March 28-30, 2019 Seattle, WA

KAPPA THERAPEUTICS CONFERENCE

March 2019

PROGRAM BOOK

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- **Elyssa B. Margolis** (University of California, San Francisco)
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Sponsors

We are grateful for the generous support of these sponsors, who have helped make this conference possible. The Program content is the sole responsibility of the speakers and does not necessarily reflect the views of our sponsors.

General Information

The 5th Conference on the Therapeutic Potential of Kappa Opioids in Pain and Addiction.

Conference Venue

Kane Hall
University of Washington
Seattle, WA

Internet Access in Kane Hall Meeting Rooms

WiFi: UW NetID: event0076 Password: 5k3H/3d6A/2x4E

Badges

Every registered participant will receive a name badge that must be worn to gain access to scientific sessions and meals/coffee breaks onsite.

Registration Desk

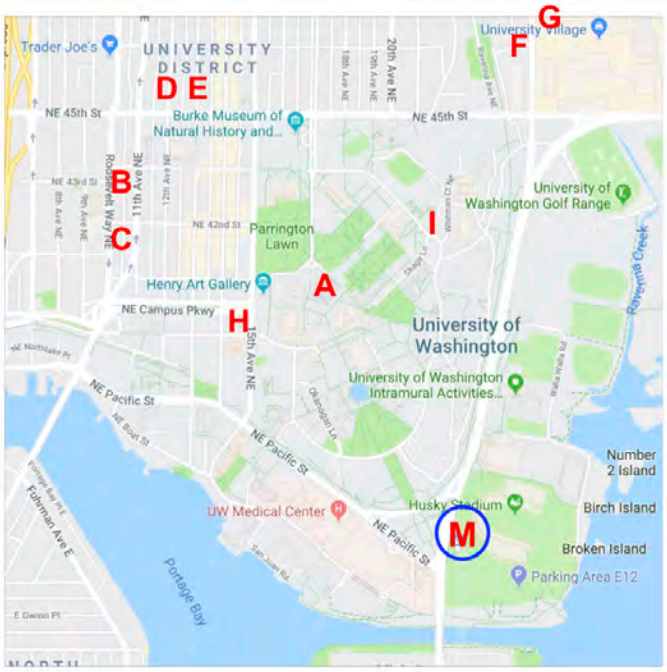
The personnel at the registration desk will assist in all conference needs. The registration desk will be located in Kane Hall and will be open:

Thursday, March 28	5 pm	-	8 pm (Walker Ames room)
Friday, March 29	7 am	-	5 pm (outside the lecture Room)
Saturday, March 30	7 am	-	5 pm (Walker Ames room)

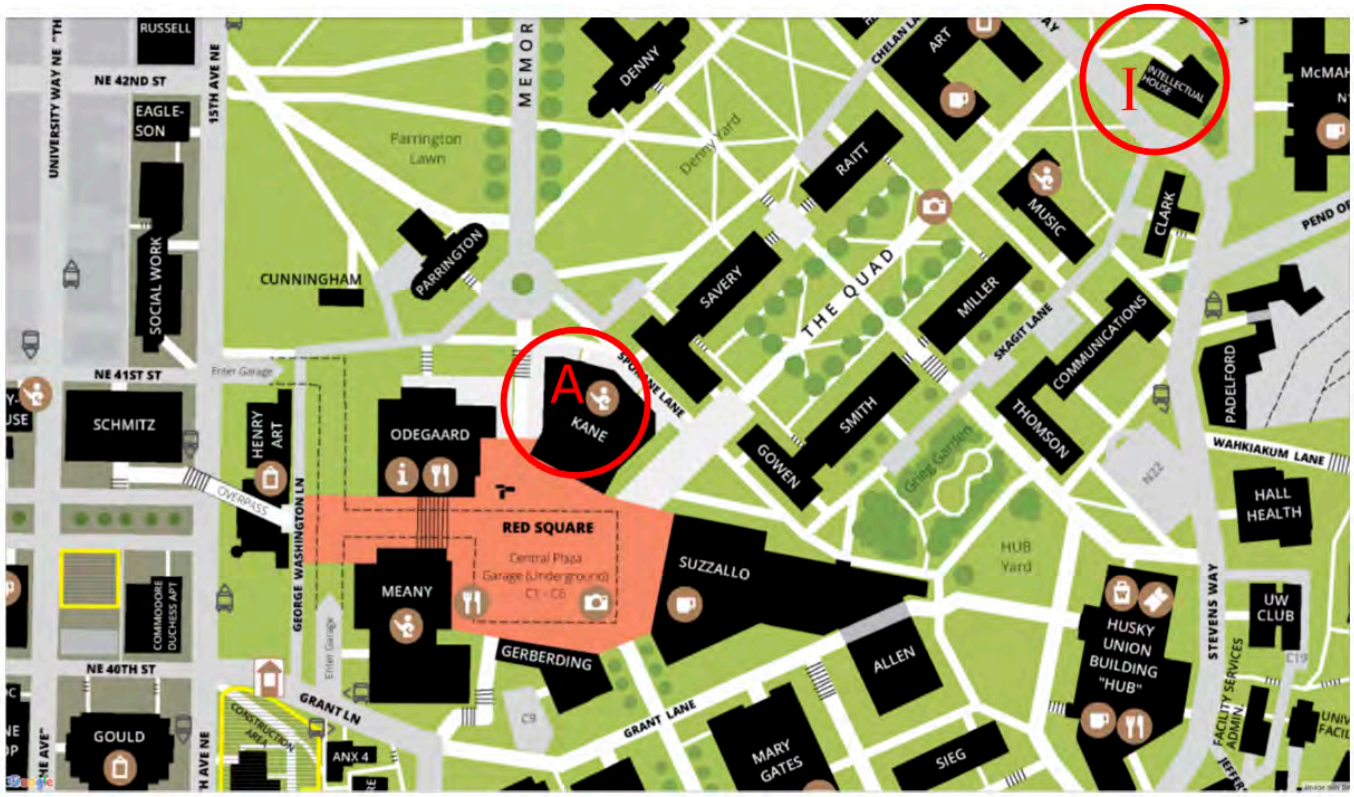
Meals

Continental Breakfast are provided at the hotels and Coffee Breaks will be provided in the Foyer. Buffet lunch will be provided on Friday at the Intellectual House (***see maps on next page***) and on Saturday in the Walker Ames room.

Map of Nearby Hotels and Conference Facilities



A. Kane Hall (opening reception) and Meeting Site
B. Watertown Hotel
C. University Inn
D. Residence Inn
E. Graduate Seattle
F. Travelodge
G. Silver Cloud
H. College Inn
I. Intellectual House (Friday lunch)
M. Metro (light rail stop)



Kappa Therapeutics Conference Code of Professional Conduct

Professional Societies (ACNP, WCBR, & SfN) have become increasingly proactive about promoting professional behavior by all participants at our meetings. We are working together to make our meetings inclusive, safe, positive and a diverse experience for everyone.

All participants are expected to treat others with appropriate respect and civility at all times. We strive to sustain an environment in which a free exchange of ideas and opinions can occur. Discrimination and harassment in any form will not be tolerated. If you witness or experience an interaction that makes you uncomfortable during the sessions or associated social events, please intervene immediately or report the incident to a member of the Program Committee (whichever you feel is appropriate).

Incidents of unprofessional conduct will be documented as completely as possible. Documented incidents will be reviewed by the Program Committee, and if the majority concur, the alleged offender will be informed, and an incident report will be forwarded to their supervisor (e.g. Dean or Department Chair) for appropriate action.

This is a proactive policy statement. We have not been informed of any previous incidents at Kappa Therapeutics Conferences, but by explicitly stating our expectations, we will hopefully reinforce everyone's positive experience.

Instructions for Presenters

Posters

Poster boards are 4 feet x 6 feet. Pushpins will be provided. Posters must be hung before lunch on Saturday, March 30.

Your poster number is listed in the Program

Oral presentations

We will have a Macintosh computer with the latest Operating System and Microsoft Office software. All talks **must** be loaded onto the conference computer the morning of the talk (i.e. during breakfast or the morning coffee break) at the latest. Talks can be emailed or brought to our A/V specialists (to be announced) for uploading at the registration desk.

5th Conference on the "Therapeutic Potential of Kappa Opioids"

March 28-30, 2019
University of Washington, Seattle, WA

Thursday, March 28th

5 - 8 PM Registration (Kane Hall Walker Ames Room - 2nd floor)

6 - 8 PM Opening Reception (Kane Hall Walker Ames Room - 2nd floor) (Kane Hall room 225)

Friday, March 29th

7 - 8 AM Coffee & Registration (Kane Hall Foyer)

8:00 AM Welcome: Charles Chavkin (Kane Hall room 210)

Oral Session 1: Novel Kappa Ligands (Bill Carlezon, Chair) (Kane Hall room 210)

8:15 AM Lori Jean Van Orden, Miguel Guerrero, Mariangela Urbano, Kerensa Saljooqi, Annette Madrid, Atul R. Mahableshwarkar, Steven Smith, Philip Mathew, Edward Roberts (BlackThorn Therapeutics) *The Discovery and Initial Human Pharmacokinetics of BTRX-335140, a Selective Kappa Opioid Receptor (KOR) Antagonist*.

8:35 AM Vsevolod Katritch, Saheem Zaidi, Nilkanth Patel, Xi-Ping Huang, Tao Che, Bryan L. Roth, Susruta Majumdar (University of Southern California) *Rational design of new chemical probes for opioid receptors*.

8:55 AM F. Ivy Carroll, Chad M. Kormos, Pauline W. Ondachi, Scott P. Runyon, James B. Thomas, S. Wayne Mascarella, Ann M. Decker, Hernán A. Navarro, Timothy R. Fennell, and Rodney W. Snyder (Research Triangle Institute) *Advancement of the development of tetrahydroisoquinoline kappa opioid receptor antagonists*.

9:15 AM Aubrie A. Harland, Tarsis Brust, Huiyong Ma, Kimberly M. Lovell, Kevin J. Frankowski, Laura M. Bohn, and Jeffrey Aubé (University of North Carolina, Chapel Hill) *Structural modification of ML139, a potent and selective kappa opioid receptor agonist.*

9:35 AM Andrea Bedini, Rossella De Marco, Luca Gentilucci, Santi Spampinato (University of Bologna) *Biased agonism and analgesic effects of CL39, a novel kappa opioid receptor selective ligand.*

9:55 AM Coffee Break (Kane Hall Foyer)

Oral Session 2: Kappa Drug Development (Elyssa Margolis, Chair) (Kane Hall room 210)

10:15 AM Charles Chavkin (University of Washington) *Why Kappa Opioid Receptor Antagonists May Fail in Clinical Trials (lessons learned from CRF-R1).*

10:35 AM Jennifer M. Bossert, Hannah Korah, Jennifer K. Hoots, S. Stevens Negus, Bruce E. Blough, and Yavin Shaham (NIDA-IRP) *Modeling opioid maintenance therapy in rats: Effects of chronic buprenorphine and the biased mu-opioid receptor agonist TRV130 on relapse to oxycodone seeking.*

10:55 AM James O. Fajemiroye, Adam W. Keasling, Pankaj Pandey, Robert J. Doerksen, Gustavo R. Pedrino, Elson A. Costa, Hoang V. Le, Nicole Ashpole, Jordan K. Zjawiony (University of Mississippi) *MOP and 5-HT1A receptors mediate antinociceptive and antidepressant-like activities of Salvindolin.*

11:15 AM Open Discussion

11:35 AM Adjourn to lunch

11:45 AM -1:45 PM Buffet Lunch, Intellectual House (see Map on page 6)

Oral Session 3: Kappa Receptor Expression and Structure (Sara Jones, Chair) (Kane Hall 210)

2:00 PM Tao Che, Susruta Majumdar, EIs Pardon, Jan Steyaert, Ivy F. Carroll, Gavril Pasternak, Bryan L. Roth (University of North Carolina) *Structural Studies of Distinct Kappa-Opioid Receptor States*.

2:20 PM Tijana Jovanović-Talisman, Steven J. Tobin, Devin L. Wakefield, Lars Terenius, Vladana Vukojević (Beckman Research Institute, City of Hope) *Ethanol and naltrexone affect the nano-organization of kappa opioid receptors in the plasma membrane*.

2:40 PM Sho Oasa, Aleksandar J. Krmpot, Stanko N. Nikolić, Claudio D'Addario, Lars Terenius, Rudolf Rigler, Vladana Vukojević (Karolinska University*) Characterizing the dynamic spatio-temporal organization of opioid receptors in live cells by functional Fluorescence Microscopy Imaging (fFMI).*

3:00 PM Lee-Yuan Liu-Chen, Chongguang Chen, Alex H. Willhouse, Peng Huang, Nora Ko, Melody Huang, Yujun Wang, Bin Xu, and Mary F. Barbe (Temple University) *Characterization of a knockin mouse line expressing KOR-tdTomato fusion protein.*

3:20 PM Rajani Maiya, Matthew B. Pomrenze, Thi Tran, Gayatri N. Tiwari, R. Dayne. Mayfield, and Robert O. Messing University of Texas, Austin) *The Transcription Cofactor Lim-only 4 (LMO4) is a Novel Regulator of Kappa Opioid Receptor Expression and Alcohol Consumption.*

3:40 PM Coffee Break

Oral Session 4: Kappa Receptors and Disease States (Elena Chartoff, Chair) (Kane Hall 210)

4:10 PM Amie L. Severino, Steve Liu, Isabel Bishop, Sarah Pickens, Caroline E. Bass, F. Ivy Carroll, Frances Leslie, Christopher J. Evans and Catherine M. Cahill (University of California, Los Angeles) *Kappa opioid receptors on dopaminergic projections from the ventral tegmental area mediate distinct aversion behaviors in chronic pain.*

4:30 PM Christoph Schwarzer, Alexandra Agostinho, Mario Mietzsch, Anna Mutti, Larissa Kraus, Luca Zangrandi, Pawel Fidzinski & Regine Heilbronn (University of Innsbruck) *Preclinical evidence for an AAV-based gene therapy for temporal lobe epilepsy targeting kappa opioid receptors.*

4:50 PM Sam Clark, Jared Van Snellenberg, Anissa Abi-Dargham (Columbia University) *Opioid antagonists are effective treatments for the positive and negative symptoms of schizophrenia: A meta-analysis.*

5:10 PM Nicolas Massaly, Bryan A. Copits, Adrianne R. Wilson-Poe, Lucia Hipólito, Tamara Markovic, Hye Jean Yoon, Shiwei Liu, Marie C. Walicki, Brendan M. Walker, Catherine M. Cahill, Kooresh I. Shoghi, Robert W. Gereau, IV, Jordan G. McCall, Ream Al-Hasani, Michael R. Bruchas, Jose A. Morón (Washington University, St Louis) *Pain-induced Negative Affect is Mediated via Recruitment of the Nucleus Accumbens Kappa Opioid System.*

Student / Postdoc Mixer (6 – 7:30 PM) *(Shultzy's Pub, 10 min walk) 4114 Univ Way NE*

Dinner (no host, maps to local restaurants provided)

Saturday, March 30th

7 - 8 AM Coffee & Registration (Kane Hall Foyer)

Oral Session 5: Kappa Receptor Signaling (Christoph Schwarzer, Chair) (Kane Hall room 210)

8:00 AM Michael S. Placzek, Hsiao-Ying Wey, Fredrick T. Schroeder, Tao Che, Ramesh Neelamegam, Genevieve C. Van de Bittner, Bryan L. Roth, Jacob M. Hooker (Harvard University) *Investigating kappa opioid receptor drug occupancy in the living brain with positron emission tomography.*

8:20 AM Jeffrey J. Liu, Yi-Ting Chiu, Luca Zangrandi, Chongguang Chen, Sean J. Humphrey, Kirti Sharma, Mariana Spetea, Lee-Yuan Liu-Chen, Christoph Schwarzer, Matthias Mann (Max Planck, Martinsried) *In vivo Brain KOR Signaling Elucidated by Phosphoproteomics.*

8:40 AM Elyssa B. Margolis (University of California, San Francisco) *Stress reverses the valence of KOR signaling from inhibitory to excitatory in dopamine neurons.*

9:00 AM Selena S. Schattauer, Andrea Bedini, Floyd Summers, Aiden Reilly-Treat, Mackenzie M. Andrews, Zeena M. G. Rivera, Jennifer S. Steger, Benjamin B. Land, Charles Chavkin (University of Washington) *Ligand directed activation of c Jun Kinase signaling by Gi/o protein coupled receptor agonists revealed by fluorescent sensors.*

9:20 AM Elizabeth K. Unger, Grace O. Mizuno, Lin Tian (University of California, Davis) *New fluorescent sensors for dynorphin and serotonin.*

9:40 AM Lee-Yuan Liu-Chen, Jeffrey J. Liu, Yi-Ting Chiu, Kelly M. DiMattio, Chongguang Chen, Peng Huang, Taylor A. Gentile, John W. Muschamp, Alan Cowan, and Matthias Mann (Temple University) *Phosphoproteomic approach for agonist-specific signaling in mouse brains: mTOR pathway is involved in kappa opioid aversion.*

10:00 AM Coffee Break

Oral Session 6: Stress Behaviors (Brendan Walker, Chair) (Kane Hall room 210)

10:20 AM Tanya L. Wallace, William J. Martin, and Amy F.T. Arnsten (BlackThorn Therapeutics) *BTRX-335140, a novel and selective kappa opioid receptor antagonist, protects working memory performance from mild stress exposure in rhesus monkeys.*

10:40 AM Antony D. Abraham, Sanne M. Casello, Mackenzie M. Andrews, Brenden Wong, Harrison M. Fontaine, Benjamin B. Land, Charles Chavkin (University of Washington) *A dynorphin projection from the dorsal raphe nucleus to the ventral tegmental area mediates stress-potentiated cocaine reward in mice.*

11:00 AM Harold L. Haun, Marcelo F. Lopez, William C. Griffin and Howard C. Becker (Medical University of South Carolina) *Dynorphin/KOR Signaling in the Extended Amygdala Contributes to Stress-Enhanced Escalated Alcohol Drinking in Dependent Mice.*

11:20 AM Katherine M. Holleran & Sara R. Jones (Wake Forest School of Medicine) *Alterations in nucleus accumbens kappa opioid receptor-mediated dopamine inhibition in response to stress and chronic alcohol exposure.*

11:40 AM William Carlezon, Kenneth McCullough (McLean Hospital, Harvard) *Induction of stress-like effects on sleep architecture by selective alterations in the activity of dopamine D1 receptor-expressing medium spiny neurons in the nucleus accumbens.*

11:40 AM **Discussion** / Data Blitz (C Chavkin, Chair)

[Participants wanting to present are welcome to show a data blitz slide]

12-2 PM Buffet Lunch (Walker Ames Room) Set up Poster Session (Walker Ames Room)

Oral Session 7: Reward Systems (Elena Chartoff, Chair) (Kane Hall room 210)

2:00 PM Sara R. Jones and Paige M. Estave (Wake Forest School of Medicine) *Stress and Cocaine Self-Administration Induced Alterations in Kappa Opioid Receptor Regulation of Dopamine in the Nucleus Accumbens.*

2:20 PM Ryan D. Farero, Lauren M. Burgeno, Antony D. Abraham, Nicole M. Murray, Jennifer S. Steger, Larry S. Zweifel, Charles Chavkin, Paul E.M. Phillips (University of Washington) *Interaction of kappa opioid receptor activation and dopaminergic signaling in the nucleus accumbens core mediate escalated cocaine intake.*

2:40 PM Christian E. Pedersen, Daniel C. Castro, Michael R. Bruchas (University of Washington) *Reward Value Encoding of Dynorphin and Enkephalin Neurons in Nucleus Accumbens.*

3:00 PM Brendan M. Walker (Washington State University) *The role of the dynorphin / kappa-opioid receptor system in alcohol use disorder.*

3:20 PM Coffee Break (with Posters)

Oral Session 8: Dynorphins in Disease (Lee-Yuan Liu-Chen, Chair) (Kane Hall room 210)

3:40 PM Thomas L. Kash, Lara S. Hwa, Sofia Neira, Melanie M. Pina, Dipanwita Pati, Rachel Calloway, Morgan Bowling, Daniel Bloodgood (University of North Carolina) *Excessive alcohol drinking disrupts stress reactions through alterations in BNST dynorphin in mice.*

4:00 PM Renata C. N. Marchette, Brendan Tunstall, Adriana Gregory-Flores, Agnieszka Sulima, Kenner Rice, Leandro F. Vendruscolo and George F. Koob (NIDA-IRP) *Kappa-opioid receptor antagonism reverses allodynia induced by heroin withdrawal.*

4:20 PM Elena Chartoff, Sineadh Conway, Daniel Puttick, Shayla Osmond, Mitchell Roitman (McLean Hospital, Harvard University) *Kappa opioid receptor-mediated depressive-like states and suppression of nucleus accumbens dopamine release are blunted in female rats.*

4:40 PM Abigail G Schindler, Erin Cooper, Elaine Peskind, Paul EM Phillips, David G Cook (University of Washington & Seattle VA) *Understanding physical vs psychological effects of repetitive blast exposure: role for kappa opioid receptor antagonism.*

5 – 7 PM Poster Session & Closing Reception (wine & cheese) Walker Ames Room (Kane Hall room 225)

7:00 PM Presentation of the ***2019 Toni Shippenberg Young Investigator Awards*** (C Chavkin)

[mentors may nominate trainees by e-mail to CC; awards will be selected by vote of the Program Committee]

Dinner (no-host, maps to local restaurants provided)

Sunday, March 31st. Checkout & Departure

Posters (Walker Ames Room)

Double-sided 4' x 6' corkboards and push-pins will be provided. Please put up your poster on Saturday before lunch and take it down at the end of the Closing Reception session <7:30PM.

POSTER

1. Michael Soeberdt, Maria Schneeweiss, Ann-Christin Lüdiger, Natia Chartolani, Ulrich Knie, Christoph Abels, Karin Loser (Dr. August Wolff GmbH & Co & University of Munster) Topical treatment with WOL071-007, a newly developed kappa-opioid receptor agonist, ameliorates ongoing atopic dermatitis in mice and humans.

2. Madison A Baird, Larry S Zweifel (University of Washington) Characterization of CeA dynorphin neurons and their role in cued fear learning.

3. Vadim Yuferov, Matthew Randesi, Eduardo R Butelman, Wim van den Brink, Peter Blanken, Jan M. van Ree, Jürg Ott, Mary Jeanne Kreek (Rockefeller University) Genetic association of variants of prodynorphin promoter 68 bp repeats in Caucasians with opioid dependence diagnosis: Effect on age trajectory of heroin use.

4. Gengze Wei, Sunil Sirohi, Alexandra K Fraser, Brendan M Walker (Washington State University) Role of medial prefrontal cortex kappa opioid receptors in alcohol dependence-induced working memory deficits.

5. Daniel C Castro, Eric T Zhang, Jose Moron-Concepcion, Michael R Bruchas (University of Washington) Mu-opioid receptors in nucleus accumbens mediate stress enhanced motivated behaviors.

6. Sho Oasa, Aleksandar J. Krmpo, Stanko N. Nikolić, Lars Terenius, Rudolf Rigler, Vladana Vukojević (Karolinska University) Mapping local heterogeneity in kappa-opioid receptor lateral dynamics.

7. Grace E. Shinn, Gengze Wei, Bok Soon Go, Michael R. Bruchas and Brendan M. Walker (Washington State University) Recapitulating phenotypes of alcohol dependence via Oprk1 overexpression in non-dependent TH::Cre rats.

8. Paige M. Estave, Anushree N. Karkhanis, Sara R. Jones (Wake Forest School of Medicine) Influence of nucleus accumbens DAT-KOR interactions on cocaine effects in naïve and self-administering rodents.

9. Moriah L. Jacobson, Hildegard A. Wulf, Caroline A. Browne, Irwin Lucki (Uniformed Services University) Antidepressant activity of JNJ-67953964 in mice: Sex differences and reversal of stress-induced behaviors.

10. Andrew T. Luskin, Dionnet L. Bhatti, Christian E. Pederson, Kate Kimbell, Hannah Oden-Brunson, Rob W. Gereau, Michael R. Bruchas (University of Washington) Extended amygdala projections to parabrachial dynorphin neurons alter threat perception and encode feeding behaviors.

11. Zeena M. G. Rivera, Antony D. Abraham, Joshua H. Cohen, Benjamin B. Land, Charles Chavkin (University of Washington) Estrogen regulated G-protein Receptor Kinase 2 (GRK2) inhibits both KOR agonist and antagonist effects.

12. Manish K. Madasu, Loc V. Thang, Sasha Singh, Tayler D. Sheahan, Jordan G. McCall, Ream Al-Hasani (Washington University in St. Louis) Activation of kappa opioid receptor potentiates cold sensation.

13. Lindsey B. Kuiper, Anushree N. Karkhanis, Jeffrey L. Weiner, Sara R. Jones (Wake Forest School of Medicine) Kappa opioid mechanisms underlying increased ethanol intake and nucleus accumbens dopamine dysregulation induced by early life stress in rats.

14. Antony D. Abraham, Sanne M. Casello, Zeena M. G. Rivera, Selena S. Schattauer, Mackenzie M. Andrews, Benjamin B. Land, Charles Chavkin (University of Washington) Prefrontocortical kappa opioid receptor activation disrupts working memory maintenance and increases reactive oxygen species generation.

15. Katie L. Reichard, Paulo Sotero de Menezes, Charles Chavkin (University of Washington) norBNI Does Not Act as a Long-Acting Antagonist in Nucleus Accumbens Dopamine Terminals.

16. Harrison Fontaine, Sanne Cassello, Antony Abraham, Michael Bruchas, Benjamin B. Land, Charles Chavkin (University of Washington) Stress acts through dynorphin/KOR system to decrease 5-HT tone in the NAcSh and potentiate cocaine seeking by allostasis at 5-HT_4R.

ABSTRACTS for ORAL PRESENTATIONS

The Discovery and Initial Human Pharmacokinetics of BTRX-335140, a Selective Kappa Opioid Receptor (KOR) Antagonist

Lori Jean Van Orden[1], Miguel Guerrero[2], Mariangela Urbano[2], Kerensa Saljooqi[1], Annette Madrid[1], Atul R. Mahableshwarkar[1], Steven Smith[3], Philip Mathew[4], Edward Roberts[2]

[1]BlackThorn Therapeutics, Inc., San Francisco CA; [2]Department of Molecular Medicine, The Scripps Research Institute, La Jolla, CA; [3]DMPK Consultant working on behalf of BlackThorn Therapeutics; [4]Celerion, Lincoln, Nebraska

Preclinical and clinical data implicate dynorphin and the kappa opioid receptor (KOR), through which it acts, in a variety of disorders such as anxiety and depression. While the potential therapeutic utility of KOR antagonists has been long-recognized, several challenges limited the development of this class of molecules including lack of selectivity for KOR over the closely homologous mu opioid receptor (MOR), a long pharmacodynamic duration of action which exceeded pharmacokinetic exposure as well as non-mechanism-based toxicological findings. Here, we report on the identification of novel KOR antagonists that exhibit potency, selectivity and medication-like duration of action suitable for clinical development.

One particular compound, 1-(6-ethyl-8-fluoro-4-methyl-3-(3-methyl-1,2,4-oxadiazol-5-yl)quinolin-2-yl)-*N*-(tetrahydro-2H-pyran-4-yl)piperidin-4 amine (**BTRX-335140**), has been discovered as a potent and selective KOR antagonist, endowed with favorable *in vitro* ADMET and *in vivo* pharmacokinetic profiles and medication-like duration of action in mouse, rat and non-human primate pharmacodynamic models.

BTRX-335140 has been evaluated in Phase 1 single and multiple dose studies aimed at understanding the safety, tolerability, and pharmacokinetic profile of the molecule in healthy human volunteers. The occupancy-exposure-dose relationships of **BTRX-335140** are currently being studied in a positron-emission tomography (PET) study. The results generated to date support continued assessment of **BTRX-335140** in translational and clinical studies. The continued development of **BTRX-335140** may provide an additional treatment option for those suffering from neurobehavioral disorders.

Source of research support: BlackThorn Therapeutics and NIH Blueprint Network Program grant number 1-UH3-NS093030-01

Conflict of interest statements: LJV, AM, ARM, and KS receive salaries and are shareholders of BlackThorn Therapeutics. ER is a shareholder of BlackThorn Therapeutics. SS is a paid consultant for BlackThorn Therapeutics.

Rational design of new chemical probes for opioid receptors

Vsevolod Katritch[1], Saheem Zaidi[1], Nilkanth Patel[1], Xi-Ping Huang[2,4], Tao Che[2], Bryan L. Roth[2,3,4], Susruta Majumdar[5]

[1]Departments of Biological Sciences and Chemistry, Bridge Institute, University of Southern California, Los Angeles, California 90089, USA [2]Department of Pharmacology and [3]Division of Chemical Biology and Medicinal Chemistry and [4]National Institute of Mental Health Psychoactive Drug Screening Program, University of North Carolina Chapel Hill Medical School, 4072 Genetic Medicine Building, Chapel Hill, North Carolina 27514, USA [5]Center for Clinical Pharmacology, St. Louis College of Pharmacy Washington University School of Medicine St. Louis, MO.

Structural information available for all opioid receptors in both active and inactive-states presents a unique opportunity for in silico discovery of new lead chemotypes and new probes with desired selectivity and functional profiles. Based on our recently solved structure of KOR active state complex with MP1104, we performed an extensive structure-guided screening for derivatives with dual MOR/KOR G-protein biased profile. The resulting compounds demonstrate effective analgesia, while lacking adverse side effects and tolerance in mice models. Other rational design examples include bitopic ligands that combine orthosteric morphinan moiety with an extension targeting conserved sodium allosteric pocket deep in the 7TM bundle of the receptor. The bitopic compounds in these series demonstrate strong G-protein biased functional profile in KOR, with potential application as a general concept for design of functional bias in class A GPCRs.

Supported by P01 DA035764 and R21/R33 DA038858 from NIDA.

Disclosure: The authors have no conflicts of interest to disclose.

Advancement of the development of tetrahydroisoquinoline kappa opioid receptor antagonists

F. Ivy Carroll, Chad M. Kormos, Pauline W. Ondachi, Scott P. Runyon, James B. Thomas, S. Wayne Mascarella, Ann M. Decker, Hernán A. Navarro, Timothy R. Fennell, and Rodney W. Snyder

Research Triangle Institute, 3040 Cornwallis Road, P.O. Box 12194, Research Triangle Park, NC

The abuse of opioids is currently a major public health concern. Approximately three million people in the United States and sixteen million worldwide have a current or past opioid use disorder (OUD). Drug overdose currently accounts for more deaths in the United States than traffic deaths and suicides and is now the leading cause of death for people under 50 years old. According to data from the Center for Disease Control and Prevention, over 64,000 people died from opioid overdose in 2016. Given the current opioid crisis, new pharmacotherapies for treating OUD are urgently needed. At present methadone, buprenorphine, and naltrexone are the only FDA approved pharmacotherapies to assist persons suffering from opioid abuse. Even though these therapies are effective, their use is not optimal. Reported studies from different laboratories have shown that KOR antagonists would likely be useful in treating OUD and other substance use disorders (cocaine, nicotine, and alcohol) as well as depression and anxiety disorders, which are often comorbid with substance abuse (opioid, cocaine, nicotine, and alcohol). At the 2017 Kappa Therapeutics Conference we presented the start of a structure activity relationship (SAR) study directed towards the design and development of a new structural type tetrahydroisoquinoline kappa opioid receptor antagonists. A continuation of these studies led to the selection of eight (8) compounds as potential leads for further development. Pharmacokinetic (PK) studies were conducted on four of the lead compounds. In addition, the eight lead compounds were screened by Cerep Panlabs for potential side effects at 62 receptors, 6 enzymes, and 3 uptake assays. In addition, the eight lead compounds were tested for binding at three serotonin receptors, four dopamine receptors, and at the dopamine serotonin, and norepinephrine transporters. The results from the SAR studies, calculated physiochemical properties, PK studies, and side effect profiles of the new tetrahydroisoquinoline kappa opioid antagonist will be presented.

Supported by R01 DA 09045 from the National Institute on Drug Abuse (NIDA).

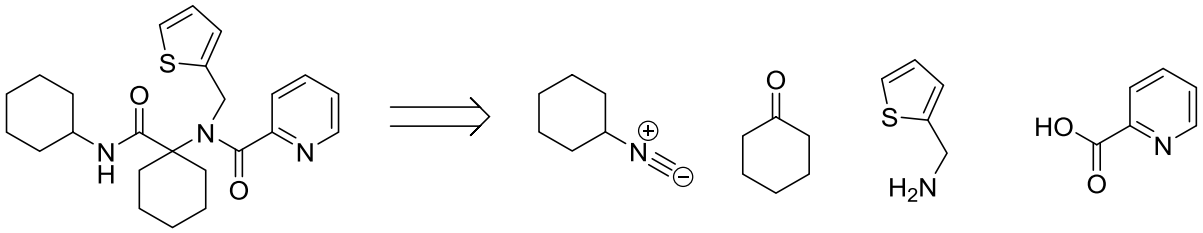
Disclosure: The authors have no conflicts of interest to disclose.

Structural modification of ML139, a potent and selective kappa opioid receptor agonist

Aubrie A. Harland[1], Tarsis Brust[2], Huiyong Ma[1], Kimberly M. Lovell[2], Kevin J. Frankowski[1], Laura M. Bohn[2], and Jeffrey Aubé[1]

[1]UNC Eshelman School of Pharmacy, Division of Chemical Biology and Medicinal Chemistry, University of North Carolina, Chapel Hill, NC 27599; [2]Departments of Molecular Therapeutics and Neuroscience, The Scripps Research Institute, Jupiter FL 33458

ML139 is a kappa opioid receptor (KOR) agonist with a structurally distinct scaffold lacking the basic nitrogen common among many KOR chemotypes. Here we report a structure–activity relationship (SAR) study of ML139 analogues, enabled by a one-pot, Ugi multicomponent reaction. Using this route, we were able to methodically modify all areas of the ligand resulting in >100 ML139 analogues, revealing structural elements essential for KOR activity and several trends associated with structural modifications. This presentation will review the SAR trends uncovered in the course of our studies, as well as, the determination of functional selectivity for G-protein activity or β arrestin recruitment in select analogues.



Support:
Supported by a grant from NIH/NIDA (2R01DA031927to L.M.B. and J.A.)

Disclosure:
The authors have no conflicts of interest to disclose.

Biased agonism and analgesic effects of CL39, a novel kappa opioid receptor selective ligand

Andrea Bedini[1], Rossella De Marco[2], Luca Gentilucci[2], Santi Spampinato[1]

[1]Department of Pharmacy and Biotechnology (FaBiT) – University of Bologna, Italy
[2]Department of Chemistry "G. Ciamician" – University of Bologna, Italy

Kappa opioid receptor (KOR) agonists are investigated as alternatives to mu opioid receptor (MOR) analgesics for their low abuse potential and reduced gastrointestinal and respiratory toxicity; nonetheless, relevant adverse effects, including dysphoria, sedation, astrocyte activation, have limited their clinical use as pain killers.
KOR biased agonists may activate, in a pathway-specific manner, G protein-mediated signaling, that produces analgesia, over arrestin 3-dependent induction of p38MAPK, which preferentially contributes to adverse effects; thus representing an intriguing opportunity to selectively induce KOR-dependent analgesia while limiting the onset of KOR-mediated toxicity.
Within this frame we investigated functional selectivity and antinociceptive effect of CL39 (H-Tyr-Amo-Trp-PheNH_2), an endomorphin-1 analogue with high affinity, selectivity and partial agonist activity at KOR that we recently identified; U50,488 was employed as reference KOR agonist in all experimental settings employed in this study.
CL39 significantly inhibited forskolin-induced cAMP accumulation in HEK-293, stably transfected to express KOR (IC_{50} = 0.22 nM), and in U87-MG human glioblastoma cells, endogenously expressing kappa receptor (IC_{50} = 0.16 nM). Moreover, in both cell models, CL39 significantly increased early (15-30min), G protein-dependent component of ERK1/2 phosphorylation without activating either late (60-120 min), arrestin-dependent component of ERK1/2 or p38MAPK phosphorylation. On the contrary, U50,488 activated all the above mentioned signaling pathways. Interestingly, CL39 determined a very weak arrestin 3 recruitment at KOR, as assessed via arrestin complementation assay in U2OS cells and by means of a BRET assay carried out in HEK-293 cells; on the contrary, U50,488 promoted a relevant arrestin recruitment in both assays. Consistently, calculated bias factor for U50,488 and CL39 were equal to 1 and 65, respectively.
Furthermore, U50,488 induced a significant and p38MAPK-dependent increase in cell proliferation levels in U87-MG cells, as assessed by [^{3}H]-thymidine incorporation, whereas CL39 did not.
In vivo, both CL39 and U50,488 (0-30 mg/kg; 0-60 min; i.p.) displayed a significant and dose-dependent antinociception in the warm-water tail-withdrawal test, displaying CL39 a similar potency (CL39-ED_{50}=10.10±0.11mg/kg; U50,488-ED_{50}=9.93±0.37mg/kg) and a lower E_{max} (CL39-E_{max}=59.9±3.49%; U50,488-E_{max}=88.2±14.0%) as compared to U50,488.
CL39-mediated analgesia was prevented by i.p. administering the KOR-selective antagonist norBNI (10 mg/kg; 30 min prior to CL39).
These findings point out CL39 as a potential new G protein-biased, KOR selective partial agonist with favorable pharmacological profile *in vitro* and antinociceptive effects *in vivo*; thus providing an interesting starting point to develop innovative therapeutics targeting kappa receptor.

Support:
Work supported by RFO2017, RFO2018 and FARB from University of Bologna; Fondazione "Umberto Veronesi" Grant (Tryptoids Project); FFABR2017 from Italian Ministry of University and Research (MIUR).

Conflict of interest:
The authors have no conflicts of interest to disclose.

Why Kappa Opioid Receptor Antagonists May Fail in Clinical Trials (lessons learned from CRF-R1).

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Kappa opioid receptor (KOR) antagonists show considerable promise for the treatment of certain forms of stress disorder. While the physiological stress response is broadly protective and promotes adaptive responses necessary for survival, chronic pervasive stress exposure can precipitate pathological mood disorders including depression and anxiety syndromes. Stress exposure can also promote addictive drug use (self-medication of mood disorder using alcohol, nicotine or opioids for their anxiolytic or euphorigenic effects) and is a risk factor for developing severe substance use disorder (drug addiction). In addition, the withdrawal syndrome during abstinence in individuals physically dependent on these addictive substances can be profoundly stressful and can trigger drug craving and drug seeking behaviors (precipitates relapse). The neurochemistry of the stress response is complex and includes the endocrine effects orchestrated by corticotropin releasing factor (CRF), adrenocorticotropic hormone (ACTH), and glucocorticoid release. Most of the acute physiological effects of these stress hormones are positively adaptive and cause the metabolic and cardiovascular responses necessary for survival. However, CRF also stimulates dynorphin release in brain, and dynorphin activation of KOR contributes to the dysphoric, anxiogenic and cognitive disrupting effects of the stress response. Preclinical studies have established that kappa receptor antagonists promote stress resilience and show promise as therapeutics, but the successful development of these medications requires a careful consideration of the physiology of the dynorphin – kappa opioid system and an understanding of the unique pharmacological properties of kappa antagonist drugs. Three classes of KOR antagonists have been distinguished: 1) ***non-selective antagonists*** that bind to other receptors besides kappa (e.g. buprenorphine, naltrexone, naloxone and nalmefene), 2) short-acting ***selective competitive antagonists*** that specifically inhibit kappa receptor activation (e.g. PF-04455242, LY2456302 (also called CERC-501, JNJ-67953964) and BTRX-335140), or 3) kappa selective ***receptor-inactivating*** antagonists that produce a long-lasting structural change in the kappa receptor signaling complex by a recently defined c-Jun Kinase mechanism. My conjecture is that receptor inactivating kappa antagonists might be safer and more effective than non-selective or competitive kappa antagonists if they can be administered at very low doses that produce accumulating receptor inactivation with minimal off-target effects; however, which would be the most effective medication has not been established. Therapeutic efficacy of antagonists requires adequate receptor blockade to prevent the endogenous agonist effects. While the degree of receptor block required for kappa system is not yet known, clinical trials of conventional competitive CRF R1 receptor antagonists failed to demonstrate efficacy, and I suggest that this may have been a consequence of R1 receptor occupancy by the antagonist doses used that was insufficient to block endogenous CRF effects. Because dynorphin is a highly efficacious agonist and spare kappa receptors are evident (Chavkin & Goldstein, 1981), dynorphin may be able to produce robust dysphoric effects at very low receptor occupancy. If true, complete kappa receptor inactivation may be necessary for clinical efficacy.

Support:
Supported by P50 MH106428 and R01 DA030074 from NIH.

Disclosure:
The author has no conflicts of interest to disclose.

Modeling opioid maintenance therapy in rats: Effects of chronic buprenorphine and the biased mu-opioid receptor agonist TRV130 on relapse to oxycodone seeking

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Background: High relapse rates perpetuate opioid addiction and are a major obstacle in addressing the current U.S. opioid epidemic. Maintenance therapy with opioid agonists (buprenorphine, methadone) is an effective treatment for opioid addiction. Here, we established an experimental procedure in rats trained to self-administer the prescription opioid oxycodone to compare the efficacy of an established treatment (buprenorphine) with that of a newer biased mu-opioid receptor (MOR) agonist, TRV130.

Methods: We trained rats to self-administer oxycodone (0.1 & 0.05 mg/kg/infusion; 7 d/dose, 6-h/d) in Context A where infusions were paired with a discrete tone-light cue. We implanted Alzet osmotic pumps containing vehicle, buprenorphine (3, 6, or 9 mg/kg/d; n=11-16), or TRV130 (3, 6, or 9 mg/kg/d; n=13-14) and performed three tests: (1) responding for drug-paired discrete cues under extinction conditions in a non-drug context (Context B, 7 d), (2) context-induced reinstatement of oxycodone seeking in Context A after extinction in Context B, and (3) reacquisition of oxycodone self-administration in Context A.

Results: Chronic buprenorphine significantly decreased responding for drug-paired discrete cues in Context B under extinction conditions and reacquisition of oxycodone self-administration in Context A; chronic buprenorphine also decreased context A-induced reinstatement of oxycodone seeking but this effect did not reach statistical significance. Chronic TRV130 significantly decreased oxycodone seeking or taking on all three relapse measures.

Conclusions: We introduce a novel rat model to study the effect of agonist-based maintenance therapy on relapse to prescription opioid seeking. We showed that chronic buprenorphine significantly decreased oxycodone seeking provoked by exposure to oxycodone-associated discrete cues and by exposure to oxycodone itself, demonstrating the predictive validity of the model. More importantly, we showed that chronic TRV130 delivery significantly decreased oxycodone seeking using multiple measures of relapse. We propose that biased MORs should be considered as a novel opioid agonist maintenance treatment for addiction to prescription opioids and heroin.

Support: This work was supported by IRP/NIDA/NIH

Disclosure: The authors have no conflicts of interest to disclose

MOP and 5-HT$_{1A}$ receptors mediate antinociceptive and antidepressant-like activities of Salvindolin

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Here we present salvindolin, new semisynthetic analog of Salvinorin A, with preferential mu opioid (MOP) affinity, and promising antinociceptive, as well as antidepressant-like activities. Competitive binding studies were performed for salvindolin with KOP and MOP. The mouse model of nociception (acetic acid induced writhing, formalin and hot plate tests), depression (forced swim and tail suspension tests), and the open field test, were used to evaluate antinociceptive, antidepressant-like, and locomotion effects, respectively, of salvindolin. We built a 3-D molecular model of the KOP receptor, using a MOP X-ray crystal structure as template, and docked salvindolin into the two proteins. Salvindolin showed affinity towards KOP and MOP receptors but with 100-fold MOP preference. Tests of salvindolin in mice revealed good oral bioavailability, antinociceptive and antidepressive-like effects, without locomotor incoordination. Docking of salvindolin showed strong interactions with the MOP receptor which matched well with experimental binding data. Salvindolin-induced behavioral changes in hot plate and forced swim tests were attenuated by naloxone (nonselective opioid receptor antagonist) and/or naloxonazine (selective MOP receptor antagonist) but not by nor-binaltorphimine (selective KOP receptor antagonist). In addition, WAY100635 (a selective 5-HT1A receptor antagonist) blocked the antidepressant-like effect of salvindolin. With its significant antinociceptive and antidepressive-like activities, salvindolin has potential to be an analgesic and/or antidepressant drug candidate.

Support:
Supported by Conselho Nacional de Desenvolvimento Cientifico e Tecnológico (CNPq), Coordenação de Aperfeicoamento de Pessoal de Nível Superior (CAPES) and Fundação de Amparo à Pesquisa do Estado de Goias (FAPEG) and by Grant Number P30GM122733 from NIGMS at the NIH, Computer time from Mississippi Center for Supercomputer Research and NSF grant 1338056 MRI.

Disclosure:
The authors have no conflicts of interest to disclose.

Structural Studies of Distinct κappa-Opioid Receptor States

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The κappa-opioid receptor (KOR) mediates the actions of psychotomimetics and proposed non-addictive analgesics. A complete understanding of KOR activation is necessary to elucidate the pharmacological properties of this important drug target. Here we present two distinct crystal structures of the KOR in complex with either an active-state or inactive-state stabilizing nanobody. Comparing these multi-state structures reveals: 1) a mechanism for opioid receptor activation and ligand selectivity; 2) key residues that explain the pharmacology, function, and biased signaling of the KOR; 3) new nanobodies as useful tools to dissect κappa receptor dynamics.

Support:
Supported by P01 DA035764 from NIDA.

Disclosure:
The authors have no conflicts of interest to disclose.

Ethanol and naltrexone affect the nano-organization of kappa opioid receptors in the plasma membrane

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Alcohol (ethanol) can penetrate into the hydrophobic regions of the plasma membrane and modify the dynamic lateral organization of proteins and lipids. This process can affect the function of plasma membrane receptors. To assess the molecular distribution of receptors, we used photoactivated localization microscopy (PALM). This technique was employed to quantitatively characterize the effects of pharmacologically relevant concentrations of ethanol and naltrexone (a medication used to treat alcohol use disorders) on the lateral nano-organization of mu and kappa opioid receptors (MOR and KOR, respectively). Ethanol reduced the size and occupancy of nano-domains that harbor opioid receptors and increased the fraction of opioid receptors residing outside of nano-domains. While naltrexone marginally affected MOR density and nano-organization, it significantly increased KOR density, KOR nano-domain size and KOR nano-domain occupancy. Pretreatment with naltrexone largely protected against ethanol-induced changes in MOR and KOR lateral organization.

Support:
Supported by The Beckman Research Institute of the City of Hope, The Knut and Alice Wallenberg Foundation (grant KAW 2011.0218), The Swedish Research Council (VR 2016-01922), Foundation for Strategic Research (SBE13-0115), The Foundation Olle Engkvist Byggmästare and the FP7-Health-2013-Innovation-1 GLORIA-602919 Project.

Disclosure:
The authors have no conflicts of interest to disclose.

Characterizing the dynamic spatio-temporal organization of opioid receptors in live cells by functional Fluorescence Microscopy Imaging (fFMI)

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Dynamic regulation of opioid receptor spatio-temporal organization in the plasma membrane is a fundamental control mechanism through which opioid receptor activity is finely tuned. This intricate mechanism, which may have important pharmacological implications, is difficult to analyze nondestructively and our progress towards its clarification is tightly related to the concomitant development of advanced analytical techniques with high sensitivity, spatial and temporal resolution. Building further on our work on Fluorescence Correlation Spectroscopy (FCS), we have developed functional Fluorescence Microscopy Imaging (fFMI). This quantitative, time-resolved confocal fluorescence microscopy imaging technique without scanning can achieve the ultimate single-molecule sensitivity; it has diffraction limited spatial resolution (≈ 300 nm) and can characterize fast dynamic processes with high temporal resolution (≈ 10 μs/frame). It also allows us to measure fluorescence lifetime, thereby visualizing local differences in opioid receptor immediate surroundings. We demonstrate here how fFMI can be used to map in live cells local differences in opioid receptor surface density, lateral dynamics and embedding in different environments, which are determinants of its activity.

Support:
Support by the Swedish Research Council (grants 2016-01922 and 2018-05337), the Nakatani Foundation for Advancement of Measuring Technologies in Biomedical Engineering, ERASMUS+ European Union programme for education, training, youth and sport, and a travel grant by Yoshida Foundation for Science and Technology are gratefully acknowledged.

Disclosure:
The authors have no conflicts of interest to disclose.

Characterization of a knockin mouse line expressing KOR-tdTomato fusion protein

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To characterize the distribution of KOR protein at a resolution higher than that of receptor autoradiography, we generated and characterized a line of knock-in mice expressing KOR fused at the C-terminus with the red fluorescent protein tandem dimer Tomato (tdT) (KtdT). Treatment of homozygous (KtdT/KtdT) mice with the selective KOR agonist U50,488H inhibited scratching behaviors elicited by compound 48/80 and reduced novelty-induced locomotor activity, suggesting intact KOR neuronal circuitries. Binding of [^{3}H]U69,593 to brain membranes showed higher KOR levels in KtdT/KtdT mice than in wildtype mice. qRT-PCR results demonstrated that KOR mRNA levels in the brain were higher in KtdT/KtdT mice than in wildtype mice. IImmunohistochemistry with antibodies against tdTomato performed on coronal brain sections of KtdT/KtdT mice revealed that distribution of KOR-tdT immunoreactivity corresponded well with that of autoradiography of the selective agonist [^{3}H]U69,593 binding to the KOR. The highest levels of KtdT were found in the claustrum, endopiriform nucleus and substantia nigra pars reticulata. KOR-tdT was observed in regions known to be involved in reward and aversion, including ventral tegmental area, nucleus accumbens, anterior cingulate cortex, prefrontal cortex, medial habenula, interpeduncular nucleus, amygdala and bed nucleus of stria terminalis. KOR-tdT was found in paraventricular nucleus of thalamus and periaqueductal gray, which are involved in pain transmission. Several hypothalamic nuclei expressed KOR-tdT, including arcuate nucleus, peri- and para-ventricular nuclei, implicating KOR in endocrine regulation. Forced swim in 22ºC water for 10 min caused KOR internalization in the VTA, which was blocked by pretreatment with norBNI, indicating that forced swim stress activates the endogenous dynorphin/KOR system. These mice thus represent a powerful and heretofore unparalleled tool for accurate, high-resolution mapping of KOR throughout the nervous system and for examining its relationship to neurotransmitters and receptors of interest.

Support:
Supported by R21 DA045274 from NIDA.

Disclosure:
The authors have no conflicts of interest to disclose.

The Transcription Cofactor Lim-only 4 (LMO4) is a Novel Regulator of Kappa Opioid Receptor Expression and Alcohol Consumption

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Repeated alcohol exposure leads to changes in gene expression that are thought to underlie the transition from moderate to excessive drinking. However, the mechanisms by which these gene expression changes are mobilized to a maladaptive response are not well understood. One mechanism could involve the recruitment of transcription co-factors that bind and modulate the activity of several transcription factors. Our results indicate that the transcription co-factor LMO4 is one such candidate regulator. LMO4 does not bind DNA directly but interacts with transcription factors to either activate or repress gene expression. Mice harboring a gene trap insertion at the *Lmo4* locus (*Lmo4gt/+)* consumed significantly more and showed enhanced preference for alcohol in a 24-hour intermittent access two-bottle choice (IA) procedure. shRNA-mediated knockdown of LMO4 in the nucleus accumbens (NAc) enhanced alcohol consumption whereas knockdown in the basolateral amygdala (BLA) led to decreased alcohol consumption and preference as well as reduced conditioned place preference to alcohol. To ascertain the molecular mechanisms that underlie the contrasting effects of LMO4 knockdown in the BLA and NAc, we carried out unbiased transcriptome profiling of these two brain regions in WT and *Lmo4gt/+* mice using RNASeq. Of the 1000 differentially expressed genes identified in each brain region, only 48 were common suggesting that transcription targets of LMO4 are vastly different between the two brain regions. We focused our validation efforts on the BLA as we have previously demonstrated a role for LMO4 in the BLA in regulating fear and selective aspects of cue-reward learning as well as in modulating dopamine responsiveness of BLA pyramidal neurons. We took a systems approach to identify gene networks that were responsive to LMO4 downregulation in the BLA. Weighted gene co-expression network analysis identified 20 modules in the BLA. Of these, three modules (yellow, greenyellow, and purple) in the BLA were significantly correlated with LMO4 deficiency. The yellow module was particularly intriguing in the BLA as it contained LMO4 and the Kappa opioid receptor (KOR) gene. KOR expression was reduced by 50% in LMO4 knockdown mice in comparison to wild type controls suggesting that LMO4 is a positive regulator of KOR expression in the BLA. *In situ* hybridization analysis revealed that approximately 50% of LMO4 expressing neurons in the BLA also expressed KOR. Chromatin immunoprecipitation studies revealed that LMO4 physically bound to KOR promoter sequences in the amygdala suggesting that KOR is a direct transcriptional target of LMO4 in the BLA. Finally, infusion of Nor-BNI, a long lasting and selective KOR antagonist into the BLA significantly reduced alcohol consumption.
These results implicate LMO4-regulation of KOR expression in the BLA as a novel determinant of alcohol consumption.

Supported by NIAAA 1R21AA025244R21 (ROM and RM) and 1R01AA027293 (RM)

Disclosure: The authors have no conflicts of interest to disclose

Kappa opioid receptors on dopaminergic projections from the ventral tegmental area mediate distinct aversion behaviors in chronic pain.

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Many patients with chronic pain also present with comorbid mood disorders such as anxiety or depression, which exacerbate their pain. Kappa opioid receptors (KOR) mediate dysphoric states and contribute to affective-like anxiogenic and depressive behaviors. We recently demonstrated that KOR agonist-induced place aversion is enhanced in models of chronic pain. We hypothesize that KORs contribute to an aversive state associated with chronic pain.

We induced chronic neuropathic (NP) pain in adult male and female C57/BL6 mice using the sciatic cuff model of peripheral nerve injury (PNI), which has established affective dysregulation. Mechanical withdrawal thresholds were lower in NP pain mice compared to controls, suggesting the occurrence of mechanical allodynia. All mice exhibited dose-dependent KOR agonist U50,488-induced hypolocomotion. NP pain mice exhibited enhanced conditioned place aversion to U50,488, demonstrating enhanced KOR signaling in chronic pain.

We investigated the circuitry involved in KOR-mediated aversion using a viral approach in transgenic floxed KOR mice. We microinjected AAV2/10-TH-Cre-dsRED or control virus to eliminate KOR from dopamine neurons into the ventral tegmental area (VTA) of mice with and without PNI. After allowing 3-4 weeks for viral expression, we assessed separate cohorts of animals for U50,488-induced place aversion. Elimination of KOR from VTA TH neurons prevented U50,488 place aversion in naïve animals, but not in NP pain mice. To investigate the involvement of KORs in on-going pain aversion, we conducted single chamber conditioning. We observed that the aversive state is KOR-signaling dependent as it can be blocked with pretreatment of the selective KOR antagonist JDTic. In contrast to the KOR agonist-mediated aversion, elimination of KOR from VTA TH neurons prevented single chamber on-going pain aversion in NP pain mice. These data suggest that KORs on VTA dopamine neurons mediate a tonic aversive state in chronic pain but are not responsible for enhanced KOR agonist-induced aversion.

Support: Supported by 2T32DA024635-11 and the Shirley and Stefan Hatos Foundation

Disclosure: The authors have no conflicts of interest to disclose.

Preclinical evidence for an AAV-based gene therapy for temporal lobe epilepsy targeting kappa opioid receptors

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The high incidence of drug-resistant focal epilepsies poses a persistent challenge in medicine. Certain patients benefit from surgical removal of the epileptogenic focus. However, a large cohort of patients cannot be treated sufficiently at present. We and others have demonstrated the importance of endogenous peptides in seizure control. Since long-term treatment is needed in epilepsy, viral vector-mediated, locally restricted but long-term expression appears suitable to fill the treatment gap. We have evaluated the potential of prolonged dynorphin overexpression as treatment option of focal epilepsy in a pharmaco-resistant model of temporal lobe epilepsy (TLE).
AAV vectors expressing either human preprodynorphin (pDyn-AAV) or a truncated form of GFP (ΔGFP-AAV) were tested in a pharmaco-resistant model of TLE induced by injection of kainic acid into the dorsal hippocampus of mice and in an electrically-induced TLE model in rats. Dynorphins expressed in the epileptogenic focus suppressed generalized seizures and hippocampal paroxysmal discharges (HPDs) up to 6 months after injection (longest time interval investigated). By contrast ΔGFP-AAV continued to display 1-3 generalized seizures per day and frequent HPDs. Moreover, treatment of mice after kainic acid injection conserved or restored spatial learning ability, as tested by Barnes maze, while ΔGFP treated animals lost this ability. Seizure suppression was also observed in epileptic rats. Moreover, the effect of dynorphins on induced epileptiform activity in human resected hippocampal tissue was tested. Epileptiform activity was significantly reduced upon application of dynorphins and anticonvulsant effects were reversed applying the kappa opioid receptor antagonist 5'-GNTI.
Dynorphin expression was restricted to neurons and microdialysis experiments showed stimulation-dependent release of mature dynorphins.

Support:
Supported by the FWF grants I977-B24, W1206-B05 and DFG grant He1598/9-1

Disclosure:
CS and RH have a patent application for the use of pDyn in epilepsy pending.

Opioid antagonists are effective treatments for the positive and negative symptoms of schizophrenia: A meta-analysis

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Importance: Current treatments for the symptoms of schizophrenia, dopamine 2 receptor antagonists, are only effective for positive symptoms in some individuals, and have considerable side effects that impact compliance.
Objective: We sought to determine whether pan-opioid antagonists have therapeutic efficacy in patients with schizophrenia in a meta-analysis of randomized placebo-controlled trials of naloxone, naltrexone, nalmefene, and buprenorphine for treatment of schizophrenia.
Study Selection: English language randomized placebo-controlled clinical trials of opioid antagonists in patients with schizophrenia, schizoaffective disorder, or schizophreniform disorder that measured drug effects on positive, negative, or total symptoms of schizophrenia. resulting in 28 blinded placebo controlled trials in our final analysis.
Data Extraction and Synthesis: Data were extracted from manuscripts with the following priority: means and SDs, test statistic (t or F), P value, means and SDs or raw data estimated from figures, an in-text description of whether the findings were statistically significant, an in-text description of the direction of effect. Effect sizes for studies providing sufficient data for effect size estimation were combined in a random-effects model, which was subsequently combined with maximum-likelihood estimates of the effect size across all studies reporting only statistical significance or direction of effect using a novel random-effects bootstrapped model. Primary study outcomes were the within-subject change on any symptom assessment scale for positive, negative, or general symptoms of schizophrenia between active drug and placebo conditions.
Results: For all drugs combined we found an effect on all symptom scales combined (all positive, negative, and total scales) with both the random effects model: (g = 0.36; P = 0.004; k = 21) and the bootstrap effects model: (g = 0.33; P = 6.1532e-06; k = 28) and on all positive scales combined with both the random effects model: (g = 0.39; P = 0.046; k = 13) and the bootstrap model (g = 0.38; P = 0.002; k = 17) and an effect on all negative symptoms combined with the bootstrap model (g = 0.56; P = 0.042; k = 8).
Conclusions and Relevance: Our meta-analysis provides the first conclusive data for a new class of therapeutic for the positive and negative symptoms of schizophrenia is already FDA approved and can be applied off label. This is also is the first evidence for a class of antipsychotics that does not affect the D2 receptor. We propose that the therapeutic mechanism underlying the efficacy of pan-opioid antagonists is dependent on their ability to antagonize the kappa opioid receptor rather than the mu or delta opioid receptor. Our data is especially relevant because these kappa antagonist produced an improvement of symptoms in patients who were already managed with optimal antipsychotic treatment, and may be useful in treatment resistant patients who do not respond to D2 drugs as a possible alternative to clozapine. Finally, our data suggests that kappa antagonists may represent one of the first effective treatments for the negative symptoms of schizophrenia, of which current treatments are only minimally effective.
Support: None
Disclosures: Dr. Clark reports he has stock ownership and is a director in Storm Biosciences. Dr. Van Snellenberg reports no conflicts of interest. Dr. Abi-Dargham reports having received stock options in Storm Biosciences.

Pain-induced Negative Affect is Mediated via Recruitment of the Nucleus Accumbens Kappa Opioid System.

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Abstract
Prolonged negative affect significantly impacts quality of life for patients suffering from pain. These maladaptive emotional states can lead to severe depression, suicide, involuntary opioid overdose, and related neuropsychiatric comorbidities. The nucleus accumbens (NAc) shell, which integrates both the aversive and rewarding valence of stimuli, exhibits allostatic changes in the presence of pain. In discrete regions of this structure, activation of the kappa opioid receptor (KOR), either by dynorphin, its endogenous agonist, or pharmacological ligands, acutely decreases the reinforcing properties of rewards and induces dysphoria and aversive behaviors. Using a wide range of complementary techniques including pharmacology, optogenetics, chemogenetics, physiology, biochemistry and in vivo positron emission tomography (PET) imaging, our current findings demonstrate that *in vivo* recruitment of NAc shell dynorphin neurons, acting through KOR, is both necessary and sufficient to drive pain-induced negative affect.
Furthermore, we reveal that the presence of inflammatory pain impacts patterns of consumption of fentanyl using an intra-venous self-administration paradigm. Those particular patterns, where rats in pain display bursts of consumption interrupted by periods of "rest", could lead to the occurrence of respiratory depression and subsequent involuntary overdose. Taken together, our results provide evidence that adaptations in the kappa opioid system within the NAc shell represent a functional target for therapeutic intervention in pain that could circumvent affective disorders and may prevent life threatening episodes.

Support:
Supported by DA041781 (J.A.M.), DA042581 (J.A.M.), DA042499 (J.A.M.), DA041883 (J.A.M.), DA045463 (J.A.M.), NARSAD Independent Investigator Award (J.A.M.), DA033396 (M.R.B), DA037152 (M.R.B.), R01-NS106953 (R.W.G.), AA020394 (B.M.W.), K99/R00-DA038725 (R.A.)

Disclosure:
The authors have no conflicts of interest to disclose.

Investigating kappa opioid receptor drug occupancy in the living brain with positron emission tomography

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Kappa agonist and antagonist drug discovery has been heavily pursued for a wide range of health disorders from pain to neuropsychiatric illnesses. To support this effort, continued development and validation of tools to study KOR pharmacology *in vivo* such as positron emission tomography (PET) is essential. Several (PET) radiotracers for brain imaging have been developed to characterize the density of KORs in the living brain and its occupancy by exogenous drug-like compounds. While exploring the pharmacology of KOR tool compounds using PET in preclinical models, we observed discrepancies in the apparent competition binding as measured by changes in binding potential (BP_{ND}, binding potential with respect to non-displaceable uptake). This prompted us to systematically look at the relationships between baseline BP_{ND} maps for three common KOR PET radioligands, the antagonists [^{11}C]LY2795050 and [^{11}C]LY2459989, and the agonist [^{11}C]GR103545. We then measured changes in BP_{ND} using kappa antagonists (naloxone, naltrexone, LY2795050, JDTic, nor-BNI), and found BP_{ND} was comparable between [^{11}C]GR103545 and [^{11}C]LY2459989. Longitudinal PET studies with nor-BNI and JDTic were also examined, and we observed a persistent decrease in [^{11}C]GR103545 BP_{ND} up to 25 days after drug administration for both nor-BNI and JDTic. Competition studies with kappa agonists were also studied, and butorphan and GR89696 (racemic GR103545) impacted binding to comparable levels between the two radiotracers. Of greatest significance, kappa agonists salvinorin A and U-50488 caused dramatic reductions in [^{11}C]GR103545 BP_{ND} but did not change [^{11}C]LY2459989 binding. This discrepancy was further examined in dose−response studies with each radiotracer as well as in vitro binding experiments. Given the broad therapeutic application of KOR targeting drugs and genetic and behavior rodent models used to study KORs, it is important to understand which KOR radiotracers are best suited for measuring drug occupancy of KOR ligands.

Support:

Supported by P41EB015896 from NIBIB. SIG S10RR015728, S10RR026666, and S10RR017208, P41RR14075. MGH ECOR Scholars Program 2016A050771, NIH-NIDA T32DA015036, and NIH-NIDA K99DA037928.

Disclosure:
The authors have no conflicts of interest to disclose.

In vivo Brain KOR Signaling Elucidated by Phosphoproteomics

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A systems view of G protein-coupled receptor (GPCR) signaling in its native environment is the key in development of GPCR therapeutics with fewer side effects. Using the kappa-opioid receptor (KOR) as a model, we employed high-throughput phosphoproteomics to investigate signaling induced by structurally diverse agonists in five mouse brain regions. We employed the classic KOR agonist U50,488H as the reference and comparatively studied downstream phosphorylation changes induced by 6'GNTI and newly reported compounds in one study, and Nalfurafine in another. From these two different phosphoproteomic studies, we observed strong regional specificity of KOR signaling, due to differences in protein-protein interaction networks, neuronal contacts and the different tissues in neuronal circuitries. Agonists with distinct signaling profiles elicited differential dynamic phosphorylation of synaptic proteins, linking GPCR signaling to the modulation of brain functions. The large-scale de-phosphorylation of synaptic proteins in striatum after 5 min agonist stimulation was partially blocked by Protein Phosphotase 2A (PP2A) inhibitors, underscoring the involvement of PP2A in KOR mediated synaptic functions. Pathway analysis in both phosphoproteomic studies revealed enrichment of mTOR signaling by agonists associated with aversion. Consequently, mTOR inhibition during KOR activation abolished aversion, while preserving beneficial antinociceptive, anti-scratch and anticonvulsant effects. Our results establish high-throughput phosphoproteomics as a general strategy to investigate GPCR in vivo signaling, enabling prediction and modulation of behavioral outcomes.

Stress reverses the valence of KOR signaling from inhibitory to excitatory in dopamine neurons

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KOR activation in the ventral tegmental area (VTA) is required for the aversiveness of KOR agonists given systemically and KOR expression specifically in midbrain dopamine neurons is necessary for this aversion (Ehrich et al., 2015). Furthermore, blockade of KORs in the VTA prevents stress induced drug seeking (Graziane et al., 2013). Here I report that a single foot shock session is sufficient to drive a change in KOR signaling in a subset of dopamine neurons from inhibitory to excitatory, as measured with whole cell slice electrophysiology recordings. This switch does not generalize to other GPCRs in the same neurons, as dopamine D2 receptor coupling to inhibitory K^+ channels does not appear to change. Briefly pretreating VTA slices from control animals with corticotrophin releasing factor (CRF) was sufficient to mimic the foot shock-induced switch in signaling, consistent with prior reports that foot shock drives CRF release into the VTA. This switch in KOR signaling is enriched VTA dopamine neurons that project to the medial prefrontal cortex, previously demonstrated to be a required circuit connection in stress induced relapse to drug seeking (McFarland et al., 2004). Together, these observations indicate that a stressor that drives CRF release in the VTA also produces a shift in signaling at a subset of KORs in the VTA, and raise the intriguing possibility that a KOR induced excitation of cortically projecting dopamine neurons is a critical contributor to the stress induced relapse to drug seeking behaviors.

Support:
Funds provided by the State of California for medical research on alcohol and substance abuse through the University of California, San Francisco.

Disclosures:
Research collaboration with BlackThorn Therapeutics, Inc.

Ligand directed activation of c Jun Kinase signaling by Gi/o protein coupled receptor agonists revealed by fluorescent sensors

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c-Jun N-terminal Kinase (JNK) MAPK activation by the Gi/o protein coupled kappa opioid, mu opioid and D2 dopamine receptors stimulates peroxiredoxin 6 (PRDX6)-mediated production of reactive oxygen species (ROS). ROS production by kappa opioid receptor (KOR) inactivating antagonists (norBNI and JDTic) blocks G protein activation, but signaling mechanisms and consequences of JNK activation by KOR agonists were not characterized. In this study, we found that the KOR agonists U50,488 and dynorphin B stimulated biphasic activation of JNK, with an early arrestin-independent phase, requiring the small G protein rac1 and Protein Kinase C, and a later arrestin-dependent phase, requiring rac1 and rho kinase. Activation of JNK by U50,488 and dynorphin B also stimulated PRDX6-dependent ROS production, but with an inverted U-shaped dose response. ROS generation by KOR agonists resulted from the early arrestin-independent phase of JNK activation, and the suppression of ROS response was caused by arrestin-dependent activation of p38 MAPK. The apparent balance between p38 MAPK and JNK/ROS signaling has important physiological implications for understanding of dynorphin actions during the stress response. As a means to visualize these actions, KOR agonist activation of ROS was detected in live transfected HEK293 cells by two fluorescent sensors: CellRox Green and HyPerRed. KOR agonist-dependent HyPerRed fluorescence was also increased after viral gene expression in midbrain dopamine neurons. These findings establish an additional branch of GPCR signaling that can be used to visualize endogenous dynorphin function and suggest that ROS induction may be part of the physiological response to stress-induced dynorphin release.

Support:

Supported by USPHS grants RO1DA030074 from NIDA and P50MH106428 from NIMH

Disclosure:

The authors have no conflicts of interest to disclose.

New fluorescent sensors for dynorphin and serotonin

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The Tian lab is focused on creating fluorescent tools for realtime optical monitoring of neurotransmission and neuromodulation. Two of the latest tools include a dynorphin sensor and a serotonin sensor. The dynorphin sensor was built from the kappa opioid receptor and maintains many of the same properties as the receptor itself, including ligand specificity, agonism and antagonism, as well as dimerization. Therefore the sensor could be used as a reporter for kappa opioid receptor activation. The serotonin sensor was engineered from a bacterial periplasmic binding protein, rather than a GPCR, and is therefore insensitive to manipulations designed to alter serotonin dynamics. The sensor was engineered from a choline binding protein, and a machine learning approach to directed evolution was developed in order to redesign the binding pocket. We demonstrate that the sensor is useful for in vivo fiber photometry, as well as an in vitro assay designed to test the functionality of the human serotonin transporter in the presence of pharmacological challenges.

Phosphoproteomic approach for agonist-specific signaling in mouse brains: mTOR pathway is involved in κ opioid aversion

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Kappa opioid receptor (KOR) agonists produce analgesic and anti-pruritic effects, but their clinical application was limited by dysphoria and hallucinations. Nalfurafine, a clinically used KOR agonist, does not cause dysphoria or hallucinations at therapeutic doses in humans. We found that in CD-1 mice nalfurafine produced analgesic and anti-scratch effects dose-dependently, like the prototypic KOR agonist U50,488H. In contrast, unlike U50,488H, nalfurafine caused no aversion, anhedonia, sedation or motor incoordination at the effective analgesia and anti-scratch doses. Thus, we established a mouse model that recapitulated important aspects of the clinical observations. We then employed a phosphoproteomics approach to investigate mechanisms underlying differential KOR-mediated effects. A large-scale mass spectrometry (MS)-based analysis on brains revealed that nalfurafine perturbed phosphoproteomes differently from U50,488H in a brain-region specific manner after 30-minute treatment. In particular, U50,488H and nalfurafine imparted phosphorylation changes to proteins found in different cellular components or signaling pathways in different brain regions. Notably, we observed that U50,488H, but not nalfurafine, activated the mammalian target of rapamycin (mTOR) pathway in the striatum and cortex. Inhibition of the mTOR pathway by rapamycin abolished U50,488H-induced aversion, without affecting analgesic, anti-scratch, and sedative effects and motor incoordination. The results indicate that the mTOR pathway is involved in KOR agonist-induced aversion. This is the first demonstration that phosphoproteomics can be applied to agonist-specific signaling of G protein-coupled receptors (GPCRs) in mouse brains to unravel pharmacologically important pathways. Furthermore, this is one of the first two reports that the mTOR pathway mediates aversion caused by KOR activation.

Support:
Supported by R01 DA041359 from NIDA.

Disclosure:
The authors have no conflicts of interest to disclose.

BTRX-335140, A Novel and Selective Kappa Opioid Receptor Antagonist, Protects Working Memory Performance from Mild Stress Exposure in Rhesus Monkeys

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Higher order executive processes are readily impaired with uncontrollable stress exposure and are dysregulated in many neurobehavioral disorders. In particular, working memory, mediated through the dorsolateral prefrontal cortex (dlPFC), is highly sensitive to stress-related impairments following either psychological or pharmacological induction. Of interest, the engagement of the kappa opioid receptor (KOR) system occurs under conditions of stress, and although KORs are expressed throughout mesolimbic and mesocortical pathways and have been investigated extensively for their role in reward processing, their involvement in higher cortical cognitive functions is less well understood. The intent of the present study was to examine the effects of a novel KOR antagonist, BTRX-335140, on stress-induced working memory deficits in rhesus monkeys. BTRX-335140 is a potent (K_i = 1.5nM), selective and brain-penetrant molecule that exhibits activity in multiple biochemical and rodent-based behavioral assays. Importantly, BTRX-335140 exhibits a medication-like duration of action unlike the long-lasting effects of prototypic KOR antagonists (e.g., norBNI). In the current study, we used FG7142, a benzodiazepine inverse agonist, known to produce a mild stress response and impair working memory function in rhesus monkeys. A dose of FG7142 was identified that specifically impaired response accuracy in each animal without compromising the animal's ability to complete the task. BTRX-335140 (0, 0.1, 0.3, 1.0 mg/kg, intramuscular) was co-administered with FG7142 or vehicle 30 min before testing. BTRX-335140 had no significant effect of its own on working memory performance under nonstress conditions. However, BTRX-335140 was able to protect working memory performance from the detrimental effects of FG7142, with no evidence of side effects. Given that stress-induced PFC dysfunction is a risk factor for a host of mental disorders, there is a great need for nonaddictive treatments that can lessen the stress response and protect higher order cognitive functions. These data in rhesus monkeys encourage the utility of the KOR antagonist BTRX-335140 for the treatment of stress-related neurobehavioral disorders.

Support:
All work was funded by BlackThorn Therapeutics.

Disclosures:

T.L. Wallace is an employee of BlackThorn Therapeutics and has ownership interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds) in BlackThorn Therapeutics.; W.J. Martin is an employee of BlackThorn Therapeutics and has ownership interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds) in BlackThorn Therapeutics.; A.F.T. Arnsten has a Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants) of BlackThorn Therapeutics; and has ownership interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds) from the royalties from USA sales of Intuniv (non-generic) from Shire Pharma.

A dynorphin projection from the dorsal raphe nucleus to the ventral tegmental area mediates stress-potentiated cocaine reward in mice

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We hypothesized that increased reward valuation following repeated stress is likely to occur through dynorphin/KOR actions on dopamine neurons. Using immunohistochemistry, we showed that a majority of dopamine neurons express KORs somatically in the ventral tegmental area (VTA). VTA-projecting dynorphin neurons were identified in the dorsal raphe nucleus (DRN) and prefrontal cortex (PFC) using a retrograde fluorescent virus in prodynorphin-Cre (pdyn-Cre) mice. Optical stimulation of dynorphin neurons in the DRN, but not PFC, produced KOR phosphorylation in the VTA that could be blocked by pre-treatment with a KOR antagonist. Deletion of dynorphin from the DRN, but not PFC, blocked stress-induced increases in cocaine reward. These effects were mediated by arrestin-dependent KOR activation on dopamine neurons, as conditional deletion of KOR or the arrestin-dependent p38α MAPK from dopamine neurons blocked stress-induced increases in cocaine preference. Using in vivo fiber photometry in the VTA of DAT-Cre mice expressing GCaMP6m, we observed that during periods of swim stress, mice showed a decrease in calcium activity that is likely related to aversive processing. When removed from the stress, calcium activity in dopamine neurons significantly increased compared to baseline responsivity, likely representing a 'relief from punishment' signal. Repeated cycles of forced swim stress prior to cocaine conditioning caused an overall increase in dopamine neuron calcium activity that could promote cocaine-mediated associative learning and increase expression of drug preference. Conditional deletion of KORs from dopamine neurons blunted both decreases and increases in dopamine neuron activity during and following swim stress. KOR activation is hypothesized to acutely inhibit electrophysiological activity of dopamine neurons, and we modeled the effect of KOR activation on cocaine CPP potentiation using an inhibitory opsin, Step Waveform inhibitory Channelrhodopsin (SwiChR). Optical inhibition of dopamine neurons was sufficient to produce avoidance in a real-time place preference assay. Similar to effects previously observed with KOR activation, optical inhibition prior to conditioning potentiated cocaine preference, whereas concurrent inhibition of dopamine neurons with cocaine treatment ablated conditioned place preference. In summary, we found that dynorphin release from the DRN activates VTA KORs and enhances cocaine reward in an arrestin-dependent manner. Activation of the p38 MAPK may lead to long-lasting changes in the excitability of dopamine neurons, altering reward processing after stress and increasing reward-seeking behaviors.

Support:

Supported by NIH grants P50-MH106428, R01-DA030074

Disclosure:

The authors have no conflicts of interest to disclose.

Dynorphin/KOR Signaling in the Extended Amygdala Contributes to Stress-Enhanced Escalated Alcohol Drinking in Dependent Mice.

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Chronic stress is associated with excessive alcohol intake in humans, non-human primates and rodents. We have previously implicated the dynorphin/kappa opioid receptor (DYN/KOR) system in excessive alcohol drinking, showing that systemic administration of a KOR antagonist selectively decreased alcohol intake in mice with a history of repeated forced swim stress (FSS) and alcohol dependence achieved through chronic intermittent ethanol (CIE) exposure. The central nucleus of the amygdala (CeA) and bed nucleus of the stria terminalis (BNST) contain a high density of DYN/KOR and are implicated in stress and excessive drinking. Studies were conducted to examine the contribution of DYN/KOR signaling within CeA and BNST in modulating FSS-enhanced escalated alcohol drinking in CIE-exposed mice. Using our established Stress-CIE Drinking model, *Pdyn* mRNA expression increased in CeA 30-min after FSS only in mice with a history of CIE+FSS exposure, with levels normalizing at 24-hr post-FSS. Given this increase in *Pdyn* mRNA, Pdyn-containing neurons in CeA were targeted with an inhibitory DREADD and silenced with clozapine-*N*-oxide (1mg/kg; ip.), resulting in a selective reduction in excessive drinking in CIE+FSS exposed mice. Dynorphinergic neurons in CeA synapse locally as well as send projections to BNST. Next, we determined whether KOR signaling in CeA and/or BNST contributes to excessive drinking in this model. Infusion of the KOR antagonist nor-binaltorphimine (nor-BNI; 2.5µg/side) into CeA or BNST significantly reduced drinking in CIE and CIE+FSS exposed mice. Together, these data suggest that Pdyn-containing neurons in the CeA are activated in mice with a combined history of stress (FSS) and dependence (CIE). Furthermore, DYN-mediated signaling at KOR within CeA and BNST play a role in stress-enhanced drinking in CIE-exposed mice. Future studies will interrogate this circuitry by examining the effects of selective chemogenetic inhibition of DYN-containing neurons in the CeA→BNST pathway in the Stress-CIE Drinking model.

Support:
NIH grants (U01 AA014095, U24 AA020929, P50 AA010761, T32 AA007474, F31AA027420-01) and VA Medical Research (BX000813).

Disclosure:
The authors have no conflicts of interest to disclose.

Alterations in nucleus accumbens kappa opioid receptor-mediated dopamine inhibition in response to stress and chronic alcohol exposure

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Alcohol use disorders (AUDs) are made especially pernicious by their high comorbidity with disorders of negative affect, such as depression and anxiety disorders. Though such disorders can predispose individuals to AUD development, emerging evidence has demonstrated the inverse - exposure to chronic alcohol may drive development of mood disorders. Here, we explored alterations in stress-related neurobiological systems that may contribute to behaviors observed during protracted withdrawal from chronic intermittent ethanol (CIE). Specifically, we focused on the ability of the KOR agonist U50,488 to decrease dopamine release in the nucleus accumbens, a characteristic that is enhanced following acute withdrawal from chronic exposure to ethanol and is believed to contribute to anhedonia during withdrawal. We exposed mice to four weeks of CIE, and subsequently examined negative affect-like behavior and alterations in KOR-mediated dopamine release inhibition using voltammetry in brain slices following two weeks of abstinence. CIE-exposed mice displayed an increased latency to feed in the novelty-suppressed feeding paradigm, indicating negative affect-like behavior. Though CIE-exposed animals showed decreased basal stimulated dopamine release, we did not observe increased KOR-mediated inhibition of dopamine release. We hypothesized that the inherent stress of the fasting period involved in the novelty-suppressed feeding paradigm may drive an underlying vulnerability to negative affect-like behaviors in animals with a history of CIE, through modulation of the dopamine system by KORs. We tested acute exposure to two stressors, a fasting stress or exposure to 16hr ethanol vapor, in animals with and without a history of CIE. We found that both stressors were able to acutely augment KOR agonist-mediated dopamine inhibition in the nucleus accumbens of ethanol-naïve animals. Further, ethanol pretreatment on ethanol-naïve slices augmented dopamine release inhibition effects of KOR agonist application. Interestingly, we found that animals with a history of CIE were insensitive to augmented KOR functioning in response to acute ethanol after two weeks of ethanol abstinence. Together, these data indicate that chronic exposure to ethanol may alter stress responsivity, resulting in tolerance to ethanol-mediated KOR sensitivity, and increased vulnerability to heterotypic stressors, such as food deprivation, possibly conferring susceptibility to stress-induced ethanol seeking during abstinence.

Induction of stress-like effects on sleep architecture by selective alterations in the activity of dopamine D1 receptor-expressing medium spiny neurons in the nucleus accumbens

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Background: Stress plays a critical role in the neurobiology of mood and anxiety disorders. Sleep is commonly dysregulated in these conditions: however, some people sleep more, whereas others sleep less. We recently showed that chronic social defeat stress (CSDS) in mice causes persistent alterations in sleep architecture, producing increases in time spent in paradoxical sleep (PS) as well as increases in the number of PS bouts that persisted after cessation of the stressor. Previous work shows that stress activates the transcription factor CREB and elevates target gene expression within the nucleus accumbens (NAc), and that non-selective elevations in NAc CREB function produce depressive-like effects whereas disruptions in CREB function produce antidepressant- and anxiolytic-like effects. Elevated NAc CREB function is associated with increased expression of dynorphin, an endogenous agonist at kappa-opioid receptors (KORs) that is co-expressed with GABA in dopamine D1 receptor-expressing medium spiny neurons (MSNs). Dynorphin, in turn, produces feedback inhibition via KORs expressed on the cell bodies and terminals of ventral tegmental area (VTA) dopamine neurons. Implicating dynorphin in the effects of CSDS on sleep, administration of the KOR antagonist JDTic (10 mg/kg, IP) mitigated CSDS-induced alterations in PS. Several lines of evidence suggest that susceptibility to CSDS is accompanied by reduced activity of D1-MSNs in the NAc; however, the ways in which this neural population contributes to the persistent effects of stress on sleep have not been thoroughly explored. Here we examined how selective manipulation of D1-MSNs affects sleep architecture, and the degree to which it can recapitulate effects of CSDS on sleep-related endpoints.

Methods: We used a wireless EEG system that enables continuous data collection in freely-moving male mice over a period of weeks. To examine mechanisms of stress-induced sleep changes, we used viral vectors to express excitatory (hM3Dq) or inhibitory (hM4Di) DREADDs (or mCherry control) in the NAc of mice expressing cre-recombinase in D1-MSNs (GENSAT FK-150). Mimicking the design of our previous CSDS study, after a 5-day baseline, all mice received clozapine (0.3 mg/kg/day) in their drinking water for 10 days, followed by a 5-day washout.

Results: Chronic inhibition of D1-MSNs (via hM4Di) produced CSDS-like increases in PS time without affecting slow wave sleep (SWS) or wakefulness (W) times. In contrast, chronic activation of D1-MSNs (via hM3Dq) produced decreases in PS time, also without affecting SWS or W times. These effects persisted following a 5-day DREADD ligand washout, suggesting that even transient activation or inhibition of this neuronal population can produce long-lasting effects on sleep.

Conclusions: Alterations in the function of NAc D1-MSNs produces complex effects on sleep and wakefulness. Chronic inhibition of D1-MSNs is sufficient to mimic effects of CSDS on key sleep-related endpoints. When considered together, our findings suggest a circuit model whereby stress effects on the NAc lead to KOR-mediated reductions in the function of midbrain dopamine systems, decreased activity of D1-MSNs, and disruptions in sleep architecture that resemble those seen in people with mood and anxiety disorders.

Support: MH063266 (to WAC)
Disclosures: Patents on KOR ligands, paid consultant for Psy Therapeutics in the last 2 years.

Stress and Cocaine Self-Administration Induced Alterations in Kappa Opioid Receptor Regulation of Dopamine in the Nucleus Accumbens

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Chronic stress and high-dose cocaine self-administration both reduce overall dopamine (DA) neurotransmission, contributing to negative affective states during withdrawal from drug exposure. One of the mechanisms driving hypodopaminergia may be cocaine-induced hyperactivity of brain stress systems, including the dynorphin/kappa opioid receptor (KOR) system. KORs are present on dopamine axon terminals in the nucleus accumbens, and when activated by dynorphin or an exogenous agonist, they robustly inhibit dopamine release. Several studies have shown that KOR activity modulates dopamine uptake through its transporter (DAT), and recent literature suggests that DATs and KORs form a physical complex, and KOR activation promotes DAT-KOR interactions. This study focuses on how activation of the KOR influences DAT function. After male Sprague-Dawley rats self-administered cocaine (1.5 mg/kg/infusion, FR1) for five consecutive days, we examined electrically-stimulated dopamine release and subsequent uptake in brain slices containing the core of the nucleus accumbens using fast-scan cyclic voltammetry. We found that the KOR agonist U50,488 inhibited dopamine release more robustly in cocaine self-administering rats, and that dopamine uptake was either not changed or only modestly altered by acute KOR activation in the slice. In addition, as we have seen before, cocaine-induced inhibition of dopamine uptake was reduced after cocaine self-administration, indicating DAT 'tolerance' to cocaine effects. To examine DAT-KOR interactions, we pre-treated slices with U50,488 and then applied cumulatively increasing concentrations of cocaine. In naïve rats, KOR activation induced cocaine 'tolerance' without prior cocaine exposure, while there were no changes in the already-'tolerant' cocaine effects in self-administering rats. These findings suggest that KORs influence DAT activity in nucleus accumbens core and further, that DAT-KOR interactions influence cocaine pharmacology.

Support:
Supported by P50-DA006634 (SRJ).

Disclosure:
The authors have no conflicts of interest to disclose.

Interaction of kappa opioid receptor activation and dopaminergic signaling in the nucleus accumbens core mediate escalated cocaine intake

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Alterations of dopaminergic signaling are hypothesized to be associated with several aspects of drug addiction. Cues that are repeatedly paired with delivery of drugs of abuse can become conditioned stimuli (CS) and are capable of driving dopamine release in the nucleus accumbens core (NAc). Recent work in the Phillips' lab has demonstrated that an attenuation of CS driven dopaminergic signaling in the NAc is correlated with escalated consumption of cocaine. Systemic administration of L-DOPA restored this neurochemical changed and returned drug consumption to pre-escalated levels. In the current data we test if temporally precise increases in dopamine transmission during CS presentation decrease drug consumption. We accomplished this by injecting a viral vector that expresses channelrhodopsin2 under the control of the CaMKIIδ promoter (AAV1-CaMKIIδ-ChR2-mCherry) bilaterally into the ventral tegmental area, and implanted optic fibers in the NAc of male Wistar rats. Photostimulation (6 pulses, 30 Hz) paired with response-contingent drug cues decreased drug consumption in animals that escalate daily drug intake ($p<0.01$), indicating that escalation of consumption is driven by low phasic DA. Thus, preventing the attenuation of dopamine transmission in the NAc through pharmacological intervention would inhibit the development of escalated cocaine consumption. One potential mechanism promoting the attenuation of dopamine signaling is dynorphin via action on Kappa opioid receptors (KOR). To test this, we locally injected the KOR antagonist, norbinaltorphimine (norBNI), into the NAc. Animals treated with norBNI had no significant increase in drug intake across weeks ($p>.05$), whereas animals treated with vehicle showed significant escalation ($p<.05$). We further examined the mechanism in which norBNI blocks development of escalated cocaine intake with *in vivo* fast-scan cyclic voltammetry. Utilizing this technique we found that animals treated with norBNI showed no significant decrease in dopaminergic signaling, while saline treated animals showed attenuation over weeks of cocaine self-administration. These data support the hypothesis that NAc KOR activation attenuates response-contingent CS dopaminergic signaling to promote escalated cocaine consumption. Therefore, the development of KOR antagonists as a pharmacological therapy may have harm-reduction benefits for persons living with addiction.

Support:
Work is supported by DA039687, and The Alcohol & Drug Abuse Institute, University of Washington

Conflict of interest:
The authors declare no conflict of interest.

Reward Value Encoding of Dynorphin and Enkephalin Neurons in Nucleus Accumbens

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In vivo electrophysiology experiments have previously revealed that neural activity of medium spiny neurons (MSNs) in the medial shell of nucleus accumbens correlates to the palatability of reward. The use of cre-driver lines and optogenetics has recently enabled the cell-type specific dissection of medial shell MSN circuits in modulating reward behavior. It has been found that genetically distinct MSN cell types can have opposite effects on valence and reinforcement, suggesting that individual cell types have diverse function. Advances in *in vivo* 2-photon calcium imaging through endoscopic lenses allows for the measurement of neural activity in deep brain structures with single cell resolution. Here we leverage this approach to observe ensemble neural activity of functionally diverse Dynorphinergic and Enkephalinergic MSNs across multiple days while animals consume rewards of varied value and learn associations between rewards and reward predictive cues. Specifically, we imaged the calcium dynamics of dozens of MSNs simultaneously while animals consumed 4 different concentrations of sucrose reward and tracked changes in single cell activity throughout 7 days of Pavlovian conditioning where animals learned auditory cues predicted subsequent reward delivery.

Support: Supported by R37 DA033396 from NIDA.
Disclosure: The authors have no conflicts of interest to disclose.

The role of the dynorphin / kappa-opioid receptor system in alcohol use disorder

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Our laboratory and others have identified that dysregulation of the dynorphin (DYN) / kappa-opioid receptor system contributes to the formation and perpetuation of alcohol use disorder (AUD), as well as neuropsychiatric conditions such as depression. A driving hypothesis has been that under conditions of heightened negative affective-like states produced by stress or dependence-induced withdrawal, increased signaling through the KOR drives maladaptive behaviors such as excessive alcohol self-administration in order to alleviate such negative states. Within the extended amygdala (bed nucleus of the stria terminalis – BNST, central nucleus of the amygdala – CeA, and nucleus accumbens shell – NAc), we have systematically investigated DYN / KOR dysregulation during acute withdrawal and shown that KOR antagonists attenuate both escalated alcohol self-administration and negative affective-like behavior in dependent rodents with dissociable effects of KOR antagonism in the CeA / NAc vs BNST for reducing physiological withdrawal symptoms. In addition, we have also evaluated cortical dysregulation of KOR signaling in the regards to compromised executive function in alcohol dependence. Using a combination behavioral and molecular approaches, we assessed medial prefrontal cortex (mPFC) KOR-mediated contributions to working memory deficits during acute withdrawal in alcohol-dependent rats. Following the identification that site-specific mPFC KOR activation can induce deficits in working memory, the results of these experiments show that during acute withdrawal in alcohol dependent rats, KOR function is increased in the mPFC compared to non-dependent rats, and that deficits in working memory can be rescued by site-specific mPFC KOR antagonism. Furthermore, in continuation of our investigations into DYN / KOR dysregulation in alcohol dependence, we conducted a comprehensive evaluation of *Oprk1* (KOR gene) mRNA expression and DYN A-stimulated GTPγS data in multiple limbic and cortical nuclei of non-dependent and alcohol dependent rats. These data implicated neuroadaptations involving mesolimbocortical dopamine (DA) projections originating in the ventral tegmental area (VTA) as a basis for multiple phenotypes of alcohol dependence. To test the hypothesis that KOR dysregulation occurs in VTA DAergic neurons to increase alcohol self-administration, we utilized transgenic TH::Cre rats that have Cre recombinase under the control of the tyrosine hydroxylase (TH) promotor in conjunction with a floxed *Oprk1* viral construct for inducible and conditional overexpression of *Oprk1* in VTA DA neurons. Following optimization of site-specific VTA viral infusions, including confirmed increases in VTA *Oprk1* mRNA expression, male and female TH::Cre rats were trained for operant alcohol self-administration, separated into groups matched for alcohol self-administration and site-specifically infused with the floxed *Oprk1* viral construct to overexpress *Oprk1* or a control construct. Beginning four weeks after viral infusion, the non-dependent TH::Cre rats were again allowed to self-administer alcohol and those TH::Cre rats infused with the floxed *Oprk1* viral construct in the VTA escalated their operant alcohol self-administration over a two-week period of testing when compared to control construct-infused rats. Collectively, the data to be presented show that DYN / KOR dysregulation occurs in cortical regions to produce deficits in executive function and suggests that *Oprk1* and KOR dysregulation of mesolimbocortical DAergic transmission contributes to the development and perpetuation of AUD.

Support: R01 AA020394 from NIAAA (to BMW) and the WSU Alcohol and Drug Abuse Research Program according to the State of Washington Initiative Measure No. 171 (to BMW).

Disclosure: The authors have no conflicts of interest to disclose.

Excessive alcohol drinking disrupts stress reactions through alterations in BNST dynorphin in mice.

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Chronic, excessive alcohol intake can lead to dynamic changes in stress coping behavior and stress-related neural mechanisms. We aim to explore how long-term intermittent alcohol (IA) changes how mice react to a variety of stressors and if the dynorphin (DYN)/kappa opioid receptor (KOR) system influences these aberrant behaviors. After eight weeks of IA, C57BL/6J mice show reduced ability to cope with repeated forced swim stress, deficits in active coping in response to TMT predator odor, and altered escape strategy when faced with an overhead looming disc threat compared to H2O-drinking mice. Further, we found that KOR antagonist norBNI could restore stress coping in the repeated forced swim and responses to predator odor, but not the looming disc. To next determine which DYN/KOR populations may drive these altered stress reactions, whole-brain *c-Fos* mapping in a transgenic DYN reporter line was conducted to reveal the dorsal bed nucleus of the stria terminalis (dBNST) contained the highest *c-Fos* interaction between alcohol history and stress. We next performed synaptic transmission experiments using whole cell patch clamp recordings of dBNST DYN neurons. IA robustly silenced synaptic drive while the combination of IA and stress significantly increased glutamatergic activity in DYN-containing cells in the dBSNT. Knockdown of BNST DYN also partially restored the stress reaction in IA mice. Ongoing studies using Fos-inducible targeted recombination are in progress to locate which TMT-induced inputs to the dBNST may change after IA. Also, chemogenetic manipulations and genetic knockout of BNST KORs remain as future directions to further alter TMT coping behavior during protracted withdrawal after IA. Altogether, this research shows a behavioral representation of an allostatic shift of stress coping after long-term alcohol. The imbalance of stress neuropeptide signaling may ultimately underlie this complex relationship between alcohol and stress.

Support:
Supported by P60 AA011605 and UO1 AA020911 from NIH.

Disclosure:
The authors have no conflicts of interest to disclose.

Kappa-opioid receptor antagonism reverses allodynia induced by heroin withdrawal

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Although opioids are potent analgesics, chronic opioid use leads to allodynia (defined as pain due to a stimulus that is not normally painful) and hyperalgesia (defined as increased pain response from a stimulus that normally provokes pain) that are observed during withdrawal. There is evidence supporting the involvement of kappa opioid receptors (KOR) in allodynia and hyperalgesia in chronic pain models. Our aim was to investigate the potential of a KOR antagonist to reverse the hyperalgesia induced by heroin withdrawal. First, we investigated the selectivity of 5'-guanidinonaltrindole (GNTI) in antagonizing KOR analgesic response in the tail flick test. Rats that received GNTI did not display analgesia induced by U50,488, a selective KOR agonist. To investigate the potential of GNTI to reverse the allodynic response in opioid withdrawal, male Wistar rats received daily injections of heroin (diamorphine hydrochloride, 2 mg/kg, s.c.) and were tested for mechanical sensitivity with an electronic von Frey (eVF) 4-6 h into heroin withdrawal. Heroin-treated rats exhibited reduced paw withdrawal thresholds compared with the saline group, indicating the development of allodynia. Next, the rats received saline (0.1 mL/100 g of body weight, s.c.) 30 min before eVF testing and on the next day, GNTI (30 mg/kg, s.c.), also 30 min before eVF testing. Paw withdrawal thresholds were significantly higher after GNTI treatment, indicating a functional role of KOR in the allodynia induced by heroin withdrawal.

Support:
This work was supported by the NIDA intramural research program.

Conflict of Interest:
The authors declare no conflict.

Kappa opioid receptor-mediated depressive-like states and suppression of nucleus accumbens dopamine release are blunted in female rats

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Using intracranial self-stimulation (ICSS), which measures brain stimulation reward, we previously found that gonadally intact female rats are less sensitive than males to the depressive-like effects of the KOR agonist U50,488—regardless of estrous cycle stage. To examine activational effects of gonadal hormones on aversive responses to U50,488, we gonadectomized rats that had previously been trained in ICSS. After five weeks, during which plasma sex hormones decreased, baseline ICSS responding was similar across groups (gonadectomized and sham; N=6-7/group). Rats were treated with U50,488 (0.0, 2.5, 5.0, and 10.0 mg/kg, IP) and stimulation thresholds compared. No significant differences to U50,488-induced increases in ICSS thresholds were detected between sham and gonadectomized rats. These data suggest that sex differences in KOR-mediated depressive-like states are not due to circulating gonadal hormones. Using qRT-PCR, we found no sex differences in mRNA levels for dynorphin or KOR in the nucleus accumbens or ventral tegmental area (VTA). However, the level of tyrosine hydroxylase (TH) mRNA was higher in female compare to male VTA. This suggests that females have an increased capacity to produce dopamine, which might protect them from the depressive-like consequences of KOR activation by blunting KOR-mediated suppression of dopamine release. Using fast scan cyclic voltammetry in intact male and female rats (N=5-7), we stimulated the medial forebrain bundle at different frequencies (5 – 60 Hz) to mimic ICSS. We recorded dopamine release in the nucleus accumbens after systemic treatment with U50,488 (0.0 or 5.0 mg/kg). There were no sex differences in baseline stimulated dopamine release. However, as predicted, U50,488-induced suppression of dopamine release was significantly blunted in female compared to male rats. Neither sex nor U50,488 altered dopamine reuptake. These data suggest that females are less sensitive to KOR-mediated suppression of dopamine release and are therefore less sensitive to the depressive-like effects of KOR activation.

Support: DA033526 to EC and DA025634 to MR

Conflict of interest:
The authors declare no conflict of interest

Understanding physical vs psychological effects of repetitive blast exposure: potential role for kappa opioid receptor antagonism

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Repetitive mild traumatic brain injury (mTBI/concussion) and post-traumatic stress disorder (PTSD) have been called the "signature injuries" of military personnel serving in the Iraq and Afghanistan wars, and are major sources of morbidity among Veteran patients enrolled in the VA health care system. Blast exposure (via detonation of high explosives) is a primary source of mTBI (75% of all TBIs reported by Veterans) and gives rise to a multi-factorial behavioral and pathophysiological syndrome that is highly comorbid with PTSD. Correct attribution of adverse blast-induced outcomes to TBI vs PTSD remains a challenge, engendering added difficulty for subsequent clinical diagnosis and treatment. Preclinical research efforts using rodent models can provide much needed insight into underlying mechanisms by which blast exposure produces subsequent dysfunction.

Using an electronically-controlled pneumatic shock tube that models battlefield-relevant open-field blast forces generated by detonation of high explosives, we found that blast exposure chronically (>3 months post-blast) increases a variety of maladaptive outcomes related to either/both mTBI and PTSD (e.g. aversion to blast-related environments, increased alcohol sensitivity, hyperactivity, anxiety, dysphoria, and aggression). In preliminary studies utilizing the pharmacological agent norBNI (a selective kappa opioid receptor (KOR) antagonist which works in part to block the dysphoric/aversive component of stress exposure), we have begun to dissociate the physical and psychological components of blast exposure in our animal model. Preliminary results suggest that norBNI administration prior to blast exposure blocks many but not all adverse behavioral outcomes following blast, highlighting both unique and common underlying mechanisms and outcomes.

Support:
VA BLR&D 1IK2BX003258NIAAA, VA Seed (AGS), NIH NIDA (PEMP), the Department of Veterans Affairs Office of Research and Development Medical Research Service (DGC), VA Rehabilitation Research & Development Service (ERP), University of Washington Friends of Alzheimer's Research (DGC, ERP), UW Royalty Research Fund (DGC); Northwest Network Geriatric Research, Education and Clinical Center (DGC, CCW), Northwest Network Mental Illness Research, Education and Clinical Center (ERP).

Disclosure:
The authors have no conflicts of interest to disclose.

ABSTRACTS for POSTER PRESENTATIONS

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Topical treatment with WOL071-007, a newly developed kappa-opioid receptor agonist, ameliorates ongoing atopic dermatitis in mice and humans

Opioids can induce analgesia without central adverse reactions by binding to peripheral opioid receptors. Kappa-opioid receptor agonists (KORA) are of particular interest for dermatological diseases since in addition to analgesic effects they exhibit anti-pruritic and even anti-inflammatory effects. In contrast to agonists of other opioid receptors, KORA are not associated side effects like euphoria and dependence. Kappa-opioid receptors (KOR) are expressed on keratinocytes as well as on immune cells and are up-regulated upon inflammation. In line with this we detected up-regulated mRNA as well as protein levels of KOR in lesional skin biopsies from patients with atopic dermatitis (AD) or psoriasis compared to healthy human skin.
Here, we investigated the anti-inflammatory potential of the newly developed KORA WOL071-007, belonging to the structural class of decahydroquinoxalines in murine and human immune cells as well as in mouse models for inflammatory skin diseases such as AD and psoriasis. *In vitro*, WOL071-007 down-regulated the expression of pro-inflammatory cytokines associated with AD or psoriasis development and progression, such as IL-4, IL-13, IFN-γ or IL-17 in activated primary mouse and human immune cells. Moreover, systemic treatment with WOL071-007 significantly reduced ongoing skin inflammation in imiquimod-induced psoriasiform dermatitis and in ovalbumin-induced AD as shown by a decreased clinical score or the down-regulated infiltration of inflammatory cells into lesional skin of WOL071-007-treated mice compared to vehicle-treated controls. For clinical use in AD or psoriasis, WOL071-007 would largely benefit from the possibility of topical application. Therefore, and to avoid systemic effects of WOL071-007, a topical formulation was developed for clinical use in AD patients containing 1% of the KORA in an oil-in-water emulsion. Interestingly, the topical application of the WOL071-007-containing formulation markedly reduced ongoing AD-like skin inflammation in mice as evidenced by a decreased clinical score, the down-regulated infiltration of Th2 and mast cells into lesional skin and the reduced erythema formation. Since AD is associated with severe itch we investigated whether WOL071-007, besides having anti-inflammatory capacities, also modulated itch. Notably, in mice with AD-like skin inflammation, which were topically treated with the WOL071-007-containing formulation, the scratching frequency as well as the expression of the itch-associated cytokine IL-31 were dramatically decreased compared to controls. Of note, the anti-inflammatory and anti-pruritic effects of WOL071-007 are mediated by binding to KOR since WOL071-007did not ameliorate skin inflammation or itch in atopic KOR-deficient mice.
To investigate the safety, tolerability and PK of WOL071-007 in humans a phase 1b trial in AD patients was initiated, in which its anti-inflammatory and anti-pruritic potential was assessed as secondary objective. Interestingly, we could detect a trend towards a reduction in the local SCORAD (scoring atopic dermatitis) index in WOL071-007-treated compared to placebo-treated patients. Together, our data clearly demonstrate that topical application of WOL071-007 significantly ameliorates ongoing skin inflammation and itch in a mouse model of AD as well as in AD patients, thus strongly suggesting kappa-opioid receptor agonists as a potential target for further clinical development.

Funding: Work supported by Dr. August Wolff GmbH & Co. KG Arzneimittel.
Disclosure: Christoph Abels, Ulrich Knie and Michael Soeberdt are employees of the sponsor.

Characterization of CeA dynorphin neurons and their role in cued fear learning

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Within the amygdala, the central amygdala (CeA) has emerged a dynamic population critical for cued fear. This GABAergic region is highly heterogeneous and is known to express a variety of stress-associated neuropeptides including corticotropin releasing factor (CRF), neurotensin, neurokinin B, and dynorphin. Little is known about the dense population of CeA dynorphin-expressing (CeA^{dyn}) neurons, and while CeA CRF and dynorphin are hypothesized to interact, the nature of the interaction and the underlying circuitry is not understood.

First, we histologically confirmed the presence of CeA^{dyn} nueurons in the lateral and medial subdivisions of the CeA. To identify CeA^{dyn} projections, we labeled CeA^{dyn} terminals by injecting AAV-DIO-synaptophysin-GFP into CeA of dynorphin-cre animals. We found that CeA^{dyn} neurons display dense projections throughout the CeA and the bed nucleus of the stria terminalis (BNST). Using the RiboTag technique of cell-type specific RNA isolation, we discovered that CeA^{dyn} neurons also co-express CRF, Tac2 (NKB gene), Nts, Crh receptor 1 and the kappa opioid receptor (KOR). Next we explored the necessity of CeA^{dyn} neurons in fear learning and anxiety. Our findings suggest that silencing of CeA^{dyn} neurons blocks low intensity discriminative fear learning without inducing changes in anxiety. Alternatively, conditional knockout of local CeA dynorphin results in little or no disruption of fear learning or anxiety, possibly due to compensation by other sources of dynorphin from outside the CeA.

We are currently investigating the role of the CeA^{dyn} population in fear learning at various threat intensities and the role of KOR in cued fear learning. Additionally, we are working to better understand the overlap and connections in the CeA between local and distal inputs expressing CRF, dynorphin, and KOR. Characterization of these circuits provides insight into stress-related peptide regulation of fear learning, and could potentially aid in the identification of novel therapeutic targets for fear disorders.

Support:
Supported by 5P50MH106428 from NIMH.
Disclosure:
The authors have no conflicts of interest to disclose.

Genetic association of variants of prodynorphin promoter 68 bp repeats in Caucasians with opioid dependence diagnosis: Effect on age trajectory of heroin use

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The dynorphin/kappa opioid receptor (Dyn/KOR) system is involved in reward processing and dysphoria/anhedonia. Exposure to mu-opioid receptor agonists such as heroin increases expression of the prodynorphin gene (PDYN) in the brain. In the present study of a Caucasian population, we examine the association of a functional polymorphism in the *PDYN* gene 68-bp repeats with particular aspects of the opioid dependence process. In this case-control study, 554 subjects with Caucasian ancestry (142 healthy controls, 153 opioid exposed, but never opioid dependent, and 259 with an opioid dependence diagnosis, OD) were examined for association of the *PDYN* 68-bp repeats with the categorical diagnosis of opioid dependence (DSM-IV criteria), with a dimensional measure of heroin exposure (KMSK scale), and age trajectory parameters of heroin use (age of heroin first use, and age of onset of heaviest use). The *PDYN* 68-bp repeat genotype (classified as: high expression “short-short”, 1-2 repeats [SS], low expression “long-long”, 3-4 repeats [LL], and heterozygous “short-long” [SL], based on the number of repeats, was not associated with categorical opioid dependence diagnoses. However, the LL genotype was associated with later age of first heroin use than the SS+SL genotype (19 versus 18 years; $p<0.01$). This was also confirmed by a significant positive correlation between the number of repeats and the age of first use of heroin, in volunteers with OD (Spearman $r=0.16$; $p=0.01$). This suggests that the functional PDYN 68-bp repeat SS+SL genotype, which results in relatively high levels of *PDYN* expression, is associated with earlier age of heroin first use in Caucasians.

Support: This study was supported by grants from the Dr. Miriam and Sheldon G. Adelson Medical Research Foundation, NIDA grant P50-DA005130 (MJK), a special supplement to R01-DA012848 (MJK) and a grant from the Netherlands Ministry of Health, Welfare and Sports.

Disclosure: The authors have no conflict of interest to disclose.

Role of medial prefrontal cortex kappa opioid receptors in alcohol dependence-induced working memory deficits

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A fundamental characteristic of alcohol use disorders is the loss of control over alcohol consumption that facilitates the progression to alcohol-dependence. Given the comorbidity of alcohol dependence and disorders of affect such as depression is extremely high, it has been posited that self-medication of negative affective states contributes to continued excessive alcohol use and relapse. Furthermore, negative affective states produced by chronic alcohol exposure can influence the neurocircuitry of cognitive control systems to perpetuate further excessive alcohol use. Once that degree of dysregulation is reached, components of the dependence cycle serve to facilitate each other in a manner that is extremely deleterious. Impaired working memory is one symptom contributing to compromised executive function in alcohol dependence. Dysregulation of cortical dynorphin (DYN) and κ-opioid receptors (KORs) has been implicated in alcoholism-induced impairment in executive function. The present experiments test the hypothesis that medial prefrontal cortex (mPFC) KORs contribute to impaired working memory in alcohol dependence. Alcohol dependence was induced in male Wistar rats via chronic intermittent alcohol vapor exposure prior to training / testing in an mPFC-dependent working memory task (delayed nonmatching-to-sample task; DNMST) during acute withdrawal with somatic withdrawal signs and escalated alcohol self-administration measured to confirm the presence of a dependence-like state. In addition, mPFC KOR function was assessed in non-dependent and alcohol-dependent Wistar rats during acute withdrawal using a DYN A-stimulated [35S]GTPγS coupling assay to identify alcohol dependence-induced mPFC KOR dysfunction. Lastly, a functional role for mPFC KORs in the regulation of working memory deficits in alcohol dependence was assessed through intra-mPFC infusions of a KOR agonists (in alcohol naïve rats) and antagonists (in alcohol-dependent rats) prior to assessment in the DNMST. In alcohol-dependent rats displaying somatic signs of withdrawal, impaired DNMST performance confirmed compromised working memory that was paralleled by site-specific intra-mPFC KOR activation in alcohol-naïve animals. Furthermore, it was determined that DYN A-stimulated mPFC KOR function was significantly increased during acute withdrawal in alcohol-dependent rats when compared to non-dependent rats. Importantly, mPFC KOR involvement in alcohol dependence-induced working memory deficits was functionally confirmed by intra-mPFC KOR antagonism that ameliorated impairments in working memory during acute withdrawal. Regulation of working memory by mPFC KORs and alcohol dependence-induced dysregulation of mPFC KOR function identify a novel therapeutic target to treat neuropsychiatric disorders defined by symptoms of working memory impairment.

Support:

Supported by R01 AA020394 from NIAAA (to BMW).

Disclosure:

The authors have no conflicts of interest to disclose.

Mu-opioid receptors in nucleus accumbens mediate stress enhanced motivated behaviors.

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The endogenous opioid system is involved in motivation for both natural and drug rewards, and ample evidence suggests that specific opioids may play particular functions (e.g., rewarding mu and aversive kappa). One important site of action for opioids is nucleus accumbens medial shell (NAc mShell). Here, both mu and kappa opioid receptors (MORs and KORs) have been shown to modulate motivated behaviors. Previous work in our lab has demonstrated that the local dynorphin signaling on KORs can have opposing functions depending on the anatomical site being stimulated MORs have also been shown to be multimodal in function, but the mechanisms underlying these MOR-specific mechanisms remain poorly understood. Here, we sought to determine when MORs are recruited to modulate behavior, what cell types they act on in the NAc, and what is the source of their endogenous ligand by systematically disrupting or restoring MOR function during food intake or elevated zero maze tasks. Using a series of pharmacological and genetic knockout models, we demonstrate that MORs are not necessary for baseline appetitive intake nor baseline open arm avoidance, but are selectively necessary for enhancing motivated behaviors when animals are in a stressed state (i.e., increased intake after food deprivation or open arm avoidance after restraint stress). Furthermore, we show that MORs appear to act on presynaptic enkephalinergic NAc afferents increase motivation to consume food. Using genetic rescue and opto-XR approaches, we go on to show that selective activation of MORs on a dorsal raphe enkephalinergic projection to NAc is sufficient to drive MOR-dependent changes on motivation. Lastly, using a combination of DREADD and caspase approaches, we show that local NAc enkephalin (but not NAc dynorphin, arcuate POMC, or other sources of enkephalin) is the source of the endogenous ligand that acts on dorsal raphe terminal MORs. Looking forward, we will use fiber photometry and *in vivo* calcium imaging through an endoscopic GRIN lens to determine how MORs modulate neuronal activity.

Support:
This work was supported by grants F32DA043999-01A1 and R01DA042499.

Disclosure:
The authors have no conflicts of interest to disclose.

Mapping local heterogeneity in kappa-opioid receptor lateral dynamics

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The plasma membrane is a crowded and complex dynamic assembly of lipids and proteins through which energy and matter are continuously being exchanged. Cell surface receptors are important conduits for transferring specific information across the plasma membrane, and their immediate environment is a key determinant of their function. Live cells therefore tightly control the lateral organization of cell surface receptors into functional units (*e.g.* monomers, homo- and heterodimers) and compartmentalize signaling pathways by dynamically regulating the plasma membrane composition. To characterize in live cells location-specific differences in the immediate surrounding of cell surface receptors and the effects of these local differences on receptor surface density and lateral dynamics, fast and sensitive methods are needed. To enable such studies, we have integrated massively parallel Fluorescence Correlation Spectroscopy with Fluorescence Lifetime Imaging Microscopy (mpFCS/FLIM). We use here this advanced technique to map in live cells local differences in kappa-opioid receptor (KOP) immediate environment, surface density and lateral mobility, and to characterize how these features are affected by selected substances.

Support:
Support by the Swedish Research Council (grants 2016-01922 and 2018-05337), the Nakatani Foundation for Advancement of Measuring Technologies in Biomedical Engineering, ERASMUS+ European Union programme for education, training, youth and sport, and a travel grant by Yoshida Foundation for Science and Technology are gratefully acknowledged.

Disclosure:
The authors have no conflicts of interest to disclose.

Recapitulating phenotypes of alcohol dependence via *Oprk1* overexpression in non-dependent TH::Cre rats

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The kappa-opioid receptor (KOR) that has dynorphin (DYN) as an endogenous ligand is an important regulator of dopamine (DA) neurotransmission implicated in motivation, emotion and executive function. Our laboratory and others have identified that alcohol dependence dysregulates DYN and the KOR in a manner that promotes multiple symptoms of dependence including escalated alcohol self-administration. Based on our assessment of *Oprk1* (gene for the KOR) mRNA expression and DYN A-stimulated GTPγS data in non-dependent and alcohol dependent rats, neuroadaptations involving mesolimbocortical DA projections originating in the ventral tegmental area (VTA) are implicated as a basis for escalated alcohol self-administration during acute withdrawal. To test the hypothesis that KOR dysregulation occurs in VTA DAergic neurons to increase alcohol self-administration, we utilized transgenic TH::Cre rats that have Cre recombinase under the control of the tyrosine hydroxylase (TH; catecholamine synthetic pathway enzyme) promotor in conjunction with use of a floxed *Oprk1* viral construct to capitalize on Cre-Lox gene manipulation technology. Initially, we phenotyped male and female TH::Cre rats for operant alcohol self-administration and confirmed normal non-dependent alcohol self-administration, as well as normal dependence-induced escalation of self-administration during acute withdrawal following intermittent alcohol vapor exposure. These results positioned us to test whether inducible overexpression of *Oprk1* in non-dependent TH::Cre rats, conditionally in VTA DAergic neurons, recapitulates phenotypes of alcohol dependence such as escalated operant alcohol self-administration. Following optimization of viral infusions involving confirmation of VTA TH+ immunostaining / viral EYFP overlap via fluorescent microscopy and, importantly, RT-qPCR confirmation of viral construct-induced increases in *Oprk1* mRNA expression in the VTA of both male and female TH::Cre rats, we tested the hypothesis that overexpression of *Oprk1* in the VTA of male and female alcohol non-dependent TH::Cre rats would increase operant alcohol self-administration. Male and female TH::Cre rats were trained for operant alcohol self-administration until stable responding during 30-min limited-access sessions was achieved, separated into groups matched for alcohol self-administration and site-specifically infused with the floxed *Oprk1* viral construct (AAV5-Ef1a-OPRK1-DIO-EYFP) or a control construct (AAV5-Ef1a-DIO-EYFP). Beginning four weeks after viral infusion, the non-dependent TH::Cre rats were again allowed to self-administer alcohol in 30-min limited access sessions. The results indicated that both male and female non-dependent TH::Cre rats infused with the floxed *Oprk1* viral construct in the VTA demonstrated escalated operant alcohol self-administration over a two-week period of testing when compared to those TH::Cre rats infused with the control construct. These data support the hypothesis that dysregulation of KOR signaling within the mesolimbocortical DA system is an important contributor to maladaptive symptoms of alcohol dependence.

Support: R01 AA020394 from NIAAA (to BMW) and the WSU Alcohol and Drug Abuse Research Program according to the State of Washington Initiative Measure No. 171 (to BMW) and R37 DA0333396 (to MRB).

Disclosure: The authors have no conflicts of interest to disclose.

Influence of nucleus accumbens DAT-KOR interactions on cocaine effects in naïve and self-administering rodents

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Chronic cocaine self-administration reduces overall dopamine (DA) neurotransmission, resulting in a hypodopaminergic state as well as a hyper-functioning kappa opioid receptor (KOR) system. When the KOR is activated, the KOR system exerts inhibitory control on the ventral striatum and results in inhibition of DA release. Recent literature suggests that the dopamine transporter (DAT) and the KOR form a physical complex, and KOR activation promotes further DAT-KOR assembly. Thus, this study focuses on how activation of the KOR influences DAT function. Male Sprague Dawley rats were used to determine the effects of chronic cocaine self-administration on the DA and KOR systems. After rats self-administered forty infusions of cocaine (1.5 mg/kg/infusion) on a fixed-ratio 1 schedule for five consecutive days, rats were used for *ex vivo* fast scan cyclic voltammetry in the nucleus accumbens core to examine DA dynamics. We found that cocaine exposure reduced stimulated DA release and attenuated cocaine potency at the dopamine transporter. Additionally, we examined KOR function through activation of KORs using the agonist U50,488, and these responses to U50,488 were augmented post-cocaine exposure. To study the interactions between the DA and KOR systems, slices were pretreated with an IC50 concentration of U50, 488 (or cocaine), followed by a cocaine (or U50, 488) concentration response curve. When KORs were pre-activated in naïve animals, animals became less sensitive to cocaine effects at the DAT, while pre-treatment with cocaine further enhanced U50, 488 effects. Taken together, these data support that activation of KORs may have a direct interaction with cocaine effects at the DAT in cocaine-naïve animals, and alterations in the DAT-KOR interaction may modulate cocaine self-administration behaviors.

Support:
Supported by F30- DA048575, T32-DA041349, P50-DA006634.

Disclosure:
The authors have no conflicts of interest to disclose.

Antidepressant activity of JNJ-67953964 in mice: Sex differences and reversal of stress-induced behaviors

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Major depressive disorder (MDD) is a leading cause of disability worldwide that is precipitated and/or exacerbated by stress exposure. Dysregulation of the endogenous opioid system is implicated in the emergence of MDD. Stress increases dynorphin-induced activation of kappa opioid receptor (KOR) signaling, which is known to produce negative affect, dysphoria, and aversion. KOR blockade produces behavioral effects in rodent tests used to screen novel antidepressant compounds that are indicative of potential antidepressant-like activity. JNJ-67953964 (previously LY2456302 and CERC-501) is the only selective KOR antagonist currently in clinical trials for MDD, yet there are no preclinical studies systematically evaluating this compound in animals exposed to chronic stress. In these studies, two strategies were employed to investigate the ability of JNJ-67953964 to ameliorate the behavioral deficits produced by stress: 1) suppression of nest building behavior following exposure to the KOR agonist U50,488 (U50), and 2) reversal of the behavioral deficits produced by exposure to a chronic mild stress paradigm. Mice ordinarily build nests that may serve for thermoregulation, social attraction, or protection. Nest building may be impaired by KOR activation or by stress and this may serve as a behavioral indictor of distress or an anhedonic state. In Experiment 1, alterations in nest building behavior was the primary endpoint, and in Experiment 2, nesting was repeatedly evaluated throughout the stress paradigm, where it was used in conjunction with the induction of increased passive coping ("immobility") in the forced swim test (FST) and reductions of sucrose drinking behavior, to confirm a stress-induced phenotype in mice. In Experiment 1, nesting was assessed in adult male and female C57BL/6J mice by providing individual mice with compressed square cotton nestlet material. The quality of the nest was scored using a scale of 1 to 5 every half hour for up to 5 hours. The primary measures included the time it took to reach a criterion nest score and the final nest score. First, we found that U50 (5 or 10 mg/kg, i.p.) suppressed nesting behavior, but females required a higher dose than males. Second, JNJ-67953964 (1, 3, or 10 mg/kg, i.p.) given 24 hours prior to testing, blocked the effects of U50 on nesting. Females again required higher doses than males. In Experiment 2, adult male C57BL/6J mice were subjected to four weeks of unpredictable chronic mild stress (UCMS) and nesting was tested weekly during stress presentation and up to 3 weeks after stress exposure. After three weeks of stress, JNJ-67953964 (10 mg/kg) was administered daily for 12 days. Exposure to UCMS suppressed nesting, increased immobility on the FST, and reduced sucrose consumption. JNJ-67953964 reversed all of these deficits. Both the effects of stress and JNJ-67953964 administration on nesting persisted during the 3-week recovery period. Overall, our results demonstrate that nesting is a useful assay for compounds that engage with KORs. Our data showing that a KOR antagonist counteracted the behavioral effects of UCMS also encourages further clinical development of JNJ-67953964 as a therapeutic for stress-related disorders. These data also support the existence of sex differences in KOR activation and sensitivity, and indicate that females will potentially require a different dosing regimen than males to produce corresponding behavioral effects.

Support:
Supported by R01 MH92412 and R01 MH105623.

Disclosure:
The authors have no conflicts of interest to disclose.

Extended amygdala projections to parabrachial dynorphin neurons alter threat perception and encode feeding behaviors

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In order to survive, an animal must seek and consume food while avoiding environmental threats. In mammals, food consumption is heavily influenced by the parabrachial nucleus (PBN), a pontine structure that integrates visceral information to encode metabolic needs. Peptidergic neurons in the PBN, including dynorphin-expressing neurons, receive top-down input from the bed nucleus of the stria terminalis (BNST), an extended amygdala structure that encodes affective and threat information. These dense projections to the PBN enable a potential circuit that may be involved in the complex integration of environmental threat evaluation with an animal's own homeostatic or hedonic motivations for feeding. *We hypothesize that BNST-PBN circuits integrate environmental threat information to modulate food-seeking behaviors such as exploration and consumption.* Here we used complementary tracing, electrophysiological, and biochemical techniques to identify and characterize distinct excitatory and inhibitory BNST-PBN circuits. In order to assess the role of these distinct circuits, we selectively targeted GABAergic and glutamatergic BNST-PBN circuits to monitor and manipulate circuit activity during behaviors associated with affective motivation, such as hedonic and homeostatic feeding and threat perception. We found that vGAT- and vGluT2-expressing BNST-PBN circuits have divergent roles in valence-encoding, threat, and homeostatic and hedonic feeding behaviors. When activated, vGlut2+ BNST-PBN circuits cause aversion, operant negative reinforcement, anxiogenesis, and reduced feeding behavior. vGAT+ BNST-PBN circuits drive preference, operant positive reinforcement, anxiolysis/exploratory behavior, and increased feeding behavior. Using calcium imaging, we uncover divergent endogenous excitatory and inhibitory BNST-PBN circuit dynamics during feeding behavior, showing that inhibitory BNST-PBN projections are most active during feeding. Rabies tracing suggests that these populations are monosynaptically connected to a population of dynorphin neurons in the PBN that encode negative valence, decrease exploration, and reduce food consumption, consistent with both inhibitory and excitatory GABAergic and glutamatergic BNST input. Together, our findings characterize distinct BNST-PBN circuits, revealing their opposing roles in threat perception and feeding behaviors to describe a mechanism by which animals evaluate environmental threat to enable feeding and promote survival.

Support:
NIMH-1R01MH112355-01 (MRB), NIDA-R21DA035144 (MRB), NIGMS-5T32GM008151-33 (ATL), NIGMS-5T32GM008151-34 (ATL)

Disclosure:
The authors have no conflicts of interest to disclose.

Estrogen regulated G-protein Receptor Kinase 2 (GRK2) inhibits both KOR agonist and antagonist effects.

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We previously found that female mice in high estrogen phases of the estrus cycle are less sensitive to both kappa agonism and antagonism than males. U50,488 does not produce a consistent increase in tail-flick or hot-plate analgesia in normal C57Bl/6 females; however, ovariectomized female mice responded with similar analgesic effects to U50,488 as males. Consistent with published reports (Laman-Maharg, 2018), intact females do not show consistent long-lasting KOR antagonism after norBNI treatment; whereas ovariectomized mice do. The mechanism of estrogen effect was determined to be a consequence of increased phosphorylation of G protein-coupled receptor kinase 2 (GRK2) (Abraham 2018). Estrogen-stimulated phosphorylation of GRK2 increased its sequestration of Gβγ, thereby preventing the Gβγ signaling required for the analgesic response. When the GRK2/3 inhibitor CMPD101 (15 mg/kg) was given prior to U50,488, intact female C57BL/6 mice showed equivalent analgesic responses to males. Similarly, when CMPD101 was given with norBNI to female mice, the analgesic response to U50,488 in the tail flick assay was blocked. Two classes of selective kappa antagonists have been distinguished: competitive (readily reversible) and non-competitive (receptor-inactivating); however, which would be the more effective medication has not been established. To assess the utility of receptor-inactivating antagonists, we tested the effects of a range of doses in both male and female mice. As previously established, the antinociceptive effects of the kappa agonist U50,488 were blocked by a single injection of the long-acting antagonist norBNI (10 mg/kg i.p.). Ten to 20-fold lower doses of norBNI were ineffective after a single administration, but daily administration of 1.0 or 0.5 mg/kg for 5 days completely blocked U50,488 antinociceptive effects. Daily administration of 0.1 mg/kg norBNI produced slowly accumulating inhibition and completely blocked the antinociceptive effect of U50,488 after 20-30 days. Thirty days of 0.1 mg/kg norBNI also completely blocked U50,488 analgesia in ovariectomized mice. Receptor inactivation in both male and female mice treated for 30 days with 0.1 mg/kg norBNI persisted for greater than 1-week. The enhanced safety of this low-dosing protocol has important clinical implications if receptor-inactivating kappa antagonists advance in medication development. Based on these preclinical findings, we predict that women will respond to low doses of receptor-inactivating KOR antagonists during low estrogen periods in their menstrual cycle and that accumulating receptor inactivation may still be therapeutically effective because of the low turnover rate of KOR.

Support:
Supported by P50 MH106428, R01 DA030074 from NIH.

Disclosure:
The authors have no conflicts of interest to disclose.

Activation of kappa opioid receptor potentiates cold sensation

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Noxious cold sensation is commonly associated with peripheral neuropathies, however there has been limited progress in understanding the mechanism of cold pain. Here we investigate the role of transient receptor potential ankyrin 1 (TRPA1) channels in mediating cold sensation and whether the kappa opioid receptor (KOR) modulates such an effect.
Wildtype mice (WT) injected with U50,488 (U50) (KOR agonist, 5mg/kg i.p) show significant potentiation in the number of jumps on the cold plate compared to controls at 3˚C. NorBNI (KOR antagonist) attenuates U50-induced nocifensive responses. In the thermal plantar assay, WT mice treated with U50 actively avoid the cold zone when compared to their respective controls. The hyperresponsivity to cold temperatures is higher at 3^0C vs. 10^0C. Using fluorescent *in situ* hybridization (FISH), we show that *Oprk1* RNA colocalize with the *Trpa1* in dorsal root ganglion (DRG). To determine the calcium dynamics in DRG neurons, simultaneous application of the TRPA1 agonist mustard oil (100μM), and U50 (10 μM) potentiated Ca^{2+} responses compared to mustard oil alone. This result suggests crosstalk between receptors in the DRG. Together, these findings identify a novel role for the kappa opioid receptor system in the potentiation of cold sensation through TRPA1 signaling.

Support:
Supported by R00-DA038725 from NIDA

Disclosure:
The authors have no conflicts of interest to disclose.

Kappa opioid mechanisms underlying increased ethanol intake and nucleus accumbens dopamine dysregulation induced by early life stress in rats

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Early life stress, such as social isolation during adolescence, increases vulnerability to the later development of alcohol use disorder. In our laboratory, rats that are socially isolated during adolescence display increased levels of alcohol intake in a two-bottle choice paradigm. Along with greater drinking, we have found that there is an enhanced ability of the kappa opioid receptor (KOR) agonist U50,488 to inhibit dopamine (DA) release in the nucleus accumbens (NAc), indicating greater KOR responsivity. Further, early life stress led to reduced basal levels of DA in the extracellular space, which was rescued by administration of the KOR antagonist norBNI. Social isolation-reared rats also decreased their intake of alcohol after norBNI. We hypothesize that reduced DA signaling in the nucleus accumbens sets the stage for a long-term negative affective state, leading to increased ethanol drinking. The current study investigates the pharmacological mechanisms altered by adolescent social isolation. Together, our findings further demonstrate that KOR signaling plays a major role in the hypodopaminergic state associated with early life stress. These findings implicate long-lasting KOR activity alterations in the development of addiction-like behavior and underlying neurobiological changes after exposure to adolescent social isolation stress.

Support:
Supported by P50 AA12298125 (JLW, SRJ).

Disclosure:
The authors have no conflicts of interest to disclose.

Prefrontocortical kappa opioid receptor activation disrupts working memory maintenance and increases reactive oxygen species generation

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Major depressive disorder is characterized by affective and cognitive dysfunction that is hypothesized to occur as a consequence of increased dynorphin/KOR activity. We aimed to determine the cellular and molecular mechanisms underlying KOR-mediated cognitive disruptions in the prefrontal cortex. We observed that a majority of KOR-expressing neurons in the prefrontal cortex (PFC) co-expressed Ca2+/calmodulin-dependent kinase II (CamKII) and that systemic administration of a KOR agonist increased phosphorylation of KOR in the PFC. To examine the role of KORs in the PFC in working memory, we trained male C57BL/6J mice in an operant delayed alternation task to make a response on one retractable lever, wait a specified delay for reinsertion of the levers, and then respond on the alternate lever. Mice were trained until reaching stable performance with a 10s delay for reinsertion and then were injected in the PFC with either artificial cerebrospinal fluid (ACSF) or the long-lasting (~3 weeks) KOR antagonist, norBNI (1.25 µg in 0.5 µL vehicle). Following recovery from surgery, mice were treated with saline or KOR agonist (U50,488; 5 or 10 mg/kg i.p.) immediately before a delayed alternation session. Saline administration did not change performance in delayed alternation. Mice with PFC ACSF injections showed decreased correct responses following systemic KOR activation compared to a baseline day, and these disruptions were blocked with local PFC norBNI microinjection. To identify the molecular actions of KOR in the PFC, we measured the activation of a novel genetically encoded reactive oxygen species (ROS) sensor, HyPerRed, which we have previously shown fluoresces in response to recruitment of the c-Jun N-terminal Kinase (JNK) pathway by KOR. HyPerRed fluorescence in the PFC was increased by U50,488 treatment, and norBNI pre-treatment decreased agonist-mediated increases in HyPerRed fluorescence. Future studies will determine the specific cellular and molecular substrates involved in KOR-mediated cognitive disruptions.

Support:

Supported by NIH grants P50-MH106428 and R01-DA030074

Disclosure:

The authors have no conflicts of interest to disclose.

norBNI Does Not Act as a Long-Acting Antagonist in Nucleus Accumbens Dopamine Terminals

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The kappa opioid receptor antagonist Norbinaltorphimine (norBNI), produces a prolonged inactivation of the KOR by activating JNK, leading to ROS-mediated disruption of G-protein signaling. This mechanism has been demonstrated in HEK293 cells and in mice, but it is unknown if norBNI causes long-lasting receptor inactivation in all neuronal subcompartments and neuronal subtypes. Because VTA dopamine neurons are necessary for several norBNI-sensitive behavioral effects of KOR agonists, we investigated if KOR can activate JNK-mediate ROS in both cell bodies and terminals and cause long-lasting inactivation. To test this, we recorded KOR-mediated inhibition of presynaptic dopamine release, in nucleus accumbens slices by fast-scan cyclic voltammetry. One-week post-treatment, norBNI no longer blocks KOR inhibition of dopamine release, but it can acutely block KOR-mediated inhibition. The long-acting covalent antagonist, beta-chlornaltrexamine does block KOR-inhibition of terminals for a week, indicating that receptor recycling rates are not shifted in terminals and that there must be signaling differences in terminals as opposed to soma. A one-week pretreatment was sufficient to block KOR-activated GIRK currents recorded by whole-cell voltage clamp electrophysiology in VTA dopamine neuron cell bodies and was sufficient to block U50,488 tail flick. These results indicate that norBNI does not act as a long-acting antagonist in dopamine terminals, and this is not because of an artifact of slicing or different rates of receptor recycling. Future studies will investigate if these differential durations of action are due to signaling differences downstream of norBNI in the JNK-PRDX6-ROS species pathway, due to differences in receptor-G protein precoupling, or due to another phenomenon.

Support:
Supported by P50 MH106428 and R01 DA030074 from NIH.

Disclosure:
The authors have no conflicts of interest to disclose.

Stress acts through dynorphin/KOR system to decrease 5-HT tone in the NAcSh and potentiate cocaine seeking by allostasis at 5-HT_4R

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Stress engages kappa opioid receptor (KOR) system and potentiates the preference of a variety of drugs of abuse. Prior work indicates KOR signaling decreases serotonin tone, resulting in a potentiation of subsequent drug preference. Here, we show conditional excision of KOR from serotonin (SERT expressing) neurons or dynorphin from the nucleus accumbens (NAc) abolishes repeated forced swim stress potentiation of cocaine conditioned place preference (cocaine CPP). To mimic the effects of stress on serotonin tone, we inhibited DRN^{SERT} neurons prior to cocaine CPP conditioning. Prior inhibition of DRN^{SERT} neurons in the DRN or at terminals within the medial NAc shell increased subsequent cocaine CPP, suggesting that a prior hyposerotonergic state within the accumbens is sufficient to potentiate cocaine preference. Lastly, we demonstrate that administration of a KOR agonist prior to 5-HT_4 receptor activation potentiates the effect of 5-HT_4R activation on neuronal activity in the medial NAc shell. These data indicate that stress induces a hyposerotonergic state in the NAc that is sufficient for stress potentiation of drug preference, and indicate a compensatory upregulation of postsynaptic 5-HT_4 receptors may mediate this effect. Together, these studies reveal a Dyn- KOR- 5-HT_4R axis within the accumbens that is reshaped by stress to increase drug preference.

Support:

Supported by P50 MH106428 from NIMH.

Disclosure:

The authors have no conflicts of interest to disclose.

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Kappa 2019 Post-Meeting Survey – *turn this in at the Registration Desk after the close*

[Thanks for completing this survey. The aggregate information is necessary for our final report to NIH and Donors. Your anonymity is assured. Please provide comments, suggestions and feedback on the reverse side that would help the program committee understand your answers to Q2-8.]

1. Demographics (check the choices that apply):
   - Male
   - Female
   - Non-binary
   - Under 40 yo
   - Underrepresented Minority

2. How worthwhile did you find the meeting?
   - Extremely
   - Very
   - Not very

3. Describe the quality of the Oral Presentations:
   - Generally outstanding
   - Mostly fine
   - Mostly disappointing

4. Describe the quality of the Poster Session:
   - Generally outstanding
   - Mostly fine
   - Mostly disappointing

5. Describe your overall impression of the general format of the conference (did you feel that you had an adequate opportunity to contribute/participate):
   - Generally outstanding
   - Mostly fine
   - Not very effective

6. Kappa-2019 Program Committee posted our explicit expectations that the meeting be inclusive and free of sexual harassment. Did you experience anything that made you feel excluded or uncomfortable?

   Yes
   No

   (if yes, please describe the incident with as much detail as you are comfortable in providing on the back of this form. Or if you prefer, please approach a member of the Program Committee and describe the circumstances.)

7. How likely are you to attend Kappa-2021?
   - Very likely
   - Possibly
   - Not likely

8. Would you prefer Kappa-2021 to be in:
   - Bethesda, MD (standard Kappa Mtg format; Irwin Lucki & Hugo Tejeda offered to co-host)
   - Montreal, CA (satellite of CPDD & INRC in June; Louis Gendron will lead INRC)
   - Other Location (describe on reverse)
   - No preference