Kappa Therapeutics

Seattle, Washington - 2011

"Therapeutic Potential of Kappa Opioids in Pain and Addiction"

July 10th-13th, 2011

Edgewater Hotel & Conference Center

Seattle, WA

Supported by:

NIDA NATIONAL INSTITUTE ON DRUG ABUSE
The Science of Drug Abuse & Addiction

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Program Committee:

Charles Chavkin (University of Washington)
Bill Carlezon (McLean Hospital & Harvard University)
Ivy Carroll (Research Triangle Institute)
Alan Cowan (Temple University)
Howard Fields (UCSF & Gallo Inst)
Toni Shippenberg (NIDA-IRP)

Contact Information:

http://depts.washington.edu/kappa11/index.html

Presentations

Bring your powerpoint on a USB drive and give to the projectionist at least 30 minutes before your session. If you have a complex video presentation, you can attach your laptop during the question period immediately prior to your talk.

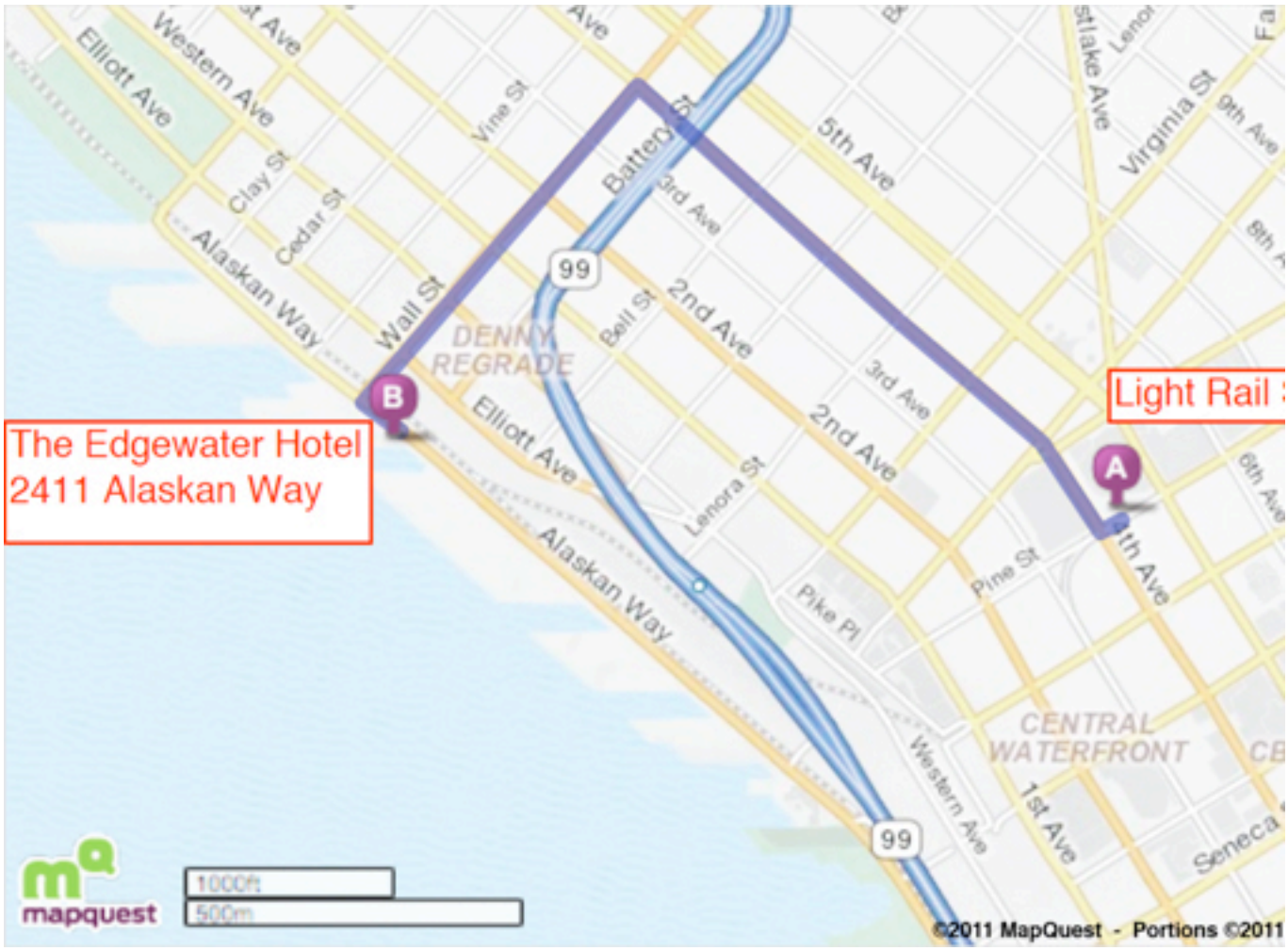
Poster boards measure 4'x8'. Set up your poster (pins will be available) on Monday after lunch in the Cascade room (2nd floor). The poster session will be on Monday 4-6PM, and your poster can remain up until the end of the meeting (Wed 6PM).

Travel Advice

Taxis and limos are available at the Airport (follow signs).

Shuttle Express offers van rides to the Edgewater Hotel (206-246-7675) or http://shuttleexpress.com/seatac-airport/default

Light Rail is convenient and costs $2.75. Follow signs at airport to "Link to Light Rail." After you leave baggage claim, go up to the Ticketing level (4th floor), walk North (car traffic on the airport drive goes South), you'll see the walkway to the light rail station opposite the United Airlines desks. Get a ticket to the "Westlake Station." From the Westlake Station, it's a 16 min walk (0.9 mi) or a short cab ride to the hotel.



Local Attractions (walking distance)

- Olympic Sculpture Garden - 2901 Western Avenue (8 minute walk from Hotel)
- Seattle Aquarium - 1483 Alaskan Way (10 minute walk from Hotel)
- Pike Place Market - 1531 Western Avenue (12 minute walk from Hotel)
- Seattle Center (Space Needle, Pacific Science Center, Experience Music Project, Monorail, Seattle Guided Tours) (19 minute walk from Hotel)
- Bainbridge Island Ferry (1 mile south on Alaskan Way) (19 minute walk from Hotel)
- Pioneer Square (30 minute walk from Hotel)
- Underground Seattle Tour - In Pioneer Square
- Theo's Chocolate - 3400 Phinney Avenue N (short cab ride, factory tours available by reservation; among the best chocolate truffles/caramels in the world)

A Few Favorite Restaurants (within walking distance)

(ask the Concierge or use http://www.opentable.com or http://www.yelp.com/ for recommendations & reservations)

- Shiro's - 2401 2nd Avenue (great sushi)
- Wild Ginger - 1401 3rd (very popular Asian fusion)
- Travolata - 23232 2nd Avenue (recommended Italian)
- Restaurant Zoe - 2137 2nd Avenue (American)
- Café Campagne - 1600 Post Alley (great French Bistro)
- Waterfront Seafood Grill - 2801 Alaskan Way (expensive fish)
- El Gaucho - 2505 1st Avenue (expensive steak)
- Local 360 - 1st and Bell (Northwest-Local)
- Palace Kitchen - 2030 5th Avenue (Northwest)
- Matts in the Market - 94 Pike Street (Northwest)
- RN74 - 1433 4th Avenue at Pike Street (French/local seasonal)
- Salumi Deli - 309 3rd Avenue S (Lunch Only - Mario Batali's family owns)
- Serious Pie - 316 Virginia (great thin crust pizza)
- Steelhead Diner - 95 Pine Street (Northwest Diner)
- Spur - 113 Blanchard (Gastro Pub)
- Umi Sake House - 2230 1st Avenue (Japanese Izakaya/Sushi)
- Maximillian in the Market - 81 Pike Street (great French)

A Few Favorite Restaurants (within short cab ride distance)

- Skillet Diner - 14th and Union (Creative Diner) (cab)
- Sitka & Spruce (highly recommended west-Northwest Local) (cab)
- Poppy - 622 Broadway E (great Asian Indian-inspired small plates) (cab)
- Crush - 2319 E Madison Street (expensive, but great local food) (cab)

Pubs/Local Breweries/Cocktails:

- Elysian Fields Brew Pub - 542 1st Avenue S & 1221 E Pike Street (Alternate location in Capitol Hill)
- Pikes Brewery - 415 1st Avenue
- Pyramid Brewery - 201 1st Avenue South
- ZigZag - 1501 Western (award winning cocktail lounge)
- Vessel - 1312 5th Avenue (craft cocktails)
- Artusi - 1435 14th Avenue (cab)

"Therapeutic Potential of Kappa Opioids in Pain and Addiction"
Edgewater Hotel and Conference Center
Seattle, WA July 10-13, 2011

SUNDAY **July 10, 2011** Registration 12-5 PM in Foyer, Opening reception 4-6 PM in Lobby Bar

MONDAY **July 11, 2011**

7-8 AM **Continental Breakfast / Registration** (Terrace Room 4th floor)

8:15 AM **Welcome (Charles Chavkin, Program Chair)** (Olympic Ballroom)

Oral Session 1 **Medicinal Chemistry (Charles Chavkin, Session Chair)** (Olympic Ballroom)

8:30 Carroll FI, Rehder K, Fennell TR, Pollard GT, Howard JL. **Does the duration of action of the antagonism of kappa agonist-induced diuresis by kappa opioid receptor antagonists depend on their pharmacokinetic properties?**

8:50 Mitch C, Quimby S, Diaz N, Pedregal C, De La Torre M, Shi Q, McKinzie D, Statnick M, Rash K, Barth V. **Discovery of Kappa Selective Opioid Receptor Antagonists: Application to Preclinical Evaluation of New Receptor Occupancy Tracers.**

9:10 Melief EJ, Miyatake M, Carroll FI, Béguin C, Potuzak JA, Carlezon Jr WA, Cohen BM, Grimwood S, Mitch CH, Rorick-Kehn L, Chavkin C. **Duration of action of a broad range of selective kappa opioid receptor antagonists is positively correlated with c-Jun N-terminal Kinase-1 activation.**

9:30 Prisinzano TE, Aubé J, Hedrick MP, Gosalia P, Frankowski K, Shi S, Schoenen F, Su Y, Vasile S, Sergienko E, Gray W, Hariharan S, Slausen S, Ghosh P, Milan L, Heynen-Genel S, Chung TDY, Caron M, Bohn LM Barak LS. **Discovery and preliminary evaluation of five new kappa opioid receptor antagonist and agonist scaffolds.**

9:50 Husbands SM, Cueva JP, Kumar V, Clark M, Traynor JR. **Selectively promiscuous kappa opioid receptor *antagonists*.**

10:10 Aldrich JA, Kulkarni SS, Senadheera SN, Patkar KA, Ross NC, Reilley KJ, Eans S, Ganno ML, Zhang Y, Kreek MJ, McLaughlin JP. **Peptide Kappa Opioid Receptor Ligands: Challenges in Development and Successes.**

10:30-11AM **Discussion / Morning Coffee Break** (Terrace Room)

11:00 Cashman JR, Ghirmai S, Kalisiak J, Azar MR, Wee S, Koob G. **Efficacious kappa antagonists for decreasing cocaine and alcohol self-administration**.

11:20 McCann D. **The discovery and development of JDTic: A NIDA perspective**

11:40 Zjawiony JK, Polepally PR, Setola V, Vardy E, and Roth BL. **Michael acceptor approach to the design of new salvinorin A - based high affinity ligands to the kappa-opioid receptor.**

12:00-2:00 PM **Buffet Lunch** (Terrace Room)

Oral session 1 (con't) **(Ivy Caroll & Alan Cowan, Session Co-Chairs)**

2:00 Neumeyer JL, Sromek AW, Zhang T, Bidlack JM. **Aminothiazole-modified morphinans with potent kappa and mu activity.**

2:20 Kivell BM, Morani A, Simonson B, Prisinzano T, Shippenberg TS, Miller JH, Schenk S, **Anti-addiction effects of Salvinorin A and novel analogues.**

2:40 – 4:00 **Medicinal Chemistry Workshop** (Data blitz – 1 slide per speaker) (Olympic Ballroom)

Additional speakers are invited to volunteer. As of 6/20, the following participants are listed:

John E. Mendelson, "Human Trials with Salvinorin A"
Jordan K. Zjawiony, "Salvinorin-based affinity labels for KOR"
Philip D. Mosier, "KOR-selective *N*-alkyl-octahydroisoquinolin-1-one-8-carboxamide agonists"
Stephen M. Husbands, "Selectively promiscuous kappa *agonists*"

Ron Dolle, "Medicinal chemistry of peripheral KOR agonists"
John R. Cashman, “Decreasing hepatotoxicity of kappa antagonists”

4:00 – 6:00	**Poster Session / Wine & Cheese**	(Cascade Ballroom 2nd floor)
7-9 PM	Dinner (no host)	(Local Restaurants, walk or short cab ride)
9-11 PM	Informal discussion in Pub	(Lobby Bar)

TUESDAY July 12, 2011

7-8:15 AM **Continental Breakfast** (Terrace Room)

Oral Session 2: **Addiction Models & Treatment (Toni Shippenberg, Session Chair)** (Olympic Ballroom)

8:15 Koob G. **A conceptual framework for the role for dynorphin in the motivational effects of drug dependence**.

8:30 Maldonado R. **Involvement of kappa opioid receptors in drug addiction**

8:50 Damaj MI, Carroll FI, Negus SS, Jackson KJ. **Effect of kappa opioid receptor antagonists in mouse models of nicotine dependence.**

9:10 Smith JS, Schindler AG, Gustin RM, Bruchas MR, Neumaier JN, Chavkin C**. Kappa opioid receptor antagonists: a promising pharmacotherapy to treat stress-induced increase in nicotine reward and dependence**

9:30 Walker BM. **The role of extended amygdala kappa-opioid receptors in escalated operant ethanol self-administration during acute withdrawal in ethanol-dependent rats.**

9:50 Rorick-Kehn L, Crile R, Eberle E, Shannon H, Sahr A, McKinzie J, Statnick M, Need A, Hart J, Li X, Witkin J, Kahl S, Quimby S, Diaz N, Mitch C, McKinzie D. **Preclinical pharmacological characterization of a structurally-unique, potent, kappa opioid receptor antagonist, (S)-3-fluoro-4-(4-((2-(3-fluorophenyl)pyrrolidin-1-yl)methyl)phenoxy)benzamide (FP3FBZ) in animal models of alcohol dependence and mood disorders**

10:10 McLaughlin JP, Ganno ML. **Kappa opioid receptor signaling mediates stress-induced reinstatement and potentiation of ethanol seeking behavior.**

10:30-11AM **Discussion / Morning Coffee Break** (Terrace Room)

11:00 Schlosburg JE, Vendruscolo LF, Park PE, Whitfield TW, & Koob GF. **Long-term antagonism of kappa opioid receptors prevents escalation of, and increased motivation for, heroin intake.**

11:20 Whitfield Jr. TW, Wee S, Gould AT, Schlosburg JE, Vendruscolo LF, Koob GF. **Kappa Receptor Activation Underlies Compulsive Methamphetamine Intake**

11:40 Saxon AJ, Mooney LJ, Ling W. **Cocaine Use Reduction with Buprenorphine (CURB)**

12:00-2 PM **Buffet Lunch** (Terrace Room)

Oral Session 3: **Pain, Inflammation & Epilepsy (Alan Cowan, Session Chair)** (Olympic Ballroom)

2:00 Ko MC. **The role of kappa opioid receptor in the modulation of pain and itch in primates.**

2:20 Stein C. **Peripheral kappa receptors mediating antinociceptive and anti-inflammatory effects.**

2:40 Menzaghi F. **Analgesic and morphine-sparing effects of the kappa opioid agonist CR845 after IV administration in women undergoing laparoscopic hysterectomy**.

3:00 Spencer R. **Anti-inflammatory effects of the peripheral kappa opioid receptor agonist CR845: a novel approach for the treatment of inflammatory disease.**

3:20 **Discussion / Afternoon Coffee Break**

3:40 Cowan A, Menzaghi F, Spencer RH, Inan S. **Kappa opioid receptor ligands and the development of antipruritics.**

4:00 Dolle RE. **Discovery and Pharmacological Evaluation of Putative Peripheral Kappa Opioid Agonists ADL0101 and ADL0116.**

4:20 Schwarzer C, Schunk E, Loacker S. **The dynorphin / kappa opioid receptor system in temporal lobe epilepsy.**

4:40 Bruchas MR, Schindler A, Shankar H, Messinger D, Miyatake M, Land BB, Lemos JC, Hagan C, Neumaier JN, Quintana A, Palmiter RD, Chavkin C. **Biased signaling at Kappa-opioid receptors distinguishes analgesic from dysphoric behavioral responses.**

5:00-6:30 PM **Discussion / Wine & cheese** (Terrace Room)

7-9 PM Dinner (no host) (Local Restaurants, walk or short cab ride)

9-11 PM Informal discussion in Pub (Lobby Bar)

WEDNESDAY July 13, 2011

7-8:15 AM **Continental Breakfast** (Terrace Room)

Oral session 4: **Kappa Regulation of Amine Function: Implications for Behavior** (Olympic Ballroom)
(Howard Fields, Session Chair)

8:30 Shippenberg, T, Sitte, H, Kivell, B, Chefer, V, Ramamoorthy S. **Kappa Opioid Receptor Regulation of Dopamine Transporter Function: Cellular Mechanisms and Physiological Relevance.**

8:50 Ramamoorthy S, Mannangatti P, Jayanthi LD Shippenberg T. **Kappa-Opioid Receptor- Mediated Regulation of Serotonin Transporter Function, Trafficking, Phosphorylation and Protein-Protein Interactions.**

9:10 Schindler AG, Shankar H, Messinger D, Miyatake M, Gustin RM, Hagan C, Neumaier JN, Chavkin C. **Stress-induced activation of the dynorphin/kappa opioid system increases serotonin reuptake through a p38alpha MAPK mechanism and underlies prodepressive and proaddictive responses.**

9:30 Valentino RJ, Van Bockstaele EJ. **Regulation of the rat brain norepinephrine system by the dynorphin-kappa opiate receptor system.**

10:00-10:30 **Discussion / Morning Coffee Break** (Terrace Room)

Oral Session 5: **Stress, Affect, Cognitive Function and Treatment** (Olympic Ballroom)
(Howard Fields, Session Chair)

10:30 Carlezon Jr. WA. **Roles for dynorphin and kappa-opioid receptors in the effects of stress**.

10:50 Chartoff E, Sawyer A, Rachlin A, Potter D, Pliakas A, Carlezon Jr. WA. **Blockade of kappa-opioid receptors attenuates cocaine withdrawal-induced negative affective states.**

11:10 Lucki I, Carr GV, Bangasser D, Valentino RJ. **Antidepressant-like Effects of Kappa Opioid Receptor Antagonists and Buprenorphine in WKY Rats.**

11:30 Kreek MJ, Zhang Y, Schlussman S, Zhou Y, Butelman B. **Bidirectional translational research of the kappa opioid receptor and dynorphin systems: Implications for human disease and therapeutics**

11:50 Yuferov V, Ho A, Morgello S, Proudnikov D, Levran O, Kreek MJ. **Human molecular genetics of *PDYN* and *OPRK1* and expression of those genes and chemokine receptors in postmortem brain of HIV infected and HIV negative subjects.**

12:00-2:00 PM **Buffet Lunch** (Terrace Room)

Oral Session 6 **Cellular & Molecular Receptor Mechanisms** (Olympic Ballroom)
(Bill Carlezon, Session Chair)

2:00 Vukojević V, Ming Y, Gruol D, Terenius L. **Live cell study of kappa opioid receptor (KOR) dynamics in the plasma membrane and interactions with dynorphins.**

2:20 Portoghese PS, Yekkirala AS. **Selective activation of kappa opioid receptor heteromers by exogenous and endogenous ligands in HEK-293 cells**

2:40 Clarke WP, Rowan MP, Sanchez TA, McGuire BA, Portoghese PS, Hargreaves KM, Berg KA. **Interactions between KOR and DOR in peripheral sensory neurons**

3:10 Yadav PN**,** Whistler J, Roth BL. **Cellular mechanisms of Salvinorin A action *in vivo*.**

3:30-4:00 **Discussion / Afternoon Coffee Break**

Oral session 7: **Developmental & Sex Differences**
(Bill Carlezon, Session Chair)

4:00 Varlinskaya E, Anderson R, Morales M, Truxell E, Spear LP. **Is the endogenous kappa opioid system implicated in adolescent-typical insensitivity to ethanol?**

4:20 Wei LN. **Physiological function of kappa opioid receptor in development.**

4:40 Coscia CJ, Croskey W, Kim EN, Hahn JW, Jagwani, S, Miller K, Belcheva MM. **Developmental stage dependent kappa opioid modulation of embryonic stem cell differentiation via MAP kinases.**

5:10 Liu-Chen LY. Wang Y, Rasakham K, Chudnovskaya D, Cowan A, and Huang P. **Sex Difference in KOPR-Mediated Behaviors and Brain KOPR level and KOPR-mediated [35S]GTPγS Binding in the Guinea Pig.**

5:20 Murray TF, Kelamangalath L, Dravid SM,George J, Aldrich JV. **Kappa-opioid receptor inhibition of calcium oscillations in spinal cord neurons.**

5:40 **Closing Remarks**

7-9 PM Dinner (no host) (Local Restaurants, walk or short cab ride)

POSTERS Monday 4-6PM

Kash TL, Li CJ, Pleil KE, Stamatakis AM. Stuber GD. **Presynaptic inhibition of GABA release in the BNST by kappa opioid receptor signaling.**

Kapusta DR, Hoffman E, Zieske A, Varner KJ. **Kappa opioid agonists protect against acute kidney injury in a rat model of anesthesia, surgery and hemorrhage.**

Pintar J, Ansonoff M. **The pattern of ultrasonic vocalizations in altered in neonatal KOR-1 KO mice.**

Béguin C, Potuzak J, Xu W, Liu-Chen L-Y, Potter DN, Goodman T, Carlezon, Jr. WA, Cohen BM. **Diaryl ether containing kappa opioid receptor antagonists: synthesis and preclinical evaluation of novel candidate drugs for mood disorders.**

Mosier PD, Koparde VN, Westkaemper RB, Roth BL. **Potential interaction modes of *N*-alkyl-octahydroisoquinolin-1-one-8-carboxamides with the κ-opioid receptor.**

Nizhnikov ME, Pautassi R, Varlinskaya EI, Rahmani P, Spear NE. **Ontogenetic differences in kappa opioid mediation of ethanol's motivation properties.**

Schweitzer P, Roberto M, Gilpin N. **The kappa opioid receptor system modulates ethanol effects on inhibitory transmission in rat Central Amygdala.**

Harkey S, Maina FK, Kuhnmuench M, Hambro C, Mathews TA, Aragona BJ **Interactions between the dopamine and the dynorphin/kappa opioid receptor system regulate pair bond behavior in prairie voles**

Patkar KA, Sherwood ML, Singh HD, Ross NC, McLaughlin JP. **Detection of nor-BNI in mouse brain weeks after administration using LC-MS/MS.**

Tejeda HA, Schultz-Kuszak KN, Counette D, O'Donnell P, Chefer V, Shippenberg T. **Kappa-opioid receptor modulation of dopaminergic and amino acid neurotransmission in the medial prefrontal cortex.**

Morales M, Anderson R, Varlinskaya E, Spear, LP. **Pharmacological blockade of kappa opioid receptors and ethanol intake: Impact of age and sex.**

Anderson RI, Agoglia AE, Morales M, Varlinskaya EI, Spear LP. **Kappa agonist-induced conditioned taste aversion in male Sprague-Dawley rats: Impact of age and stress**

Nguyen K, Tseng A, Marquez P, Hamid A, Friedman TC, Lutfy K. **The role of endogenous dynorphin/kappa opioid receptor system in alcohol reward**

Marquez P, Hamid A, Friedman TC, Lutfy K. **The role of endogenous dynorphin in cocaine-induced motor stimulation and locomotor sensitization**

Berg KA, Rowan MP, Sanchez TA, Hargreaves KM, and Clarke WP. **Inflammatory mediator regulation of KOR in peripheral sensory neurons.**

Navarro H, Butala E, Gilmour B, Carrol FI. **The selective kappa antagonist, JDTic, but not RTI-240 and 241, is a noncompetitive inhibitor of the human kappa opioid receptor.**

Liu-Chen, LY, Aldrich, JV, Yakovleva, T, Huang, P. **Zyklophin, a systemically active selective peptide KOPR antagonist with short duration of action, has anxiolytic-like effects in mice.**

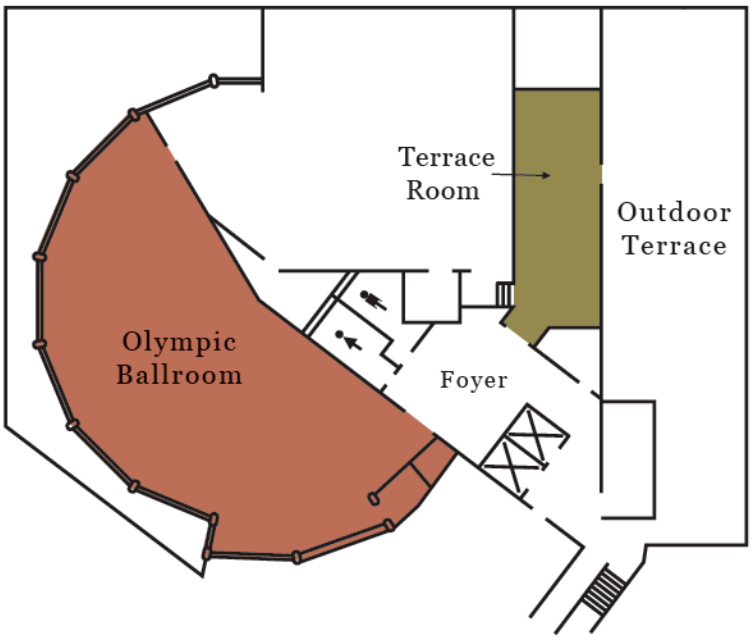
Ehrich JM, Zietz CC, Evans SB, Phillips PE, Chavkin C. **Interacting effects of U50488 and cocaine on dopamine release in the nucleus accumbens *in vivo*.**

Gustin RM, Smith JS, and Chavkin C. **Understanding the role of kappa-opioid receptor (KOR) modulation in the response to stress and the therapeutic potential of long-term KOR antagonists.**

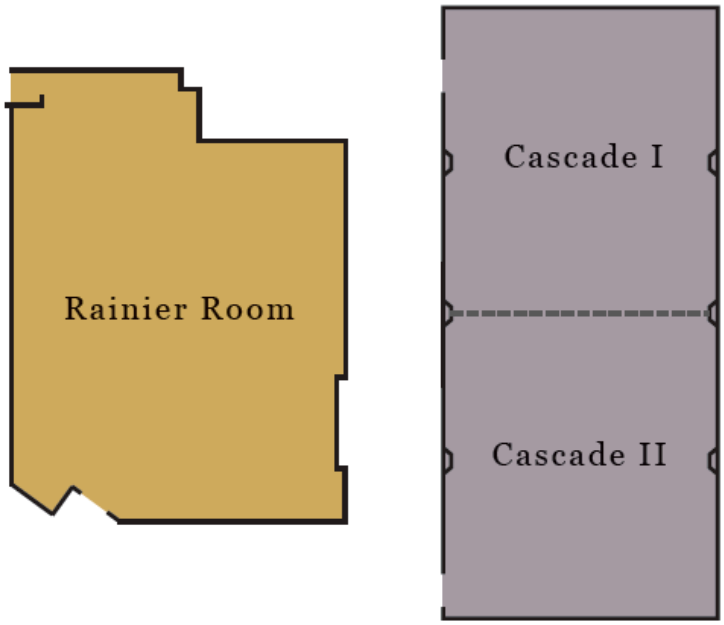
Schattauer S, Shankar S, Tarabochia M, Liu-Chen LY, Chavkin C. **Arrestin-dependent p38 activation by the human kappa opioid receptor.**

Lemos JC, Roth CA, Phillips PEM, Chavkin C**. Kappa opioid regulation of serotonergic dorsal raphe neuronal excitability in naïve and stress-exposed mice**

FOURTH FLOOR



SECOND FLOOR



Monday Talks

Carroll FI[1], Rehder K[1], Fennell TR[2], Pollard GT[3], Howard JL[3]

Center for Organic and Medicinal Chemistry, Research Triangle Institute, Research Triangle Park, NC, USA[1]; Drug Metabolism and Pharmacokinetics, Research Triangle Institute, Research Triangle Park, NC, USA[2]; and Howard Associates LLC, Research Triangle Park, NC, USA[3]

Does the duration of action of the antagonism of kappa agonist-induced diuresis by kappa opioid receptor antagonists depend on their pharmacokinetic properties?

Results from several animal studies suggest that kappa opioid receptor antagonists might have use for the treatment of depression, anxiety, schizophrenia, addiction, and eating disorders. JDTic is an orally active kappa opioid receptor developed in our laboratory. Preclinical studies for JDTic have been completed, and an IND has been submitted to the FDA for phase I clinical studies. In a separate presentation, Dave McCann (NIDA) will present JDTic's development status. Recent structure activity studies have led to several JDTic analogs that possess K_es comparable to those of JDTic at the kappa opioid receptor in the [^{35}S]GTPγS in vitro efficacy assays and greater than 100-fold selectivity for the kappa opioid receptor relative to the mu and delta opioid receptors. The potency and duration of action of several analogs to antagonize U50,488-induced diuresis were determined and compared to similar data for JDTic. In addition, data from PK studies of each analog were determined and compared to data for JDTic. The data suggest that even though PK properties are important to their duration of action, other factors are likely involved.

Supported by R01 DA09045 from NIDA.

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

Charles Mitch, Steven Quimby, Nuria Diaz, Conchi Pedregal, Marta De La Torre, Qing Shi, David McKinzie, Michael Statnick, Karen Rash and Vanessa Barth.

Eli Lilly and Company, Indianapolis, IN 46285

Discovery of Kappa Selective Opioid Receptor Antagonists: Application to Preclinical Evaluation of New Receptor Occupancy Tracers.

Antagonism of Kappa Opioid Receptors may be therapeutically useful for the treatment of a number of disorders, including anxiety, depression, drug abuse and alcohol dependence. Investigation of an aminobenzyloxyarlyamide scaffold resulted in the discovery of antagonists selective for the Kappa Opioid Receptor. Characterization of Kappa selective antagonism and utility in preclinical models of anxiety, depression and alcohol dependence will be presented. Use of LC/MS methodology for evaluation of non-radiolabeled compounds for potential use as PET tracers for the Kappa Opioid Receptor will also be included.

This work was carried out as part of the authors' employment with Eli Lilly and Co.

The authors have no conflicts of interest to disclose.

Melief EJ[1], Miyatake M[1], Carroll FI[2], Béguin C[3], Potuzak JA[3], Carlezon Jr WA[3], Cohen BM[3], Grimwood S[4], Mitch CH[5], Rorick-Kehn L[5], Chavkin C[1]

Department of Pharmacology, University of Washington, Seattle WA[1], Center for Organic and Medicinal Chemistry, Research Triangle Institute, North Carolina[2], Department of Psychiatry, Harvard Medical School, McLean Hospital, Belmont, MA[3], Pfizer Inc., Groton, CT[4], Lilly Research Laboratories, Eli Lilly and Company, Indianapolis, IN[5]

Duration of action of a broad range of selective kappa opioid receptor antagonists is positively correlated with c-Jun N-terminal Kinase-1 activation

The kappa opioid receptor is a widely expressed GPCR that has been implicated in biological responses to pain, stress, anxiety, and depression, and its potential as a therapeutic target in these syndromes is becoming apparent. It has long been known that the prototypical antagonist for KOR, norBNI, has a long duration of action which limits its use as a therapeutic in humans. It has also been shown that these long-lasting effects are dependent on activation of c-Jun N-terminal kinase (JNK). We used C57Bl/6 wild type mice to determine the duration of action of 12 selective KOR antagonists and examine their efficacy in activating JNK in spinal cord and transfected HEK293 cells. Of the compounds tested, 5 had long duration of action (>1d) that positively correlated with increases in pJNK-ir. Inhibiting JNK protected G-protein binding at the KOR following norBNI treatment as well as prevented long duration of action. Long lasting antagonism and JNK activation were not seen in animals lacking the JNK1 isoform, indicating that JNK1 is required for persistent inactivation of the kappa receptor. These results indicate the duration of action of small molecule KOR antagonists is determined by their efficacy in activating JNK.

Supported by R37 DA011672 and K05 DA 020570 from NIDA

Disclosures: WA Carlezon has a patent (US 6,528,518; Assignee: McLean Hospital) related to the use of kappa-opioid antagonists for the treatment of depressive disorders. Corporate affiliations of authors are listed. The other authors have no conflicts of interest to disclose.

Prisinzano TE[1], Aubé J[1], Hedrick MP[2], Gosalia P[2], Frankowski K[1] Shi S[2], Schoenen F[1], Su Y[2], Vasile S[2], Sergienko E[2], Gray W[2], Hariharan S[2], Slausen S[1], Ghosh P[1], Milan L[2], Heynen-Genel S[2], Chung TDY[2], Caron M[3], Bohn LM[4] Barak LS[3].

University of Kansas, Lawrence, Kansas[1]; Burnham Center for Chemical Genomics, La Jolla, CA[2]; Duke University Medical Center, Durham, NC[3]; and Scripps Research Institute, Jupiter, FL[4]

Discovery and preliminary evaluation of five new kappa opioid receptor antagonist and agonist scaffolds

A growing body of evidence has indicated that modulating kappa opioid receptors (KOR) offers an excellent platform for potentially treating a number of CNS disorders including drug abuse. Antagonists have been shown to prevent reinstatement of drug taking behavior in animal paradigms thought to model relapse. Partial agonists could well represent alternatives for the same indication. An important goal of our team is to identify subtype specific small molecule modulators of the human KOR that represent different chemical scaffolds than the many existing potent and selective literature antagonists.

Five new chemotypes of KOR ligands have been identified through two different high-throughput screening exercises. In one, a library prepared as part of the KU Chemical Methodologies and Library Development center was screened for binding to a set of central nervous system targets maintained at the Psychoactive Drug Screening Program (University of North Carolina, Bryan Roth, director). In addition, a separate program toward the identification of agents with affinity and varying efficacy at kappa receptors was pursued in the context of the Molecular Libraries Probe Production Center Network. In that work, both agonist and antagonist assay paradigms were utilized (beta-arrestin recruitment) to screen approximately 299K compounds. Secondary screens investigated potency and efficacy using G protein coupling and ERK activation assays in both agonist and antagonist modes. Together, these programs have resulted in the identification of five new scaffolds comprising activities that range from full agonists to full antagonists. Secondary assays reveal that several of the scaffolds display binding potencies of <10 nM and selectivities >100 versus the MOR and DOR countertargets.

This presentation will summarize the results of the screening along with preliminary chemical optimization and pharmacological evaluation.

Supported by 5U54HG005033-03 (John Reed, PI) and 5U54HG005031-03 (JA) from the Molecular Libraries Initiative, 5P50GM069663-08 (JA) from NIGMS, 1X01DA026208-01 (LSB) from NIDA, and a grant from the Institute for Advancing Medical Innovation (JA).

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

Husbands SM[1], Cueva JP[1], Kumar V[1], Clark M[2], Traynor JR[2].

Department of Pharmacy and Pharmacology, University of Bath, Bath, UK[1]; and Department of Pharmacology, University of Michigan, Ann Arbor, MI[2].

Selectively promiscuous kappa opioid receptor antagonists

The use of buprenorphine, a mu opioid receptor (MOR) partial agonist, kappa opioid receptor (KOR) antagonist and NOP receptor partial agonist, in the treatment of opiate abuse and dependence by detoxification, substitution and maintenance, is the most noteworthy recent addition to the repertoire of methods available for the treatment of substance abuse disorders. Furthermore, buprenorphine in combination with naltrexone has been shown, in open-label studies, to be effective in reducing relapse to opioid use in patients who also used cocaine, significantly lowering positive urine tests for morphine and cocaine metabolites, as well as reducing levels of dysphoria and craving. The combination presumably provides KOR/MOR antagonist, NOP receptor partial agonist activity and so there is interest in developing single compounds with this general profile. Despite the predominant KOR agonist activity of buprenorphine-like compounds, we have developed structure activity relationships that allow prediction of low efficacy/antagonism at KOR. This has allowed the development of series of ligands with KOR antagonist activity, combined with MOR antagonism or partial agonism and significant activity at NOP receptors. The on-going development and evaluation of these new ligands will be presented.

Supported by R01 DA07315 from NIDA.

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

Aldrich JA[1], Kulkarni SS[1], Senadheera SN[1], Patkar KA[2], Ross NC[2], Reilley KJ[2], Eans S[2], Ganno ML[2], Zhang Y[3], Kreek MJ[3], McLaughlin JP[2].

Department of Medicinal Chemistry, The University of Kansas, Lawrence, KS, USA[1]; and Torrey Pines Institute for Molecular Studies, Port St. Lucie, FL, USA[2]; Laboratory of the Biology of Addictive Diseases, Rockefeller University, New York, NY, USA[3]

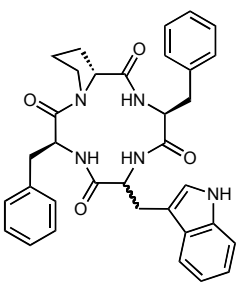
Peptide Kappa Opioid Receptor Ligands: Challenges in Development and Successes

Challenges in developing opioid peptides as potential therapeutic agents include their metabolic stability and their distribution following systemic administration, particularly their ability to cross the blood-brain barrier. By incorporating appropriate structural modifications such as cyclization we have identified kappa opioid receptor (KOR) peptide ligands, both agonists and antagonists, that exhibit activity *in vivo* after systemic administration. The dynorphin analog zyklophin (Patkar *et al.*, *J. Med. Chem.* 2005, *48*, 4500), which contains several structural modifications, including a cyclic constraint that enhances metabolic stability, appears to cross the blood brain barrier, and blocks stress-induced reinstatement of cocaine-seeking behavior after systemic administration, while also exhibiting a finite duration of KOR antagonist activity (Aldrich *et al.*, *Proc. Natl. Acad Sci. U.S.A.* 2009, *106*, 18396). Because of the modulatory effect of KOR signaling on dopaminergic neurotransmission, concerns have been raised that KOR antagonists might themselves be rewarding or that they could potentially increase cocaine-seeking behavior in subjects who were not yet abstinent from cocaine. Zyklophin, however, did not induce conditioned place preference (CPP) alone, nor did it enhance the CPP response induced by cocaine. Moreover, in a microdialysis study acute administration of zyklophin did not significantly increase striatal dopamine levels, consistent with the behavioral results. Together these results suggest that this KOR antagonist alone is not rewarding, nor does it enhance the rewarding effects of cocaine.

A second class of peptide KOR ligands with the potential for drug development are cyclic tetrapeptides based on the natural product CJ-15,208 (Saito *et al.*, *J. Antibiot.* 2002**,** *55*, 847). The cyclic structure of these peptides should confer resistance to proteolytic degradation, while their hydrophobicity should facilitate penetration of the blood-brain barrier. Like zyklophin, [D-Trp]CJ-15,208 exhibits KOR antagonist activity of finite duration (< 24 h). Furthermore, pretreatment with this cyclic tetrapeptide blocks stress-induced reinstatement of cocaine seeking behavior following oral administration, suggesting it could be useful in the treatment of drug abuse. In contrast, the L-Trp isomer demonstrated mixed agonist/KOR antagonist activity in mice in testing with the 55^{o}C warm-water tail withdrawal assay. This peptide did not exhibit significant tolerance in a model of acute antinociceptive tolerance, nor did it significantly affect respiration rate at a dose that produced potent antinociceptive activity, both in marked contrast to morphine. This peptide is also active after oral administration, suggesting that a cyclic tetrapeptide could induce potentially useful analgesia in the clinic with decreased liabilities compared to current narcotic analgesics such as morphine. The oral activity of both these cyclic tetrapeptides make them promising lead compounds for potential therapeutic development.

$PhCH_2$-Tyr-Gly-Gly-Phe–D-Asp-Arg-Arg-Dap–Arg-Pro–LysNH_2

zyklophin



cyclic tetrapeptides

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

Cashman JR[1], Ghirmai S[1], Kalisiak J[1], Azar MR[2], Wee S[3], Koob G[3]

Human BioMolecular Research Institute, San Diego, CA[1]; Behavioral Pharma, Inc., La Jolla, CA[2] and The Scripps Research Institute, La Jolla, CA[3]

Efficacious kappa antagonists for decreasing cocaine and alcohol self-administration.

Several series of substituted aryl amide derivatives of 6-naltrexamine showing kappa opioid receptor antagonism were synthesized and tested in vitro and in vivo. The chemical synthesis was an efficient one utilizing naltrexone as a starting material. SG-II-39 and SG-II-49 were designed to be metabolically stable and non-hepatotoxic. Binding assays showed that SG-II-39 (like SG-II-49) had subnanomolar K_i values for μ and κ opioid receptors. Functional assays for stimulation of [^{35}S]GTPγS binding showed the compounds acted as partial or inverse agonists and antagonists of the μ, δ, κ opioid or NOP receptors. The compounds showed considerable stability in the presence of rat, mouse or human liver preparations and NADPH and only modest inhibition of CYP3A4, CYP2C9 and CYP2C19 was observed. One agent decreased cocaine self-administration and another agent potently decreased alcohol self-administration in rat models of substance overuse. Rats self-administered 0.5 mg/kg/injection of cocaine on a fixed-ratio (FR) schedule in either one-hour (short access, ShA) or six-hour (long access, LgA) sessions. After cocaine intake in the LgA rats increased to a maximum, the effects of three kappa (κ) opioid receptor antagonists were tested on cocaine intake in ShA and LgA rats. Cocaine self-administration increased under FR and progressive-ratio (PR) schedules in LgA rats. Nor-BNI, a κ receptor antagonist, decreased cocaine intake in LgA rats under a PR schedule whereas naltrexone and SG-II-49, a nonselective opioid receptor antagonist and a partial agonist, respectively, decreased cocaine intake in both groups. The present study showed that inhibition of κ opioid receptors attenuated only the increased cocaine intake in LgA rats under a PR schedule whereas inhibition of μ and κ receptors decreased cocaine intake in both ShA and LgA groups. The data suggest that increased motivation for cocaine in rats with extended access may be related to increased κ opioid activity and may contribute to compulsive use. As a representative example, radiolabelled SG-II-49 was examined *in vivo* and showed reasonable brain penetration. The inhibition of ethanol self-administration in rats trained to self-administer a 10 % (w/v) ethanol solution, utilizing operant techniques showed SG-II-39 to have very potent efficacy (ED_{50} value of 19 μg/kg).

Supported by NIH Grant R44 AA018066

McCann D

Division of Pharmacotherapies and Medical Consequences of Drug Abuse, National Institute on Drug Abuse, National Institutes of Health, Bethesda MD

The discovery and development of JDTic: A NIDA perspective

In 1989, the U.S. Congress statutorily mandated that a Medications Development Program (MDP) be established within the National Institute on Drug Abuse (NIDA). In 1990, to operationalize the goals of the MDP, NIDA created the Medications Development Division, which, through multiple NIDA reorganizations, became part of today's Division of Pharmacotherapies and Medical Consequences of Drug Abuse. The NIDA MDP is modeled after a typical pharmaceutical company, with the ability to conduct all phases of medications development: from the synthesis and screening of new chemical entities, to preclinical and clinical safety testing, to Phase II and III clinical trials. Accomplishments include multiple NDA approvals for the treatment of opioid use disorders, advancement of a nicotine vaccine to late stage clinical development, and advancement of medication candidates to multi-site trails for the treatment of cocaine and methamphetamine use disorders.

One of the most promising new molecular entities to advance through IND-enabling safety testing within the NIDA MDP is JDTic, a highly selective *kappa*-opioid receptor antagonist which may be useful for the treatment of multiple substance use disorders, including cocaine dependence. NIDA initiatives to stimulate the discovery and development of such a compound date back to 1993. Through coordinated grant and contract efforts, JDTic emerged as a medication candidate with desirable pharmacodynamic properties and an attractive preclinical safety profile. The presentation will provide a brief background on the NIDA MDP, a historical perspective on initiatives that lead to the discovery of JDTic, data highlights from preclinical testing, and an update on JDTic's development status. Presentation content will be coordinated with other meeting presenters who plan to cover portions of the JDTic story, avoiding overlap while drawing from the content of preceding presentations to develop a comprehensive picture.

Support: As a NIDA employee, the author directed related research under multiple NIDA contracts but did not receive funding in support of this work.

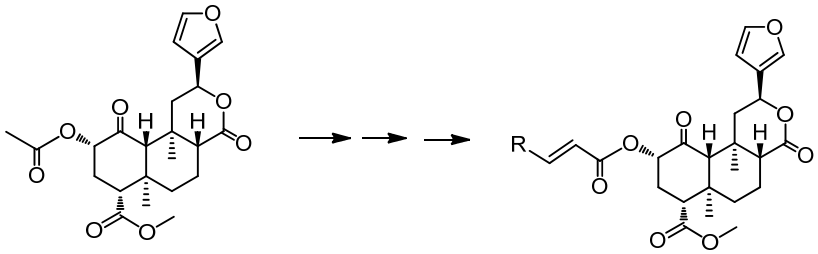
Disclosure: The author has no conflict of interest to disclose.

Zjawiony JK[1], Polepally PR[1], Setola V[2], Vardy E[2], and Roth BL[2].

Department of Pharmacognosy, and Research Institute of Pharmaceutical Sciences, School of Pharmacy, University of Mississippi, University, MS[1]; and Department of Pharmacology, School of Medicine, and NIMH Psychoactive Drug Screening Program, University of North Carolina, Chapel Hill, NC[2]

Michael acceptor approach to the design of new salvinorin A - based high affinity ligands to the kappa-opioid receptor.

Psychoactive natural products play an important role in the discovery and development of new drugs for the treatment of central nervous system (CNS) disorders. Our studies are focusing on identification of plant metabolites responsible for CNS activity and designing new ligands with high affinity to CNS receptors. Salvinorin A, the most potent naturally occurring hallucinogen isolated from the plant *Salvia divinorum*, has received great attention since the kappa-opioid receptor (KOR) was identified as its principal molecular target. Previously, extensive efforts were made to understand how salvinorin A binds to and activates KOR [1-4]. Our goal was to design a series of ligands with high affinity to KOR to further explore the ligand-receptor interactions at molecular level. Following the synthesis of 22-thiocyanatosalvinorin A [5], the first irreversible salvinorin-derived KOR ligand, we now report the synthesis and biological evaluation in vitro of new salvinorin A derivatives with Michael acceptor-type functional groups.



salvinorin A

Supported by grant R01DA017204 from NIDA

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

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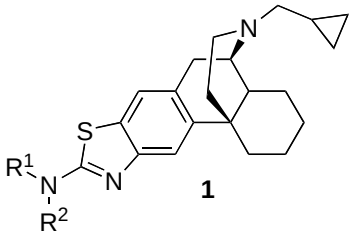
Neumeyer, J. L.;[1] Sromek, A. W.; [1] Zhang, T.; [1] Bidlack, J. M. [2]

Alcohol and Drug Abuse Research Center, McLean Hospital, Harvard Medical School, Belmont, MA[1] and School of Medicine and Dentistry, University of Rochester, Rochester, NY[2]

Aminothiazole-modified morphinans with potent κ and μ activity

We have synthesized a series of aminothiazole-modified morphinans (**1**) for expanding the structure-activity relationships of these novel compounds.

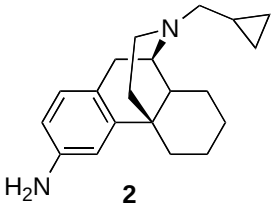


1

1a) **MCL-147.** $R^1 = R^2 = H$
(*K*i nM) κ = 0.049, μ = 1.5

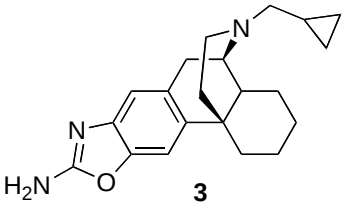
b) **MCL-442.** R^1 = Me, $R^2 = H$
(*K*i nM) κ = 0.066, μ = 3.0

c) **MCL-439.** R^1 = Et, $R^2 = H$
(*K*i nM) κ = 1.5, μ = 4.7



2

(*K*i nM) κ = 0.19, μ = 1.3



3

(*K*i nM) κ = 52, μ = 1.5

Binding assays and [^{35}S]GTPγS functional assays showed that the aminothiazolomorphinans were κ agonists with mixed agonist and antagonist activity at the μ opioid receptor. **MCL-147** (ATPM) was demonstrated to yield more potent antinociceptive effects than (-)U50,488 with an ED_{50} value of 2.68 mg/kg in mice (hot plate).

We have also examined **MCL-147** for its behavioral effects and its ability to alter the reinforcing effects of cocaine in Rhesus monkeys, suggesting that **MCL-147** reduces both self-administration at doses that also reduce food-maintained responding. These novel aminothiazole-derived morphinans may be valuable for the development of drug abuse medications.

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

Kivell BM[1], Morani A[1], Simonson B[1], Prisinzano T[2], Shippenberg TS[3], Miller, JH[1], Schenk S[1].

Victoria University of Wellington, New Zealand[1], University of Kansas[2], NIH/NIDA Intramural Research Program[3].

Anti-addiction effects of Salvinorin A and novel analogues

Acute kappa-opioid receptor (KOPr) activation by both traditional agonists and Salvinorin A (Sal A) have been shown to attenuate drug-seeking behaviour in animal models of addiction. However, adverse side effects such as sedation and depression have limited their therapeutic development. By understanding the behavioural and cellular mechanism of action of these novel KOPr compounds our aim is to screen for potential anti-addiction compounds with reduced side effects. In this study we screened the behavioural effect of Sal A and several analogues on cocaine self-administration using a drug prime induced reinstatement model and expression of cocaine sensitization in rats. Acute exposure to Sal A (0.3, 1.0 mg/kg) or analogues DS1 and MOB Sal B (0.3 mg/kg), significantly attenuated cocaine prime induced reinstatement ($p<0.05$) and expression of cocaine behavioural sensitization (0.3 mg/kg; $p<0.05$). However, the more potent analogue EOM Sal B and mixed Mu-opioid receptor (MOPr)/KOPr agonist, herkinorin had no effect. No significant changes in cocaine induced hyperactivity were seen for any of the KOPr compounds tested. However, Sal A and DS1 caused a significant reduction in swimming behaviour and increased immobility in the forced swim test model of depression. Sal A and novel analogues all increase dopamine transporter function in rat striatal and accumbens tissue and in cells transiently transfected with YFP-hDAT and myc-rKOPr. This data will help to understand the mechanism of action of these novel compounds, and may aid in developing new therapeutic compounds for treating drug addiction.

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

POSTERS

Anderson RI, Agoglia AE, Morales M, Varlinskaya EI, Spear LP

Department of Psychology, Center for Development and Behavioral Neuroscience, Binghamton University, Binghamton NY. Developmental Exposure to Alcohol Research Center, Binghamton NY, Cortland NY, and Syracuse NY.

Kappa agonist-induced conditioned taste aversion in male Sprague-Dawley rats: Impact of age and stress

Evidence supports the involvement of the endogenous kappa opioid system in aversive/dysphoric properties of drugs of abuse. Given that adolescents commonly experience attenuated aversive effects of drugs of abuse (including ethanol and cocaine) relative to adults, we hypothesized an ontogenetic difference in responsiveness to pharmacological activation of kappa opioid receptors by the selective kappa agonist U62,066. Our previous work using a conditioned taste aversion paradigm in water-deprived rats indicated that adolescents were dramatically less sensitive to kappa agonist-induced aversion than adults. Given that water deprivation may act as a stressor, the present study was designed to determine the influence of stress on kappa activation-induced conditioned taste aversion in both adolescent and adult male Sprague-Dawley rats. A highly palatable solution, chocolate Boost, was selected as the conditioned stimulus in order to allow for testing in non water-deprived animals. On postnatal day (P) 21 (adolescents) or P61 (adults), subjects were re-housed with a non-littermate. Animals assigned to the stress condition were subjected to restraint stress for 90 min for five consecutive days beginning on P29 or P69. Twenty-four hours after the final restraint stress exposure, each housing pair was separated by a mesh divider in order to allow for assessment of individual fluid intake. For conditioning, all subjects were given access to chocolate Boost for one hour, immediately followed by injection of one of four doses of U62,066 (0, 0.3, 0.4, or 0.5 mg/kg, s.c.). Twenty-four hours later, subjects were again separated by a mesh divider for testing. Each subject was given one bottle of Boost and one bottle of water for one hour. Analysis of the test day Boost intake revealed kappa agonist-induced aversion (i.e., a decrease in Boost intake relative to saline-injected animals) at all doses tested for both adolescents and adults in the non-stressed condition. Marked attenuations in the aversiveness of U62,066 were seen in stressed animals, with this effect more pronounced among adolescents. In summary, in contrast to the lack of age differences in sensitivity to kappa agonist-induced aversions seen in non-stressed animals, adolescents were found to be more sensitive than adults to stress-induced attenuation of taste aversions induced by pharmacological activation of kappa opioid receptors. Adolescence can often be a stressful period of transition. To the extent that kappa receptor activation contributes to aversive effects of ethanol that serve as feedback cues to attenuate ethanol intake, age differences in the interaction of stress and kappa activation may contribute to elevated ethanol consumption frequently reported in adolescent animals relative to their adult counterparts.

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

Béguin C[1], Potuzak J[1], Xu W[2], Liu-Chen L-Y[2], Potter DN[1], Goodman T[1], Carlezon, Jr. WA[1], Cohen BM[1].

McLean Hospital, Belmont, MA[1]; and Temple University School of Medicine, Philadelphia, PA[2]

Diaryl ether containing kappa opioid receptor antagonists: synthesis and preclinical evaluation of novel candidate drugs for mood disorders.

A series of recent preclinical studies have consistently shown that selective kappa opioid receptor (KOR) agents strongly affect behaviors in rodents believed to model mood in humans. Taken as a whole, these findings suggest that KOR antagonists would be effective antidepressants, KOR agonists might be useful antimanic agents, and partial agonists might be mood stabilizers. Many selective KOR agonists are available for preclinical studies. Selective KOR antagonists (norBNI and analogues and JDTic) exist as well, but have some undesirable properties in vivo: including slow onset (24-48 h) and long duration of action (several weeks). This unusual time course of effects can be a limitation for both preclinical and clinical studies. We are currently studying a class of diaryl ether containing agents with non-selective opioid antagonist properties. We have tested the lead compound in vivo to determine potency and time course of blockade of salvinorin A's KOR agonist effects. The findings, which suggest more rapid onset and offset of action, will be presented. We are currently designing analogues with the goal of increasing KOR selectivity. We will include the in vitro KOR potency and selectivity of the lead compound and select analogues.

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

Berg,K.A., Rowan, M.P., Sanchez, T.A, Hargreaves, K.M. and Clarke, W.P

University of Texas Health Science Center, San Antonio, TX

Inflammatory mediator regulation of KOR in peripheral sensory neurons.

We studied regulation of KOR responses in primary cultures of sensory neurons and in a rat model of thermal allodynia. Under basal conditions, application of the KOR agonist, U50488, did not inhibit adenylyl cyclase (AC) activity in primary cultures of peripheral sensory neurons and did not inhibit PGE_2-stimulated thermal allodynia following intraplantar (i.pl.) injection in the rat hindpaw. However, following pre-treatment with bradykinin (BK, 15 min), U50488 became capable of inhibiting AC activity, CGRP release and thermal allodynia. The effect of U50488 in BK-primed tissue was blocked by the KOR antagonist, nor-BNI, both *in vitro* and *in vivo*. The effect of BK *in vitro* was blocked by either indomethacin or bis-indolylmaleimide, suggesting that an arachidonic acid (AA) metabolite and protein kinase C (PKC) activation mediate BK-induced regulation of KOR. Further, KOR function in BK-treated tissue was blocked by a soluble integrin-blocking peptide (GRGDSP), but not the inactive reverse sequence peptide (GDGRSP), suggesting that in addition to AA and PKC, RGD-binding integrins participate in the regulation of KOR signaling. However, not all signaling pathways coupled to KOR required an inflammatory stimulus for functional competency. For example, in the absence of BK, U50488- produced a time-dependent activation of Extracellular Signal-Regulated Kinase (ERK) in sensory neuron cultures. Interestingly, the duration of U50488-induced anti-allodynia was inversely related to dose, which was reversed by the MEK inhibitor, U0126 (i.e., inhibition of ERK activity). Understanding the mechanisms by which inflammation and/or tissue damage regulates the efficacy of KOR drugs along with the roles of each of the multiple signaling pathways coupled to peripheral KOR may lead to improved pharmacotherapy for treatment of pain with reduced adverse effects.

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

Ehrich JM[1], Zietz CC[1], Evans SB[2], Phillips PE[2], Chavkin C[1]

Department of Pharmacology[1], Psychiatry & Behavioral Sciences[2], University of Washington, Seattle, WA

Interacting effects of U50488 and cocaine on dopamine release in the nucleus accumbens *in vivo*.

In humans, exposure to stress increases the propensity for drug addiction and the risk of relapse to drug abuse. Work in animal models suggests that this effect is mediated by stress-induced release of dynorphin and subsequent activation of the kappa opioid receptor (KOR), which has been shown to increase the rewarding effects of cocaine, increase drug self-administration, and reinstate extinguished drug seeking behaviors. Further, although pretreatment with U50,488 60 minutes prior to cocaine potentiates cocaine reward, a 15 minute pretreatment with U50,488 blocks both the rewarding and reward-sensitizing properties of cocaine. These effects have been attributed to shifts in the reward valence of cocaine due to KOR-induced dysphoria and aversion. Since KOR activation alone leads to a reduction in accumbal dopamine, we investigated if interactions between KOR and cocaine mechanisms are mediated by convergent effects on synaptic dopamine levels using fast-scan cyclic voltammetry (FSCV). Urethane-anesthetized mice were administered the KOR agonist U50,488 (5 mg/kg, i.p.) or saline 15 or 60 minutes prior to subsequent administration of 15 mg/kg s.c. cocaine while receiving repeated stimulations to either the medial forebrain bundle (MFB) or the pedunculopontine tegmental nucleus (PPTg), a glutamatergic nucleus which projects to the VTA. U50,488 administration induced a rapid and significant (> 40%) decrease in peak dopamine release. Intriguingly, pretreatment with U50,488 had no effect on cocaine-induced increases in peak MFB-stimulated dopamine currents at both the 15 and 60 minute pretreatment time points. However, cocaine-induced increases in PPTg-stimulated dopamine currents were inhibited by pretreatment with U50,488 at 15 minutes but not 60 minutes prior to cocaine. This suggests that potentiation could be partially mediated by interactions between cocaine and a KOR-induced signaling cascade at the level of the VTA which is activated at 60 minutes after U50,488 but not at 15 minutes. Further experiments will attempt to confirm that KOR expression solely in VTA neurons is sufficient to reproduce this phenomenon while exploring the role of KOR-induced activation of p38 MAPK.

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

Gustin RM, Smith JS, and Chavkin C

Department of Pharmacology, University of Washington, Seattle WA

Understanding the role of kappa-opioid receptor (KOR) modulation in the response to stress and the therapeutic potential of long-term KOR antagonists

Stress is a complex, biological response that can alter an individual's perception, leading to anxious or depressive mood states in otherwise benign settings. Faced with repeated or chronic stressors, an individual's responses to stress can become physically and mentally debilitating, in many cases, manifesting as long-term neurodevelopment, neurodegenerative, and/or psychological disorders. The kappa-opioid receptor (KOR) system has been shown to modulate several behavioral responses to a variety of stressors. Recent studies have shown that systemic pretreatment with the KOR-antagonist, norbinaltrophimine (norBNI), can block the potentiation of cocaine conditioned place preference induced by repeated forced swim stress (FSS) (Schindler, et al. 2010), as well as block anxiety-like behavior due to an acute FSS on the elevated-plus maze (Bruchas, et al, 2009). Similarly, norBNI administered prior to an acute social defeat stress (SDS) prevents a decrease in social interaction seen in a social avoidance assay (Bruchas, et al., In preparation). We hypothesized that the dysphoric component of stress is responsible for the observed decrease in social interaction, whereas the stress-induced anxiety-like phenotypes can be observed in the elevated-plus maze. Therefore, we wanted to determine the importance of context in the presentation of a stressor in elucidating different behavioral responses, in attempts to differentiate the neuronal circuitry responsible for driving the anxiety-like versus dysphoric-like behaviors. Furthermore, we will test the therapeutic potential of norBNI in reversing these stress-induced behaviors. To this end, we have used SDS and FSS paradigms that have both been shown to induce anxiety-like behavior on the elevated-plus maze. We are able to show that SDS, not FSS, leads to a significant reduction of social interaction compared to control animals. The social defeat induced reduction in social interaction is dependent on KOR expressed in the dorsal raphe nucleus (DRN), as norBNI stereotactic microinjections into the DRN prevent SDS-induced social avoidance. Interestingly, the dysphoric behavioral component leading to social avoidance appears to be circuit dependent due to the fact that norBNI microinjections into the basolateral amygdala (BLA) do not block the SDS-induced social avoidance. These data suggest that using targeted inhibition of KOR, we can determine neuronal pathways that are necessary for manifestation of KOR dependent behaviors. Before determining if KOR-antagonists, such as norBNI, have the therapeutic potential to reverse chronic stress-induced behaviors, we first tested if norBNI pretreatment has the ability to block social avoidance behaviors following repeated social defeat stress. Consistent with our hypothesis, norBNI pretreatment prevented the social avoidance, while having no effect on the social interaction in control animals, due to the repeated social defeat sessions. Moving forward, norBNI will be administered following the repeated social defeat stress to establish if long-term KOR antagonists can reverse the social interaction deficits following social defeat stress. In all, these results extend our understanding of the neurocircuitry involved in stress-induced behaviors, and further implicate the KOR system as a potential target for the development of pharmacotherapies for the treatment of anxiety and depression disorders.

Supported by DA030074 and DA 020570

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

Harkey S[1], Maina FK[3], Kuhnmuench M[2], Hambro C[2], Mathews TA[3], Aragona BJ[2]

Neuroscience Graduate Program, University of Michigan, Ann Arbor MI[1]; Department of Psychology, University of Michigan, Ann Arbor MI[2]; and Department of Chemistry, Wayne State University, Detroit MI[3]

Interactions between the dopamine and the dynorphin/kappa opioid receptor system regulate pair bond behavior in prairie voles

The dynorphin/kappa opioid receptor (KOR) system is known to modulate striatal dopamine (DA) transmission as well as play a substantial role in the neural processing of aversive stimuli and stress. For example, social stress leads to an up-regulation of KOR signaling systems located throughout reward processing regions of the brain, such as the mesolimbic DA systems. Our previous work has shown that DA transmission within the nucleus accumbens (NAc) shell is critical for social attachment and most recent data from our lab demonstrates that KOR's within the shell, but not the core, are also necessary. Specifically, both D1-like DA receptors and KORs in the NAc shell regulate the characteristically intense aggression displayed by the socially monogamous prairie vole that maintains the pair bonds that are the foundation of the species' social organization. The necessity of both these receptor systems is hypothesized to be the result of D1-mediated increases in dynorphin, the endogenous ligand of KORs. Preliminary data from our lab indicates that D1-mediated selective aggression is indeed due to downstream activation of KORs as blockade of D1-like receptors (previously shown to inhibit selective aggression) while simultaneously activating KORs in the NAc shell fails to inhibit selective aggression. Finally, we have begun to use in vitro fast-scan cyclic voltammetry in striatal slice preparations to directly measure interactions between the mesolimbic DA system and dynorphin/KOR system in sexually naïve and pair bonded prairie voles. Data from these studies indicates that DA release throughout the striatum is enhanced following pair bond formation, which may be the result of alterations in the dynorphin/KOR system. This hypothesis is currently being tested through bath application of a KOR agonist while measuring DA release properties in striatal slice preparations.

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

Daniel R. Kapusta, Ph.D.[1,2], Ernesto Hoffman, M.D.[3], Arthur Zieske[3] and Kurt J. Varner, Ph.D. [1,2]

Louisiana State University Health Sciences Center, Departments of Pharmacology[1] and Pathology[3], and the Cardiovascular Center of Excellence[2]

Kappa opioid agonists protect against acute kidney injury in a rat model of anesthesia, surgery and hemorrhage.

We and other investigators have demonstrated that kappa opioid agonists produce a marked water diuresis (aquaresis) in conscious rats. In the present study, we examined if the kappa opioid agonist, U-50,488H (U50), which is known to attenuate ischemic brain injury, prevents renal injury caused by the combination of gaseous anesthesia, surgery and severe hemorrhagic trauma (ASH). **Methods.** Sprague-Dawley rats received a continuous intravenous (i.v.) infusion (55 µl/min) of isotonic saline vehicle alone (SAL; n=4) or plus U50 (100 µg/kg/min, n=4). Conscious rats were infused for 15 minutes and then were anesthetized (thiopental/isoflurane) and subjected to periods of surgery (chronic bladder catheterization; 30-min), hemorrhage (20 cc/kg; 45-min), and recovery (blood replacement; 120-min). Consecutive 10-miin urine samples were collected throughout, then rats were allowed to recover and followed for 7 days. **Results**. In SAL rats, urine flow rate (V) and urinary sodium excretion (UNaV) decreased to near 0 during anesthesia/surgery; in contrast U50 rats maintained enhanced V, but decreased UNaV (i.e., sodium-free water diuresis). Subsequent to hemorrhage, V slowly increased to 40 µl/min with variable increases in UNaV. In contrast, V increased promptly to >100 µl/min, while UNaV remained low (sustained water diuresis) in U50 treated rats. Post-ASH, SAL rats developed oliguria, hematuria and /or azotemia (Serum creatinine from 0.5 to max 6.7 mg/dl) by day 3; all SAL rats died by day 5. U50 rats also developed oliguria but recovered quickly; none became azotemic and all survived. SAL rats were autopsied promptly and U50 rats were sacrificed on day 7 for renal histology. Histologic examination of kidneys, compared to an untreated control, showed foci of severe tubular damage (necrosis of epithelial cells, granularity of cytoplasm and loss of brush borders) in >50% of fields in SAL rats. U50 treated rats showed foci of tubular damage in <20% of fields. **Conclusions.** Kappa opioid agonist (U50) treatment protected kidneys from ischemic acute kidney injury (AKI) and increased survival in this model of severe ischemia/reperfusion injury.

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Presynaptic inhibition of GABA release in the BNST by kappa opioid receptor signaling

Thomas L. Kash[1,2], Chia J. Li[1,3], Kristen E. Pleil[1,2], Alice M. Stamatakis[3] Garret D. Stuber[4],

[1] Bowles Center for Alcohol Studies
[2] Department of Pharmacology
[3] Curriculum in Neurobiology
[4] Departments of Psychiatry & Cell and Molecular Physiology, School of Medicine, University of North Carolina Chapel Hill, Chapel Hill, NC, USA.

Abstract

Endogenous stress and anti-stress systems co-exist in mammalian organisms. The kappa opioid receptor (KOR) and its endogenous agonist, the neuropeptide dynorphin, are a critical component of the 'stress' system. Both dynorphin and KOR are expressed in the bed nucleus of the stria terminalis (BNST), a brain region associated with anxiety and stress. This suggests that KOR activation in this region may play a role in the regulation of emotional behaviors. However, the impact of KOR activation on synaptic transmission in this region has not been characterized. Using whole-cell voltage clamp recordings in an *ex vivo* mouse brain slice preparation, we investigated the effect of KOR activation on inhibitory transmission in the BNST. We found that activation of KOR reduced GABAergic transmission through a presynaptic mechanism. Futhermore, we examined the signal transduction pathways that mediate this inhibition, and found that ERK, but not p38, was required for this effect. Next, using pathway-specific optogenetic manipulations knock-into selectively stimulate GABAergic fibers from the central nucleus of the amygdala (CeA) to the BNST we found that KOR signaling robustly reduced inhibitory synaptic transmission in this pathway. Finally, we found that repeated, but not acute, stress altered KOR mediated inhibition of GABAergic function in the BNST. Together, these results demonstrate that KOR provide important inhibitory control over presysnaptic GABAergic signaling within the BNST and this form of modulation is modulated by exposure to stress.

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Julia C. Lemos[1,2,3], Clarisse A. Roth[1], Paul E.M. Phillips[1,2,3] and Charles Chavkin[1,3]

1. Department of Pharmacology 2. Department of Psychiatry & Behavioral Sciences and 3. Program in Neurobiology & Behavior, University of Washington, Seattle, WA 98195.

Kappa opioid regulation of serotonergic dorsal raphe neuronal excitability in naïve and stress-exposed mice

Several GPCRs couple to G-protein coupled inwardly rectifying potassium channels (GIRK) to suppress the excitability of the cell. Stress-induced dynorphin release and subsequent kappa opioid receptor (KOR) activation in the DRN has been shown to produce an aversive state. Using an antibody directed toward dynorphin-activated, phosphorylated KOR, immunohistochemical analysis demonstrated that repeated swim stress increased phospho-KOR immunoreactivity similar to that caused by acute pharmacologic activation. To define the cellular effects of KOR activation and the functional role of KOR in modulating excitability in the DRN, whole cell voltage clamp recordings were obtained in 5-HT and non 5-HT containing neurons in DRN, and KOR modulation of post-synaptic GIRK currents were evaluated. Cells were filled with biocytin and identified as 5-HT or non 5-HT post-hoc using a standard IHC procedure with an anti-TPH primary antibody. Bath application of the selective KOR agonist U69,593 (1 μM) significantly enhanced a BaCl2 sensitive inward rectifying K+ conductance in both 5-HT and non 5-HT neurons. This effect was blocked when cells were pre-treated with the selective antagonist Norbinaltorphimine (NorBNI) (1 μM). In DRN cells from animals exposed to a two day repeated swim stress protocol, the U69,593 response was reduced to approximately half of that seen in the naïve animals, suggesting stress-induced desensitization. Moreover, stress-induced desensitization of the KOR-mediated enhancement in GIRK current in the DRN was not apparent in mice lacking preprodynorphin. Stress-induced KOR desensitization would contribute to an increase in neuronal excitability of 5-HT neurons. In contrast to the stress-induced KOR desensitization, sub-chronic stress causes a sensitization of the 5-HT1A mediated increase in GIRK current. In naïve mice, 5-HT1A activation by 5-CT produced a concentration dependent increase in GIRK current. In DRN neurons from animals exposed to a two day repeated swim stress protocol, there was no difference in maximum 5-CT effect at saturating concentrations (30 nM), however, there was significant sensitization of the 5-CT response at sub-saturating concentrations (1 nM). Consistent with other reports, following chronic stress (7 day swim stress) the responses to 3, 10 and 30 nM 5-CT were desensitized. The 5-HT1A sensitization may be a compensatory effect caused by the hyperexcitability of DRN neurons following stress exposure, a state that may be due, in part, to desensitization of KOR.

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DISCLOSURE/CONFLICT OF INTEREST

The authors report no conflict of interest regarding the contents of this abstract.

Lee-Yuan Liu-Chen[1], Jane V. Aldrich[2], Tatyana Yakovleva[2], and Peng Huang[1]

1 Department of Pharmacology and Center for Substance Abuse Research, Temple University School of Medicine, Philadelphia, PA
2 Department of Medicinal Chemistry, The University of Kansas, Lawrence, KS

Zyklophin, a systemically active selective peptide KOPR antagonist with short duration of action, has anxiolytic-like effects in mice

It has been demonstrated that KOPR antagonists have antidepressant- and anxiolytic-like effects in animal models. However, the KOPR antagonists used have slow onsets (with peak at 24-48 h) and extraordinarily long durations of action (weeks to more than a month), which may limit their further development. In addition, it is not clear if the exceptionally long-lasting activity of the KOPR antagonists is essential for their effects in animal models. The cyclic peptide zyklophin {[*N*-benzylTyr^1,*cyclo*(D-Asp^5,Dap^8)-dynorphin A-(1–11)NH_2, Patkar KA, et al. (2005) *J Med Chem* 48: 4500–4503} is a selective peptide KOPR antagonist and blocks U50,488H-induced antinociception in mice. It is active following systemic administration and has a duration of action of <12h [Aldrich JV, et al. (2009) *PNAS* 106: 18396-18401]. The aim of the current study is to investigate the effects of zyklophin vs norBNI on two anxiety-like behaviors in male CD-1 mice: the novelty-induced hypophagia (NIH) and the elevated plus-maze (EPM) tests. Zyklophin (3 mg/kg, s.c.) produced strong anixolytic-like effects 1 h following drug administration, similar to norBNI (10 mg/kg, i.p.) 48 h after injection, in the NIH test. In addition, in the EPM test mice demonstrated significant anixolytic-like responses to norBNI (10 mg/kg, i.p.). Experiments are being conducted to investigate (1) dose effects of zyklophin in the NIH test; and (2) effects of zyklophin in the EPM test.

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Marquez P[1,2], Hamid A[1,2], Friedman TC[2], Lutfy K[1,2]

[1]College of Pharmacy, Western University of Health Sciences, Pomona CA 91766 and [1,2]The Charles Drew University of Medicine & Sciences, Los Angeles, CA 90059

The role of endogenous dynorphin in cocaine-induced motor stimulation and locomotor sensitization

Previous studies have reported that dynorphin and other kappa opioid receptor agonists attenuate locomotor sensitization induced by cocaine and other psychostimulants. Furthermore, there is a body of literature demonstrating that repeated treatment with addictive drugs increases the level of dynorphin and its precursor molecule, raising the possibility that endogenous dynorphins may be important in cocaine-induced locomotor sensitization. Thus, in the present study, we assessed whether mice lacking dynorphin compared to their wild-type littermate/controls would exhibit altered locomotor sensitization. Mice were habituated to motor activity chambers for 1 h, injected with saline or cocaine (15 or 30 mg/kg, i.p.) and motor activity was recorded for 1 h. The same treatment was given for 3 consecutive days and mice were tested for sensitization on day 8. On this day, mice were habituated to the test chambers for 1 h, injected with cocaine (15 mg/kg) and motor activity was recorded for 1 h. Given that compensatory changes could occur in knockout mice, we also studied the effect of nor-binaltorphimine (nor-BNI), a kappa opioid receptor antagonist, on the phenomenon of sensitization. Mice were treated with nor-BNI (10 mg/kg), 2 h prior to saline or cocaine (15 mg/kg, i.p.) on day 1. On days 2 and 3, mice were injected only with their respective saline or cocaine treatment and tested for sensitization on day 8, as described above. Our results illustrated that the effect of low dose cocaine (15 mg/kg) was reduced in knockout mice. On the other hand, nor-BNI treatment appeared to increase the motor stimulatory effect of this dose of cocaine. Nevertheless, there was no difference in cocaine-induced motor stimulation between wild-type and knockout mice at a higher dose of cocaine (30 mg/kg). Likewise, the magnitude of cocaine-induced locomotor sensitization was not different between the two genotypes. Similarly, locomotor sensitization was not altered in wild-type mice treated with nor-BNI compared to their saline-treated controls. Together, these results suggest that cocaine dose-dependently increased locomotor activity and induced locomotor sensitization in both genotypes but the endogenous dynorphin/kappa opioid receptor system may be involved in other aberrant behavioral responses that develop following repeated cocaine treatment.

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Disclosures: The authors have no conflicts of interest to declare

Morales M, Anderson R, Varlinskaya E, Spear, L.

Department of Psychology, Center for Development and Behavioral Neuroscience, Binghamton University, Binghamton, NY. Developmental Exposure to Alcohol Research Center, Binghamton NY, Cortland NY, and Syracuse NY.

Pharmacological blockade of kappa opioid receptors and ethanol intake: Impact of age and sex.

As the endogenous kappa opioid system has been implicated in the aversive properties of ethanol (EtOH), the present experiment sought to examine both age- and sex-related differences in sensitivity to pharmacological blockade of kappa opioid receptors following a single injection of the selective kappa antagonist, nor-binaltorphimine (nor-BNI) at doses of 0, 2.5, 5 and 10 mg/kg. Given that adolescents typically consume more EtOH than their adult counterparts, and are less affected by the adverse effects of EtOH, we hypothesized that the nor-BNI would be more effective in increasing EtOH intake in adults than in adolescents. Similarly, because adult females consume greater quantities of EtOH compared to adult males, we hypothesized that they too, would be less affected by the intake-enhancing effects of kappa blockade than their male counterparts. On postnatal day (P) 25 (adolescent) or P67 (adult), male and female Sprague-Dawley rats were individually housed (experimental Day 1 and given ad-libitum access to food and water). Every other day from Day 4-14, rats were exposed to one bottle of supersaccharin (SS) solution with 10% EtOH for 30 min during the light part of their light/dark cycle. On Day 9, rats were injected subcutaneously with one of the doses of nor-BNI and left undisturbed until subsequent drinking sessions (days 10, 12, and 14). EtOH intake (g/kg) on Days 10, 12 and 14 were converted to %baseline scores relative to intake on Day 8 (the last pre-treatment intake session). As commonly seen, adolescents consumed more EtOH on a g/kg base than did adults, and females demonstrated higher EtOH intake than males. In both male and female adolescents, nor-BNI failed to alter their EtOH intake. In adults, however, an opposite sex-related pattern was observed: EtOH intake was elevated by nor-BNI in males as hypothesized, whereas females surprisingly consumed significantly less EtOH relative to their baseline following nor-BNI administration. These results suggest that adolescents are less sensitive than adults to pharmacological blockade of the kappa opioid system, a finding which corresponds to their insensitivity to some of the negative effects associated with EtOH. Among adults, observed sex differences may be related to the fact that overall, females consumed more EtOH than males—differences in consumption that may be due to the anxiety/stress associated with isolate-housing which may have affected the kappa opioid system differently in adult male and females.

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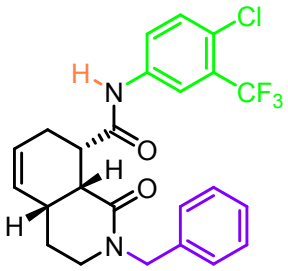
Mosier PD[1], Koparde VN[1], Westkaemper RB[1], Roth BL[2].

Virginia Commonwealth University, Richmond VA[1], and University of North Carolina, Chapel Hill NC[2]

Potential interaction modes of *N*-alkyl-octahydroisoquinolin-1-one-8-carboxamides with the κ-opioid receptor.

A novel series of *N*-alkyl-octahydroisoquinolin-1-one-8-carboxamides was recently reported to act as full agonists at the κ-opioid receptor (KOR) [Frankowski et al., ACS Med. Chem. Lett. *1*, 189, **2010**]. These compounds were also found to be selective for the KOR, with some possessing moderate to high affinity (K_i = 300–5 nM at KOR).



K_i = 5 nM (KOR)
K_i = 3550 nM (MOR)
K_i = > 10,000 nM (DOR)

This series of compounds representing a new class of KOR ligands also lacks basic amine functionality, a trait shared with salvinorin A, another well-known small molecule that is a selective full agonist at KOR. To explore the potential interaction modes of these novel non-basic compounds at KOR, homology models of the human KOR receptor were generated and the isoquinolinone carboxamide series was subsequently docked to the models using automated docking routines. A comparison of the resulting putative receptor–ligand interaction models with experimentally-guided salvinorin A–KOR interaction models shows that the isoquinolinone carboxamides are able to interact with some of the same residues that have been shown to be important for the binding of salvinorin A. To provide further rationale for the KOR selectivity of these compounds, an analysis of the corresponding similarities and differences among the amino acids comprising the intrahelical binding crevice for each of the three main opioid receptor subtypes was performed. Combined with the docking results, the analysis shows that the isoquinolinone carboxamides, like salvinorin A, may interact with binding site residues unique to KOR.

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Navarro H, Butala E, Gilmour B, Carrol F

Discovery Sciences, Research Triangle Institute, Research Triangle Park, NC

The selective kappa antagonist, JDTic, but not RTI-240 and 241, is a noncompetitive inhibitor of the human kappa opioid receptor.

The functionally selective kappa opioid antagonist, JDTic, exhibits in vivo effects that persist long after what would be predicted by its pharmacokinetic profile. One possibility is that JDTic depots in cell membranes, leading to prolonged inhibition of kappa opioid receptors. In keeping with this, repeated washing of whole CHO cells overexpressing the human kappa opioid receptor and the parent cell line preincubated with [^{3}H]JDTic failed to wash out the radioligand in either cell line. If JDTic were tightly associated with the cell membrane, the potential exists for it to interact noncompetitively with the kappa receptor. To investigate this, we preincubated CHO cell crude membrane homogenates that overexpress the hKOR with JDTic and used them in the [^{35}S]GTPγS functional binding assay. Under these conditions, JDTic appeared to noncompetitively inhibit U69,593-stimulated radioligand binding. This effect was not observed with membrane homogenates overexpressing the hMOR. JDTic also noncompetitively inhibited U69,593-mediated release of internal calcium stores in CHO cells overexpressing the promiscuous G_q, $G_{\alpha 16}$, and the hKOR. However, this could simply be explained by receptor kinetics because of the short assay time. To test this, we evaluated naltrexone, norBNI, and two JDTic analogs with similar K_e values (~0.02 nM) to JDTic, RTI-240 and RTI-241, short- and long-acting antagonists of U50,488-stimulted diuresis in rats, respectively. All test compounds except naltrexone were noncompetitive inhibitors in this assay. These same compounds and JDTic were tested in a β-arrestin recruitment assay for U69,593 activation of hKOR, an assay with a 90-min incubation time at RT. In this assay, only JDTic and norBNI noncompetitively inhibited hKOR activation. Similar levels of inhibition were observed for these two compounds after washing the cells with assay buffer prior to agonist stimulation. These results suggest that the in vivo effects of JDTic are not solely related to its low K_e because similar high affinity, selective kappa antagonists failed to noncompetitively inhibit hKOR in the β-arrestin assay. Moreover, JTDic could be altering hKOR function through interactions with the receptor at an as yet undefined site that is resistant to washing. These results also indicate the β-arrestin assay for hKOR activation could be useful in differentiating JDTic analogs with different in vivo activities.

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Nguyen K[1], Andy Tseng[1], Marquez P[1,2], Hamid A[1,2], Friedman TC[2], Lutfy K[1,2]

[1]College of Pharmacy, Western University of Health Sciences, Pomona CA 91766 and [1,2]The Charles Drew University of Medicine & Sciences, Los Angeles, CA 90059

The role of endogenous dynorphin/kappa opioid receptor system in alcohol reward

Previous studies have demonstrated that dynorphin and other kappa opioid receptor agonists reduce the rewarding action of alcohol, as measured by the conditioned place preference (CPP) paradigm. Additionally, repeated alcohol treatment has been shown to increase the level of dynorphin, raising the possibility that endogenous dynorphins may be involved in alcohol reward and reinforcement. Thus, in the present study, we determined whether ethanol-induced CPP would be altered in mice lacking dynorphin compared to their wild-type littermate/controls. Mice were tested for baseline place preference on day 1, received morning/afternoon saline/ethanol (2 g/kg, i.p.) or ethanol/saline conditioning on days 2-4 and then tested for postconditioning place preference on day 5. Mice were also tested for state-dependent CPP following a challenge dose of ethanol (1 g/kg) on day 8. On each test day, mice were placed in the central neutral chamber of a 3-chambered CPP apparatus and allowed to freely explore all the CPP chambers. The amount of time that mice spent in each chamber was recorded and used for data analysis. The effect of nor-binaltorphimine (nor-BNI), a kappa opioid receptor antagonist, was also studied on ethanol-induced CPP. Mice were injected with saline or nor-BNI (10 mg/kg, i.p.) on day 1, mice then received their twice daily conditioning with saline/ethanol (2 g/kg, i.p.) or ethanol/saline on days 2-4 and then tested for CPP under a drug-free state on day 5 and for state-dependent CPP on day 8, as described above. Our results revealed that ethanol induced a robust CPP in both genotypes but the magnitude of this response was not different between the wild-type and knockout mice. However, when mice were challenged with ethanol on day 8, there was a significant increase in ethanol CPP in mice lacking dynorphin compared to wild-type mice. A similar result was observed in wild-type mice treated with nor-BNI compared to their saline-treated controls. Overall, these results demonstrate that the endogenous dynorphin/kappa opioid receptor system may negatively regulate ethanol-induced state-dependent CPP.

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Michael E. Nizhnikov, Ricardo Pautassi, Elena I, Varlinskaya, Pouyan Rahmani & Norman E. Spear

Center for Developmental Psychobiology, Binghamton University – SUNY

Ontogenetic differences in kappa opioid mediation of ethanol's motivation properties.

Adult rodents exhibit aversions to conditioned stimulus (CS) paired with moderate to high doses of ethanol. Conversely, rats one-week old or younger or tend to exhibit appetitive conditioned responses to these same CSs (Cheslock et al., 2000; Petrov et al., 2003; Nizhnikov et al., 2006a). This ethanol-mediated appetitive conditioning seems to fade during the second postnatal week. Akin to the results found with ethanol, our recent work has shown that neonate rats find kappa opioid stimulation appetitive (Petrov et al., 2006) while in adults kappa agonists induce robust aversions (Mucha & Herz, 1985; Hippenberg &Herz, 1986). Altogether these results suggest that the dynorphin/kappa opioid receptor system switches during early development from mediating appetitive to aversive reinforcement. It has been clearly demonstrated that the opioid system is involved in the reinforcing properties of EtOH (Vengeliene et al., 2008; Sommer et al., 2006; Méndez et al., 2008), with mu-delta and kappa receptors apparently mediating the appetitive and aversive motivational properties of ethanol in adults respectively (Mitchell et al., 2005; Logrip et al., 2009; Sandi et al., 1988; Matsuzawa et al., 1999). Data also suggest that EtOH exposure has a profound effect on the kappa opioid system and its mediation of EtOH intake changing receptor denseties and even the function of the kappa opioid system (Rosin et al., 1999; Lindholm et al., 2007; Walker and Koob 2008). The present set of experiments tested the reinforcing properties of ethanol and kappa opioid agonists across infancy using a classical taste conditioning model on one or two-week old pups(PD 4 vs PD12). Furthermore we examined the effects of kappa opioid antagonists on ethanol reinforcement across the same ages. The overarching hypothesis is that at least part of the differential effects of ethanol across age might be mediated by the kappa opioid system. The results indicate that 4 day-old rat pups find stimulation by ethanol and kappa opioid agonists positively reinforcing. Conversely, older infants (PD12) find kappa opioid agonists aversive. Furthermore, kappa opioid antagonists have opposite effects on ethanol's reinforcing properties across age. Older subjects pretreated with nor-BNI (kappa opioid antagonist) prior to ethanol reinforcement exhibit appetitive responding to a flavor paired with ethanol compared to their saline controls. On the other hand, younger subjects no longer found ethanol positively reinforcing.

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Patkar KA., Sherwood ML., Singh HD., Ross NC., McLaughlin JP.

Torrey Pines Institute for Molecular Studies, Port St. Lucie, Florida 34987

Detection of nor-BNI in mouse brain weeks after administration using LC-MS/MS.

Therapeutic development of the kappa opioid receptor (KOR) selective antagonist nor-BNI has been tempered by prolonged pharmacological activity (days to weeks), the mechanisms of which are currently being investigated. We evaluated the potential accumulation of nor-BNI in brain tissue following direct intracerebroventricular (i.c.v., 1-100 nmol) or systemic intraperitoneal (i.p., 1-50 mg/kg) administration to C57Bl/6J mice, determining if prolonged presence of this compound in brain matched the long duration of KOR antagonist activity *in vivo*. Additional mice were administered saline or the short acting, non-selective opioid antagonist naloxone (i.c.v., 100 nmol) alone and in presence of nor-BNI (i.c.v., 30 nmol). In the mouse warm-water tail-withdrawal assay, a single administration of nor-BNI (i.p., 10 mg/kg or greater) significantly antagonized U50,488-induced antinociception for at least 7 d, whereas naloxone- (i.c.v., 100 nmol or i.p., 10 mg/kg) mediated antagonism lasted less than 24 h. The mice were euthanized at various time points ranging from 30 min to 21 days post administration, and brain extracts prepared from isolated tissues using a simple organic extraction for LCMS/MS analysis. The analysis was carried out using 'multiple reaction monitoring' (MRM). MRM is a mass spectrometric technique that employs selective monitoring of the forced fragmentation of the ions of interest (precursor ions) in the mass spectrometer. A precursor-product ion pair is referred to as a "transition". Selecting such unique transitions ensures maximum specificity for the analyte of interest. Nor-BNI and naloxone also undergo spontaneous degradation in the electrospray source of the mass spectrometer giving rise to unique fragments (in source decay). We chose these 'in source decay' fragments of nor-BNI and naloxone in addition to the parent ions for the MRM experiment to improve overall sensitivity of the assay. Nor-BNI was detected in the brain up to 21 days after a single i.c.v. injection of 30 or 100 nmol, and up to 24 h following administration of a 10 nmol dose. Likewise, nor-BNI was detected in brain up to 21 days after an i.p. administration of 50 (but not 1) mg/kg. In contrast, naloxone was not detected in brain after 6 h. Currently, we are investigating the length of time over which nor-BNI is detected in mouse brain after an i.p. administration of therapeutically relevant dose (10 mg/kg). Data will be presented quantitating the amount of nor-BNI retained in the mouse brain over time following either i.c.v. or i.p. administration. Additionally, we will present a comparison of nor-BNI detected in both brain and plasma collected from the same animal over the course of the experiment. In conclusion, to the best of our knowledge, this is the first direct physical determination of nor-BNI in brain after a single administration. The continued presence of nor-BNI (but not naloxone) in mouse brain days after a single injection correlates with the prolonged antagonism of U50,488, and may offer insight into the long duration of KOR antagonism mediated by nor-BNI.

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Pintar, J. [1] and Ansonoff, M.[1]

Dept. Neuroscience and Cell Biology, Robert Wood Johnson Medical School-UMDNJ, Piscataway, NJ 08854[1]

The pattern of ultrasonic vocalizations in altered in neonatal KOR-1 KO mice.

The study of mouse ultrasonic vocalizations (USVs) has recently emerged as an important index of social interactions occurring not only in wild-type mice but also in mutant strains exhibiting altered behavioral traits potentially related to human psychiatric disease. USVs emitted by neonatal rodents are thought to guide pup retrieval and influence maternal behavior while adult vocalizations appear to be involved in several aspects of social interaction. In addition quantitative and qualitative differences in the patterns of USVs in both pups and adults of different background strains have been correlated with differences in social behavior. Thus, examination of ultrasonic vocalizations appears to be a valid measure of both the magnitude and quality of social interactions.

We have begun to determine whether neonatal KOR-1 KO pups show qualitative or quantitative differences from WT in ultrasonic vocalizations following maternal separation. We have quantitated both total USVs as well as the relative prevalence of ten waveforms that have been identified and characterized in both neonatal and adult mice. Our data to date indicate that both the number and types of ultrasonic vocalization calls differs significantly in KOR-1 KO pups compared to wild type following maternal separation. Specifically, while overall USV number is decreased in KOR-1 KO mice, two relatively rare individual waveforms of the ten analyzed ("upward" and "downward"), show significant and opposing quantitative alterations in the KOR-1 KO. Since different call types in adult rodents have been associated with specific behaviors such as mating, feeding, aggression and fear, these data suggest that the altered USV patterns exhibited by KOR-1 neonatal mice may reflect an altered response to maternal separation.

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Schattauer S[1], Shankar S[1], Tarabochia M[1], Liu-Chen LY[2], Chavkin C[1].

Department of Pharmacology[1] University of Washington, Seattle, WA; Department of Pharmacology and Center for Substance Abuse Research, Temple University School of Medicine, Philadelphia, PA.

Arrestin-dependent p38 activation by the human kappa opioid receptor

Activation of the mouse and rat kappa opioid receptor leads to p38 phosphorylation in a GRK/arrestin dependent manner. In mice, phosphorylation of p38 is required for the aversive but not analgesic properties of KOR agonism. However, the GRK phosphorylation site is not conserved between species, and in the human KOR (hKOR) GRK phosphorylates Ser358 (rather than Ser369 as in mice and rats). We used western blot analysis of HEK293 stably expressing hKOR to determine whether activation of hKOR causes arrestin-dependent p38 phosphorylation. U50,488 caused a significant and dose dependent increase in p38 phosphorylation in cells expressing rat KOR (rKOR) or hKOR, but not HEK293 expressing hKOR(S358N). U50,488 caused a significant and dose dependent increase in ERK1/2 phosphorylation in cells expressing rKOR, hKOR, and hKOR(S358N), as expected based on previous studies showing rapid G protein mediated phosphorylation of ERK1/2 after KOR stimulation. While ERK1/2 phosphorylation was only elevated at 5 min after stimulation of hKOR(S358N), ERK1/2 phosphorylation was still significantly elevated at 1 hour after stimulation of hKOR; this suggests that activation of hKOR causes a second, arrestin dependent, phase of ERK1/2 phosphorylation, as previous studies have established with rKOR. Knockdown of arrestin 3 by siRNA prevents p38 phosphorylation following hKOR activation and reduces ERK1/2 phosphorylation at 30 minutes. These data show that while the sites of GRK phosphorylation are not conserved across species, activation of human KOR leads to the same arrestin mediated signaling pathways which have been previously established in rat and mouse models and further suggest that arrestin mediated p38 phosphorylation also underlies the dysphoric effects of KOR activation in humans.

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Schweitzer P, Roberto M, Gilpin N

Neurobiology of Addictive Disorders, The Scripps Research Institute, La Jolla CA

The kappa opioid receptor system modulates ethanol effects on inhibitory transmission in rat Central Amygdala.

Considerable evidence suggests that kappa opioid receptors (KORs) and their natural ligand, dynorphin, are involved in ethanol related behaviors. The central amygdala (CeA) is strongly implicated in anxiety and alcohol use disorders. Dynorphin expression is activated in the amygdala during acute and chronic administration of alcohol, and the selective KOR antagonist nor-binaltorphimine (nBNI) blocks ethanol self-administration in alcohol-dependent animals. In the CeA, ethanol augments GABAergic responses but the modulation of such effects by the KOR system is mostly unknown. Here we used intracellular recording techniques in rat CeA slices to examine the effects of KOR agonists and antagonists on evoked $GABA_AR$-mediated inhibitory postsynaptic potentials (IPSPs) and their interaction with ethanol.

With glutamatergic and $GABA_BR$-mediated transmission blocked, dynorphin dose-dependently diminished IPSPs with a maximum decrease of 21%. We observed a similar effect upon application of the synthetic KOR agonist U69593. The diminution of IPSPs by KOR agonists appeared to occur at a presynaptic site, as evidenced by increases in paired-pulse ratio. The effects of dynorphin and U69 were prevented by the KOR antagonist nBNI. Surprisingly, nBNI applied alone increased GABAergic transmission by 36%, revealing a tonic endogenous KOR activity that suppresses inhibition in CeA. Ethanol applied alone augmented IPSPs by 48%. The effect of ethanol was reduced but not prevented by addition of dynorphin or U69 in the superfusate. Pretreatment of the slices with nBNI, however, completely prevented the ethanol-elicited augmentation of IPSPs.

Our results reveal an intricate role of the dynorphin/KOR system in the regulation of inhibitory transmission in the CeA. Activation of KORs by natural and synthetic agonists reduced inhibitory transmission, an effect opposite to that of ethanol. KOR agonists attenuated but did not prevent ethanol effects. Endogenously formed KOR ligands (likely dynorphin) appear to tonically depress inhibitory transmission in CeA, and KOR blockade greatly interfered with the action of ethanol. Collectively, these data suggest a role for the dynorphin/KOR system in the reinforcing properties of alcohol mediated by the CeA.

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Hugo A. Tejeda[a,b], Kristin N. Schultz-Kuszak[a], Danielle Counette[b], Patricio O'Donnell[b], Vladmir Chefer[a], and Toni Shippenberg[a]

[a]Integrative Neuroscience Section, Integrative Neuroscience Branch, NIDA IRP

[b]Department of Anatomy and Neurobiology, University of Maryland, Baltimore

Kappa-opioid receptor modulation of dopaminergic and amino acid neurotransmission in the medial prefrontal cortex.

Dysfunction of medial prefrontal cortical (mPFC) dopamine (DA) and amino acid neurotransmission is implicated in aberrant cognitive, affective, and motivational behavior that occur in various psychiatric disorders including addiction and depression. Kappa-opioid receptors (KORs) and dynorphins are enriched in the mPFC and dysregulation of this opioid system has been implicated in mediating behavioral alterations observed in addiction and depression. The present in-vivo microdialysis study examined the role of KOR systems in modulating mPFC DA, glutamate, and GABA transmission. Adult S/D rats were implanted with guide cannula aimed at the mPFC. Microdialysis was conducted 5-7 d later. Reverse dialysis of the selective KOR agonist U69,593 and antagonist nor-Binaltorphimine (nor-BNI) into the mPFC decreased and increased basal extracellular DA levels, respectively. These results suggest activation of KORs inhibits DA overflow and this system tonically inhibits DA overflow. Since nor-BNI attenuates mu-opioid receptor (MOR)-mediated analgesia for several hours after systemic nor-BNI administration, we determined whether a nor-BNI concentration that increased extracellular DA would block the effects of the selective MOR agonist DAMGO on mPFC DA dialysate levels. Reverse dialysis of DAMGO produced a robust elevation in extracellular mPFC DA which was not altered by nor-BNI pretreatment, suggesting mPFC nor-BNI administration does not antagonize local MOR function. Reverse dialysis administration of U69,593 or nor-BNI were without effect on basal mPFC dialysate GABA and glutamate levels. However, local administration of trans-PDC, a glutamate reuptake blocker, unmasked an inhibitory effect of U69,593 on glutamate overflow. Moreover, trans-PDC produced a U69593-reversible elevatation of extracellular GABA levels. On-going work is aimed at determining the mechanism by which U69,593 inhibits glutamate overflow using electrophyiological techniques. Collectively, these results suggest that mPFC KOR systems negatively modulate local extracellular DA, glutamate, and GABA levels in naïve rats. This provides a framework whereby dysregulation of cortical KOR system function may result in aberrant behaviors in psychiatric disorders.

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Tuesday Talks

George F. Koob

Committee on the Neurobiology of Addictive Disorders, The Scripps Research Institute, La Jolla, CA

A conceptual framework for the role for dynorphin in the motivational effects of drug dependence.

Drug addiction has been defined as a chronically relapsing disorder characterized by (1) compulsion to seek and take the drug, (2) loss of control in limiting intake, and some have included (3) emergence of a negative emotional state (e.g., dysphoria, anxiety, irritability), reflecting a motivational withdrawal syndrome, when access to the drug is prevented (defined here as dependence). The compulsivity associated with drugs of abuse has been hypothesized to derive from several mechanisms, one of which involves increased drug seeking that results from negative reinforcement. Here, the emergence of a negative emotional state with the development of dependence provides the aversive stimulus to drive drug seeking to remove the negative emotional state (negative reinforcement). Relapse in drug addiction has a major stress component, which presumably reactivates aspects of the negative emotional state associated with dependence. Animal models of excessive drug taking associated with dependence and animal models of relapse have been developed, in which animals increase drug seeking with extended access/exposure and reinstate responding to stressors. One hypothesis is that this excessive drug taking reflects dysregulation of brain stress-aversive systems, such as dynorphin. Multiple mechanisms can be envisioned to explain the role of kappa systems in the compulsivity associated with addiction, including interactions with the dopamine system (a within-system neuroadaptation in which dynorphin drives decreases in dopamine function) and interactions with other brain stress systems (between-system neuroadaptation in which dynorphin activates or is activated by corticotropin-releasing factor). Different brain areas may be responsible for these differential effects of activation of the kappa opioid system in the transition to addiction, and kappa antagonists may have selective and effective actions in decreasing the excessive drug taking and relapse associated with the stressful effects of dependence and relapse.

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The author reports no conflicts of interest.

Involvement of kappa opioid receptors in drug addiction

Rafael Maldonado

Laboratori de Neurofarmacologia. Facultat de Ciències de la Salut i de la Vida. Universitat Pompeu Fabra. Carrer Dr. Aiguader 80, 08003 Barcelona, Spain.

Drug addiction is a chronic brain disorder leading to complex adaptive changes within the brain reward circuits that involve different components of the endogenous opioid system. Several studies have revealed that the dynorphin/κ-opioid receptor system participates in the addictive processes induced by several drugs of abuse. Pharmacological studies using selective κ-opioid receptor ligands and genetic studies using knockout mice deficient in the different components of the endogenous opioid system have demonstrated that the dynorphin/κ-opioid receptor system is involved in the rewarding effects produced by psychostimulants, cannabinoids, nicotine and ethanol. The dynorphin/κ-opioid receptor system would play an opposite role to the other components of the endogenous opioid system. Indeed, the different drugs of abuse that enhance mesolimbic dopamine levels also increase dynorphin content leading to a homeostatic mechanism that opposes the effects of drugs on the reward circuitry. This dynorphinergic activation may serve as a feedback mechanism to counteract the high levels of dopamine released by drugs of abuse. Therefore, the dynorphin/κ-opioid receptor system represents a promising target for the treatment of drug addiction.

Damaj MI[1], Carroll FI[2], Negus SS[1], Jackson KJ[1,3]

Department of Pharmacology and Toxicology, Medical Campus, Virginia Commonwealth University, Richmond, VA 23298[1]; Center for Organic and Medicinal Chemistry, Research Triangle Institute, Research Triangle Park, NC 27709[2]; Department of Psychiatry, Virginia Institute of Psychiatric and Behavioral Genetics, Virginia Commonwealth University, Richmond, VA 23298[3]

Effect of kappa opioid receptor antagonists in mouse models of nicotine dependence

Several lines of evidence support a role for the endogenous opioid system in mediating behaviors associated with drug dependence. Specifically, recent 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. In this study we determined the involvement of the KOR in the initial behavioral responses of nicotine, nicotine reward, and nicotine withdrawal using selective KOR antagonists JDTic and nor-BNI. KOR antagonists were administered subcutaneously (s.c.) at different doses 18 h prior to nicotine treatment.

JDTic dose-dependently blocked acute nicotine-induced antinociception in the tail-flick but not the hot-plate test and did not significantly attenuate morphine's antinociceptive effect in either the tail-flick or hot-plate test. Furthermore, JDTic (8 and 16 mg/kg, s.c.) failed to block or enhance development of nicotine reward as measured by the conditioned place preference model. In contrast, JDTic and the KOR antagonist norBNI attenuated the expression of both the physical (somatic signs, hyperalgesia) and affective (anxiety-related behavior, conditioned place aversion) nicotine withdrawal signs.

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 selective KOR antagonists may be useful smoking cessation pharmacotherapies.

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Smith JS[1], Schindler AG[1], Gustin RM[1], Bruchas MR[1], Neumaier JN[2], Chavkin C[1]

Departments of Pharmacology[1], Psychiatry & Behavioral Sciences[2], University of Washington, Seattle, USA

Kappa opioid receptor antagonists: a promising pharmacotherapy to treat stress-induced increase in nicotine reward and dependence

Every year, more than five million people die from the consequences of smoking tobacco, yet only 5% of smokers are able to quit their habit. Nicotine, the primary addictive component of tobacco, plays a key role in maintaining tobacco dependence. Growing evidence suggests that signaling through the kappa opioid system, comprised of the kappa opioid peptide receptor (KOR) and its endogenous ligand dynorphin, increases the immediate rewarding properties of nicotine.

C57Bl/6 mice developed dose-dependent nicotine conditioned place preference (CPP). Kappa receptor activation, either by repeated forced swim or systemic U50,488 administration, prior to the second preference test significantly potentiated the magnitude of the CPP, similar to the effect of KOR activation previously demonstrated on cocaine and ethanol preference. The increase in nicotine CPP was blocked by systemic norBNI pretreatment and also by local injection of norBNI in the lateral amygdala. Mice administered U50,488 showed heightened anxiety-like behavior in two assays, the elevated plus maze and latency to explore a novel object. Both of the observed increases in anxiety-like behavior were prevented by nicotine.

Mice were made nicotine dependent by repeated injection of nicotine for 6 days in a neutral environment and then conditioned with nicotine in the place preference apparatus. Similar to nicotine-naïve mice, nicotine-dependent animals showed a dose-dependent nicotine CPP, but the dose-response was shifted to the right, consistent with acetylcholine receptor desensitization. A dose of nicotine (1 mg/kg) that produced aversion in drug-naïve mice produced a preference in nicotine dependent animals. Interestingly, this rightward potency shift was dependent on kappa opioid system signaling, as mice pretreated with norBNI prior to chronic nicotine treatment no longer showed nicotine preference compared to vehicle controls.

As withdrawal from nicotine is mediated in part by serotonergic systems, we investigated if KOR signaling during withdrawal might alter 5-HT tone. Indeed, synaptosomes of nicotine-dependent mice ~44 hours withdrawn from nicotine showed increased 5-HT uptake by the serotonin transporter. Interestingly, this increased rate of 5-HT uptake was blocked by systemic norBNI pretreatment. These findings elucidate a possible KOR signaling mechanism that may contribute to the negative affective state of nicotine withdrawal. Our data suggest that kappa opioid receptor antagonism might be a novel pharmacotherapy to prevent both stress-induced and withdrawal-induced nicotine seeking behavior.

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Brendan M. Walker, Ph.D. Laboratory of Alcoholism and Addictions Neuroscience, Department of Psychology and Graduate Program in Neuroscience, Washington State University, Pullman, WA, USA.

The role of extended amygdala kappa-opioid receptors in escalated operant ethanol self-administration during acute withdrawal in ethanol-dependent rats.

The opioid peptide dynorphin (DYN) has been implicated in escalated ethanol consumption produced by ethanol dependence. DYN is the endogenous peptide for the kappa-opioid receptor (KOR) and stimulation of KORs is associated with depression and dysphoria. Previous research indicates that DYN systems are upregulated by chronic ethanol exposure and administration of a KOR antagonist reduces escalated ethanol consumption associated with dependence. The primary focus of these experiments was to test the hypothesis that KORs in the extended amygdala mediate escalated ethanol self-administration in dependent rats. Thus, the effect of nor-binaltorphimine (nor-BNI; a KOR antagonist), following site-specific intra-extended amygdala infusions, was evaluated for the ability to attenuate ethanol self-administration in dependent and non-dependant Wistar rats. The ethanol-dependent group was subjected to a four week intermittent vapor exposure period. Following dependence induction, the vapor-exposed animals displayed escalated responding while the non-dependent animal's responding remained consistent with their baseline levels. Once animals showed stable post-dependence induction self-administration during acute withdrawal following sham and subsequent artificial cerebrospinal fluid infusions, nor-BNI was infused into extended amygdala nuclei prior to self-administration sessions. Nor-BNI did not impact ethanol self-administration in non-dependent animals, but significantly reduced ethanol self-administration in dependent animals. These data support the hypothesis that antagonism of KOR receptors selectively decreases self-administration of ethanol in dependent rats and suggest that upregulation of DYN contributes to escalated ethanol consumption. Since DYN has been shown to contribute to negative affective states (i.e., depressive- and anxiety-like behaviors), targeting KOR receptors may attenuate the negative affect that accompanies ethanol dependence, and therefore, may be an improved pharmacotherapeutic strategy for the treatment of alcoholism.

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Linda Rorick-Kehn, Robert Crile, Elizabeth Eberle, Harlan Shannon, Allison Sahr, Jamie McKinzie, Michael Statnick, Anne Need, John Hart, Xia Li, Jeffrey Witkin, Steven Kahl, Steven Quimby, Nuria Diaz, Charles Mitch, David McKinzie

Lilly Research Laboratories, Eli Lilly and Company, Indianapolis, IN 46285

Preclinical pharmacological characterization of a structurally-unique, potent, kappa opioid receptor antagonist, (S)-3-fluoro-4-(4-((2-(3-fluorophenyl)pyrrolidin-1-yl)methyl)phenoxy)benzamide (FP3FBZ) in animal models of alcohol dependence and mood disorders

Background: Alcohol dependence is a chronic relapsing disorder characterized by a compulsion to seek and consume alcohol, loss of control in limiting intake, and development of a negative affective state during alcohol withdrawal and protracted abstinence. Kappa opioid receptors and their endogenous neuropeptide ligand, dynorphin A, are densely localized in limbic and cortical areas comprising the brain reward system, and play a key role in modulating stress and mood. Prolonged activation of kappa receptors by dynorphin (resulting from chronic stress or repeated drug use) leads to a pro-depressive state and promotes further drug use. A growing literature indicates that kappa receptor antagonists may be beneficial in the treatment of mood and addictive disorders.
Method: (S)-3-fluoro-4-(4-((2-(3-fluorophenyl)pyrrolidin-1-yl)methyl)phenoxy)benzamide (FP3FBZ) was tested in *in vitro* binding and functional assays using CHO cells transfected with human recombinant mu, kappa, and delta opioid receptors. Selectivity against various non-opioid receptors was determined using radioligand binding assays. FP3FBZ was tested for central opioid receptor occupancy and brain/plasma exposure, as well as microdialysis, rat formalin, and prepulse inhibition assays as pharmacodynamic measures. Behavioral assays used to determine *in vivo* efficacy included oral and operant ethanol self-administration in alcohol-preferring (P) rats, C57BL/6 mice, and kappa receptor knockout and wildtype mice. Antidepressant- and anxiolytic-like efficacy was determined using forced swim test and stress-induced hyperthermia assays, respectively.
Results: FP3FBZ is a structurally-unique, potent, kappa receptor antagonist with selectivity over mu/delta and other non-opioid receptors. Pharmacokinetic and pharmacodynamic studies indicate that it exhibits canonical properties that are favorable for clinical development. In behavioral models, it potently reduces high ethanol self-administration behavior in alcohol-preferring (P) rats and, unlike naltrexone, does not exhibit significant tolerance with repeated dosing. Moreover, it attenuates excessive ethanol intake in C57BL/6 and wild-type, but not kappa receptor knockout, mice. FP3FBZ reverses kappa-agonist-induced disruption of prepulse inhibition without producing effects on its own. Additionally and, in contrast to naltrexone, FP3FBZ also produces antidepressant- and anxiolytic-like effects in rodent models.
Discussion: Collectively, the preclinical data suggest that FP3FBZ is a centrally-penetrant, potent, selective kappa receptor antagonist with efficacy in animal models of alcohol dependence. In contrast to naltrexone, it may provide additional benefit with respect to comorbid depressive and/or anxious symptomatology.

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

Kappa opioid receptor signaling mediates stress-induced reinstatement and potentiation of ethanol seeking behavior.

McLaughlin JP, Ganno ML.

Torrey Pines Institute for Molecular Studies, Port St. Lucie FL.

Exposure to chronic stressors activates the kappa opioid receptor (KOR), contributing to maladaptive behavioral responses such as the increase of cocaine-seeking behavior. We hypothesized that signaling of the kappa opioid receptor contributes significantly to the forced swim stress (FSS)-induced potentiation of ethanol reward and self-administration, as well as reinstatement of extinguished ethanol-seeking behavior.

Male C57Bl/6J and prodynorphin gene-disrupted (Dyn -/-) or wildtype littermate mice were exposed to repeated forced swim stress (FSS). A biased ethanol conditioned place preference (CPP) and two-bottle free choice (TBC) assays were then used to measure the effects of stress-induced KOR signaling on ethanol reward and self-administration, respectively. Additionally, the mediating effect of KOR signaling on stress-induced reinstatement of extinguished ethanol-seeking behavior was assessed with a modified conditioned place preference assay. To determine the role of the KOR in the resulting behaviors, KOR agonists U50,488 (10 mg/kg) or Salvinorin A (3 mg/kg), and antagonist nor-binaltorphimine (nor-BNI, 10 mg/kg) were administered prior to parallel testing. Data were then compared to saline-treated, control mice.

Mice exposed to repeated FSS 5 min prior to daily place conditioning with ethanol (0.8 g/kg) demonstrated a 4.4-fold potentiation of ethanol-CPP and increased the consumption of 10% (v/v) ethanol by 19.3% in the TBC assay in a nor-BNI sensitive manner as compared to unstressed mice. Moreover, pretreatment with U50,488 90 min prior to daily ethanol place conditioning substituted for FSS exposure, resulting in a 2.8-fold potentiation of ethanol-CPP, and increased consumption of 10% (v/v) ethanol. Dyn -/- mice did not demonstrate significant stress-induced increases in ethanol consumption. Additionally, exposure to FSS resulted in the reinstatement of an extinguished ethanol CPP response that was prevented by nor-BNI pretreatment. Interestingly, treatment with the KOR agonist U50,488, but not Salvinorin A, induced reinstatement of ethanol CPP, consistent with the ability of these KOR agonists to activate ERK1/2 MAP kinase. Notably, U50,488-induced reinstatement was prevented by pretreatment with either nor-BNI or the ERK1/2 MAP kinase inhibitor SL-327.

These data demonstrate that KOR signaling contributes to a stress-induced potentiation of the rewarding effects and self-administration of ethanol, as well as the reinstatement of extinguished ethanol-seeking behavior. Moreover, the data highlights the importance of downstream signaling resulting from KOR activation, e.g. ERK1/2 MAP kinase, in mediating drug-seeking behavior, suggesting both additional insights into the neurobiology of stress and alcoholism, and new therapeutic approaches for treatment.

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Schlosburg JE[1], Vendruscolo LF[1], Park PE[2], Whitfield TW[1], & Koob GF[1]

[1]Committee on the Neurobiology of Addictive Disorders, The Scripps Research Institute, La Jolla, CA; [2]Skaggs School of Pharmacy & Pharmaceutical Sciences, UCSD, San Diego, CA

Long-term antagonism of kappa opioid receptors prevents escalation of, and increased motivation for, heroin intake

The abuse of opiate drugs, both illicit and prescription, is a persistent problem in the United States, accounting for over 1.2 million users requiring treatment each year. Current treatment paradigms rely on suppressing immediate withdrawal symptoms, and replacing illicit drug use with long-acting opiate drugs. However, the mechanisms that lead to opiate dependence, and potential therapeutic targets for preventing opiate dependence, are still poorly understood. We hypothesized that kappa opioid receptor (KOR) activation during chronic opioid intake and withdrawal may contribute to the negative affective state associated with heroin withdrawal, and motivation to take increasing amounts of drug.

Using a twelve hour long-access model of heroin self-administration (60 µg/kg/infusion), rats show progressive escalation of heroin intake over a period of several weeks. This escalation is accompanied by tolerance, spontaneous withdrawal, and disruptions in both food and sleep patterns that effectively recapitulate many aspects of drug addiction. A single, systemic high-dose (30 mg/kg) pretreatment with the KOR antagonist norbinaltorphimine was administered, after baseline training and prior to prolonged drug-access during the escalation phase of the experimental paradigm. While the kappa antagonist did not suppress initial heroin intake in long-access rats or in rats stably taking heroin on a one-hour short-access schedule, the escalation curve of long-access heroin rats was significantly blunted. This was paralleled by reduced motivation to respond for heroin infusions in a progressive ratio test. These effects persisted for at least a month, and rats previously treated with norbinaltorphimine demonstrated normal escalation of heroin intake and increased progressive ratio responding after a month-long abstinence and drug washout period.

Following the heroin escalation paradigm, rats going through acute 24-hour withdrawal from heroin were tested for mechanical sensitivity and anxiety-like behavior in the Von Frey and elevated plus maze tests, respectively. Long-access heroin rats had greater mechanical sensitivity and far lower open-arm time than controls. However, norbinaltorphimine was only able to suppress the anxiety component of withdrawal, and in agreement with known literature, was pronociceptive. This data suggests that chronic blockade of kappa opioid receptors, while not blocking the normal intake of heroin, is able to suppress increasing responding for heroin towards excessive levels. This may be due, in part, to its ability to suppress negative affective states during withdrawal. Future studies will evaluate the effectiveness of KOR antagonists to attenuate re-escalation following abstinence, as well as examining dynorphin distribution and content in brain following heroin escalation.

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Whitfield Jr. TW, Wee S, Gould AT, Schlosburg JE, Vendruscolo LF, Koob GF

Committee on the Neurobiology of Addictive Disorders, The Scripps Research Institute, La Jolla, CA

Kappa Receptor Activation Underlies Compulsive Methamphetamine Intake

Methamphetamine abuse is a chronically relapsing disorder that affects approximately 1.2 million individuals nationwide. It is characterized by a preoccupation with obtaining the drug, compulsive seeking and taking of the drug, and a loss of control of drug intake. There is substantial evidence that the kappa opioid receptor (KOR) system is involved in the central effects of psychostimulants, however the involvement of this system in compulsive methamphetamine intake has not yet been investigated. In the current work, we evaluated the effect of the KOR antagonist norbinaltorphimine (NorBNI) on methamphetamine self-administration in an animal model of extended access to methamphetamine, which produces excessive ("compulsive") drug intake. Rats were trained to self-administer methamphetamine in 1-h sessions. Upon stable responding, rats were treated with NorBNI (30 mg/kg) or vehicle and split into two groups: long access (LgA; 6 h sessions, which produces escalation of drug intake) and ShA (ShA; 1 h sessions, which produces stable levels of drug intake). NorBNI decreased methamphetamine intake (fixed-ratio 1) and blocked escalation of methamphetamine intake in the LgA group, but had no effect on ShA rats. After a period of abstinence (17 days), NorBNI persistently attenuated re-escalation of methamphetamine intake, demonstrating that a single dose of NorBNI produces long-lasting behavioral effects. NorBNI-treated rats were also tested under a progressive ratio (PR) schedule of reinforcement several times during the course of the study. In this test the workload to receive a drug infusion increases progressively, which was used to evaluate motivation for methamphetamine. The results showed that NorBNI decreased motivation for methamphetamine in both ShA and LgA conditions, but the effects were more dramatic and sustained in the LgA group. Taken together, these results indicate that the dynorphin/KOR system is critically involved in the emergence of compulsive methamphetamine seeking and taking behavior, and may become sensitized in rats with a history of extended access. Ongoing experiments are evaluating prodynorphin expression in relevant brain areas in these rats, and site-specific injections of NorBNI are also being tested. The goal of these experiments will be to elucidate the molecular neuroadaptations in the KOR/dynorphin system that underlie the escalation of intake and motivation to seek methamphetamine.

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Saxon, A.J.[1,2], Mooney, L.J.[3], Ling, W.[3]

Center of Excellence in Substance Abuse Treatment and Education, VA Puget Sound Health Care System, Seattle, WA[1], Department of Psychiatry & Behavioral Sciences, University of Washington, Seattle, WA[2], Integrated Substance Abuse Programs and Department of Psychiatry & Biobehavioral Sciences, David Geffen School of Medicine at UCLA, Los Angeles, CA[3]

Cocaine Use Reduction with Buprenorphine (CURB) Study

Individuals with cocaine addiction exhibit reduced dopaminergic function which may contribute to dysphoria and relapse. Agonism at kappa receptors may in part cause and/or exacerbate this dysfunction. Animal models show that antagonism at kappa receptors blocks stress induced re-instatement of cocaine self-administration. Buprenorphine is one widely prescribed, FDA approved medication that has kappa antagonist properties. Concern over inducing opioid dependence through buprenorphine's partial mu-agonist action has dampened enthusiasm for testing it as a cocaine addiction pharmacotherapy in humans. However, recent evidence suggests that oral naltrexone can largely block buprenorphine's mu effects and that the combination is well tolerated and may reduce cocaine use. NIDA plans a multisite clinical trial in which patients with a history of opioid use and current cocaine addiction will be tapered off opioids, inducted onto extended-release injectable naltrexone, and then randomly assigned to daily 1) placebo, 2) buprenorphine 4mg per day, or 3) buprenorphine 16 mg per day for 8 weeks with a second naltrexone injection after week 4. Patients will provide urine toxicology specimens 3 times weekly and report on their cocaine use for every day of the 8 week period. Number of cocaine use days during the final 30 days of the study will be compared across study conditions.

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Ko MC

Department of Pharmacology, University of Michigan Medical School, Ann Arbor, MI

The role of kappa opioid receptor in the modulation of pain and itch in primates.

Pruritus (itch sensation) is a significant clinical problem, but little effort has been made to understand the receptor mechanisms underlying itch sensation and to identify the receptor targets as potential development of antipruritics. The kappa opioid receptor (KOP) may be a prominent therapeutic target because several studies suggest that agonists at this receptor may be useful for treating refractory itch. This presentation provides an overview of various behavioral effects of KOP agonists and antagonists in non-human primates. We have established different experimental pain models (i.e., acute, neurogenic, and inflammatory pain) and itch models (i.e., centrally and peripherally evoked itch/scratching) in monkeys. The potency and effectiveness of non-peptidic KOP agonists vary against different forms of nociceptive stimuli. However, antinociceptive doses of these KOP agonists produced different degrees of sedation. In contrast, KOP agonists are significantly more potent to attenuate scratching responses elicited by different pruritogens without producing observable side effects. Anti-scratching effects of KOP agonists can be completely blocked by pretreatment with KOP antagonists. More importantly, endogenous KOP-preferring opioid peptide, dynorphin-A, can also attenuate centrally-evoked scratching responses following intrathecal administration. These pharmacological studies not only elucidate the receptor mechanisms underlying pruritus and antipruritics in primates, but also offer functional evidence for KOP agonists as a new generation of antipruritics in humans.

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Stein C

Department of Anesthesiology and Critical Care Medicine, Freie Universität Berlin, Charité Campus Benjamin Franklin, Germany

Peripheral kappa receptors mediating antinociceptive and anti-inflammatory effects

Kappa opioid receptors are present and upregulated on peripheral sensory neurons ("nociceptors"), and opioid peptides (including dynorphin) are expressed in immune cells within injured tissue. Exogenous and endogenous opioids can produce analgesia and antiinflammatory effects by inhibiting excitability/sensitization of these neurons and the release of proinflammatory neuropeptides. We have examined G-protein coupling and signaling of opioid receptors in sensory neurons, opioid peptide processing, release and extracellular degradation, as well as mechanisms governing the migration of opioid containing cells to inflamed tissue. Clinical studies have shown that small doses of opioids applied into inflamed knee joints can not only produce long lasting pain relief but also decrease synovial inflammation. Efficacy, tolerance and disease modifying effects of peripherally acting opioid compounds in animal models, human arthritis and other inflammatory diseases will be discussed.

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Menzaghi F, Spencer RH, O'Connor SO, Labissiere G, Gardell LR, and Chalmers DT.
Cara Therapeutics, Inc., 1 Parrott Drive, Shelton CT

Analgesic and morphine-sparing effects of the kappa opioid agonist CR845 after IV administration in women undergoing laparoscopic hysterectomy.

CR845 is a potent full agonist at human kappa opioid receptors (EC_{50} = 0.16 nM) with greater than 30,000-fold selectivity over human mu and delta opiate receptors with comparable activity at rodent opioid receptors, and no detectable activity at other receptors, ion channels, transporters or enzymes. Its unique peptidic structure significantly differs from the small molecules kappa agonists developed thus far which, for the most part, are CNS-active. Due to its physicochemical properties, CR845 has limited membrane permeability by passive diffusion which limits its access to the CNS and thus preferentially activates kappa receptors located outside the CNS.

Based on non-clinical pharmacological studies, it is anticipated that CR845 could produce a combined analgesic, anti-itch and anti-inflammatory effect. It has been shown to decrease pain related to activation of nociceptors and/or nerve injury, to decrease itch and to reduce the production and release of pro-inflammatory mediators with an improved safety profile over current clinically used analgesics.

To date, CR845 has been evaluated in 146 subjects (i.e. 65 healthy subjects, 18 end-stage renal disease patients and 63 post-surgical patients) in three Phase 1 studies and one proof of concept Phase 2 study, with mean CR845 plasma maximum concentrations (C_{max}) up to 276 ng/mL and AUC_{inf} up to 563 ng·h/mL after IV administration. With respect to plasma protein binding, the calculated unbound fraction of CR845 is greater than 75%. To the best of our knowledge, concentrations achieved in these studies are the highest currently reported in the literature for a kappa agonist.

A disconnect between the pharmacokinetic properties and pharmacodynamic effects of CR845 has been observed both preclinically and clinically, where the terminal elimination half-life ($T_{1/2z}$) of the compound has been estimated at approximately 2 hours while efficacy has been recorded for at least 16 hours after a single dose. This extended pharmacological action could be, in part, related to the slow dissociation rate of CR845 at the kappa receptor. No metabolites of CR845 have been identified and the compound is mostly excreted renally.

In patients undergoing laparoscopic hysterectomy CR845 was demonstrated to significantly reduce pain intensity and morphine use after a single IV dose of 0.040 mg/kg administered post-surgery. This was accompanied by a significant decrease in the incidence of adverse events commonly associated with morphine use (i.e., vomiting, nausea and pruritus).

Overall, CR845 has been shown to be safe and tolerated with no dysphoric activity after single or repeated IV dosing over 24 hours. The most commonly observed effect of CR845 has been an aquaretic effect (i.e., free water loss with electrolyte sparing). As CR845 administration has not been associated with significant changes in plasma vasopressin, it is likely that this is a direct effect of CR845 at the level of the kidney.

All together, these data suggest that CR845 represents a novel therapeutic class with significant potential in postoperative pain management.

Disclosure: Authors are employees of Cara Therapeutics, Inc.

Spencer RH, Gardell LR, and Menzaghi F.
Cara Therapeutics, Inc., 1 Parrott Drive, Shelton CT

Anti-inflammatory effects of the peripheral kappa opioid receptor agonist CR845: a novel approach for the treatment of inflammatory disease.

Kappa opioid receptors (KORs) are known to be expressed on a variety of immune cells including T-cells, macrophages, and neutrophils. KORs can also be found on fibroblast-like synoviocytes from patients with osteoarthritis (OA) and rheumatoid arthritis (RA). CR845 is a selective, peripherally-restricted kappa opioid agonist that has analgesic and morphine-sparing effects in patients post-surgery. The goal of the present study was to utilize preclinical models and ex-vivo human studies to determine if CR845 could also exhibit direct anti-inflammatory activity.

CR845 reduced inflammatory pain and also inhibited the development of hind paw edema induced by carrageenan in rat. In the mouse lipopolysaccharide (LPS) model of acute inflammation, driven in part by activated macrophages, CR845 significantly reduced the levels of several proinflammatory cytokines, including TNFα. In addition, CR845 was also effective at reducing changes in weight bearing in the rat monoiodoacetate model of osteoarthritis.

To extend these observations to humans, we evaluated the effect of CR845 on primary human macrophages activated by LPS and interferon-gamma (INF-γ). Similar to the effects observed in rodents, CR845 significantly reduced the release of TNFα, IL-1β, IL-6, IL-8 and G-CSF from activated human macrophages with an EC_{50} of ~1 nM.

To determine whether this anti-inflammatory action of CR845 could be disease-relevant, we evaluated the anti-inflammatory potential of CR845 on stimulated synoviocytes and chondrocytes derived from RA and OA patients, respectively. CR845 dose-dependently suppressed the evoked release of TNFα, and matrix metalloproteinases 1 and 3 (MMP1, MMP3) from RA synoviocytes stimulated with IFNγ and anti-CD40. In addition, the spontaneous proliferation of RA-derived synoviocytes – a major feature of rheumatoid arthritis, was also dose-dependently inhibited by CR845. Chondrocytes from OA patients produce elevated levels of MMP-13 (collagenase 3) resulting in articular cartilage degeneration. The production of MMP-13 from human OA chondrocytes stimulated with IL-1β and oncostatin M was potently suppressed by CR845 with a comparable level of effect to that observed with corticosteroid treatment. All of the anti-inflammatory effects of CR845 were shown to be reversible by nor-BNI suggesting a direct role of kappa receptor activation.

In summary, CR845 exhibits potent anti-inflammatory activity in both rodent and human models suggesting that it may be effective in the treatment of inflammatory diseases.

Disclosure: Authors are employees of Cara Therapeutics, Inc.

Cowan A[1], Menzaghi F[2], Spencer RH[2], Inan S[1]

Department of Pharmacology and Center for Substance Abuse Research, Temple University School of Medicine, Philadelphia, PA[1] and Cara Therapeutics Inc., Shelton, CT[2]

Kappa opioid receptor ligands and the development of novel therapies for the treatment of itch

Itch is an orphan symptom of several systemic diseases that affects approximately 10% of people worldwide. Although itch has long been viewed as a milder form of pain, new research has shown that it has a unique sensory modality that is similar to yet distinct from the transmission of pain. Recently, spinal gastrin-relasing peptide has been suggested to act as a common itch neurotransmitter by relaying information to the somatosensory cortex in response to an array of pruritic stimuli in mice. These stimuli included known chemical inducers of itch behavior (chloroquine and compound 48/80) which cause compulsive hindleg scratching of the neck in this species.

We have found that 5´- guanidinonaltrindole (GNTI), a kappa opioid receptor antagonist, also provokes the same frenzied, repetitive scratching behavior when injected subcutaneously (s.c.) behind the neck in male Swiss Webster mice, suggesting this may be a useful animal model for the development of novel antipruritic agents. Scratching induced by GNTI is dose-related (0.03-1 mg/kg), persists uniformly for at least 30 minutes, and can also be elicited by norbinaltorphimine (1-10 mg/kg), another kappa antagonist. Nalfurafine, the centrally active kappa agonist, attenuates the scratching caused by a standard dose of GNTI (0.3 mg/kg, s.c.), when given s.c. either before (0.001-0.03 mg/kg) or after (0.01-0.03 mg/kg) GNTI challenge. Since nalfurafine is the first kappa opioid agonist to be approved for the treatment of pruritus (in 2009 in Japan for uremic pruritus in hemodialysis patients) this suggests a critical pharmacological link to new therapies for itch with proven clinical efficacy.

To further understand the role of kappa receptors in the modulation of itch, we have focused on the ability of the peripherally restricted kappa agonist, CR845, to suppress scratching behavior in mice. CR845 is a synthetic D-amino acid tetrapeptide with limited CNS penetration. When dosed intravenously, pretreatment with CR845 produced a dose-dependent inhibition of scratching against GNTI (A50 = 0.05 mg/kg) or compound 48/80 (A50 = 0.08 mg/kg), with a duration of action of at least 12 hours (CR845 at 0.3 mg/kg vs. compound 48/80). Comparable suppression of scratching was noted after *subcutaneous* administration of CR845 in the dose range, 0.3-3 mg/kg. These results suggest that peripheral kappa opioid receptors play an important role in the modulation of itch signals and represent a target for the development of novel antipruritic agents. (Funded by DA013429 and Cara Therapeutics, Inc).

Dolle, R E*

Adolor Corporation, 700 Pennsylvania Drive, Exton, PA 19341

Discovery and Pharmacological Evaluation of Putative Peripheral Kappa Opioid Agonists ADL0101 and ADL0116

Centrally-acting kappa opioid agonists display exceptional analgesic properties but their therapeutic utility is hampered by mechanism-based central nervous system (CNS) side effects including sedation, dysphoria, hallucinations, and diuresis. Because opioid antinociception can be mediated by activation of opioid receptors outside the CNS, there is continuing interest in identifying peripherally-restricted kappa opioid agonists. In theory, such agents would retain the desired analgesic action and eliminate the central side effects. Using the CNS active kappa opioid agonist ICI 199441 as a starting point, an analgesic discovery program was initiated to peripheralize this lead molecule. Two agents, ADL0101 (IV administration) and ADL0116 (oral administration) were ultimately advanced into clinical trials. A brief outline of the discovery strategy and full pharmacological characterization of these agents will be presented as well as highlights of the human trial results and lessons learned. The structures of the clinical candidates are revealed for the first time.

*Current affiliation: Cubist Pharmaceuticals, 65 Hayden Avenue, Lexington, MA 02421

Schwarzer C.[1], Schunk E.[1], Loacker S.[1]

Dept. Pharmacology, Medical University Innsbruck, Austria

The dynorphin / kappa opioid receptor system in temporal lobe epilepsy

Epilepsies are among the most common serious disorders of the brain. Presently a number of drugs for the treatment of epilepsies are available, mostly targeting the GABAergic system. Still, depending on the type of epilepsy, up to 70/80% of patients are refractory to pharmacological treatment. Presently, such patients face surgical removal of the epileptic focus as only treatment option. Thus, there is tremendous need for novel drug targets in this field. Increasing evidence suggests that neuropeptides, particularly the opioids, play an important role in epilepsy. However, little is known about the functions of the endogenous opioid system in epileptogenesis and epilepsy. Therefore, we investigated the role of endogenous prodynorphin-derived peptides in acute seizure behavior, epileptogenesis and epilepsy in prodynorphin-deficient mice ($dyn^{(-/-)}$).
Compared to wildtype littermates ($dyn^{(+/+)}$), $dyn^{(-/-)}$ mice showed a significantly reduced seizure threshold as assessed by tail-vein infusion of the $GABA_A$ receptor antagonist pentylenetetrazole. This phenotype could be rescued entirely by the kappa opioid receptor (KOP) specific agonist U-50488H, but not the mu opioid receptor specific agonist DAMGO. The delta opioid receptor specific agonist SNC80 decreased the seizure threshold in both genotypes. Pre-treatment with the kappa selective antagonist GNTI completely blocked the rescue effect of U-50488H in $dyn^{(-/-)}$ mice and mimicked the knockout phenotype in wild-type mice. Consistent with the reduced seizure threshold, $dyn^{(-/-)}$ mice showed faster seizure onset and a prolonged time of seizure activity after intracisternal injection of the glutamate analogue kainic acid. In the classic pentylenetetrazole kindling model, $dyn^{(-/-)}$ mice showed a significantly faster kindling progression than wild-type mice. Local injection of kainic acid into the stratum radiatum of CA1 of the dorsal hippocampus resulted in markedly increased neuronal damage in $dyn^{(-/-)}$ mice during the acute phase. This phenotype was fully rescued by pretreatment of $dyn^{(-/-)}$ mice with the KOP selective agonist U-50488H and mimicked in $dyn^{(+/+)}$ mice by pretreatment with GNTI. U-50488H had no effect on acute neuronal damage in wild-type mice. Interestingly, $dyn^{(+/+)}$ mice displayed more progressive neuronal loss in the subchronic phase (1-3 weeks) than $dyn^{(-/-)}$ mice. In this phase the difference between the two genotypes gradually decreased. Noteworthy, during this phase the expression of prodynorphin mRNA is decreased in the hippocampus while KOP are still present. We hypothesized that these receptors might be a target for antiepileptic treatment. Indeed, progressive neurodegeneration could be blocked by treatment with the KOP agonist U-50488H starting one week after kainiate treatment in most regions investigated. The suppression of progressive neurodegeneration was accompanied by reduced expression of NPY immunoreactivity in mossy fibres, suggesting decreased seizure activity.
Taken together, our data strongly support a critical role for dynorphin in the regulation of hippocampal excitability, indicating an anticonvulsant role of kappa opioid receptors, thereby providing a potential target for antiepileptic drugs. We presently investigate, whether such protective effects can be achieved in a way avoiding severe dysphoric side effects. On the other hand our data suggest that antidepressant treatment with KOP antagonist may cause severe side effects in people with predisposition for epileptic seizures.

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Bruchas MR[1,2], Schindler A[1], Shankar H[1], Messinger D[1], Miyatake M[1], Land BB[3], Lemos JC[3], Hagan C[4], Neumaier JN[5], Quintana A[6], Palmiter RD, [6] Chavkin C[1].

[1]University of Washington, Pharmacology[1], Neurobiology and Behavior[3], Comparative Medicine[4], Psychiatry and Behavioral Neuroscience[5], HHMI & Biochemistry[6], Seattle, WA; [2]Current Address: Washington University School of Medicine, Anesthesiology and Anatomy/Neurobiology, St. Louis, MO.

Biased signaling at Kappa-opioid receptors distinguishes analgesic from dysphoric behavioral responses.

Prior studies have shown that p38 mitogen-activated protein kinase (MAPK) and ERK 1/2 activated by the dynorphin kappa opioid (KOR) system are key mediators of the behavioral responses to stress. Numerous groups have demonstrated that KOR antagonists are effective in blocking social defeat stress (SDS)-induced immobility, swim-stress-induced immobility and stress-induced reinstatement to drug and alcohol seeking. KOR agonists are also effective analgesic compounds but are not well tolerated because of their hallucinogenic and dysphoric effects. We recently established a role for the KOR system in dorsal raphe (DR) serotonergic nucleus in stress-induced aversive and analgesic behaviors and have found that p38 MAPK activation is required. We developed lentiviral KOR expression techniques and p38alpha conditional knockout (CKO) mice for inactivation and rescue of KOR signaling. Injection of AAV1-cre into DR of p38alphaCKO mice selectively disrupted p38alpha expression and blocked both KOR-mediated conditioned place aversion (CPA) and social defeat stress (SDS)-induced reinstatement of cocaine CPP. We found that mice expressing mutant KOR receptors that cannot activate p38 MAPK signaling still cause KOR-mediated analgesia and activate ERK signaling, but show no place aversion or dysphoria-like behavioral responses. Similarly, serotonergic deletion of p38alpha blocked CPA, produced an antidepressant-like effect in the forced swim test, and blocked stress-induced reinstatement, yet had no effect on Kappa-opioid mediated analgesia. These results suggest that KOR-selective partial agonists that do not effectively activate p38 MAPK may be useful analgesics without producing the unwanted dysphoric effects of high efficacy KOR agonists.

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Wednesday Talks

Shippenberg, T[1], Sitte, H[2], Kivell, B[1], Chefer, V[1], Ramamoorthy,S[3]

NIH/NIDA Intramural Research Program [1], Medical University of Vienna[2] and Medical University of South Carolina[3]

K- Opioid Receptor Regulation of Dopamine Transporter Function: Cellular Mechanisms and Physiological Relevance

K-opioid receptors (KORs) are enriched in the mesocorticolimbic dopamine (DA) system where they regulate the basal activity of DA neurons projecting to both the nucleus accumbens and prefrontal cortex. Until recently, these effects were attributed to activation of KOR on DA and glutamatergic terminals and the resultant inhibition of neurotransmitter release. Importantly, however, extracellular DA concentrations are rapidly regulated by the DA transporter (DAT), a transmembrane protein that uptakes DA released into the extracellular space. Given previous data showing that KOR are apposed to DAT in rat mesocorticolimbic DA terminals, we have investigated whether KOR agonists regulate DAT function in heterologous expression systems and native tissue, the cellular mechanisms of this effect and whether the functional interaction of KOR with DAT is of physiological relevance. Fluorescence resonance energy transfer microscopy (FRET) in live cells revealed that KOR and DAT are associated in HEK cells expressing both proteins as evidenced by an increase in plasma membrane $N_{FRET.}$ Furthermore, the association of these proteins is significantly increased upon acute addition of either U69593 or salvinorin A. Using live cell imaging to quantify DAT function in real time, we have found that KOR activation induces a rapid, nor-binaltorphimine-reversible up-regulation of DAT function. This effect, which is associated with an increase in ERK phosphorylation, is blocked by ERK kinase inhibition or mutations of ERK consensus sites located on the N-terminus of both human and rat DAT. A similar KOR-mediated, ERK-dependent up-regulation of DAT is observed in synaptosomes of the ventral striatum and medial prefrontal cortex. Up-regulation is associated with increased phosphorylation of DAT and inhibited by manipulations that selectively prevent the interaction of ERK with DAT. The physiological relevance of the KOR regulation of DAT is indicated by findings that preventing the interaction of KOR with DAT in the ventral striatum attenuates the behavioral effects of KOR agonists. Together these data demonstrate that KOR regulates the function and phosphorylation state of the DAT and delineate a previously, unidentified cellular mechanism by which KOR agonists affect mesocorticolimbic neurotransmission and DA-dependent behaviors.

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Ramamoorthy, S[1] Mannangatti, P[1], Jayanthi, L.D[1] and Shippenberg, T[2]

[1]Department of Neurosciences, Medical University of South Carolina and [2]NIH/NIDA Intramural Research Program.

Kappa-Opioid Receptor- Mediated Regulation of Serotonin Transporter Function, Trafficking, Phosphorylation and Protein- Protein Interactions.

Kappa opioid receptor (KOR) agonists produce dysphoria and psychotomimesis. They produce pro-depressant like effects in rodents. In contrast, KOR antagonists produce anti-depressant-like effects in rodent models. The cellular mechanisms and downstream effector(s) by which KOR ligands produce these effects are not known. KOR agonists modulate serotonin (5-HT) transmission in brain regions implicated in the regulation of mood and motivation. This effect has been attributed to inhibition of 5-HT release. Whether KOR ligands modulate the serotonin transporter (SERT) is unknown. Such information, however, is important in view of the postulated role of this protein in affective disorders. The present studies used *in vivo/ex vivo* preparations to identify the influence of KOR agonists and antagonists on SERT functional regulation followed by heterologous expression systems to delineate the molecular basis for KOR-mediated SERT regulation. Exposure of rat brain striatal synaptosomes to the KOR agonists, U69593, U50488 or salvinorin A produced concentration- and time- dependent decreases in 5-HT uptake. KOR-mediated inhibition of 5-HT uptake was sensitive to the KOR antagonist nor-binaltorphimine. Treatment of U69593 for 5 min or less resulted in a rapid inhibition of SERT activity involving trafficking independent changes in intrinsic activity of the transporter followed by decreases in surface SERT levels at later time points. U69593 and salvinorin A triggered SERT phosphorylation and decreased SERT association with PP2Ac and syntaxin 1A. Analogous to *ex vivo* data, in heterologous expression system, the KOR agonists, U69593 and salvinorin A reduced 5-HT uptake in KOR and SERT coexpressing cells, but not in SERT expressing cells. KOR mediated SERT down regulation was sensitive to calcium removal. Inhibition of CAMK but not p38 MAPK, PKC or ERK attenuated KOR-agonist induced SERT functional down-regulation. Together these data reveal that SERT function is regulated by KOR-signaling cascades in a biphasic manner involving both trafficking dependent and independent mechanisms. Interestingly, in contrast to SERT, dopamine transporter function was upregulated by KOR agonists while norepinephrine transporter function was unaltered. We hypothesize that the differential effect of KOR agonists on biogenic amine clearance and subsequent modulation of monoamine neurotransmission may contribute to the pro-depressant and psychotomimetic effects of these agents.

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Schindler AG[1], Shankar H[1], Messinger D[1], Miyatake M[1], Gustin RM[1], Hagan C[2], Neumaier JN[3], Chavkin C[1].

Departments of Pharmacology[1], Comparative Medicine[2], Psychiatry & Behavioral Sciences[3], University of Washington, Seattle, USA

Stress-induced activation of the dynorphin/kappa opioid system increases serotonin reuptake through a p38alpha MAPK mechanism and underlies prodepressive and proaddictive responses.

Stress-exposure increases the risk of addictive drug use in human and animal models of drug addiction by mechanisms that are not understood. Prior studies have demonstrated that activation of the dynorphin-kappa opioid receptor (KOR) system by stress exposure or agonist treatment produces conditioned place aversion (CPA), potentiates cocaine-conditioned place preference (CPP), and induces reinstatement of cocaine-CPP. Additionally, repeated, but not acute stress or KOR activation stimulates p38 MAPK, and p38 MAPK has been shown to increase the function of the serotonin transporter (SERT) *in vitro*. The goal of the current study was to understand the mechanisms underlying these KOR-induced behaviors by determining the role of KOR-activated p38alpha MAPK. Using rotating disk electrode voltammetry (RDEV) to measure serotonin uptake, we found that synaptosomes isolated from animals previously exposed to either repeated forced swim stress (FSS) or direct KOR activation by U50,488 demonstrated a significant increase in serotonin uptake by SERT in a norBNI dependent manner. This repeated stress effect was recovered 24 hours following stress exposure, and was not apparent after a brief acute stress exposure. Unlike SERT, repeated stress did not affect dopamine transporter (DAT) or low-affinity, high-capacity transporter function. Michaelis-Menten kinetic analysis showed that repeated FSS increased SERT Vmax without affecting SERT Km. Furthermore, repeated FSS increased the surface expression of SERT without changing total SERT levels. The stress-induced increase in surface SERT expression was brain region specific, with increased surface SERT expression seen in the ventral striatum but not the hippocampus. Finally, this KOR-mediated increase in uptake was not apparent in animals lacking GRK3 or animals lacking p38alpha MAPK in serotonergic expressing cells. Likewise, stress-induced immobility and potentiation of cocaine-CPP was not seen in GRK3 knockout mice or in mice lacking p38alpha MAPK in serotonergic cells. Together these results support the hypothesis that p38alpha MAPK in serotonergic nuclei regulates SERT function to produce adverse effects following stress.

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Valentino RJ, Van Bockstaele EJ.

The Children's Hospital of Philadelphia, Philadelphia, PA; Thomas Jefferson University, Philadelphia, PA

Regulation of the rat brain norepinephrine system by the dynorphin-kappa opiate receptor system

The norepinephrine nucleus, locus coeruleus (LC), is important in initiating arousal and shifting the mode of attention in response to salient stimuli. The LC is regulated by excitatory amino acid afferents, which promote phasic discharge in response to sensory stimuli. The LC is also regulated by afferents containing corticotropin-releasing factor (CRF), which is released during stress to tonically activate the LC. Enkephalin-containing afferents to the LC can act on inhibitory μ-opiate receptors that are highly expressed in LC neurons. Evidence suggests that these are engaged during or immediately following stress to oppose the excitatory effects of CRF and to return LC discharge to a pre-stress level when the stressor is terminated. Recent anatomical and electrophysiological studies from our laboratories suggest a novel role for the dynorphin-kappa opiate receptor (κ-OR) system in regulating LC activity through effects on LC afferents. Dynorphin-containing axon terminals were found to target the LC, forming predominantly asymmetric (excitatory-type) synapses with LC dendrites. Immunoelectron microscopy revealed a prominent localization of κ-ORs in axon terminals in the LC that also contained dynorphin, the vesicular glutamate transporter or CRF, suggesting a presynaptic site of action. Additionally, dynorphin-containing axon terminals often co-localized CRF or glutamate, suggesting that the dynorphin-κ-OR system can influence activity of the LC-norepinephrine system by regulating its major afferents. This hypothesis was tested using electrophysiological recordings of LC neurons. Intra-LC administration of dynorphin or the κ-OR agonist, U50488, inhibited LC discharge evoked by phasic sensory stimuli (presumably glutamate-mediated) while having no effect on spontaneous LC discharge rate. This was observed in both the anesthetized state with repeated sciatic nerve stimulation as the sensory stimulus and in the unanesthetized state using auditory stimulation. This effect of U50488 was prevented by pretreatment with a selective κ-OR antagonist, norbinaltrorphamine. LC activation during opiate withdrawal is in part mediated by glutamatergic afferents to the LC and this was also greatly attenuated by intra-LC administration of U50488. In addition to attenuating the influence of glutamate on LC neurons, κ-OR agonist activation in the LC decreased the magnitude of activation of LC neurons elicited by hypotensive stress, an effect known to be mediated by CRF afferents to the LC. Together, these results indicate that κ-ORs are poised to presynaptically inhibit diverse afferent signaling to the LC. This is a novel and potentially powerful means of regulating the LC-NE system that can impact on forebrain processing of stimuli and the organization of behavioral strategies in response to environmental stimuli.

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Disclosure: The authors have no conflicts to disclose

William A. Carlezon Jr., Psychiatry, Harvard Medical School, McLean Hospital, Belmont MA, USA

Roles for dynorphin and kappa-opioid receptors in the effects of stress

Stress can induce profound changes in the brain that have immediate and long-lasting effects on behavior. We have shown that various stressors activate the transcription factor CREB in the nucleus accumbens (NAs). Using viral vectors, we have shown that elevated CREB activity in the NAs causes signs characteristic of depression (anhedonia) and anxiety (resistance to extinction of fear), producing a phenotype similar to that seen in people with post-traumatic stress disorder (PTSD). In contrast, disruption of CREB activity in the NAs has antidepressant-like effects. CREB may produce these effects by regulating the firing rate of NAs neurons that provide feedback inhibition of mesolimbic dopamine neurons, which in turn send projections to areas more classically implicated in stress responsiveness (amygdala, prefrontal cortex). Our work suggests that CREB regulation of dynorphin, an endogenous ligand at KOR receptors, plays a key role in this process. CREB-mediated elevation of dynorphin tone leads to increases in the stimulation of KORs located on mesolimbic dopamine neurons, an effect that decreases the activity of this system and produces depressive-like behaviors. In support of this model, we now have considerable evidence that (depending on the methods used) disruption of KORs can prevent, attenuate, and reverse stress effects on behavior. KOR antagonists produce antidepressant-like effects in the forced swim test regardless of whether they are given before or after exposure to stress. Likewise, KOR antagonists have acute anxiolytic-like effects in the elevated plus maze, and administration of these drugs before fear conditioning can prevent the development of PTSD-like changes in behavior. New data indicate that KOR antagonists reduce the disruptive effects of stress on attention in rats in the 5-choice serial reaction time task. Collectively, these data suggest that KOR antagonists might be particularly useful for producing protective effects in cases where it is possible to predict when stress will occur.

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Dr. Carlezon has a patent (US 6,528,518; Assignee: McLean Hospital) related to the use of kappa-opioid antagonists for the treatment of depressive disorders.

Elena Chartoff[a], Allison Sawyer[a], Anna Rachlin[a], Dave Potter[a], Andrea Pliakas[a], William A. Carlezon, Jr.[a]

[a]Behavioral Genetics Laboratory, Department of Psychiatry, Harvard Medical School, McLean Hospital, 115 Mill Street, Belmont, MA 02478, USA

Blockade of kappa-opioid receptors attenuates cocaine withdrawal-induced negative affective states

Drug dependence is characterized by dysregulation of brain reward systems and increased sensitivity to stress. Chronic exposure to drugs of abuse is associated with increased expression of the neuropeptide dynorphin, the endogenous ligand for kappa opioid receptors (KORs). Activation of KORs causes depressive- and aversive-like responses in rodents, raising the possibility that drug-induced upregulation of dynorphin plays a role in dependence-associated negative states. Here we used "binge" exposure to cocaine (3 daily intraperitoneal injections of 15 mg/kg for 14 days) to examine the development of dependence-like behavior in the intracranial self-stimulation (ICSS) test and the forced swim test (FST). When rats were tested immediately before their first scheduled injection of each day—a period of drug withdrawal corresponding to 20 hr after their last injection on the previous day—there were exposure-dependent increases in ICSS thresholds (a putative indicator of anhedonia) and latencies to immobility in the FST (a putative indicator of behavioral despair). Administration of the long-lasting KOR antagonist norBNI (20 µg, intracerebroventricular) before the beginning of the binge regimen attenuated the development of cocaine withdrawal-induced anhedonia in the ICSS test. In contrast, administration of norBNI in the midst of the binge regimen had no effect on expression of cocaine withdrawal-induced anhedonia in the ICSS test, although it did attenuate despair-like behavior in the FST. These data raise the possibility that KOR antagonists may be useful for treating the anhedonia that can promote self-medication with psychostimulants as well as for rehabilitating addicts who are at risk for stress-induced relapse.

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Dr. Carlezon has a patent (US 6,528,518; Assignee: McLean Hospital) related to the use of kappa-opioid antagonists for the treatment of depressive disorders.

Lucki I[1], Carr GV[1], Bangasser D[2], Valentino RJ[2]

Department of Psychiatry, University of Pennsylvania, Philadelphia PA; and Children's Hospital of Philadelphia, Philadelphia PA

Antidepressant-like Effects of Kappa Opioid Receptor Antagonists and Buprenorphine in WKY Rats

Testing potential antidepressant compounds in rodent genetic models may better address the unique aspects of comorbid depression and anxiety that are resistant to treatment with conventional antidepressants. The Wistar-Kyoto (WKY) rat strain displays a unique behavioral phenotype characterized by increased sensitivity to stress, as shown by increased immobility in the forced swim test (FST), increased learned helplessness, increased stress-induced ulceration, increased anxiety-like behaviors, and prolonged elevation of corticosterone secretion. WKY rats demonstrate increased KOR gene expression and dynorphin protein levels compared with Sprague-Dawley rats, which may be associated with their increased sensitivity to stress.

Recently, we showed that the kappa opioid receptor (KOR) antagonist nor-BNI was effective at reducing immobility and increasing swimming behavior in the FST in WKY rats. The effects were measured 24 h after a single injection, suggesting that the KOR antagonists may exert a longer duration of action than conventional antidepressants that normally require 3 injections in 24 hours to be effective in the FST. Regional changes in cfos indicated potential involvement of the piriform cortex and nucleus accumbens that was confirmed by the local injection of nor-BNI. In addition to nor-BNI, the mixed mu opioid receptor partial agonist and KOR antagonist, buprenorphine, was active in the FST when given to WKY rats. In contrast, both KOR antagonists and buprenorphine were ineffective in Sprague-Dawley (SD) rats when tested at the same doses. These data support the importance of genetic background or stress levels in revealing antidepressant-like behavioral effects of KOR antagonists. These results in animal studies support the utility of developing KOR antagonists as effective antidepressant compounds that may be particularly useful in subject that are resistant to conventional antidepressants.

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Mary Jeanne Kreek, MD[1], Yong Zhang, PhD[1], Stefan Schlussman, PhD[1], Yan Zhou, PhD[1], Eduardo Butelman, MD[1]

Laboratory of the Biology of Additive Diseases, The Rockefeller University, New York, NY[1]

Bidirectional translational research of the kappa opioid receptor and dynorphin systems: Implications for human disease and therapeutics

After our successful initial research and translational research leading to the prompt development of methadone maintenance treatment nationwide in the mid-1960s, attempts at developing other alternative treatments for opiate addiction were launched. Two candidate compounds, cyclazocine and ethylketocyclazocine were studied by a few groups. When these were given to heroin addicts, it was found that they initially caused some adverse side-effects, including psychomimetic and dysphoria symptoms, along with frank depression in some individuals. These unpleasant effects disappeared with time, and the primary mu opioid partial agonist effects were beneficial just as methadone, a mu opioid full agonist, was beneficial. Later studies using increasingly modern techniques showed that these two compounds were mu opioid partial agonists, but with some kappa agonist activity, a profile identical to buprenorphine, and parallel to two compounds which have primarily mu opioid receptor antagonism, nalmefene and naltrexone, but which also has some partial kappa agonism. Studies conducted by my group in the late 1980s and early 1990s showed that both dynorphin and kappa opioid receptor gene expression, are significantly altered by acute, but also subacute and chronic, “binge pattern” cocaine administration, in an intermittent and recurrent pattern (dynorphin) or a persistent pattern (kappa opioid receptors). Similar findings were made by two other groups in early contemporaneous studies. We found in microdialysis studies in rats that chronic cocaine administration led to a significant lowering of basal dopamine levels and, further, that administration of cocaine caused a rise in dopamine levels which were reduced with chronic administration. We postulated and showed that this is due to a direct dynorphin effect. We have found in mice that dopaminergic tone is reduced after chronic cocaine administration. In both rats and mice, we have shown that when the natural kappa opioid receptor ligand dynorphin $A_{1\text{-}17}$ is instilled directly into the striatum, there is a dose-dependent reduction in dopamine levels. We have found this in rats and in mice. We have found identical results when selective synthetic kappa agonists or the natural plant-derived non-nitrogen-containing hallucinogen-producing kappa agonist, salvinorin. We have shown that high dose of dynorphin will block the dopamine-increased surge following cocaine administration. Our group and many others have shown for years that depressive symptoms and frank depression frequently occurs during chronic cocaine addiction. We have postulated that this may, in part, be due to increased dynorphin/kappa activity. We have conducted several extensive studies in human subjects using dynorphin $A_{1\text{-}13}$, which is natural sequence, but shortened by four residues. We have been able to show a dynorphin-induced, dose-dependent increase in prolactin levels in healthy humans. Also, we have found that long-term methadone maintained patients have a significantly reduced response to dynorphin. Since around 2000, we have suggested that a kappa partial agonist might be desirable in management of long-term cocaine and other simulant dependency. A full agonist might yield undesirable lower dopaminergic tone, which could contribute to dysphoria and depressive symptoms; conversely, a kappa antagonist may modestly enhance dopamine tone, and have no effect during cocaine use. We postulate that a partial agonist would allow modest changes in dopamine tone in the setting of “natural rewards” such as food, but block surges in dopamine tone caused by unnatural rewards such as cocaine.

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Yuferov V[1], Ho A[1], Morgello S[2], Proudnikov D[1], Levran O[1], Kreek MJ[1]

Laboratory of the Biology of Addictive Diseases, The Rockefeller University, New York, NY[1], USA, Pathology and Neuroscience, Mount Sinai Medical Center, New York, NY, USA[2]

Human molecular genetics of *PDYN* and *OPRK1* and expression of those genes and chemokine receptors in postmortem brain of HIV infected and HIV negative subjects

Previously our laboratory reported data on variations in coding and regulatory regions of the human PDYN and OPRK1 genes. We have identified the fourth exon in the 5' UTR of the *OPRK1*, and found SNP 36G>T in exon 2 to be associated with vulnerability to develop opiate addiction. In the PDYN gene, we showed significant associations of SNPs in 3'UTR region with cocaine dependence and cocaine/alcohol codependence. Using allele-specific gene expression assay, we found that the minor alleles implicated in vulnerability to develop cocaine dependence were associated with lower *PDYN* expression. Analysis of total *PDYN* expression in 43 postmortem brains also showed significantly lower levels of *PDYN* mRNA in subjects having the risk genotype.

We extended our studies to expression of *PDYN* and *OPRK1* in post mortem brain tissues of HIV+ and HIV- subjects. An interaction of opioid and chemokine receptors has been shown to be implicated in neuronal functions and immune responses, including the co-receptor-dependent HIV-1 infection and in effects of drugs of abuse on HIV-1 neuropathogenesis. Recent studies have demonstrated cross-desensitization between opioid and chemokine receptors in both *in vitro* and in nociceptive tests in rats. The relationship between opioid and chemokine receptors in brain of HIV infected human subjects is not yet understood. The aims of the study were to examine (1) expression of the *OPRM1, OPRK1* and *PDYN* mRNAs as well as chemokine receptors *CCR5* and *CXCR4* mRNAs in the caudate and anterior cingulate in HIV positive and HIV negative subjects; (2) whether there is a correlation between mRNA levels of the opioid and chemokine receptors, or other markers of glial and neuronal cells. Tissues from postmortem brain of 24 HIV+ and 14 HIV- subjects were obtained from the Manhattan HIV Brain Bank (The Mount Sinai Medical Center, New York, NY). Quantification of the specific mRNA levels was performed using SYBR Green RT-PCR. Copy number of cDNA transcripts was expressed normalized to *GAPDH* cDNA. We have found higher expression of *OPRK1* in the anterior cingulate in HIV+ brains, but not in the caudate. No difference in the *OPRM1* expression between HIV+ and HIV- subjects in either region was found. There were significantly higher levels of *GFAP, CD163* and *CD68* mRNAs in HIV+ subjects compared to HIV- ($p<0.05$). No correlation was found between levels of *OPRM1* and *CCR5* mRNA or *OPRK1* and *CXCR4* in either HIV+ or HIV- subjects. However, there was significant correlation in expression of *OPRK1* and microglial/macrophage marker *CD163* in HIV+ subjects and of *PDYN* and microglial/ macrophage marker *CD68* in the caudate in both HIV- and HIV+ brains. The results suggest that the *OPRK1*/dynorphin system is implicated in HIV-induced gliosis and inflammation in human brain in a region-specific manner, and may provide a new strategy for studies in the field of neuroimmune pharmacology.

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Vukojević V[1], Ming Y[1], Gruol D[2], Terenius L[1].

Department of Clinical Neuroscience, Karolinska Institute, Stockholm, Sweden[1]; and The Scripps Research Institute, La Jolla CA[2]

Live cell study of kappa opioid receptor (KOR) dynamics in the plasma membrane and interactions with dynorphins.

Surface density, spatial distribution and mobility in the cell membrane are determinants of receptor activity. Quantitative studies of receptor distribution and the kinetics of ligand–receptor interactions in live cells are challenging. Using high-resolution fluorescence imaging methods with single-molecule sensitivity (APD imaging), Fluorescence Correlation Spectroscopy (FCS) and Fluorescence Cross-Correlation Spectroscopy (FCCS) we have quantified the surface density and movement of the kappa-opioid receptor (KOR), measured the kinetic rate constants and equilibrium association constants of KOR-dynorphin complexes and their internalization dynamics. We have identified two principal components of KOR with distinct diffusion properties, and are presently investigating the effects of KOR partitioning in the plasma membrane on its binding properties. Parallel studies using electrophysiological and Ca^{2+} imaging techniques have been used to monitor physiological consequences of these interactions.

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Portoghese PS, Yekkirala, AS

Department of Medicinal Chemistry and Department of Pharmacology, Academic Health Center, University of Minnesota, Minneapolis, MN

Selective activation of kappa opioid receptor heteromers by exogenous and endogenous ligands in HEK-293 cells

Burgeoning evidence for heteromers of G protein-coupled receptors (GPCRs) in cultured cells, together with the finding that signaling and trafficking may be modified by hetero-oligomerization, raises the likelihood that both heteromers and homomers may be targets of ligands that activate or antagonize GPCRs in vivo. In this regard, over a dozen heteromers have been reported in cultured cells for the opioid receptor family. In view of the existence of kappa-mu and kappa-delta heteromers, we have investigated the activity of exogenous and endogenous opioid ligands in HEK-293 cells expressing either homomeric or heteromeric opioid receptors using established methods. These studies were conducted on the standard kappa agonist, U69593, and on the clinically employed mixed agonist-antagonist analgesics (pentazocine, nalbuphine, butorphanol) that were thought to act at homomeric mu and kappa opioid receptors. Our data has revealed that these ligands often more potently activate kappa heteromers than kappa homomers. Similar cell-based studies of dynorphin A (1-13), beta-endorphin, and endomorphins indicated that they also selectively activate kappa heteromers. In this regard, beta-endorphin and endomorphin-1 selectively activated delta-kappa and mu-kappa heteromers. In contrast, endomorphin-2 was non-selective for mu, mu-kappa, and delta kappa heteromers. These results are inconsistent with the perception that the dynorphins act primarily via kappa homomers and that endomorphins activate mu homomers. The results of the present study, when compared to the classical view based on homomeric receptors, have profound implications with respect to the interpretation of in vivo data.

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Clarke, W.P.[1], Rowan, M.P.[1], Sanchez, T.A.[1], McGuire, B.A.[1], Portoghese, P.S.[2], Hargreaves, K.M.[1] and Berg, K.A.[1]

University of Texas Health Science Center, San Antonio, TX[1] and University of Minnesota, Minneapolis, MN [2]

Interactions between KOR and DOR in peripheral sensory neurons

There is evidence that KOR can form heteromers with DOR. As drug targets, heteromeric receptors offer an additional level of selectivity and, in part as a consequence of allosteric interactions between protomers, functionality. Here we report that occupancy of KOR with selective antagonists differentially altered the potency and/or efficacy of DOR agonists in primary cultures of adult rat peripheral sensory neurons and in a rat behavioral model of thermal allodynia. In vitro, the KOR antagonist, nor-BNI, enhanced the potency of DPDPE, decreased the potency of DADLE, and decreased the potency and efficacy of SNC80 to inhibit prostaglandin E_2 (PGE_2)-stimulated adenylyl cyclase activity. In vivo, intraplantar (i.pl.) injection of nor-BNI enhanced the effect of i.pl. DPDPE and decreased the effect of i.pl. SNC80 to inhibit PGE_2-stimulated thermal allodynia in the rat hindpaw. In contrast to the potentiating effect of nor-BNI on responses to DPDPE, the KOR antagonist, 5'-GNTI, reduced the effect of DPDPE both in cultured neurons and *in vivo*. Such ligand-dependent effects between KOR antagonists and DOR agonists are consistent with allosteric interactions between the protomers of DOR-KOR heteromers. To determine directly if KOR and DOR form heteromers in peripheral sensory neurons, co-immunoprecipitation experiments were conducted with primary sensory neuron cultures. Following cell surface crosslinking and immunoprecipitation with KOR antibody, a single, 120kd immunoreactive band for DOR was visualized with western blot. Moreover, the DOR-KOR heteromer selective agonist, 6'-GNTI, was effective at inhibiting adenylyl cyclase activity in primary sensory neuron cultures as well as inhibiting PGE_2-stimulated thermal allodynia in the rat hindpaw, with effects blocked by DOR or KOR antagonists. Taken together, these data suggest that DOR-KOR heteromers are functional in rat primary sensory neurons and that allosteric interactions between the protomers may allow for enhanced pharmacological control of peripheral pain mechanisms.

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Prem N. Yadav[*], Jennifer Whistler[#] and Bryan L. Roth[*].

[*]Department of pharmacology, University of North Carolina at Chapel Hill, Chapel Hill, NC.
[#]Ernest Gallo Clinic and research Center at UCSF.

Cellular mechanisms of Salvinorin A action *in vivo*.

Compelling evidence indicates that kappa opioid receptor (KOR) agonists induce perceptual distortions and modulate learning processes, although the mechanistic role of this receptor in these complex behaviors remains elusive and controversial. Because an abnormality in informational processing is hypothesized to underlie several psychiatric disorders, the present study has been designed to identify the neuronal types and assess the roles of KOR in modulation of sensorimotor gating. First, to determine the neuronal identity of KOR expressing neurons in various brain region, we bred the Floxed KOR mice with Emx1 cre (fore brain glutamate neurons), vGATcre (GABA neurons) or vGlut2cre (glutamate neurons) driver mouse line. We discovered that KOR expression in cortex was completely eliminated in mice with FKOR-emx1cre and FKOR-vGlut2cre mice, with no change in KOR expression in dorsal and ventral striatum of these mice. Moreover, we found that cortical KOR expression was unchanged in FKOR-vGATcre mice, but completely eliminated in striatum and other midbrain regions such as in hypothalamic and sub-thalamic nuclei. To determine the functional relevance of cortical and striatal KOR in sensorimotor gating, we tested the effect of selective KOR agonist and psychomimetic agent salvinorin A (Sal A) on prepulse inhibition (PPI). Interestingly, we found that deletion of KOR expression either in striatum (with vGATcre) or cortex (with vGlut2cre) blunted the SalA induced PPI disruption in mice. These results suggest that KOR expressing glutametergic neurons in cortex and medium spiny GABA neurons in the striatum play critical roles in the modulation of sensory processes.

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Varlinskaya E, Anderson R, Morales M, Truxell E, Spear L.

Department of Psychology, Center for Development and Behavioral Neuroscience, Binghamton University, Binghamton, NY. Developmental Exposure to Alcohol Research Center, Binghamton NY, Cortland NY, and Syracuse NY.

Is the endogenous kappa opioid system implicated in adolescent-typical insensitivity to ethanol?

An attenuated sensitivity to adverse effects of ethanol presumed to serve as feedback cues to terminate a drinking episode could be a permissive factor for heavy and problematic drinking during adolescence. Animal studies have shown that adolescent rats are less sensitive than adults to a number of ethanol effects, including its socially anxiogenic and aversive properties, while also showing relatively high levels of ethanol intake. Given a substantial role of the endogenous kappa opioid system in mediation of adverse ethanol effects, we used a psychopharmacological approach to test the hypothesis that the enhanced intake of and relative insensitivity to ethanol observed in adolescent rats is related, at least in part, to adolescent-specific insensitivity to activation of the kappa opioid system. Specifically, we assessed the effectiveness of a selective kappa antagonist, nor-binaltorphimine (nor-BNI) in reversing ethanol effects in adolescent and adult rats and compared age-related effects of a selective kappa agonist (U62,066) with previously characterized ontogenetic patterns of ethanol responsiveness. Our initial hypothesis was confirmed by a number of experimental findings. For instance, ethanol-induced social anxiety was seen in adolescent animals at higher doses than in their adult counterparts and was diminished by pharmacological blockade of kappa opioid receptors, with adolescents requiring higher doses of nor-BNI than adults. Analogously to ethanol, the kappa agonist induced social anxiety and taste aversions in an age-dependent fashion, with adolescents being less sensitive to these effects than more mature animals. Furthermore, the relatively high ethanol intake seen in adolescent rats was little affected by nor-BNI, whereas this kappa antagonist increased the initially low ethanol intake seen in adult males. These ontogenetic patterns suggest that the approach for pharmacological treatments of alcohol-related problems that target the kappa opioid system will likely differ between adolescents and adults.

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Li-Na Wei

Dept. Pharmacology, University of Minnesota Medical School, Minneapolis, MN 55455, USA.

Physiological function of kappa opioid receptor in development

The expression of kappa opioid receptor (KOR) begins early in developmental stages, long before the maturation of the nervous systems in animals. The physiological role for KOR has remained a topic of debate. Our recent studies reveal extensive regulation of KOR expression by various RNA-based mechanisms including targeted mRNA transport of its different mRNA isoforms and specific growth factor-triggered local translation in neurons. The tight regulation of KOR expression in selected neuronal compartments implicates certain functions of KOR in specific physiological contexts. In KOR gene-knockout primary neurons, EGF-stimulated neurite extension is retarded, suggesting a functional role for KOR in neurite extension during developmental stages. This defect can be rescued by expressing specific KOR-expression vector that carries the untranscribed regions (UTRs) of its mRNA, supporting that mRNA transport-coupled local translation is critical for the manifestation of the physiological activity of KOR in modulating neurite extension. Other potential actions of KOR will also be discussed.

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Coscia CJ[1], Croskey W[1], Kim EN[1], Hahn JW[1], Jagwani, S[1], Miller K[1], Belcheva MM[2].

St. Louis University, St. Louis, MO[1]; and Washington University, St. Louis, MO[2]

Developmental stage dependent kappa opioid modulation of embryonic stem cell differentiation via MAP kinases

In prior studies, we discovered that functional κ opioid receptors are present in blastocyst-derived mouse embryonic stem cells (ESCs) and in neural progenitors that are generated from these cells by retinoic acid. We found that the prototypic κ opioid agonist, U69,593 promoted ESC proliferation and retinoic acid-induced ESC differentiation to neural progenitors by biochemical and immunofluorescence microscopic techniques. Moreover, U69,593 inhibited terminal differentiation of neural progenitors to neurons and astrocytes while promoting oligodendrogenesis via ERK and/or p38 MAPKs. The κ opioid receptor specific antagonist, nor-binaltorphimine, reversed all of these U69,593 actions. In ESCs and early stage neural progenitors, κ and μ (as measured with the prototypic μ opioid agonist, DAMGO) opioid signaling induced remarkably similar time courses of ERK activation and lineage outcomes. We now show that only in a later phase of neural progenitor differentiation and ultimate maturation did κ opioid signaling differ from μ. Time course experiments in which opioid modulated retinoic acid-induction of ESCs to astrocytic or neuronal progenitors support this dynamic pattern. Both μ and κ opioids induced ESC differentiation to SOX-1$^+$ neural progenitors more rapidly than vehicle. After 2, 4 and 6 days of exposure to opioids, comparable effects of U69,593 and DAMGO were mediated by ERK. On day 8, both κ and μ opioids lost their ability to coax ERK mediated total SOX-1$^+$ neural progenitor formation and they inhibited differentiation to astrocytic progenitors. In contrast, U69,593 alone promoted neuronal progenitor programing in an ERK-independent manner on day 8. Similarly, U69,593 but not DAMGO inhibited neural progenitor differentiation to mature astrocytes by an ERK-independent mechanism and promoted astrogenesis via p38. In summary, κ and μ opioid signaling stimulated comparable cell lineage programing via ERK, exclusively during ESC differentiation. However in discrete stages of neural progenitor differentiation and in mature neural cells, κ opioid signaling selectively elicited inhibition or potentiation of lineage commitment in an ERK and/or p38-dependent manner.

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Lee-Yuan Liu-Chen, Yujun Wang, Khampaseuth Rasakham, Darina Chudnovskaya, Alan Cowan, and Peng Huang

Department of Pharmacology and Center for Substance Abuse Research, Temple University School of Medicine, Philadelphia, PA

Sex Difference in KOPR-Mediated Behaviors and Brain KOPR level and KOPR-mediated [^{35}S]GTPγS Binding in the Guinea Pig

We examined if sex differences in KOPR pharmacology exists in the guinea pig by investigating the effects of the KOPR selective agonist U50,488H on abnormal postures/immobility, antinociception and modulation of cocaine-induced hyperactivity. We used guinea pigs since this species is more similar to humans in the expression level and distribution of KOPR in the brain than rats and mice. U50,488H produced a dose-dependent increase in abnormal postures and immobility with greater effects in males than females. Males also showed greater U50,488H-induced antinociception than females in the paw pressure test. Notably, KOPR antagonist norBNI pretreatment blocked U50,488H-induced abnormal body postures and antinociception. In contrast to abnormal postures/immobility and antinociception, inhibition of cocaine-induced hyperactivity by U50,488H was more effective in females than males. These studies demonstrate that sex differences in the pharmacological effects of the KOPR agonist U50,488H are endpoint-dependent. Sex differences in KOPR level and KOPR-mediated G protein activation in distinct brain regions may contribute to the observed differences. Thus, we examined [^{3}H]U69,593 binding and U50,488H-stimulated [^{35}S]GTPγS binding in male and female guinea pig brains using quantitative *in vitro* autoradiography. Compared to females, males exhibited greater [^{3}H]U69,593 binding in the deep layers of somatosensory and insular cortices, claustrum, endopiriform nucleus, periaqueductal gray, and substantial nigra. Concomitantly, U50,488H-stimulated [^{35}S]GTPγS binding was greater in males than females in the superficial and deep layers of somatosensory and insular cortices, caudate putamen, claustrum, medial geniculate nucleus and cerebellum. In contrast, compared with males, females had higher U50,488H-stimulated [^{35}S]GTPγS binding in the dentate gyrus and a trend of higher KOPR activation in the hypothalamus. These data demonstrate that males and females differ in KOPR expression and KOPR-mediated G-protein activation in distinct brain regions. Together, our data demonstrate that endpoint-dependent differences in KOPR-mediated behavior pharmacology between males and females are at least in part due to sex differences in KOPR expression and function in distinct brain regions.

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Murray TF[1], Kelamangalath L[1], Dravid SM[1], George J[1], and Aldrich JV[2]

1. Department of Pharmacology, School of Medicine, Creighton University, Omaha, NE 68178
2. Department of Medicinal Chemistry, School of Pharmacy, University of Kansas, Lawrence, KS 66045

Kappa-opioid receptor inhibition of calcium oscillations in spinal cord neurons.

Mouse embryonic spinal cord neurons in culture exhibit spontaneous calcium oscillations from day in vitro (DIV)-6. This spontaneous activity in developing spinal cord neurons contributes to the maturation of synapses and development of pattern generating circuits. Here we demonstrate that these calcium oscillations are regulated by kappa opioid receptors (KORs). The kappa opioid agonist dynorphin [Dyn-A (1-13)] suppressed calcium oscillations in a concentration-dependent manner. The KOR selective agonist U-69593 mimicked the effect of Dyn-A (1-13) on calcium oscillations. Both the nonselective opioid antagonist naloxone and the kappa-selective blocker nor-BNI blocked Dyn-A (1-13)-induced suppression of calcium oscillations. The kappa-selective peptide antagonist, zyklophin, was also able to prevent the suppression of calcium oscillations caused by Dyn-A (1-13). The spontaneous calcium oscillations were blocked by 1 μM tetrodotoxin indicating a dependence on action potentials. Although the L-type voltage-gated calcium channel blocker nifedipine did not suppress calcium oscillations, the N-type calcium channel blocker ω-conotoxin inhibited these spontaneous calcium transients. Blockers of ionotropic glutamate receptors, NBQX and MK-801, also suppressed calcium oscillations demonstrating the involvement of glutamate receptor signaling. We next determined the effect of Dyn-A (1-13) on spinal neuron mEPSCs. In the presence of TTX, mEPSC frequency and amplitude were 27.8 ± 2.7 events/second and 6.7 ± 0.53 pA, respectively. Bath application of 1 μM Dyn A (1-13) produced a significant reduction in both the frequency and amplitude of mEPSCs (44.4 ± 5% reduction in frequency and 20.8 ± 2.8% reduction in amplitude compared to control). These data indicate that Dyn A (1-13) regulates excitatory neurotransmission in spinal neurons by reducing quantal release of glutamate from presynaptic nerve endings. Further investigation indicated that KORs are colocalized in the presynaptic compartment with glutamatergic spinal neurons. The KOR mediated inhibition of spontaneous calcium oscillations may therefore be a consequence of presynaptic inhibition of glutamate release.

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	First	Last	
1	Christoph	Abels	Dr. August Wolff GmbH & Co. University of Regensburg
2	Jane	Acri	NIDA/NIH
3	Jane	Aldrich	University of Kansas
4	Rachel	Anderson	Binghamton University
5	Derrick	Arnelle	Alkermes, Inc.
6	Najib	Babul	Theraquest, Inc
7	Patrick	Beardsley	Virginia Commonwealth Univ
8	Katherine	Beebe	Titan Pharmaceuticals
9	Kelly	Berg	University of Texas Health Sciences Center
10	Laura	Bohn	Scripps Florida
11	Michael	Bruchas	Washington University
12	Lauren	Burgeno	University of Washington
13	William A.	Carlezon	McLean Hospital & Harvard Univ
14	F. Ivy	Carroll	Research Triangle Inst
15	John	Cashman	Human BioMolecular Research Institute
16	Elena	Chartoff	Harvard University
17	Charles	Chavkin	University of Washington
18	William	Clarke	University of Texas Health Science Center
19	Carmine	Coscia	St Louis University
20	Alan	Cowan	Temple University
21	Conrad	Cowan	Trevena
22	Andre	Curtis	Children's Hospital of Philadelphia
23	Seth M.	Davis	Washington State University
24	Robert	Day	SAMA Foundation
25	Reginald	Dean	Alkermes, Inc.
26	Roland	Dolle	Cubist Pharmaceuticals
27	Jonathan	Ehrich	University of Washington
28	Margolis	Elyssa	Gallo Institute
29	Howard L	Fields	UCSF & Gallo Inst
30	Kevin	Frankowski	Kansas State University
31	Richard	Goehring	Purdue Pharma
32	Danielle	Graham	EMD Serono Research Institute
33	Peter	Groblewski	University of Washington
34	Richard	Gustin	University of Washington
35	Shanna	Harkey	University of Michigan
36	Yasmin	Hurd	Mt Sinai
37	Stephen	Husbands	University of Bath
38	Olav	Hustveit	University of Olso
39	Lankupalle	Jayanthi	Medical University of South Carolina
40	Reese T.	Jones	UCSF
41	Daniel	Kapusta	Louisiana State University
42	Thomas	Kash	University of North Carolina, Chapel Hill
43	Linda	Kehn	Eli Lilly and Company
44	Bronwyn	Kivell	Victoria University of Wellington
45	Mei-Chuan	Ko	University of Michigan
46	George	Koob	Scripps Research Institute
47	Mary Jeanne	Kreek	Rockefeller University
48	Nathan	Lautermilch	Purdue Pharma
49	Julia	Lemos	University of Washington
50	Jamie	Levin	University of Washington
51	Renyu	Liu	Univ of Pennsylvania
52	Lee-Yuan	Liu-Chen	Temple University
53	Irwin	Lucki	University of Pennsylvania
54	Kabirullah	Lutfy	Western University of Health Sciences
55	Damaj	M. Imad	Virginia Commonwealth University
56	Rafael	Maldonado	Universitat Pompeu Fabra

57	David	McCann	NIDA/NIH
58	Jay	McLaughlin	Torrey Pines Institute for Molecular Studies
59	Erica	Melief	University of Washington
60	John	Mendelson	California Pacific Medical Center Research Institute
61	Frederique	Menzaghi	Cara Therapeutics
62	Daniel	Messinger	University of Washington
63	Charles	Mitch	Eli Lilly and Company
64	Mayumi	Miyatake	University of Washington
65	Melissa	Morales	Binghamton University
66	Philip	Mosier	Virginia Commonwealth University
67	Thomas	Murray	Creighton University
68	Hernan	Navarro	Research Triangle Institute
69	John	Neumeyer	McLean Hospital Harvard University
70	Danielle	Newcomer	University of Washington
71	Michael	Nizhnikov	Binghamton University
72	Kshitij	Patkar	Torrey Pines Institute for Molecular Studies
73	Paul	Phillips	University of Washington
74	John	Pintar	Robert Wood Johnson Medical School-UMDNJ
75	Philip	Portoghese	University of Minnesota
76	Thomas	Prisinzano	University of Kansas
77	Sammanda	Ramamoorthy	Medical University of South Carolina
78	Daniel	Reis	Washington State University
79	Andrew	Saxon	University of Washington
80	Selena	Schattauer	University of Washington
81	Abigail	Schindler	University of Washington
82	Joel	Schlosburg	Scripps Research Institute
83	Christoph	Schwarzer	Medical University Innsbruck
84	Paul	Schweitzer	Scripps Research Institute
85	Haripriya	Shankar	University of Washington
86	Toni S	Shippenberg	NIDA-IRP/NIH/DHHS
87	Sunil	Sirohi	Washington State University
88	Jeff	Smith	University of Washington
89	Michael	Soeberdt	Dr. August Wolff GmbH & Co.
90	Robert	Spencer	Cara Therapeutics
91	Karin	Sperling	Mundipharma Research
92	Christoph	Stein	Free Univ Berlin
93	Hugo	Tejeda	NIDA-IRP/NIH/DHHS
94	Lars	Terenius	Karolinska Institute
95	Gregory	Terman	University of Washington
96	Rita	Valentino	Children's Hospital of Philadelphia
97	Elena	Varlinskaya	Binghamton University
98	Frank	Vocci	Friends Research
99	Brendan	Walker	Washington State University
100	Bradford	Walters	Research Triangle Institute
101	Li-Na	Wei	University of Minnesota
102	Timothy	Whitfield Jr.	Scripps research Institute
103	Angela	Williams	Washington State University
104	Conrad	Wong	Eli Lilly and Company
105	Prem	Yadav	University of North Carolina
106	Vadim	Yuferov	Rockefeller University
107	Chad	Zietz	University of Washington
108	Jordan	Zjawiony	University of Mississippi