Carolina Cannabinoid Collaborative

October 27–29, 2017

Hilton Durham, Durham, NC

RTI INTERNATIONAL

Conference Host

RTI International is pleased to host the 2017 Carolina Cannabinoid Collaborative Conference. RTI is an independent, nonprofit research institute dedicated to improving the human condition. We combine scientific rigor and technical expertise in social and laboratory sciences, engineering, and international development to deliver solutions to the critical needs of clients worldwide.

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The Carolina Cannabinoid Collaborative would like to thank our sponsors for their generous support of the 2017 CCC Conference.

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welcome

Welcome to the 2017 Carolina Cannabinoid Collaborative!

It is an exciting time in the field of cannabinoid and cannabis research. With ever-changing policies and therapeutic potentials, the need for such research has never been more important. In this spirit, the 2017 CCC conference will showcase recent cutting-edge data from some of today's top researchers. We hope that all participants learn something new, make connections, and even develop collaborations during the conference.

The technical program focuses on emerging therapeutic trends in cannabinoid research, abuse liability research, impacts and concerns about synthetics, and the need for sex differences in cannabinoid research at all levels. Featured speakers include Michelle Glass, Bill Fantegrossi, Ryan Vandrey, Rebecca Craft, Tom Gamage, and Camille Gourdet. A poster session will highlight recently collected data and insights, while other talks explore the human research and policy implications related to medicinal and recreational marijuana use.

The schedule also includes a cocktail reception Friday evening, dinner on Saturday, and breaks throughout the conference to maximize your chances to mingle with one another. These will be great opportunities to chat with your colleagues, delve deeper into a presentation you heard, or meet someone new. The conference will wrap up with awards for best student presentations (oral and poster) and a prize drawing for attendees.

We would like to thank our generous sponsors: RTI International, La Jolla Alcohol Research, Inc. (LJARI), the Lambert Center for the Study of Medicinal Cannabis & Hemp at Thomas Jefferson University, and the North Carolina Biotechnology Center. Without their support, and the assistance of our event managers, this conference would not have been possible.

Enjoy your time here in Durham, NC, and thank you for attending the 2017 CCC conference.

Sincerely,

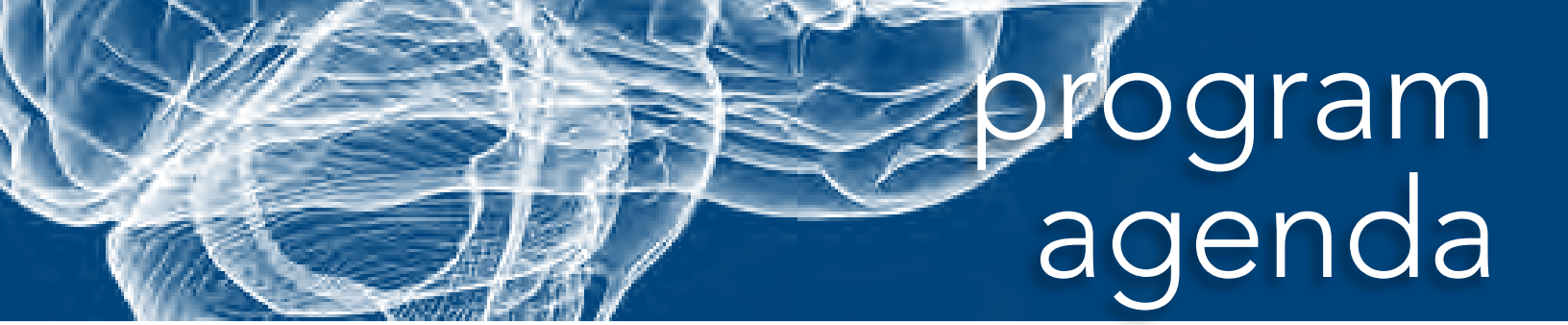
Brian Thomas, Tim Lefever, and Tom Gamage
2017 Carolina Cannabinoid Collaborative Planning Team
RTI International

Friday, October 27

TIME	ACTIVITY
6:00–9:00 p.m.	**Registration, Networking Reception**

Saturday, October 28

TIME	ACTIVITY	SPEAKERS
7:00–8:15 a.m.	**Registration, Continental Breakfast**	
8:15–8:30 a.m.	**Welcome**	Brian Thomas
Drug Abuse and Reward		
8:30–9:15 a.m.	**Design, synthesis, and biological evaluation of aminoalkylindole derivatives as potential therapeutics for substance use disorders**	Bill Fantegrossi
9:15–9:35 a.m.	**Endocannabinoid synthesis by dopamine neurons controls cue-directed reinforcement and motivation**	Dan Covey
9:35–9:55 a.m.	**The FAAH knockout rat: Biochemical validation and initial opiate intake studies**	Joel Schlosburg
9:55–10:15 a.m.	**THC withdrawal activates the neuroendocrine stress response and alters emotionality**	Kristen Trexler
10:15–10:25 a.m.	**Break**	
Development and Learning and Memory		
10:25–10:45 a.m.	**Cannabinoids exacerbate alcohol teratogenesis by disrupting Sonic Hedgehog signaling through a novel interaction between the CB1 receptor and Smoothened**	Scott Parnell
10:45–11:15 a.m.	**Cannabinoid and ethanol co-exposure: Impacts on neurogenesis and hippocampus-dependent functions in adolescent and adulthood**	Somnath Mukhopadhyay
11:15–11:35 a.m.	**Diacyglycerol lipase-α disruption impairs spatial learning and memory and changes search strategy in C57BL6/J mice**	Lesley Schurman
11:35–11:55 a.m.	**Cannabidiol improves recovery of vocal behavior following damage to pre-vocal-motor cortex**	Ken Soderstrom
Noon–1:00 p.m.	**Lunch**	
12:30–2:15 p.m.	**Poster Session**	



program agenda

Saturday, October 28 *(continued)*

TIME	ACTIVITY	SPEAKERS
Chemistry and Molecular Pharmacology		
2:15–3:00 p.m.	**The role of arrestins in regulation of CB1 function, and implications of high-efficacy arrestin recruitment by toxic synthetic cannabinoids**	Michelle Glass
3:00–3:20 p.m.	**Interpreting GPCR functional selectivity: A CB1 cannabinoid receptor case study**	David Finlay
3:20–3:50 p.m.	**CB1 allosteric modulator analogues with augmented binding cooperativity**	Tom Gamage
3:50–4:00 p.m.	**Break**	
4:00–4:20 p.m.	**Novel diarylurea-based allosteric modulators of the cannabinoid CB1 receptor**	Yanan Zhang
4:20–4:40 p.m.	**Development of a peripherally restricted CB1 receptor antagonist for alcoholic steatosis**	George Amato
4:40–5:00 p.m.	**Structural modification of the endocannabinoid anandamide: Designing chemotherapeutics and investigating metabolites**	Andrew Morris
6:00–9:00 p.m.	**Dinner**	
6:30 p.m.	**Sex differences in cannabinoid analgesia**	Rebecca Craft

Sunday, October 29

TIME	ACTIVITY	SPEAKERS
7:00–8:30 a.m.	**Continental Breakfast**	
Pain		
8:30–8:50 a.m.	**The therapeutic potential of cannabinoids for the treatment of dental pain**	Ron Tuma
8:50–9:10 a.m.	**Continuous morphine infusion deteriorates locomotor and bladder recovery and enhances chronic neuropathic pain in spinal cord injury mice: Effects of CBD or beta-caryophyllene treatment**	Sara Jane Ward
9:10–9:30 a.m.	**The MAGL inhibitor JZL184 prevents the development of paclitaxel-induced mechanical allodynia**	Zachary Curry
9:30–9:50 a.m.	**Chemotherapy-induced peripheral neuropathy: The role of diacylglycerol lipases in reversing mechanical allodynia**	Giulia Donvito
9:50–10:00 a.m.	**Break**	

Sunday, October 29 *(continued)*

TIME	ACTIVITY	SPEAKERS
Human and Policy		
10:00–10:30 a.m.	**Emerging areas of marijuana policy: Edible product labeling, advertising restrictions, and the testing of pesticides**	Camille Gourdet
10:30–10:50 a.m.	**Neuropsychological effects of ZYN002 (synthetic cannabidiol) transdermal gel in healthy subjects and epilepsy patients: Two phase 1, randomized, double-blind, placebo-controlled studies**	Marcel Bonn-Miller
10:50–11:35 a.m.	**Human laboratory studies of cannabis effects: comparisons of route of administration and dose**	Ryan Vandrey
11:35 a.m.–Noon	**Business Meeting**	

featured speakers

Rebecca Craft

PhD, Washington State University

Dr. Craft earned a PhD in experimental and biological psychology at the University of North Carolina at Chapel Hill (1991), completed post-doctoral training in the department of pharmacology at the University of Arizona (1993), and was hired as an assistant professor of psychology at Washington State University in 1993. She was tenured in 1998 and promoted to professor in 2005. From 2007 to 2011, she served as Director of Graduate Training in experimental psychology, and from 2011 to 2015 she served as Psychology Department Chair. She is currently a Herbert L. Eastlick Distinguished Professor of Psychology and also serves in a halftime appointment as Associate Dean for Research and Graduate Education in the College of Arts and Sciences. The primary focus of Dr. Craft's psychopharmacology laboratory is characterizing sex differences in the behavioral effects of psychoactive drugs. Over the past 20 years, her laboratory has determined that there are sex differences in analgesic, reinforcing, sedative, discriminative, and other effects of several classes of abused drugs, particularly opioids and cannabinoids. She has published more than 75 peer-reviewed articles and book chapters, and mentored approximately 20 MS and PhD students.

Bill Fantegrossi

PhD, University of Arkansas for Medical Sciences

Dr. Fantegrossi trained as a behavioral pharmacologist at the University of Michigan and the Yerkes National Primate Research Center. When not lifting weights or slaying imaginary dragons, his research focuses on the behavioral pharmacology of emerging drugs of abuse, including designer psychostimulants ("bath salts"), cannabinoids ("K2/Spice" products), opioids, and hallucinogens. His lab employs a variety of in vivo assays to study drug actions, including biotelemetry, intravenous drug self-administration, conditioned place preference, drug discrimination, operant behavior, antinociception, and drug-elicited behaviors. The ultimate goal of these studies is to better understand the abuse-related effects of novel pharmacological entities in order to inform clinicians, develop therapeutic strategies, and guide legislation.

Tom Gamage

PhD, RTI International

Dr. Gamage received his PhD in pharmacology and toxicology at Virginia Commonwealth University under the mentorship of Dr. Aron Lichtman, where he studied the behavioral pharmacology of CB1 allosteric modulators and the effects of endocannabinoid catabolic inhibitors in opioid dependence. In 2014, Dr. Gamage began his postdoctoral training in the laboratory of Dr. Mary Abood at the Center for Substance Abuse Research at Temple University, where he studied the effects of CB1 allosteric modulators on ERK signaling. In 2016, Dr. Gamage joined RTI International's Discovery Science unit to examine the molecular pharmacology of novel CB1 allosteric modulators and abused synthetic cannabinoids under the mentorship of Dr. Brian Thomas.

Michelle Glass

PhD, University of Auckland

Dr. Glass is a molecular neuropharmacologist with a strong interest in the area of therapies targeting G-protein coupled receptors. She has a particular interest in the cannabinoid receptors and endocannabinoid system and their role in neurodegenerative disease and, more recently, cancer. Her research team at the University of Auckland is interested in how current molecular pharmacology paradigms such as allosterism and biased signaling could be exploited to develop therapies that minimize adverse effects. She also has an interest in method development and optimization and improved experimental design. Her research portfolio consists of more than 80 research articles. She collaborates widely, at Auckland working with researchers from the Centre for Brain Research, Maurice Wilkins Centre, and Auckland Cancer Society Research Centre, and internationally with researchers from both academia and the pharmaceutical industry in the United States, Australia, Switzerland, the Netherlands, and France.

Camille Gourdet

JD, RTI International

Camille Gourdet is a research public health analyst in RTI International's Center for Health Policy Science and Tobacco Research. She has nearly 7 years of experience in evaluating tobacco-related policies at the state level to help measure their impact on public health outcomes. She has done extensive primary legal and policy research, synthesis, and data abstraction of relevant and existing state-level policies that pertain to medicinal and recreational marijuana use, child obesity, and tobacco control. At RTI, she conducts primary policy research and analysis, manages a nationwide study aimed at decreasing tobacco use among certain populations, and contributes to study designs and protocols. She has also supported innovative social media recruiting efforts by using targeted ads on Facebook and Instagram to recruit certain groups of respondents.

Ryan Vandrey

PhD, Johns Hopkins University

Dr. Vandrey is an experimental psychologist with degrees from the University of Delaware (BA) and the University of Vermont (PhD). He is currently an associate professor at the Johns Hopkins University Behavioral Pharmacology Research Unit. Dr. Vandrey's research focuses primarily on the behavioral pharmacology of cannabis (marijuana) and includes controlled laboratory studies with adult research volunteers, clinical trials, web-based survey research, and natural history studies with patient populations using cannabis/cannabinoids for therapeutic purposes. He has published 60 peer-reviewed journal articles and 10 book chapters. His work helped characterize the cannabis withdrawal syndrome, has provided novel data about the comparative pharmacokinetics and corresponding pharmacodynamics of cannabinoids across routes of administration, explored medications that are potential adjuncts to behavior therapy to improve rates of abstinence among individuals trying to quit using cannabis, examined the effects of cannabis on sleep, and is involved with multiple studies evaluating the risks and benefits of medicinal use of cannabis/cannabinoids for various health conditions.

speaker abstracts

Design, synthesis, and biological evaluation of aminoalkylindole derivatives as potential therapeutics for substance use disorders

Fantegrossi, B.

University of Arkansas for Medical Sciences

Attenuation of increased endocannabinoid signaling with a CB1R neutral antagonist, or activation of CB2 receptors with an agonist, might offer a new therapeutic direction for treatment of substance abuse. We have previously reported that a monohydroxylated metabolite of the synthetic aminoalkylindole cannabinoid JHW-073 exhibits neutral antagonist activity at CB1Rs and partial agonist efficacy at CB2Rs, and thus may serve as a promising lead for the development of novel substance abuse therapies. In drug development efforts, we show that systematic modification of an aminoalkylindole scaffold identified novel compounds with dual CB1R antagonist/CB2R agonist activities. Similar to the CB1R antagonist/inverse agonist rimonabant, two of these compounds decreased oral ethanol self-administration in mice without affecting total fluid intake and blocked the development of ethanol-conditioned place preference. The cannabinoid tetrad confirmed that two lead compounds were devoid of CB1R agonist effects in vivo at behaviorally relevant doses, and neither of the novel compounds induced significant rimonabant-like scratching. Generalized aversive effects of each drug were assessed using an operant-conditioned taste aversion procedure, where rimonabant and one lead induced taste aversion, but the other lead compound did not. Finally, schedule-controlled responding was used to assess suppression of response rates following administration of cumulative rimonabant or one of the lead compounds in mice chronically treated with either high-efficacy CB1R agonist JWH-018 or vehicle. Both drugs dose-dependently suppressed rates, but the dose-effect curve for our lead compound, but not for rimonabant, was shifted to the left in JWH-018–treated mice compared with vehicle-treated controls. These findings suggest differences in withdrawal-related and direct adverse effects elicited by rimonabant and our lead compounds, which could relate to neutral CB1R antagonism, CB2R partial agonism, or their combination. Both mechanisms should be explored and exploited in future drug design efforts to develop pharmacotherapies for substance abuse.

The role of arrestins in regulation of CB1 function, and implications of high-efficacy arrestin recruitment by toxic synthetic cannabinoids

Glass, M.,[1] Finlay, D., Manning, J., Ibsen, M., Grimsey, N.

[1]University of Auckland

Like most GPCRs, CB1 is thought to be phosphorylated by G-protein–coupled receptor kinases and internalized following arrestin recruitment. In our studies of allosteric modulators, we have come to question the importance of arrestin-mediated signaling to CB1 regulation. In this talk, I will describe our studies that show that in HEK cells the internalization of CB1 is not arrestin-mediated. Utilizing an arrestin translocation assay, we demonstrate that CB1 is a weak recruiter of β-arrestin-2, and we look at the ability of a range of

ligands to drive this pathway. Furthermore, our assays show that with only one exception, agonist-driven recruitment of β-arrestin-1 is not detected, although constitutive activity of the receptor is altered by β-arrestin-1 expression.

Recently, the "zombie epidemic" has made international headlines—individuals suffering essentially cataleptic responses and other severe toxicity following recreational consumption of high-potency/high-efficacy cannabinoid drugs. In New Zealand, approximately 20 synthetic cannabis-related deaths have been reported since June . I will present very recent data from my group that shows enhanced signaling and a much more pronounced recruitment of arrestin-2 than other traditional CB1 agonists assayed to date.

CB1 allosteric modulator analogues with augmented binding cooperativity

Gamage, T.F., Farquhar, C.E., Blough, B.E., Zhang, Y., Thomas, B.F., Wiley, J.L.

RTI International

CB1 allosteric modulators represent a promising avenue in the development of potential cannabinoid pharmacotherapeutics because they bind a distinct site on the receptor and could stabilize novel receptor species with altered affinity for endogenous ligands or bias signaling toward pathways of therapeutic relevance.

PSNCBAM-1 is a CB1 allosteric antagonist that exhibits positive binding cooperativity with the synthetic cannabinoid CP55,940. We have recently reported structural analogues to PSNCBAM 1 that exhibit differential binding cooperativity with CP55,940. RTICBM-28, which contains a cyano substitution at the 4-chlorophenyl position, exhibited enhanced binding cooperativity with CP55,940 as demonstrated by increased specific [3H]CP55,940 binding and CP55,940 EC50 shifts in agonist-stimulated [35S]GTPγS binding at lower concentrations as compared to the parent PSNCBAM-1. In contrast, RTICBM-15, which contains a dimethyl substitution at the 2-pyrrolidinyl position, exhibited lower maximal increases in [3H]CP55,940 binding and produced EC50 shifts of CP55,940 in [35S]GTPγS at higher concentrations than PSNCBAM-1. Furthermore, RTICBM-28 exhibited greater inhibitory potency than PSNCBAM-1 in [35S]GTPγS binding, whereas RTICBM-15 exhibited less potency.

ZCZ011 is a CB1 positive allosteric modulator that enhances the potency and/or efficacy of CB1 agonists and acts as an agonist on its own. We have observed that ZCZ011 enhances the potency of endocannabinoid anandamide at lower concentrations than those that produce leftward shifts in 2-AG's concentration response curve in [35S]GTPγS binding. Furthermore, in [3H]SR141716 competition binding, ZCZ011 produced leftward shifts in anandamide's Ki at lower concentrations than those that affected 2-AG's Ki. Therefore, ZCZ011 appears to exhibit probe bias for the endocannabinoid anandamide over 2-AG. Among the ZCZ011 analogues, we have characterized CAL010, which exhibits greater efficacy than ZCZ011 and increased specific [3H]CP55,940 binding, suggesting that CAL010 is stabilizing a greater proportion of receptors into an active conformation. These studies demonstrate that structural changes to CB1 allosteric modulators can yield compounds with different binding cooperativities, suggesting that further bias can be introduced into this system.

Sex differences in cannabinoid analgesia

Craft, R.

Washington State University

The prevalence of many types of pain, including headache, orofacial, musculoskeletal, and abdominal, is two to three times greater in women than in men. Thus, there is a particular need to develop more effective analgesics for women. There are now approximately a dozen clinical trials demonstrating that cannabinoids can reduce chronic pain, but only three studies comparing the analgesic effects of cannabinoids in women and men, and these are equivocal in their findings. Our research using tests of acute and chronic inflammatory pain in rats show that cannabinoids may be more potent and efficacious in females compared to males, but sex differences may depend on the type of cannabinoid, the doses examined, and the pain test used. When THC is administered repeatedly, females develop more tolerance than males do, using acute pain tests but not using a model of chronic inflammatory pain. When THC is administered locally (e.g., into an inflamed hind paw), it produced greater anti-allodynia in females, but greater reduction in edema in males. As has been shown for morphine, very low doses of THC also appear to prevent conditioned place aversion produced by formalin paw injection, at least in males. Sex differences in THC's antinociceptive effects are due to sex differences in (and gonadal hormone modulation of) multiple pharmacokinetic and pharmacodynamic (and possibly immune) mechanisms. For example, females given THC produce significantly more 11-OH-THC, the primary active metabolite of THC, than males do, and greater active metabolite production contributes to females' greater acute responses to THC as well as their greater development of tolerance. In future studies, we will determine whether another primary cannabinoid in cannabis, cannabidiol, produces differential effects in females and males, and whether it augments THC effects to the same extent in both sexes.

This research was supported by NIDA DA016644 (J. Wiley, PI) and by funds provided for biological and medical research by the State of Washington Initiative Measure 171.

Emerging areas of marijuana policy: Edible product labeling, advertising restrictions, and the testing of pesticides

Gourdet, C., Peiper, N.

RTI International

Even though the cultivation, manufacturing, distribution, and sale of marijuana products remain illegal under federal law, 29 states have legalized medicinal marijuana and eight of those have additionally legalized recreational marijuana. In the absence of federal guidance, each of these states has had to create a regulatory framework that limits and enforces how marijuana products can be cultivated and distributed. Three areas of emerging marijuana policy that are key to protecting public health, and which vary widely across the states, are product labeling, advertising, and pesticides.

The lead author conducted primary legal research in the LexisNexis legal database to identify and compile state-level statutes and regulations that regulate edible labeling, advertising restrictions and the use of pesticides.

Edible package labeling requirements vary across states and may include the physical demarcation of single servings, warnings about the potential for delayed intoxicating effects, the number of servings contained within the package, and the total amount of THC per individual serving. Several states prohibit advertising content that is false or misleading and restrict how a dispensary or recommending physician can advertise online, in print, outdoors, or within the retail environment. Most of the states set limits on the amount or type of pesticides that can be used in the cultivation of marijuana for medicinal or recreational purposes and require regular testing to ensure that the use of pesticides is within those limits.

State lawmakers need evidence-based science to effectively and sensibly regulate the cultivation, distribution, and sale of medicinal and recreational marijuana. While some states have taken steps to standardize edible labeling, restrict advertising, or regulate pesticides, additional research is needed to understand whether homogenization requirements for edibles are adequate, current THC limits are acceptable, existing testing or analytical methods are rigorous enough to confirm that a product's composition matches its product label, and what the health implications of short- and long-term ingestion of marijuana products may be. Interdisciplinary efforts are necessary to determine if advertising restrictions and warning labels should differ for medicinal and recreational marijuana users or for users who consume marijuana occasionally but not regularly. More studies are needed to assess whether there are different health effects from exposure to pesticides based on whether a marijuana product is inhaled, ingested, or absorbed topically.

Human laboratory studies of cannabis effects: comparisons of route of administration and dose

Vandrey, R.,[1] Schlienz, N.J.,[1] Herrmann, E.S.,[2] Bigelow, G.E.,[1] Mitchell, J.M.,[3] Flegel, R.,[4] LoDico, C.,[4] Cone, E.J.

[1]Johns Hopkins University School of Medicine, [2]Battelle Memorial Institute, [3]RTI International, [4]Substance Abuse and Mental Health Services Administration

In the United States, a burgeoning marketplace exists that offers a growing number of consumable cannabis goods and products intended for use via a variety of methods of administration. Few controlled studies have been conducted using administration methods other than smoking. We conducted a series of studies to directly compare the dose effects of cannabis following oral, smoked, and vaporized routes of administration.

The comparative pharmacokinetic and pharmacodynamic effects of cannabis were assessed in healthy adults at 0, 10mg, and 25mg THC doses administered via oral ingestion, vaporization, and smoke. Seventeen participants completed all doses within each route. Participants had a history of cannabis use, but had not used cannabis for at least 1 month prior to randomization. Outpatient sessions were conducted 1 week apart. Pharmacokinetic and pharmacodynamic assessments were obtained at baseline and for 8 hours following drug exposure. Analyses compared active dose conditions with placebo, and the same doses were compared across routes of administration. Correlations between behavioral measures and cannabinoids in blood and oral fluid were evaluated.

Dose-dependent drug effect ratings, cardiovascular effects, and cognitive performance effects were observed across routes of administration. Peak drug effects occurred within 15 minutes for inhaled cannabis, followed by a gradual return to baseline. In contrast, oral cannabis drug effects onset +60 to 90 minutes post-administration and remained at peak levels longer. Cardiovascular and cognitive performance effects had a different time course, with cardiovascular effects occurring more immediately (peak effect at +90min) compared with subjective drug effects (peak effect at +180min) and cognitive performance impairment (peak effect at +300min). The magnitude of peak drug effects was comparable across smoked and oral routes, but greater following vaporized cannabis administration. Blood cannabinoid levels were significantly correlated with subjective drug effect ratings and inversely correlated with some performance tasks. Oral fluid THC was significantly correlated with self-reported Drug Effect following smoked ($r = 0.21$) and vaporized ($r = 0.26$) cannabis, but not oral cannabis. Oral fluid THC was not significantly correlated with psychomotor, divided attention, or working memory performance following any route of administration. Pharmacodynamic effects were significantly greater in magnitude for females than males on a subset of outcome measures.

Significant variability in the pharmacodynamic effects of cannabis was observed across doses and as a function of route of administration. At the same doses, the magnitude of peak drug effects was comparable for both oral and smoked, but higher for vaporization, suggesting it is a more efficient route of cannabis delivery. Blood cannabinoids were better biomarkers of acute drug effects than oral fluid cannabinoids, but neither was consistently well correlated with performance on cognitive tasks.

oral presentation abstracts

DRUG ABUSE AND REWARD

THC withdrawal activates the neuroendocrine stress response and alters emotionality

Trexler, K.R., Kinsey, S.G.

West Virginia University

Δ^9-tetrahydrocannabinol (THC) withdrawal alters somatic as well as emotionality-like and motivational behaviors. Previous work from our lab and others suggests that THC withdrawal leads to agitation and alters behavior in common drug screens for anxiolytic drugs. The goals of the present study were to (1) establish behavioral paradigms that are altered by cannabinoid withdrawal, (2) identify the effects of cannabinoid withdrawal on corticosterone, and (3) determine the extent to which the stress response alters cannabinoid withdrawal-induced behaviors.

Male C57BL/6J mice were administered THC (10 mg/kg, b.i.d., s.c.) or vehicle for 6 days, then withdrawal was precipitated using rimonabant (3 mg/kg, ip). THC withdrawal induced paw tremors and head twitches, increased struggling in the tail suspension test and suppressed marble burying. In a separate group of mice, plasma was collected 30 minutes after rimonabant treatment, and corticosterone was quantified by ELISA. Precipitated THC withdrawal significantly increased corticosterone, which was unaffected by repeated THC or rimonabant treatment. To test the hypothesis that either corticosterone or catecholamines contributed directly to THC withdrawal-induced behaviors, we administered mifepristone (25 mg/kg) or propranolol (1 and 10 mg/kg), respectively. Neither antagonist affected THC withdrawal-induced marble burying or struggling. Thus, cannabinoid withdrawal activates the hypothalamic-pituitary axis, but the resulting corticosterone release does not mediate emotionality-related behaviors.

Endocannabinoid synthesis by dopamine neurons controls cue-directed reinforcement and motivation

Covey, D.

University of Maryland School of Medicine

Mounting clinical and preclinical work demonstrates that manipulations of endocannabinoid (eCB) signaling potently alter pathological forms of reward seeking and may represent a powerful treatment target for motivational disorders. While mesolimbic dopamine (DA) projections from the ventral tegmental area (VTA) to nucleus accumbens (NAc) control the conditioned reinforcing properties of reward-predicting cues and motivation, and are modulated by eCB manipulations, the endogenous mechanisms by which eCBs shape DA function and reward pursuit are not known.

To elucidate how the eCB 2-arachidonoylglycerol (2-AG) controls reinforcement and DA function, we selectively deleted the 2-AG synthesizing enzyme diacylglycerol lipase α (DGLα) from VTA DA neurons. DGLα deletion was accomplished by infusing an adeno associated virus expressing Cre under the tyrosine hydroxylase promoter into the VTA of mice expressing two loxP sites flanking the DGLα gene. Real-time DA dynamics in the NAc were monitored using fast-scan cyclic voltammetry during sucrose reinforcement.

Conditional, targeted deletion of DGLα from VTA DA neurons disrupts the ability of predictive cues to guide reinforcement and dramatically alters how DA release in the NAc encodes cues and rewards. As control mice learn the cue-reward contingency, NAc DA release transfers from reward delivery to cue onset, a well-characterized phenomenon that adheres to classic learning theories and economic models. In contrast, DGLα deletion disrupts this pattern of DA release, such that DA predominantly responds to reward delivery and does not transfer to cue onset. Deficits in operant responding and DA dynamics further increased as the response ratio (i.e., lever presses) escalated. Alternatively, DGLα deletion has no effect when reward receipt is not reliant on cue processing and response cost is minimal.

These findings demonstrate that 2-AG mobilization from VTA DA neurons controls dopaminergic encoding of reward predictive cues and effortful responding, providing mechanistic insight into a well-established but poorly understood property of DA neurons and motivated behavior and their susceptibility to eCB manipulations.

The FAAH knockout rat: Biochemical validation and initial opiate intake studies

Schlosburg, J.E.,[1] Karnati, A.R.,[1] Vendruscolo, L.F.,[2] Cravatt, B.F.,[3] Koob, G.F.[2]

[1]Department of Pharmacology & Toxicology, Virginia Commonwealth University, [2]Neurobiology of Addiction Section, NIDA Intramural Program, [3]Department of Molecular Medicine, The Scripps Research Institute

Based on an underpinning of previous studies examining the role of opiate dependence altering brain stress systems, and that chronic stressors result in upregulation of fatty acid amide hydrolase (FAAH) activity, we have previously presented data showing chronic FAAH inhibition by PF-3845 can blunt the escalation of intake and diminish motivation for heroin in rat self-administration models. To facilitate more efficient examination of the role of FAAH in response to chronic stressors, opiate intake, and prolonged withdrawal, a Wistar rat deficient in FAAH activity was generated using a zinc-finger nuclease targeted deletion around an end region of exon 1. As of this year's ICRS conference, we had initial behavioral screening suggesting some similar anxiolytic effects under high-stress conditions, like that seen in the FAAH knockout mouse. However, there was also some concern over an apparent duplication of the FAAH gene specific to the laboratory rat genome. We have since confirmed that the duplicate gene, even if downstream of the original FAAH gene, does not produce measurable mRNA or functional enzyme activity.

We have also performed initial studies, in both males and females, looking for differences in opioid intake under extended-access self-administration conditions. FAAH knockout rats show similar acquisition of opioid self-administration, with preliminary data suggesting a trend toward decreased intake of opioids in FAAH deficient rats. Additional self-administering cohorts are currently being tested, along with saccharin/sucrose preference and quinine sensitivity tests.

Cannabinoids exacerbate alcohol teratogenesis by disrupting Sonic Hedgehog signaling through a novel interaction between the CB1 receptor and Smoothened

Fish, E.W.,[1] Murdaugh, L.B., Boschen, K.E.,[1] Mendoza-Romero, H.N., Tarpley, M., Chdid, L., Zhang, C.,[2] Boa-Amponsem, O.,[2] Mukhopadhyay, S.,[1,2] Cole, G.J., Williams, K.P.,[2] **Parnell, S.E.**[1]

[1]University of North Carolina at Chapel Hill, [2]North Carolina Central University

Alcohol and cannabinoids (CBs) are two of the most widely used drugs during pregnancy. While fetal alcohol exposure can harm many aspects of development, the effects of fetal CB exposure alone or in combination with alcohol are poorly understood. We have recently demonstrated that exposure to CP-55,940 during early gestation causes defects of the face and brain, similar to those observed in fetal alcohol syndrome. We hypothesize that Smoothened (Smo) may function as an allosteric modulator of CB1, allowing the CB1-Smo complex to bind Gαs. The activity of alcohol on the morphogen Sonic Hedgehog (Shh), combined with CB inhibition of Smo and the actions of CB1 through Gαs, are hypothesized to act together to reduce Shh signaling, a critical regulator of embryonic and fetal development. Our studies establish a novel link between two distinct signaling pathways and have implications not only for development, but also for diseases such as addiction and cancer.

Employing in vivo and in vitro techniques, the present study determines whether CBs potentiate alcohol-induced defects and explores the underlying mechanisms for these effects. Using a mouse model of neurulation stage drug exposure, we demonstrate that HU-210, cannabidiol, and Δ9-THC dose-dependently affect craniofacial and ocular development to a similar degree as a high-dose alcohol exposure. Moreover, in both mouse and zebrafish embryos, low to moderate doses of CBs potentiate the effects of alcohol beyond an additive interaction and phenocopy a loss of function of the morphogen Sonic Hedgehog (Shh). The role of Shh in mediating these effects is supported by studies in vitro demonstrating that CBs reduce Shh signaling by inhibiting Smoothened (Smo), the primary mediator of the Shh pathway. Further demonstrations in the mouse embryo confirm that CP-55,940 exposure down regulates Shh pathway gene expression; while in zebrafish embryos, injection of Shh mRNA blocks the synergistic effects of alcohol and CP-55,940. A mechanistic role for the CB1 receptor is demonstrated in mice by the finding that the CB1 receptor antagonist, SR 141716A, dose dependently reduces the teratogenic effects of CP-55,940, suggesting a link between CB1 and the Shh pathway. Co-immunoprecipitation experiments reveal for the first time that, in the embryo, CB1 receptors and Smo form heteromers that are coupled to both Gαi and Gαs proteins and that alcohol exposure alters this interaction.

We hypothesize that Smo may function as an allosteric modulator of CB1, allowing the CB1-Smo complex to bind Gαs. The activity of alcohol on Shh, combined with CB inhibition of Smo and the actions of CB1 through Gαs, are hypothesized to act together to reduce Shh signaling, a critical regulator of embryonic and fetal development. Our studies establish a novel link between two distinct signaling pathways and have implications not only for development, but also for diseases such as addiction and cancer.

Cannabinoid and ethanol co-exposure: Impacts on neurogenesis and hippocampus-dependent functions in adolescent and adulthood?

Khatri, D.,[1] Olivares, A.,[1] Vaidyanathan, M.,[1] Crews, F.T.,[2,3]
Mukhopadhyay, S.[1,3,4]

[1]Neuroscience Research Program, Biomedical Biotechnology Research Institute, [3]Department of Chemistry and Biochemistry, North Carolina Central University, [2]Bowles Alcohol Research Center, [4]Department of Pharmacology, University of North Carolina at Chapel Hill School of Medicine

A large body of research has indicated the deleterious effects of both ethanol and cannabinoids on adolescent brain development and behavior. However, despite the increasing trend of co-exposure to ethanol and cannabinoid during adolescence, very little is known about the effects of this combined exposure during adolescence on adult brain neurogenesis and hippocampal-related learning and memory tasks in adulthood. The purpose of the current study is to determine (a) if adolescence and adult co-exposure to ethanol and cannabinoid cause a significant change in neurogenesis and hippocampus-related functions, (b) if the effects during adolescent exposure persists (locked in) during adulthood, and (c) the role of endocannabinoid system in the regulation of these processes.

Adolescent male Wistar rats at post-natal day (PND) 28 were treated with vehicle or ethanol (3 mg/kg/day, ig) or CB1R agonist ACEA (0.3 mg/kg/day, ip,) or JZL195(0.3 mg/kg/day, ip; dual inhibitor of endocannabinoid degrading enzymes FAAH& MAGL), on a 2 day on/2 day off paradigm alone (AIE: adolescent Intermittent ethanol (AIE) and AIC (adolescent Intermittent cannabinoid) or in combination (AIE+AIC) till PND 48 (11 treatments) in the presence and absence of CB1R antagonist SR141716 (SR1;0.3 mg/kg/day, ip). For combination treatment, ethanol and cannabinoid drug doses were reduced to half and SR1 was administered 20 minutes prior to ethanol, ACEA or JZL195 treatment. Body weights were taken every alternate day and 24 hours following the last treatment; animals were subjected to Y-maze or Novel Object Recognition (NOR) tasks, respectively, using standard protocols. The animals were then allowed to grow to adulthood (PND 90) without any drug treatment except food and water ad libitum. At PND 91, the animals were again subjected to Y-maze or NOR tasks. After 24 hours of the last trial for both the tests, animals were sacrificed and brains were collected. Immunohistochemical analysis was carried out to assess for changes inneurogenesis, (DCX+IR), cell proliferation (Ki67) and apoptosis (cleaved caspase-3). For chronic studies in adult, male Wistar rats at post-natal day (PND) 80 (body wt. 400–450 gm) were treated with vehicle or ethanol (5 mg/kg/day, ig) or CB1R agonist ACEA (3 mg/kg/day, ip,) or JZL195 (3 mg/kg/day, ip; dual inhibitor of endocannabinoid degrading enzymes FAAH& MAGL), for 10 days (till PND 90) in the presence and absence of CB1R antagonist SR141716 (SR1;3 mg/kg/day, ip). For combination treatment, ethanol and cannabinoid drug doses were reduced to half, and SR1 was administered 20 minutes prior to ethanol, ACEA, or JZL195 treatment. Body weight was taken every alternate day and 24 hours following the last treatment, animals were subjected to Y-maze or NOR tasks, as mentioned earlier.

We found that adolescent exposure to ethanol, cannabinoids, or their combination significantly impaired both spatial memory performance (as indicated by the arm entry and dwell time in the novel arm in a Y-maze test) and novel object recognition ability (indicated by total exploration time) immediately following the test period during adolescence, and these effects remain persistent (locked-in) when the same tests were performed during adulthood following 6 weeks of abstinence. We also found that ethanol or CB1R agonist ACEA or endocannabinoid inactivation enzyme inhibitor alone or in combination significantly reduced doublecortin positive neurons (DCX+IR) with concomitant increase in cleaved caspase-3 positive cell in dentate gyrus of hippocampus. Pretreatment with CB1R antagonists SR141716 significantly blocked the ethanol/cannabinoid-induced impairment of spatial memory function and NOR ability and reversed ethanol-induced inhibition of adult neurogenesis and cell death. Interestingly, CB1R antagonist did not produce any effect when administered alone. For adult studies, we found that chronic co-exposure to ethanol, CB1R agonist ACEA, or endocannabinoid inactivation enzyme inhibitor (JZL195) alone or in combination significantly impaired both spatial memory (as indicated by the arm entry and dwell time in the novel arm in a Y-maze

test) and NOR ability (total exploration time). We also found that these treatments significantly reduced adult neurogenesis (DCX+IR), which was accompanied by increased cell death (i.e., cleaved caspase-3 + IR cells) and pretreatment with CB1R antagonists SR141716 significantly ameliorated the ethanol/cannabinoid–induced impairment of spatial memory function and NOR ability and reversed ethanol-induced inhibition of adult neurogenesis and cell death.

Diacyglycerol lipase-α disruption impairs spatial learning and memory and changes search strategy in C57BL6/J mice

Schurman, L.D.,[1] Carper, M.C.,[1] Liu, Q.S.,[2] Ogasawara, D.,[3] Cravatt, B.F.,[2] Lichtman, A.H.[1]

[1]Virginia Commonwealth University, [2]Medical College of Wisconsin, [3]Scripps Research Institute

A growing body of evidence implicates the importance of the endogenous cannabinoid 2-arachidonoyl glycerol (2-AG) in the regulation of learning and memory. 2-AG biosynthesis occurs through diacylglycerol lipase (DAGL), in which DAGL-α is the principal regulator on neurons. Given that DAGL-$\alpha^{-/-}$ mice show decrements in synaptic plasticity and compromised hippocampal neurogenesis, the present study used complementary pharmacologic and genetic approaches to examine if DAGL-α disruption impairs the acquisition of spatial memory in the Morris water maze (MWM) in C57BL/6J mice. Furthermore, we evaluated if performance deficits were accompanied by changes in search strategy, as well as cellular alterations in long-term potentiation.

To assess MWM acquisition and search strategy; DAGL-$\alpha^{-/-}$, -$\alpha^{+/-}$, and -$\alpha^{+/+}$ mice received 10 Fixed Platform MWM training days, and C57BL6/J mice received a 2 h pretreatment of either the DAGL inhibitor DO34[4] (0.3, 3, and 30 mg/kg), or vehicle (VEH). To assess cellular plasticity, hippocampi were dissected in DAGL-$\alpha^{-/-}$, -$\alpha^{+/-}$, and -$\alpha^{+/+}$ mice, and 2 h following 30 mg/kg DO34 or VEH. Field excitatory postsynaptic potentials were recorded from the CA1 region by evoked stimulation of the Schaffer collateral/commissural pathway, and LTP was induced by theta burst stimulation (TBS).

While both DAGL-$\alpha^{-/-}$ and DO34 disrupted TBS-induced LTP in CA1 of the hippocampus, only DAGL-$\alpha^{-/-}$ mice failed to acquire the MWM Fixed Platform task, whereas DO34-treated mice displayed a main effect of drug impairment at 30 mg/kg. DAGL-$\alpha^{-/-}$ mice used more non-spatial and thigmotaxic swim paths and spent more time in the MWM outer ring than DAGL-$\alpha^{+/+}$ mice. Although DO34 (30 mg/kg) did not significantly alter swim paths, it significantly increased time spent in the outer ring. As such, the poor MWM performance of DAGL-$\alpha^{-/-}$ mice was accompanied by extensive changes in search strategy, whereas the modest DO34 performance deficits produced modest search strategy changes. This pattern of results suggests that MWM performance deficits of DAGL-α disruption are a result of less efficient search strategies. The lower efficiency search strategies, as well as deficits in cellular correlates of learning and memory, point to the regulatory and perhaps developmental importance of DAGL-α in spatial learning and memory.

Cannabidiol improves recovery of vocal behavior following damage to pre-vocal-motor cortex

Soderstrom, K.,[1] Wooley-Roberts, M.,[2] Brodie, J.,[2] Alalawi, A.[1]

[1]Department of Pharmacology and Toxicology, ECU Brody School of Medicine, [2]GW Pharmaceuticals

In most cannabis strains, cannabidiol (CBD) is the second most abundant active constituent. Unlike delta-9-THC, CBD has very low cannabinoid receptor (CBR) affinity and its psychoactivity is subtle and

attributable to interactions with non-CBR cellular targets. In clinical trials, CBD shows clear efficacy in treating intractable seizures in children, a condition associated with developmental delays, including language deficits. Anecdotal evidence suggests CBD may improve vocal learning in these children. To begin to test for efficacy to improve vocal development, we have evaluated CBD in a vocal learning animal: a songbird, the zebra finch.

In an initial dose-response experiment, four dosage groups (0, 1, 10, 100 mg/kg/day CBD) and three surgical conditions (HVC microlesion [ML], sham-ML, no-ML) were employed with $n = 6–7$ animals/group. HVC is a pre-vocal-motor cortical region, and ML of this region (about 10% of HVC destroyed) temporarily impairs vocal phonology, syntax, and the amount of vocal output. These impairments normally resolve over about 1 week. Experiments spanned 20 days, with animals housed in chambers for continuous recordings. Baseline recordings were made over the first 3 days. Daily CBD treatments (injected in 50µl IM) began on Day 4 and continued through the experiment. HVC ML, sham-ML or no-ML procedures were done on Day 10, and animals were followed for an additional 10 recovery days.

Results demonstrated that 10 and 100 mg/kg/day CBD improved recovery time and reduced the magnitude of disruptions to vocal phonology. These dosages were also effective in improving recovery of syntax. 10 mg/kg/day CBD improved recovery of vocal output. As vocalizations do not improve following HVC MLs in deafened birds, recovery requires auditory-guided sensorimotor re-learning. Thus, CBD appears to improve this learning process. In addition to demonstrating CBD efficacy, results from this initial dose-response study indicate songbirds may be used as an animal model to evaluate drugs for efficacy to promote a form of vocal learning. As only a few groups of animals are vocal learners, and only songbirds are well-suited to laboratory experimentation, the utility of this preclinical model is unique.

CHEMISTRY AND MOLECULAR PHARMACOLOGY

Interpreting GPCR functional selectivity: A CB1 cannabinoid receptor case study

Finlay, D.B.,[1] Zhu, X.,[2] Duffull, S.B.,[2] Glass, M.[1]

[1]Department of Pharmacology and Clinical Pharmacology, Faculty of Medical and Health Sciences, University of Auckland, [2]School of Pharmacy, University of Otago

In G Protein-Coupled Receptor (GPCR) pharmacology, the ability of ligands to differentially affect a receptor's signaling responses has become a popular theme—functional selectivity. Substantial literature is available that describes CB1 cannabinoid receptor functional selectivity. However, these studies tend to rely on isolated ligand descriptions between pathways, rather than constituting systematic ligand/pathway comparisons using established methods of bias quantification.

The current study utilized a panel of six CB1 agonists to characterize activity in three pathways: receptor internalization, and the downstream cAMP and pERK signaling pathways. Receptor internalization was characterized using two quantitative immunocytochemistry protocols, cAMP signaling was observed by real-time BRET biosensor, and pERK signaling was observed using PerkinElmer AlphaLisa. A systematic assessment of CB1 functional selectivity was then performed using current methods of quantification. The impact of differing pathway kinetics on the interpretation of functional selectivity was also considered.

The experimental method for characterizing agonist-driven internalization was found to substantially affect conclusions about agonist efficacy. In conflict with current literature, operational analysis did not indicate bias for any agonist/pathway comparison. Challenges will also be discussed, particularly as concerns a methodological limitation of operational analysis when applied to pathways which do not have an equilibrium time point.

Novel diarylurea-based allosteric modulators of the cannabinoid CB1 receptor

Nguyen, T.,[1] Decker, A.,[1] Gamage, T.,[1] Farquhar, C.,[1] Li, J.X.,[2] Thomas, B.F.,[1] Wiley, J.L.,[1] Kenakin, T.,[3] **Zhang, Y.**[1]

[1]RTI International, [2]Department of Pharmacology and Toxicology, University at Buffalo, [3]Department of Pharmacology, University of North Carolina at Chapel Hill

Several allosteric modulators of the CB1 receptor, including Org27569 and PSNCBAM-1, have been recently reported and they display pharmacological characteristics distinct from those of orthosteric agonists and antagonists. Given the psychoactive effects commonly associated with orthosteric ligands, allosteric modulators may offer a much-needed alternative strategy to modulate the important CB1 signaling for therapeutic benefits.

Our group has been developing diarylureas derived from PSNCBAM-1 as CB1 receptor allosteric modulators. Structure–activity relationship studies around these diarylureas revealed that the 4-chlorophenyl moiety preferred electron-deficient groups, whereas the pyrrolidinyl ring was not required for activity, and the pyridinyl ring can be replaced by a variety of other aromatic rings, including substituted phenyl and five-member ring heterocycles. While they increased the binding of the CB1 orthosteric agonist CP55,940, these diarylureas attenuated CP55,940-induced effects in the calcium mobilization and [35S]GTP-γ-S binding assays, as expected for negative allosteric modulators. These compounds showed high selectivity against the CB2 receptor and did not have any agonist activities on their own. Among these, RTICBM-74 showed similar CB1 potency as PSNCBAM-1 in several in vitro assays, but was significantly more stable in the rat liver microsomes. Importantly, RTICBM-74 was more potent in attenuating prime induced reinstatement of cocaine-seeking behavior in rats than PSNCBAM-1. These findings suggest that diarylureas represent a promising candidate for the treatment of cocaine addiction and related conditions.

Development of a peripherally restricted CB1 receptor antagonist for alcoholic steatosis

Amato, G., Maitra, R., Manke, A., Wiethe, R., Snyder, R., Vasukuttan, V., Cortes, R., Lefever, T., Wiley, J., Harris, D., Runyon, R.

RTI International

Antagonists of peripheral CB1 receptors have potential utility in the treatment of obesity, liver disease, metabolic syndrome and dyslipidemias. Inhibition of CB1 receptors in the central nervous system (CNS), however, produces adverse effects, including depression, anxiety, and suicidal ideation. To avoid these central adverse effects, there is a strong interest in identifying CB1 antagonists that do not penetrate the CNS.

In this study, novel analogues of otenabant were explored, in which the 4,4-disubstituted piperidine group was switched to a smaller monosubstituted piperazine group. To access a lipophilic binding site, the nitrogen of the piperazine was functionalized with alkyl, cycloalkyl, and aryl groups, both with and without a spacer. By varying the spacer, both neutral and mildly basic compounds were prepared. To balance potency with drug-like properties, the groups were decorated with heteroatoms. Potent and selective hCB1 receptor antagonists were discovered. Some of these compounds were predicted to have limited brain permeability based on an in vitro predictive model (penetration of MDCK-mdr1 cell monolayers). The 2-chlorobenzyl piperazine antagonist from this series of compounds was found to have favorable properties for continued development and assessment in efficacy studies. This compound demonstrated good solubility in acidic media, stability in human microsomes and no hERG liability. It was shown to be an inverse agonist of hCB1 receptors. In mice, this compound demonstrated excellent oral absorption and peripheral selectivity. Oral administration of this compound to mice failed to reverse cannabinoid-induced hypothermia, confirming little to no brain penetration. Finally, the CB1 inverse agonist was tested in a mouse model of alcoholic liver steatosis, induced

by feeding a Lieber DeCarli diet containing alcohol for 4 weeks. At an oral twice daily dose of 1.25 mg/kg, this peripherally selective compound demonstrated significant efficacy in blocking liver steatosis. In conclusion, an advanced candidate targeting CB1 receptors in the liver and other peripheral tissues has been identified for further development to treat alcoholic liver disease.

Structural modification of the endocannabinoid anandamide: Designing chemotherapeutics and investigating metabolites

Morris, A.,[1] Van Dross, R.,[2] Burns, C.[1]

[1]Department of Chemistry, East Carolina University, [2]Department of Pharmacology and Toxicology, East Carolina University

Many epithelial cancers overexpress cyclooxygenase-2 (COX-2), an enzyme responsible for metabolizing anandamide (AEA) to prostamides. AEA causes cell death in COX-2 overexpressing cancers via its metabolism to novel J-series prostamides, namely 15d-PMJ2. By understanding COX-2 metabolism of AEA, derivatives of AEA can be synthesized that have greater potency and efficacy against cancer. To examine the effects of altering polarity and steric bulk on AEA-mediated cytotoxicity, we investigated AEA derivatives with functional groups that vary in these properties. Arvanil and R1-methanandamide (m-AEA) add steric bulk to the molecule via aromatic rings and additional methyl functional groups. Arachidonoyl glycine (NAGly) substitutes the terminal EA alcohol with a carboxylic acid increasing polarity. NAGly carries a charge in biological systems, making it unique to all other AEA derivatives and NAGly is also a known COX-2 substrate. To determine which structural modifications improve AEA-mediated cytotoxicity, JWF2 tumorigenic keratinocytes and HCA-7 colon cancer cells, which overexpress COX-2, were exposed to AEA and AEA analogs and cell viability was measured by conducting MTS assays. AEA, Arvanil, NAGly, and m-AEA significantly reduced cell viability, indicating that functional groups that contribute steric bulk and polarity do not inhibit the cytotoxicity of AEA. MTS assays were also conducted in cancer cell lines with low COX-2 expression. AEA, Arvanil, and NAGly were less cytotoxic, indicating that COX-2 metabolizes cannabinoids with polar groups and steric bulk to cytotoxic products. These findings suggest that modulation and substitution to the non-active site ethanolamine arm of AEA can be accomplished while keeping or improving cytotoxicity of the molecule.

PAIN

The MAGL inhibitor JZL184 prevents the development of paclitaxel-induced mechanical allodynia

Curry, Z.A.,[1] Toma, W., Meade, J.A., Wilkerson, J.L., Damaj, M.I., Lichtman, A.H.

[1]Virginia Commonwealth University

Paclitaxel chemotherapy is widely used for the management of ovarian, breast and lung cancers. However, the use of paclitaxel is limited by the development of a disabling chemotherapy-induced peripheral neuropathy (CIPN) in the hands and feet. Because there are no effective treatments preventing paclitaxel CIPN, reduction of paclitaxel use remains the standard of care but hinders effective tumor treatment. As paclitaxel CIPN results from both neuronal injury and inflammation, an effective treatment strategy would need to protect against both mechanisms of neuropathy. The enzyme monoacylglycerol lipase (MAGL), which degrades

the endogenous cannabinoid (eCB) 2-arachidonoylglycerol (2-AG), is a useful target for the prevention of paclitaxel CIPN. Inhibition of MAGL with JZL184 increases 2-AG levels and stimulates both cannabinoid (CB) receptors type 1 and 2, dampening both nociceptive signaling (CB1) and inflammation (CB2) in rodent models of neuropathic pain. Here, we hypothesize that MAGL inhibition will prevent the development of paclitaxel CIPN in a mouse model. Treatment of C57Bl/6J mice with paclitaxel (8 mg/kg, i.p.) on 4 alternate days induced a mechanical allodynia as assessed using von Frey filaments. In separate cohorts of mice, JZL184 (4 or 40 mg/kg, i.p.) was given for 3 days prior to the start of a cycle of paclitaxel. During the cycle, JZL184 was administered alone or in conjunction with paclitaxel. On the day after the last paclitaxel treatment, paw withdrawal thresholds were assessed, and mice were treated with one final injection of JZL184. Our results demonstrate that 40 mg/kg JZL184 prevents the development of paclitaxel allodynia 1, 8, and 15 days after the last paclitaxel treatment. Treatment with 4 mg/kg does not prevent allodynia 1 day after the paclitaxel cycle, but causes early resolution of allodynia by day 8.

Chemotherapy-induced peripheral neuropathy: The role of diacylglycerol lipases in reversing mechanical allodynia

Donvito, G.,[1] Mischel, R.,[1] Wilkerson, J.L.,[1] Ogasawara, D.,[2] Akbarali, H.,[1] Hsu, K.L.,[3,4] Cravatt, B.F.,[2] Lichtman, A.H.[1]

[1]Department of Pharmacology and Toxicology, Virginia Commonwealth University, [2]The Skaggs Institute for Chemical Biology and Department of Chemical Physiology, The Scripps Research Institute, [3]Department of Chemistry, University of Virginia, [4]Department of Pharmacology, University of Virginia

Diacylglycerol lipases (DAGLs)-α and -β, the main biosynthetic enzymes for the endogenous cannabinoid 2-arachidonoylglycerol (2-AG), represent potential targets for the treatment of painful conditions such as chemotherapy-induced peripheral neuropathy (CIPN), which is resistant to traditional analgesics. Intraperitoneal administration of the DAGL inhibitors DO34 and KT109 dose-dependently reversed paclitaxel-induced allodynia. However, neither DAGL-α (-/-) mice nor DAGL-β (-/-) mice showed a protective phenotype against paclitaxel-induced nociceptive behavior.

While these findings suggest that these enzymes are dispensable for the development of paclitaxel-induced allodynia, genetic deletion of these enzymes across ontogeny may have led to compensatory changes underlying the pathology. Nonetheless, these transgenic mice represent a useful tool for screening the selectivity of inhibitors targeting both enzymes. Both KT109 and DO34 fully reversed paclitaxel-induced allodynia in wild-type mice and DAGL-α (-/-) mice but did not elicit antinociceptive effects in DAGL- β (-/-) mice, suggesting that DAGL-β plays a necessary role in the antinociceptive effects of both inhibitors. To test whether the antinociceptive effects of KT109 undergo tolerance, mice were given a daily injection of KT109 (40 mg/kg) for 6 days. The antinociceptive effects of KT109 administered repeatedly were maintained at least 24 hours after the last administration of the drug, whereas the antinociceptive effects of a single injection of KT109 last no more than 8 hours. In the final set of experiments, we examined potential neural mechanisms mediating these effects and found that repeated KT109 administration ameliorates paclitaxel-induced hyperexcitability of primary afferent neurons isolated from dorsal root ganglia innervating the lumbar level of the spinal cord.

Overall, these findings suggest the following: (1) DAGLs are dispensable for the development of paclitaxel-induced allodynia, but DAGL inhibitors reverse allodynia through a DAGL-β site of action, (2) DAGL-β inhibition reduces paclitaxel-induced neuronal hyperexcitability, and (3) DAGL-β inhibitors represent a promising strategy to treat neuropathic pain associated with chemotherapy.

The therapeutic potential of cannabinoids for the treatment of dental pain

Tuma, R.F., Laks, E., Ward, S.J.

Center for Substance Abuse Research, Lewis Katz School of Medicine, Temple University

Previous investigators have shown that cannabinoid CB2 knockout mice demonstrate increased pain and anxiety following tooth pulp exposure as compared to wild-type animals. The phytocannabinoid cannabidiol (CBD) has been shown to attenuate the development of neuropathic pain, potentially through both peripheral effects and effects on the central nervous system. In addition, activation of the cannabinoid CB2 receptor is also anti-inflammatory and potentially anti-neuropathic. The goal of the present study was to determine if the administration of CBD or the naturally occurring CB2 receptor agonist terpene molecule beta caryophyllene (βCP) would attenuate the development of facial touch sensitivity and inflammation following pulp exposure and to investigate the potential mechanisms of action.

Both drugs were administered ip. Coronal pulpotomies were performed on the mandibular left first molar of anesthetized mice using a slowly rotating dental bur with a #1 round tip. The animals were then allowed to recover from the surgery. CBD (5mg/kg) or βCP (30mg/kg) was administered 1 hour prior to pulp exposure, 24 hours post exposure, 3 days post exposure and then twice weekly. Von Frey filaments were used to assess facial allodynia daily following pulp exposure. Food intake was monitored, and the animals were weighed daily. Coronal sections of the trigeminal nucleus were made and stained with Iba1 for microglial activation.

While pulp exposure increased allodynia on the ipsilateral side, animals treated with either CBD or βCP demonstrated a reduction in facial allodynia. Body weight was not influenced by pulp exposure in either group. Examination of the trigeminal nucleus showed enhanced microglial activation following pulp exposure and modification of this response by both CBD and βCP.

Continuous morphine infusion deteriorates locomotor and bladder recovery and enhances chronic neuropathic pain in spinal cord injury mice: Effects of CBD or beta-caryophyllene treatment

Ward, S.J., Li, H., Tuma, R.F.

Center for Substance Abuse Research, Lewis Katz School of Medicine, Temple University

The opioid analgesic morphine is widely used in the clinic to treat acute and chronic pain. However, morphine not only has high potential for addiction but may also worsen recovery and pain through proinflammatory mechanisms. We recently determined that cannabidiol (CBD), the predominant non-psychoactive phytocannabinoid, can ameliorate thermal sensitivity and decrease neuroinflammation in a mouse model of spinal cord injury (SCI), while synthetic cannabinoid CB2 receptor agonism also improves recovery from SCI while attenuating inflammation.

Our central hypothesis was that co-administration of CBD or the CB2 receptor agonist terpene beta caryophyllene (βCP) with morphine would attenuate the poorer treatment outcomes observed with morphine infusion alone.

Female C57/BL6 mice were exposed to sham or contusion injury to the thoracic spinal cord. Osmotic pumps containing 98.8ul morphine in 2.52mg/100ul, 3.78mg/100ul concentration or control vehicle solution were implanted subcutaneously at the time of impact, infusing morphine 0.5u per hour, 24 hours continuously for 7 days. A subset of spinal cord injured mice with 3.78mg/100ul mini pumps were given vehicle, CBD, or βCP treatment (30 mg/kg IP) 1 hour post injury and every 24 hours for 7 days by intraperitoneal injection.

Changes in locomotor and bladder function and hindpaw thermal and tactile sensitivity in sham and injured mice were evaluated.

Results: (1) Morphine infusion with 2.52mg/100ul and 3.78mg/100ul mini-pump for 7 days deteriorated locomotor and bladder function in spinal cord injured mice. (2) Morphine infusion was associated with mechanical and thermal allodynia in sham-injured animals, and a trend was observed in this direction in spinal cord–injured mice. (3) CBD but not βCP treatment can ameliorate neuropathic pain in morphine+SCI mice. Both CBD and βCP failed to improve locomotor and bladder recovery.

HUMAN AND POLICY

Neuropsychological effects of ZYN002 (synthetic cannabidiol) transdermal gel in healthy subjects and epilepsy patients: Two phase 1, randomized, double-blind, placebo-controlled studies

Bonn-Miller, M.,[1] Gutterman, D.,[1] O'Neill,[1] C., Messenheimer, J.,[2]

[1]Zynerba Pharmaceuticals, Inc., [2]Moncure, NC

Clinical and nonclinical data suggest that cannabidiol (CBD), a cannabinoid derived from Cannabis, may have dramatic therapeutic effects in many chronic medical conditions, including epilepsy and pain, without the deleterious neuro-cognitive effects associated Δ9-tetrahydrocannabinol (THC). ZYN002 is a synthetic form of CBD formulated as a transdermal gel at 1% and 2.5% CBD. No human clinical studies of ZYN002 have been previously published.

Two Phase 1, randomized, double-blind, placebo-controlled studies were conducted to determine the neuropsychological effects of ZYN002. One study was a 7-day multiple-dose study in which 24 adult subjects were treated daily with ZYN002 200 mg ($n = 6$), 250 mg ($n = 6$), 500 mg ($n = 6$), or placebo ($n = 5$). The second study was a 7-day study of adults with epilepsy in which 12 subjects were treated daily with either ZYN002 500mg ($n = 9$) or placebo ($n = 3$). Assessments included the following: Trail Making, Paced Auditory Serial Addition Test (PASAT), Divided Attention Test (DAT), Positive and Negative Affect Schedule (PANAS), and Inventory of Depression and Anxiety Symptoms (IDAS).

Repeated measures ANOVAs were conducted for each dependent variable to determine main effects of dose, time, and dose x time interactions. Main effects of time were observed in both studies for the trail making task, PASAT, PANAS, and IDAS. Furthermore, a main effect for dose was observed in both studies for the IDAS. No dose by time interactions were observed for any of the measures among healthy subjects. However, a significant trend ($p = .052$) was observed for the PASAT (total correct) among epilepsy patients, where improved performance was demonstrated over time among those who received ZYN002.

Results indicate that ZYN002 does not produce impairment in critical areas of cognitive functioning or psychological health often impacted by CNS drugs. In fact, results highlight the potential for ZYN002 to improve some domains of cognitive functioning among patients with epilepsy. Unlike THC, ZYN002 may provide therapeutic benefit for chronic medical conditions while minimizing neuropsychological risks.

poster abstracts

1. The anti-neoplastic effect of novel endocannabinoid metabolite, 15dPMJ$_2$, on human colon cancer cells

Albassam, H.M.,[1] Ladin, D.A.,[1] Van Dross, R.[1,2]

[1]Department of Pharmacology and Toxicology, Brody School of Medicine, East Carolina University, [2]Center for Health Disparities, East Carolina University

The endoplasmic reticulum (ER) is a cellular organelle that is primarily responsible for oxidative protein folding. When the protein folding load in cells exceeds the protein folding capacity, ER stress occurs. The ER stress pathway is a prominent regulator of cancer cell death and as a result, ER stress inducers are being exploited pharmacologically. An important regulator of the cytotoxic ER stress pathway is the transcription factor, C/EBP homologous protein 10 (CHOP10). Previously, we showed that 15deoxy, $\Delta^{12,14}$ prostamide J$_2$ (15dPMJ$_2$)-induced apoptosis occurred in an ER stress-dependent manner. However, the role of CHOP10 and its downstream transcriptional products in ER stress-apoptosis is unclear. We also demonstrated that 15dPMJ$_2$ caused ER stress apoptosis in tumorigenic keratinocytes and melanocytes; however, the mechanism of 15dPMJ$_2$ apoptosis in colon cancer cells has not been investigated.

The goal of this study is to determine the requirement for CHOP10 and its transcripts in 15dPMJ$_2$-mediated apoptosis in colon cancer cells. The data show that 15dPMJ$_2$ was a potent inducer of apoptosis in the human colon cancer cell line, HCT116, whereas it was threefold less effective in inducing apoptosis in the non-tumorigenic colon cell line, FHC. 15dPMJ$_2$ also caused a concentration-dependent increase in CHOP10 expression. Pharmacological inhibition of the ER stress pathway with 4-phenylbuterate (PBA) or salubrinal prevented apoptosis, indicating that cell death is reliant upon ER stress. To determine if CHOP10 was required for 15dPMJ$_2$ apoptosis and to identify CHOP10 transcriptional products that mediate this effect, CRISPR/Cas9 CHOP10 knockout HCT116 cells (CHOP10-KO-HCT116) were generated. 15dPMJ$_2$ increased CHOP10 expression in wt-HCT116 cells but not in CHOP10-KO-HCT116 cells. Studies are underway to define the role of CHOP10 and its transcriptional products in apoptosis. Gaining an understanding of mechanisms of 15dPMJ$_2$ apoptosis will allow us to better predict methods by which adverse effects, drug resistance and synergistic tumor cytotoxicity can occur.

2. Oxidative Stress Mediates Ethanolamide Conjugated D-series Prostaglandins-Induced Apoptosis in Skin Tumor

Elhassanny, A.E.M.,[1] Ladin, D.A.,[1] Soliman, E.,[2] Albassam, H.,[1] Morris, A.,[3] Danell, A.S.,[3] Van Dross, R.[1,4]

[1]Department of Pharmacology and Toxicology, Brody School of Medicine, East Carolina University, [2]Department of Pharmacology and Toxicology, Faculty of Pharmacy, Zagazig University, [3]Department of Chemistry, and [4]Center for Health Disparities, East Carolina University

Non-melanoma skin cancer (NMSC) is the most prevalent cancer in the United States. Our previous data showed that arachidonoyl ethanolamide (AEA)-induced apoptosis in NMSC cells occurred as a consequence of COX-2-metabolism of AEA to PGD$_2$-EA and then to 15dPGJ-EA$_2$ (15dPMJ$_2$). We also found that AEA-

induced cell death was dependent on reactive oxygen species (ROS) production but was independent of CB1, CB2, and TRPV1.

To understand the role and source of ROS in AEA-mediated apoptosis, the activity of its downstream metabolite, PGD_2-EA, was examined. PGD_2-EA caused a concentration-dependent increase in apoptosis in NMSC cells. The induction of ROS and apoptosis by PGD_2-EA was prevented by the antioxidant Trolox but not by D-type prostaglandin receptor antagonists. Oxidative stress occurs due to an imbalance between ROS generating and neutralizing molecules. Reduced glutathione (GSH), thioredoxin (TXN), and heme oxygenase-1 (HO-1) are important for ROS neutralization while NADPH oxidase (NOX) and uncoupled endothelial nitric oxide synthase (eNOS) are critical for ROS generation. GSH, TXN, and HO-1 were required for protection against the oxidative stress-induced apoptosis mediated by PGD_2-EA. In contrast, pro-oxidant enzymes did not play a role in PMD_2-EA-mediated apoptosis. These findings demonstrate that PGD_2-EA reduces the cell's ability to neutralize ROS, thereby increasing ROS generation and promoting tumor cell death. Because promising chemotherapeutic outcomes have been observed with ROS inducing agents, AEA or PGD_2-EA may be an effective approach for eliminating cancer.

3. Novel endocannabinoid metabolite, 15-deoxy-Δ12,14 prostamide J2, displays activity against melanoma in vitro and in vivo: Potential role of endoplasmic reticulum stress and calcium signaling

Ladin, D.A.,[1] Yang, L.V.,[2] Fitzgerald, T.L.,[3] Van Dross, R.,[1,4]

[1]Department of Pharmacology and Toxicology, [2]Department of Internal Medicine, [3]Department of Surgery, [4]Center for Health Disparities—Brody School of Medicine, East Carolina University

Melanoma is the most aggressive cutaneous neoplasm in the United States, representing a major clinical challenge. Our lab showed that the endocannabinoid, arachidonoyl ethanolamide (AEA), induced cell death in non-melanoma skin cancer (NMSC) cells through the COX-2 mediated formation of novel J-series prostamides (PMJs). We were the first to chemically synthesize the primary metabolite, 15-deoxy-Δ12,14 prostamide J2 (15d-PMJ2), which displayed potent and selective toxicity in NMSC cells.

As such, we hypothesize that the selective toxicity of 15d-PMJ2 would be observed in other forms of skin cancer, including melanoma. B16F10 murine melanoma cells and non-tumorigenic Melan-A cells were treated with various concentrations of 15d-PMJ2, and cell viability was measured using MTS assays. At 5μM, 15d-PMJ2 decreased viability by 63% in B16F10 cells, while Melan-A viability was not affected. To verify that cell death was apoptotic, the cleavage of caspase-3 and PARP was examined by conducting Western blot analysis. 15d-PMJ2 markedly increased caspase-3 and PARP cleavage only in B16F10 melanoma cells.

Previous studies in NMSC indicated that 15d-PMJ2 induced ER-stress and apoptosis. To investigate the mechanism of 15d-PMJ2-mediated death in melanoma, we examined ER-stress responses. Melan-A and B16F10 melanoma cells were treated with 5μM 15d-PMJ2 and evaluated for CHOP10 and p-PERK expression. B16F10, but not Melan-A cells, exhibited a notable increase in CHOP10 and p-PERK expression. ER-stress is known to induce apoptotic Ca+2 signaling, and, as such, we investigated this mechanism by pretreating cells with the Ca+2 channel inhibitors ruthenium red and 2-APB. Both inhibitors decreased cell death, suggesting that Ca+2 channel activation plays an important role in 15d-PMJ2 mediated tumor cell death.

To determine the anti-melanoma activity of 15d-PMJ2 in vivo, B16F10 allograft tumors grown in C57BL/6 mice were dosed subcutaneously with 0.5 mg/kg 15d-PMJ2. Tumors treated with 15d-PMJ2 exhibited significantly reduced growth and weights compared to vehicle and untreated animals. TUNEL analysis of

tumor tissues indicated a large presence of cell death in 15d-PMJ2-treated tumors compared to control tumors. To determine whether 15d-PMJ2 induced ER-stress in vivo, tumors were assayed for p-PERK and CHOP10 levels by immunohistochemistry (IHC). These markers were elevated in 15d-PMJ2-treated tumors. Similarly, the viability of primary patient-derived melanoma cells was significantly decreased by 15d-PMJ2. These findings suggest that the novel endocannabinoid metabolite, 15d-PMJ2, possesses potent and selective anti-melanoma activity in vitro and in vivo.

4. Immune cell mediated death as an approach for NMSC therapy

Hess, J.,[1] Allen, A.,[1] Ladin, D.,[2] Elhassanny, A.,[2] Murray, G.,[3] **Van Dross, R.**,[2,4]

[1]Medical Program, [2]Department of Pharmacology and Toxicology, [3]Department of Pathology, [4]Center for Health Disparities—Brody School of Medicine, East Carolina University

Non-melanoma skin cancer (NMSC) is the most common malignancy in the United States, with more than 3.3 million new cases diagnosed each year. NMSC therapy includes surgical techniques and topical therapeutics that are associated with serious adverse effects and tumor recurrence. As such, highly effective therapeutics that are cancer-directed and produce durable responses are needed to improve treatment outcomes. Damage-associated molecular pattern (DAMP)-immunogenic cell death (ICD) is an immunostimulatory signaling pathway that is now a target for drug development. DAMP activation provokes dendritic cell phagocytosis of the tumor, cytotoxic T cell activation, and memory T-cell production that ultimately immunizes the host against the tumor. DAMP activation is characterized by translocation of intracellular proteins to the cell surface [e.g., calreticulin (CALR) and heat shock proteins] and the release of intracellular molecules into the extracellular space [e.g., ATP and non-histone chromatin-binding protein high-mobility group box 1 (HMGB1)].

Most clinical chemotherapeutics fail to activate DAMPs, except for a select group of agents (e.g., oxaliplatin and doxorubicin) that induce endoplasmic reticulum (ER) stress, a process required for DAMP translocation. In different cancer cell types, favorable responses to DAMP-inducing agents have been associated with elevated intracellular DAMP expression. However, DAMP levels and ER stress have not been examined in NMSC.

To evaluate the potential responsiveness of NMSC to DAMP-inducing agents, DAMP and ER stress expression were detected in patient NMSC and normal skin samples. In addition, the effect of DAMP-inducers on CALR translocation was determined in a human NMSC cell line. Samples of verified NMSC tissues and noncancerous epidermis were obtained from the Department of Dermatology (UMCIRB 16-001098) and the North Carolina Tissue Consortium (NCTC) of ECU. To quantify levels of DAMPs (CALR and HMGB1) and ER stress (PDI) in each tissue section, immunohistochemical analysis was performed. To detect cell surface CALR expression in cultured NMSC cells, flow cytometric analysis was conducted on cells that were exposed to DAMP-inducing agents.

The results showed that, in NMSC tissues, CALR, HMGB1, and PDI expression were significantly elevated compared to the noncancerous epidermis. In addition, pharmacological DAMP inducers increased cell surface CALR expression in cultured NMSC cells. DAMP inducers may be effective immunotherapeutics for NMSC. Additional in vitro and in vivo studies are needed to determine if these agents will provide potent, selective, and durable responses against NMSC.

5. Evaluating the ability of cannabinoids and prostaglandins to activate damage-associated molecular patterns in cancer

Escobedo, R.,[1] Neill, N.E.,[2] Elhassanny, A.,[1] Van Dross, R.[1,3]

[1]Department of Pharmacology & Toxicology, [2]Medical Doctor Program, and [3]Center for Health Disparities, Brody School of Medicine, East Carolina University

Cancer is the second leading cause of death in the United States. Many of the chemotherapeutic agents and surgical procedures that eliminate cancer can cause severe adverse effects because they also damage normal cells. In addition, some treatments are inefficient in completely removing the tumor, which may lead to cancer recurrence. Therefore, improved chemotherapeutic treatments are needed to reduce cancer mortality and recurrence. Agents that induce damage-associated molecular patterns (DAMPs) are being targeted for development due to their ability to initiate immune cell-mediated tumor death. DAMP signals include cell surface calreticulin (ecto-CRT), as well as the release of ATP and HMGB1. 15d-PMJ_2 is an endocannabinoid metabolite that induces tumor cell death in an ER stress-dependent manner. Interestingly, agents that induce DAMPs require ER stress.

The goal of this study is to determine if the prostamide 15d-PMJ_2, other prostaglandins, and cannabinoids are potent inducers of DAMP expression. B16F10 melanoma cells were treated with different concentrations of prostaglandins (15d-PMJ_2 and PGE2), cannabinoids [arachidonoyl ethanolamide (AEA), met-AEA, Win55,212-2, arvanil and CBD], commercially utilized chemotherapeutics (doxorubicin, mitoxanthrone, oxaliplatin and cisplatin) or vehicle. Cell viability was then determined by conducting MTS assays.

Of the agents tested, only 15d-PMJ_2 (IC50 = 4.5 μM), CBD (IC50 = 9.74 μM), arvanil (IC50 = 14.4 μM) and doxorubicin (IC50 = 3 μM) were potent inducers of melanoma cell death. To determine if these agents increased the expression of ecto-CRT, cells were treated with each agent for different periods of time, and CRT expression was detected by conducting flow cytometric analysis. Of the drugs tested, 15d-PMJ_2, CBD and doxorubicin significantly increased ecto-CRT expression. Next, the ability of 15d-PMJ_2 to induce ATP secretion was evaluated compared to oxaliplatin. Cells were treated with drugs or vehicle, and ATP was measured by a luminescence assay. 15d-PMJ_2 was more potent than oxaliplatin at inducing ATP secretion. These results suggest that 15d-PMJ_2 and CBD will effectively stimulate antitumor immune responses in vivo.

6. Assessment of CB1 and CB2 receptors in head and neck squamous cell carcinoma

Daniels, K.E., Luginbuhl, A.J., South, A.P.

Thomas Jefferson University

The endocannabinoid signaling system is involved in many cellular processes, including those that regulate cell growth and apoptosis through the PI3K/Akt pathway. Many of these pathways are also implicated in squamous cell carcinoma growth and treatment targets. Marijuana users have a known increase in relative risk to develop human papilloma virus (HPV)–related head and neck squamous cell carcinoma (HNSCC).

The ESS has been implicated for its role in the pathogenesis of various cancers. However, there is limited research exploring its activity in HNSCC. This research characterizes the expression of the two primary receptors in this pathway, CB1 and CB2, in HNSCC versus healthy tissue. Further analysis of patient demographics and outcomes will be correlated to receptor expression levels.

IF imaging indicated positive expression of CB1 in all HNSCC and NHK cell lines, and minimal to no expression of CB2. Qualitatively, CB2 exhibited greater expression in NHK cells. Expression of CB1 was confirmed by WB where all cell lines yielded a positive band for CB1 at 60kDa. Quantitative density analysis of WB showed that, on average, HNSCC exhibited increased expression of CB1 over NHK ($n = 7$). In isogenic

pairs of HNSCC and NHK cells, there is a greater than 50% increase in expression of CB1 in HNSCC over NHK. HPV status yielded variable expression of CB1 between cohorts. Qualitative and quantitative analysis of IHC in marijuana users and non-users is pending completion.

In conclusion, CB1 is consistently present on HNSCC cells and above the level of expression on NHK from the same patient. IHC images will be assessed and presented looking at qualitative and quantitative parameters to determine differential expression between marijuana users and non-users.

7. Male Tat transgenic mice show inhibitory control deficits using go no-go task

Jacobs, I.R.,[1] Hermes, D.J.,[1] Antonucci, A.G.,[1] Ferguson, A.B.,[1] Manjarres, C.,[1] Sabater, S.M.,[1] Mackie, K.,[2] Lichtman, A.,[3] Ignatowska-Jankowska, B.,[4] Fitting, S.[1]

[1]Department of Psychology and Neuroscience, University of North Carolina at Chapel Hill, [2]Department of Psychology and Brain Science, Indiana University, [3]Department of Pharmacology and Toxicology, Virginia Commonwealth University, [4]Edmond and Lily Safra Center for Brain Science, Hebrew University of Jerusalem

Using our Tat transgenic mouse model, the present study investigated the effects of HIV-1 Tat on operant conditioning by using the behavioral Go/No-Go (GNG) task. Previous in vitro research in our lab demonstrated Tat-induced deficits in dendritic growth and morphology of cultured primary prefrontal cortex (PFC) neurons. The PFC is involved in regulating attention and inhibition; therefore, the GNG task, which places demands on those systems, served as an appropriate task to further investigate our hypothesis in vivo.

We hypothesized that HIV-1 Tat interferes with attention, with Tat expressing mice (Tat+) showing less inhibition than their wild-type counterparts (Tat-). Twenty-six Tat transgenic mice (thirteen females) were free-fed a doxycycline (DOX) diet for 2 weeks to activate Tat expression. During the following week, mice were food-deprived to 85% of their initial body weights, where they remained throughout the rest of the study. Using standard MED operant chambers, experimenters trained mice (Tat+/Tat-) on the GNG task for three months over four phases. At test, a conditional discrimination revealed male Tat+ animals showed significantly less inhibitory control than male Tat+ animals. In other words, male Tat+ mice were less likely to inhibit their response on a No-Go trial than male Tat- mice.

8. Developmental manipulation of endocannabinoid signaling persistently alters reinforcing properties of abused drugs

Aldhafiri, A., Soderstrum, K.

Department of Pharmacology and Toxicology, East Carolina University Brody School of Medicine

The endocannabinoid system (ECS) is involved in a variety of physiological processes, including appetite, pain-sensation, mood, and memory. Given clear evidence that the ECS is important to establishment of neural circuits, altering ECS activity during adolescence, a period associated with brain maturation, is of particular concern. To better understand how manipulation of the ECS may alter learning that is dependent on CNS maturation, we have used a zebra finch model of developmental psychopharmacology. Zebra finches are one of few natural vocal learners and have thalamocortical-basal ganglia circuity similar to mammals, including dopaminergic input from VTA to striatum essential for incentive learning. We have previously found that cannabinoid exposure during the sensorimotor vocal learning period alters song patterns produced in

adulthood. To test whether altered vocal development is associated with changes in other types of incentive learning, we have developed a conditioned place preference method to study cocaine reinforcement. The CPP paradigm is a standard preclinical behavioral model used to assess preferences for environmental stimuli associated with a positive or a negative reward. Animals were chronically treated once daily for 25 days during their sensorimotor stage of vocal learning and allowed to mature to adulthood before cocaine reinforcement experiments. Developmental treatments (in 50 µl IM) were 3mg/kg of THC (partial CB1 agonist), 6 mg/kg of SR141716A (CB1-selective antagonist/inverse agonist) and 4 mg/kg of MAG lipase inhibitor (JZL184). Both developing and adult animals were employed.

Results demonstrated that daily developmental THC treatments increased time spent in cocaine-paired chambers. In contrast, developmental JZL184 treatments produced avoidance of cocaine-paired chambers. Interestingly, these effects were not observed following treatment of adults, demonstrating distinct developmental sensitivity. Contrasting efficacy of direct vs. indirect cannabinoid agonism suggests the ECS is active within distinct brain regions relevant to negative reinforcement during development.

9. Effect of PF3845 on GABAergic neurotransmission in Tat transgenic mice

Xu, C.,[1] Xu, C.,[1] Hermes, D.J.,[1] Jacobs, I.R.,[1] Niphakis, M.J.,[2] Cravatt, B.F.,[2] Mackie, K.,[3] Lichtman, A.,[4] Ignatowska-Jankowska, B.,[5] Fitting, S.[1]

[1]Department of Psychology and Neuroscience, University of North Carolina at Chapel Hill, [2]Department of Chemical Physiology, Scripps Research Institute, [3]Department of Psychology and Brain Science, Indiana University, [4]Department of Pharmacology and Toxicology, Virginia Commonwealth University, [5]Edmond and Lily Safra Center for Brain Science, Hebrew University of Jerusalem

In the era of combined antiretroviral therapy (cART), human immunodeficiency virus type 1 (HIV-1) is now considered a chronic disease that specifically targets the brain and causes HIV-1-associated neurocognitive disorders (HAND). Endocannabinoids exhibit neuroprotective and anti-inflammatory properties in several neurodegenerative disease models. Preliminary in vitro studies from our laboratory demonstrate protective effects of PF3845 (a selective fatty acid amide hydrolase (FAAH) catabolic enzyme inhibitor) against HIV-1 Tat-induced neuronal injury. However, the exact mechanism of its neuroprotective action is not clear.

To address this issue, ex vivo whole-cell recordings were performed on GABAergic neurotransmission in the medial prefrontal cortex (mPFC) using brain slices of HIV-1 Tat transgenic mice. Results indicate significant increases in the mean frequency but not the mean amplitude of GABAergic currents for transgenic Tat(+) brain slices compared to control Tat(-) tissue for both sex (females and males). PF3845 inhibited GABAergic neurotransmission via a Ca2+-mediated signaling pathway and CB1R-related pathway. Immunohistochemistry confirmed the uniform distribution of CB1Rs on the cytoplasm and dendrites, indicating that abundant CB1R is present in our mPFC Tat transgenic mouse tissue. Exploring the effect of PF3845 on GABAergic neurotransmission in Tat transgenic mice will give us important insights into the potential mechanisms related to the neuroprotective actions of endocannabinoids in the context of neuroAIDS.

10. PF3845 alters neurotoxic microglial responses to HIV-1 Tat exposure

Hermes, D.J.,[1] Xu, C.,[1] Meeker, R. B.,[2] Niphakis, M.J.,[3] Cravatt, B.F.,[3] Mackie, K.,[4] Lichtman, A.,[5] Ignatowska-Jankowska, B.,[6] Fitting, S.[1]

[1]Department of Psychology and Neuroscience, University of North Carolina at Chapel Hill, [2]Department of Neurology, University of North Carolina at Chapel Hill, [3]Department of Chemical Physiology, Scripps Research Institute, [4]Department of Psychology and Brain Science, Indiana University, [5]Department of Pharmacology and Toxicology, Virginia Commonwealth University, [6]Edmond and Lily Safra Center for Brain Science, Hebrew University of Jerusalem

The HIV-1 virus is known to affect microglial immune responses which likely play a paramount role in the development of chronic neuroinflammatory conditions and neuronal damage found in HIV-1–associated neurocognitive disorders (HAND)–affected brains. The HIV-1 transactivator of transcription (Tat) protein is detectable in the brains of AIDS patients receiving combined antiretroviral treatment (cART), and has been shown to be neurotoxic in in vitro and in vivo assays. Drugs targeting the degradative enzymes of endogenous cannabinoids have shown promise in reducing pain and inflammation with minimal side effects in rodents.

Here we demonstrate that inhibiting the degradation of anandamide, one of two major endogenous cannabinoid ligands, with PF3845 in murine PFC neuron culture, blunts the neurotoxic effects of Tat. Ca^{2+} imaging and dendritic process volumetric analysis with IMARIS show that neurons incubated with PF3845 before Tat exposure show less Ca^{2+} equilibrium dysregulation and dendritic degeneration compared to Tat-treated neurons. We found the former effect to be mediated by CB1R activity while the latter was mediated by CB2R. Considering that markers of neuroinflammation are among the best correlates of loss of cognitive function in AIDS patients, we also wanted to assess the effect of PF3845 on microglial release of neurotoxic soluble factors. We exposed cultured murine microglia to Tat with or without incubation of PF3845. After 24 hours, microglial conditioned media was collected for neuron exposure experiments. DIV8-10 neuron cultures were then exposed to diluted microglial-conditioned media, and neurotoxicity was assessed using Ca^{2+} imaging. Data collection is still in progress. Preliminary data suggest that PF3845 attenuates the microglial response to Tat, resulting in lower neurotoxicity.

11. Cannabinoid receptor CB1R but not CB2R increases neurite extension in human neuroblastoma

Lyons, E.,[1] Kabler, S.,[1] Kovach, A.L.,[2] Thomas, B.F.,[2] Howlett, A.C.[1]

[1]Integrated Physiology and Pharmacology, Wake Forest School of Medicine, [2]RTI International

Despite neurodegenerative diseases affecting more than 5 million Americans, there is not yet sufficient understanding of the underlying mechanism of disease to cure or even halt progression. Transplanted neurons may represent a potential treatment if neurons could integrate successfully with the host tissue to mitigate symptoms. Both CB1 and CB2 receptors are localized in the neuronal growth cone membranes, implying a role for cannabinoid signaling in neurite extension or guidance. Cannabinoid signaling has been shown to be active in neuronal extension, with the differences between CB1R and CB2R signaling remaining unclear.

We have created a novel stable overexpression model to investigate the role of cannabinoid signaling in human neuronal extensions. We quantified the endogenous production of cannabinoid ligand 2-Arachidonoyl Glycerol (2-AG) in SHSY5Y human neuroblastoma at an abundance of 4,000 nanograms 2-AG per million cells. This 2-AG was likely produced by DAGL enzyme activity because calcium stimulation by Oxotremorine M increased 2-AG abundance to 6,000 nanograms per million cells while inhibition of DAGL by THL decreased 2-AG abundance to less than 100 nanograms per million cells.

We transfected SHSY5Y cells with either CB1 or CB2 receptors, purified and maintained the transfection using Geneticin selection, and isolated clonal cell lines that stably overexpress either CB1R (CB1XS) or CB2R (CB2XS). Stable overexpression of cannabinoid receptors did not alter mRNA abundance of the enzymes that synthesize and degrade the endogenous cannabinoids 2-AG and AEA.

Stable overexpression of CB1R but not CB2R increased the extension length from 30 to 200 micrometers per neurite. Our research suggests a role for CB1R but not CB2R mediated cannabinoid signaling in neurite development that may provide insights in development of therapies that support functional integration of transplanted neurons into host tissue.

12. Discriminative stimulus effects of inhaled synthetic cannabinoids

Barrus, D., Pulley, N., Lefever, T., Wiley, J.

RTI International

Amidst the rapidly changing regulatory environment for cannabinoids like Δ^9-THC, the primary psychoactive component of cannabis, synthetic cannabinoids have risen in popularity, frequently under misleading names like "fake marijuana" or "spice." These compounds, generally far more potent agonists of cannabinoid receptors than Δ^9-THC, are often administered in aerosol using electronic cigarette (e-cigarette) devices, but only a few of these compounds have been extensively studied for behavioral and pharmacological effects.

We characterized the in vivo discriminative stimulus effects of three synthetic cannabinoids (CP55,940, AB-CHMINACA, and MMB-FUBINACA) administered via injection and aerosol using an e-cigarette device. Male and female C57BL/6J mice were trained to discriminate between injected Δ^9-THC and vehicle in the drug discrimination assay. After animals demonstrated reliable discrimination and completed a typical injected Δ^9-THC dose-response curve, a range of injected and aerosol concentrations of synthetic cannabinoids were tested for Δ^9-THC substitution. In males, all synthetic cannabinoids tested produced substitution along a characteristic sigmoidal dose-response curve for both injected and aerosol administration. In females, however, full substitution was only observed for injected AB-CHIMINICA and aerosol CP55,940. The present findings provide further evidence of the efficacy of aerosol administration as a translationally relevant method of preclinical drug delivery.

13. Pregnenolone inhibition and the affective properties of Δ9-tetrahydrocannabinol

Hempel, B.J.,[1] Crissman, M., Riley, A.L.

[1]American University

Pregnenolone is a neurosteroid precursor recently shown to function as a negative allosteric modulator at the CB1 receptor. Interestingly, injections of Δ9-tetrahydrocannabinol induce a surge of endogenous pregnenolone in reward-related brain areas and mice trained to self-administer the synthetic cannabinoid WIN 55,212-2 decrease responding after an injection of pregnenolone. Taken together, these findings suggest that pregnenolone may be part of a negative feedback system that responds to perturbations of the CB1 receptor. These findings are particularly noteworthy in light of the mixed and weak evidence of THC's rewarding and reinforcing properties in pre-clinical rodent models. The negative feedback loop described may be responsible for the difficulties encountered in modeling the rewarding effects of THC. As such, blocking the surge of pregnenolone following cannabinoid administration could enable a display of THC-induced reward.

Male Long-Evans rats were administered aminoglutethimide (AMG; 0 or 50 mg/kg; SC), an inhibitor of pregnenolone synthesis, prior to place conditioning with THC (0, 0.15, 1.5 or 15 mg/kg; IP). Subjects underwent a baseline assessment of side preference in the CPP apparatus (pre-test). They received

four pairings of THC with one CPP chamber and four pairings of vehicle with the opposite chamber, counterbalanced across 8 days, followed by a post-test assessment of chamber preference.

THC induced significant place aversions at 1.5 and 15 mg/kg, collapsed across pre-exposure group (AMG or vehicle). No aversions or preferences were evident at 0.15 mg/kg THC. Student t-tests at each pre-exposure and THC dose group revealed significant place aversions only in the AMG pre-exposed subjects at the two highest THC doses.

AMG potentiated the aversive properties of THC at an intermediate and high dose. These results suggest that inhibiting the surge of pregnenolone following THC administration enhances THC's subjective effects, potentially by prolonging drug action. No evidence of THC-induced reward was observed with or without AMG pre-exposure, likely indicating that THC is not rewarding at these doses, or in general, within the place preference preparation.

14. Motivation is disrupted during Δ^9-THC withdrawal

Eckard, M. L.,[1] Anderson, K. G., Kinsey, S. G.

[1]West Virginia University

Dependence on cannabis can result in withdrawal following abstinence, which increases the likelihood of relapse. The primary psychoactive component of cannabis is Δ^9-tetrahydrocannabinol (THC), and behavioral models of THC withdrawal can aid in identifying therapeutics to attenuate withdrawal symptoms. The current study used a progressive ratio schedule to evaluate disruptions in motivation in mice during THC withdrawal.

Following baseline stability, mice were administered THC (10 mg/kg) or vehicle (n = 9) every 12 hours for 6 days. THC withdrawal was either precipitated using the CB1 antagonist rimonabant (SR141716; 2 mg/kg) or allowed to develop spontaneously following abrupt cessation of THC administration. Precipitated THC withdrawal decreased break point and response rate, whereas spontaneous THC withdrawal had no detectable effects. Implications of these results suggest THC withdrawal may induce blunted motivation, as observed in clinical populations. These results also necessitate more sensitive behavioral screens for future cannabinoid withdrawal testing.

15. Convulsant and seizure-like effects of synthetic cannabinoids JWH-018 and 5F-AB-PINACA in mice: Observational signs and in vivo electroencephalography

Wilson, C.D., Cozart, M.A., Zheng, F., Fantegrossi, W.E.

Department of Pharmacology and Toxicology, University of Arkansas for Medical Sciences

Synthetic cannabinoids (SCBs) are man-made chemical compounds which bind to cannabinoid type-1 (CB1) receptors, typically with higher affinity and efficacy than Δ^9-THC, the main psychoactive constituent in cannabis. Abuse of SCBs is associated with more frequent and severe adverse events than use of marijuana, and convulsions and seizures are documented in the clinical literature following exposure to SCBs.

Recently, SCBs have been demonstrated to induce convulsant activity in mice. In this study, we used an observational scoring procedure and in vivo electroencephalography (EEG) to assess the convulsant and seizure-like effects of SCBs from two different "generations" of abused substances representing distinct structural classes: JWH-018, a first-generation indolyl-based SCB, and 5F-AB-PINACA, a third-generation indazole-based SCB. For observational studies, drugs were injected intraperitoneally (i.p.) into mice, which were immediately placed into 500 ml Pyrex beakers with mesh covering. Animals were observed continuously for convulsant effects, and scored in two separate 15-minute intervals. Both SCBs elicited convulsant effects,

which were blocked by prior treatment with the CB1 antagonist / inverse agonist rimonabant. In separate studies, mice received a headmount device and EEG recording leads implanted onto the skull. After surgery, mice recovered and adapted to the headmount for 4 to 7 days. Mice were then placed into a round EEG recording cage, in which the headmount attached to a preamplifier and digitizer to record the EEG signals. The preamplifier and cable connecting to the digitizer were suspended from above on a swivel arm to allow the mice free movement. Prior to the experiment, baseline data of brain activity per mouse was collected and recorded for 24 hours. Afterward, mice were injected i.p. with either JWH-018 or 5F-AB-PINACA, and EEG signals were recorded for 4 hours post-injection.

Results from preliminary EEG data show seizure-like activity following injection of 5F-AB-PINACA, while studies with JWH-018 are ongoing. Comparisons among drugs, doses, and observable signs versus EEG recordings will be presented.

16. Effects of cannabidiol (CBD) on vocal learning and recovery from CNS damage

Alalawi, A.,[1] Woolley-Roberts, M.,[2] Brodie, J.,[2] Soderstrom, K.[1]

[1]Department of Pharmacology and Toxicology, ECU Brody School of Medicine,
[2]GW Pharmaceuticals, Cambridge, UK

The major non-psychoactive ingredient derived from cannabis sativa is CBD. It has many cellular targets, including 5-HT1A receptors, GPR55, α3 and α1 glycine receptors and adenosine receptors. It holds promise as an anxiolytic, anti-inflammatory, and in the treatment of schizophrenia and seizure disorders. The anti-seizure properties of CBD have been evaluated in children who suffer from intractable seizures with promising results, including the suggestion that CBD may improve vocal learning.

To test the ability of CBD to influence vocal learning and recovery from CNS damage, a vocal learning animal model was employed: a songbird, the zebra finch. The capacity of CBD to improve recovery of vocal behavior in these animals following partial electrolytic ablation of a region of pre-motor-cortex was evaluated (HVC, approximately 10% destroyed). Four groups of adult animals were treated with 0, 1, 10, and 100 mg/kg CBD once daily via intramuscular (IM) injection; no-surgery and sham control groups were also evaluated at all four doses. Experiments were done over 20 days. Birds were recorded over this 20-day period to analyze vocal phonology, syntax and production. The first 3 days were baseline recordings with no treatment. Drug treatment period began at day 4. On day 10, HVC lesions (or sham lesions) were done and treatments and recording were continued for 10 recovery days.

We found that 10 and 100 mg/kg/day CBD improved recovery time and reduced the magnitude of lesion effects on phonology. As the recovery of phonology depends upon auditory feedback, this process involves sensorimotor learning that is improved by CBD. These results indicate that CBD can mitigate effects of CNS damage on phonology and improves vocal learning. The mechanism for this effect remains unknown, but may likely involve several cellular targets. Results from this project also demonstrate the utility of a songbird model in evaluating drug effects on vocal behavior.

17. The orphan receptor GPR6: Structural understanding and its relation with the cannabinoids

Isawi, I.,[1] Morales, P.,[1] Laun, A.,[2] Hurst, D.,[1] Song, Z.H.,[2] Reggio, P.[1]

[1]Chemistry and Biochemistry Department, University of North Carolina at Greensboro,
[2]Pharmacology and Toxicology Department, University of Louisville

The orphan G protein coupled receptor 6 (GPR6) is a constitutively active, Gαs protein coupled receptor that raises intracellular formation of cyclic adenosine-3',5'-monophosphate (cAMP). It has been reported that

GPR6 participates in both neurite outgrowth and instrumental learning. GPR6 is highly expressed in the striatopallidal region of the basal ganglia and it has been shown that depletion of GPR6 in that region causes both a striatal cAMP decrease and a dopamine increase. This event increases motor activity and decreases abnormal uncontrollable movement. Therefore, the results suggest that GPR6 may serve as a therapeutic target for the treatment of Parkinson's disease.

The orphan receptors GPR6, GPR3, and GPR12 share more than 50% identity and 65% similarity at the amino acid level. They belong to the GPCR Class A MECA cluster of receptors (melanocortin receptors (MCRs), endothelial differentiation G-protein coupled receptors, cannabinoid receptors (CNRs), and adenosin binding receptors (ADORAs)).

We recently found that cannabidiol (CBD), a non-psychoactive phytocannabinoid, acts as an inverse agonist at GPR6 in a β-arrestin2 recruitment assay. In addition, the Takeda Pharmaceutical Company has developed ligands with a pyridopyrazine scaffold that modulate GPR6.

The goal of the current study is to develop a computer model of GPR6. We began by comparing the sequence of GPR6 to other MECA cluster receptors and the cannabinoid-related receptors GPR55 and GPR18 to identify similarities and differences. We also evaluated GPR6 species differences. Since TMH6 changes conformation upon ligand activation, and because GPR6 has been shown to have a high level of constitutive signaling, we focused first on TMH6. Our comparisons indicated that GPR6 TMH6 may have a unique conformation because it contains five helix bending Gly residues (G6.33, G6.35, G6.42, G6.45 and G6.58). To investigate the conformational consequences of these Gly residues in TMH6, we used the Monte-Carlo, simulated annealing method, Conformational memories. The output of this method is a set of low free energy TMH6 conformations. Conformational Memories results for GPR6 TMH6 will be presented. These results shed light on the origin of the high G-protein dependent constitutive activity of GPR6.

18. Identification of novel GPR55 thienopyrimidine derivatives

Morales, P.,[1] Hurst, D.P.,[1] Console-Bram, L.M.,[2] Zhao, P.,[2] Abood, M.E.,[2] Reggio, P.H.[1]

[1]Center for Drug Discovery, Department of Chemistry and Biochemistry, University of North Carolina at Greensboro, [2]Center for Substance Abuse Research, Department of Anatomy and Cell Biology, Lewis Katz School of Medicine, Temple University

The putative cannabinoid receptor, GPR55 represents a possible target for the treatment of various diseases. Its role in physiopathological conditions—such as cancer, inflammatory and neuropathic pain, metabolic disorders, regulation of vascular functions, bone physiology, or motor coordination—has been reported.

The lysophospholipid, LPI (lysophosphatidylinositol) has been proposed to be the endogenous ligand for GPR55. In addition, diverse chemical entities—endogenous, plant-derived, and synthetic cannabinoid ligands among them—have been shown to modulate this receptor. Nonetheless, pharmacological inconsistencies, and the lack of potent and selective GPR55 ligands, are delaying the exploitation of such a promising therapeutic target.

To address this need, we have designed and synthesized a series of novel thienopyrimidine derivatives on the basis of the structural features identified for GPR55 ligands in our published homology models. The thienopyrimidine ML192 (CID1434953) was previously discovered as a GPR55 antagonist in a high-throughput, high-content screen. Docking studies in the GPR55 inactive state model allowed us to design optimized derivatives of this scaffold. These compounds were synthesized and evaluated using a β-arrestin recruitment assay in CHO cells overexpressing human GPR55 and βarr2-GFP. Interestingly, several compounds revealed increased potency and efficacy as GPR55 antagonists when compared with the hit ML192.

In summary, we show here that the combination of structure-activity relationship development and molecular modeling studies has permitted the identification of novel GPR55 antagonists that may serve as new tools for characterizing this putative cannabinoid receptor.

19. Parameterization and all atom molecular dynamics simulation of SR141716A in POPC bilayer

Al Zoubi, R., Lynch, D., Reggio, P.

Center for Drug Discovery, University of North Carolina At Greensboro

The biarylpyrazole, 5-(4-Chlorophenyl)-1-(2,4-dichloro-phenyl)-4-methyl-N-(piperidin-1-yl)-1H-pyrazole-3-carboxamide (SR141716A, Rimonabant [SR]), is the first identified CB1 receptor antagonist/inverse agonist. SR is CB1-selective with high potency, and is widely used in pharmacological experiments and in binding assay experiments due to the availability of its tritiated version.

The two inactive-state crystal structures for CB1 resolved in complex with AM6538 and Taranabant show a transmembrane portal between helix one and seven at the extracellular domain of the receptor. This conforms with the notion that, unlike ligands that access the binding site directly from bulk water, lipid-derived ligands for GPCRs such as SR diffuse from the lipid bilayer to the binding site through a transmembrane portal. Thus, the depth and orientation of cannabinoid ligands, as well as their dynamics in the membrane, will affect their binding affinity or their binding mode to the receptor.

We report here, the development of missing CHARMM force field parameters for SR based on a previously published parameterization method for drug-like molecules, and an all atom MD simulation study of SR in a fully hydrated POPC bilayer. Generated parameters were successful in reproducing target data from SR crystal structures equilibrated at MP2/6-31G* level of theory. Results from MD simulations show that SR can adopt multiple orientations in the lipid bilayer, it can rotate in all directions and move freely between leaflets.

20. Development of diarylureas as cannabinoid CB1 receptor allosteric modulators for treatment of drug addiction

Nguyen, T.,[1] Decker, A.,[1] Gamage, T.,[1] Li, J.X.,[2] Thomas, B.F.,[1] Wiley, J.L.,[1] Kenakin, T.,[3] Zhang, Y.[1]

[1]RTI International, [2]Department of Pharmacology and Toxicology, University at Buffalo, [3]Department of Pharmacology, University of North Carolina at Chapel Hill

The cannabinoid system has been demonstrated to be involved in many important physiological processes, including drug dependence, pain regulation, appetite control, learning, and memory. The CB1 signaling pathway appears as a valuable target for the treatment of drug addiction. In clinics, the CB1 receptor antagonist SR141716A has shown efficacy for obesity treatment and smoking cessation. However, it has been withdrawn from the market due to adverse effects, such as depression and suicidal ideation. To avoid the psychoactive effects commonly associated with orthosteric ligands, we aimed to develop CB1 negative allosteric modulators to target this important CB1 signaling pathway for the treatment of drug addiction.

A series of diarylureas derived from PSNCBAM-1 were designed, synthesized, and characterized by 1H and ^{13}C NMR, MS, and HPLC. These compounds increased binding affinity of CB1 orthosteric agonist [^{3}H]CP55940. In functional assays, these diarylureas reduced the Emax of CP55940 in calcium mobilization assays, as expected with negative allosteric modulators (NAMs). Most compounds possessed low nanomolar IC_{50} values at CB1 receptor without any significant activities at the CB2 receptor. Their potencies in antagonizing CP55940-

induced GTP-γ-[^{35}S] binding were consistent with calcium mobilization. Our novel compound RTICBM-74 demonstrated high metabolic stability in rat liver microsomes. RTICBM-74 attenuated prime induced reinstatement of cocaine-seeking behavior with greater efficacy than PSNCBAM-1 in rats. These results will facilitate the development of potent and selective CB1 receptor modulators as potential medications for the treatment of drug addiction and related conditions.

21. Toxic thermolytic degradants of carboxamide-type synthetic cannabinoids

Kevin, R.C.,[1] **Kovach, A.L.**,[2] McGregor, I.S.,[1] Thomas, B.F.[2]

[1]School of Psychology, The University of Sydney, [2]RTI International

The use of novel synthetic cannabinoids as intoxicants continues despite the health risks associated with these substances. These compounds are typically used via smoking and inhalation, yet many synthetic cannabinoids contain chemical moieties with questionable thermal stability. For example, UR-144 forms ring-opened thermal degradants that retain efficacy at cannabinoid receptors, while other compounds, like JWH-018, may also produce potentially toxic degradants such as naphthalene. Recently, large numbers of synthetic cannabinoids containing a carboxamide moiety have been detected in recreational products, some of which have produced toxicity in humans. However, to our knowledge, the thermal stability and thermolytic degradants of these compounds have not been characterized.

In this study, six carboxamide-type synthetic cannabinoids (CUMYL-PICA, 5F-CUMYL-PICA, AMB-FUBINACA, MDMB-FUBINACA, NNEI, and MN-18) were heated sequentially to 200, 400, 600, and 800 °C using a thermolysis/pyrolysis probe. Heating was conducted under an ambient airflow, which was passed through a charcoal trap held at 50 °C. The trap was then rapidly heated under helium flow and analyzed via GC-MS.

Generally, each synthetic cannabinoid degraded to its indole or indazole amide when heated to 400 °C, which was then dehydrated to the corresponding nitrile. At 600 °C, the nitrile-free indole or indazole compound was observed. Some synthetic cannabinoids formed specific thermal degradants; methylbenzene (toluene) and α-methylstyrene were liberated from CUMYL-PICA and 5F-CUMYL-PICA, 1-napthylamine was produced from NNEI, and napthalene was formed from both NNEI and MN-18. This data also suggested that thermolytic liberation of a cyanide anion (and subsequent production of hydrogen cyanide) could occur; this was subsequently verified via LC-MS/MS. A single milligram of a given synthetic cannabinoid was found to release micrograms of cyanide at 800 °C—approaching levels that could produce toxicity in humans. Verification of this phenomenon in vivo in blood or urine is ongoing.

22. Pain management, gender, and quality of life in cancer patients

Buhmeyer, J.

Walden University

The type of cancer pain management used may have an effect on the quality of life (QOL) of cancer patients. Researchers have determined that cancer patients are inadequately treated for pain and pain management is an essential determinant of patient survivability and QOL. Numerous clinical studies have been accomplished concerning opioid administration and noncancer and cancer pain management. Previous studies have examined the relationship between cannabinoid products, noncancer pain, cancer pain, and related QOL for patients but have not focused on the QOL of cancer patients while also moderating for gender. These relationships were investigated using the health belief model.

The cancer pain management treatments (opioids and/or marijuana [cannabis]) and QOL—measured with World Health Organization Quality of Life Survey (WHOQOL-BREF)—of 236 cancer patients were analyzed using analysis of variance (ANOVA), planned contrasts, post hoc tests, and moderated ANOVA (PROCESS tool) in the causal–comparative research. Research findings indicated significant benefit in cancer patient physical and psychological QOL in participants using marijuana when compared to participants using opioids and physical QOL for participants using marijuana over participants using both opioids and marijuana combined. Enhanced pain management options for cancer patients to reduce opioid side effects, increase pain treatment effectiveness, and improve patient QOL could yield positive social change. Growing rates of opiate addiction, abuse, and mortality are public health concerns, and cannabis may be an effective pain treatment to reduce these social costs. This research may be of use to legislators considering rescheduling marijuana to less than Schedule I.

23. Gender-specific relationships between plasma levels of endocannabinoids and vagal and sympathetic control of heart rate in normotensive obese older adults

Schaich, C.L.,[1] Shaltout, H.A.,[1, 2] Kovach, A.,[3] Grabenauer, M.,[3] Thomas, B.F.,[3] Brinkley, T.E.,[4] Nicklas, B.J.,[4] Diz, D.I.[1]

[1]Department of Surgery, Hypertension and Vascular Research, Cardiovascular Sciences Center, Wake Forest School of Medicine, [2]Department of Obstetrics and Gynecology, Wake Forest School of Medicine, [3]RTI International, [4]Department of Internal Medicine—Gerontology and Geriatric Medicine, Wake Forest School of Medicine

Previous studies in obese individuals indicate higher circulating endocannabinoids anandamide (AEA) and 2-arachidonoylglycerol (2-AG) than in lean counterparts. However, the association between plasma endocannabinoids and autonomic control of blood pressure and heart rate has not been assessed in obesity.

In a sample of normotensive, obese older adults we analyzed plasma content of the endocannabinoids AEA and total AG using mass spectrometry and examined correlations with various indices of spontaneous sympathovagal activity. Spontaneous baroreflex sensitivity (BRS) for heart rate control was calculated by spectral analysis of arterial pressure (AP) time (Sequence [Seq] Up, Seq Down and Seq All) and frequency (low-frequency [LF] and high-frequency [HF] α) domains from continuous resting AP recordings. In addition, time domain analysis was used to calculate heart rate variability (HRV) and blood pressure variability, indices of cardiac vagal tone and vascular sympathetic tone, respectively.

The sample included 8 males and 17 females with a mean age of 68.4 ± 0.6 years, a mean body mass index of 35.0 ± 0.8 kg/m2, and mean AP of 101.0 ± 2.2 mmHg. Across the complete sample, we report a significant inverse correlation between plasma AG content and HFα, an index of the vagally-mediated parasympathetic spontaneous BRS (r = -0.50, $P < 0.05$). We further report a significant inverse correlation between plasma AG and the vagal spontaneous BRS (Seq Up) in males (r = -0.87, $P < 0.01$) but not in females. However, in females but not males we found significant positive relationships between AEA and LFα, an index of sympathetic spontaneous BRS (r = 0.49, $P < 0.05$), and AEA and HRV (r = 0.50, $P < 0.05$). These results are consistent with a role for the endocannabinoid system to modulate autonomic control of the circulation in populations at risk for hypertension and cardiovascular disease, and suggest gender differences that have yet to be elucidated.

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