APRIL 2015

KAPPA THERAPEUTICS

CONFERENCE

PROGRAM BOOK

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Bill Carlezon (Harvard Medical School, McLean Hospital)
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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 3rd Conference on the Therapeutic Potential of Kappa Opioids in Treating Pain and Addiction.

Conference Venue

The Carolina Inn – Chapel Hill, NC

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 the Hall (outside of General Sessions in Ballroom) and will be open

Monday, April 20	5 pm - 7 pm
Tuesday, April 21	7 am - 5 pm
Wednesday, April 22	7 am - 5 pm
Thursday, April 23	7 am - 5 pm

Meals

Continental Breakfast will be provided in South Hall of the John Sprunt Hill Grand Ballroom. Lunch will be provided in the Old Well room at the Carolina Inn for all registrants.

Social Program

Monday April 20	Opening Reception (Hill Ballroom, Carolina Inn, 7-9pm)
Tuesday April 21	Student/Postdoc Mixer (Tyler's Taproom, Carborro, 6-7pm)
Wednesday April 22	Wine and Cheese Poster Session (Old Well Room, Carolina Inn, 5-7pm)

Instructions for Presenters Posters

Poster boards are 4 feet x 8 feet. Pushpins will be provided. Posters must be hung after lunch on Wednesday, April 22.

Your poster number is listed in the Program.

Oral presentations

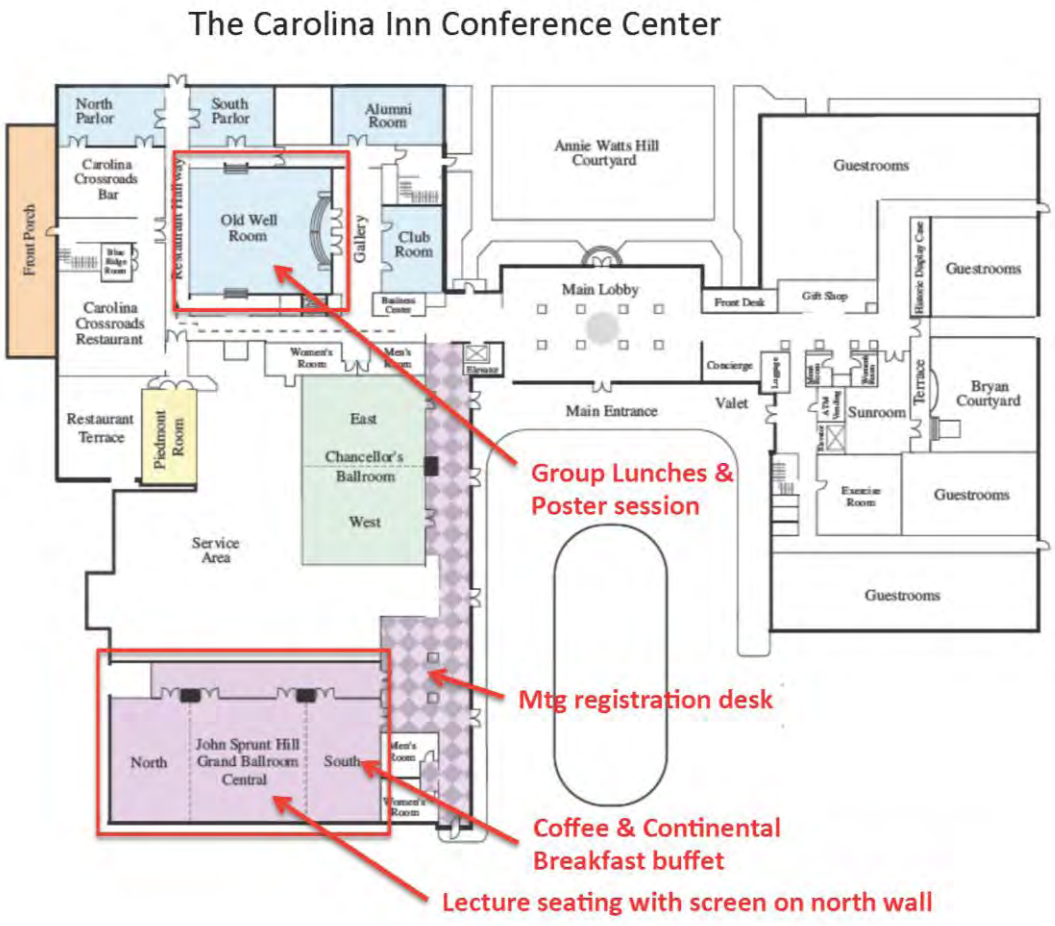
We will have both Macintosh and Windows computers with the latest Operating System and Microsoft Office software. All talks **must** be loaded onto the conference computer the morning of the talk (i.e. during breakfast or the morning coffee break) at the latest. Talks can be emailed or brought to our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

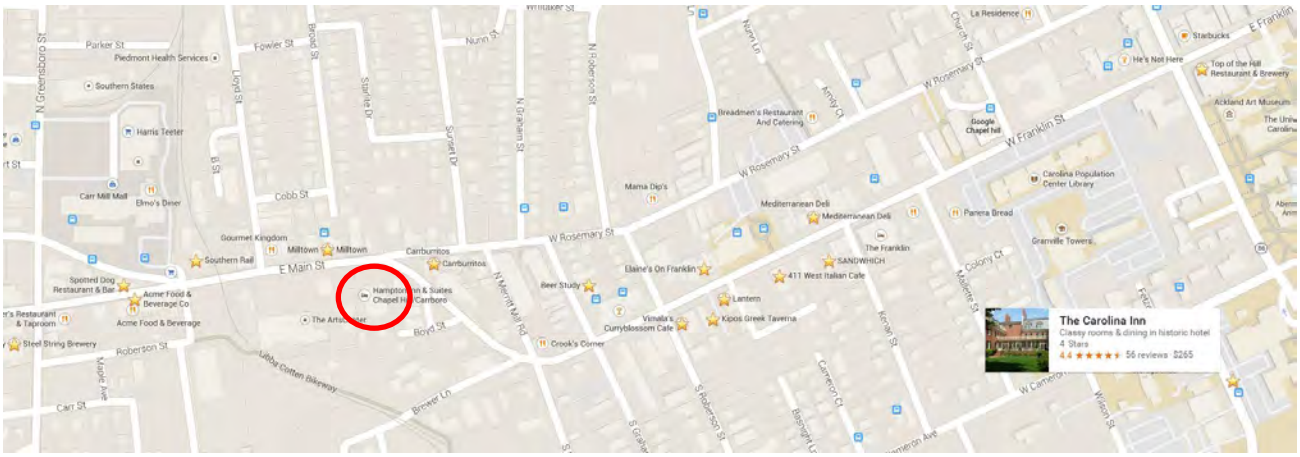
Hotel Accommodations

The Carolina Inn
211 Pittsboro St
Chapel Hill, NC 27516
(919) 933-2001

Hampton Inn & Suites Chapel Hill-Carrboro/Downtown
370 E Main St #100
Carrboro, NC 27510
(919) 969-6988

Floorplan





Hotels and Transportation:

The Hampton Inn (circled in red) and **The Carolina Inn** (shown on right) are under 1 mile from each other – we recommend walking and enjoying the Chapel Hill spring weather! Stars indicate some recommended restaurants in Chapel Hill and Carborro (next page) within 1 mile of The Carolina Inn.

If you would prefer not to walk, some options:

-The Carolina Inn has a complementary shuttle to anywhere within 5 miles of the hotel

-Cabs and independent driver services (Uber and Lyft) are both very active in the area

-There is a great public transportation system. The **CM** (every 30 minutes) and the **F** (every 60 minutes) routes connect the two hotels. You can pick up maps at the hotel, or download the app NextBus (http://www.nextbus.com/wirelessConfig/index.shtml) for easy live access to the schedule. It's really user friendly, will notify you of the closest closest bus stop as well as route times (also web accessible).

-The closest bus stop to The Carolina Inn in **S. Columbia at Abernethy Hall** (as well as S. Columbia at Sitterson Hall).

-The closest bus stop to The Hampton Inn is **E Main St. at Arts Center Plaza**, just outside the hotel

Food and Drinks - Chapel Hill

Food

Lantern | Asian fusion with local ingredients | http://www.lanternrestaurant.com/

Jujube | Asian fusion | http://www.jujuberestaurant.com/

The Pig | NC BBQ with veg-friendly options | http://www.thepigrestaurant.com/

Mama Dip's | Famous homestyle southern food | http://www.mamadips.com/

Carolina Brewery | Local beer and pub food | http://carolinabrewery.com/

Top of the Hill | Local beer and Tarheel propaganda|
http://www.thetopofthehill.com/#toporestaurant

Cholanad | Contemporary Indian | http://cholanad.com/

Vimala's Curryblossom Cafe | Locally-sourced Indian, veg/vegan options |
http://curryblossom.com/

Mediterranean Deli | Mediterranean food, GF options|
http://www.mediterraneandeli.com/

Sandwich | locally sourced burgers, BLTs | sandwhich.biz

Kippos | greek food & entertainment |http://giorgiosgroup.com/Restaurants/Kipos

Drinks

West End Wine Bar | Fancy drinking | http://www.westendwinebar.com/

Beer Study | Craft beer hangout | http://www.beerstudy.com/

Tyler's Tap room | beer and food | tylerstaproom.com

Food and Drinks - Carrboro

Food
Milltown | Quality beers on draft, Belgian frites |
http://www.dininganddrinking.com/

Venable | Modern southern food and a cool bar | http://venablebistro.com/

Crook's Corner | Southern comfort food | http://www.crookscorner.com/

Acme | Upscale southern fusion | http://acmecarrboro.com/home/

Drinks
Carrburritos | Better burritos than Chipotle |
http://www.carrburritos.com/carrboro/index.html

Steel String Brewery | Small local brewery | http://steelstringbrewery.com/

Open Eye Café |Local coffee roasters, art, music | http://openeyecafe.com/

Weaver St. Market | Actually a grocery co-op but you can enjoy food and drink on the lawn, and let the locals entertain you | http://www.weaverstreetmarket.coop/

Food and Drinks- Downtown Durham
(all these restaurants will require a car, but are highly recommended)

Food

Dos Perros | Upscale Mexican | http://dosperrosrestaurant.com/

Dame's Chicken and Waffles | … Chicken and Waffles | http://www.dameschickenwaffles.com/

Original Q Shack | North Carolina style barbeque | http://www.theqshackoriginal.com/

Mateo | Spanish tapas | http://mateotapas.com/

Watts Grocery | local farm-to-table | http://www.wattsgrocery.com/

JuJu | Asian tapas | www.jujudurham.com

Pizzeria Toro | woodfired pizza | pizzeriatoro.com

Nana's | French | nanasdurham.com

Drinks

Fullsteam | Local brewery with a large hangout space | http://www.fullsteam.ag/

Geer St. Garden | outdoor seating, American food | http://geerstreetgarden.com/

Motorco | local beers, cocktails, live music &food | motorcomusic.com

Bar Virgile | cocktails | too cool for a website

Alley 26 | lounge | http://alleytwentysix.com/mobile/

Bar Lusconi | wine bar | http://www.barlusconi.com/

3rd Conference on the "Therapeutic Potential of Kappa Opioids"

April 20-24th, 2015
Carolina Inn, Chapel Hill, NC

Monday, April 20th

5 - 7 PM Registration

6:30 PM Terry Kenakin (UNC) Plenary Talk (Introduction, Bryan Roth)
Biased Receptor Signaling: Receptors go from being switches to microprocessors.

7 - 9 PM Opening Reception

Tuesday, April 21st

7 - 8 AM Continental Breakfast & Registration

8:00 AM Charles Chavkin Welcome

8:10 AM Ray Stevens (USC) Plenary Talk (Introduction, Charles Chavkin)
The amazing diversity of the 7-transmembrane receptor superfamily.

Oral Session 1: Structure Based Design (Bryan Roth, Chair)

8:45 AM Seva Katrich (USC) **Conserved sodium site in class A GPCRs and its implications for □-OR function**.

9:05 AM Ming-Yue Lee (USC) **Structural studies of the human kappa opioid receptor conformation states upon activation.**

9:25 AM Vadim Cherezov (USC) **Structural basis for bi-functional peptides recognition at human opioid receptors.**

9:45 AM Ivy Carroll (RTI) **Design, synthesis, and biological evaluation of JDTic analogs**.

Coffee Break

Oral Session 2: Medicinal Chemistry of Novel Ligands (Terry Kenakin, Chair)

10:30 AM Laura Bohn (Scripps) **Towards understanding the impact of biased agonism in vivo: an evaluation of the physiological effects of G protein/βarrestin2- biased KOR agonists**.

10:50 AM Bernard Wunsch (Münster) **Conformationally restricted □appa-opioid receptor agonists: Stereoselective synthesis and pharmacological evaluation.**

11:10 AM Jane Aldrich (Florida) **Liability profiles and activity in different pain models of analogs of the macrocyclic tetrapeptide CJ-15,208**.

11:30 AM Thomas Murray (Creighton) **Quantification of kappa opioid receptor ligand potency, efficacy and desensitization using a real-time membrane potential assay.**

Workshop / Discussion 1: Structure-Based Design (Terry Kenakin, moderator)

Data Blitz / Short talks (11:50-12:30 PM):

Sarah Scarry (Kansas) **Synthetic efforts toward kappa opioid receptor antagonists.**

Kate White (UNC) **A G Protein-biased κ-Opioid Receptor agonist is analgesic with a unique spectrum of activities in vivo**.

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

Buffet Lunch 12:30 – 2 PM (Old Well Room)

Oral Session 3: Mechanisms of KOR Antagonists (Ivy Carroll, Chair)

2:00 PM Linda Rohrick-Kehn (Lilly) **The novel, potent, short-acting kappa opioid receptor antagonist, LSN2444296, modulates mesoaccumbens dopamine neurotransmission and dopamine-mediated behaviors.**

2:20 PM Raehannah Jamshidi (UTexas-SA) **The long-lasting reduction in KOR function by norBNI in peripheral sensory neurons requires protein synthesis.**

2:40 PM Selena Schattauer (Univ Wash) **Proteomic analysis of the norBNI regulated KOR signalosome.**

3:00 PM Blaine Jacobs (UTexas-SA) **Allosteric interactions within DOR-KOR heteromers in peripheral sensory neurons.**

3:20 PM Zoe Hughes (Pfizer) **Characterization of kappa opioid receptor antagonists for the treatment of aberrant positive and negative valence domains**.

Break

Oral Session 4: Sex Differences in Response (Lee-Yuan Liu Chen, Chair)

4:00 PM Brian Trainor (UC-Davis) **Possible antidepressant effects of kappa opioid receptor agonists following social defeat stress.**

4:20 PM Elena Chartoff (McLean) **Sex differences in the depressive-like effects of kappa opioid receptor activation in rats**

4:40 PM Anna Taylor (UCLA) **Kappa-opioid mediated antinociception can be stress-mediated and some agonists show marked sex differences.**

Workshop / Discussion 2: Best Practices for Responding to the NIH Mandate to Incorporate Females into all Funded Studies (Elena Chartoff, Chair)

Data Blitz / Short talks (5-5:30 PM):

Abigail Laman-Maharg (UC-Davis) **Social defeat stress reverses the effects of a kappa opioid receptor agonist on social interaction behavior in female but not male California mice.**

Maria Mavrikaki (McLean) **Sex differences in kappa opioid receptor-mediated negative affective states in rats.**

[Participants wanting to share 'best practices' are welcome to show a data blitz slide]

Student / Postdoc Mixer (Tyler's Taproom in Carrboro 6 – 7 PM)

Dinner (no host, maps to local restaurants provided)

Wednesday, April 22nd

7 - 8 AM Continental Breakfast & Registration

8 AM Bryan Roth (UNC) Plenary Talk (Introduction, Tom Kash)
Genome wide-approaches for GPCR Interrogation

Oral Session 5: Translational Human Studies (Brad Walters, Chair)

8:35 AM Andrew Saxon (VA Puget Sound & Univ Wash**) Cocaine use reduction with buprenorphine (CURB) preliminary study findings.**

8:55 AM Eliot Ehrich (Alkermes) **Evaluation of balanced agonist-antagonist opioid modulation with ALKS-5461 in Major Depressive Disorder.**

9:15 AM Joseph Stauffer (Cara) **CR845, A novel peripherally-acting kappa opioid receptor agonist, has low abuse potential compared with pentazocine.**

9:35 AM Diana Martinez (Columbia) **Imaging kappa receptors in cocaine abuse**.

Break

Workshop / Discussion 3: How Do We Improve the Predictive Utility of Animal Models for the Treatment of Psychiatric Disorders? (Diana Martinez, Chair)

Data Blitz / Short talks (10:30 - 11 AM):

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

Oral Session 6: Biased Agonists (Michael Bruchas, Chair)

11:00 AM Christoph Schwarzer (Innsbruck) **The G-Protein biased kappa opioid receptor agonist 6'-GNTI blocks paroxysmal discharges without inducing aversion**.

11:20 AM Larry Toll (TPRI) **PPL-101 A high affinity kappa partial agonist is a potent analgesic with low abuse potential but without dysphoria.**

11:40 AM Gavril Pasternak (Sloan Kettering) **Genetic dissociation of the analgesic and aversive actions of the kappa drug U50,488H.**

12:00 PM Lee-Yuan Liu-Chen (Temple) **Nalfurafine shows lower propensity to cause aversion in mice and produces lower KOPr phosphorylation than U50,488H and MOM-Sal B.**

Buffet Lunch 12:30 – 2 PM (Old Well Room)

Oral Session 7: KORs in Withdrawal & Dependence Behaviors (Brendan Walker, Chair)

2:00 PM Renyu Liu (Penn) **Dezocine and buprenorphine reduces withdrawal syndrome in a morphine dependent rat model.**

2:20 PM Rachel Anderson (MUSC) **Kappa opioid receptors and stress-induced enhancement of ethanol dependence-related drinking in C57BL/6J mice**.

2:40 PM Aishwarya Vijay (Yale) **Effects of kappa opioid receptor availability on effectiveness of naltrexone in alcohol-dependent individuals.**

3:00 PM Imad Damaj (VCU) **Effect of orally-bioavailable short-acting kappa-selective antagonist LY2456302 on nicotine withdrawal in mice.**

Break

3:50 PM Cody Siciliano (Wake Forest) **Voluntary ethanol intake predicts kappa opioid receptor supersensitivity and regionally distinct dopaminergic adaptations in macaques.**

4:10 PM Nicole Crowley (UNC) **Chronic intermittent ethanol exposure (CIE) modulates BNST GABA and glutamate transmission and social behavior in a kappa opioid receptor (KOR) dependent manner.**

Workshop / Discussion 4: possible Late Breaking Abstract Talks (C Chavkin, moderator)

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

Poster Session (Old Well Room) 5 – 7 PM

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

Thursday, April 23rd

7 - 8 AM Continental Breakfast & Registration

8:00 AM Mary Jeanne Kreek (Rockefeller) Plenary Talk (Introduction, Bill Carlezon)
The role of kappa opioid receptor-directed compounds in treatment of specific addictive diseases and affective disorders.

Oral Session 8: KOR Effects on Neural Circuits (Bill Carlezon, Chair)

8:40 AM Ream Al-Hasani (Wash U) **Distinct subpopulations of nucleus accumbens dynorphin neurons drive aversion and reward.**

9:00 AM Charles Chavkin (Univ Wash) **Presynaptic inhibition of dopamine release is not required for kappa opioid receptor mediated aversion**.

9:20 AM Brendan Walker (Wash State Univ) **Medial prefrontal cortex kappa-opioid receptors and working memory.**

9:40 AM Anushree Karkhanis (Wake Forest) **Adolescent social isolation sensitizes the kappa opioid receptor system affecting dopamine regulation in the nucleus accumbens.**

Break

Discussion (Bill Carlezon, moderator)

Short talks

John Pintar (Rutgers) **Cortical Alterations In KOR-1 KO Mice**.

Stephanie Nygard (Wash U) **Activation of the kappa-opioid receptor system is both necessary and sufficient for reinstatement of nicotine place preference**.

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

Oral Session 9: KOR Effects On Pain & Itch (Alan Cowan, Chair)

11:10 AM Irene Choi (Nektar) **"Kapp-ing" Visceral Pain: A novel orally efficacious kappa opioid receptor agonist with reduced CNS side-effects.**

11:30 AM Rafael Maldonado (Pompeu Fabra**) Involvement of the kappa/dynorphin system in the emotional manifestations of osteoarthritic pain.**

11:50 AM Jose Moron-Conception (Columbia) **Inflammatory pain impacts motivation for heroin self-administration in dependent rats: a possible role for kappa opioid receptors.**

Buffet Lunch 12:30 – 2 PM (Old Well Room)

Oral Session 9 (continued): KOR Effects on Pain & Itch (Alan Cowan, Chair)

2:00 PM Mei-Chuan Ko (Wake Forest) **Differential functions of dynorphin a, β-endorphin, and gastrin-releasing peptide in regulating itch and pain in the spinal cord of primates.**

2:20 PM Jordan Zjawiony (Mississippi) **Salvinorin A analogs, PR-37 and PR-38, attenuate 48/80-induced Itch responses in mice.**

2:40 PM Lindsey Snyder (Pittsburgh**) Genetic identification of somatosensory neurons that express the kappa opioid receptor.**

Break

Oral Session 10: Role Of Dynorphin / KOR In Stress & Depression (C Chavkin, Chair)

3:30 PM Shivon Robinson (Penn) **Pharmacological mechanisms underlying the antidepressant and anxiolytic effects of buprenorphine.**

3:40 PM Audrey Wells (McLean) **Involvement of kappa opioid receptors in the effects of chronic social defeat stress on sleep, body temperature, and motor activity in mice.**

4:00 PM Abigail Polter (Brown) **Acute stress induces persistent alterations in VTA kappa opioid receptors.**

4:20 PM Yan Zhou (Rockefeller) **Potential involvement of kappa opioid receptors (KOP-r) in PTSD: animal modeling.**

Presentation of the ***2015-Toni Shippenberg Young Investigator Award*** (C Chavkin)

Closing at 5:30 PM

Dinner (no-host)

Friday, April 24th

Checkout & Departure

Posters (Wednesday, 5-7 PM) (Old Well Room)

Double-sided 4′ x 8′ corkboards and push-pins will be provided. Please put up your poster during a break in the program after lunch on Wednesday before 5PM and take it down at the end of the session.

Refreshments will be provided.

1. Abraham AD, Land BB, Gandy JL, Chavkin C. **Kappa opioid receptor activation disrupts interval timing.**

2. Bedini A, De Marco R, Gentilucci L, Spampinato S. **Identification of a novel, selective kappa opioid receptor agonist by screening a library of CJ-15,208/C[Ypwfg] hybrids**.

3. Berg KA, Jacobs BA, Clarke WP. **6' –Guanidinonaltrindole (6'-GNTI) targets DOR-KOR heteromers In peripheral sensory neurons.**

4. Browne CA, Smith T, Lucki I. **Antidepressant-like effects of the κ-opioid receptor partial agonist nalmefene.**

5. Burgeno L, Abraham A, Murray N, Chavkin C, Phillips P**. Kappa-receptor blockade by nor-BNI prevents escalation of cocaine intake.**

6. Clarke WP, Jamshidi RJ, Chavera TA, Prisinzano TE, Berg KA**. KOR agonist functional selectivity in peripheral sensory neurons.**

7. Cowan A, Inan S, DiMattio KM, Carroll FI, Liu-Chen L-Y **JDtic and analogs: probing possible relationships between repetitive scratching episodes in mice and duration of action as kappa receptor antagonists.**

8. Dixit V, Brew C, Ali C, Choi I, Duarte D, Zhang W, Addepalli M, Gogas K, Harrison S, Gursahani H. **In vitro characterization of kappa opioid receptor agonists reveals several distinct pharmacological profiles.**

9. Dolle RE, Hipkin RW, Xiao Y. **2,3-Dimethyl-3-(3-hydroxyphenyl)-1-aminocyclopetanes: new opioid**

receptor ligands.

10. Fajemiroye OJ, Polepally PR, Roth BL, Costa EA, Zjawiony JK. **New insight to the catecholamine-mediated antidepressant-like mechanism of 22-azidosalvinorin A - a compound with high affinity for kappa-opioid receptor.**

11. Frankowski KJ, Lovell K, Slauson SR, Porubsky PR, Scarry SM, Streicher JM, Zhou L, Phillips A, Day VW, Prisinzano, TE, Bohn LM, Aubé J. **New SAR exploration of isoquinolinone-derived kappa opioid receptor agonists.**

12. Gomez AM, Bruchas MR. **Activation of the kappa-opioid receptor system is sufficient for reinstatement of nicotine-self-administration in mice.**

13. Hanks AN, Hedde J, Rosenstein T, More AI, Sawant-Basak A, Hughes ZA. **Using progressive ratio and in vivo binding to characterize novel kappa opioid receptor antagonists for the treatment of motivational deficits.**

14. Huang P, Tunis J, Parry C, Tallarida R, Liu-Chen LY. **Is there a synergism for antidepressant-like effect between a kappa opioid antagonist (LY2444296) and a delta opioid agonist (ADL5859) in the mouse forced swim test?**

15. Kishioka S, Kiguchi N, Saika F, Yamamoto C, Ko M-C. **Restorable effects of kappa opioid receptor antagonist on the attenuation of the development of physical dependence on morphine by formalin-induced pain in mice.**

16. Kuhar JR, Schattauer SS, Chavkin C. **c-Jun N-terminal Kinase (JNK) mediated inactivation of opioid receptors.**

17. Laman-Maharg A, McMackin MZ, Sanchez EO, Campi KL, Trainor BC. **Social defeat stress reverses the effects of a kappa opioid receptor agonist on social Interaction behavior in female but not male California mice.**

18. Lansu K., Kroeze W., Roth, B.L. **Ligand discovery and functional analysis of the novel opioid orphan receptor MRGPRX2.**

19. Liu JJ, Humphrey S, Steger M, Mann M. **Phosphoproteomic survey of KOR-mediated signal transduction**.

20. Ma H, Frankowski K, Slauson S, Lovell KM, Zhou L, Prisinzano TE, Bohn LM, Aubé J. **Development of novel kappa opioid receptor agonists based on the bisamide scaffold**.

21. Mavrikaki M, Mays J, Puttick D, Chartoff E**. Sex differences in kappa opioid receptor-mediated negative affective states in rats**.

22. Morgenweck J, You IJ, Frankowski K, Yoo E, Lovell KM, Schmid CL, Zhou Z, Prisinzano T, Aubé J, Bohn LM. **Functionally selective kappa opioid receptor agonist efficacy in vivo**.

23. Mosier PD, Zaidi SA, Roth BL. **Computational modulation of the kappa-opioid receptor by sodium: structural implications for receptor activation and ligand binding**.

24. Nygard SK, Al-Hasani R, Hourguettes NJ, Spangler SM, Bruchas MR. **Activation of the kappa-opioid**

receptor system as both necessary and sufficient for reinstatement of nicotine place preference.

25. O'Connor C, Milon A, Didenko T, Stevens RC, Wuthrich K. **NMR characterization of structure and conformational dynamics in the dynorphin-KOR-Gi system**.

26. Patel N, Fenalti GW, Giguere PM, Han GW, Cherezov V, Roth BL, Stevens RC Katritch V. **Molecular modeling studies of sodium ion binding pocket suggest a mechanism of biased signaling in □-OR**.

27. Pintar JE, Viljetic B, Wijerante S, Pintar M, Ansonoff M, Rasin R. **Cortical alterations in KOR-1 KO mice**.

28. Placzek MS, Van de Bittner GC, Wey HY, Lukas SE, Hooker JM. **Immediate and persistent effects of Salvinorin A on the kappa opioid receptor in rodents, monitored in vivo with PET**.

29. Reed B, Hillman J, Dunn A, Deutsch-Feldman M, Butelman ER, Kreek MJ. **U69,593 and Nalmefene stimulate prolactin release, with modulation by Mu and/or Kappa blockade, in mice.**

30. Ross SE, Cai X, Snyder LM. **Genetic identification of the CNS neurons that express the kappa opioid receptor.**

31. Scarry SM, Lovell K, Frankowski KJ, Slauson SR, Ma H, Streicher JM, Zhou L, Phillips A, Prisinzano TE, Laura M. Bohn LM, Aubé J. **Synthetic efforts toward kappa opioid receptor antagonists**.

32. Sullivan LC, Jamshidi RJ, Chavera TA, Lococo PM, Berg KA, Clarke WP. The Effect Of Aging On **Peripheral kappa opioid receptor function.**

33. Valenza M, Picetti R, Yuferov V, Butelman ER, Kreek MJ. **Basal and cocaine-induced prodynorphin and kappa opioid receptor gene expression may predispose Lewis but not Fischer rats to escalate cocaine consumption.**

34. White KL, Robinson JE2, Zhu H, Diberto JF, Polepally PR, Zjawiony JK, Nichols DE, Malanga CJ, & Roth BL. **A G Protein-biased κ-opioid receptor agonist is analgesic with a unique spectrum of activities in vivo.**

Bias and Beyond

Terry Kenakin Ph.D., Department of Pharmacology, UNC School of Medicine, Chapel Hill, NC

It is now evident that ligands for seven transmembrane receptors (7TMRs) can stabilize a range of tertiary conformations and that these can go on to differentially activate different cytosolic signaling proteins. This has the effect of 'biasing' the responses to these ligands toward certain signaling pathways over others. This effect can be used for therapeutic advantage (medicinal chemistry may be used to customize signaling repertoires of ligands) but also can lead to capricious potency ratios when cellular hosts utilize biased receptor stimuli in unpredictable ways. This presentation will review the current methods available to quantify signaling bias with scales that can be used to optimize these effects. A further discussion of these scales will consider their use to also quantify receptor selectivity and the effects of receptor point mutation. Secondly, this presentation will consider the 'elephant in the room' of cell-based perturbation of measured *in vitro* bias when ligands are tested *in vivo* (our *in vitro* scales may be meaningless for therapeutic predictions). Positive applications of cell-based bias will be discussed for screening and also the identification of pathology-specific signaling. Finally, concepts related to the use of bias signaling measurements in selection of drug candidates, with special reference to efficacy-dominant vs affinity-dominate bias, will be considered. These ideas naturally evolve to a relatively as yet unexplored pharmacological area of biased antagonism.

The Amazing Diversity of G Protein-Coupled Receptors

Raymond Stevens, Seva Katrich, Vadim Cherezov

Department of Biological Sciences and Chemistry, The Bridge Institute, University of Southern California, Los Angeles, California, USA

G protein-coupled receptors (GPCRs) constitute one of the largest protein families in the human genome and play essential roles in normal cell processes, most notably in cell signaling. After 20 years of technology innovation, the benefits of the technology efforts are now paying off. Delivering GPCR structure-function data in close collaboration with the community experts on specific receptor systems is of immense value to the basic science community interested in cell signaling and molecular recognition, as well as the applied science community interested in drug discovery. To date, we have determined high resolution structures of 18 of the 26 – notably the adrenergic, adenosine, histamine, dopamine, chemokine, S1P, opioid, serotonin, AT1, LPA1, class B (glucagon), C (mGluR1) and F (smoothened) receptor systems with new structures continuing to be deposited at a strong rate.

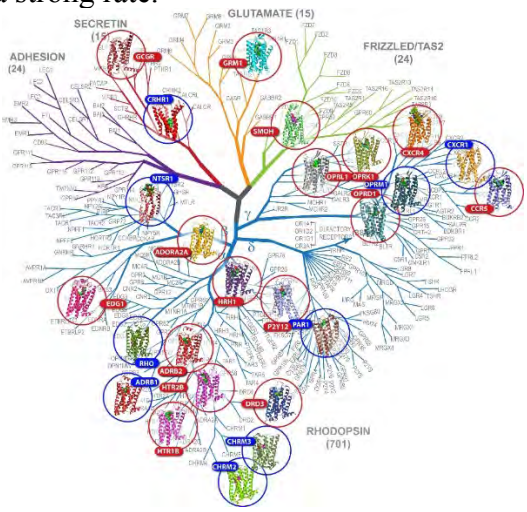


Fig. 1. GPCR phylogenetic tree with solved structures highlighted (adapted from V. Katritch, V. Cherezov, R.C. Stevens, Diversity and modularity of G protein-coupled receptor structures, Trends Pharm Sci 33 (2012) 17-27.

This work is being followed up with additional biophysical characterization including ^{19}F NMR spectroscopy/HDX to understand signaling pathways and community wide assessments with computational biology groups throughout the world. We are now observing both common and different structural features in these receptors. The scope of structural diversity of GPCRs at different levels of homology provides insight into our growing understanding of the biology of GPCR action and their impact on drug discovery. The latest high resolution structures are providing new insight including allosteric control of the receptors by cholesterol and sodium, as well as novel hypotheses of the receptors having channel or transporter activity. The rapidly expanding repertoire of GPCR structures provide a solid framework for experimental and molecular modeling studies, and helps to chart a roadmap for comprehensive structural coverage of the whole superfamily and a more thorough understanding of GPCR chemistry and biology. The most advanced drug discovery molecule connected to this effort is a $S1P_1$ allosteric agonist that has entered two Phase III clinical trials (RPC1063).

Through the generous support of an NIH P01 NIDA grant (DA035764), chemistry and pharmacology research on kappa opioid receptor with leading experts Bryan Roth, Ivy Carroll, and Charles Chavkin are further exploiting the structural data to better understand the selectivity and signaling of this important subfamily of GPCRs.

Katritch V[1], Fenalti GW[1], Giguere PM[2], Han, GW, Patel N[1], Cherezov V[1], Roth BL[2] and Stevens RC[1].

[1]Departments of Biological Sciences and Chemistry, Bridge Institute, University of Southern California, Los Angeles, California 90089, USA
[2]National Institute of Mental Health Psychoactive Drug Screening Program and Department of Pharmacology, University of North Carolina Medical School, Chapel Hill, North Carolina 27599, USA.

Conserved sodium site in class A GPCRs and its implications for κ-OR function

Allosteric effects of monovalent cations on agonist binding was discovered in opioid receptors more than 4 decades ago[1], and even harnessed to help differentiate opioid agonists from antagonists; however, the mechanism of this effect remained enigmatic. Recently, the sodium binding pockets have been characterized in high resolution crystal structures of adenosine A_{2A} and δ-opioid receptors[2,3], while structural bioinformatics and molecular modeling suggested functional importance of this highly conserved site for a vast majority of other class A GPCRs[4]. This talk will describe how these new atomistic insights advance our understanding of signaling mechanisms of κ-OR mediated by G-protein and arrestin pathways. We will also discuss novel approaches to modulating κ-OR signaling via residue mutations and bitopic small molecule ligands targeting the sodium binding site.

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

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Structural studies of the human kappa opioid receptor conformation states upon activation.

The crystal structure of the human kappa opioid receptor (hKOR) in complex with antagonist JDTic had been previously solved to 2.9 Å (Wu et al, 2012, Nature 485, 327-332). From this crystal structure key receptor-ligand interactions were observed and extended via modeling to GNTI, β-NNTA, and other hKOR ligands. However, a major hurdle still remains in elucidating the structure of hKOR in different states of activation. Having this information would lead to a more complete understanding of hKOR activation and function, informing design of more efficient analgesics with reduced side effects. Here we present our work in progress on the design of new hKOR constructs and characterization of their complexes with potent agonists, antagonists, and biased ligands.

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

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Structural basis for bi-functional peptides recognition at human opioid receptors

Bi-functional opioid receptor (OR) ligands represent potential therapeutic alternatives to alkaloid opiate analgesics with diminished side effects. We solved the structure of human δ-OR bound to the bi-functional δ-OR antagonist/μ-OR agonist tetrapeptide H-Dmt(1)-Tic(2)-Phe(3)-Phe(4)-NH_2 (DIPP-NH_2) solved by serial femtosecond crystallography at an X-ray Free-Electron Laser, revealing a *cis*-peptide bond configuration between H-Dmt(1) and Tic(2), and the structural requirements for DIPP-NH_2 recognition. Together with the previously reported structures of δ-, μ- and κ-ORs, the observed in this structure receptor-peptide interactions are critical for both understanding the pharmacological and selectivity profiles of opioid peptides, and the development of improved analgesics.

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Disclosure: The authors have no conflicts of interest to disclose

Design, synthesis, and biological evaluation of JDTic analogs

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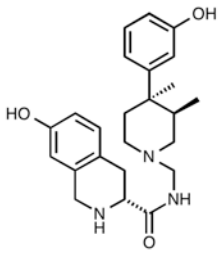
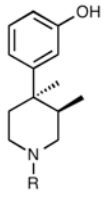
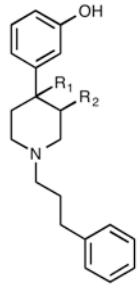
The design and discovery of JDTic as a potent and selective kappa opioid receptor antagonist used the *N*-substituted *trans*-3,4-dimethyl-4-(3-hydroxyphenyl)piperidine (**1**) pharmacophore as the lead structure. In a recent study we reported that *N*-phenylpropyl 4-methyl-4-(3-hydroxyphenyl)-piperidine (**2a**) where the 3-methyl group is removed from **1** and *N*-phenylpropyl-4-(3-hydroxyphenyl)-piperidine (**2b**), where both the 3- and 4-methyl groups were removed from **1** were still pure opioid receptor antagonists. In another recent study we reported that the replacement of the hydroxy group in the 4-(3-hydroxyphenyl) group with a hydrogen or fluoro group of JDTic gave compounds **3a** and **3b** both of which were potent and selective kappa opioid antagonists. As a continuation of our structural activity relationship studies directed toward the kappa opioid receptor as well as the development of potential pharmacotherapies for CNS disorders, compounds **4a-e** were synthesized and evaluated for their in vitro opioid receptor and ADME properties. A comparison of their in vitro antagonist activity and ADME properties to those of JDTic and compounds **3a** and **3b** will be presented.



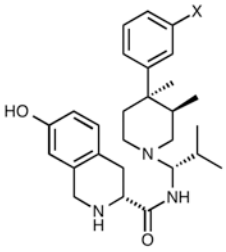
JDTic



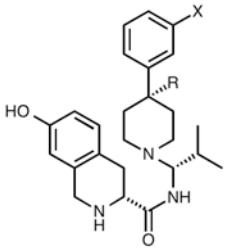
1



2a, R_1 = CH_3, R_2 = H
b, R_1 = R_2 = H



3a, X = H
b, X = F



4a, X = OH, R = H
b, X = H, R = H
c, X = F, R = H
d, X = OH, R = CH_3
e, X = F, R = CH_3

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Laura M. Bohn[1], Jenny Morgenweck[1], In-Jee You[1], Kimberly Lovell[1], Lei Zhou[1], Jamie Rose[2], Stephen Slauson[3], Euna Yoo[3], Kevin Frankowski[3], Thomas Prisinzano[3], Sara Jones[2], and Jeffrey Aubé[3]

Towards understanding the impact of biased agonism in vivo: an evaluation of the physiological effects of G protein/βarrestin2- biased KOR agonists.

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As part of a collaborative probe discovery program, we have developed a series of agonists at KOR with diverse pharmacological properties. Using cell-based assays to assess [^{35}S]GTPγS binding and βarrestin2 recruitment, we identified certain agonists that promote active signaling in G protein binding yet produce little activity for βarrestin2 recruitment (Zhou et al., 2013, JBC). After confirming that these compounds are stable and brain penetrant, we have compared their pharmacological profiles across different behavioral and physiological assays that are usually attributed to KOR activation and have asked what behavioral outputs are conserved and which differ, compared to U50,488H. In this presentation, we describe the results obtained with one of our high affinity, highly selective, triazole compounds. We find that its antinociceptive potency is similar to U50,488H as well as its ability to suppress an itch response induced by chloroquine phosphate. However, the compound does not suppress locomotor activity nor does it suppress the release of dopamine in nucleus accumbens (as tested by ex vivo cyclic voltammetry). By the time of the conference, we hope to report on its effects in conditioned place aversion studies. Overall, while we cannot fully predict what degree of bias will be necessary for in vivo physiologies to be separated; these early studies may provide direction in translating bias observed in cell-based assays to predicted physiological response profiles.
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The authors have no conflicts to disclose.

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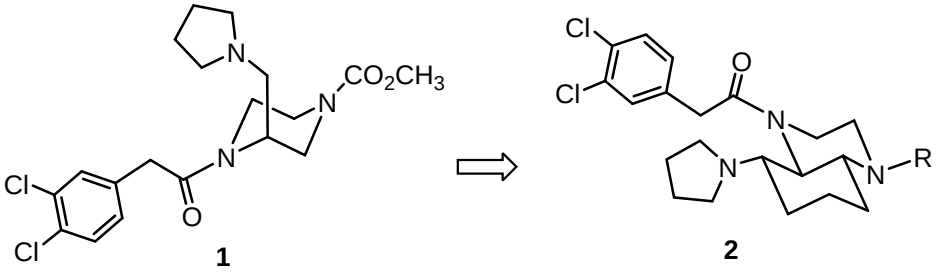
Conformationally restricted κ-opioid receptor agonists: Stereoselective synthesis and pharmacological evaluation

Strong analgesia results from activation of μ-, δ-, and κ-opioid receptors. The potent μ-opioid receptor agonists, which are clinically used as strong analgesics, are generally associated with severe side effects like respiratory depression, constipation, euphoria and development of tolerance and dependency. Activation of κ receptors is not associated with the dangerous μ agonist side effects, such as respiratory depression and addiction. However, κ agonists lead to centrally mediated dysphoria, sedation and strong diuresis. Therefore, our interest has been focused on κ agonists, which are restricted to the periphery by inhibition of the passage of the blood-brain barrier. Peripherally restricted κ agonists can be used for the treatment of pain as well as inflammatory and itching skin diseases.

The piperazine derivative **1** belonging to the ethylenediamine class of κ agonists (K_i = 0.36 nM) represents the starting point of this project. The conformational flexibility of the side chain will be restricted by incorporation into a bicyclic framework. The additional N-atom, which is located outside of the ethylenediamine κ-pharmacophore, allows the attachment of various substituents. Therefore, we hypothesized that this structural element should not affect the interaction with the κ-opioid receptor but the pharmacokinetic properties (e.g. passage of the blood brain barrier) of the novel ligands. In the Scheme the quinoxaline based κ agonists **2** derived from **1** are shown.



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

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Liability profiles and activity in different pain models of analogs of the macrocyclic tetrapeptide CJ-15,208.

The macrocyclic tetrapeptide CJ-15,208 (*cyclo*[Phe-D-Pro-Phe-Trp]) exhibits mixed opioid agonist/kappa opioid receptor (KOR) antagonist activity *in vivo* in the 55^{o}C warm water tail withdrawal antinociception assay and is a promising lead compound for developing novel therapeutics for the treatment of pain and drug abuse. We synthesized analogs of this lead peptide, varying the aromatic residues, to explore the structure-activity relationships for opioid receptor-mediated antinociception in multiple mouse models of pain, and to explore the potential liabilities (respiratory depression, hyperlocomotion and sedation) of analogs with different opioid activity profiles.

The series of analogs all exhibited significant opioid receptor-mediated antinociception in the 55^{o}C warm-water tail withdrawal assay, but differed markedly in their opioid antagonist activity. Of interest, one analog also produced antinociception in the acetic acid stretching and formalin assays, but exhibited no evidence of liability of use following oral administration (10 mg/kg p.o.). Two other peptides partially alleviated hyperalgesia in the chronic constriction injury (CCI) model of neuropathic pain following oral administration (10 mg/kg p.o.). In contrast, CJ-15,208 did not display significant antinociception at this dose in the CCI assay. Together, these data suggest that these novel opioid peptides hold promise as lead compounds for potentially safer new analgesics effective against multiple nociceptive modalities, including neuropathic pain.

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Disclosure: A patent application has been submitted on these compounds.

Quantification of kappa opioid receptor ligand potency, efficacy and desensitization using a real-time membrane potential assay

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We sought to explore the utility of the FLIPR Membrane Potential (FMP) assay as a method to assess kappa opioid receptor (KOR)-induced hyperpolarization resulting from coupling to G-protein-coupled inwardly rectifying potassium channels (GIRKs). The FMP blue dye was used to measure fluorescent signals reflecting changes in membrane potential in KOR expressing CHO (CHO-KOR) cells. Treatment of CHO-KOR cells with the kappa opioid agonists U50488 (IC_{50} = 0.25 ± 0.03 nM) or dynorphin (Dyn)-A(1-13) NH_2 (IC_{50} = 0.30 ± 0.03 nM) produced rapid and concentration-dependent decreases in FMP emission intensity reflecting membrane hyperpolarization. Both the nonselective opioid antagonist naloxone and the κ-selective blockers norbinaltorphimine (nor-BNI) and zyklophin produced rightward shifts in the U50488 concentration-response curves consistent with competitive antagonism of the response. The decrease in fluorescent emission produced by U50488 was blocked by overnight pretreatment with pertussis toxin (PTX, 100 ng/mL), indicating the requirement for PTX-sensitive G proteins in KOR activation of GIRKs. We directly compared the potency of U50488 and Dyn-A(1-13)NH_2 in the FMP and [^{35}S]GTPγS binding assays, and found that both were approximately 10 times more potent in the cellular fluorescence assay. The assay also allowed detection of acute KOR desensitization to the responses of U50488 and Dyn-A(1-13)NH_2. The maximum responses of both U50488 and Dyn-A(1-13)NH_2 declined following repeated additions over a 20 minute assay. We assessed the efficacy and potency of an array of structurally distinct KOR small molecule and peptide ligands. The FMP assay reliably detected small molecule partial agonists and stereoselectivity. Using KOR selective peptides with varying efficacies, we found that the FMP assay allowed high throughput quantification of peptide efficacy. These data demonstrate that the FMP assay is a sensitive and robust method for assessing κ-opioid receptor induced hyperpolarization, and represents a useful approach for quantification of the potency, efficacy and desensitization of KOR ligands.

The authors declare no conflict of interest.

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The novel, potent, short-acting kappa opioid receptor antagonist, LSN2444296, modulates mesoaccumbens dopamine neurotransmission and dopamine-mediated behaviors

Mounting evidence suggests that kappa opioid receptor antagonists play a critical role in stress and mood regulation. It is well-established that dopamine pathways are key components of established mood circuits in the brain, and that activation of kappa receptors, via stress or kappa receptor agonists, suppresses dopamine release in the nucleus accumbens (NAcc), a major terminal region of ventral tegmental area (VTA) dopamine neurons. Recently, improved pharmacological tools exhibiting canonical pharmacokinetic-pharmacodynamic (PK-PD) properties have become available which will facilitate interrogation of the neurobiological mechanisms by which kappa antagonists modulate stress and mood. We report here that LSN2444296 is a selective kappa opioid receptor antagonist, with rapid brain penetration and occupancy of kappa, but not mu or delta, opioid receptors in the brain, making it an excellent tool compound. We used in vivo electrophysiological and neurochemical techniques to demonstrate that although LSN2444296 did not strongly stimulate dopaminergic transmission by itself, it completely blocked kappa-agonist-induced suppression of VTA dopamine cell firing and subsequent dopamine release in the NAcc. Moreover, LSN2444296 demonstrated efficacy in several preclinical models known to be dependent on dopamine neurotransmission. Specifically, LSN2444296 blocked kappa-agonist-induced disruption of conditioned avoidance responding and prepulse inhibition. Further, LSN2444296 attenuated ethanol self-administration and the motivation to consume ethanol in Alcohol-Preferring (P) rats, without affecting general consummatory behavior. LSN2444296 blocked compulsive ethanol drinking in wild-type mice, but the effect was lost in mice lacking kappa opioid receptors. Together, the data provide evidence that LSN2444296 modulates dopamine neurotransmission and exhibits canonical PK-PD properties, making it an excellent preclinical tool compound for evaluating kappa opioid receptor pharmacology.

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Disclosure: All authors are employees of, and shareholders in, Eli Lilly and Co.

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The long-lasting reduction in KOR function by norBNI in peripheral sensory neurons requires protein synthesis

KOR couples to a variety of intracellular signaling cascades, including inhibition of adenylyl cyclase (AC), activation of Extracellular Signal-Regulated Kinase (ERK), and activation of c-Jun N-terminal Kinase (JNK). Previous studies with norbinaltorphimine (norBNI) show that this selective KOR "antagonist" is an "agonist" for activation of JNK. Activation of JNK by norBNI results in a long-term reduction in KOR function in HEK cells and in brain following a single administration. Similarly, we have found that local injection of norBNI into the rat hindpaw leads to long-lasting, JNK-mediated, reduction of KOR function in peripheral sensory neurons. Using a rodent behavioral model of thermal nociception, we found that peripheral KOR-mediated antinociception in the ipsilateral paw was abolished 2 and 7 days following a single intraplantar (i.pl.) injection of norBNI. The KOR responsiveness of the contralateral paw was unaffected. The long-term inhibition of KOR-mediated antinociception was completely abolished by the JNK inhibitor, SP600125, injected i.pl., prior to norBNI. Long-term inhibition of KOR function by norBNI also occurred when norBNI was applied directly to adult rat peripheral sensory neurons in culture (ex vivo). Treatment of neuronal cultures with norBNI for 1h, followed by washing, abolished KOR-mediated inhibition of AC activity (in a JNK-sensitive manner), however KOR-mediated activation of ERK was unaffected.

Since JNK is a well-known activator of transcription factors, ultimately leading to protein synthesis, we sought to determine if protein synthesis was required for the long-term effects of norBNI. In hindpaws treated (i.pl.) with the protein synthesis inhibitor, cycloheximide (CHX), prior to norBNI administration (i.pl.), the long-term effect of norBNI on peripheral KOR-mediated antinociception was completely abolished. Similarly, the inhibitory effect of norBNI on KOR-mediated inhibition of AC activity in peripheral sensory neuron cultures was abolished completely with CHX pretreatment. Additionally, inhibiting protein translation with rapamycin, an mTOR inhibitor, also completely abolished the long-term effects of norBNI in vivo and ex vivo. Taken together, these results suggest that activation of JNK by norBNI leads to increased protein synthesis of an unknown protein that subsequently leads to prolonged reduction in some, but not all, KOR functional responses in peripheral sensory neurons. Moreover, these data provide strong evidence that norBNI is a protean ligand that has powerful capacity to regulate peripheral KOR function.

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

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Proteomic analysis of norBNI regulated KOR interacting proteins

NorBNI treatment was previously shown to produce long-lasting KOR inactivation through a JNK-mediated mechanism (Bruchas 2007, Melief 2010, Melief 2011). To identify KOR-interacting proteins regulated by norBNI, stable isotope labeling by amino acids in cell culture (SILAC) in combination with mass spectrometry was used to analyze myc-immuno-isolates from mycKOR expressing HEK293 cells. For this study, the comparison groups were cells treated with 10μM norBNI 5 hr, vehicle 5hr, and cells treated with norBNI but co-incubated on anti-myc beads with excess myc peptide as a control for nonspecific hits. KOR was identified with a posterior error probability (PEP) of $1.9x10^{-10}$. KOR was enriched ~3-8 fold relative to negative controls. Proteins significantly enriched by 30% or greater, as calculated by the quantitative proteomics p-value calculator (QPPC). In addition to KOR, 65 proteins were identified as basal KOR interactors. Gαi2/3, Gβ1, and TFCP2 were enriched in norBNI treatment. Other identified proteins not altered by norBNI treatment included TSC1, TSC2, and TBC1D7 (components mTOR regulatory complex), Lyn (tyrosine kinase), RAPGEF2 and RAPGEF6 (GEFs), and various endoplasmic reticulum and golgi proteins. To confirm the finding that Gαi was increased by norBNI in mycKOR pulldowns, as analyzed by mass spectrometry, flag-Gαi3 expressing HEK293 cells were transiently transfected with mycKOR, and treated with vehicle, norBNI 5hr, naloxone 5h, or 500nM SP6100025 (JNK inhibitor) + norBNI 5 hr before cell lysis and extracting KOR complexes by immunoprecipitation with anti-myc. Western blot analysis indicates a 50% increase in Gαi immunoprecipated with KOR under norBNI treatment alone but not naloxone or norBNI combined with SP6100025. In the complementary coIP with flag-GNAI3, an SP6100025-sensitive increase in KOR immunoreactivity is also observed following norBNI. To investigate the possibility that Gαi may be a JNK substrate with receptor association regulated by phosphorylation, we used an in vitro kinase assay to assess the ability of isolated JNK1 enzyme to induce phosphorylation of flag-Gαi3 immunoprecipitates. Phosphorylation was measured by immunoblotting for pan-phospho-Ser/Thr. No evidence of Gαi phosphorylation was observed, but JNK induced phosphorylation of higher molecular weight proteins in the immunoprecipitate was observed. A phospho-band of similar size was also observed in flag-Gαi3 immunoprecipitates of cells treated with norBNI. This study has identified several potential members of the KOR signalosome. Additional studies will be required to validate these proteins and identify their potential roles in KOR function. This study further identified Gαi as a potential target of JNK regulation via phosphorylation of an unidentified interacting protein. Future studies will assess the functional role of these changes for G protein signaling.

Funded by NIH grants DA035764 and DA020570.

The authors declare no competing financial interests.

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Allosteric interactions within DOR-KOR heteromers in peripheral sensory neurons.

G-protein coupled receptor (GPCR) heteromers often display unique pharmacological properties, signaling characteristics and anatomical specificity. Thus, GPCR heteromers may provide unique therapeutic targets for a variety of disease states. We have reported previously that DOR-KOR heteromers are expressed in peripheral nociceptors (i.e., pain-sensing neurons) and, when activated, produce robust antinociceptive responses (Berg et al., 2012, *Mol Pharmacol* 81:264-272). Further, selective KOR antagonists differentially alter the potency and efficacy of DOR agonists in both a rat behavioral model of thermal nociception and in primary cultures of peripheral nociceptors (i.e., ex vivo model). Here we sought to further demonstrate that DOR-KOR heteromers are functional in nociceptors by examining bidirectional allosteric interactions between protomers of DOR-KOR heteromers. First, we determined if the selective DOR antagonist naltrindole (NTI) altered responses to the KOR agonist ICI 199441 ex vivo and in vivo. In primary cultures of rat peripheral sensory neurons, ICI 199441- mediated inhibition of PGE_2-stimulated cAMP accumulation was determined in the presence and absence of NTI (2nM, 100 x Ki). Neither basal nor PGE_2-stimulated cAMP levels were altered by NTI, however, the concentration response curve to ICI 199441 was shifted to the left by more than 100-fold; the EC50 for ICI 199441 was 15 nM versus 0.12 nM in the absence or presence of NTI, respectively (n=4). Similarly, NTI shifted the dose response curve for ICI 199441 -mediated inhibition of PGE_2-induced thermal nociception to the left by 10-fold; the ED_{50} ICI 199441 was 2.7ug versus 0.24ug in the absence or presence of NTI, respectively (n=6 animals per group). Next, we tested if DOR occupancy by the antagonist, NTI altered responses to a different KOR agonist, U50488. Consistent with the ligand-dependence of allosteric interactions, we found that NTI did not alter U50488 –mediated responses ex vivo nor in vivo. However, occupancy with the DOR antagonist 7-Benzylidenenaltrexone (BNTX) enhanced U50488-mediated inhibition of PGE_2-induced thermal nociception by 100-fold.

In summary, we have found that in peripheral sensory neurons, KOR antagonists differentially regulate DOR agonist responses and DOR antagonists differentially modulate KOR agonist responses both in vivo and ex vivo. This demonstration of ligand-dependent, bidirectional allosteric interactions between the protomers of DOR-KOR heteromers provides further support for functional DOR-KOR heteromers in peripheral nociceptors. We propose that the DOR-KOR heteromer may be a valuable target for pain pharmacotherapy.

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

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Characterization of Kappa Opioid Receptor Antagonists for the Treatment of Aberrant Positive and Negative Valence Domains

Given the limited progress in discovering novel treatments for psychiatric disorders, there is a drive to place greater emphasis on the pathophysiology of psychiatric diseases. The NIMH has launched the RDoC initiative which considers domains of function and how these are affected in different disorders. The five domains are Negative Valence Systems, Positive Valence Systems, Cognitive Systems, Systems for Social Processes, and Arousal/Regulatory Systems. The kappa opioid receptor (KOR) system has been shown to modulate many of these domains in humans and animals. In particular, KOR agonists such as salvinorin A produce dysphoria, cognitive impairment and anxiety symptoms. Stressors cause release of dynorphin, and subsequent activation of KORs. Conversely, KOR antagonists have been reported to improve mood and reduce anxiety.

To test the effects of KOR antagonists on negative valence we have used an in vivo electrophysiological assay in anesthetized rats to measure brainstem stimulated hippocampal theta oscillations. PF-04455242 (10 mg/kg, sc) decreased the frequency of theta oscillations in a dose-dependent manner, comparable to the benzodiazepine anxiolytic, diazepam (0.32 mg/kg, sc). As a measure of positive valence, the effects of KOR ligands were evaluated in the progressive ratio assay. PF-04455242 (17.8 mg/kg, sc) or LY2456302 (0.1 mg/kg, sc) fully reversed the deficit in motivation caused by the KOR agonist, spiradoline (3.2 mg/kg, sc). To incorporate an aspect of disease relevance paradigms involving stress effects in preclinical models of substance use disorders have also been assessed. The KOR antagonist, LY2456302 (0.1 -3 mg/kg, sc) attenuated stress-induced reinstatement of fentanyl seeking in rats, indicating potential benefit of KOR antagonism in relapse prevention.
Overall these data support the potential broad efficacy of selective KOR antagonists in treating deficits in negative and positive valence which can be tested in transdiagnostic early clinical development studies.

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Disclosures: All authors are current or former Pfizer employees.

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Possible antidepressant effects of kappa opioid receptor agonists following social defeat stress

The aversive properties of kappa opioid receptors (KOR) are well established, and there is strong evidence that psychosocial stress induces activation of KOR. Activation of KOR also mediates behavioral responses to stress, which suggests that KOR antagonists could have important therapeutic properties. In addition to short term effects on brain and behavior, psychosocial stress also induces long term changes in brain function that can take several days to manifest. Evidence from several sources suggest these long term changes have important implications for KOR function. We examined the effects of social defeat stress in California mice, a monogamous species in which social defeat can be applied in both males and females. Our studies suggest that two weeks after social defeat stress, the behavioral effects of KOR activation are profoundly altered in females but not males. A dose of the KOR agonist U50,488 that induced place aversion in control females did not induce aversion in stressed females. Further, in the social interaction test U50,488 had antidepressant-like effects when administered to females exposed to social defeat. One possible mechanism contributing to this effect is KOR modulation of serotonergic signaling. Previous studies in males showed that KOR activation inhibited serotonergic activity. Serotonin neurons in the dorsal raphe stained for phosphorylated extracellular signal regulated kinase (pERK) as an indirect marker of cellular activity. Cell count data suggest that females exposed to defeat have hyperactive serotonin neurons and that acute U50,488 treatment blunts this effect. These data suggest that KOR agonists could be beneficial for counteracting long term effects of psychosocial stressors.

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The authors have nothing to disclose.

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Sex differences in the depressive-like effects of kappa opioid receptor activation in rats

There are pronounced sex differences in behavioral responses to stress. For example, females are more sensitive to the aversive effects of drugs of abuse and stress-induced relapse. Using intracranial self-stimulation (ICSS), we previously found that gonadally intact female rats are less sensitive than males to the depressive-like effects of the KOR agonist U-50,488, regardless of estrous cycle stage. U50,488 induced sex-dependent elevations in c-Fos expression in the paraventricular nucleus of the hypothalamus (PVN) and the bed nucleus of the stria terminalis (BNST), two stress-responsive regions that express corticotropin releasing factor (CRF). We hypothesized that the effects of KOR activation on reward depend on interactions between circulating gonadal hormones and CRF. To examine the activational effects of gonadal hormones on aversive responses to U50,488, we gonadectomized male and female rats that had previously been trained in ICSS. After five weeks, during which plasma sex hormones decreased baseline ICSS responding was similar across groups (male and female, gonadectomized and sham). 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 quantitative real-time RT-PCR, we found higher basal levels of prodynorphin mRNA in the female PVN, BNST, and amygdala, and lower KOR mRNA in the BNST. Finally, levels of CRF receptor 1 (CrfR1) mRNA were lower in the amygdala and BNST of intact female compared to male rats. These findings raise the possibility that elevated dynorphin tone in females occludes the effects of KOR agonists, and that KOR-mediated activation of CRF systems is blunted in females due to decreased CrfR1. This underscores the importance of understanding KOR function in both sexes such that pharmacotherapeutics targeting mood disorders can be rationally designed.

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Taylor AMW[1], Arnold A[2], Cahill CM[3], Evans CJ[1]

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Kappa-opioid mediated antinociception can be stress-mediated and some agonists show marked sex differences.

Kappa opioid agonists are both aversive and analgesic. Previously, we have shown, using a low-stress protocol and tail immersion assay at 49^{o}C, that the antinociceptive effects of systemic U50,488H is partially mediated via a stress-induced mechanism (Taylor et al 2014 Br J Pharmacol). This kappa-induced antinociception could be blocked pre-treatment with a delta opioid agonist, SNC80, and another anxiolytic, diazepam. IBNtxA is a derivative of naltrexone that has been reported to produce analgesia in the absence of reward via interaction with a six-transmembrane mu opioid receptor splice variant generated from exon 11 and exon 2 (Majumdar et al 2011 Proc Natl Acad Sci U S A). IBNtxA also has exceptionally high affinity for the kappa opioid receptor (Grinnell et al 2014 J Pharmacol Exp Ther). The present study uses the low-stress tail immersion protocol to determine the contribution of kappa-mediated effects produced by IBNtxA.

We found that systemic IBNtxA (1mg/kg, i.p.) produced robust antinociception in male C57BL/6J mice. Pretreatment with the kappa opioid antagonist, JDTic, effectively blocked IBNtxA mediated thermal antinociception. We next tested IBNtxA antinociception in male C57Bl/6 mice lacking the exon 2 of the mu opioid receptor (MOP KO). In MOR KO male mice, IBNtxA antinociception was only slightly reduced, but again was completely blocked by pretreatment with JDTic in these null mice. Likewise, in a chronic pain state, IBNtxA (1mg/kg, i.p.) attenuated both mechanical allodynia and thermal hyperalgesia. Pretreatment with JDTic effectively blocked this IBNTxA analgesia in chronic pain animals. Furthermore, IBNtxA was mildly aversive in both control and chronic pain animals. Interestingly, female WT mice did not exhibit IBNtxA-mediated antinociception in either acute or chronic pain models. We next explored the nature of the sex difference to IBNtxA antinociception. We used a genetic approach (Four Core Genotype model) in which the testis-determining gene, *Sry*, is removed from the Y chromosome to an autosome producing XX and XY mice with testes and XX and XY mice with ovaries. IBNtxA antinociception was different between XX and XY mice with the same gonad, either testes or ovaries, but not different between mice with different gonads but the same sex chromosomes. The result suggests the sex difference in IBNtxA antinociception is due to chromosomal, rather than hormonal, effects.

Our data suggest that using a low-stress antinociceptive assay IBNtxA appears to act as a potent kappa opioid agonist without requirement of exon 2 of the mu opioid receptor locus. This identifies potential kappa-mediated analgesic mechanism for this compound.

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Genome-wide approaches for interrogating GPCRs

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In this talk I will focus on two emerging areas which are revolutionizing my lab's understanding of GPCRs in health and disease.

The first part will be devoted to a genome-wide technology my lab has developed which facilitates the unbiased interrogation of the druggable (e.g. non-olfactory) GPCR-ome. By way of background I will briefly review studies my lab has recently completed which show that a large proportion of the druggable GPCR-ome is understudied from biological and chemistry perspectives. I will then describe our new open-source resource which will allows virtually any lab the ability to elucidate the potential actions of perturbants (e.g. small molecules, biologics, genomic technologies) on the entire GPCR-ome in an unbiased fashion. I will show how this platform has allowed us to discover a new family of GPCRs which are modulated by opioid ligands. This platform has also allowed us to uncover a heretofore subterranean pharmacology associated with FDA approved medications. The implications of these findings for enhancing drug discovery will be highlighted.

The second part of the talk will be devoted to a genome-wide approach we have adapted which facilitates the unbiased elucidation of kinase activation following cellular perturbation (e.g. via small molecules, biologics and genomic technologies). Using a serotonin receptor as an initial model system I will show how large families of kinases show differentially modulated activity following activation by unbiased and highly functionally selective ligands. I will show that our previous understandings of ligand-receptor-signaling networks merely scratches the surface with regard to potential signaling nodes essential for the actions of GPCRs and other signaling molecules. The implications of these findings for basic and translational research will be highlighted.

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Saxon, A.,[1,2] Ling, W.,[3] Mooney, L.,[3] Hillhouse, M.,[3] Thomas, C.,[3] Hasson, A.,[3] Ang, A.,[3] Nielsen, D.[4,5]

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Cocaine Use Reduction with Buprenorphine (CURB) Preliminary Study Findings

Buprenorphine is the only medication approved for human use that has kappa antagonist properties. Therefore, this study, conducted under the auspices of the National Drug Abuse Treatment Clinical Trials Network, investigated the safety and effectiveness of buprenorphine/naloxone (BUP, as Suboxone®) provided on a platform of extended-release injectable naltrexone (XR-NTX, as Vivitrol®) for reducing cocaine use in participants who met DSM-IV criteria for cocaine dependence and past or current opioid dependence or abuse. With naltrexone blockade of the mu receptor, buprenorphine becomes a *de facto* kappa antagonist. This multi-centered, double-blind, placebo-controlled study provided XR-NTX to all participants before random assignment to one of three daily BUP conditions: 4mg BUP (BUP4, n=100), 16mg BUP (BUP16, n=100), and no BUP/placebo (PLB, n=102). Participants received pharmacotherapy for 8 weeks, with thrice-weekly clinic visits for observed dosing, provision of take-home medication, urine drug screening (UDS), and assessments. Follow-up assessments occurred at 1 and 3 months post-intervention. Planned primary analyses of self-reported days of cocaine use during the last 4 weeks of medication corrected by UDS found no difference in cocaine use between placebo (m=7.7) and each BUP group (BUP4 [m=6.6] vs PLB, p=0.143; BUP16 [m=7.2] vs PLB, p=0.165). Longitudinal analysis of number of cocaine negative UDS during last 4 weeks using GEE found a significant difference between BUP16 (m=5.8) and PLB (m=4.9; p=0.030), but no difference between BUP4 (m=5.5) and PLB (p=0.529). No differences across groups were found for adherence, retention, or adverse events. Days of self-reported opioid use decreased from baseline during the active medication period and throughout the follow-up interval. DNA was obtained from 277 participants. Eight genes were genotyped for a total of 18 variants. Treatment response was evaluated by the percent cocaine negative urines per total possible urines over each 2 week interval of the full 8 week treatment period (treatment effectiveness score, TES). An increase in TES was observed in BUP16, compared to PLB, but not in BUP4. Using repeated measures ANOVA, significant interactions of variant x treatment were observed for two variants of the *preprodynorphin* (*PDYN*) gene (experiment-wise P = 0.0022 for rs1022563 and P = 0.024 for rs1997794). BUP16 increased TES from 21% to 54% and PLB increased TES from 35% to 37% in the *PDYN* rs1022563 A allele-carrier group. No difference was observed in the GG genotype group between PB and BUP16. The combination of XR-NTX and BUP was acceptable to participants and well tolerated without evidence of increased opioid use after discontinuation. Though the pre-specified primary outcome did not demonstrate an effect of buprenorphine on cocaine use, a modest signal for possible efficacy was detected in secondary analyses, and this efficacy was significantly associated with gene variants known to be relevant to kappa opioid activity.

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Ehrich E.

Alkermes, Inc., Waltham, MA

Evaluation of Balanced Agonist-Antagonist Opioid Modulation with ALKS-5461 in Major Depressive Disorder

Major depressive disorder (MDD) has been associated with dysregulation of the endogenous opioid system. Opioid dysregulation, however, is not addressed by existing antidepressant medications. ALKS 5461, a balanced agonist antagonist opioid modulator combines buprenorphine (BUP), a μ opioid partial agonist and κ opioid partial agonist with low intrinsic activity, together with samidorphan (SAM), a potent μ opioid antagonist. ALKS 5461 is intended to treat endogenous opioid dysregulation while avoiding the key limitation of exogenous opioids, their potential for abuse and dependence. The safety and efficacy of ALKS 5461 was evaluated as adjunctive therapy in subjects with MDD and an inadequate response SSRI or SNRI therapy. In both initial pilot study and a follow-up phase II study utilizing sequential parallel comparison design (SPCD), ALKS 5461 demonstrated statistically significant and clinically important antidepressant efficacy versus placebo as assessed by the Hamilton and Montgomery–Åsberg Depression Rating Scales (HAM-D17, MADRS). ALKS 5461 was well tolerated with the most common adverse events being nausea, vomiting, and dizziness. There was no evidence of opioid withdrawal following treatment. Overall the results indicate balanced agonist-antagonist opioid modulation with ALKS 5461 is a novel and promising new treatment approach for patients with an inadequate response to standard antidepressants. Large phase III studies are ongoing.

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CR845, a novel peripherally-acting kappa opioid receptor agonist, has low abuse potential compared with pentazocine

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CR845, a potent, peripherally-acting, selective kappa opioid receptor agonist, is being developed for the treatment of acute and chronic pain. This study examined the relative abuse potential of CR845 compared to placebo or pentazocine, a schedule IV opioid analgesic. Recreational polydrug users with experience with opioids and hallucinogenic agents were enrolled in this single-center, randomized, double-blind, active- and placebo-controlled study. Thirty-nine subjects received a single bolus IV dose of the following 4 treatments in random order: CR845 5 mcg/kg (therapeutic dose), CR845 15 mcg/kg (supra-therapeutic dose), placebo, and pentazocine 0.5 mg/kg. Treatments were separated by 48-hour washout period. Drug Liking bipolar Visual Analog Scale (VAS) was the primary measurement, and was assessed periodically between 5 minutes and 8 hours after dosing. This trial met the primary endpoint with drug liking scores for pentazocine significantly greater than that of placebo and either doses of CR845 (P<0.0001 for each comparison to pentazocine). The least squares mean for the maximum drug liking VAS (E_{max}) scores (±standard error of LS mean) were 87.6±1.9 for pentazocine, 65.3±1.9 for CR845 5 mcg/kg, 66.9±1.9 for CR845 15 mcg/kg, and 52.4±1.9 for placebo, indicating that both doses of CR845 had significantly lower drug liking response compared to pentazocine. Additional bipolar VAS measurements were lower for CR845 compared with pentazocine for “overall drug liking” (P<0.0001 for both doses of CR845) and “take drug again” (P<0.0003 for CR845 5 mcg/kg and P<0.0001 for CR845 15 mcg/kg). These VAS scores for CR845 were equivalent for both doses and were similar to those of placebo. These results suggest that the novel and selective peripherally acting kappa agonist CR845 presents a low risk for abuse liability in humans.

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Imaging kappa receptors in cocaine abuse

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Postmortem studies in cocaine dependence have shown that the kappa receptor and dynorphin are upregulated in the human brain, and animal studies show that binge cocaine administration results in a significant upregulation of dynorphin. Using the kappa receptor PET radiotracer ([11C]GR103545), we investigated the kappa receptor system in human cocaine abusers and healthy controls. Both cocaine abusers and healthy controls underwent PET scans to measure kappa receptor binding (volume of distribution, Vt). Our hypothesis was that cocaine abusers would have a higher kappa receptor availability compared to the control group. After this first scan, the cocaine abusers underwent cocaine self-administration sessions following a stressor (cold pressor test), in order to investigate the correlation between kappa receptor Vt and stress-induced choices for cocaine. The cocaine abusers then self-administered binge doses of cocaine (300mg/day for 3 days) and were scanned a second time with [11C]GR103545. We hypothesized is that kappa receptor BPND will be reduced following the cocaine binge.

Data is presently available for 14 cocaine abusers and 10 controls. The cocaine abusers were heavy, chronic users of smoked cocaine, and the controls were matched for age, gender, ethnicity, and smoking status. The results of the study are as follows. 1) No difference in [11C]GR103545 Vt in any brain region was seen between the cocaine dependent and healthy control subjects. 2) A positive correlation was seen between kappa receptor Vt in the striatum and stress-induced cocaine self-administration: higher values of Vt were associated with more choices for cocaine following the cold pressor test. 3) In the cocaine dependent group, a significant decrease (-17. 7% ± 2.3%) in Vt was measured in the stratum when comparing the baseline and post-binge scans. Similar decreases were seen in other brain regions (-12.9% ± 5.1%)

Therefore, contrary to our hypothesis, we did not see a difference in kappa receptor Vt between the cocaine abusers and healthy control subjects. However, there was a positive correlation between striatal kappa receptor Vt and cold-pressor induced cocaine self-administration, showing that higher kappa receptor binding is associated with a greater vulnerability to stress induced cocaine consumption. Lastly, the decrease in [11C]GR103545 measured following binge dosing of cocaine suggests that levels of dynorphin are significantly increased in this condition, which replicates in humans the finding that cocaine increases brain levels of dynorphin.

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The G-protein biased kappa opioid receptor agonist 6'-GNTI blocks paroxysmal discharges without inducing aversion

With a prevalence of 1–2%, epilepsies belong to the most frequent neurological diseases worldwide. Although antiepileptic drugs are available since several decades, the rate of patients refractory to medication is still over 30 %. The antiepileptic potential of kappa opioid receptor (KOR) agonists is known since the early 1980s. However, clinical use of their potential was hampered by dysphoric side effects. b-arrestin activation was suggested to mediate the aversive effects of KOR activation. In recent years, G-protein biased KOR agonists became available, which show little activation of the b-arrestin pathway in-vitro. However, in-vivo data for such agonists are missing.

Therefore, we investigated the effects of the full KOR agonist U-50488H and the G-protein biased KOR agonist 6'-GNTI in models of acute seizures and drug resistant temporal lobe epilepsy and in the conditioned place preference test. Like previously shown for U-50488H, 6'-GNTI markedly increased the threshold for pentylenetetrazole induced seizures in prodynorphin deficient mice. This effect was competitively blocked by the KOR antagonist 5'-GNTI. Subchronically epileptic (unilateral intrahippocampal injection of kainiate) mice displayed significant reduction of paroxysmal discharges upon treatment with 6'-GNTI (10 - 30 nmoles, icv) or U-50488H (6 - 20 mg / kg, ip). Moreover, 6'-GNTI did not induce conditioned place avoidance, while U-50488H did.

Our data provide the proof of principle that anticonvulsant / antiepileptic and aversive effects can be pharmacologically separated. Thus, G-protein biased KOR agonists may open a novel avenue in the treatment of drug resistant epilepsies.

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PPL-101 a high affinity kappa partial agonist is a potent analgesic with low abuse potential but without dysphoria

PPL-101 (α-methyl-cyclopropylmethyl-normorphine) is an opiate derivative that binds with high affinity to mu and kappa receptors, with slightly lower affinity to delta receptors. The α-methyl constituent produces two diasteriomers and constrains the cyclopropylmethyl moiety into an R or S configuration. Previous studies have demonstrated that the R configuration has higher affinity for the opiate receptors. [^{35}S]GTPγS binding studies have indicated that PPL-101 has partial agonist activity at kappa receptors, with lower efficacy at delta and very low efficacy (roughly 10%) at mu receptors. This compound is a potent analgesic with an ED_{50} in tail flick of less than 1 mg/kg, approximately 3 times more potent than morphine. Despite kappa agonist activity, PPL-101 induces no dysphoria and in fact, displays a significant CPP in mice at 3 mg/kg. In contrast to CPP data, in rats PPL-101 was not self-administered using either fixed ratio or progressive ratio schedules. PPL-101 also blocked morphine self-administration at 3 mg/kg. In order to determine whether PPL-101 was not self-administered because kappa agonist activity blocked mu reward or because mu efficacy was too low to mediate the reward, self-administration studies were carried out in the presence of JDTic to block kappa receptor activity. A single dose of JDTic (10 mg/kg) neither influenced morphine self-administration nor induced self-administration of PPL-101, suggesting the mu efficacy is too low to induce reward. When combined with older studies demonstrating that PPL-101 substituted for morphine and thereby blocked withdrawal symptoms in addicted monkeys these results demonstrate that PPL-101 has an unusual profile that could be ideal as an analgesic with low abuse potential or potentially as a drug abuse medication.

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Genetic dissociation of the analgesic and aversive actions of the kappa drug U50,488H

Kappa receptors are one of the three members of the opioid receptor family. Like the others, compounds acting through kappa receptor are able to elicit analgesia. However, kappa drugs have a number of undesirable side-effects that have seriously limited their clinical utility. Thus, dissociating their analgesic actions from the others would facilitate efforts to develop kappa agents. U50,488H analgesia is lost in animals with the disruption of the kappa receptor KOR-1, but retained in an exon 1 mu receptor knockout (E1/MOR-1 KO), establishing the importance of the kappa receptor, but not the traditional mu receptors, in its analgesic actions since morphine was inactive in the E1/MOR-1 KO mice. While the disruption of exon 1 in the E1/MOR-1 KO eliminated all the full length traditional mu receptors, a series of truncated variants associated with exon 11 continued to be expressed. We recently generated an exon 11 mu receptor knockout mouse (E11/MOR-1 KO). While the exon 11-associated variants were lost, the mice continued to express full length traditional MOR-1 variants and morphine analgesia was retained in these animals. Although morphine was active, U50,488H analgesia was lost in the E11/MOR-1 KO mice following either subcutaneous, supraspinal or spinal administration. Unlike analgesia, U50,488H aversion in a conditioned place preference assay and its locomotor activity were retained in the E11/MOR-1 KO mice. These observations suggest that U50,488H analgesia, but not its aversive activity, is dependent upon expression of truncated exon 11-associated mu receptor splice variants.

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Nalfurafine shows lower propensity to cause aversion in mice and produces lower KOPR phosphorylation than U50,488H and MOM-Sal B

KOPR agonists are potentially useful as antipruiritic agents, analgesics and water diuretics; however, their development has been limited by dysphoria. Nalfurafine, U50,488H and methoxymethyl salvinorin B (MOM-SalB) are structurally distinct selective KOPR agonists. Nalfurafine is used in Japan for treatment of uremic pruiritis in hemodialysis patients; however, surprisingly, at the therapeutic doses, nalfurafine does not produce dysphoria. Here we examined the three compounds for inhibition of compound 48/80-induced scratching, antinociceptive effect in the formalin test and aversive effect in conditioned place aversion (CPA) in male CD-1 mice to determine if there were dose differences among the three tests for each compound. The three compounds produced antinociception and anti-scratching effects in dose-dependent fashion with antinociception A_{50} values of 5.8 μg/kg (nalfurafine), 17 μg/kg (MOM-SalB) and 0.58 mg/kg (U50,488H)and the corresponding A_{50} values for antiscratching effect of 8.0 μg/kg, 70.2 μg/kg and 2.07 mg/kg, respectively. Significant aversion was seen at all doses of MOM-Sal B (10-300 μg/kg) and U50,488 (0.25-10 mg/kg), whereas nalfurafine only produced significant aversion at the 20 μg/kg. Thus, U50,488H and MOM-SalB produced CPA at lower doses than those required for antinociceptive and anti-scratching effects, whereas the reverse is true for nalfurafine. In neuro2A mouse neuroblastoma cells expressing the mouse KOPR (mKOPR), the three compounds were full agonists in promoting [^{35}S]GTPγS binding. While U50,488H and MOM-SalB were full agonists in promoting KOPR internalization, nalfurafine showed lower efficacy. All three compounds induced mKOPR phosphorylation at S356/T357, T363 and S369, but nalfurafine caused T363 phosphorylation to a much lower extent. These results indicate that nalfurafine acts on the KOPR in a different manner from U50,488H and MOM-SalB, which may account for the unique in vivo pharmacological profile. In addition, our mouse models may be useful for screening for KOPR agonists with lower propensity to cause dysphoria.

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Dezocine and buprenorphine reduces withdrawal syndrome in a morphine dependent rat model

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Abstract: Opioid dependence continues to be a major public health issue without optimal therapeutic. Based on our recent discovery of the unique pharmacological profile of dezocine as a non-addictive opioid, a partial mu agonist and kappa antagonist, similar to that of buprenorphine, we hypothesized that dezocine could be used to manage opioid dependence. In the present study, the effects of dezocine and buprenorphine on morphine withdrawal syndrome were compared in a rat morphine dependence model. Daily intraperitoneal injection of dezocine markedly reduced morphine withdrawal syndrome similar to that of buprenorphine. Astrocyte activation in nucleus accumbens after opioid exposure was observed in the morphine dependent rats, and such astrocyte activation was significantly inhibited in the presence of dezocine and buprenorphine. The molecular target profiling for both dezocine and buprenorphine was performed. Dezocine interact with sigma 1 receptor, while buprenorphine has no interaction with sigma 1 receptor. These findings suggested that dezocine could be an alternative medication for opioid addiction management similar to that of buprenorphine. The advantage of dezocine over buprenorphine for opioid dependence management is proposed.

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Kappa opioid receptors and stress-induced enhancement of ethanol dependence-related drinking in C57BL/6J mice

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Our laboratory has previously demonstrated that daily forced swim stress (FSS) prior to ethanol drinking sessions facilitates enhanced ethanol consumption in mice with a history of chronic intermittent ethanol (CIE) vapor exposure (a model of ethanol dependence) without altering ethanol intake in air-exposed controls (CTL). Because both stress and chronic ethanol exposure can influence the dynorphin/kappa opioid receptor (KOR) system, the present series of experiments was designed to explore a potential role for KORs in modulating stress effects on ethanol consumption in the CIE model of dependence and relapse drinking. After stable baseline ethanol intake was established in adult male C57BL/6J mice, subjects received chronic intermittent exposure (16 hr/day x 4 days/week) to ethanol vapor (CIE group) or air (CTL group). Weekly cycles of inhalation exposure were alternated with 5-day drinking test cycles. Each day of the drinking test weeks, mice were either exposed to a 10-min FSS (4 hour prior to drinking) or left undisturbed. In one experiment, CIE and CTL mice were administered the short-acting KOR antagonist LSN2444296 after FSS (5 mg/kg, ip; 30 min prior to drinking). The FSS-induced increase in ethanol consumption observed in CIE mice was blocked by administration of the KOR antagonist. In a second experiment, CIE and CTL mice were administered one of four doses of U50,488 (0,1.25, 2.5, 5.0 mg/kg) one hour prior to each daily drinking test (in lieu of FSS). All doses of U50,488 increased ethanol consumption in both CIE and CTL mice, although there was some evidence that the CIE mice were more sensitive to the effects of U50,488. Our results demonstrate that the KOR system contributes to the stress enhancement of ethanol dependence-related drinking. Studies are currently underway to more fully characterize sensitivity to KOR activation and blockade in CIE and CTL mice, and to explore interactions of the KOR system with CRF.

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The authors have no conflicts of interest to disclose.

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Effects of kappa opioid receptor availability on effectiveness of naltrexone in alcohol-dependent individuals

Objectives: Kappa-opioid-receptor (KOR)-selective drugs have been explored for controlling alcohol-seeking behavior. Naltrexone (NTX), which has affinity for KOR, may be preferentially effective in reducing drinking in men [1]. Our aim in the present study was to determine if KOR availability predicts efficacy of NTX in altering drinking in alcohol-dependent (AD) subjects.

Methods: Eighteen AD non-treatment seeking participants (15 males and 3 females) underwent 2 PET scans on the HRRT with [^{11}C]-LY2795050, a kappa-selective antagonist tracer [2], and two alcohol drinking paradigms (ADP) [3], at baseline and after 7.67 ± 0.94 days of treatment with 100 mg/day of naltrexone. The primary behavioral outcome of ADP was total number of drinks consumed. Tracer was administered as a bolus and PET data were collected for 90 min. Partial volume correction (to correct for effects of atrophy) was applied to the data [4]. Time-activity data were fitted with the multilinear analysis model (MA1) [5] to estimate regional volumes of distribution (V_T) as the imaging outcome. Given uniform nonspecific binding of tracer, V_T represents density of available KOR. Regional baseline V_T values were correlated with absolute change in the number of drinks consumed from first to second ADP, and significance was defined as $p<0.05$.

Results: Correlation between V_T and change in number of drinks consumed in the ADP was negative in all regions and significant in 5/15 regions (uncorrected for multiple comparisons).

Conclusions: This is the first report to examine the correlation between baseline KOR availability and the effect of NTX on drinking behavior. Negative correlation in all regions of interest indicates that a lower baseline KOR availability may predict higher NTX efficacy. Standard PET imaging does not allow us to differentiate between low receptor density and high baseline dynorphin levels. Nevertheless, these are important preliminary steps in understanding the effectiveness of medications for alcoholism that target the KOR.

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Effect of orally-bioavailable short-acting kappa-selective antagonist LY2456302 on nicotine withdrawal in mice.

Several lines of evidence support a role for the endogenous opioid system in mediating behaviors associated with drug dependence. Specifically, initial findings suggest that the kappa opioid receptor (KOR) may play a role in aspects of nicotine dependence, which contribute to relapse and continued tobacco smoking. We recently reported that the selective long-acting KOR antagonists JDTic and nor-BNI given systemically attenuated the expression of both the physical and affective nicotine withdrawal signs in mice (Jackson et al., 2011). Therefore, the purpose of the present study was to investigate the effect of the short-acting kappa-selective antagonist LY2456302 in both physical (somatic and hyperalgesia) and affective (anxiety-like effects) components of nicotine withdrawal after oral administration in the mouse.

Mice were implanted with 14-day osmotic minipumps containing either saline or nicotine (24 mg/kg/day). On Day 15, minipupms were removed and 16 hr later, mice were pretreated either with LY2456302 (1, 3, and 10mg/kg po) or vehicle (i.p.) 60 min before assessing anxiety-like responses (plus-maze test), somatic withdrawal signs (paw tremors, body shakes, backing and other signs) and hyperalgesia (hot-plate test). LY2456302 was able to dose-dependently block all three signs. We then assessed LY2456302 effect in nicotine aversion withdrawal sign using the conditioned-place preference (CPA) model. Mice were rendered dependent on nicotine by implantation of 28-day minipump for 2 weeks. During testing nicotine continued to remain on board. LY2456302 was also able to completely block mecamylamine induced aversion.

Our findings clearly show that the KOR is involved in mediating the withdrawal aspects of nicotine dependence. The results from this study suggest that blockade of the KOR by short-acting KOR antagonists may be useful smoking cessation pharmacotherapies.

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Voluntary Ethanol Intake Predicts Kappa Opioid Receptor Supersensitivity and Regionally Distinct Dopaminergic Adaptations in Macaques

The dopaminergic projections from the ventral midbrain to the striatum have long been implicated in mediating motivated behaviors and addiction. Recently, it has been demonstrated that kappa opioid receptor (KOR) signaling in the striatum plays a critical role in the increased reinforcing efficacy of ethanol following extensive ethanol vapor exposure in rodent models. Although rodents have been used extensively to determine the neurochemical consequences of chronic ethanol exposure, establishing high levels of voluntary drinking in these models has proven difficult. Conversely, non-human primates exhibit similar intake and pattern to humans in regard to drinking, and a subset of nonhuman primates develop signs of dependence. Here we examined the effects of chronic voluntary ethanol self-administration on dopamine neurotransmission and the ability of KORs to regulate dopamine release in the dorsolateral caudate (DLC) and nucleus accumbens (NAc) core. Using voltammetry in brain slices from cynomolgus macaques after 6 months of voluntary ad lib ethanol drinking, we found increased KOR sensitivity in both DLC and NAc. The magnitude of ethanol intake predicted increases in KOR sensitivity in the NAc core, but not DLC. Additionally, ethanol drinking increased dopamine release and uptake in the NAc but decreased both of these measures in the DLC. These data suggest that chronic daily ethanol drinking may result in regionally distinct disruptions of striatal outputs. Further, the strong relationship between KOR activity and ethanol intake suggests that increased KOR sensitivity may drive increased ethanol drinking, and targeting KORs may provide a promising target for pharmacotherapies for alcoholism.

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Chronic Intermittent Ethanol Exposure (CIE) modulates BNST GABA and Glutamate Transmission and social behavior in a kappa opioid receptor (KOR) dependent manner.

Kappa opioid receptors (KORs) have been shown to be involved in alcohol consumption and withdrawal both in humans and rodents. Recently, a greater emphasis has been placed on the role of KORs in ethanol withdrawal as a potential therapeutic target. DBA/2J mice were exposed to chronic intermittent ethanol vapor for 16hrs/day for 5 days, and then 24 hours following the final exposure, mice underwent a social approach test as a proxy for ethanol-induced anxiety and social deficits. We found that alcohol exposed mice show decreased social preference for a novel mouse as compared to air-exposed controls. This behavior is normalized by administration of the KOR antagonist JDTic (10mg/kg). In order to understand the neurocircuitry underlying this KOR-mediated social behavior, we conducted slice electrophysiology experiments in the bed nucleus of the stria terminalis (BNST), a region implicated in alcohol withdrawal induced anxiety. We found that CIE exposed mice have decreased KOR modulation of evoked excitatory post-synaptic currents (eEPSCs) as compared to air-exposed controls. In contrast, CIE exposed mice have greater KOR modulation of evoked inhibitory post-synaptic currents (eIPSCs) as compared to air-exposed controls. In addition, in CIE-exposed mice, eIPSCs show a potentiation by the KOR antagonist norBNI, indicating there may be tonic KOR activation at these synapses following ethanol exposure. Taken together, this work supports KORs as a therapeutic target for alcohol addiction and related behaviors.

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The role of kappa opioid receptor-directed compounds in treatment of specific addictive diseases and affective disorders

In 1992, my laboratory and others found that activation of the kappa opioid receptor system by its endogenous ligand, dynorphin, modulates dopaminergic tone and thus countermodulates the reward effects of drugs of abuse, mediated through dopamine and activation of the mu opioid receptor system. Observations in the clinic suggested that medications with kappa partial agonism could cause modest psychotomimesis, apparently less in persons with addictive diseases than in healthy people. Our laboratory and others found direct and indirect indications that induced stress, as well as chronic exposure to drugs of abuse, lead to excess dynorphin-kappa activation, which in turn can lead to dysphoria and depression. These findings suggest that medications directed specifically at the kappa opioid receptor (agonists, antagonists, or agonists) would be able to modulate the rewarding effects of specific drugs of abuse, such as cocaine, alcohol, and possibly opiates, and further might modulate or treat resultant withdrawal, dysphoria, depressive symptoms, as well as unipolar depression and post-traumatic stress disorder, where recapitulation of initial stressors contributes to the pathophysiology of the disease.

Studies have shown that acute, subacute, and chronic binge-pattern cocaine, investigator-administered or self-administered, cause an increase of dynorphin gene expression, and further that this increase is recurrent and persistent. We also have found that non-drug-related stressors, such as the forced swim test, cause an increase in dynorphin gene expression, and thus an increase in dynorphin kappa activation. Selective kappa antagonists, such as the very long-acting norBNI, prevent the depressive symptoms of forced swim test, such as immobility; gene expression is not altered in any way.

Currently, with our investigator-initiated IND, we are conducting studies of a selective kappa antagonist initially developed for treatment of depression, provided by Lilly, in volunteer subjects, including healthy volunteers and persons with history of severe cocaine addiction in various stages of abstinence. Findings from selected laboratory studies, as well as completed and ongoing basic clinical research studies, will be presented.

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Distinct subpopulations of nucleus accumbens dynorphin neurons drive aversion and reward

The dynorphin/kappa opioid system is implicated in stress and vulnerability to drug abuse. It is thought that stress causes dynorphin release activating kappa-opioid receptors (KOR) within both dopaminergic and serotonergic nuclei and their ventral striatal targets. Consequently, much attention has focused on these systems in the modulation of KOR-mediated responses. Despite our current knowledge of central dynorphinergic cell body populations, a clear description of the axonal projections of these neurons is unknown. To address this we crossed the Cre-dependent tdTomato (Ai9) reporter mouse to a mouse expressing Cre recombinase under the same promoter as dynorphin (Dyn-Cre) so only dynorphinergic cells express tdTomato, allowing complete visualization of dynorphinergic circuitry throughout the brain. We show robust dynorphin expression in cell bodies throughout the brainstem and forebrain. We were also able to use these mice in conjunction with viral retrograde approaches to isolate and identify NAc dynorphinergic projections throughout the brain. Prior studies have shown that KOR agonists inhibit dopamine and serotonin release in the nucleus accumbens (NAc), which regulates aversive behaviors. Therefore, we investigated whether specific modulation of dynorphinergic neuronal firing in the NAc is sufficient to induce aversive behaviors. We virally targeted channelrhodopsin-2 to striatal dynorphinergic neurons and using optogenetics photo-activated neuronal populations in both the dorsal and ventral NAc shell. Activation of dorsal NAc shell induced a place preference and was positively reinforcing in an FR1 operant stimulation paradigm. However, activation of ventral NAc shell drove conditioned and real-time aversive behavior, which was blocked by local infusion of NorBNI, suggesting it is KOR dependent. Using wireless optogenetics and bidirectional control in the same mouse we were also able to modulate the preference and aversion in a bimodal manner. Furthermore, photoactivation of dynorphinergic neurons in the ventral NAc increased dynorphin release, as measured using opto-microdialysis with mass spectroscopy. Understanding the mechanisms by which the dynorphin/kappa opioid system regulates negative affective behaviors provides valuable insight into potential treatments for drug abuse and depression. Work supported by NIDA R01DA033396 (M.R.B), NIMH F31MH101956 (to J.G.M).

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Presynaptic inhibition of dopamine release is not required for kappa opioid receptor mediated aversion

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The dynorphin-kappa opioid receptor (KOR) system encodes the dysphoric component of the stress response. While inhibition of dopamine release from ventral tegmental area (VTA) nerve terminals in the nucleus accumbens (NAc) is widely presumed to be the mechanism for KOR-induced dysphoria, this has not been directly demonstrated. In addition, strong evidence supports a role for dynorphin inhibition of the serotonergic input to NAc in KOR-induced aversion. Understanding how KOR agonists produce dysphoria is key to the development of better opioid analgesics and antidepressants. Here, we report that p38α MAPK activation in *either* the VTA dopaminergic neurons or dorsal raphe (DRN) serotonergic neurons was required for KOR-induced conditioned place aversion (CPA). Repeated KOR activation stimulated p38α MAPK in DRN-5HT neurons, which increased serotonin transporter function and dysregulated G-protein gated potassium channel activation. KOR activation by U69593 or U50488 significantly reduced dopamine release measured using fast scan cyclic voltammetry in NAc both *in vivo* (urethane-anesthetized mice) and in brain slices of male C57Bl/6 mice. Conditional deletion of KOR in DAT-Cre mice blocked KOR-induced inhibition of dopamine release. Conditional deletion of p38α from VTA dopamine neurons blocked KOR-induced CPA, but did not block KOR-mediated inhibition of dopamine release. U50488-induced CPA was blocked by local norBNI injection into VTA, blocked by conditional deletion of KOR from VTA dopamine neurons, and rescued by selective expression of KOR in VTA DA neurons of KOR-/- mice. Pharmacological inhibition of p38 MAPK by SB203580 also failed to affect KOR-induced inhibition of dopamine release. Thus, contrary to the prevailing view, direct inhibition of mesolimbic dopamine release does not mediate the aversive effects of KOR activation.

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Medial prefrontal cortex κ-opioid receptors and working memory

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Deficits in executive function, including working memory, are symptoms of aging, alcohol and drug dependence and numerous neuropsychiatric disorders. Evidence implicates dysregulation of cortical dynorphin / κ-opioid receptors (KOR) in executive function deficits produced by alcohol dependence. To evaluate the role of medial prefrontal cortex (mPFC) KORs in alcohol dependence-induced working deficits, behavior was assessed using a measure of working memory following alcohol vapor exposure-induced dependence induction and following site-specific mPFC KOR agonist and antagonist administration. In addition, mPFC KOR function was assessed using a dynorphin A-stimulated GTPγS assay. The results suggest that chronic alcohol exposure-induced deficits in working memory are related to mPFC KOR functional activity that declines with age in older rats, but is 'reawakened' during withdrawal when alcohol dependent. Providing functional confirmation for a role of mPFC KORs in the regulation of working memory, site-specific intra-mPFC KOR agonist infusion-induced working memory deficits were rescued by mPFC KOR antagonism. These findings underscore the importance of the mPFC DYN/KOR system in working memory regulation and the deficits that occur in alcohol-dependent populations; positing mPFC KORs as a novel therapeutic target for neuropsychiatric disorders defined by symptoms of deficient working memory and impaired executive function.

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Adolescent social isolation sensitizes the kappa opioid receptor system affecting dopamine regulation in the nucleus accumbens.

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Chronic early life stress, such as neglect during childhood, results in increased risk for alcohol use disorders during adulthood. Similarly, rats reared in social isolation (SI) during adolescence show increased ethanol (EtOH) intake compared to group housed (GH) rats. We investigated whether kappa opioid receptor modulation of dopamine release alters following a 6-week protocol in which rats were either housed in groups (4 rats/cage) or individually (1 rat/cage), rats underwent a 2-bottle choice intermittent access EtOH drinking protocol for 7 weeks. NorBNI (10 mg/kg; i.p.) was administered 24 hrs prior to the test day and SI, but not GH rats, showed attenuated EtOH intake. The sensitivity of KORs in NAc core and shell slices of SI and GH rats was assessed by measuring the effects of U50,488 (10-1000 nM), a KOR agonist, on DA release. The inhibitory effects of U50,488 on DA release were enhanced in both NAc core and shell of SI compared to GH rats, suggesting that chronic stress sensitizes KORs. Baseline and acute EtOH (1 g/kg, i.p.)-induced changes in DA levels in the NAc were measured before and after norBNI treatment using microdialysis in freely moving rats. The baseline levels of DA were significantly lower in SI compared to GH rats. KOR blockade increased DA levels only in SI rats. Acute EtOH augmented DA responses in the NAc of SI rats pre-treated with norBNI compared to untreated rats; no difference was observed in GH rats. These data suggest that KORs are functionally hyperactive following SI. The increased sensitivity of KORs explains the hypodopaminergic state of SI rats, possibly resulting in anhedonia, further driving the increased EtOH intake. The ability of nor-BNI to reverse chronic stress-mediated hypodopaminergia suggests that KOR antagonists could potentially be used as therapeutics to treat chronic stress associated alcoholism.

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“Kapp-ing” Visceral Pain: A novel orally efficacious kappa opioid receptor agonist with reduced CNS side-effects.

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The clinical development of kappa opioid receptor (KOR) agonists for treatment of pain has been hampered by the CNS liability of dysphoria. Evidence suggests that peripheral kappa opioid receptor agonists may be effective analgesics but to date there are no approved small molecule kappa opioid receptor agonists that target peripheral receptors with no associated CNS liabilities. We are using our proprietary polymer technology to develop potent, orally efficacious small molecule KOR agonists, with superior separation from efficacy and CNS-related side-effects.

To evaluate the analgesic and CNS effects of our novel compounds, we utilized murine models of rodent acute visceral pain (mouse acetic acid writhing) and locomotor activity. In vivo target engagement was assessed using urine output, a known KOR agonist related effect.

Nektar’s novel KOR agonists were orally efficacious, producing dose-related analgesic effects at doses lower than or comparable to known KOR agonists (asimadoline, CR845 and U62066). In addition, our novel KOR agonists displayed up to 20-fold improved analgesia vs. CNS side-effect ratios compared to other KOR agonists. The effects of Nektar KOR agonists on urine output were dose-related and consistent with activation of KOR in vivo.

Overall, we have successfully used Nektar’s proprietary polymer technology to develop novel KOR agonists with superior separation between efficacy and side-effect, compared to purported peripherally-restricted compounds (asimadoline and CR845), in these preclinical rodent models. This wide separation between analgesic and side-effect profile suggests peripheral restriction of Nektar’s kappa agonists, where analgesia is mediated from peripheral activity and limited blood brain barrier penetration results in reduced centrally-mediated side-effects. Future efforts will focus on evaluating our KOR agonists in a rodent aversion model to provide surrogate data supporting the separation of analgesic efficacy from dysphoric potential.

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All authors are full time employees at Nektar Therapeutics.

Involvement of the kappa/dynorphin system in the emotional manifestations of osteoarthritic pain

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Osteoarthritis is a degenerative joint disease associated with articular cartilage degradation. The major clinical outcome of osteoarthritis is a complex chronic pain state that includes both nociceptive and emotional manifestations. Emotional alterations, such as anhedonia and other depressive-like symptoms, have been widely reported to be associated with chronic pain, which may increase the pain experience impairing life quality (Campbell et al., 2006). The aim of our study was to evaluate the possible involvement of the endogenous κ-opioid receptor (KOR-KO)/dynorphin system in the nociceptive and emotional manifestations of osteoarthritic pain. The murine model of monosodium iodoacetate was used to induce osteoarthritis, as previously reported (La Porta et al, 2013), in knockout mice for κ-opioid receptor (KOR-KO) and prodynorphin (PDYN-KO). Wild type (WT) mice, as well as KOR-KO and PDYN-KO mice developed mechanical allodynia in the ipsilateral paw after intra-articular injection of monosodium iodoacetate, without any nociceptive alteration in the contralateral side. This allodynia was significantly increased in KOR-KO animals. Moreover, the anhedonic state related to chronic pain was also evaluated by measuring the preference for palatable drink (sucrose), using a highly sensitive behavioral device developed in our laboratory (Bura et al., 2010). Concurrently to nociceptive behavior, the anhedonic state was significantly increased in both KOR-KO and PDYN-KO mice, when compared to WT mice. These findings reveal a specific involvement of the KOR/dynorphin system in the emotional manifestations associated to chronic osteoarthritic pain.

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Inflammatory pain impacts motivation for heroin self-administration in dependent rats: A possible role for kappa opioid receptors

Pain management in opioid abusers raises many ethical and practical difficulties for clinicians, resulting in a general under-treatment of pain in this population. In the present study, we investigated the effect of inflammatory pain on i.v. heroin self-administration under progressive ratio (PR) schedule of reinforcement. We selected the complete Freund's adjuvant (CFA) rat model of inflammation to assess the effect of inflammatory pain on heroin seeking. In addition, we investigated the neurochemical changes induced by inflammatory pain on DA transmission within the mesolimbic pathway by conducting the *in vivo* microdialysis studies. Biochemical and GTPγS analyses were conducted to examine a role for kappa opioid receptors expression and function. First we analyzed the effect of inflammatory pain on motivation for heroin and sucrose intake measured in a progressive ratio (PR) schedule of reinforcement. Rats were trained to self-administer 50 µg/kg/infusion heroin or sucrose pellets under FR1, FR2 and FR5. Afterwards, animals underwent a heroin (50 or 200 /kg/infusion) or sucrose PR baseline session to measure initial ordinal value of the final ratio to which the animal responded (PR step number). Then, a second PR session was performed 48 hours after CFA or saline injection in the hindpaw. Inflammatory pain reduced PR step number from baseline to 72% with sucrose as the reward. In animals trained with 50 µg/kg/infusion (dependent rats) the presence of inflammatory pain reduced the PR step number from baseline to 63% with 50 µg/kg/infusion heroin as the reward. Interestingly, this effect of inflammatory pain on motivated behavior for heroin was completely reversed when the heroin dose was increased up to 200 µg/kg/infusion. Overall, our data show that there is a robust decrease in the overall motivational state during inflammatory pain and that this effect is completely reversed when the dose of heroin is increased. We next investigated the effect of inflammatory pain on heroin-evoked dopamine (DA) release in the nucleus accumbens (NAc) given the fact that DA levels influence the motivation for drug intake measured in a PR schedule of reinforcement. Interestingly, we observed that whereas the administration of i.v. heroin (75 µg/kg) triggered an increase in extracellular DA levels, this same dose of heroin only elicited a much smaller increase in DA levels compared to baseline in CFA-treated animals. However, higher dose of heroin (150 µg/kg, i.v.) induced a significant increase of DA release in both control animals and in CFA-treated animals. These data strongly suggest that the presence of inflammatory pain selectively impacts the sensitivity to the rewarding properties of heroin. Finally, given the role of kappa opioid receptors (KORs) in motivational states, we analyzed expression of these receptors in the mesolimbic dopaminergic system and found that KOR expression was significantly increased in the NAc but was not altered in the VTA. More interestingly, in VTA and NAc membranes obtained from CFA-injected rats, a significant increase in dynorphin A-stimulated GTPγS binding observed as compared to the control rats. We are currently conducting studies to examine whether activation of KORs in the reward pathway is responsible for this pain-induced alteration in motivation for heroin and natural reinforcers. The results presented here reveal that the presence of inflammatory pain impacts the reinforcing effects of heroin in dependent rats probably via activation of kappa opioid receptors. Taken together, our data also suggest that the presence of pain impacts the effects of opioids (and natural reinforcers) on the reward pathway such that higher doses of the opioid are necessary to produce neurochemical and positively reinforcing behavioral effects.

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Differential functions of dynorphin A, β-endorphin, and gastrin-releasing peptide in regulating itch and pain in the spinal cord of primates

Neuropeptides have been investigated for regulating itch and pain in the spinal cord of rodents. However, there is no functional evidence of neuropeptides delivered spinally in primates. By examining behavioral and pharmacological factors in awake monkeys, here we elucidate the functional roles of spinal opioid peptides and gastrin-releasing peptide (GRP) in the somatosensory function. Following intrathecal administration, both β-endorphin (10-100 nmol) and GRP (1-10 nmol) dose-dependently elicit the same degree of robust itch scratching responses, which can be merely inhibited by mu opioid receptor and GRP receptor antagonists, respectively. Unlike β-endorphin eliciting itch scratching and attenuating inflammatory pain, GRP only elicits itch scratching. In contrast, dynorphin A (10-100 nmol) dose-dependently attenuates both β-endorphin- and GRP-elicited robust scratching without affecting pain processing. The anti-scratching effects of dynorphin A can be reversed by a kappa opioid receptor antagonist nor-binaltorphimine (3 mg/kg). This invaluable nonhuman primate behavioral model with spinal delivery of neuropeptides provides the physiological relevance to patients with changed sensory modalities, namely an imbalance between dynorphin A-kappa opioid receptor and GRP-GRP receptor and/or β-endorphin-mu opioid receptor systems. More importantly, it serves as a pharmacological foundation to facilitate the development of kappa opioid receptor agonists as antipruritics in humans.

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Salvinorin A analogs, PR-37 and PR-38, attenuate 48/80-induced itch responses in mice

It has been shown that selective opioid receptor agonists exert potent inhibitory action on pruritus and pain. This study describes the antipruritic properties of two salvinorin A analogs, methyl salvinorin B-2-*O*-malonate (PR-37) and 2-*O*-cinnamoylsalvinorin B (PR-38). Both compounds displayed high affinity at KOR *in vitro* with K_i values of 2.0 ± 0.9 and 9.6 ± 2.0 nM and potently inhibited adenylate cyclase in live HEK293 cells with EC_{50} values of 137 ± 15 and 7 ± 1 nM, respectively.
To examine the antipruritic activity of PR-37 and PR-38 the mouse model of 48/80-induced pruritus was used. In order to elucidate the mechanism of action of tested compounds, specific antagonists of opioid and cannabinoid receptors were used. The effect of PR-37 on the central nervous system was assessed by measuring motoric parameters and exploratory behaviors in mice. Previously it was found that the PR-38 does not affect the CNS system in mice model.
PR-37 and PR-38 (s.c.) significantly reduce the number of 48/80-induced scratching behavior in mice in dose- and time-dependent manners. PR-38 is also active when orally administered. The antipruritic activity of PR-37 was blocked by the selective KOR-antagonist, nor-binaltorphimine, and that of PR-38 by the selective MOR-antagonist, β-funaltrexamine. In conclusion, a novel framework for the development of new antipruritic drugs derived from salvinorin A is provided.

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Genetic identification of somatosensory neurons that express the kappa opioid receptor

Opioids are known to modulate the activity of somatosensory neurons. Recently, the specific afferent subtypes that express the delta and mu opioid receptors have been characterized. However, the identity of sensory neurons that express the kappa-opioid receptor (KOR) are unclear. We have now generated a *KOR-cre* knockin allele, thereby allowing the genetic marking of KOR-expressing cells. Using this allele we discovered that, in the dorsal root ganglia (DRG), *KOR-cre* marks two populations of primary afferents: a myelinated population that terminates in the deep dorsal horn and an unmyelinated population that terminates in the superficial dorsal horn. Here, we analyze the functional properties of *KOR-cre* expressing sensory neurons and characterize these cells by single-cell RT-PCR and immunohistochemistry. Moreover, we assess the functional role of KOR in somatosensory neurons using optogenetic, electrophysiology, and behavior approaches. These findings provide key insight into the specific somatosensory sub-modalities that would be modulated by therapeutic treatment with peripherally selective kappa agonists.

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Pharmacological Mechanisms Underlying the Antidepressant and Anxiolytic Effects of Buprenorphine

Recently published clinical studies suggest that buprenorphine (BPN), a drug with high affinity for opioid receptors, may be effective in alleviating symptoms in treatment-resistant depressed patients. BPN has mixed pharmacology at opioid receptors, acting as a partial agonist at mu opioid receptors (μ-ORs), and antagonist at kappa opioid (κ-ORs) and delta opioid receptors. In these studies, the forced swim test (FST) and novelty-induced hypophagia (NIH) test, two behavioral tests sensitive to conventional antidepressant drugs, were used to probe the pharmacological mechanisms associated with the antidepressant and anxiolytic effects of BPN, respectively. BPN (0.25 mg/kg) and selective κ-OR antagonist nor-BNI (10 mg/kg) reduced immobility in the FST in C57BL/6J mice when tested 24 h post injection, a time at which locomotor activity is unaffected (Falcon et al., 2014). Interestingly, BPN did not reduce immobility in C57BL/6J mice with genetic deletion of κ-ORs (Oprk1$^{-/-}$), but was effective in mice with genetic deletion of either μ-ORs (Oprm1$^{-/-}$) or delta opioid receptors. In the NIH test, BPN reduced approach latencies for palatable food in a novel arena (Falcon et al., 2014), a response similar to that produced by chronic treatment with antidepressants or acute anxiolytic drugs. However, in contrast to the FST, nor-BNI was not effective in reducing approach latencies in the NIH test. Moreover, behavioral response to BPN in the NIH test was blocked in Oprm1-/- mice, but not altered in Oprk1-/- mice. These data suggest that κ-ORs mediate the antidepressant-like effect of BPN measured in the FST whereas μ-ORs mediate the anxiolytic effect of BPN measured in the NIH test. In summary, the pharmacological mechanisms associated with the behavioral effects of BPN in measures of affective behavior are test-dependent and involve both κ-ORs and μ-ORs. These results support further investigation into the role of κ-ORs versus μ-ORs in depressive and anxiety-like behaviors.

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Involvement of Kappa Opioid Receptors in the Effects of Chronic Social Defeat Stress on Sleep, Body Temperature, and Motor Activity in Mice

Stress is a critical component in the etiology of many psychiatric illnesses, including Major Depressive Disorder and Post-Traumatic Stress Disorder. Our understanding of the effects of stress has benefitted from the development of the chronic social defeat stress (CSDS) paradigm, an ethologically-relevant model of social stress which engenders a long-lasting depressive-like phenotype in mice. However, it is unknown whether this model affects sleep and circadian rhythms, functions that are routinely dysregulated in individuals with stress-related disorders and in rodents following acute stress. In the present study, we evaluated the extent to which CSDS produces alterations in sleep and circadian amplitude of body temperature and motor activity. As adaptations in the kappa opioid receptor (KOR) system putatively underlie some of the long-term consequences of stress, we also assessed the involvement of KORs in CSDS-related changes in sleep and circadian function.

To address these questions, adult male C57BL6/J mice were surgically implanted with telemetry transmitters that enable continuous wireless recording of cortical EEG, neck EMG, body temperature, and activity. Following recovery from surgery, recordings were obtained for 5 baseline days, 10 days of the CSDS (or control) regimen, and for up to 10 days post-defeat. Before the onset of social defeat, mice received intraperitoneal injections of JDTic (30 mg/kg), a long-acting KOR antagonist with putative antidepressant properties, or saline vehicle (VEH). On each day of social defeat, 1 h into the light cycle (zeitgeber time 1), defeated mice were exposed to a novel, aggressive CD-1 mouse for 10 min, followed by continuous, protected sensory exposure. Control mice were similarly housed opposite a conspecific with continuous, protected sensory exposure, but were never exposed to defeat stress.

Examination of body temperature amplitude in our pilot studies revealed a decrease in defeated mice, relative to controls. This is a reliable indicator of chronic stress effects and importantly, is capable of revealing susceptible and resilient subpopulations. Examination of sleep architecture in these subpopulations revealed that CSDS preferentially increased NREM time, but not bouts (number of discrete episodes), in susceptible mice. This effect persisted after CSDS. CSDS selectively increased REM time and bouts in susceptible mice during, but not following, CSDS. Notably, CSDS failed to alter these measures in resilient mice at any time point, compared to controls. Both resilient and susceptible mice were sensitive to the effects of CSDS on circadian amplitude of body temperature and activity during CSDS, but only susceptible mice continued to exhibit enduring deficits during the post-defeat recording period. Pretreatment with JDTic facilitated recovery of NREM sleep following CSDS, prevented CSDS-related increases in REM time and bouts, and ameliorated the effects of CSDS on activity amplitude. In conclusion, these findings suggest that KOR antagonists may have therapeutic efficacy in the treatment of physiological abnormalities that arise from chronic stress.

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Disclosures: Dr. Carlezon and McLean Hospital are co-owners of a patent on the use of KOR antagonists to treat depressive disorders.

Acute stress induces persistent alterations in VTA kappa opioid receptors

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Stressful experiences drive many adaptive and maladaptive behaviors, and even acute stressors can have lasting behavioral consequences. Emerging evidence shows that dopaminergic neurons in the ventral tegmental area (VTA) are an important locus in stress. We previously identified a long-term potentiation of GABAergic synapses onto these neurons (LTP_{GABA}) that is blocked by acute stress (Graziane et al, Neuron, 2013). This block of plasticity is prevented by administration of a kappa opioid receptor (κOR) antagonist norBNI. Intra-VTA injection of this antagonist also prevents reinstatement of cocaine seeking by acute stress, suggesting that κOR-mediated regulation of VTA inhibitory plasticity may play a role in stress-induced drug seeking.

Our recent work shows that a single five minute cold water swim stress blocks LTP_{GABA} for at least five days. Surprisingly, post-stress antagonism of κORs with norBNI is effective at recovering LTP_{GABA} and preventing reinstatement of cocaine self-administration even when administered well after the stress has occurred (Polter et al, Biological Psychiatry, 2014). These results demonstrate that kappa opioid receptors are persistently activated for several days following stress. This persistent activation is observable in brain slices, as bath application of norBNI to slices prepared from animals 24 hours after stress rescues LTP_{GABA} (LTP magnitude:136±18% of baseline). In contrast to norBNI, which demonstrates inverse agonist activity at κORs, bath application of the neutral κOR antagonist 6-β-naltrexol to slices from stressed animals failed to rescue LTP_{GABA} (LTP magnitude:99±8% of baseline). The differential effects of these ligands suggest that an acute stressor induces a persistent change in the basal activity level of κORs rather than increased levels of the endogenous κOR agonist dynorphin. Therefore, a single exposure to acute stress may cause days-long alterations in κOR activity. These alterations in κOR signaling and GABAergic plasticity in the VTA provide a mechanism by which acute stressors could induce lasting changes in behavior.

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

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Potential involvement of kappa opioid receptors (KOP-r) in PTSD: Animal modeling

Post-Traumatic Stress Disorder (PTSD) is unique among psychiatric diseases in that the primary eliciting factor – the experience of a severe, life threatening trauma – is explicitly environmental. One well-documented biological change in response to episodic stress is increased signaling of KOP-r through activation by endogenous dynorphins in brain circuits related to stress-responsive behaviors. KOP-r activation produces anxiety-/depressive-like behaviors in rodent models and anhedonia/dysphoria in humans. Our rodent studies indicate that KOP-r activation results in negative motivational behaviors via interaction of KOP-r with noradrenergic (NA) system in central nucleus of amygdala, a brain region known to play a critical role in PTSD-like behaviors. In order to model aspects of human PTSD, we have developed a dual-stress regimen to examine PTSD-like behaviors. Mice were briefly exposed to forced swim stress followed by conditioned place avoidance (CPA) training by contextual pairing of a specific environment with chemical stressor yohimbine (Yoh), an alpha-2 NA antagonist that produces anxiety in humans. After repeated exposure to the dual-stress regimen with contextual pairing, mice showed an extended CPA to the stress-paired environment.This stress-CPA paradigm has several translational features, potentially matching major aspects of human PTSD: a) Individual differences in vulnerability; the CPA persisted for 4 weeks in more “vulnerable” mice (21% of all subjects), showing impaired extinction (long-lasting “memory” effect); b) KOP-r mediated long-lasting CPA and its retrieval by mild stress (modeling flashbacks); blockade of KOP-r by nor-BNI reduced the prolonged impairment of extinction in the vulnerable mice. When the CPA was no longer expressed in all mice, Yoh or the KOP-r agonist U69,593 (trauma-related or unrelated stressors, respectively) reinstated the CPA, with further long-lasting responses in the vulnerable mice. Our results suggest that persistent hyper-responsivity in dynorphin/KOP-r systems plays roles in extinction- and stress-induced negative affective states, resulting in retrieval of long-lasting PTSD-like behaviors in mice.

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Poster Session Abstracts

Wednesday 5-7PM

Kappa opioid receptor activation disrupts interval timing

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Chronic unpredictable stress can lead to disruptions in a variety of cognitive domains, including attention, response selection, short- and long-term memory, learning, and decision-making (Mendl, 1999). A fundamental component of these behaviors is the development and maintenance of temporal associations between stimuli to properly guide responding (Gallistel & Balsam, 2014). The dynorphin/kappa opioid receptor (KOR) system, which encodes dysphoric components of stress (Land et al., 2008), may play a role in altering behavioral timing, as pharmacological KOR activation in humans is hallucinogenic and disrupts attention, time perception, and cognition (Johnson et al., 2011). The goal of our experiments is to assess the contribution of KOR activation to interval timing. C57BL/6 mice were trained in a 20s fixed interval (FI) task, during which reinforcers were only delivered for responses 20s following the previous reinforced response. There was no consequence for a premature response, and following training, mice showed a peak in responding surrounding the 20s time point. Administration of a KOR agonist (U50,488; 5 mg/kg) led to a significant decrease in response efficiency during a 60-min test session caused by increased nonreinforced responding during the 20s trial interval. There was no alteration in total reinforced responses or magazine entries, indicating that the effect was specific to behavioral timing rather than general locomotor disruption. This effect was also replicated when the time interval was expanded to a 40s FI period. Together, these results suggest decreased inhibitory control of responding during the fixed interval trial. Current studies are exploring the role of G-protein receptor kinase 3 (GRK3) signaling in KOR mediated timing alterations and future studies will determine whether the observed disruption of timing behavior is dependent on dopamine or serotonin signaling through selective knockout of KOR in dopaminergic or serotonergic neurons.

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Identification of a novel, selective kappa opioid receptor agonist by screening a library of CJ-15,208/c[YpwFG] hybrids

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Selective kappa opioid receptor (KOR) agonists are being explored as therapeutic alternatives to mu opioid receptor (MOR) analgesics, for their low abuse potential and less gastrointestinal and respiratory side effects. Clinical relevance of KOR agonists, however, is still limited due to dysphoric and stress-related effects, which seem to be connected to the GRK3-, arrestin 3-dependent activation of MAPKs, including p38. KOR agonists biased towards G protein coupling and displaying a limited activation of arrestin 3-dependent signaling may represent innovative analgesics.
In this frame, we screened a novel series of opioid peptide hybrids of CJ-15,208 and c[YpwFG]; to circumvent rapid degradation of peptides *in vivo*, a series of chemical modifications, such as cyclization, were introduced. c[YpwFG], an endomorphin-1 cyclic analogue that we previously developed, has high MOR affinity and selectivity, agonist activity *in vitro* and antinociceptive effects in different models of pain. CJ-15,208 (c[FpFW]), on the other hand, is a KOR natural ligand showing antagonist activity *in vitro* and both dose-dependent antinociception and KOR antagonist activity *in vivo*; interestingly, it displays significant structural similarities with c[YpwFG]. Starting from these two cyclic peptides, we synthesized and characterized a novel library of hybrids of both sequences.
Competition binding assays and inhibition of forskolin-induced cAMP accumulation were performed in HEK-293 cells expressing MOR, DOR or KOR. Out of the tested compounds, we identified one MOR selective (EDO43; K_i = 4 nM) and one KOR selective (LOR17; K_i = 1.2 nM) ligand. Interestingly, LOR17 inhibited forskolin-induced accumulation of cAMP, thus confirming a KOR agonist profile. Experiments supporting the hypothesis that it may activate KOR in a biased manner are in progress and will be presented at the Conference. We propose LOR17 as a new and selective KOR cyclic peptide, a useful tool for exploring ligand-directed signaling at KOR.

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The authors have no conflicts of interest to disclose

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6' –Guanidinonaltrindole (6'-GNTI) targets DOR-KOR heteromers in peripheral sensory neurons.

Originally developed as a KOR agonist, 6'-GNTI has been shown to have agonist activity at KOR for Gi-protein-mediated signaling in brain and HEK cells and affinity (but no efficacy) for DOR. However, in peripheral nociceptors, we have found that 6'-GNTI agonist activity for Gi-mediated responses requires expression of both KOR and DOR. In primary cultures of peripheral sensory neurons, 6'-GNTI inhibited adenylyl cyclase activity by 65%. However, following siRNA knockdown of DOR, 6'-GNTI had no effect on adenylyl cyclase activity but antagonized the response to the KOR agonist, U50488. Similarly, when KOR expression was reduced with siRNA treatment, 6'-GNTI had no effect on adenylyl cyclase activity, but antagonized the response to the DOR agonist, DPDPE. Thus, 6'-GNTI has affinity, but not efficacy (i.e. acts as an antagonist) when either DOR or KOR expression is reduced. Importantly, these data suggest that 6'-GNTI efficacy requires activation of DOR-KOR heteromers in peripheral nociceptors.

Because we have demonstrated that allosteric interactions between protomers of DOR-KOR heteromers can regulate DOR and KOR agonist potency and efficacy in peripheral nociceptors, (Berg et al., 2012, *Mol Pharmacol* 81:264-272; and Jacobs et al this meeting), we next tested the hypothesis that 6'-GNTI occupancy of the DOR protomer of DOR-KOR heteromers allosterically enhances its own efficacy at KOR. In peripheral sensory neuron cultures, we measured 6'-GNTI-mediated responses in the presence of several different selective DOR antagonists. Both inhibition of adenylyl cyclase activity as well as antinociception in response to 6'-GNTI were reduced in the presence of the DOR antagonists naltrindole (2nM, 100x Ki) or 7-Benzlidenealtrexone (1nM, 100x Ki). By contrast, naltriben (NTB, 1nM, 100x Ki), fully substituted for 6'-GNTI occupancy of DOR. The concentration response curves of 6'-GNTI for inhibition of adenylyl cyclase activity were superimposable in the absence (DOR occupancy by 6'-GNTI) and presence of NTB (DOR occupancy by NTB). Similarly, in a behavioral model of thermal allodynia, 6'-GNTI produced the same robust antinociceptive response in the presence or absence of NTB. These data are consistent with the hypothesis that 6'-GNTI occupancy of DOR augments its own efficacy at KOR through allosteric interactions between DOR and KOR within the DOR-KOR heteromer.

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Antidepressant-like effects of the κ-opioid receptor partial agonist nalmefene

Compounds that exhibit mixed effects at mu opioid receptor (μ-OR) and kappa opioid receptors (κ-ORs) are currently being explored as novel therapeutics for major depressive disorder. Nalmefene, a μ-OR antagonist with partial agonist activity at κ-ORs, is now medically available for the treatment of alcohol use disorder in Europe. Therefore, these studies sought to evaluate the behavioral effects of nalmefene in rodent tests relevant to anxiety and depression.

First the behavioral effects of nalmefene were characterized using the forced swim test (FST) in C57BL/6J mice, a test associated with antidepressant drugs. Nalmefene (5 mg/kg) significantly reduced immobility 1 h but not 24 h following a single injection. There was no gender difference in response to acute administration of nalmefene in the FST. The effects of nalmefene were also examined at marble burying and in the light/dark box, two behavioral tests associated with anxiolytic drugs. Nalmefene (5 mg/kg) reduced the number of marbles buried in the marble burying task, and the latency to emerge into a brightly lit arena in the light-dark box 1 h post administration. Finally, the effects of chronic nalmefene (5 mg/kg/day administered for 14 days by mini-osmotic pumps), were assessed. Nalmefene significantly decreased immobility scores in the FST, reduced the latency to emerge in the light/dark box and the number of marbles buried in the marble burying task.

Next the behavioral effects of acute nalmefene administration were evaluated using a genetic model of anxiety and depression, the Wistar Kyoto (WKY) rat strain. WKY rats exhibited dose-dependent decreases in immobility scores in the modified rat FST studied 1 h following nalmefene treatment; the most efficacious dose was 2.5 mg/kg.

The next set of studies ascertained the pharmacological mechanisms underlying the behavioral effects of nalmefene in the FST, using female C57BL/6J mice with genetic deletion of either the *Oprk1* gene (*Oprk1*$^{-/-}$ mice), or the *Oprm1* gene (*Oprm1*$^{-/-}$ mice). Immobility scores of wild type but not *Oprm1-/-* mice were significantly reduced following nalmefene treatment, suggesting involvement of μ-ORs in this behavioral effect. In addition, nalmefene significantly reduced the immobility scores of wild type but not *Oprk1*$^{-/-}$ mice, suggesting that κ-ORs also mediate the antidepressant-like effects of nalmefene in mice in the FST.

As a partial k-OR agonist, nalmefene may produce its behavioral effects either by activating k-ORs, or blunting the effects of its endogenous ligand dynorphin. In order to examine this, the effects of nalmefene on k-OR-mediated extracellular dopamine release in the nucleus accumbens was evaluated using in vivo microdialysis. Administration of the κ-OR agonist, U50, 488 (5 mg/kg) reduced extracellular dopamine levels to 30% below baseline. Similarly, acute administration of nalmefene decreased dopamine levels to 25% below baseline. To determine whether nalmefene might desensitize κ-ORs after its prolonged administration, the effects of U50, 488 were measured in mice following chronic treatment with nalmefene (5 mg/kg/day via osmotic minipump for 14 days).

In summary, these are the first data to indicate antidepressant-like and anxiolytic-like effects of nalmefene. Furthermore, both k-ORs and m-ORs appear to be involved in producing its behavioral effects in the FST an assay for antidepressant-like effects. Nalmefene continued to produce behavioral effects following chronic administration. The mechanism of action of chronic nalmefene involving changes in the efficacy of κ-ORs and dopamine release is being investigated. These data support further investigations of nalmefene as a potential therapeutic for the treatment of depression and anxiety.

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Kappa-receptor blockade by nor-BNI prevents escalation of cocaine intake

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Several studies have shown that chronic drug use results in altered dopamine signaling within the striatum (DiChiara & Bassareo, 2007). Recent work from the Phillips laboratory has demonstrated a causal link between decreases in ventral striatal dopamine release and the escalation of cocaine intake observed when animals are given protracted access to drug (Willuhn, et al., 2014). In these studies, administration of the dopamine precursor, L-dopa, prevented decreases in dopamine release during long-access self-administration and blocked escalation of drug intake as a result. One possible mediator of these observed decreases in dopamine release could be the dynorphin system. Numerous studies have demonstrated that kappa opioid receptor (KOR) activation decreases dopamine release in the striatum (Lemos et al., 2012; Schlosser et al., 1995; Mulder, et al., 1984). Additionally, elevated levels of dynorphin in the striatum have been observed following chronic cocaine exposure (Daunais et al., 1994; Hurd & Herkenham, 1993). Furthermore, long-term KOR antagonism both prevents the escalation of heroin intake (Schlosburg et al., 2013), and reduces motivation to obtain drug (Schlosburg et al., 2013; Groblewski et al., 2014). The aim of the current work is to determine whether alterations in the ventral striatal dynorphin system during long-access drug taking are responsible for producing escalation of cocaine intake. To this end we trained male Wistar rats to self-administer cocaine (FR1 schedule, 0.5mg/kg/infusion) during one hour sessions. Once we established baseline drug intake (7-10 sessions total) we administered the long lasting kappa antagonist, norbinaltorphimine (norBNI), or vehicle within the ventral striatum, and switched all animals to six hour daily access sessions to assess the extent of escalation of drug intake. Preliminary results suggest that long-term KOR blockade in the ventral striatum prevents the escalation of cocaine intake. Future studies will determine whether nor-BNI prevents escalation via inhibition of kappa mediated decrease of dopamine release.

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KOR agonist functional selectivity in peripheral sensory neurons

Functional selectivity, also known as biased agonism, is a term used to describe the ability of drugs to differentially activate signaling cascades coupled to a single receptor subtype. In this study, we compared the agonist activity of the highly selective KOR agonist, Salvinorin-A (Sal-A), to that of its analogue, ethoxymethyl (EOM)-Sal-A. We show here that the addition of an ethoxymethyl group resulted in pronounced differences in KOR-mediated regulation of cellular signaling cascades in peripheral sensory neurons in culture and markedly improved antinociceptive efficacy.

In primary cultures of adult rat peripheral sensory neurons, Sal-A inhibited PGE_2-stimulated cAMP accumulation and increased the activity of c-Jun N-terminal kinase (JNK), but not that of extracellular signal-regulated kinase (ERK). In a rodent behavioral model of thermal nociception, intraplantar injection of peripherally-restricted doses of Sal-A into the hindpaw produced robust antinociception. However, the dose response curve (DRC) had an inverted U-shape. The descending limb of the DRC was sensitive to inhibition of JNK activity. Addition of an ethoxymethyl group to Sal-A increased potency (consistent with increased KOR affinity), but not efficacy, of Sal-A to inhibit PGE_2-stimulated cAMP accumulation. Interestingly, unlike Sal-A, EOM-Sal-A increased ERK activation (via a pertussis-toxin insensitive mechanism), but only weakly stimulated JNK activity. When tested in vivo, EOM-Sal-A produced strong antinociception with a similar peak magnitude as that of Sal-A; however, consistent with weak JNK activation, the DRC to EOM-Sal-A was monotonic (not an inverted U-shape). These data strongly support the idea that ligand efficacy for specific signaling pathways can be finely tuned by structural modifications to a ligand, leading to improved therapeutic profiles for the treatment of pain.

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JDTic and analogs: Probing possible relationships between repetitive scratching episodes in mice and duration of action as κ receptor antagonists.

We call attention to another class of kappa opioid receptor antagonist that precipitates compulsive auto-scratching and excessive body grooming within 2-4 minutes of subcutaneous injection behind the neck of male Swiss Webster mice: the *N*-substituted *trans*-3,4-dimethyl-4-(3-hydroxyphenyl)piperidines. Thus, JDTic, RTI-5989-194 and RTI-5989-240 (each at 0.30-10 mg/kg) join norbinaltorphimine (1-10 mg/kg), 5´-guanidinonaltrindole (0.03-1 mg/kg) and zyklophin (0.10-1 mg/kg) in eliciting this dramatic behavior in mice. The slower onset and long duration of action of JDTic as a kappa receptor antagonist in, for example, the mouse tail flick test is well-known (Carroll et al, 2004) but contrasts with the almost immediate onset and short duration (~15 min) of neck scratching in this species. We also observed quick onset/offset of scratching with both a “short-term antagonist” (RTI-5989-240) and a “long-term antagonist” (RTI-5989-194) (Melief et al, 2011). JDTic, RTI-5989-240 and RTI-5989-194 acted similarly in our animal model of itch. Thus, these compounds cause scratching regardless of their durations of action as kappa receptor antagonists. The molecular processes behind such a prominent and measurable behavior, induced by chemically diverse kappa opioid receptor antagonists, have yet to be defined.

Carroll I et al (2004) Eur J Pharmacol 501:111-119

Melief EJ et al (2011) Mol Pharmacol 80:920-929

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The authors declare no conflict of interest.

The endogenous opioid system regulation in alcoholism: evaluation of ethanol mechanisms by molecular imaging, genetic and epigenetic analysis

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Alcohol induces changes in the central nervous system by altering, directly or indirectly various neuromodulatory and neurotransmitter systems. A reasonable body of evidence indicates a linkage of the endogenous opioid system (EOS) to the development and/or maintenance of alcoholism. We here study mechanisms of adaptive transformations evoked by alcohol in the EOS at (1) cellular and (2) genetic levels .

(1) Advanced fluorescence imaging by Confocal Laser Scanning Microscopy and Fluorescence Correlation Spectroscopy are used to study ethanol effects on MOP, KOP and NOP in live PC12 cells. We observed that ethanol (20 mM) differentially alters opioid receptor mobility and surface density in the plasma membrane, whereas pre-exposure to naltrexone partially counteract these effects.

(2) We analysed genetic association of Single-Nucleotide Polymorphisms (SNPs) in the prodynorphin (PDYN) gene with alcoholism in 744 alcoholic Swedish patients and we have observed that SNP rs2235751 and rs10854244 are associated with alcohol-dependence. Moreover, after methylation analysis in a subset of individuals, we also report an increase in DNA methylation at PDYN gene promoter in alcoholics compared to healthy controls. By analysing the connection between genetic variants and DNA methylation, we have observed that the presence of the minor allele in both SNPs is linked with a higher DNA methylation of PDYN gene promoter.

The focus on alcohol effects on the dynamical aspect of adaptive transformations, as well as the gene x environment effects, might be of relevance to a better understanding of the role of the EOS in alcoholism and be of help in search for new treatments.

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Conflict of interest:

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Presentation preference: poster

In Vitro Characterization of Kappa Opioid Receptor Agonists Reveals Several Distinct Pharmacological Profiles

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Agonists at the Kappa Opioid Receptor (KOR) can differentially promote G protein coupling, β-arrestin2 recruitment and multiple downstream signaling pathways that may contribute to their diverse therapeutic and side effect profiles. To determine if differences in cellular signaling are observed for known kappa agonists, we characterized the in vitro signaling properties and functional selectivity of known agonists with diverse chemical structures.

To examine the effects of KOR agonists on G protein signaling and β-arrestin2 recruitment pathways, we used two approaches: an equi-active bias comparison, and a multi-dimensional analysis to qualitatively compare curves from G protein, β-arrestin, and KOR internalization assays. The equi-active approach classified compounds as biased or not, but the latter approach identified 4 categories of compounds. First, the endogenous ligand Dynorphin A was equipotent in assays measuring G protein activation, β-arrestin2 recruitment, and internalization. The second group, comprising arylacetamides, showed little bias for G-protein versus arrestin pathways, and recruitment of β-arrestin2 was comparable to receptor internalization. The third class, including ketazocine and Salvinorin A, showed potent G-protein signaling and β-arrestin2 recruitment, but low receptor internalization. The final class of compounds, represented by partial agonists such as 6'-GNTI, showed a pattern of potent yet partial agonism in the G-protein assays, and low β-arrestin recruitment and internalization, suggesting G-protein bias. To characterize the compounds for activation of MAPK signaling pathways, we examined ERK and p38, involved in G-protein and β-arrestin signaling. All compounds were found to activate ERK and p38 with comparable potencies. However, we observed significantly reduced maximal response of p38 phosphorylation after treatment with the partial agonists. Using the equi-active bias comparison for phospho-ERK vs phospho-p38, only 6'-GNTI showed >5-fold pathway bias towards G protein signaling. Most significantly, these analyses showed a relationship between structurally related scaffolds studied and their clustered signaling profiles.

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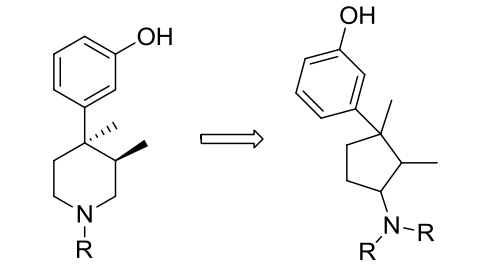
2,3-Dimethyl-3-(3-hydroxyphenyl)-1-aminocyclopetanes: New Opioid Receptor Ligands

The *trans*-3,4-dimethyl-4-(3-hydroxyphenyl)piperidines (**1)** are a well known class of pure opioid receptor antagonists. The uniquely substituted piperidine is present in the commercially successful drug ENTEREG®, a peripheral MOR antagonist, and the clinical candidate, JDTic, a highly selective centrally-acting KOR antagonist. Since its disclosure in 1978, hundreds of N-substituted analogs of (**1**) have been synthesized to develop and understand the SAR at this position. Much less common are modifications to the core piperidine scaffold itself. In this regard, we prepared and examined the opioid receptor affinity and selectivity of a prototypical set of aminocyclopentanes (**2**). The agents were conceptually designed by contracting the 6-membered piperidine ring into a 5-membered cyclopentane ring with concomitant extrusion of the nitrogen atom. Potent dual KOR/MOR affinity was observed for certain compounds. The activity was linked to rather stringent requirements of relative stereochemistry about the substituents in (**2**). This is further illuminated in the computed low energy conformations of these novel molecules.



1
Piperidine class

2
New cyclopentanes

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

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New SAR exploration of isoquinolinone-derived kappa opioid receptor agonists

We have developed a kappa opioid (KOR)-selective chemotype based on an isoquinolinone carboxylic acid scaffold. Numerous amide derivatives of this scaffold possess high affinity for the KOR receptor, low affinity for the other opioid receptors and, in contrast to many KOR receptor ligands, possess a nonbasic, nitrogen-containing architecture. Representative analogues of this series have been shown to behave as full to partial agonists at the KOR receptor. Functional assays comparing the β arrestin recruitment, ^{35}S GTP conversion and ERK phosphorylation revealed that a select subset of active analogues were biased in favor of the G-protein pathway. Such compounds could be utilized to further elucidate KOR signaling pathways and as probes to test whether such biased compounds could lead to KOR-based analgesics. In an effort to expand the versatility of this series, we have further examined three additional aspects of this series: 1) ester replacement of the amide functional group, constructing a set of six novel analogues that were screened for KOR agonism and plasma stability. The ester functional group would be expected to have far less stability in a physiological-like environment and function as a short acting KOR agonist. 2) eutomer/distomer determination through chiral chromatographic separation, screening of the isolated enantiomers and X-ray crystallographic structural determination. 3) major scaffold manipulations such as appending additional rings and incorporation of covalent modifiers such as epoxides. The later could be useful tools toward obtaining a crystal structure of the KOR receptor bound to an agonist ligand.

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Activation of the kappa-opioid receptor system is sufficient for reinstatement of nicotine-self-administration in mice.

Nicotine is the most widely used addictive substance, and its use is accompanied by a high propensity for relapse. However, the neurobiological mechanisms underlying nicotine relapse/reinstatement remain unclear. Prior studies have shown that in rodents, activation of the kappa-opioid receptor (KOR) system via stress-induced dynorphin release elicits reinstatement of drug-seeking behaviors. Therefore, our goal is to establish the role of the dynorphin/KOR system in stress-induced reinstatement of nicotine-seeking. We first trained male C57BL/6 mice to self-administer nicotine intravenously (0.03 mg/kg/infusion, 60 min sessions) on a fixed ratio-5 schedule of reinforcement for a minimum of 14 days. After stable levels of nicotine intake were established, mice underwent extinction training until criterion was reached (≤ 20% of responding compared to last nicotine self-administration session). We then investigated whether activation of KORs were sufficient to induce reinstatement of nicotine-seeking. Indeed, mice showed a robust reinstatement response after administration of the KOR agonist, U50,488 (2.5-5.0 mg/kg, i.p., 30 mins prior to reinstatement test). This data suggests that activation of the KOR system is sufficient to cause nicotine-seeking in mice. Future follow-up studies will examine whether systemic KOR selective antagonists attenuates stress-induced reinstatement of nicotine-seeking in mice, as well as determine the neural circuitry involved in KOR-mediated reinstatement of nicotine-seeking.

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Using Progressive Ratio and In Vivo Binding to Characterize Novel Kappa Opioid Receptor Antagonists for the Treatment of Motivational Deficits

Kappa opioid receptors (KOR) have been implicated in controlling mood, anxiety and addiction. It is suggested that KOR antagonists may treat deficits in motivation in disorders such as depression. We characterized the ability of the KOR antagonist, PF-04455242 to restore the deficit caused by the KOR agonist, spiradoline using the progressive ratio assay. Furthermore, we used in vivo receptor binding to determine the level of KOR occupancy in the brain required to elicit these effects.

To assess motivation levels, food restricted C57Bl6/j mice were trained to nose poke for a food reward. For progressive ratio responding, the number of nose pokes required to obtain a reward increased with each trial (PR = [5 * e^(n*0.24)]-5). Spiradoline (0.32-10 mg/kg, sc) dose-dependently decreased responding ($F_{5,66}$=42.77; P<0.01). Co-administration of PF-04455242 (10 & 17.8 mg/kg, sc) attenuated the spiradoline (3.2 mg/kg) induced deficit (SPIR vs PF + SPIR: P<0.01). In fixed ratio responding, PF-04455242 (32 mg/kg) or spiradoline (0.32-10 mg/kg, sc) produced significant deficits ($F_{4,33}$=10.84 P<0.01 and $F_{4, 52}$=15.07 P<0.01, respectively). In vivo target occupancy was assessed by measuring displacement of [^{3}H]PF-04767135 (100 μCi/kg, i.v.). Spiradoline inhibited 50% KOR binding at a calculated dose of 8.9 ± 3.3 mg/kg and PF-04455242 at a dose of 12.6 ± 1.5 mg/kg.

Spiradoline produced a consistent deficit in progressive ratio responding which was reversed by PF-04455242. At high doses both spiradoline (≥40% RO) and PF-04455242 (at ≥75% RO) reduced fixed ratio responding, indicating potential off-target effects. These data suggest that 40% RO by a KOR agonist, produced a robust deficit which could be restored by administration of doses of KOR antagonist that blocked >60% RO. These results indicate that the progressive ratio and in vivo receptor occupancy assays can be used to characterize potent KOR antagonists in vivo.

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Is there a synergism for antidepressant-like effect between a kappa opioid antagonist (LY2444296) and a delta opioid agonist (ADL5859) in the mouse forced swim test?

Kappa opioid antagonists and delta opioid agonists have antidepressant-like effects in animal models and they may be useful for treatment-resistant depression in humans. However, it is unknown whether the combination of a kappa antagonist and a delta agonist would produce a better than additive effect (i.e. synergy). LY2444296, an analogue of LY2456302, is a novel short-acting kappa opioid antagonist. ADL5859 is a delta opioid agonist which produces antidepressant-like effects without inducing seizures and EEG disturbances in rodents. Here each compound and combinations of the two were examined in the mouse forced swim test (FST), a screening test for antidepressant-like effect. Adult C57BL/6 mice (Jackson Lab) were administered subcutaneously (s.c.) with vehicle (saline) or LY2444296 at doses of 3, 10 and 30 mg/kg and 60 min later examined in the FST. The two higher doses significantly decreased immobility time in a dose-dependent manner, and the lowest dose had no effect. Intraperitoneal (i.p.) injections of ADL5859 also reduced the immobility time dose-dependently at doses of 3 and 10 mg/kg compared with vehicle (H_2O), but had no effect at 1 mg/kg. Four combinations of the two compounds at a fixed dose ratio were tested in mice. A synergy analysis was conducted using the method of dose equivalence. Preliminary results revealed a tendency suggestive of synergism for combining LY02444296 and ADL5859. More dose combinations are being tested.

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Restorable effects of kappa opioid receptor antagonist on the attenuation of the development of physical dependence on morphine by formalin-induced pain in mice

Physical dependence on morphine is clarified by the spontaneous or antagonist-precipitated morphine withdrawal. We have reported that serum corticosterone (SCS) increase is a sensitive and quantitative indicator of morphine withdrawal. In this study, we examined the effects of kappa opioid receptor antagonist, nor-binaltorphimine (nor-BNI), on the attenuation of the development of physical dependence on morphine by formalin-induced pain in ICR mice. SCS increase was employed as an indicator of the intensity of morphine withdrawal, and SCS level was estimated by fluorometrical method. Mice were treated with morphine (10 mg/kg, s.c., twice a day) for 6 days, and were injected formalin (2%, 0.02 ml, intraplantar) on day 1 (right side) and day 4 (left side). Nor-BNI (20 mg/kg, s.c.) was injected 24 hr before the first morphine treatment. On day 7, morphine withdrawal was precipitated by naloxone (5 mg/kg, s.c.), and blood sample was collected at 30 min after naloxone. Naloxone-induced SCS increase was inhibited by the intraplantar injection of formalin, indicating that formalin-induced pain attenuated the development of physical dependence on morphine. The inhibition of SCS increase was restored by the pretreatment with nor-BNI. These results suggest that kappa opioid system may involve in the chronic inflammatory pain-induced attenuation of development of physical dependence on morphine.

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

c-Jun N-terminal Kinase (JNK) Mediated Inactivation of Opioid Receptors

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The development of spinally-mediated tolerance following administration of the mu opioid receptor (MOR) agonist morphine requires activation of c-Jun N-terminal Kinase 2 (JNK2), and JNK-dependent kappa opioid receptor (KOR) inactivation has also been found to be responsible for long-acting kappa KOR antagonist actions (Bruchas et al., 2007; Melief et al., 2010). The durations of action of a broad range of KOR antagonists, including norBNI, positively correlate with the ability of the antagonist to activate JNK1 (Melief et al., 2011), whereas there is no correlation between duration of antagonist action and *in vivo* drug clearance rate (Munro et al., 2012). *In vivo* treatment with morphine for 4 hours or norBNI for 7 days causes a JNK-dependent reduction in agonist stimulation of [^{35}S]GTPγS binding in isolated spinal cord membranes. This suggests that JNK activation ultimately results in receptor uncoupling, rather than JNK activation blocking a down stream effect on the behavioral response to opioid receptor activation. We hypothesized that JNK activation phosphorylates a component of the receptor-signaling complex, but alternative sites of action are not excluded. Indirect effects would include changes in gene expression or effects on downstream signaling cascades, whereas direct effects would include phosphorylation of the receptor itself or components of the receptor-signaling complex. To distinguish between direct and indirect effects responsible for opioid receptor uncoupling, we tested whether an *in vitro* kinase treatment could affect opioid stimulated [^{35}S]GTPγS binding. First, we confirmed that a 4 hour morphine treatment of mice caused a reduction in subsequent DAMGO stimulated [^{35}S]GTPγS binding in spinal cord membranes, confirming previous findings. Morphine-pretreatment also reduced U69,593 stimulated [^{35}S]GTPγS binding in spinal cord membranes, consistent with prior results showing cross desensitization. Next we isolated spinal cord membranes from untreated mice and incubated them for 30 min with JNK1α1. We found that DAMGO stimulated [^{35}S]GTPγS binding was significantly reduced by *in vitro* JNK treatment, and that this inhibition required both kinase and ATP. Similarly, U69,593 stimulated [^{35}S]GTPγS binding in mouse spinal cord membranes was also blocked by *in vitro* JNK treatment in an ATP-dependent manner. These results suggest that the JNK-phosphorylated substrate responsible for opioid receptor inactivation is present in washed membranes and that JNK activation directly desensitizes opioid receptor signaling. Identification of the phosphorylated component of the opioid receptor-signaling complex is ongoing.

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Social defeat stress reverses the effects of a kappa opioid receptor agonist on social interaction behavior in female but not male California mice

Psychosocial stress leads to activation of kappa opioid receptors (KOR) which in turn facilitate depressive-like behaviors. This has generated a strong interest in the development of KOR antagonists as a potential novel class of antidepressant. However, most studies showing stress-induced activation of KOR focus on more short-term effects of stress and only study male subjects. New evidence suggests that long-term effects of stress (over weeks or months) have important implications for KOR function, and that there may be sex differences. We examined the effects of social defeat stress on KOR action using California mice (*Peromyscus californicus*), a monogamous species in which defeat can be studied in both males and females. Behavioral observations were conducted two weeks after three episodes of social defeat or control conditions. A 10 mg/kg i.p. injection of KOR agonist U50,488 reduced social interaction behavior in females naïve to defeat. Females exposed to defeat and injected with vehicle had reduced social interaction behavior, as previously described. However, in stressed females U50,488 exerted an antidepressant effect, increasing social interaction to the level of controls treated with vehicle. In males there was a trend for U50,488 to reduce social interaction behavior in stressed males, suggesting a sex specific impact of social defeat. These results indicate that defeat stress may induce long-term changes in KOR activation in females. Ongoing studies are investigating potential mechanisms underlying this effect. These data suggest that KOR agonists should be further evaluated for potential antidepressant effects in the context of stress-induced psychiatric disorders.

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Ligand discovery and functional analysis of the novel opioid orphan receptor MRGPRX2

Orphan G protein-coupled receptors have no known endogenous ligands and make up approximately 30% of the non-olfactory GPCRs. These understudied receptors have potential pharmacological and therapeutic relevance. Although orphan GPCRs are expressed throughout the body, several subtypes are expressed exclusively in the brain and/or spinal cord and may be tractable targets for the treatment of pain. One such class of receptors is the Mas-Related G Protein-Coupled Receptor X (MRGPRX) family of orphan GPCRs that are expressed (or just "express") in the dorsal root ganglion and mast cells. These receptors have few known ligands and have poorly characterized function *in vivo*. We used a β-arrestin recruitment assay to screen approximately 6,800 compounds against the MRGPRX family of receptors and discovered that many opiate compounds (eg. morphinans, benzomorphans) activate MRGPRX2 but not MRGPRX1 or MRGPRX4. We confirmed these activities using calcium flux and PI hydrolysis assays and created preliminary structure-activity relationship (SAR) for the MRGPRX2 receptor. Our data indicate that MRGPRX2 is activated by opiate drugs and may be a novel Gαq-coupled opioid receptor with physiological relevance.

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

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Phosphoproteomic Survey of KOR-mediated Signal Transduction

A comprehensive survey of kappa opioid receptor (KOR)-mediated signal transduction at the systems level is lacking. In particular, functional selectivity of KOR in a cellular context has been largely underexplored beyond the initial G-protein or arrestin recruitment and mitogen-activated protein kinases (MAPKs) activation. To fill this gap, we employ quantitative high-resolution mass-spectrometry to examine the hypothesis that the components of KOR-mediated signal transduction can be discovered through phosphorylation events by applying unbiased phosphoproteomics on the human KOR transiently expressed in the Human Embryonic Kidney (HEK) cells. Specifically, combination of the stable isotope labeling by amino acid in cell culture (SILAC), which is a powerful technique to yield precise quantification, an internally-developed 96-well format phosphopeptide enrichment, and the state of art high-field orbitrap mass-spectrometry yields, on average, over 7000 quantifiable phosphorylation events per sample on a two-hour measurement. From this approach, we observed that phosphorylation of MAPKs, such as p38alpha, increases in a dosage-dependent manner. The abovementioned high-throughput approach thus enables a proteomic-based investigation of KOR pharmacology on a systems level.

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Development of Novel Kappa Opioid Receptor Agonists Based on the Bisamide Scaffold.

The kappa opioid receptor (KOR) belongs to the superfamily of G protein-coupled receptors and regulates a wide range of physiological functions including reward, depression, and pain relief. In pursuit of the therapeutic potential of KOR agonists, we aimed to investigate new structural classes of KOR ligands seeking selective and potent compounds with fewer side effects.

The bisamide class of KOR agonist in this work was originally discovered in a high-throughput screening campaign using the Molecular Libraries compound collection. The chemotype possesses an attractively simple and highly modular peptide-like scaffold. To efficiently explore the potential of this new series, we have developed a Ugi multicomponent reaction to afford analogues in a single step. Sixty-two analogues were synthesized and screened in an initial round of SAR, revealing two examples with single digit nanomolar potency. In addition, several discrete SAR trends emerged from analysis of the data set. The most recent results obtained from this line of reasoning will be presented.

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

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Sex differences in kappa opioid receptor-mediated negative affective states in rats

Dynorphin, an endogenous ligand at kappa opioid receptors (KOR), is required for the expression of stress-induced depressive-like states in male rats. Previously we demonstrated that female rats are less sensitive to the depressive-like effects of KOR activation in the intracranial self-stimulation (ICSS) paradigm. The present study was designed to assess the role of KORs in the ability of an ethologically relevant stressor, social isolation (SI), to produce negative affective states in male and female rats. Adult male and female rats were either group housed (GH) or socially isolated (SI) for 5 weeks. The elevated plus maze (EPM) was used to assess the effects of SI on anxiety. Place conditioning was used to assess the effects of SI on the aversive effects of the KOR agonist U50,488. In separate rats, effects of SI on plasma corticosterone levels and brain region-specific levels of dynorphin and KOR gene expression were measured. SI reduced the amount of time spent in the open arms of the EPM in both males and females, an anxiogenic effect. SI female rats were less sensitive than males to U50,488-induced conditioned place aversions. SI rats had lower corticosterone levels compared to GH rats. Finally, KOR mRNA levels were reduced in the bed nucleus of the stria terminalis (BNST) of female compared to male rats and were lowest in SI female rats. Our results are consistent with previous work demonstrating depressive-like effects of SI and they suggest that SI acts to decrease sensitivity to KOR activation—particularly in females. SI- and sex-dependent modulation of KOR expression in the BNST may contribute to sex differences in stress-induced depressive-like states. Taken together, our findings raise the possibility that novel, KOR ligands could be used as effective, gender-specific pharmacotherapies for anxiety and depressive disorders.

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

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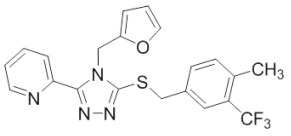
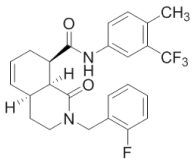
Functionally selective kappa opioid receptor agonist efficacy *in vivo*

Through a collaborative probe discovery program we have developed a series of KOR agonists that promote active signaling in G protein binding with little activity for βarrestin2 recruitment (Zhou et al., 2013, JBC). We characterized these compounds in various behavioral assays to evaluate whether distinct behavioral profiles that differ from the reference KOR agonist, U50,488H, exist. Here, we describe behavioral profiles for Triazole 1.1 and Isoquinolinone 2.1 compared to U50,488H in antinociception, locomotor activity, acute pruritus, and are beginning to investigate differences in conditioned place aversion. While the antinociceptive and anti-pruritic potency of our novel KOR agonists are similar to U50,488H, our observations show that Triazole 1.1 does not suppress locomotor activity. Treatment of pruritus is of particular interest since the only clinically utilized KOR agonist on the market used effectively in the treatment of pruritus is partial agonist Nalfurafine. The question remains as to whether more efficacious KOR agonists can be developed for itch or pain without sedating effects and whether the recruitment of βarrestin2 may serve as a pivotal point to consider in drug design.



Triazole 1.1



Isoquinolinone 2.1

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Disclosure: The authors have nothing to disclose.

Computational Modulation of the Kappa-Opioid Receptor by Sodium: Structural Implications for Receptor Activation and Ligand Binding

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The functional activities of the mu, delta and kappa opioid receptors (MOP, DOP and KOP, respectively) are modified by sodium ions. High-resolution crystal structures of recently released GPCRs, including DOP, have produced valuable insights into the mechanism by which sodium allosterically modulates the function of these complex signaling proteins. $N141^{3.35}$ has been postulated to play an important role in receptor activation and is located in the critical sodium-binding pocket that is conserved in opioid and other GPCRs. Exploration of the effect of sodium on KOP ligand binding was conducted via conventional molecular dynamics (cMD) simulation of unliganded KOP and KOP in complex with either the long-acting antagonist JDTic or the highly affine and non-basic agonist salvinorin A (SalA). Each model was constructed either with or without a Na^+-occupied sodium binding pocket. Analyses of the obtained trajectories revealed that the KOP–JDTic and KOP–JDTic-Na^+ model showed very limited differences. The JDTic binding mode was conserved as well as a number of conserved water sites. These two models were in turn similar to the *apo*-KOP-Na^+ model. However, the SalA–KOP-Na^+ model showed significant differences compared to the SalA–KOP model. Strikingly, SalA was no longer attached favorably the protein pocket and floated away from the pocket during the simulation. These results suggested that the presence of the sodium ion in its allosteric binding pocket caused conformational changes (or conformational retention) amenable to interactions with antagonists, but that are not suitable for interaction with agonists. As a corollary, the KOP–SalA possessed features suitable for interaction with agonists, while the other models exemplified antagonistic conformations. We present similarities and differences among key conformational features of these models that elucidate the role of sodium as an important determinant of molecular recognition by KOP, and likely by other opioid receptors.

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Activation of the kappa-opioid receptor system is both necessary and sufficient for reinstatement of nicotine place preference.

The Kappa-opioid receptor (KOR) system has been implicated in stress-induced reinstatement of drug seeking behavior for nearly every major drug of abuse, attesting to dynorphin/KOR's conserved role in this behavioral response. Due to nicotine's high propensity for stress-induced relapse, we hypothesized that stress would also induce reinstatement of nicotine seeking behaviors in a KOR-dependent manner. Using an unbiased, counterbalanced conditioned place preference (CPP)/reinstatement protocol, mice were pre-tested on day1, and conditioned on days2-3, with saline treatment during AM sessions and nicotine (0.5mg/kg) in PM sessions (20min). On day 4, they were tested for nicotine place preference, determined by time spent in the drug-paired chamber post-test minus pre-test. Animals were extinguished for 2 days prior to the reinstatement phase. We found that the widely used pharmacological stressor Yohimbine (Yoh) (2mg/kg) 5 minutes prior to reinstatement post-testing causes reinstatement of nicotine CPP. This reinstatement of nicotine CPP is NorBNI sensitive, indicating that KOR activity is necessary for Yoh-induced nicotine CPP reinstatement. To determine if KOR activation alone is sufficient for reinstatement of nicotine CPP, we injected KOR agonist U50,488 (5mg/kg) 30 minutes prior to the reinstatement post-test, and found that KOR activation was sufficient to reinstate nicotine place preference. Two hours following reinstatement, mice were perfused to examine the effects on Yoh on neuronal activation (c-fos) in the presence and absence of KOR signaling. We visualized robust c-fos expression in the Basolateral Amygdala (BLA) and Central Amygdala (CeA) following Yoh treatment. However, c-fos expression was significantly reduced in these regions in mice pre-treated with norBNI . Follow-up studies were conducted to locally inactivate KOR or neuronal activity in these regions, to assess the influence of KOR-expressing cells and neural circuits on nicotine CPP. These data suggest a role for KOR activity in these regions with the expression of stress-induced nicotine seeking behaviors.

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NMR Characterization of Structure and Conformational Dynamics in the Dynorphin–KOR–Gα_i System

Signal transduction across the plasma membrane through G-protein coupled receptors is an inherently dynamic process. The next generation of rationally designed kappa opioid receptor (KOR)-targeting drugs will therefore benefit from knowledge of atomic-scale conformational dynamics.

Solution NMR spectroscopy was used to study KOR reconstituted in detergent micelles and apolipoprotein A nanodiscs. The structure of the endogenous peptide agonist dynorphin was determined in a low affinity KOR-bound state. The dynorphin peptide structure consists of two turns of helical secondary structure formed by residues L5 to R9, which are bounded by flexibly disordered N- and C-terminal peptide segments with residues Y1 to F4, and L10 to K13, respectively. Changes to this dynorphin conformation were observed upon addition of Gα_i, to KOR indicating transitions between different KOR states of dynorphin.

KOR conformational dynamics were directly measured by fluorine NMR spectroscopy. Trifluoroethanethiol was attached to cysteine residues as a ^{19}F-NMR probe. We investigated how ligands, solution conditions, and protein binding partners affected the local dynamics, which yielded indications of a complex conformational landscape for KOR reconstituted in detergent micelles

Ongoing investigations are aimed at mapping the contact surface of Gα_i when bound to KOR reconstituted in nanodiscs.

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Molecular modeling studies of Sodium ion binding pocket suggest a mechanism of biased signaling in κ-OR

Conformational changes required in class A GPCR signaling are believed to be mediated by highly conserved sodium ion binding site[1]. Recently resolved crystal structures of opioid receptors and related chemical genetic studies provide insights into Na+ binding pocket geometry, which further substantiates its putative role in ligand binding and downstream biased signaling[2,3]. κ-OR, like the other opioid receptors, has a distinct Na+ pocket side chain residue $N^{3,35}$, which plays an important role in the coordination of sodium ion in the cavity by replacing a water molecule. Furthermore, mutation associated with Na+ binding pocket residue $N^{3,35}$-A, in δ-OR (which shares similar Na+ binding pocket with κ-OR) resulted in transformation of an inhibitory effect of several antagonists in WT into biased β-Arrestin signaling in the mutant[4]. We hypothesize that this sodium ion –mediated mechanism could play a pivotal role for observed bias in κ-OR signaling. We will discuss results from molecular modelling studies and structural analysis of Na+ pocket of κ-OR and δ-OR, which will shed more light on this mechanism at molecular level.

Disclosure: The authors declare no conflict of interest.

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Cortical alterations in KOR-1 KO mice.

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We have previously reported that KOR-1 KO mice exhibit altered ultrasonic vocalization patterns both as neonates and as young adults. We have here begun to determine whether any alterations in brain circuitry occur in KO-1 KO mice that potentially could be related to altered behavior. In these initial studies, we have examined cortical organization in 129S6 WT and 129S6 KOR-1 KO as well as compared the 129S6 WT pattern to that of C57Bl6/J mice. Cortical layer organization was assessed using the upper and lower layer markers CDP and TLE4, respectively, while gliogenesis was assessed using APC. Several significant results have arisen from the analysis to date. First, there is a significant decrease in the ratio of upper and lower layer neuronal markers in KOR-1 KO mice compared to isogenic 129S6 WT mice. Second, this change appears to primarily result from a decrease in the number of CDP neurons. Third, there is a dramatic decrease in the ratio of upper to lower layer markers in C57Bl6/J WT mice compared to 129S6 mice. Fourth, the distribution of CDP neurons is restricted to most upper cortical layers in C57WT mice compared to 129S6. Fifth, the extent of glia presence is similar in all three genotypes examined. Together, these data provide the first evidence that neuronal organization within the neocortex is altered not only between distinct mouse strains but also in KOR-1 KO mice.

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None

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Immediate and Persistent Effects of Salvinorin A on the Kappa Opioid Receptor in Rodents, Monitored *in vivo* with PET

Monitoring changes in opioid receptor function with positron emission tomography (PET) could lead to a better understanding of tolerance and addiction since altered opioid receptor dynamics following acute or chronic exposure to agonists has been linked to tolerance mechanisms. PET imaging of neurotransmitter systems allows the mapping of changes in both receptor occupancy and density following acute or chronic administration of exogenous ligands. We have studied changes in kappa opioid receptor function *in vivo* with positron emission tomography (PET) following kappa opioid agonist administration. Male Sprague-Dawley rats (n=30) were anesthetized and treated with the kappa opioid receptor (KOR) agonist Salvinorin A (0.032 – 1.8 mg/kg, i.v.) prior to administration of the KOR selective radiotracer [^{11}C]GR103545. This is the first report of assessing tracer uptake and kinetics of [^{11}C]GR103545 via dynamic PET imaging in rodents. We observed specific binding in regions of highest kappa opioid receptor density in, nucleus accumbens, caudate-putamen, amygdala, hypothalamus, and midbrain. When Salvinorin A was administered 1 min prior to injection of the radiotracer, [^{11}C]GR103545 specific binding was decreased in a dose-dependent manner, indicating receptor binding competition. In addition, the unique pharmacokinetics of Salvinorin A (half-life ~3 min in rats) allowed us to study the drug's residual impact on KOR after the drug was eliminated from the brain. Salvinorin A was administered up to 5 h prior to [^{11}C]GR103545 and the changes in specific binding were compared to baseline, 2.5 h and 1 min pretreatment times. Our results indicate that Salvinorin A causes a persistent decrease in KOR function as indicated by decreased [^{11}C]GR103545 specific binding, well after Salvinorin A had been eliminated from the brain.

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U69,593 and Nalmefene stimulate prolactin release, with modulation by mu and/or kappa blockade, in mice.

The kappa opioid receptor has recently emerged as a prominent target in the search for viable addiction pharmacotherapeutics. On one hand, antagonists have been shown to result in attenuation of stress-induced reinstatement of cocaine self-administration, whereas kappa agonists result in decreased reward association in conditioned-place-preference models. We hypothesize that a drug which can function as both a kappa antagonist and agonist, namely a kappa opioid receptor *partial* agonist, will yield serve as a suitable pharmacotherapeutic. While to date no *selective* kappa partial agonist has been developed for human use, nalmefene has kappa partial agonist properties, as well as mu antagonism. The effects of nalmefene as a kappa partial agonist have not been explored extensively *in vivo* in animal models. We compared nalmefene with U69,593 on prolactin response in male C57BL/6J mice. Using a commercial sandwich enzyme linked immunosorbent assay, we determined that serum levels of prolactin were increased by U69,593 in a dose-dependent fashion, with a half-maximal dose of approximately 0.32 mg/kg. Nalmefene (10 mg/kg) alone did not induce prolactin release in mouse blood sampled 1 hour following nalmefene administration. However, pretreatment with clocinnamox (10 mg/kg, in 25% DMSO, 1 hour prior to drug treatment), an irreversible mu antagonist, resulted in nalmefene induced prolactin release. This increase was attenuated by 24 hour pretreatment with the long-acting kappa antagonist JDTic. JDTic also blocked U69,593 induced prolactin increase, as expected, whereas clocinnamox had no effect on U69,593 induced prolactin release. The results demonstrate a complicated effect of the mixed kappa-partial agonist/mu-antagonist nalmefene in animal models. Prior irreversible antagonism of the mu opioid receptor with clocinnamox “unmasks” at least the prolactin-releasing kappa opioidergic effects of nalmefene. This suggests that nalmefene may serve as a model kappa partial agonist in animal models lacking the mu opioid receptor (e.g. constitutive or inducible mouse knockout strains).

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Genetic identification of the CNS neurons that express the kappa opioid receptor

The kappa opioid receptor (KOR) is known to be distributed broadly throughout the nervous system. However, the specific cell types that express KOR are poorly understood. We have now generated a KOR-cre knockin allele, thereby allowing the genetic marking of KOR-expressing cells. Here, we validate this mouse and characterize its expression pattern, with the hope that others in the greater research community may find it a useful reagent.

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The authors have no conflicts of interest to disclose.

Sarah M. Scarry[1], Kimberly Lovell[2], Kevin J. Frankowski[1], Stephen R. Slauson[1], Huiyong Ma[1], John M. Streicher[2], Lei Zhou[2], Angela Phillips[2], Thomas E. Prisinzano[1], Laura M. Bohn[2] and Jeffrey Aubé[1]

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Synthetic Efforts toward Kappa Opioid Receptor Antagonists.

There is considerable interest in the discovery and development of selective kappa opioid receptor (KOR) antagonists. It has been suggested that KOR-selective antagonists have potential therapeutic use for treatment of depression, anxiety, compulsive disorders, and substance abuse. In addition to their clinical significance, KOR antagonists serve as valuable chemical tools to interrogate KOR mediated processes. Several canonical KOR antagonists have been shown to possess an extremely long duration of action in animal models (> 3 weeks), which may limit their utility in vivo. Thus, the development of structurally distinct KOR-selective antagonists remains an ongoing focus of our research group. The pyridopyrrolopyrazinone and sulfonamide chemotypes were discovered as highly selective KOR antagonists with modest potency through a high-throughput screening campaign under the NIH Molecular Libraries Program. Initial structure-activity relationship (SAR) studies led to the pyridopyrrolopyrazinone and sulfonamide probe molecules ML190 and ML140, respectively. SAR exploration around the pyridopyrrolopyrazinone scaffold was greatly limited by the lengthy synthetic route and scarcity of available starting materials. We have developed an efficient and streamlined synthetic route to access novel analogues of this series. Recent synthetic efforts on both chemotypes have afforded a structurally diverse set of new analogues, including chimeric exemplars. These analogues have been evaluated for KOR antagonism leading to a more thorough understanding of the structural features critical for KOR potency.

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Disclosure: The authors declare no conflicts of interest.

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The effect of aging on peripheral kappa opioid receptor function

The management of pain in the elderly is a major medical challenge. Targeting peripheral opioid receptors could provide effective and safer medications for the elderly. However, little is known regarding the effects of aging on nociceptor (pain-sensing neuron) responsiveness or the function of opioid receptors expressed on nociceptors. Here, we compared the effects of the sensitizing agent, bradykinin (BK), and the kappa opioid receptor (KOR) agonist, Salvinorin A, on nociceptors in young (4-months-old) and aged (26-months-old) Fisher x Brown Norway rats. Behavioral responses to noxious heat, cold, or mechanical stimulation were measured following intraplantar injections of BK. For all three stimuli, aged rats displayed a greater allodynic response to BK. In primary cultures, activation of phospholipase C in nociceptors from aged rats was more sensitive to BK than those from young rats. In addition, local intraplantar administration of the KOR agonist Salvinorin A produced greater antinociceptive effects in aged rats compared to young rats. Similarly, opioid receptor-mediated inhibition of adenylyl cyclase activity was greater in nociceptors from aged rats. Overall, our results indicate that aging enhances allodynic effects of the inflammatory mediator, BK, as well as increases antinociceptive effects of a kappa opioid agonist. We propose that peripherally-restricted KOR agonists may be a valuable analgesic strategy for treating pain in the elderly.

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The authors declare no conflict of interest.

Basal and cocaine-induced prodynorphin and kappa opioid receptor gene expression may predispose Lewis but not Fischer rats to escalate cocaine consumption

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Endogenous opioids have important roles in mediating the reinforcing properties of drugs of abuse, including cocaine. Recently we reported that Lewis rats progressively escalate cocaine consumption compared to Fischer rats in a new paradigm of cocaine self-administration, in which rats can select the unit dose to self-administer during daily 18h sessions, which may more closely model the human condition of cocaine exposure.
The aim of the present study was to investigate the cocaine-induced changes in opioid system components expression in the dorsal striatum (DS), Nucleus Accumbens (Nacc) shell and core, and to determine if strain differences could contribute to relative vulnerability to escalate cocaine intake. We therefore compared Fischer and Lewis rats in prodynorphin (pDyn) and kappa opioid receptor (KOR) gene expression after exposure to 2 weeks of either cocaine self-administration or yoked-saline. We then performed a correlation analysis between the mRNA level observed and cocaine intake during the last operant session.
We found in DS that pDyn mRNA was higher in Fischer versus Lewis yoked-saline rats. pDyn gene expression was increased by chronic cocaine in both strains, and the percent increase seen in Lewis was greater than in Fischer, in parallel with the greater intake escalation in the former strain. Furthermore, in DS the pDyn and KOR mRNA levels were positively correlated with cocaine intake in Fischer, but not in Lewis rats. In the Nacc shell Fischer, but not Lewis rats, displayed a difference in pDyn due to cocaine exposure. However, in the NAcc shell and core there was no strain difference in either baseline pDyn or KOR and no correlation of gene expression level with cocaine intake.
Results presented here corroborate the hypothesis that baseline genetic factors in dorsal striatal pDyn and KOR, as well as their responsivity to cocaine self-exposure, could predispose subjects to escalate cocaine consumption.

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A G Protein-biased *κ* -Opioid Receptor agonist is analgesic with a unique spectrum of activities *in vivo*

The use of functionally selective G protein–coupled receptor (GPCR) agonists as safer therapeutics has revitalized interest for many GPCR targets. In particular, the *κ*-opioid receptor (KOR) represents an attractive target for developing biased therapies. KOR agonists are analgesic with a low risk of dependence and abuse, but their use is limited by a propensity to induce sedation, motor incoordination, hallucinations, and dysphoria-like states. Several laboratories have produced a body of work suggesting that G protein–biased KOR agonists might be analgesic with fewer side effects. Although that has been an intriguing hypothesis, suitable KOR-selective and G protein–biased agonists have not been available to test this idea until very recently. We provide data using a G protein–biased agonist, RB-64 (22-thiocyanatosalvinorin A), which suggests that KOR-mediated G protein signaling induces analgesia and aversion, whereas *β*-arrestin-2 signaling may be associated with motor incoordination. Additionally, unlike unbiased KOR agonists, the G protein–biased ligand RB-64 does not induce sedation and does not have anhedonia-like actions, suggesting that a mechanism other than G protein signaling mediates these effects. Our findings provide evidence for a highly selective and G protein–biased tool compound that produces less negative side effects than unbiased KOR agonists. These results suggest that KOR biased ligands may be useful as therapeutics, yet prior to developing these therapies we must better understand the complex KOR signaling networks beyond G protein and *β*-arrestin-2 signaling and their roles in physiology, and assemble a clear structure/function relationship for a range of biased and unbiased ligands.

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LATE ABSTRACT SUBMISSION

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New insight to the catecholamine-mediated antidepressant-like mechanism of 22-azidosalvinorin A - a compound with high affinity for kappa-opioid receptor

Chemical modifications of salvinorin A (SA), a potent selective kappa opioid agonist and psychoactive constituent of *Salvia divinorum*, seems to be prerequisite to its potential therapeutic applications. The present study sought to investigate 22-azidosalvinorin A (ASA) - new SA derivative in the animal model of depression-forced swimming test (FST). In FST, an increase in swimming time without alteration in locomotor activity was observed with oral administration of ASA. Unlike *p*-chlorophenylalanine (serotonin depletor), prazosin (selective α1-receptor antagonist) or WAY100635 (selective serotonin 5-HT_{1A} receptor antagonist) pretreatments, α-methyl-*p*-tyrosine (catecholamine depletor) pretreatment attenuated the antidepressant-like effects of ASA. Though the antidepressant-like effect of ASA suggests the involvement of catecholamines, it is still unclear to relate this effect with its high KOR binding affinity (K_i=5.43nM). Hence, subsequent study will focus on the clarification of this biological activity and underlining neural mechanism.

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