE HYDROLASE**

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The body regulates many of its physiological responses such as sleep-wake cycles, body temperature, metabolism, and cell cycle progression through transcriptional control that has a circadian rhythm. When circadian patterns are disrupted, physiological functions are altered. The endocannabinoid system is important in a variety of physiological functions which show regulation over a 24 hour cycle, such as body temperature and feeding. *N*-Arachidonylethanolamine (AEA) is an agonist of CB1 cannabinoid receptors; and an activator of TRPV1 and PPAR signaling systems. The tissue concentrations of AEA are regulated by the relative rates of its synthesis and degradation by fatty acid amide hydrolase (FAAH). We have carried out studies to test the hypothesis that AEA signaling is circadian and, as such, it contributes to the circadian patterns of several physiological processes. Tissue contents of AEA and two additional *N*-acylethanolamines (NAEs), palmitoylethanolamide (PEA) and oleoylethanolamide (OEA) were measured in lipid extracts from brains harvested from adult male mice at 300, 700, 1100, 1500, 1900 and 2300. Mice were maintained on a 12 hour light cycle with lights on at 600. We also measured FAAH activity at the same time points. In the prefrontal cortex, PEA and OEA exhibited strong circadian rhythms, with peak values at 1500 and nadir values at 300. The catalytic efficiency for FAAH had the opposite relationship. In the striatum, PEA and AEA exhibited similar patterns, with peak contents at 1900 and nadir contents at 0700 for PEA and 1100 for AEA. OEA contents were not as rhythmic. The catalytic efficiency of FAAH did not vary significantly with time of day, suggesting that synthetic processes alone drive the rhythms in NAEs in the striatum. In the hippocampus, all three NAEs exhibited strong rhythmic patterns, reaching a nadir in the first half of the light phase. The catalytic efficiency of FAAH peaked at 1100, suggesting that it contributed to the suppression of NAE contents in the light phase. The most strongly rhythmic NAE in the hypothalamus was AEA, which exhibited a large change between 1100 (its peak) and 1500 (its nadir). FAAH catalytic efficiency was highest at 1500, which is consistent with low AEA content at that time. Taken together, our data indicate that NAE contents exhibit a circadian rhythm in many brain regions and FAAH activity likely contributes to the patterns but does not explain all of the variation. (Supported by Research for a Healthier Wisconsin)

BRAIN TRANSPORT AND METABOLISM STUDIES WITH 2-ARACHIDONOYLGLYCEROL (2-AG) AND FATTY ACID ETHANOLAMIDES

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We previously evaluated the use of tritiated anandamide (AEA) to image local activities of fatty acid amide hydrolase (FAAH) in mouse brain (Glaser et al. *J. Pharmacol. Exp. Ther.* 2006, 316, 1088-97). This technique depends on the generation of radiolabeled arachidonic acid that is then locally incorporated into membrane phospholipids in the brain with hydrolysis-resynthesis turnover times of several hours, as had been previously shown by Rapoport and co-workers (*J. Neurosci. Res.* 1989, 24, 413-23) using labeled arachidonic acid with ex vivo autoradiography. We are extending our previous studies by evaluating the regional brain transport and disposition of radiolabel after intravenous administration to mice of other endocannabinoid and endocannabinoid-like radiotracers, including [arachidonoyl-³H]2-arachidonoylglycerol ([³H]2-AG). In this case, regional disposition of radiolabel is anticipated to primarily reflect local activities of monoacylglycerol lipase (MGL). However, studies using high specific activity [³H]2-AG have up to now been problematical because of the difficulty of procuring and using this tracer, which has not been commercially available in acceptable purity as it is highly unstable to rearrangement.

We have addressed this problem by the development of a novel methodology to conveniently synthesize pure 2-AG with tritium labeling of the arachidonic acid moiety (Duclos et al. *J. Org. Chem.* 2011, 76, 2049-2055). Ethanolysis of the tritiated endocannabinoid protected as the 1,3-dibutyrate in the presence of acrylic beads containing *Candida antarctica* lipase B gave [³H]2-AG without any rearrangement to [³H]1(3)-AG. The flash cartridge filtration purification gave a 50% radiochemical yield of [³H]2-AG at a specific activity of 200 Ci/mmol with less than 4% rearrangement to [³H]1(3)-AG. Storage in argon-degassed toluene for five months at -80 degrees C resulted in only a further 3% rearrangement to [³H]1(3)-AG. This strategy utilizes a stable labeled triglyceride precursor that can be readily synthesized from tritiated arachidonic acid and distributed to other laboratories. The method allows pure [³H]2-AG to be generated immediately before use.

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THE EFFECT OF POLYUNSATURATED FATTY ACIDS ON THE ENDOCANNBINOID SYSTEM IN NEURAL STEM/PROGENITOR CELLS

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Neurogenesis is an essential constituent of neuronal plasticity in the adult brain for homeostasis and recovery from brain injuries. The process consists of the maintenance of neural stem/progenitor cells (NSPCs), their proliferation, differentiation, migration, and integration into neuronal circuits. The endocannabinoid system was suggested to be involved in adult neurogenesis through the analysis of genetically modified rodents and pharmacological studies. Polyunsaturated fatty acid (PUFA) treatment enhances brain plasticity, including neurogenesis. Since endocannabinoids are arachidonic acid derivatives derived from omega-6 polyunsaturated fatty acids, it is conceivable that the endocannabinoid system is affected by the availability of polyunsaturated fatty acids in the microenvironment. Based on this background, we examined the effect of PUFAs (linoleic acid and alpha-linolenic acid) on the fate of NSPCs *in vitro*. Cell viability and mRNA levels of endocannabinoid system-related genes were examined by MTT assay and quantitative PCR respectively. The levels of endocannabinoids in these cells were measured by isotope dilution LC-MS analyses. Results show that the administration of these molecules (1-5 μM) to NSPC cultures directly affects the proliferation and survival of NSPCs in a dose-dependent manner. Furthermore, we observed alterations in the mRNA levels of the enzymes involved in endocannabinoid biosynthesis and degradation, whereas data on endocannabinoid levels in these cells are currently being determined. In conclusion, our results show that the administration of PUFAs to NSPCs in culture directly affect the endocannabinoid system in, and the preservation of, these cells.

CHARACTERIZATION OF ANANDAMIDE SYNTHESIS INHIBITORS

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The endocannabinoid, *N*-arachidonyl ethanolamine (anandamide; AEA) is regulated by many physiological processes, including stress and pain. Anandamide is synthesized from the precursor phospholipid, *N*-arachidonylphosphatidyl ethanolamine (N-ArachPE) via at least three enzymatic pathways. Our ability to understand the mechanisms of regulation of anandamide in vivo is hampered by a lack of pharmacological inhibitors of its synthesis.

We have developed a method to assay the conversion of radiolabeled N-ArachPE to anandamide by brain membranes using thin layer chromatography to separate intermediate products. This method has the advantage that it is unbiased with respect to the pathway of conversion. We have used this assay to screen novel compounds. These compounds (whose structures will be provided at the meeting) inhibit the synthesis of anandamide by approximately 45% at concentrations of 100 μ M.

These data serve as a starting point for the development and optimization of inhibitors of anandamide synthesis.

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DELTA-9-TETRAHYDROCANNABINOL DOES NOT REDUCE TNF- α PRODUCTION IN *EX VIVO* LPS- AND SEB-CHALLENGED WHOLE BLOOD

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Introduction: Delta-9-tetrahydrocannabinol (THC) binds to CB₁ and CB₂ cannabinoid receptors. CB₂ receptors are expressed by immune cells, such as B cells, NK cells and monocytes, and to a lesser extent on macrophages, T cells and endothelial cells. This may qualify cannabinoids as immune modulators. With the addition of the CB₂ receptor agonist, WIN 55,212-2, a reduction in TNF- α production in peripheral blood mononuclear cells (PBMCs) was induced in a previous study. Therefore, the aim of the research was to investigate whether THC was also capable of inhibiting the TNF- α immune response to Staphylococcal Enterotoxin-B (SEB) and Lipopolysaccharide (LPS) through the CB₂ receptor *ex vivo*.

Methods: Twenty millilitres of whole blood of four healthy male volunteers, aged between 20 and 23, was incubated (37°C, 95% O₂, 5% CO₂) for 30 minutes with exponentially increasing concentrations of THC (0 – 1000 ng/mL) or placebo, both dissolved in 0.5% ethanol. The CB₂ antagonist, SR144528 (1.5 μ g/mL), was added to the samples. LPS (5 ng/mL) and SEB (10 ng/mL) stimulations were performed. TNF- α concentrations were measured by ELISA-methods.

Results: TNF- α increased upon addition of LPS (200-fold, from 11.2 pg/mL to 2213 pg/mL) or SEB (8-fold, from 11.2 pg/mL to 83.4 pg/mL). However, THC addition did reduce the TNF- α concentration after LPS (2070 pg/mL) and SEB (391 ng/mL) addition. Furthermore, the CB₂ antagonist did not show an opposite effect to THC on the TNF- α production.

Conclusions: THC demonstrates no reduction of TNF- α production *ex vivo* in LPS- and SEB-challenged blood. These findings do not support an anti-inflammatory role of THC in human blood. It is also possible that the anti-inflammatory response or the affinity of THC for the CB₂ receptor was not optimal under these experimental conditions, which included low levels of the solvent ethanol. This is considered less likely however, since the solvent did not affect the pro-inflammatory actions of LPS or SEB.

CANNABINOID INHIBITION OF MACROPHAGE-LIKE CELL MIGRATION TO THE TAT TRANS-ACTIVATING PROTEIN OF HIV-1 IS LINKED TO THE CB₂ CANNABINOID RECEPTOR

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Macrophages and microglia, the resident macrophages of the central nervous system, serve as major targets for the human immunodeficiency virus-1 (HIV-1). These cells are productive hosts for the virus and elicit a plethora of cytokines, monokines, and neurotoxic factors in response to infection. Included among these are the virus envelope glycoprotein gp120 and the trans-activating protein Tat. Both proteins can activate uninfected macrophage-like cells resulting in an expansive release of inflammatory and cytotoxic factors. However, in contrast to gp120, the production of Tat is not affected by currently available anti-retroviral drugs, which target the reverse transcriptase and protease enzymes of HIV. Tat also harbors a β -chemokine receptor ligand motif and exerts robust chemotactic properties and, in this capacity, may recruit uninfected immunocytes to focal areas of HIV-1 infection. These observations, taken together with the fact that Tat is released extracellularly, articulate a rationale for assessment of its role in HIV pathogenesis in the current highly-active antiretroviral therapy (HAART) era.

Select cannabinoids modulate the functional activities of macrophage-like cells, most of which are inhibitory and mediated through the CB₂ cannabinoid receptor (CB₂R). Thus, this receptor has the potential to serve as a cell-selective molecular target for ablating untoward immune responses including those engendered by Tat. In the present study we demonstrate that the exogenous cannabinoids delta-9-tetrahydrocannabinol (THC) and CP55940 significantly inhibited migration of human U937 macrophage-like cells and mouse BV-2 microglial-like cells to Tat. The CB₁ receptor (CB₁R)-selective agonist ACEA had no effect on Tat-mediated migration while the CB₂R-selective ligand O-2137 exerted a concentration-related inhibition of U937 cell migration in response to Tat. Pharmacological blockage of CB₁R signaling using the antagonist SR141716A had no effect on CP55940-mediated inhibition of migration to Tat, whereas treatment with the CB₂R antagonist SR144528 reversed this inhibition. In addition, THC had no inhibitory effect on U937 cell migration to Tat after small interfering RNA knockdown of the CB₂R. These results were replicated using the BV-2 microglial-like cells that also showed a diminution in expression of the β -chemokine receptor CCR3. Collectively, the data suggest that cannabinoid-mediated modulation of macrophage-like cell migration to the HIV-1 Tat protein is linked to the CB₂R. This linkage, at least for microglial-like cells, may involve “cross-talk” with chemokine receptors. Furthermore, the results suggest that the CB₂R has potential to serve as a therapeutic target for ablation of untoward HIV-1-associated inflammatory responses.

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CHRONIC Δ^9 -THC MODULATION OF SIMIAN IMMUNODEFICIENCY VIRUS INFECTION

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With the advent of active antiretroviral therapy, human immunodeficiency virus (HIV) infection has become a chronic disease frequently co-existing with chronic drug abuse, including that of marijuana. Cannabinoids, the principal chemical constituents of marijuana, exert neurobehavioral effects and in addition have the potential of affecting the immune system. Because of the multiple systemic effects of cannabinoids, understanding their impact, particularly of Δ^9 -tetrahydrocannabinoid (Δ^9 -THC), the major psychoactive cannabinoid in marijuana, on metabolic, immune and neurobehavioral function in HIV infection are essential. This is particularly relevant, as cannabinoids have been approved as appetite-stimulating therapy in AIDS patients while having the potential of modulating immune function. We have used a systems approach to examine the impact of chronic Δ^9 -THC administration (0.32mg/kg i.m., 2 X daily) on behavioral, anthropometric, biochemical, and immune parameters in SIV_{mac251} (100 TCID₅₀/ml, i.v.)-infected non-human primates. In addition, the in vitro cannabinoid effects on viral infectivity were examined in a lymphocyte cell line. Our studies indicate that chronic Δ^9 -THC treatment attenuates viral load and tissue inflammation in SIV-infected non-human primates, significantly decreasing morbidity and mortality from SIV infection. Chronic Δ^9 -THC produced tolerance to its behaviorally disruptive effects on complex tasks. In addition, Δ^9 -THC decreased viral replication in an in vitro assay. While the ability of cannabinoids to suppress inflammation and viral replication has been reported by others and confirmed by our ongoing studies, the mechanisms involved are not known. Our recent studies clearly suggest that the overall mechanisms mediating the protective effects of cannabinoids involve novel epigenomic regulatory factors/mechanisms underlying changes in the transcriptome in need of systematic investigation. Supported by NIDA-020419 and NIDA030053.

EFFECTS OF CANNABINOID RECEPTOR MODULATION ON LEUKOCYTE-ENDOTHELIAL INTERACTIONS IN ENDOTOXIN-INDUCED UVEITIS

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Introduction: Uveitis represents inflammation of the eye which can become severe and lead to blindness. Experimentally, uveitis can be induced by systemic endotoxin administration (endotoxin-induced uveitis, EIU). The endotoxin lipopolysaccharide (LPS) causes leukocyte activation leading to leukocyte-endothelial interactions. The endocannabinoid system (ECS) modulates immune cell activation. The purpose of this study was to investigate the effects of cannabinoid receptor modulation on leukocyte-endothelial interactions in the rat iridial microcirculation using intravital microscopy (IVM).

Methods: Five groups of Lewis rats (n=5-8 in each group) were studied: control, endotoxemia (20 mg LPS/kg i.v.), LPS + CB1R/CB2R agonist (1 mg/kg WIN55212-2 i.v) and LPS + WIN55212-2 + CB1R or CB2R antagonist, (2.5 mg/kg AM281 / AM630 i.v.). All drug treatments were given 15 min after LPS administration. IVM of the iridial microcirculation was carried out at 0, 1, and 2 h post-LPS administration. Rolling and firm adhesion of leukocytes to the endothelium was measured offline in a blinded manner.

Results: A baseline for leukocyte-endothelial rolling and adhesion was initially established for all groups at 0 h. In comparison to the control group, the LPS-treated group had a significant increase in leukocyte adhesion 2 h after induction of endotoxemia in iridial microvessels ($p < 0.001$ in vessels $> 25 \mu\text{m}$ and $< 25 \mu\text{m}$). Administration of the CB1R/CB2R agonist, WIN55212-2, significantly decreased leukocyte adhesion in vessels $> 25 \mu\text{m}$ and $< 25 \mu\text{m}$ ($p < 0.01$ and $p < 0.001$, respectively). The combination of WIN55212-2 and the CB1R antagonist, AM281, significantly attenuated leukocyte adhesion in vessels $> 25 \mu\text{m}$ and $< 25 \mu\text{m}$ at 2 h ($p < 0.01$ and $p < 0.05$). The treatment combination of WIN55212-2 and the CB2R antagonist, AM630, significantly decreased leukocyte adhesion in vessels of $< 25 \mu\text{m}$ at 2 h after endotoxemia induction ($p < 0.01$), but failed to reduce leukocyte adhesion in vessels $> 25 \mu\text{m}$ ($p > 0.05$). There was no significant decrease in leukocyte rolling in vessels of $< 25 \mu\text{m}$ or $> 25 \mu\text{m}$ in any of the drug treatment groups ($p > 0.05$).

Conclusion: Activation of CB1R and CB2R by WIN55212-2 significantly attenuates leukocyte adhesion in vessels of all diameters 2 h after EIU. This effect was mostly due to CB2R activation. These results indicate that future drugs targeting CB2R in the iridial microcirculation may have potential benefits in treating ocular inflammation.

EFFECT OF NON-PSYCHOTROPIC CANNABINOIDS ON NITRITE PRODUCTION IN MOUSE PERITONEAL MACROPHAGES

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Introduction. The plant *Cannabis sativa* contains over 100 well-characterized compounds known as phytocannabinoids. Among them, Δ^9 -tetrahydrocannabinol and cannabidiol are the best studied (Izzo et al., Trends Pharmacol Sci. 30 (2009) 515-27). However, many other phytocannabinoids might contribute to several therapeutic benefits ascribed to *Cannabis*. Here, we have investigated the possible anti-inflammatory effects *in vitro* of cannabichromene (CBC), cannabidivarin (CBDV), cannabigerol (CBG) and Δ^9 -tetrahydrocannabivarin (THCV) on nitrite levels, the stable metabolites of NO, in the supernatant of LPS-stimulated mouse peritoneal macrophages.

Methods. Neutral Red assay was used to measure macrophages viability; nitrite levels were evaluated using a fluorescent assay.

Results. CBC, CBDV, CBG and THCV (up to 1 μ M) exhibited no significant cytotoxic effect in macrophages after 18-hour exposure. However, these phytocannabinoids (0.001-1 μ M) caused a significant reduction of LPS-stimulated nitrite levels. Experiments are in progress to verify if cannabinoid receptors or transient receptor potential channels are involved in these effects.

Conclusions. CBC, CBDV, CBG and THCV inhibit LPS-stimulated nitrite production in macrophages. These results open the way to investigate the effect of such non-psychoactive phytocannabinoids in experimental models of inflammation *in vivo*, including inflammatory bowel disease.

AN *IN VITRO* IMMUNE RESPONSE MODEL MIMICKING EARLY-STAGE HIV INFECTION FOR ELUCIDATING MECHANISMS OF CANNABINOID-INDUCED IMMUNE MODULATION

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Here we present a novel *in vitro* model of HIV infection using mouse splenocytes and engineered cell lines to study the immune modulatory effects of the phytocannabinoid, Δ^9 -tetrahydrocannabinol (Δ^9 -THC), and the endocannabinoid, anandamide (AEA) on the anti-viral immune response. In this model, a dendritic cell line (DC2.4) and a T cell line (EL4) have been engineered using a lentiviral system to express the HIV envelope protein, gp120, making cell lines DC2.4_{gp120} and EL4_{gp120}. In each cell line, the viral gp120 protein is processed into antigenic peptides that are then expressed on major histocompatibility complex I or II. Hence both cell lines serve as surrogates for HIV infected cells. The model proceeds in two stages. In the first (elicitation) stage, DC2.4_{gp120} is co-cultured with mouse splenocytes for five days to induce an antigen-specific anti-gp120 immune response. In the second (effector) stage, the gp120-induced splenocytes are restimulated with either EL4_{gp120} (expressing gp120-derived antigens) or wild type EL4 cells (EL4_{wt}), which serve as a negative control, for an additional 12 to 24 hours. Exogenously added compounds, such as cannabinoids, may be added at any time during either the elicitation and/or effector stage. Currently, activation of CD8⁺ T cells (increased proliferation and CD69 expression) has been demonstrated using flow cytometry in the elicitation stage. In the effector stage, specificity of the anti-gp120 response was demonstrated in EL4_{gp120}-restimulated CD8⁺ T cells as evidenced by enhanced interferon- γ (IFN γ) production, when compared to EL4_{wt}-restimulated CD8⁺ T cells. Moreover, addition of Δ^9 -THC (1 μ M to 15 μ M) was observed to suppress as well as enhance the gp120-specific anti-viral CD8⁺ T cell response as measured by effector stage IFN γ production. Further examination of these results revealed a striking correlation between the magnitude of T cell activation and whether Δ^9 -THC was immune enhancing or immune suppressive. Specifically, under conditions of modest T cell activation Δ^9 -THC enhanced the T cell anti-viral response while under conditions of strong T cell activation Δ^9 -THC suppressed the T cell anti-viral response. In addition, when the anti-gp120 CD8⁺ T cell response was enhanced by Δ^9 -THC, a parallel increase in proliferation and CD69 was also observed. In contrast, AEA (1 μ M to 10 μ M) was observed to consistently enhance the anti-gp120 CD8⁺ T cell response as measured by IFN γ production in the effector stage.

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SATIVEX SAFETY PROFILE IS IMPROVING OVER TIME

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Introduction: Sativex® (USAN, nabiximols) is a highly standardized oromucosal cannabinoid spray combining a CB₁ partial agonist (THC, 2.7 mg/spray) with an endocannabinoid system modulator (CBD, 2.5 mg/spray), alongside minor cannabinoids and terpenoids plus ethanol and propylene glycol excipients and peppermint flavoring. It is a licensed pharmaceutical for spasticity in multiple sclerosis (MS) in the UK, Spain, Canada, and New Zealand, and has a Notice of Compliance with conditions in Canada for symptomatic relief of neuropathic pain in MS, and treatment of cancer pain unresponsive to optimized opioid therapy. This study examines the adverse event (AE) profiles for Sativex employed adjunctively in randomized clinical trials (RCT) in treatment of intractable pain and MS symptoms.

Methods: Company Core Safety Information from 16 advanced phase randomized clinical trials (RCT) of Sativex vs. placebo was examined. Patient exposure to Sativex represents over 7500 patient-years of experience. AEs are presented as the percentage classified as having a plausible causal relationship to medication.

Results: Categories were divided into MS patients (805 Sativex/ 741 placebo), non-MS patients (425 Sativex/ 419 placebo) and cancer pain patients (328 Sativex/ 150 placebo). Patients generally titrated to a therapeutic response at an individualized stable dosage within 7-10 days, usually between 8 and 10 Sativex sprays per day. Dizziness was the most common AE in RCT (24.8% Sativex/ 7% placebo in MS; 31.5% Sativex/ 7.6% placebo in non-MS; and 15.9% Sativex/ 8.0% placebo in cancer pain), but this was an early and mostly a transient complaint rarely leading to discontinuation. Most other CNS AEs occurred with less than 10% incidence, e.g., somnolence (8.1% Sativex/ 1.9% placebo in MS; 6.8% Sativex/ 2.9% placebo in non-MS; 12.5% Sativex/ 6.7% placebo in cancer pain). Within the gastrointestinal system, the most common AE was nausea (7.6% Sativex/ 3.6% placebo in MS). In the general category, the only complaint exceeding 10% incidence was fatigue (11.1% Sativex/ 6.6% placebo in MS; 10.1% Sativex/ 3.6% placebo in non-MS; 2.1% Sativex/ 2.7% placebo in cancer pain). Psychiatric AEs affected 5.8% or less, as did other AE such as vertigo, blurred vision, and falls. When a slower titration schedule was introduced in a Phase III study of spasticity in MS, the incidence of more common AEs (dizziness, fatigue, somnolence and nausea) was markedly reduced despite the fact that the final Sativex dose attained was unchanged from earlier RCTs. In MS patients, only 9.8% withdrew due to AEs (vs. 4.7% of controls on placebo). This compares favorably to other drugs used for spasticity or neuropathic pain: gabapentin, 16%; pregabalin, 13.5%; tizanidine, 13%. The clinical safety profile of Sativex suggests a lack of clinically important drug-drug interactions.

Conclusion: The instigation of a slower initial dose titration has significantly improved the AE profile of Sativex. Withdrawal rates due to Sativex AE compare favorably with those reported in association with other available medicines for the target conditions.

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