30th 1999 INRC

Conference Program

Plenary lectures, symposia and oral presentations will be held in the Grand Ballroom of the Saratoga Springs Sheraton. Posters should be mounted before the plenary lecture on Sunday morning (July, 11, 1999). The presenting author should be present at the posters during the appropriate viewing time.

Saturday, July 10, 1999

13:00 – 19:30	Registration		
19:30 – 21:30	Reception		

Sunday, July 11, 1999

07:00 – 08:30	Put up posters. Posters will remain up until Thursday, July 15.		
07:00 – 08:30	Breakfast		
08:30 – 08:35	Opening Remarks		Charles Chavkin Jean Bidlack
08:35 – 09:35	**Plenary Lecture**		*Ion channels, human disease, and drug therapy*
	R. Alan North		Chair: Charles Chavkin
09:35 – 12:35	**Symposium 1**		*Dr. Sydney Archer Memorial Symposium on Medicinal Chemistry of Opioid Alkaloids and Peptides*
	Chair: Jean Bidlack		
09:35 - 9:50	Jean Bidlack	(S1-1)	*Tribute to Dr. Sydney Archer*
09:50 – 10:15	Peter Schiller	(S1-2)	*New approaches in opioid peptide based drug development*
10:15- 10:40	Philip Portoghese	(S1-3)	*New "address" moieties in the design of highly potent kappa-selective antagonists*
10:40 – 11:10	Coffee Break		
11:10 – 11:35	Victor Hruby	(S1-4)	*Exploring the differential structural requirements for opioid agonists and antagonists in conformational and topographical space*

11:35 – 12:00	Girolamo Calò	(S1-5)	*Characterization of [Nphe^1]NC(1-13)NH$_2$, a novel selective nociceptin receptor antagonist*
12:00 – 12:15	Christopher Cioffi	(O1-1)	*8-Phenylamino analogues of cyclazocine*
12:15 – 12:30	Balvinder Vig	(O1-2)	*Synthesis and pharmacological activity of cyclo[D-Asp^2, Dap^5]Dyn A (1-11)NH$_2$ analogues with substitutions at positions 1, 3 and 4*
12:30 – 14:00	Lunch		
12:30 – 14:00	INRC Executive Committee Meeting		
13:30 – 15:30	Poster Session 1		
15:30 – 16:00	Coffee break		
16:00 – 18:30	**Symposium 2**		*Consequences of repeated drug use on reward pathways*
	Chair: Toni Shippenberg		
16:00 - 16:30	Toni Shippenberg	(S2-1)	*Neurochemical consequences of stimulant abuse: Modulation by dynorphin*
16:30 – 17:00	Virginia Pickel	(S2-2)	*Targeting of opioid receptors within the dopaminergic mesostriatal circuitry*
17:00 – 17:30	William Carlezon	(S2-3)	*Cocaine reward influenced by CREB-mediated dynorphin expression*
17:30 – 18:00	John Williams	(S2-4)	*C-AMP dependent regulation of synaptic transmission during morphine withdrawal*
18:00 – 18:15	David Grandy	(O2-1)	*Modulation of the mesolimbic dopamine reward pathway by nociceptin-orphanin FQ*
18:15 – 18:30	Zhen-Xiong Xi	(O2-2)	*Effects of NMDA & non-NMDA antagonists on heroin self-administration and mesolimbic dopamine release*

Monday, July 12, 1999

07:00 – 08:30	Breakfast		

08:30 – 09:30	**Plenary Lecture**		*Drug abuse and addiction research into the 21ST century: Where are we going from here?*
	Alan Leshner		Chair: Mary Jeanne Kreek
09:30 – 12:30	**Symposium 3**		*Bench to bedside: Update on clinical and related laboratory research*
	Chairs: Mary Jeanne Kreek and Fred Nyberg		
09:30 – 10:00	Mary Jeanne Kreek	(S3-1)	*Opioids, stress responsivity and the addictions*
10:00 – 10:30	Charles O'Brien	(S3-2)	*Opioids and the management of alcoholism*
10:30 – 11:00	Coffee Break		
11:00 – 11:30	Charles Inturrisi	(S3-3)	*Interactions of glutamatergic and opioid receptors provide a basis for drug development*
11:30 – 12:00	Fred Nyberg	(S3-4)	*Anabolic androgenic steroid abuse: a gateway to substance dependence*
12:00 – 12:15	Conan Kornetsky	(O3-1)	*Opiate-induced oral stereotypy in the rat, a model for hyperkinetic movement disorders*
12:15 – 12:30	Elisa Giannini	(O3-2)	*Agonist and antagonist actions of a new buprenorphine analogue on mu, delta and kappa opioid receptors*
12:30 – 14:00	Lunch		
13:30 – 15:30	Posters Session 2		
15:00 – 15:30	Coffee Break		
15:30 – 16:30	Rita Liu and Khursheed Asghar		*Peer Review Process and Grant Support Mechanisms at NIDA*
16:30 – 18:30	**Symposium 4**		*New concepts in opioid peptide and receptors structure and processing*
	Chairs: Peter Schiller and Anna Borsodi		
16:30 – 16:50	Lakshmi Devi	(S4-1)	*Heterodimerization of G-protein coupled receptors modulates their function*
16:50 – 17:10	Gavril Pasternak	(S4-2)	*The molecular biology of morphine pharmacology: Bring in the clones…*

17:10 – 17:30	Rüdiger Schulz	(S4-3)	*Mu-Opioid receptor endocytosis under the control of phosducin and beta-arrestin*
17:30 – 17:50	Philippe Jauzac	(S4-4)	*Internalization and recycling of the delta opioid receptor are dependent on a phosphorylation-dephosphorylation mechanism*
17:50 – 18:10	Ken Kramer	(S4-5)	*Phosphorylation of tyrosine(s) in the delta opioid receptor (DOR) contributes to its rapid internalization and desensitization*
18:10 – 18:20	Thomas Koch	(O4-1)	*The C-terminus plays important role in the internalization and membranal targeting of the rat mu opioid receptor*
18:20 – 18:30	Stefan Schulz	(O4-2)	*Involvement of mitogen-activated protein kinase in agonist-induced phosphorylation of the mu-opioid receptor in HEK 293 cells*
18:30 – 19:00	Brian Cox		*Opioid receptor nomenclature and the IUPHAR receptor nomenclature committee*

Tuesday, July 13, 1999

07:00 – 08:30	Breakfast		
08:30 – 09:30	**Plenary lecture 3**		*Estrogenic effects on the preproenkephalin gene: 1 paradox and 3 solutions*
	Donald Pfaff		Chair: Charles Chavkin
09:30 – 12:25	**Symposium 5**		*Long lasting effects: Regulation of gene expression by opioids*
	Chairs: Wolfgang Sadée and Carmine Coscia		
09:30 – 09:40	Carmine Coscia	(S5-1)	*Introduction*
9:40 – 10:10	Laura Bohn	(S5-2)	*Mechanism of kappa and mu opioid modulation of C6 glioma cell proliferation*
10:10 – 10:40	Roby Polakiewicz	(S5-3)	*Protein kinase cascades in m-opioid receptor signaling and desensitization*
10:40 – 11:10	Coffee Break		
11:10 – 11:40	Danxin Wang	(S5-4)	*Nuclear signaling of the mu opioid receptor, OP_3, via calmodulin*

11:40 – 11:55	Ryszard Przewlocki	(O5-1)	*Opioid induced CREB phosphorylation in Neuro2A MOR1A cells*
11:55 – 12:10	Peter Mayer	(O5-2)	*Repeated morphine application alters the responsiveness of cerebral gene expression to various drugs of abuse*
12:10 – 12:25	Gang Pei	(O5-3)	*Differential modulation of CaMK II alpha and beta isoforms by morphine in rat hippocampus*
12:30 – 14:00	Lunch		
14:00 -	Excursions/ free afternoon		

Wednesday, July 14, 1999

07:00 – 08:30	Breakfast		
08:30 – 09:30	**Plenary lecture 4**		*Cellular trafficking of GPCR following desensitization: determinants and functional consequences*
	Marc Caron		Chair: Eric Simon
09:30 – 12:30	**Symposium 6**		*Does opioid receptor internalization have a function?*
	Chair: Chris Evans		
09:30 – 09:35	Chris Evans	(S6-1)	*Ligand-regulated receptor trafficking*
09:35 – 10:05	Jeffrey Benovic	(S6-2)	*Role of GRKs and arrestins in receptor trafficking*
10:05 – 10:35	Mark von Zastrow	(S6-3)	*Mechanisms of GPCR endocytosis*
10:35 – 11:00	Coffee Break		
11:00 – 11:30	Kenneth Mackie	(S6-4)	*Desensitization of cannabinoid signaling & cannabinoid receptor internalization*
11:30 – 12:00	Yehia Daaka	(S6-5)	*Gbg Subunit-mediated MAP kinase activation*
12:00 – 12:30	Allan Basbaum	(S6-6)	*Functional correlates of G-protein-linked receptor internalization*
12:30 – 14:00	Lunch		

14:00 – 16:05	**Symposium 7**		*Anatomy and physiology of opioid circuits in brain*
	Chair: MacDonald Christie		
14:00 – 14:05	MacDonald Christie		*Introduction*
14:05 – 14:35	Martin Wessendorf	(S7-1)	*Brainstem circuits in opiate analgesia*
14:35 – 15:05	Howard Fields	(S7-2)	*Brainstem opioid circuitry contributing to pain modulation*
15:05 – 15:35	Li-Yen Mae Huang	(S7-3)	*Mechanism underlying increased neuronal activity in the rat periaqueductal gray by a m-opioid*
15:35 – 16:05	Frank Porreca	(S7-4)	*Repeated spinal opioid administration produces abnormal pain and antinociceptive tolerance which is reversed by dynorphin antiserum*
16:05 – 16:30	Coffee Break		
16:30 – 17:30	Business Meeting		
17:30 – 18:30	Founders' Lecture		
	Eric Simon		Chair: Charles Chavkin
19:30 – 22:30	Banquet		

Thursday, July 15, 1999

07:00 – 09:00	Breakfast		
09:00 – 12:00	**Symposium 8**		*Genetic approaches to opioid controlled behavior*
	Chair: Brigitte Kieffer		
09:00 – 09:05	Brigitte Kieffer	(S8-1)	*Introduction*
09:05 – 9:35	John Pintar	(S8-2)	*Genetic and behavioral analysis of opioid system KO mice*
9:35 – 10:05	Jeffrey Mogil	(S8-3)	*Analgesic redundancy in opioid null mutants: the good news and the bad news*
10:05 – 10:35	Lisa Gold	(S8-4)	*Genetic influences on self-administration of drugs of abuse*
10:35 – 11:00	Coffee Break		

11:00 – 11:30	Laure Jamot	(S8-5)	*Opioid receptors, spatial learning and synaptic plasticity in the hippocampus*
11:30 – 12:00	Rene Hen	(S8-6)	*Animals models of vulnerability to drugs of abuse*
12:00 – 12:15	Olga Valverde	(O8-1)	*Reduction of opiate dependence but not opiate analgesia in mice lacking CB1 receptors*
12:15 – 12:30	Allan Basbaum	(O8-2)	*Neurochemical correlates of acute and persistent pain*
12:30	End of the Meeting		

Poster Session 1 (Sunday, July 11, 13:30 – 15:30)

Opioid Receptors (Structure, Function, and Distribution)

Sun01: *Determination of cysteine residues within transmembrane domains of the rat mu opioid receptor exposed in the binding pocket*, P Huang, W Xu, C Chen, JK de Riel, L-Y Liu-Chen

Sun02: *Cys7.38(315) of the human kappa opioid receptor is exposed in the binding pocket*, W Xu, C Chen, J-G Li, P Huang, JK de Riel, L-Y Liu-Chen

Sun03: *Identification of residues in the putative sixth transmembrane domain of the human delta opioid receptor exposed in the binding pocket*, L-Y Liu-Chen, C Chen, P Huang, W Xu, JK de Riel

Sun04: *Naltrindole, a delta selective opioid antagonist, displays enhanced binding affinity at single point mutants of mu and kappa opioid receptors*, TG Metzger, T Vo, MG Paterlini, DM Ferguson, PS Portoghese

Sun05: *Binding of the steroid SC17599 to the mu opioid receptor: implications for a mu pharmacophore*, IJ McFadyen, JR Traynor

Sun06: *The unireceptor hypothesis of opioid analgesia: Binding studies in amphibians*, CW Stevens, LC Newman, DR Wallace

Sun07: *Distribution of a splice variant of the mu-opioid receptor-1, MOR-1D, in the murine CNS*, C Abbadie, YX Pan, GW Pasternak

Sun08: *Cloning and expression of a novel splice variant (MOR-1F) of the mouse mu-opioid receptor (MOR-1) gene*, Y-X Pan, J Xu, A Chang, E Bolan, L Mahurter, G Rossi, GW Pasternak

Sun09: *Mutations in the delta receptor dramatically altered the agonist/antagonist property but not the binding affinity of opioid alkaloids*, F Meng, Q Wei, MT Hoversten, LP Taylor, H Akil

Sun10: *Coupling of the mu opioid receptor to the yeast pheromone signal transduction pathway*, F Meng, H Akil

Sun11: *Evidence against a random collision coupling model of G protein activation*, A Alt, IJ McFadyen, JH Woods, JR Traynor

Sun12: *Mapping the sites of d- and m-opioid receptor-G protein interface*, Z Georgoussi, G Megaritis, C Zioudrou, G Milligan, M Merkouris

Sun13: *Antagonists produce fifty percent inhibition of DAMGO-stimulated [^{35}S]GTPgS binding at different levels of receptor occupancy in rMOR-HN9.10 cells*, H Xu, QX Zheng, JS Partilla, JB Thomas, FI Carroll, KC Rice, J Lai, RB Rothman

Sun14: *Modulation of delta opioid receptor function by acute ethanol*, I Gomes, N Trapaidze, M Bansinath, H Turndorf, L Devi

Sun15: *Role of GDP in mu opioid receptor-mediated G-protein activation*, DE Selley, C-C Cao, CS Breivogel, SR Childers

Sun16: *Pharmacological profiles of kappa opioids: Correlation between [^{35}S]GTPgS binding and cyclic AMP production*, I Jadrovski, JM Bidlack

Sun17: *Antipropulsive action of dynorphin A (Dyn A) on colonic transit in mice*, SF Rittenhouse, MR Pietras, A Cowan

Sun18: *Detection of delta opioid receptor (DOR) protein in rat pituitary glands*, VJ Aloyo

Sun19: *Delta-Opioid receptors (DOR) in porcine ileum*, DR Brown, S Poonyachoti, D Townsend IV

Sun20: *Cloning and characterization of a mu opioid receptor from bovine brain*, I Onoprishvili, ML Andria, SS Vilim, JM Hiller, EJ Simon

Sun21: *Expression of mu opioid receptors in HL-60 promyelocytic leukemia cells*, H Cheng, H Rahimi, SL Chang

Sun22: *Co-Immunoprecipitation of soluble, active mu receptors from rat brain with the G proteins, Go, Gi1, and Gi3*, T Cote, B Cox, E Chalecka-Franaszek, H Weems, AT Crowder

Sun23: *Differential regulation of d-opioid receptors by peptide and alkaloid agonists*, S Allouche, A Hasbi, N Marie, Ph Jauzac

Sun24: *Coupling of rod transducin to the human d opioid receptor in Chinese Hamster Ovary (CHO) cells*, D Stropova, E Varga, T Kim, M Rubenzik, WR Roeske, HI Yamamura

Sun25: *Immunological characterization of DOR-1 in NG108-15 subcellular fractions*, CJ Coscia, EG Ignatova, MM Belcheva

Sun26: *Opioid receptors in zebrafish brain*, RE Rodriguez, A Barrallo, R González-Sarmiento, JR Traynor

Sun27: *Evidence for cross-talk between opioid and muscarinic receptors in SH-SY5Y cells*, KM Standifer, P Kelkar

Sun28: *(+)Pentazocine ((+)PTZ) and pregnenolone sulfate (PS) modulate intracellular Ca^{2+} concentration via sigma-1 receptors in NG108-15 cells*, T-P Su, T Maurice, T Hayashi

Sun29: *Differential coupling between cloned d-receptors and cloned or native Ca^{2+} channels*, TG Hales, P Safa, J Boulter

Sun30: *Genetic modification of phospholipase C beta3 expression alters cellular responses to DAMGO*, GM Samoriski, W Xie, JP McLaughlin, JM Bidlack, D Wu, RA Gross

Sun31: *The involvement of protein-tyrosine phosphorylation in the opioid receptor signaling*, M Merkouris, D Mangoura

Sun32: *Morphine translocates calmodulin to cell nucleus and regulates the phosphorylation of cyclic-AMP response element binding protein (CREB)*, D Wang, LM Tobert, W Sadée

Sun33: *Opioid-mediated activation of MAPK does not require receptor endocytosis*, N Trapaidze, I Gomes, S Cvejic, L Devi

Tolerance, Dependence and Addiction

Sun34: *Translocation of dynamin from cytosol to plasma membrane in brain of morphine-tolerant rats*, F Noble, M Szücs, E Kicsi, B Bozó, G Fabian, BP Roques

Sun35: *Regulatory changes induced by sustained mu-opioid agonist treatments in rat brains*, M Szücs, B Bozó, E Kicsi, J Farkas, G Tóth, M Mácsay, G Szabó

Sun36: *Sustained morphine exposure results in elevated dynorphin content in the lumbar/sacral spinal cord of ICR, but not 129 SvEv, mice: Possible role in opioid antinociceptive tolerance*, EJ Bilsky, ER Castro, M Ibrahim, TP Malan, F Porreca

Sun37: *Periaqueductal grey neurons withdrawn from opioids respond differently to opioids*, EE Bagley, MJ Christie

Sun38: *Chronic morphine modulates the synaptic inputs to interneurons, but not medium spiny neurons in the nucleus accumbens*, JM Brundege, B Chieng, JT Williams

Sun39: *Antagonism by pyrrolidine dithiocarbamate, an inhibitor of nuclear factor-kB, of morphine dependence. A hypothesis for the development of opioid dependence*, A Capasso

Sun40: *Expression of GDNF mRNA in spinal cord, stem and cortex during morphine withdrawal in rats*, Z Wenhua, L Huifen, X Xiaohu, Y Guodong

Sun41: *Chronic administration of morphine elevates substance P endopeptidase-like activity in rat*, Q Zhou, K Karlsson, Z Liu, P Johansson, M Thörnwall, A Kiuru, F Nyberg

Sun42: *Inhibition of CaMK II in rat hippocampus attenuates morphine tolerance and dependence*, L Ma, G Fan, L Wang, H Qiu, G Pei

Sun43: *Dextromethorphan effectively prevents adverse effects of chronic morphine treatment in mother rats to the next generation*, P-L Tao, C-H Su, G-C Yeh

Sun44: *Striatal modulation of opioid-induced feeding: Anatomical mapping studies*, M Zhang, AE Kelley

Sun45: *Brain areas involved in long lasting sensitization towards morphine*, M Erdtmann-Vourliotis, P Mayer, U Riechert, V Höllt

Sun46: *The effect of antisense to protein kinase A (PKA) on opioid analgesia, tolerance and m-opioid receptor regulation*, J Shen, BA Gomes, BC Yoburn

Sun47: *The efficacy of Jie-Du-Ling on opiate addiction in rat*, C-F Bian, X-Q Ge

Sun48: *Effects of rotundine on morphine tolerance and dependence*, Z-Q Chi, W-Q Jin

Sun49: *Inhibition of agmatine on opioid physical dependence*, J Li, X Li, G Pei, BY Qin

Sun50: *Effects of ACTH and dexamethasone on second order schedule of intravenous morphine self-administration behavior by rhesus monkeys*, Z Fuqiang, Z Wenhua, W Zhaolin, Y Guodong

Sun51: *The combined use of scopolamine, naltrexone and naloxone as a rapid, safe and effective detoxification treatment for heroin addicts*, Y Guodong, Z Wenhua, X Kun

Behavior and Circulation

Sun52: *18-MC alters the locomotor and dopamine responses to acute and chronic morphine*, KK Szumlinski, IM Maisonneuve, SD Glick

Sun53: *Where does naloxone act to block cocaine-induced conditioned place preference?* P Skoubis, NT Maidment

Sun54: *Mu Opiate receptor knockout mice have decreased responses to ethanol*, FS Hall, I Sora, X-F Li, N Karmacharya, N Goodman, GR Uhl

Sun55: *Voluntary running modulates ethanol preference*, M Werme, S Lindholm, J Franck, P Thorén, S Brené

Sun56: *Sucrose and exercise differentially alter the behavioral actions of opioid drugs*, RB Kanarek, S Mandillo, W Foulds Mathes

Sun57: *The influence of forced swimming stress on the analgesic effect of fine ceramic semiconductor*, H-Y Tsai, Y-F Chen, W-C Cheng, Y-H Chang, C-H Tsai

Sun58: *Opiate receptor blockade increases baroreflex gain in conscious dogs*, BA Barron, G Kline, D Yoshishige, R Phillips, PA Gwirtz

Sun59: *Myocardial protective effects of mu opioid agonists*, DL Wu, Y Soong, GM Zhao, J Fasolo, HH Szeto

Sun60: *Local met-enkephalin-arg-phe alters sinoatrial node responses during ischemia*, K Jackson, M Farias, A Stanfill, JL Caffrey

Sun61: *Role of delta receptor in hypertension*, SF Xu, X Yin

Nociceptin/Orphanin FQ and ORL 1

Sun62: *Modulation of long-term depression by orphanin FQ in hippocampal dentate gyrus*, W-Z Wei, CJ Evans, CW Xie

Sun63: *Analgesic and cognition-enhancing activity of retronociceptin methylester, a nociceptin antagonist*, M Yoshikawa, M Takahashi, J Yunden

Sun64: *OFQ/N and the endogenous opioids act synergistically in protecting from excessive sensation of fear and pain*, RK Reinscheid, K Knaudt, A Köster, A Montkowski, O Civelli

Sun65: *The effects of orphanin/FQ receptors ligands in the formaline assay in mice*, K Gustaw, W Zgodzinski, A Rubaj, M Sieklucka-Dziuba, Z Kleinrok

Sun66: *Peripheral orphanin FQ/nociceptin analgesia in the mouse*, Y Kolesnikov, GW Pasternak

Sun67: *Orphanin FQ produces hyperalgesia in the presence, but not absence, of naloxone in the rat hot plate test*, K Lutfy, NT Maidment

Sun68: *Antinociceptive effect of endomorphin-1 in diabetic mice is mediated by d-opioid receptors*, K Zushida, M Ohsawa, H Nagase, J Kamei

Sun69: *The influence of [Phe^1 y(CH$_2$-NH)Gly^2]nociceptin (1-13)-NH$_2$ on spontaneous alternation in mice*, A Rubaj, K Gustaw, W Zgodzinski, M Sieklucka-Dziuba, Z Kleinrok

Sun70: *Anticonvulsant effects of orphanin/FQ receptors ligands*, W Zgodzinski, A Rubaj, K Gustaw, M Sieklucka-Dziuba, Z Kleinrok

Sun71: *Intra-amygdala injection of orphanin FQ increases plasma corticosterone concentration*, DP Devine, HJ McWilliams, AM Padron, MJ Repetto

Sun72: *Orphanin FQ/Nociceptin modulation of dopamine release from midbrain primary cultures*, NP Murphy, AM Tan, HA Lam, NT Maidment

Sun73: *The anatomical relationship between orphanin FQ, the OFQ receptor and dopamine neurons*, CS Norton, S Kumar, H Akil, SJ Watson

Sun74: *Quantitative autoradiography of ORL1, mu, delta, and kappa opioid receptors in the brain of ORL1 receptor or nociceptin (OFQ) knockout mice*, S Clarke, Z Chen, MS Hsu, JE Pintar, RG Hill, I Kitchen

Sun75: *ORL-1 Receptors and ORL-1-activated G-proteins in rat cingulate cortex*, LJ Sim-Selley, L Vogt, BA Vogt, SR Childers

Sun76: *Binding and functional profile of orphanin FQ/nociceptin in BE(2)-C, human neuroblastoma cells*, JP Mathis, IE Goldberg, AH Chang, J Ryan-Moro, GF Altememi, KM Standifier, GW Pasternak

Sun77: *Mechanisms of molecular selectivity for opioid and orphanin FQ receptors*, CE Owens, F Meng, H Akil

Sun78: *Translational efficacy of various cloned human OFQ/N splice variants*, J Arjomand, CJ Evans

Sun79: *Cloning & characterization of the promoter region of the human prepronociceptin gene*, NT Zaveri, CJ Green, LR Toll

Opioid Modulation of the Immune System, Development and Apoptosis

Sun80: *Expression of Ag-NORs of T-lymphocytes from heroin addicts*, Z Yang, GX Liu, GM Gao, WN Ci

Sun81: *Electroacupuncture stimulation can induce the release of IL-1 beta and TNF-alpha in the blood of rats*, X-H Chen, EB Geller, TK Eisenstein, JJ Meissler, Jr., X Peng, MW Adler

Sun82: *Mitogen-induced activation of mouse T cells increases k opioid receptor expression in relation to phenotypic markers*, MK Abraham, JM Bidlack

Sun83: *Opioid receptor activity in the developing mouse*, JF Nitsche, JE Pintar

Sun84: *A role for dietary casein in opioid receptor development*, R Goody, I Kitchen

Sun85: *Effect of mu and delta$_2$ opioids on developing mouse granule neuron precursors in vitro*, KF Hauser, J Foldes, KM Martin, CS Turbek

Sun86: *Enkephalin gene expression during neocortical glial proliferation*, DK Meyer, M Knittel, S Quast, L Just

Sun87: *Delta opioid DADLE blocks neuronal death caused by serum deprivation in PC12 cells: An opioid receptor-dependent action*, T Hayashi, T-P Su

Sun88: *Non-opioid cytotoxic intracellular effects of dynorphins in tumor cell lines*, K Tan-No, I Gileva, T Yakovleva, K Reznikov, L Terenius, G Bakalkin

Poster Session 2 (Monday, July 12, 13:30 – 15:30)

Desensitization and Downregulation of Opioid Receptors

Mon01: *Identification of serine 261 and 266 as sites involved in agonist-induced phosphorylation of the mu-opioid receptor in HEK 293 cells*, M. Händel, H Schmidt, S Schulz, T Koch, M Klutzny, I Brüggemann, M Schreff, V Höllt

Mon02: *Study of mu opioid receptor phosphorylation by C-terminus deletion and point mutation of the receptor*, JB Wang, HB Deng, Y Pak, S George, GR Uhl

Mon03: *The C-terminus plays important role in the internalization and membranal targeting of the rat mu opioid receptor*, T Koch, S Schulz, M Klutzny, E Raulf, V Höllt

Mon04: *Involvement of mitogen-activated protein kinase in agonist-induced phosphorylation and internalization of the mu-opioid receptor in HEK 293 cells*, S Schulz, H Schmidt, M Klutzny, T Koch, M Händel, V Höllt

Mon05: *GRK2 and b-arrestin-1 are involved in human delta-opioid receptor desensitization and internalization*, A Hasbi, S Allouche, M Mirshahi, N Marie, J Polastron, Ph Jauzac

Mon06: *Phosphorylation of adenylyl cyclase VI upon chronic SNC 80 treatment in CHO cells expressing the human d opioid receptor*, E Varga, M Rubenzik, D Stropova, WR Roeske, HI Yamamura

Mon07: *Co-Expression of the human delta-opioid receptor and alpha-transducin blocks adenylyl cyclase superactivation in response to chronic agonist pretreatment*, MK Rubenzik, EV Varga, D Stropova, WI Roeske, HI Yamamura

Mon08: *Cellular models of mOR tolerance induced differentially by protein kinase A and morphine*, Z Wang, W Sadée

Mon09: *Induction of opioid dependence is not linked to a specific inhibitory G-protein*, H Ammer, R. Schulz

Mon10: *Irreversible opioid antagonists prevent opioid desensitization in SH-SY5Y neuroblastoma cells*, JP McLaughlin, JM Bidlack

Mon11: *Chronic heroin treatment produces desensitization of mu opioid-activated G-proteins in specific rat brain regions*, CE Maher, TJ Martin, SR Childers

Mon12: *Acute mu-opioid desensitization selectively affects pathways targeting calcium channels*, RA Gross, GM Samoriski

Mon13: *Effects of endocytosis inhibitors on mu opioid receptor function*, P Zaki, V Jordan, A Klaassen, C Evans

Mon14: *Regulation of opioid receptor function by dimerization*, B Jordan, S Cvejic, N Trapaidze, L Devi

Mon15: *Differential regulation of kappa opioid receptors by opiates and opioid peptides*, B Jordan, S Cvejic, L Devi

Mon16: *The proteasome and opioid receptor down-regulation*, RD Howells, K Chaturvedi, P Bandari, N Chinen

Mon17: *Mechanism mediating agonist-specificity of opioid receptor endocytosis*, JL Whistler, H-H Chuang, LY Jan, CJ Evans, M von Zastrow

Mon18: *Mechanisms of agonist-induced down-regulation of the human kappa opioid receptor*, J-G Li, JG Krupnick, JL Benovic, L-Y Liu-Chen

Mon19: *Mu-Opioid receptor desensitization and resensitization in vivo*, HH Szeto, Y Soong, J Fasolo, DL Wu

Regulation of Gene Expression, Genetics and Heredity

Mon20: *Alterations of mu opioid receptor gene in CXBK mice*, K Ikeda, T Ichikawa, T Kobayashi, T Kumanishi, H Niki, R Yano

Mon21: *Expression of multiple splice variants of the mouse mu-opioid receptor (MOR-1) gene during ontogeny*, J Xu, Y-X Pan, E Bolan, L Mahurter, SR Letchworth, GW Pasternak

Mon22: *Mu-Opioid & morphine-6-gluconuride actions in mice lacking exon 1 of MOR-1*, CW Vaughan, M Connor, EE Bagley, HQ Lin, A Schuller, J Pintar, MJ Christie

Mon23: *Retinoic acid (RA) induced expression of mu opioid receptor mRNA in a human neuroblastoma cell line, SH-SY5Y*, AE Persaud, BC Yoburn, JM Carroll

Mon24: *Gender dependent expression and binding of delta opioid receptor in mouse brain*, L Negri, L Narzi, R Lattanzi

Mon25: *Differential expression of delta-opioid receptors and mRNA during the cell cycle*, PAI Persson, T Thorlin, L Ronnback, E Hansson, PS Eriksson

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S1-1

A TRIBUTE TO SYDNEY ARCHER

J. M. Bidlack. University of Rochester, School of Medicine and Dentistry, Rochester, NY, USA

Sydney Archer, a founding member and former Secretary of INRC, extended the invitation to hold the 1999 INRC Meeting in Saratoga Springs, NY. Syd was born in New York City on January 23, 1917. With a Ph.D. in Organic Chemistry, he joined Sterling Winthrop Research Institute in 1943, where he rose from Group Leader to Associate Director and Vice President. Much of Syd's research was directed towards the development of new opioid analgesics, particularly, the synthesis of benzomorphans. Two of his best-known drugs are pentazocine and cyclazocine. In 1973, Syd left Sterling to become Research Professor of Chemistry at Rensselaer Polytechnic Institute. He established a vigorous academic research program focusing on the synthesis of long-acting opioids. In addition, he served as Dean of the School of Science at Rensselaer from 1980-1985, and was a member of numerous Study Sections and advisory committees. Syd died from a stroke in August 1996. One of Syd's greatest pleasures was to watch INRC grow from a satellite session of the IUPHAR Congress in Basel in 1969 to the large thriving organization that INRC is today.

S1-2

NEW APPROACHES IN OPIOID PEPTIDE BASED DRUG DEVELOPMENT

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Structural modification of the prototype δ antagonist TIPP (H-Tyr-Tic-Phe-Phe-OH) not only produced compounds with extraordinary δ antagonist potency and δ receptor selectivity, but also led to the discovery of a new class of potent δ agonists (e.g. H-Tyr-Tic-NH-CH_2-$CH(Ph)_2$) and of the first known compounds with a mixed μ agonist/δ antagonist profile (e.g. H-Dmt-TicΨ[CH_2NH]Phe-Phe-NH_2 [DIPP-NH_2[Ψ], Dmt = 2',6'-dimethyltyrosine] or H-Dmt-Tic-NH-$(CH_2)_3$-Ph [Ph = phenyl]). DIPP-NH_2[Ψ] showed subnanomolar binding affinity for both μ and δ receptors, was a potent analgesic and produced no physical dependence and less tolerance than morphine in rats. Cyclic β-casomorphin analogs of the type H-Dmt-c[-D-Orn-2-Nal- D-Pro-Gly-] (2-Nal = 2-naphthylalanine) represent another class of potent mixed μ agonist/δ antagonists. Recently developed dermorphin-related tetrapeptide analogs with distinct physico-chemical properties displayed very high μ agonist potency and μ receptor selectivity and were shown to have excellent potential as peripherally acting analgesics or as obstetrical analgesics.

S1-3

NEW ADDRESS MOIETIES FOR THE DESIGN OF HIGHLY POTENT KAPPA OPIOID ANTAGONISTS.

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Norbinaltorphimine (norBNI) is a prototypical kappa- selective opioid antagonist that is widely employed in opioid research. Structure-activity relationship (SAR) studies have suggested that the second naltrexone-derived pharmacophore in this bivalent ligand acts as a scaffold to direct its 17' basic nitrogen (an "address") to a unique acidic residue on the kappa receptor. These studies have provided the basis for the design of kappa antagonists that are structurally related to the delta antagonist, naltrindole (NTI), in which the indole moiety functions as a scaffold to hold a kappa "address". The "address", which includes groups such as guanidine, amidine, secondary and tertiary amines, and trimethylammonium, are all cationic at physiologic pH and are believed to be involved in ion- pairing with the carboxylate of Glu297 at the top of TM6 in the kappa receptor. The SAR of the series together with molecular modeling studies based on site-directed mutagenesis of opioid receptors will be discussed in the context of different "address" recognition loci on the kappa receptor for agonist and antagonist ligands.

S1-4

EXPLORING THE STRUCTURAL REQUIREMENTS FOR OPIOID AGONISTS AND ANTAGONISTS IN CONFORMATIONAL AND TOPOGRAPHICAL SPACE

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The natural endogenous opioid peptides such as the enkephalins, dynorphins, dermorphins, etc. tend to be conformationally flexible, readily degraded linear peptides with only modest selectivities for opioid receptors. Beginning in the early 1980s, our group and others began to explore the possibility of obtaining more potent, receptor selective, and stable peptide and peptidomimetic ligands for the mu, delta and kappa opioid receptors. Agonists, antagonists, and more recently inverse agonists were sought. Our early work concentrated on conformational constraint in phi/psi space by cyclization and transannular stabilization. More recently, we have sought further constraints in chi space (chi^1 and chi^2) to obtain a more complete topographical picture of the opioid receptor pharmacophores. This in turn has led us to *de novo* design of non-peptide ligands. The design of highly potent, opioid receptor selective and biostable opioid ligands such as DPDPE, [(2S,3R)-TMT^1]DPDPE, CTOP, TCTOP, SL-3011 and TMT-Tic will be discussed.

Supported by grants DA 06284, DA04248, and DA08657

S1-5

CHARACTERIZATION OF [Nphe^1]NC(1-13)NH_2, A NOVEL SELECTIVE NOCICEPTIN RECEPTOR ANTAGONIST

G. Calò, R. Guerrini*, R. Bigoni, A. Rizzi, G. Marzola, H. Okawa, C. Bianchi, D.G. Lambert**, S. Salvadori*, and D. Regoli. Dept. Pharmacology and *Dept. Pharmaceutical Sciences, University of Ferrara, Italy. **Dept. Anaesthesia, Leicester, U.K.**

Nociceptin (NC) is a novel neuropeptide capable of inducing a variety of biological actions via activation of a specific cell surface G-protein coupled receptor. However, the lack of a selective NC receptor antagonist has hampered our understanding of NC actions and the role of this peptide in pathophysiological states. As part of a broader programme of research geared to the identification and characterisation of NC receptor ligands we report that the novel peptide [Nphe^1]NC(1-13)NH_2 acts as the first truly selective and competitive NC receptor antagonist and is devoid of any residual agonist activity. [Nphe^1]NC(1-13)NH_2 binds selectively to recombinant NC receptors expressed in Chinese hamster ovary (CHO) cells (pKi 8.4) and competitively antagonizes the inhibitory effects of NC i) on cAMP accumulation in CHO cells (pA_2 6.0) and ii) on electrically evoked contractions in tissues of the mouse (vas deferens), rat (vas deferens) and guinea pig (ileum) with pA_2 values ranging from 6.0 to 6.4. In addition, [Nphe^1]NC(1-13)NH_2 is also active in vivo where it prevents the pronociceptive and antimorphine actions of intracerebroventricularly applied NC measured in the mouse tail withdrawal assay. Moreover, [Nphe^1]NC(1-13)NH_2 produces per se a dose dependent, naloxone resistant antinociceptive action and, at relatively low doses, potentiates morphine-induced analgesia. Collectively our data indicate [Nphe^1]NC(1-13)NH_2, acting as a NC receptor antagonist, may be the prototype of a new class of analgesics.

S2-1

NEUROCHEMICAL CONSEQUENCES OF STIMULANT ABUSE: MODULATION BY DYNORPHIN

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The repeated use of psychostimulants is associated with alterations in basal and drug-evoked dopamine dynamics within the mesolimbic system. Basal dopamine uptake and release are increased in terminal projection areas and there is a progressive increase in drug-evoked dopamine levels as the abstinence proceeds. Increases in the activity of endogenous k-opioid receptor systems as evidenced by elevations in prodynorphin gene expression and dynorphin levels have also been observed. This talk will provide evidence that changes in the activity of dynorphin systems is a compensatory response that opposes the neurochemical and behavioral sequelae that occur following the repeated administration of psychostimulants. Microdialysis studies in mice and rats will show that k-opioid receptor agonists prevent increases in basal and drug-evoked dopamine dynamics that occur during cocaine abstinence. They also attenuate reductions in basal and stimulus-evoked dopamine overflow that occur in striatal neurons after neurotoxic doses of methamphetamine. Behavioral studies will show that k-opioid agonists attenuate the reinstatement of cocaine-seeking behavior and prevent cocaine-induced sensitization. The role of the dopamine transporter in mediating the modulatory effects of k- opioid receptor agonists will be discussed.

S2-2

TARGETING OF OPIOID RECEPTORS WITHIN THE DOPAMINERGIC MESOSTRIATAL CIRCUITRY.

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The rewarding and adverse effects of opiates, as well as sensitization to psychostimulants, are largely determined by the strategic positioning of mu, delta, and kappa opioid receptors (MOR, DOR, and KOR) within the dopaminergic mesostriatal circuitry. By using electron microscopic immunocytochemistry, we have show that within the ventral tegmental area, MOR are present within many non-dopaminergic, and a few dopaminergic neurons. Within the striatum, MOR-immunoreactivity was often seen in dendritic spines that apposed dopaminergic terminals and received excitatory input from axon terminals, some of which were of cortical origin. Striatal DOR were mainly localized to morphologically heterogeneous axon terminals that apposed, but did not contain the dopaminergic markers, suggesting a major presynaptic role in release of non-dopaminergic transmitters. DOR was less frequently seen in striatal dendrites, and some of these also contained MOR. Together, the results suggest that opiates active at MOR and DOR have distinct and complementary roles in modulation of the presynaptic release and postsynaptic responses to dopamine in spiny striatal neurons. Furthermore, KOR was seen in many dopaminergic terminals, suggesting that this receptor is also targeted to key sites for opposition to the effects of MOR and, DOR agonists. (Supported by NIH grants DA04600 and HL18974).

S2-3

COCAINE REWARD INFLUENCED BY CREB-MEDIATED DYNORPHIN EXPRESSION.

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Exposure to psychomotor stimulants (cocaine, morphine) alters the levels and function of the transcription factor CREB (cAMP response element binding protein) in the nucleus accumbens. Altered CREB activity in this region could cause neuroadaptations that change sensitivity to cocaine's rewarding effects. To determine the functional role of CREB in the nucleus accumbens shell (NASh) of rats, we directly and selectively induced its overexpression in this region with a herpes simplex virus vector (HSV- CREB). With a second vector (HSV-mCREB), we overexpressed in other rats a dominant negative CREB that acts as an antagonist of endogenous CREB. Bilateral microinjections of HSV-CREB into the NASh makes low doses of cocaine aversive, and decreases the rewarding effects of higher doses of the drug. Conversely, similar treatment with HSV-mCREB increases cocaine's rewarding effects. These effects are likely caused by altered dynorphin expression: dynorphin mRNA in the NASh is increased by HSV-CREB and decreased by HSV- mCREB. Furthermore, blockade of kappa opioid receptors (the brain receptors for dynorphin) with the irreversible antagonist norBNI eliminates the negative effect of HSV-CREB on cocaine reward. These results identify a molecular cascade through which altered expression of the kappa opioid dynorphin can influence cocaine reward.

S2-4

C-AMP DEPENDENT REGULATION OF SYNAPTIC TRANSMISSION DURING MORPHINE WITHDRAWAL

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Upregulation of the cAMP dependent cascade following chronic treatment with opioids was first reported by Klee and Nirenberg in experiments using NG108-15 cells and has become one of the cellular hallmarks of opioid withdrawal. It is only recently that the consequences of that upregulation have been identified using electrophysiological methods. GABA-mediated inhibitory synaptic transmission was studied at three synapses in the mesolimbic system during acute withdrawal from morphine. At each synapse, there was evidence of an upregulation of the cAMP cascade that (1) increased GABA release and (2) increased the inhibition produced by opioid agonists. An additional result of the increased cAMP production was (3) a rise in adenosine tone. This adenosine resulted in the activation of presynaptic A1- adenosine receptors to inhibit GABA synaptic transmission during withdrawal. These results along with similar observations made in the periaqueductal grey and dorsal raphe suggest that the regulation of transmitter release is one important consequence of the upregulation of cAMP processes induced by chronic morphine treatment.

S3-1

OPIOIDS, STRESS RESPONSIVITY AND THE ADDICTIONS

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In the late 1960's, we hypothesized that an altered stress responsivity may contribute to the acquisition, persistence, and relapse to opiate addiction, and in the 70's and 80's provided evidence from clinical research to support that hypothesis. In the mid '80's we extended that hypothesis to suggest that cocaine similarly may alter stress responsivity in humans and also in animal models. Our recent studies have shown that in humans, the mu, but also kappa, opioid receptor system are involved in the normal tonic regulation of the stress responsive hypothalamic-pituitary-adrenal axis. We have developed an animal model of a "binge" pattern of cocaine administration, mimicking the most common human pattern of abuse, and have shown that mu opioid receptor gene expression, and receptor density, kappa opioid receptor gene expression, and receptor density are all altered, each in specific brain regions, and after specific patterns of acute, sub-acute, or chronic cocaine administration, along with significant alterations of gene expression and hormones of the stress responsive systems. These findings suggest that the opioid system, along with the various neurotransmitters, may participate in the modulation of stress responsivity, and thus contribute to the acquisition of addiction and relapse.

S3-2

OPIOIDS AND THE MANAGEMENT OF ALCOHOLISM

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Studies on the endogenous opioid system have had the unexpected benefit of resulting in an improved treatment for alcoholism. Blocking opiate receptors in animals was found to reduce preference for alcohol. Low doses of morphine were found to stimulate alcohol drinking while higher doses abolished alcohol preference. Human studies were undertaken and the animal models were validated. Alcoholics on naltrexone "slipped" and took at least one drink at about the same rate as placebo controls, but relapse to out of control drinking was significantly less. When they did drink, alcoholics reported less than the usual amount of pleasure from alcohol. They also reported less craving for alcohol. Subsequently, the majority of published and not yet published studies have replicated the advantage for naltrexone, most consistently on relapse rate. It was approved by the FDA as an adjunct medication in the treatment of alcoholism. The mechanism for this therapeutic effect is not clear. Another μ receptor antagonist nalmephene has also been reported to reduce relapse in alcoholics, but not all μ antagonists are effective in animal models.

S3-3

INTERACTIONS OF GLUTAMATERGIC AND OPIOID RECEPTORS PROVIDE A BASIS FOR DRUG DEVELOPMENT

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Tissue injury results in sensitization at both peripheral nociceptors and at central neurons. In the dorsal horn of the spinal cord, central sensitization is the result of an activity dependent neuronal plasticity that is mediated in part by glutamatergic (Glu) receptor systems. In animals and humans, Glu receptor antagonists have analgesic activity. For some these analgesic responses are obtained at doses that produce adverse reactions. The Glu receptor system has also been implicated in the development and maintenance of morphine tolerance and dependence. These results suggest that in addition to their use as a primary analgesic, a Glu receptor antagonist with an acceptable safety margin could be of particular clinical utility when used in combination with an opioid to increase the therapeutic range of the opioid and by providing analgesia for pain states that are less sensitive to opioids. In developing a strategy to identify suitable candidates I will discuss the concept that low affinity open channel blockers (LAOCB) such as memantine, dextromethorphan and d-methadone may have a more favorable therapeutic ratio than some of the higher affinity OCB'S. Also I will outline approaches for the production of conditional knockouts of Glu receptors that will provide a definitive test of the role of Glu receptors in nociception and tolerance. Supported by NIDA Grants DA01457, DA00198, DA07274 and DA05130.

S3-4

ANABOLIC ANDROGENIC STEROID ABUSE - A GATEWAY TO SUBSTANCE DEPENDENCE.

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In recent years the question whether use of anabolic-androgenic steroids (AAS) can result in drug dependence has received much attention. A number of reports describe euphoric and psychological effects of AAS. For instance, a study on physical and psychological dependence of AAS in weight lifters underscored the importance of diagnosing steroid dependence in clinical practice. It is further reported several individuals addicted to e.g. alcohol or amphetamine have previous abuse of AAS. In a recent survey of AAS abuse among swedish high school students it was found that the use of these compounds appears to have much in common with use of alcohol, tobacco and psychotropic drugs. In our animal studies we have recently found that daily administration of high-dose AAS during two weeks affects the brain levels of opioid peptides. Also the expression of the transcripts of the NMDA receptor subunits were affected as well as the expression of the peptides related to the HPA-axis. Studies also revealed that chronical treatment with AAS appears to sensitise the animals to other drugs, such as ethanol and amphetamine. Thus, in rats two weeks of chronic AAS followed by three drugfree weeks rendered the aminals in a condition, where they increased their behavioural response to amphetamine and alcohol. The steroid treatment resulted in an enhanced intake of alcohol and in increased aggression. All these data will be discussed in a relation to the hypothesis that AAS may be a gateway to the addiction of multiple drugs of abuse.

S4-1

HETERODIMERIZATION OF G-PROTEIN COUPLED RECEPTORS MODULATES THEIR FUNCTION

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G protein coupled receptors (GPCRs) are involved in a vast array of biological activities including drug response, hormone physiology, neurotransmission and transduction of external stimuli. Structural and biochemical studies with a variety of GPCRs indicate that a number of GPCRs exist as homodimers. Relatively few studies have examined heterodimerization between these receptors. The ability of non-functional receptor subunits to heterodimerize and form functional receptors has recently been reported. However, the ability of two fully functional receptors to heterodimerize leading to the generation of a new fully functional receptor has not yet been documented. Here we provide biochemical and pharmacological evidence for fully functional opioid receptors to heterodimerize and generate a novel binding site with unique ligand binding properties. Furthermore, the heterodimer synergistically binds highly selective agonists leading to potentiation of signal transduction. Heterodimerization also appears to alter the trafficking properties of these receptors. Thus heterodimerization of GPCRs represents a novel mechanism that modulates receptor function.

S4-2

THE MOLECULAR BIOLOGY OF MORPHINE PHARMACOLOGY: BRING IN THE CLONES...

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Numerous studies have suggested multiple subtypes of mu opioid receptors. Pharmacological differences between morphine and morphine-6 -glucuronide (M6G) raised the possibility of yet another mu receptor subtype. Yet, only one gene encoding a mu receptor has been cloned, MOR-1. Antisense mapping has confirmed the importance of MOR-1 in the actions of both morphine and M6G while also revealing striking differences which raised the possibility of splice variants. Two MOR-1 splice variants were described soon after the initial identification of MOR-1. However, the molecular biology of the MOR-1 is far more complex. Cloning studies have now identified at least 6 murine MOR-1 variants comprised of 11 exons spanning over 250kb. All show traditional mu selectivity profiles in binding assays, although there are subtle differences among them. More important, their regional expression and developmental appearance differ. These findings raise questions regarding the role of these variants and their relationship to the pharmacologically defined subtypes.

S4-3

MU-OPIOID RECEPTOR ENDOCYTOSIS UNDER THE CONTROL OF PHOSDUCIN AND BETA-ARRESTIN

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Internalization of mu-opioid receptors is controled by multiple criteria, including drug-receptor affinity and cytosolic components such as phosducin and beta-arrestin. Phosducin impairs and arrestin enhances internalization. NG 108-15 cells transiently expressing the mu-opioid receptor fused to green fluorescence protein were employed to study endocytosis of the fluorescent receptor construct in living cells by means of confocal microscopy. Etorphine (1nM) induces internalization, a process strongly reduced in cells overexpressing phosducin. However, coexpression of phosducin and arrestin reverses the blockade. In contrast to etorphine, morphine fails to internalize mu-receptors. When arrestin is overexpressed, morphine causes endocytosis of the mu-receptor, and this mechanism is not affected by phosducin. The findings suggest that arrestin, which uncouples the receptor from its G protein, is of overriding importance for receptor internalization as compared to the function of phosducin, which inhibits phosphorylation of activated receptors.

S4-4

INTERNALIZATION AND RECYCLING OF THE DELTA OPIOID RECEPTOR ARE DEPENDENT ON A PHOSPHORYLATION-DEPHOSPHORYLATION MECHANISM

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Internalization and recycling of the human δ-opioid receptor (hDOR) were studied in the neuroblastoma cell line SK-N-BE, endogenously expressing this receptor. Conventional and confocal fluorescence microscopy observations, corroborated by Scatchard analysis, indicated that, following a 100 nM etorphine treatment hDOR were rapidly internalized. This internalization was reversible for a treatment not exceeding 1h and became irreversible for prolonged treatment (4 h), leading probably to the degradation and down-regulation of the receptor. The internalization of hDOR was totally blocked in the presence of heparin, an inhibitor of GRKs, indicating that phosphorylation by these kinases is a critical step in desensitization and internalization of hDOR in this cell line. Blockade of internalization by agents not interferring with phosphorylation, as hypertonic sucrose or concanavalin A, blocked also the resensitization process. Furthermore, blockade of dephosphorylation of the internalized hDOR by okadaic acid totally suppressed its recycling to the plasma membrane and its subsequent resensitization. These results indicate that regulatory events leading to desensitization, internalization and recycling in a functional state of hDOR involve phosphorylation by a GRK, internalization via clathrin-coated vesicles and receptor dephosphorylation by acid phosphatases.

S4-5

PHOSPHORYLATION OF TYROSINE(S) IN THE DELTA OPIOID RECEPTOR (DOR) CONTRIBUTES TO ITS RAPID INTERNALIZATION AND DESENSITIZATION.

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Repeated exposure to opioid agonists leads to a progressive loss of opioid receptor function. Two separate phenomena - receptor desensitization and downregulation - appear to contribute to this decrease. Desensitization is a rapid loss of agonist function and occurs concurrently with the internalization of the receptor into clathrin-coated endosomes. Longer periods of exposure produce a downregulation of receptor binding sites. Both processes appear dependent on the phosphorylation of key amino acids in the C-terminus of the opioid receptor protein. Recent studies have suggested that protein tyrosine kinases (TYK) are among several phosphorylating enzymes that can be activated by opioids. However, the importance of tyrosine phosphorylation in opioid receptor function and regulation is poorly understood. Our laboratory has focused on determining whether the DOR acts as a substrate for TYKs, and whether this type of phosphorylation is involved in opioid receptor desensitization and/or downregulation. We will demonstrate that the delta-specific opioid agonist DSLET increases tyrosine phosphorylation within the DOR, in a time and concentration dependent manner, when both are present in Chinese hamster ovary (CHO) cells. In addition, the presence of a TYK inhibitor attenuates the rapid and reversible internalization of the DOR, which occurs concurrently with receptor desensitization. In contrast, although the DOR remains in state of hyper-tyrosine phosphorylation for up to 120 minutes, genestein does not attenuate DOR downregulation produced by a 24-hour exposure to DSLET. Finally, we will present data from mutation studies (Y318F), which suggest the importance of tyrosine, residue(s), within the C-terminus of the receptor, for DOR function and agonist-mediated receptor desensitization and internalization.

S5-1

LONG LASTING EFFECTS: REGULATION OF GENE EXPRESSION BY OPIOIDS.

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Recent studies on opioid receptors have focused on nuclear signaling pathways leading to gene expression and long-lasting, physiological effects. Speakers in this symposium will describe their findings on opioid regulation of transcription. Bohn will report on the mechanism of kappa opioid activation of MAPK, leading to cell proliferation in an astrocytic model system. Chronic mu agonists attenuated kappa mitogenic signaling and receptor endocytosis may play a role in this pathway. Polakiewicz will discuss mu opioid stimulation of 4 different PI3K cascades that activate MAPK, Akt and GSK-3 involved in apoptosis and neuronal survival. Wang will describe how calmodulin (CaM) binds to mu and delta opioid receptors and upon morphine stimulation translocates to the nucleus to activate Ca2+/CaM-dependent protein kinases and phosphatases, thereby regulating CREB phosphorylation. Przewlocki also found that morphine acutely elicits CREB phosphorylation, while chronic exposure leads to upregulation of CREB protein. Mayer will discuss effects of acute and chronic morphine on brain c-fos mRNA and the influence of chronic morphine experience on c-fos message induced in brain regions by other drugs of abuse. Pei will review effects of acute and chronic morphine on the expression of CaMK II protein and mRNA in rat hippocampus. A striking aspect of this research is the interplay between opioids and other neurotransmitters along new signaling pathways to affect long lasting changes in cellular responsiveness to drugs of abuse.

S5-2

MECHANISM OF KAPPA AND MU OPIOID MODULATION OF C6 GLIOMA CELL PROLIFERATION.

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In examining signaling mediated by the endogenous kappa opioid receptor (OR) in C6 glioma cells, an astrocytic model system, we found that the kappa-selective agonist, U69,593 stimulated phospholipase C (PLC), extracellular signal-regulated kinase (ERK) phosphorylation, PYK2 phosphorylation and DNA synthesis. U69,593-stimulated ERK activation was shown to be upstream of DNA synthesis as inhibition of pertussis toxin-sensitive G proteins, L-type Ca^{2+} channels, PLC, intracellular Ca^{2+} release, PKC and ERK kinase blocked ERK activation and cell proliferation. By overexpressing dominant-negative or sequestering mutants, we provided evidence that ERK activation is Ras-dependent and is transduced by Gβγ subunits. C6 cells also contain mu opioid receptors. Chronic treatment of C6 cells with mu agonists attenuated kappa mitogenic signaling. To investigate the role of receptor endocytosis in signaling, we examined the effects of dynamin suppressor mutant (K44A) on opioid modulation of ERK activity. K44A overexpression did not affect kappa opioid activation of ERK but it blocked the inhibitory action on kappa signaling mediated by the mu opioid receptors. Our results are consistent with a growing body of evidence of the opposing actions of mu and kappa opioids and provide insight into the role of opioid receptor trafficking in signaling. Supported by NIDA grant DA05412.

S5-3

PROTEIN KINASE CASCADES IN μ-OPIOID RECEPTOR SIGNALING AND DESENSITIZATION

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Despite the clinical importance of the μ-opioid receptor the intracellular signaling mechanisms triggered by μ-opioid agonists have not been fully described. We will present evidence indicating that μ-opioid agonists induce the activation of four different phosphoinositide 3-kinase (PI3K)-dependent signaling cascades. In addition to the activation of the MAPK cascade, which may be involved in receptor desensitization, μ-opioids stimulate the phosphorylation and activation of Akt a serine/threonine protein kinase implicated in protecting neurons from apoptosis. Downstream to Akt, μ-opioid receptor stimulation also results in phosphorylation and inactivation of glycogen synthase kinase 3 (GSK-3) α and β isoforms. Among, its many different functions. GSK-3 has been recently shown to induce apoptosis in PC 12 cells, to phosphorylate brain microtubule-associated protein Tau, and to be involved in the Wingless signaling pathway. μ-opioid agonists also induce the activation of p7O S6 kinase and the inhibition of the repressors of translation initiation factor eIF-4E, 4E-BP1 and 4E-BP2, which are involved in translational control. Thus, the μ-opioid receptor activates PI3K-dependent signaling pathways that may regulate its desensitization and are typically associated with neuronal survival and translational control. We will discuss how these signaling cascades may mediate some of the diverse physiological effects of μ-opioid receptor activation. translational control. We will discuss how these signaling cascades may mediate some of the diverse physiological effects of μ-opioid receptor activation.

S5-4

NUCLEAR SIGNALING OF THE MU OPIOID RECEPTOR, OP_3, VIA CALMODULIN

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We have previously proposed that opioid receptors contain calmodulin (CaM) binding domains in the third intracellular loop, a region commonly thought to mediate G protein coupling. Indeed, CaM inversely affects G protein coupling of mu and delta receptors (OP_3 and OP_1, respectively). Moreover, CaM was released from cell plasma membranes upon morphine stimulation in HEK 293 cells transfected with OP_3 and OP_1, suggesting that CaM could serve as a second message. In this study, we found that the CaM released from plasma membranes translocated to the cell nucleus and activated both Ca^{2+}/CaM dependent protein kinase and phosphatase (calcineurin). This in turn regulated the phosphorylation of CREB after morphine stimulation. Our results suggest that calmodulin is involved in regulation of gene expression by morphine, and this might play a role in morphine tolerance and dependence.

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S6-1

LIGAND-REGULATED RECEPTOR TRAFFICKING

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Adaptations at the receptor level such as downregulation, phosphorylation and desensitization have been the target of much research in the opioid field in an attempt to explain, at least in part, tolerance to opiate drugs. Ligand regulated receptor trafficking is clearly one adaptation that could have pronounced influence on both the acute and chronic activity of opioids, either by alteration of the number of surface receptors, resensitization of receptors via endosome recycling or redistribution of receptor and signaling proteins within the membrane. As with many G-protein coupled receptors, agonists (but in the case of opioid receptors, not all agonists) induce a rapid internalization of opioid receptors. Furthermore, antagonists induce an upregulation of surface receptors. In this symposium we will address a number of aspects concerning trafficking of G- protein coupled receptors. Initially we will focus on the mechanisms of internalization and differences in ligand-regulated receptor trafficking among members of the G-protein coupled receptor family. We will then address possible functional roles of internalization in signaling - specifically with regard the MAP kinase pathway. Finally we will focus on the internalization process in vivo and in more complex systems that express multiple receptors.

S6-2

ROLE OF GRKs AND ARRESTINS IN RECEPTOR TRAFFICKING

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A general feature of many hormonal signalling systems is that they rapidly lose their responsiveness following receptor activation. While multiple mechanisms contribute to this loss of responsiveness or desensitization, receptor phosphorylation often plays a major role. For G protein-coupled receptors (GPCRs), this phosphorylation can be mediated by G protein-coupled receptor kinases (GRKs) that specifically phosphorylate the agonist-occupied form of the receptor. Receptor phosphorylation then promotes the binding of arrestin proteins, resulting in desensitization of the agonist-activated receptor. Recent studies have implicated receptor phosphorylation and arrestin binding as also playing a role in GPCR internalization. This appears to be largely mediated by the ability of arrestins to function as adaptor proteins, being able to bind to both phosphorylated GPCRs as well as clathrin, the major protein component of coated pits. In this presentation, I will discuss the role of arrestins in mediating GPCR trafficking with particular emphasis on the specificity of the various interactions and potential mechanisms mediating subcellular localization.

S6-3

MECHANISMS OF GPCR ENDOCYTOSIS

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Our laboratory is interested in membrane trafficking mechanisms that determine the subcellular localization of signal-transducing receptors, as these mechanisms mediate diverse functions in the regulation of signal transduction and differentially regulate closely homologous subtypes of receptor co-expressed in the same cells. We have examined mechanisms that mediate and regulate the rapid endocytosis of several classes of heptahelical G protein-coupled receptor. Studies of the beta-2 adrenergic receptor have identified a multistep mechanism that regulates the ligand-dependent redistribution of receptors between distinct microdomains of the plasma membrane, thus controlling the rate of receptor endocytosis via clathrin-coated pits. Interestingly, coated pits that endocytose activated GPCRs represent a biochemically and functionally distinct subpopulation within the plasma membrane that may have specialized functions in signal transduction. Studies of D1 and D2 dopamine receptors indicate that individual subtypes of GPCR are selectively endocytosed by distinguishable molecular mechanisms and membrane pathways, and they demonstrate that this machinery is capable of physically segregating individual receptors within distinct microdomains of the plasma membrane before endocytosis occurs. Studies of opioid receptors reveal a remarkable level of agonist-selectivity in the regulation of the endocytic machinery, which we propose may have important implications for understanding the physiological effects of addictive opiate drugs.

S6-4

DESENSITIZATION OF CANNABINOID SIGNALING & CANNABINOID RECEPTOR INTERNALIZATION

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The behavioral effects of cannabinoids are mediated by a G protein-coupled receptor (GPCR), the CB1 receptor. Rapid tolerance develops during repeated administration of cannabinoids. Are desensitization and CB1 internalization related? CB1 activation of G protein-coupled inwardly rectifying potassium channels desensitized in a beta-arrestin- and GPCR kinase-dependent fashion as a consequence of phosphorylation of S426 and S430 in the C-terminus of the CB1 receptor. In a fashion similar to certain GPCR's, CB1 receptors expressed in AtT20 cells rapidly internalized in a dynamin-dependent fashion when exposed to efficacious CB1 agonists. CB1 receptor internalization was not necessary to activate MAP kinase. Mutations in the CB1 receptor preventing desensitization (e.g., S426A and S430A) had little effect on internalization. Furthermore, certain mutations preventing internalization (e.g., truncation of CB1 at V460) had no effect on desensitization. These results suggest desensitization of CB1-mediated signaling and CB1 receptor internalization are distinct processes.

S6-5

Gβγ SUBUNIT-MEDIATED MAP KINASE ACTIVATION.
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Termination of GPCR signaling is initiated via GRK-mediated phosphorylation of agonist-occupied receptors and subsequent binding of β-arrestin proteins which inhibit further receptor-G protein interaction and initiate the process of sequestration by targeting the receptor to clathrin-coated pits for internalization. Study of the mechanisms controlling growth signals by GPCRs is altering our basic understanding of GPCR signal transduction. GPCRs, like RTKs, stimulate cell proliferation in part via MAP kinase cascades. In this signal transduction pathway, Gi-coupled receptors utilize many of the same intermediates as those activated by RTKs including Ras. Ras-dependent activation of the MAP kinase pathway by GPCRs is dependent upon regulation of tyrosine protein phosphorylation. In most cell types, activation of the Src family and RTKs, via unknown effectors of Giβγ subunits, results in the formation of scaffolds for the recruitment of adaptor, proteins and subsequent recruitment of Ras guanine nucleotide exchange factors. Recently, we found that Gβγ-mediated activation of mitogenic signaling is initiated by the β-arrestin-dependent recruitment of c-Src kinase to the "desensitized" GPCR, and is dependent upon clathrin-mediated endocytosis. These observations suggest that the events which terminate receptor-G protein coupling, and induce receptor desensitization and sequestration, serve as critical initiators of a "second wave" of signal transduction in which the desensitized receptor itself comprises critical structural components of the mitogenic signaling complex.

S6-6

FUNCTIONAL CORRELATES OF G-PROTEIN-LINKED RECEPTOR INTERNALIZATION
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Although internalization of G-protein linked receptors has been monitored *in vitro and in vivo*, the extent to which this can be used to measure activity that is generated by ligand binding at these receptors is unknown. Here we compared neurokinin-1 (NK-1) receptor-mediated increases in intracellular calcium with confocal microscopy measures of internalization in sister cultures of spinal cord neurons. We found identical dose-response relationships for the two measures of activity at the NK-1 receptor. In other studies we compared DAMGO-induced internalization of the mu opioid receptor after spinal intrathecal injection with the behavioral antinociception produced by the same drug in the hot plate test. In this case, the behavioral and anatomical endpoints were highly correlated. Furthermore, the dose response curve for DAMGO-induced internalization of the mu opioid receptor in cultured spinal cord neurons correlated highly with published curves of DAMGO-induced increases in potassium current (Grundt and Williams, 1994). We conclude that internalization of the receptor not only provides a measure of the populations of neurons that are influenced under different experimental conditions, but that the magnitude of internalization also provides an excellent measure of receptor-induced signaling.
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S7-1

BRAINSTEM CIRCUITS IN OPIATE ANALGESIA

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The analgesic effects of opioids appear to be due at least in part to activation of brainstem structures such as the PAG and RVM. We have examined the relationships between the cloned opioid receptors, and brainstem neurons identified by their circuitry and neurotransmitter content. We found that DOR1- and MOR1-ir processes apposed both 5-HT and non-5-HT RVM neurons projecting to the spinal dorsal horn. However, MOR1-ir, DOR1-ir, and KOR1-ir, and MOR1 and DOR1 mRNAs were also expressed by some spinally projecting RVM neurons; some of these neurons expressed 5-HT or GABA. Similarly, in PAG, both DOR1- and MOR1-ir processes apposed neurons projecting to the RVM, and MOR1-ir was expressed by GABAergic PAG neurons that were NOT retrogradely labeled from the RVM. However, in addition MOR1 and DOR1 mRNA's were expressed by PAG neurons projecting to the RVM. We conclude that opioids may activate some descending inhibitorycircuits, but that they also appear likely to inhibit some descending inhibitory circuits.
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S7-2

BRAINSTEM OPIOID CIRCUITRY CONTRIBUTING TO PAIN MODULATION

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At the PAG and RVM, microinjection of mu opioid receptor (MOR) agonists inhibits nociceptor driven withdrawal reflexes. This effect depends on inhibition of a GABAergic inhibitory interneuron. The inhibition by PAG MOR agonists of spinal withdrawal reflexes depends on endogenous opioids acting at both MOR and delta opioid receptors (DOR) in the RVM and at MOR in the spinal cord dorsal horn. In contrast, kappa opioid receptor (KOR) agonists acting in the RVM oppose the antinociceptive action of PAG MOR agonists. The opposing behavioral actions of MOR and KOR agonists in RVM depend on the fact that they inhibit classes of neurons having opposing pain modulatory actions. In female rats, KOR agonists in RVM have a partial *antinociceptive* effect. Orphanin FQ/nociceptin hyperpolarizes all RVM cells and, when microinjected into the RVM, reduces PAG MOR agonist antinociception. CCK_4 and CCK_8 microinjected into the RVM block the antinociceptive effect of systemic morphine and PAG DAMGO. Conversely, microinjection of the CCKA receptor antagonist L-365,260 enhances the analgesic effect of PAG DAMGO suggesting either tonic or opioid induced release of endogenous CCK in the RVM. (Supported by PHS grant DA 01949).

S7-3

MECHANISM UNDERLYING INCREASED NEURONAL ACTIVITY IN THE RAT PERIAQUEDUCTAL GRAY BY A μ-OPIOID.

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Mu-opioids increase K+ currents and decrease both excitatory and inhibitory postsynaptic currents (EPSCs and IPSCs) in periaqueductal gray (PAG) neurons. To determine the overall effect of μ-opioids, we examined the actions of the μ-opioid agonist, DAMGO, on synaptic transmission in the ventrolateral PAG. Experiments were performed on rat brain slices; synaptic responses were recorded with patch electrodes. Under current clamp conditions, DAMGO (l μM) increased cell firing in many PAG neurons, although the opioid induced hyperpolarization and inhibited excitatory postsynaptic potentials in these cells. To explore the mechanism underlying the excitatory action of DAMGO, the GABA antagonist, bicuculline, was added to the external solution. Bicuculline caused cell firing, mimicking the excitatory effect of DAMGO. Unlike DAMGO, however, bicuculline depolarized PAG cells. Under voltage clamp conditions, with the same driving force, the evoked IPSCs are 2.3 times larger than the evoked EPSCs. DAMGO inhibited IPSCs by 60.7% while it inhibited EPSCs by 35.3%. The overall effect of an opioid therefore depends on the dynamic balance of its excitatory and inhibitory actions. The large blockade of the inhibitory drive of GABAergic inputs by DAMGO overcomes the DAMGO-induced hyperpolarization and inhibition of EPSCs, resulting in the excitation of PAG neurons.

S7-4

REPEATED SPINAL OPIOID ADMINISTRATION PRODUCES ABNORMAL PAIN AND ANTINOCICEPTIVE TOLERANCE WHICH IS REVERSED BY DYNORPHIN ANTISEURM.

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Sustained spinal opioid administration produces analgesic tolerance as well as unexpected abnormal pain including hyperalgesia and tactile allodynia. Animals with peripheral nerve injury show elevated levels of spinal dynorphin as well as a loss of antinociceptive actions of spinal opioids (i.e., "tolerance") which is reversed by pretreatment with MK-801 or antiserum to dynorphin. Here, we treated rats with either saline, or continuously with [D-Ala^2,NMePhe^4,Gly-ol^5]enkephalin (DAMGO), a μ opioid agonist given intrathecally via an osmotic minipump for 7 d. Possible tactile allodynia (von Frey filaments) and thermal hyperalgesia (radiant heat paw flick) were assessed on day 6 during the continuous delivery of spinal DAMGO to eliminate the possibility of withdrawal. Antinociceptive (52^{o}C hot-water tail-flick test) tolerance was measured on day 7, immediately after stopping DAMGO infusion. Spinal saline produced no changes in any test. DAMGO elicited tactile allodynia and thermal hyperalgesia during the infusion as well as antinociceptive tolerance. Lumbar dynorphin content was increased in DAMGO treated rats. Antiserum to dynorphin produced no effect in saline treated rats but reversed thermal hyperalgesia (to above baseline levels) and tactile allodynia (to baseline levels). Antiserum to dynorphin also reversed antinociceptive tolerance. The data suggest that a spinal μ opioid regulates dynorphin levels and that elevated spinal dynorphin is functionally associated with induction of abnormal pain and promoting opioid tolerance.

S8-1

GENETIC APPROACHES TO OPIOID CONTROLLED BEHAVIOR

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The opioid system controls a number of behaviors that are essential in responding to noxious stimuli and facing life-threatening situations. It regulates emotions and reward pathways, as well as cognitive functions such as learning and memory. The opioid system is also critical in the development and maintenance of drug addiction. New tools are now available to study opioid-regulated behavioral responses. Mice have been generated which lack components of the opioid system, or components of other functionally related neurotransmitter systems. Mice lacking opioid receptors exhibit dramatic modifications in their responses to opioid drugs, but otherwise do not present any obvious abnormal behavior under home cage conditions. Sophisticated paradigms need now to be developed in order to reveal subtle behavioral modifications in mutant mice. It is the purpose of this symposium to discuss: (i) the consequences of opioid receptor or peptide gene deletion in pain and stress responses, drug self-administration, learning, as well as in the adaptation to chronic opiates; (ii) the consequences of null mutations for other genes that operate in related neural pathways; (iii) gene targeting as a tool to study complex integrated responses.

S8-2

GENETIC AND BEHAVIORAL ANALYSIS OF OPIOID SYSTEM KO MICE.

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We have produced individual gene-targeted mice with mutations in the mu, delta, kappa, ORL-1, OFQ, and enkephalin genes and begun to explore several biochemical and behavioral parameters that accompany these mutations in these mice. Previously we have reported that analgesia to delta peptides administered icv, but not it, is retained in delta KO mice. We now report that DPDPE is also effective at producing analgesia in the second phase of the formalin test as it does in wild-type mice. Moreover, analgesia to a non-peptide delta preferring agonist, BW 393U69 is dramatically enhanced in delta KO mice following both icv and sc administration. To investigate whether the development of tolerance is affected in delta KO mice, the effects of daily morphine administration were examined. The development of tolerance to morphine and DPDPE were abolished in both the delta and enkephalin KO strains indicating a potential ligand-receptor system that is critical to the development of analgesic tolerance. Combinatorial mating of triple heterozygotes have produced mice of all possible genotypes, including mice lacking all three opioid receptors,. that are viable and fertile. Despite abundant expression of the OFQ gene in the fetal nervous and reproductive systems, mice with a deletion of the OFQ coding sequence exhibit no developmental abnormalities. Finally, mice doubly homozygous for mutations in the ORL-1 and OFQ loci have also been produced. These mice are also viable and thus provide a unique model system for investigating the contribution of the ORL/ORQ system to multiple physiologic responses.

S8-3

ANAGESIC REDUNDANCY IN OPIOID NULL MUTANTS: THE GOOD NEWS AND THE BAD NEWS

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Transgenic null mutant mice, or knockouts (KOs), represent an intriguing new way to study the function of opioid peptides and receptors. My laboratory is interested primarily in the phenomenon of stress-induced analgesia (SIA), the endogenous substrate on which opiates act. It has long been known that both opioid and non-opioid mechanisms exist, and these two classes can be further subdivided as well. Working with a number of opioid null mutants, we have been struck by the ubiquitous demonstration of apparent compensation in these mice. The existence of compensation, implying redundancy in analgesic mechanisms, is both good and bad. The good news is that a greater understanding of which systems substitute for which others under which circumstances may provide clinical advances. The bad news is that compensation greatly complicates the interpretation of observed KO phenotypes. I will present data obtained using β-endorphin KOs, enkephalin KOs, and β-endorphin/enkephalin double KOs tested for their sensitivity to basal thermal stimuli, morphine analgesia, swim SIA, injection-related SIA, and electroacupuncture. Data from inbred mouse strains (focusing on 129 and C57BL/6) will also be presented, since they are directly pertinent to interpretation of these and all other experiments with KO mice.

S8-4

GENETIC INFLUENCES ON SELF-ADMINISTRATION OF DRUGS OF ABUSE

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Considerable individual differences are apparent in the liability to abuse drugs in clinical populations. Mouse behavior is sensitive to both genetic and procedural influences and murine mutagenic techniques have greatly expanded the tools available for investigating the genetic and molecular bases of behavior and the behavioral effects of drugs of abuse. Procedural variables in drug self-administration include dose and drug availability, operant manipulanda and behavioral training history. Opioid peptides acting via the mu opioid receptor have been implicated in the reinforcing effects of ethanol. We have found that mu opioid receptor knockout mice show no evidence of ethanol self-administration in either two-bottle choice or operant procedures. While previous studies of opiate self-administration have focused on morphine and orally available drugs such as etonitazene, we have been developing an intravenous heroin self-administration protocol. We have evaluated heroin self-administration in C57BL/6J (B6) mice and the combination of cocaine+heroin, known as speedball, in B6 x SJL hybrid mice. Genetic vulnerability to self-administration of individual drugs was demonstrated in BALBc/ByJ mice that failed to self-administer cocaine, but self-administered heroin and the speedball combination. Continued refinement and use of drug self- administration studies will reveal the contribution of opioid peptides, receptors and related molecules to the genetic influences and molecular targets for drugs of abuse. Supported in part by PHS grants AA06420 and DA10191.

S8-5

OPIOID RECEPTORS, SPATIAL LEARNING AND SYNAPTIC PLASTICITY IN THE HIPPOCAMPUS.

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It has been postulated that opioid receptors would play an important role in the hippocampal synaptic plasticity. Mu and kappa opioid receptors (MOR and KOR) could respectively be involved in enhancement and repression of long term potentiation (LTP) in the mossy fiber region of the hippocampus (CA3). It has also been shown that pharmacological blockade of opioid receptors (presumed mu-specific or kappa-specific) entails memory impairment or enhancement respectively. We therefore have postulated that MOR activation would be a condition that is required for spatial memory, which is known to be hippocampal dependent, whereas KOR activation would inhibit it.

In order to study the effect of MOR and KOR activation in hippocampal synaptic transmission and in learning and memory, we knocked-out the MOR and KOR genes. General neuroanatomical studies as well as immunohistochemical labeling of synapses show no obvious differences between normal and knocked-out brains. However, specific labeling and quantification of the mossy fiber synapses shows a dramatically decrease of that field in MOR KO but was unaffected in KOR KO. Moreover, we have shown that the disruption of the MOR gene drastically reduces the performances of mice in spatial tasks such as the positive reinforced 8-arm radial-maze or the Morris' water-maze. Indeed, MOR KO mice constantly make more errors and need more essays to understand and acquire the task. On the other hand, the disruption of the KOR gene enhances the acquisition of learning but not the maximum plateau. KOR KO mice learn faster than wild-type ones but not better. Meanwhile the hippocampal synaptic transmission is also affected in a more subtle way than expected since MOR gene does not seem to be necessary for CA3 LTP induction. These results provide evidences that the activation of MOR and KOR is important for both spatial memory and synaptic transmission in the hippocampus.

S8-6

ANIMAL MODELS OF VULNERABILITY TO DRUGS OF ABUSE

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Using gene targeting strategies, we have generated mice that either lack or express reduced levels of the serotonin receptors 5-HT1A, 5-HT1B, 5-HT2A and 5-HT5A as well as the serotonin transporter and the dopamine transporter. We have been analyzing these animals in a number of behavioral paradigms aimed at assessing their responses to various families of abused drugs. These studies have revealed unexpected dissociation between behavioral responses. The 5-HT1B knockout mice are more responsive to cocaine in locomotor activity and self administration paradigms but less responsive in a conditioned place preference paradigm. The DAT knockdown mice are hyper- responsive to cocaine while amphetamine inhibits their activity. The 5-HT2A and 5-HT5A knockouts display altered responses to LSD. We are also currently investigating the neural circuits that are responsible for these phenotypes by analyzing conditional knockout mice that lack these receptors only in specific tissues.

O1-1

8-PHENYLAMINO ANALOGUES OF CYCLAZOCINE

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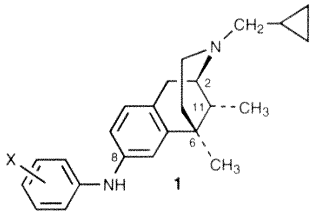
With **(±)-1** (X = H) as our lead compound, we have extended our studies to identify novel kappa agonists/mu antagonists having potential as anti-cocaine medications. The (-)- enantiomer (2*R*,6*R*, 11*R*) of **1** (X = H) displayed potent opioid binding affinity and was somewhat selective for kappa [Ki (nM) vs, mu, delta, kappa = 1.1, 5.2, and 0.54, respectively]. Using a Topliss approach, we made the racemic 4-Cl, 4-Me, 4-OMe, and 3,4-Cl_2 derivatives and found opioid binding affinity to be positively correlated to the electron donating ability of the X group. In vivo evaluation of **(±)-l** (X=H) indicate it to be a kappa agonist and mu antagonist. We prepared these compounds using a novel method which involves the Pd-catalyzed amination of the 8-triflate of cyclazocine.

(Supported by DA01674 and DA03742).



O1-2

SYNTHESIS AND PHARMACOLOGICAL ACTIVITY OF *cyclo*[D-Asp^2,Dap^5]Dyn A(1-11)NH_2 ANALOGUES WITH SUBSTITUTIONS AT POSITIONS 1, 3 AND 4.

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We are interested in developing potent and selective peptide-based ligands for kappa receptors as pharmacological tools for studying the structural and conformational requirements for interaction with kappa receptors. Although the endogenous opioid peptide dynorphin A (Dyn A) is very potent at kappa opioid receptors, it retains significant affinity for mu and delta receptors. Incorporation of a conformational constraint is an approach by which selectivity of a peptide may be increased. Various conformationally constrained analogues of Dyn A were synthesized previously in our laboratory (Arttamangkul et al., *J. Med. Chem.* **1995,** *38,* 2410-7). Of these analogues *cyclo*(D-Asp^2,Dap^5]Dyn A(1-13)NH_2 and its analogues showed the highest affinity and agonist activity at kappa receptors. We have synthesized additional (2,5) cyclic Dyn A analogues containing substitutions at positions 1, 3 and 4. Two new strategies utilizing either allyl-based or hyper-acid labile side chain protecting groups for the residues involved in cyclization were developed for the synthesis of cyclic peptides. Optimized synthetic strategies and pharmacological data of these cyclic peptides and their linear counterparts will be presented.

This work is supported by NIDA grant DA05195

O2-1

MODULATION OF THE MESOLIMBIC DOPAMINE REWARD PATHWAY BY NOCICEPTIN-ORPHANIN FQ

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Naturally rewarding stimuli such as food and sex promote locomotor activity and increase mesolimbic dopamine (DA) release in the nucleus accumbens. Addictive drugs such as the opiates produce similar effects when administered directly into the ventral tegmental area (VTA) of rats. Here we report that when the endogenous neuropeptide nociceptin-orphanin FQ (N-OFQ) is microinjected directly into the VTA it dose-dependently blocks opioid-induced locomotion in rats. Furthermore, our data indicate that N-OFQ-activated receptors (NORs) expressed in VTA DA cells cause a hyperpolarization of membrane potential. Based on our observations we propose that N-OFQ is an important endogenous neuropeptide modulator of the central DA reward circuitry and that selective NOR agonists may help to control drug self-administration.

O2-2

EFFECTS OF NMDA & NON-NMDA ANTAGONISTS ON HEROIN SELF-ADMINISTRATION AND MESOLIMBIC DOPAMINE RELEASE.

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In addition to inhibitory GABAergic modulation, VTA DA neurons also receive excitatory glutamatergic input. To examine the role of glutamate modulation in opiate reinforcement, (1) MK-801, a non-competitive NMDA blocker, significantly increased heroin SA and shifted the heroin dose-response SA curve to the right when coadministered IV (0.02 mg/kg,) or microinjected into the VTA (2 microgram). However, no significant effect on heroin-induced DA release was observed, as indicated by fast-cyclic voltammetry. MK-801 alone at 0.1 mg/kg, iv, but not 0.05 mg/kg, significantly increased basal NAcc DA release. (2) Ketamine (another non-competitive NMDA antagonist, 0.18 mg/kg) co-administered with heroin also significantly increased heroin SA behavior, but also decreased mesolimbic DA release. The same dose of ketamine alone did not maintain SA behavior in heroin trained rats. (3) DNQX (a non-NMDA antagonist) dose-dependently increased heroin SA behavior when co-administered IV (0.05-0.1 mg/kg) or administered into the VTA (2-6 microgram), and significantly blocked heroin-induced DA release when administered 5-10 min prior to heroin access. DNQX alone (0.5 mg/kg) did not maintain SA behavior in heroin trained rats. In conclusion, both the NMDA antagonist ketamine and the non- NMDA antagonist DNQX antagonize heroin reinforcement by inhibiting mesolimbic DA transmission, while MK-801 antagonism appears to be mediated by a DA-independent mechanism. (Supported by DA 09465).

O3-1

OPIATE-INDUCED ORAL STEREOTYPY IN THE RAT, A MODEL FOR HYPERKINETIC MOVEMENT DISORDERS

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Although repeated high doses of morphine cause stereotypic biting behavior in the rat little attention has been paid to this phenomenon. Not only is the behavior robust but sensitization to this effect can be demonstrated. MK801, fluoxetine, naloxone, and the D1 antagonist SCH23390 but not the D2 antagonist raclopride can block the expression of the behavior. Of these agents, only naloxone and MK801 block the development of the sensitization of this morphine-induced biting behavior. Based on these findings and that opioid peptides and opiate drugs modulate the function of dopamine neurons and can facilitate the dopamine transporter we have administered naltrexone to 20 carbidopa-levodopa treated Parkinson's disease patients who had severe hyperkinesia and painful muscle spasms resulting from the antiparkinson medication. In all of the patients some relief of the symptoms was obtained and in some, the therapeutic effect was dramatic. These clinical findings support the hypothesis that the endogenous opioid system may be involved in the modulation of the dopamine system in these patients and that the opiate-induced stereotypy in the rat may be a useful model in the study of dopamine-induced movement disorders. (Supported in part by NIDA grants DA-02326 and K05-DA-00099 to CK).

O3-2

AGONIST AND ANTAGONIST ACTIONS OF A NEW BUPRENORPHINE ANALOGUE ON MU, DELTA AND KAPPA OPIOID RECEPTORS

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In radioligand binding assays, conducted on rat membrane homogenates, the new didehydro-derivate of buprenorphine (HS-599) displayed very high affinity for the mu opioid receptors but lower affinity for the kappa and the delta opioid receptors (K_d = 0.57±0.08 nM; 8.5±0.9 nM; 32.0±4 nM). The biological activity of HS-599 was tested and compared with that of buprenorphine, in isolated tissues preparations. In the guinea pig ileum longitudinal muscle preparation (mu-receptor) HS-599 behaved as a mu agonist (IC_{50} = 6±1 nM) comparable to buprenorphine (IC_{50} = 3.8±0.4 nM). Repeated washing of the tissue failed to eliminate the inhibition of contractions, suggesting that HS-599 acted as an irreversible mu-opioid receptor ligand. HS-599 dose-dependently shifted to the right the dose-response curve of the mu-selective agonist dermorphin (pA_2 = 9.3). Naloxone (200 nM) prevented the effect of HS-599 (2 nM) but did not reverse HS-599 induced twitch inhibition. In mouse vas deferens (MVD, delta-receptor) and in rabbit vas deferens (RVD, kappa-receptor) preparations, HS-599 behaved as a delta- and kappa-receptor antagonist (pA_2 = 8.2 in MVD and 9.25 in RVD). Subcoutaneously injection of HS-599, in mice, induced naloxone sensitive analgesia. In tail-flick test HS-599 was two times more potent than buprenorphine.

O4-1

THE C-TERMINUS PLAYS IMPORTANT ROLE IN THE INTERNALIZATION AND MEMBRANAL TARGETING OF THE RAT MU OPIOID RECEPTOR.

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Internalization and recycling of G-protein coupled receptors, such as the mu opioid receptor largely depends on agonist stimulation. In previous studies we have demonstrated that the C- terminal spliced isoforms (MOR1 and MOR1B) of the rat mu opioid receptor differ in their internalization and recycling rate *(Koch et al., J. Biol. Chem. 273, 13652-13657, 1998).* These results indicate an important role of the C-terminus in the regulation of the mu opioid receptor internalization and recycling. To investigate structural domains and putative phosphorylation sites involved in this mu opioid receptor internalization, we constructed three truncation mutants (Trunc 386, Trunc 360, and Trunc 344). To permit localization of the receptor by immunocytochemistry we added an epitope tag to the N-terminus of the wildtype and mutant receptors. The immunocytochemical analyses revealed that only wildtype and truncation mutant Trunc 386 showed membranal localization. Therefore, in addition to its importance for the receptor internalization, the C-terminus of the mu opioid receptor seems to contain information for receptor targeting to the plasma membrane.

O4-2

INVOLVEMENT OF MITOGEN-ACTIVATED PROTEIN KINASE IN AGONIST-INDUCED PHOSPHORYLATION OF THE MU-OPIOID RECEPTOR IN HEK 293 CELLS

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In the present work, we show that treatment of human embryonic kidney HEK 293 cells stably transfected to express the rat mu-opioid receptor (MOR1) with DAMGO stimulated a rapid and transient (3-5 min) activation and nuclear translocation of the mitogen-activated protein kinase (MAPK). Exposure of these cells to the mitogen-activated protein kinase kinase (MAPK kinase) 1 inhibitor, PD98059, not only prevented MAPK activation but also inhibited homologous desensitization of the mu-opioid receptor. We have therefore determined the effect of PD98059 on agonist-induced mu-receptor phosphorylation. DAMGO stimulated a three-fold increase in MOR1 phosphorylation within 20 min that could be reversed by the antagonist naloxone. PD98059 produced a dose-dependent inhibition of agonist-promoted mu-receptor phosphorylation with an IC50 of 20 microM. DAMGO also induced MOR1 internalization which reached a maximum at 30 min. Confocal microscopy revealed that DAMGO-induced MOR1 internalization was also largely inhibited in the presence of PD98059. U0126, another chemically-unrelated inhibitor of the MAPK cascade, mimicked the effect of PD98059 on mu-receptor phosphorylation and desensitization. MOR1 itself, however, appears to be a poor substrate for MAPK because mu-receptors immunoprecipitated from stably transfected HEK 293 cells were not phosphorylated by exogenous ERK 2 in vitro. The fact that morphine also triggered MAPK activation but did not induce MOR1 internalization and DAMGO-induced MAPK activation was not prevented by sucrose, an inhibitor of clathrin-mediated endocytosis, indicates that receptor internalization was not required for MOR1 mediated mitogenic signaling. We conclude that MOR1 stimulates a rapid and internalization-independent MAPK activation. Activation of the MAPK cascade in turn may not only relay mitogenic signals to the nucleus but also trigger initial events leading to phosphorylation and desensitization of the mu-opioid receptor.

O5-1

OPIOID INDUCED CREB PHOSPHORYLATION IN NEURO2A MOR1A CELLS.

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Administration of opioids may regulate the neural gene expression either by changes in the amount of the transcription factor, or by altering its phosphorylation. The present study shows that acute administration of morphine as well as now highly selective ligand of μ-opioid receptor, endomorphin-1 can stimulate CREB phosphorylation (pCREB) in Neuro2a MOR1A cells expressing μ-opioid receptors. Morphine, in naloxone-reversible manner increased pCREB level, which peaked after 30min, although, after 1h of incubation declined below basal level. Prolonged (3 days) exposure to morphine caused only a slight increase in the pCREB level and upregulation of CREB protein level. The drug withdrawal elicited an increase in pCREB after 30min, which returned to the basal level after 4h. Endomorphin-1 also increased pCREB level, however in a different time-dependent manner. Thus, activation of μ-opioid receptor by morphine and endomorphin-1 stimulates CREB phosphorylation. Regulation of CREB and subsequent changes in gene expression may underlie some of the long-term effect of μ-opioid receptor activation. *Supported by KBN Nr.4.P05A.017.13 and EC CT93-0253*

O5-2

REPEATED MORPHINE APPLICATION ALTERS THE RESPONSIVENESS OF CEREBRAL GENE EXPRESSION TO VARIOUS DRUGS OF ABUSE

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Morphine given as a single dose up to 100 mg/kg in drug naive Wistar rats elicited only a weak expression of the transcription factor c-fos (and, as can be assumed, late response genes in turn) in the brain, namely in the lateral septum. In contrast, injecting the rats twice daily for ten days with morphine in ascending doses (10 mg/kg to 50 mg/kg) caused several cerebral regions (dorsal striatum, nucleus accumbens shell, cingulate cortex and medial mammillary nuclei) to respond with a strong c-fos mRNA synthesis to a test dose (10 to 100 mg/kg) of the drug. This permissive effect of repeated morphine application on drug-induced gene expression was not only, observed towards morphine itself but also to the pharmacologically different substances cocaine and MDMA ('ecstasy'). Cocaine in morphine-experience but not in naive rats caused a marked c-fos response in limbic parts of the caudate putamen, in the mammillary and in certain midbrain nuclei. The reponse to MDMA was enhanced in the central striatum. There was no such effect on LSD- or THC-induced c-fos expression. In conclusion, the pronounced co-sensitization of morphine and cocaine may reflect the highly addicting potential common to both drugs.

O5-3

DIFFERENTIAL MODULATION OF CaMK II ALPHA AND BETA ISOFORMS BY MORPHINE IN RAT HIPPOCAMPUS

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Acute morphine treatment significantly increased both Ca^{2+}/calmodulin-independent and Ca^{2+}/calmodulin-dependent activities of CaMK II in the rat hippocampus, with little alteration in the protein level of either alpha or beta isoform of CaMK II. However, chronic morphine treatment, by which rats develop apparent tolerance, significantly down-regulated both Ca^{2+}/calmodulin-independent and Ca^{2+}/calmodulin-dependent activities of CaMK II and differentially regulated the expression of alpha and beta isoforms of CaMK II at protein and mRNA levels. Application of naloxone or discontinuation of morphine treatment after chronic morphine administration, which induced the withdrawal syndrome of morphine, resulted in the overshoot of CaMK II (at both protein and mRNA levels) and its kinase activity. The phenomena of overshoot were mainly observed in the beta isoform of CAMK II but not in the alpha isoform. The effects of both acute and chronic morphine treatments on CaMK II could be completely abolished by the concomitant application of naloxone, indicating that the effects of morphine were achieved through activation of opioid receptors. Our data demonstrated that both acute and chronic morphine treatments could effectively modulate the activity and the expression of CaMK II in the hippocampus.

O8-1

REDUCTION OF OPIATE DEPENDENCE BUT NOT OPIATE ANALGESIA IN MICE LACKING CB1 RECEPTORS

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CB1 cannabinoid receptors are widely distributed in the central nervous system where they mediate most of the cannabinoid-induced responses. Recently, CB1 receptors were inactivated by invalidating its gene in a mouse model (Ledent et al., Science. 283: 401, 1999). These mutant mice constitute a unique tool to determine the physiological role of the cannabinoid system. An interaction between the opioids and cannabinoids has been proposed. Morphine-induced antinociception, as well as stress-induced analgesia were investigated in CB1 mutant mice. The antinociceptive effects of morphine in the tail-immersion and the hot-plate tests were similar in mutant mice and in their littermates. The development of morphine dependence has been also evaluated. Opiate dependence was induced after chronic morphine administration and the withdrawal syndrome was precipitated by the injection of the opiate antagonist naloxone. The behavioral expression of morphine abstinence was significantly decreased in CB1 mutant mice. Indeed, seven of the nine withdrawal signs evaluated were decreased in mutant mice. These data demonstrated that the presence of CB1 cannabinoids receptors are necessary to obtain a complete manifestation of the opiate abstinence.

O8-2

NEUROCHEMICAL CORRELATES OF ACUTE AND PERSISTENT PAIN

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Although traditional approaches to identifying the contribution of particular molecules to the processing of pain information have relied on pharmacological antagonism, there are limitations to those studies. Most importantly, selectivity of the antagonists is often called into question. Furthermore, it is often difficult to deliver antagonists for extended times, as is necessary in the study of the pain produced by persistent injury. Our laboratory has used mice in which there is a deletion of genes that encode either putative transmitters of the "pain" message or key second messenger molecules that have been implicated in the development of long term changes after injury. Our studies suggest that products of the pre-protachykinin-A gene (substance P and neurokinin A) are major contributors to the processing of acute pain messages, but are not essential for the development or the persistence of injury-associated conditions in which pain is produced by non-painful stimuli. By contrast, deletion of the gamma isoform of protein kinase C (PKCγ) dramatically reduces this condition. Of particular interest is the evidence that not all spinal "pain" pathways are regulated by the interneurons of the dorsal horn that express PKCγ. Supported by NS 14627.

Sun01

DETERMINATION OF CYSTEINE RESIDUES WITHIN TRANSMEMBRANE DOMAINS OF THE RAT MU OPIOID RECEPTOR EXPOSED IN THE BINDING POCKET

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The seven putative transmembrane domains (TMDs) of the mu opioid receptor are thought to form a binding pocket open to the extracellular medium. (2-Aminoethyl)methanethiosulfonate (MTSEA) is a hydrophilic reagent that reacts specifically with reduced sulfhydryl group. In this study, we determined sensitivity of the mu opioid receptor to MTSEA and identified the cysteine residues within the TMDs that confer the sensitivity. Pretreatment of the rat mu opioid receptor expressed in HEK293 cells with MTSEA for 5 min inhibited [^{3}H]diprenorphine binding in a dose-dependent manner with an IC_{50} value of 0.5 mM. The effect of MTSEA occurred rapidly, reaching the maximal inhibition at 2 min. (-) naloxone prevented the inhibitory effect of MTSEA, but (+) naloxone did not. Each cysteine residue in the TMDs was mutated to serine individually (Cl.43S, C3.44S, C4.48S, C5.41S, C5.57S, C6.47S, C7.38S and C7.47S) and effects of MTSEA treatment were examined. Only the mutation of C7.38S rendered the μ receptor less sensitive to MTSEA, indicating that the conserved residue C7.38 is exposed to the binding pocket. Using the C7.38S mutant as the control, we mutated each residue within the TMD6 one at a time and examined their sensitivity to MTSEA treatment to determine the residues exposed in the binding pocket. (Supported by NIH grants DA 04745 and DA11263.)

Sun02

CYS7.38(315) OF THE HUMAN KAPPA OPIOID RECEPTOR IS EXPOSED IN THE BINDING POCKET

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The binding pocket of the kappa opioid receptor is presumably formed among the transmembrane domains (TMDs) and open to the extracellular milieu. (2-Aminoethyl) methanethio-sulfonate (MTSEA) is a hydrophilic reagent that reacts specifically with reduced sulfhydryl groups. In this study, we determined sensitivity of the human kappa opioid receptor to MTSEA and identified the cysteine residues within the TMDs that confer the sensitivity. Pretreatment of the human kappa opioid receptor expressed in HEK293 cells with MTSEA for 5 min inhibited [^{3}H]diprenorphine binding in a dose-dependent manner with an IC_{50} value of 0.1 mM. The effect of MTSEA occurred rapidly, reaching the maximal inhibition at 2 min. Each cysteine in the TMDs was mutated to serine individually (C4.48S, C5.41S, C5.57S, C6.47S and C7.38S) and effects of MTSEA treatment were examined. The C7.38S mutant became resistant to MTSEA (0.1 mM - 10 mM), while all other mutants were still sensitive. Thus, C7.38, conserved among opioid receptors, is exposed in the binding pocket of the human kappa receptor. Using the C7.38S mutant as the insensitive control, we mutated each amino acid in the TMD6 to Cys to determine the residues contributing to the surface of the binding pocket. (supported by NIH grant DA 04745 and DA 11263.)

Sun03

IDENTIFICATION OF RESIDUES IN THE PUTATIVE SIXTH TRANSMEMBRANE DOMAIN OF THE HUMAN DELTA OPIOID RECEPTOR EXPOSED IN THE BINDING POCKET

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The binding pocket of the delta opioid receptors is formed by the seven putative transmembrane domains (TMDs) and accessible to ligands applied in the extracellular medium. (2-Aminoethyl) methanethiosulfonate (MTSEA) is a hydrophilic reagent that reacts specifically with reduced sulfhydryl groups. [^{3}H]diprenorphine binding to the human delta opioid receptor expressed in HEK293 cells was relatively insensitive to MTSEA with an IC_{50} value of 5 mM, compared with IC_{50} values of 0.1 mM and 0.5 mM, for kappa and mu receptors, respectively. We then used MTSEA in the substituted cysteine accessibility method to map the residues in the TMD6 of the delta receptor that contribute to the surface of the binding pocket. Each residue in the TMD6 was mutated one at a time to cysteine and effect of MTSEA treatment on [^{3}H]diprenorphine binding to each mutant was examined. Among the 24 mutants, 10 mutants were sensitive to MTSEA treatment. These results will be presented and discussed. (supported by NIH grant DA 04745 and DA 11263.)

Sun04

NALTRINDOLE, A DELTA SELECTIVE OPIOID ANTAGONIST, DISPLAYS ENHANCED BINDING AFFINITY AT SINGLE POINT MUTANTS OF MU AND KAPPA OPIOID RECEPTORS.

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Previously, it has been suggested that ligand selectivity among opioid receptors can be conferred by a mechanism of exclusion whereby particular regions of the receptor prevent binding to non-preferred types. Further, it was proposed that if such barriers were removed, selective ligands would bind in a pocket that is common among the opioid receptor types. Reported studies aimed at determining the region of the delta opioid receptor involved in enabling selective recognition of the delta opioid antagonist naltrindole (NTI) have indicated that extracellular loop 3 (EL-3) is the critical region. To test the above hypothesis, single point mutations have been made in EL-3 of the mu and kappa opioid receptors in an attempt to confer high affinity to naltrindole. In particular, residue W318 of the mu receptor, when mutated to alanine, yields a receptor that binds NTI with an affinity similar to that of the delta receptor. Mutation at the corresponding position in the kappa receptor (based on sequence alignment of EL-3 of the opioid receptors), Y312A, gives enhanced affinity for NTI relative to the kappa wild type by ~10 fold. Based on docking studies of NTI into opioid receptor models, it is proposed that the presence of large aromatic groups at this position (located at the top of putative transmembrane helix 7) inhibits binding of NTI into the binding pocket of mu and kappa opioid receptors.

Sun05

BINDING OF THE STEROID SC17599 TO THE MU OPIOID RECEPTOR: IMPLICATIONS FOR A MU PRARMACOPHORE.

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The amino-steroid 17alpha-acetoxy-6-dimethylaminomethyl-21-fluoro-3-ethoxypregna-3,5-dien-20-one hydrochloride (SC17599) binds with high affinity to the mu opioid receptor in membranes from SH-SY5Y cells (Ki 16nM). Affinity of the compound for delta and kappa receptors is low (12microM and 4.6microM, respectively). SC17599 is a mu receptor agonist and stimulates the binding of [^{35}S]GTPgammaS in SH-SY5Y cell membranes with an EC_{50} of 250nM, giving a maximal response equivalent to that seen with fentanyl. We have found no other steroids with nM affinity for the mu-opioid receptor. In contrast SC17599 does not displace [^{35}S]triamcinalone from glucocorticoid receptors expressed in sf9 cells. SC17599 possess an amino-group but lacks any aromatic moiety which is considered crucial for ligand binding to the mu opioid receptor. Comparison of the structure of SC17599 with a pharmacophore modeled using a wide variety of mu-opioid ligands shows that the aromatic tyramine or tyrosine feature in opioids of more classic structure is not an absolute requirement and can be substituted by a conjugated pi bond system in SC17599. We thank Searle for the supply of SC17599. Supported by USPHS DA 00254 and DA 07315.

Sun06

THE UNIRECEPTOR HYPOTHESIS OF OPIOID ANALGESIA: BINDING STUDIES IN AMPHIBIANS

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Previous work in our laboratory showed that selective *mu*, *delta*, and *kappa* opioid analgesics produce the same relative potency in amphibians as in mammals. This is surprising given that mammalian brain and spinal cord tissue contains three types of functional opioid receptors, called *mu*, *delta*, or *kappa* whereas the amphibian CNS appears to contain one main type of opioid binding site. Our working hypothesis is that amphibians express a primordial opioid receptor, termed the *unireceptor*, that can bind and mediate the analgesic effects of all types *(mu*, *delta*, and *kappa)* of opioid agonists. Binding studies were done by characterization and competition using the general opioid antagonist, [^{3}H]-naloxone, in brain and spinal cord tissue homogenates harvested from *Rana pipiens.* Selective opioid antagonists for *mu* (beta-funaltrexamine), *delta* (naltrindole) or *kappa* (nor-binaltorphimine) opioid receptors had identical IC50 values against labeled naloxone. These data provide further evidence that selective *mu*, *delta*, and *kappa* opioids act at a single receptor type in amphibians, and along with behavioral data, support the unireceptor hypothesis of opioid receptors.

Sun07

DISTRIBUTION OF A SPLICE VARIANT OF THE MU-OPIOID RECEPTOR-1, MOR-1D, IN THE MURINE CNS

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We have recently identified 3 splice variants of the mouse mu-opioid receptor (MOR-1) gene due to alternative splicing of additional exons (6,7,8 and 9) downstream from the original exon 3. In MOR-1D the 12 amino acids (AA) encoded by exon 4 in MOR-1 are replaced by 7 AA derived from the combination of exons 8 and 9. In the present study, we looked at the distribution of the splice variant MOR-1D in rat and mouse CNS using immunohistochemistry. Antisera were raised against a synthetic peptide corresponding to the carboxyl terminus of MOR-1D (Multiple Peptide Systems). Specificity of the antisera was verified (1) by staining of HEK 293 cells transfected with MOR-1D, (2) Western blots of membranes of transfected cells and brain tissue, (3) the absence of staining in both brain tissue and transfected cells after preabsorption of the antisera with the cognate peptide. Intense MOR-1D-like immunoreactivity (MOR-1D-LI) was found in the hippocampus (only CA 3), the nucleus of the solitary tract and laminae I and II of the medullary and spinal dorsal horn. Moderate MOR-1D-LI was observed in laminae III-VI and X of the spinal cord. MOR-1D-LI neurons seem to target their receptors preferentially to their somatodendritic domain. MOR-1D was expressed in similar structures as MOR-1, but with a more discrete distribution. However, MOR-1C-LI (encoded by exons 6, 7 and 9) is intensely expressed in the hypothalamic nuclei. Since the splice variants are derived from the same gene, the differences in regional distribution represent region-specific mRNA processing.

Sun08

CLONING AND EXPRESSION OF A NOVEL SPLICE VARIANT (MOR-1F) OF THE MOUSE MU-OPIOID RECEPTOR (MOR-1) GENE.

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Recently, increased evidence has suggested that the mouse MOR-1 gene undergoes extensive splicing. Five MOR-1 splice variants (MOR-1A, MOR-1B, MOR-1C, MOR-1D, MOR-1E), differing only at the intracellular carboxyl terminus, have been identified. Here we report isolation of an additional MOR-1 splice variant, MOR-1F, using an RT-PCR approach. Sequence analysis indicates that MOR-1F contains another new exon (exon 10) with an exon composition of 1, 2, 3, 10, 6, 7, 8 and 9. Exon 10 has been mapped between the exons 4 and 6 in the mouse genomic BAC clones containing the MOR-1 gene. Exon 10 is predicted to encode 58 amino acids in the C-terminal of the MOR-1F, which is longest among the variants. Exons 6, 7, 8 and 9 are not translated in MOR-1F. Northern blot analysis of mouse brain total RNA using an exon 10 probe showed a diffuse band, ranging in size from approximately 3 to 4 kb. Expression of the MOR-1F mRNA in different brain regions determined by RT-PCR indicated that all the regions expressed MOR-1F with higher levels in hypothalamus and PAG. Binding studies in CHO cells transfected with the MOR-1F construct suggest that MOR-1F encodes a mu opioid receptor. This work is supported by DA00296 (Y.-X. P.) and DA02615 and DA00220 (G.W.P.).

Sun09

MUTATIONS IN THE DELTA RECEPTOR DRAMATICALLY ALTERED THE AGONIST/ANTAGONIST PROPERTY BUT NOT THE BINDING AFFINITY OF OPIOID ALKALOIDS

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Our earlier study showed that by mutating five amino acid residues to those conserved in the opioid receptors, the orphanin FQ receptor could be converted to a functional receptor that bound many opioid alkaloids with nanomolar affinities. Surprisingly, when the reciprocal mutations (K→A (TM5), IHI→VQV (TM6) and I→T (TM7)) were introduced in the delta receptor, neither the individual mutations nor the various combinations of them significantly reduced the binding affinities of opioid alkaloids tested. However, many of these mutants behaved very differently from the wild type delta receptor in GTPγS binding assays. Although etorphine and SNC80 are still agonists on most mutants, the delta receptor with only the K→A mutation can no longer be activated by either of them. Interestingly, the K→A mutant retains the ability to activate G-protein(s) because ICI-174864 and BNTX are still inverse agonists on this receptor. More strikingly, on the receptors that contain the IHI→VQV and /or I→T mutations, naltrindole and naltrexone become agonists but their effects can be blocked by the peptide antagonist TIPP. Even the delta inverse agonist BNTX is now an agonist on the mutant containing both the IHI→VQV and I→T mutations. Our results indicate that the mutated residues are critically involved in the control of the delta receptor activation process. These data are also compatible with our hypothesis that a ligand may achieve high affinity binding in several different ways and each of which may have different effects on receptor activation.

Sun10

COUPLING OF THE MU OPIOID RECEPTOR TO THE YEAST PHEROMONE SIGNAL TRANSDUCTION PATHWAY

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In order to establish an efficient functional assay system for the G-protein coupled receptors, we adopt a strategy that links the growth of yeast to the activation of the target receptors. The *FAR1*gene and the *SST2* gene were deleted in the wild type yeast strain YPH499 in order to eliminate the pheromone induced growth arrest and to increase the sensitivity of the yeast toward agonists. The *HIS3* gene, an enzyme in the yeast histidine biosynthesis pathway, was inserted after the *FUS1* promoter. This modification enables the yeast to grow faster in histidine deficient media upon the activation of the pheromone signal transduction pathway. The exogenous G-protein coupled receptors can be introduced into the modified yeast cells by either an expression plasmid or by homologous recombination. We expressed the mu opioid receptor in our modified yeast strain using a plasmid and successfully observed the functional coupling of the mu receptor to the yeast pheromone signal transduction pathway. When this strain was grown in histidine deficient media, the presence of 0.5 μM etorphine increased its growth rate by about 4-fold. The mu antagonist naltrexone did not change the basal growth rate but it significantly reduced the etorphine induced growth rate increase. Fentanyl could also cause a smaller but naltrexone blockable growth acceleration. Additional modifications are being introduced into yeast in order to increase the sensitivity and suitability of this system for the study of antagonists, inverse agonists as well as agonists. This approach should also be useful for large-scale structure-function study, receptor / G-protein engineering and functional characterization of orphan G-protein coupled receptors.

Sun11

EVIDENCE AGAINST A RANDOM COLLISION COUPLING MODEL OF G PROTEIN ACTIVATION

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The [^{35}S]GTPgammaS binding assay was used to measure G protein activation in whole C6 cells expressing the mu opioid receptor. Cells were permeabilized with digitonin to allow [^{35}S]GTPgammaS entry. Relative mu agonist efficacy was determined to be, in rank order: DAMGO > etorphine > fentanyl > morphine > buprenorphine. Pentazocine showed no efficacy. In order to test the collision coupling theory of receptor-G protein interaction cells were treated with beta-funaltrexamine (beta-FNA) to reduce receptor concentration. The collision coupling model predicts that such a reduction in the number of receptors should slow down the rate of G protein activation but not the maximum number of G proteins able to be activated. Treatment of the cells with 10 nM beta-FNA reduced the number of receptors determined with [^{3}H]diprenorphine to 23 ± 3% of control with no change in affinity. A commensurate reduction (to 29 ± 10% of control) in the level of [^{35}S]GTPgammaS binding stimulated by DAMGO was observed; the rate of [^{35}S]GTPgammaS binding, however, remained unchanged. Therefore, random collision of receptor and G protein fails to occur in this permeablized whole cell preparation. It is proposed that a model which assumes some "compartmentalization", or association of G proteins with individual receptors, could better describe the activation of G proteins by receptors.

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Sun12

MAPPING THE SITES OF δ- AND μ- OPIOID RECEPTOR - G PROTEIN INTERFACE

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A wealth of information has recently shed light in our laboratory about the structural determinants involved in opioid receptor-G protein coupling domains. This was achieved using a series of receptor derived peptides corresponding to amino acid sequences of the second, third intracellular loop and the cytoplasmic tail of the murine δ-opioid receptor. In this respect we were able to determine for the first time that the amino-terminal portion of the third intracellular loop and part of the carboxyl-terminal tail serve as major contact sites with G proteins (Merkouris et al. Mol. Pharmacol. 50:985,1996; Biochim. Biophys. Acta 1359:263, 1997). To test whether these peptides could also interfere in specifying G protein-mediated effector coupling we examined the ability of these peptides to interfere in adenylate cyclase coupling in membranes from Rat-1 fibroblasts and $Neuro_{2A}$ cells stably transfected to express the δ- and μ-opioid receptor respectively. We provide evidence that indicates that the entire third intracellular loop is responsible for adenylate cyclase inhibition. Moreover, we stressed out for the first time the involvement and the significance of the βγ subunits of G proteins for these interactions. Collectively, our data provide novel information about the molecular and structural determinants governing opioid receptor/G protein/adenylate cyclase interface.

Sun13

ANTAGONISTS PRODUCE FIFTY PERCENT INHIBITION OF DAMGO-STIMULATED [^{35}S]GTP-γ-S BINDING AT DIFFERENT LEVELS OF RECEPTOR OCCUPANCY IN rMOR-HN9.10 CELLS.

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Recent work from our laboratory showed that the [^{35}S]GTP-γ-S binding assay can be used to determine opioid agonist potency, efficacy and intrinsic efficacy, the latter property being defined as the relationship between receptor occupancy and effect. The aim of this study was to compare the functional Ki values of antagonists, as determined with the [^{35}S]GTP-γ-S binding assay, with their Ki values as determined by inhibition of [^{125}I]IOXY binding under [^{35}S]GTP-γ-S binding conditions. Using cloned rat mu receptors (rMOR-HN9.10 CELLS), the functional K_i values of 10 selected opioid antagonists were determined by measuring their inhibition of DAMGO-stimulated [^{35}S]GTP-γ-S binding. The order of potency was LY255582 = RTI5989-25 > RTI5989-1 = RTI5989-23 > buprenorphine > naloxone > naltrindole > naltrexone > diprenorphine > CTAP. In this assay system, buprenorphine acted as an antagonist and inhibited DAMGO-stimulated [^{35}S]GTP-γ-S binding. Binding Ki values were determined for the antagonists, and the ratio of the binding Ki to the functional Ki calculated. (K_i(IOXY)/K_i(GTPγS)). The ratio was greatest for naltrindole (348) and least for diprenorphine (0.22). Most of agents had moderate ratios: LY255582 (109); naloxone (277); buprenorphine (151); CTAP (62); naltrexone (15). These data suggest that the concentration of each antagonist required to produce a 50% attenuation of DAMGO-stimulated [^{35}S]GTP-γ-S binding occurs at a different level of receptor occupation.

Sun14

MODULATION OF DELTA OPIOID RECEPTOR FUNCTION BY ACUTE ETHANOL

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Previous studies have shown that ethanol modulates opioid receptor neurotransmission and that the delta opioid receptor is more susceptible to its acute and chronic effects compared to mu and kappa receptors. In this study we used clinically relevant concentrations of ethanol (50-200mM) to evaluate its effects on immediate agonist induced events in N18TG2 cells expressing Flag-tagged delta receptors. We find that ethanol affects the binding of the agonist, 3H-DPDPE, but not the antagonists 3H-diprenorphine, in a dose-dependent manner. The effects on 3H-DPDPE binding were temperature dependent. Ethanol reversibly inhibits agonist stimulation of 35S-GTPγS binding and decreases the rates of receptor as well as ligand internalization. These effects were not observed when cells were pretreated with ethanol for 30 min at 37^{o}C. Taken together these results suggest that acute ethanol affects functional coupling and receptor/ligand internalization; pretreatment induces compensatory mechanisms that allow the delta receptor to function normally in its presence.

Sun15

ROLE OF GDP IN MU OPIOID RECEPTOR-MEDIATED G-PROTEIN ACTIVATION

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Opioid agonists stimulate [^{35}S]GTPγS binding in a GDP- and agonist efficacy-dependent manner. This study examined the role of GDP in regulating mu opioid receptor-stimulated [^{35}S]GTPγS binding to membranes from transfected CHO cells expressing a high level of mu receptors. In the absence of GDP, neither the full agonist DAMGO nor removal of sodium (which increases agonist-independent receptor activity) affected [^{35}S]GTPγS binding. GDP competed for high affinity [^{35}S]GTPγS binding in a biphasic manner under all conditions tested. High affinity GDP competition of [^{35}S]GTPγS binding was not significantly affected by agonist or by removal of sodium, although there was a trend towards a decrease in the number (I_{max}) of high affinity GDP sites under these conditions. In contrast, DAMGO produced a 2-fold increase in both the K_i and I_{max} values of the low affinity GDP sites. Removal of sodium increased the I_{max} of the low affinity GDP sites by 3-fold without affecting the K_i value. Examination of agonists of different efficacies at the mu receptor (DAMGO, morphine, buprenorphine and nalbuphine) revealed that agonist efficacy correlated with both the K_i and I_{max} values of the low affinity GDP sites, with partial agonists predominantly affecting the I_{max} value. These results demonstrate that mu receptors stimulate [^{35}S]GTPγS binding by modulating GDP affinity states of G-proteins. Supported by NIDA: DA-10770 (DES) & DA-02904 (SRC).

Sun16

PHARMACOLOGICAL PROFILES OF KAPPA OPIOIDS: CORRELATION BETWEEN [^{35}S]GTPγS BINDING AND CYCLIC AMP PRODUCTION.

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Kappa opioid agonists have been shown to decrease cocaine self-administration in rats and monkeys. Opioid agonists stimulate the binding of GTP to G-proteins, and subsequently, the inhibition of cyclic AMP production. Using R1E/TL8x.1.G1.OVAr.1 (R1EGO) mouse thymoma cells, a cell line expressing only κ opioid receptors, the ability of several opioid agonists and mixed agonist/antagonists to stimulate [^{35}S]GTPγS binding and inhibit cyclic AMP production was measured. The rank order of the relative E_{max} values in stimulating [^{35}S]GTPγS binding was: spiradoline = enadoline = (-)U50,488 > ethylketocyclazocine (EKC) > (-) cyclorphan = Mr2033 = bremazocine. The rank order of the relative EC_{50} values was: bremazocine = (-)cyclorphan > enadoline > EKC > Mr2033 = spiradoline > (-)U50,488. The rank order of κ agonists in producing maximal stimulation of [^{35}S]GTPγS binding did not correlate with the rank order of the potency of κ agonists. Data from adenylyl cyclase assays indicated similar trends in both efficacy and potency. These assays can be used to rapidly screen compounds to determine potency and efficacy at the κ receptor. (Supported by NIDA grants DA03742 and K05-DA00360.)

Sun17

ANTIPROPULSIVE ACTION OF DYNORPHIN A (Dyn A) ON COLONIC TRANSIT IN MICE

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Kappa agonists such as U50,488 have limited actions on small intestinal transit in rodents. Less is known about their actions in the colon where morphine is reported to slow transit and inhibit defecation (in humans). In studying the effects of kappa agonists in the mouse bead expulsion test, we included Dyn A, and now call attention to its marked antipropulsive action on colonic transit. Male Swiss mice (25-30 g; n=8-10) were injected i.v. with either saline or Dyn A (0.1-3 micromol/kg) 20 min before a glass bead (3 mm diameter) was inserted 2 cm into the rectum of each animal. Expulsion of the bead was timed over 30 min. Data were expressed as % inhibition of bead transit and antitransit-50 values obtained. Dyn A was efficacious and potent (A50=0.76 micromol/kg). The corresponding A50 value in the mouse formalin test was 0.54 micromol/kg. The antitransit action of Dyn A (2 micromol/kg) was antagonized markedly (but not completely) by naloxone (3 mg/kg, s.c.); and only partially by a neutral dose of norBNI (10 mg/kg, s.c. at -15 hr) that reversed behavioral depression. These results implicate a nonopioid component in the antitransit action of Dyn A on mouse colon.

Sun18

DETECTION OF DELTA OPIOID RECEPTOR (DOR) PROTEIN IN RAT PITUITARY GLANDS

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Previous studies reported high levels of DOR mRNA in rat pituitary. In order to directly demonstrate the expression of the DOR protein, rat pituitary extracts were subjected to Western Blot analysis. Whole rat pituitaries and the cervical segment of the spinal cord (which served as a positive control) were homogenized in sample buffer containing SDS and beta-mercapoethanol. The proteins were separated by electrophoresis on 10% PAGE SDS gels, and transferred onto nitrocellulose membranes. The membranes were probed with two separate antibodies directed against peptides derived from the N- and C- terminal regions of rat DOR. Both of these antibodies detected the putative DOR protein band at about 59 kDa in extracts of spinal cord and pituitary. In addition, there was also a prominent band at 49 kDa, which was detected by both antibodies in spinal cord tissue, but not in pituitary. These results demonstrate the presence of DOR protein in rat pituitary.

Sun19

***DELTA*-OPIOID RECEPTORS (DOR) IN PORCINE ILEUM.**

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The GI tract is a major site of opioid action, but little is known about opioid receptors expressed in enteric neural plexuses in non-rodent species. We examined the characteristics of DORs in porcine ileum. The DOR ligand ^{3}H-naltrindole bound to a single population of sites in submucosal and myenteric neural membranes with respective K_D = 30 and 70 pM. Electrical stimulation (ES) contracted circular muscle strips. *Delta*$_2$-agonists (DSLET; deltorphin II) inhibited these contractions, but *delta*$_1$-agonists (DPDPE; DADLE) had partial efficacy in this assay. DSLET action was reversed by naltriben (*delta*$_2$-antagonist), but not BNTX (*delta*$_1$-antagonist). In mucosal sheets, ES increased short-circuit current, a measure of ion transport. *Delta*$_1$ - and *delta*$_2$-agonists reduced mucosal responses to ES with similar potencies. These agonist effects were reversed in a subtype specific fashion by BNTX and naltriben. The results suggest that myenteric and submucosal DORs differ in their pharmacological characteristics.

Sun20

CLONING AND CHARACTERIZATION OF A MU OPIOID RECEPTOR FROM BOVINE BRAIN

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Bovine mu opioid receptor cDNA has been cloned and sequenced by probing a bovine brain library with conserved sequences from rat mu receptor cDNA. The partial sequence was extended by PCR to provide the full length clone. The cDNA has an open reading frame of 1203 base pairs (bp) with a 3' untranslated region of 1900 bp and a 5' untranslated region of 265 bp. The protein contains 401 amino acids and has 94% identity with the human and 91% with the rat mu receptor. It has the putative 7 transmembrane domains, characteristic of G protein-coupled receptors and contains 5 potential N-linked glycosylation sites and several potential phosphorylation sites, as well as a putative palmitoylation site. The receptor was stably expressed in HEK-293 cells. The binding profile was that of a typical mu receptor; mu agonists and antagonists, but not delta and kappa ligands, bound with high affinity. Opioid stimulation of [^{3}H]GTPγS binding and inhibition of forskolin-activated adenylyl cyclase, were also found to be highly specific for mu agonists and reversed by naloxone. Evidence is presented indicating that the cloned receptor is the same as the bovine mu receptor previously purified to homogeneity in our laboratory. No evidence was found for genes for multiple mu-type opioid receptors. Supported by NIDA/NIH grant DA00017 to EJS. We thank Ellen Unterwald and H. Kenneth Kramer for their contributions.

Sun21

EXPRESSION OF MU OPIOID RECEPTORS IN HL-60 PROMYELOCYTIC LEUKEMIA CELLS

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Expression of both classical and non-classical mu opioid receptors has been shown in immune cells, and opioid-dependent pathways are reportedly involved in immunomodulation. In this study, using RT-PCR, we examined mu opioid receptor mRNA expression in HL-60 cells with and without treatment with one of three agents -- 10 uM retinoic acid (RA), 1.5 % DMSO or 10^{-7} M 1, 25-dihydroxy Vitamin D_3. Following 5-6 days of treatment, the cells were harvested, and stored at –75^{o}C until total RNA isolation. RT-PCR was performed using the GeneAmp PCR kit (Perkin Elmer) and primers derived from the human brain mu opioid receptor, or beta-actin as a control. The PCR products were analyzed by agarose gel electrophoresis using a 123 bp DNA size marker. Expression of mu opioid receptor was demonstrated in untreated HL-60 cells, and RA-treated cells showed a similar level of mu opioid receptor mRNA expression. However, both DMSO and 1, 25-dihydroxy Vitamin D3 treatment appeared to attenuate mu opioid receptor expression. These results suggest that, while classical mu opioid receptors are expressed in HL-60 promyelocytic leukemia cells, the expression of mu opioid receptors may be modulated by agents that have been shown to induce differentiation. (This work was supported, in part, by RO1 DA 07058 to SLC).

Sun22

CO-IMMUNOPRECIPITATION OF SOLUBLE, ACTIVE MU RECEPTORS FROM RAT BRAIN WITH THE G PROTEINS Go, Gi1AND Gi3.

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Antibodies directed against the C-terminal and the N-terminal regions of the mu receptor were generated in order to identify the G-protein alpha subunits that co-immunoprecipitate with the mu receptor. Both antisera were capable of immunoprecipitating more than 60% of solubilized rat brain mu receptors as determined by [^{3}H]DAMGO saturation binding. The material immunoprecipitated with both antisera was recognized as a broad band with a molecular weight between 60 and 75 kDa when screened in a western blot. GTPγS had an EC50 of 0.4 nM in diminishing [^{3}H]DAMGO binding to the immunoprecipitated pellet. Saturation binding studies indicated that the K_D of the immunoprecipitated material for [^{35}S]GTPγS was 0.2 nM and that the ratio G proteins to mu receptors was 1 to 1. When the material immunoprecipitated with affinity purified antibody was screened for the presence of G-protein alpha subunits, it was determined that Gαo, Gαi1, Gαi3 and to a lesser extent Gαi2, but not Gαs or Gαq/11, were co-immunoprecipitated with the mu receptor. Inclusion of GTPγS during the immunoprecipitation process abolished the co-precipitation of these G proteins.

Sun23

GRK2 AND β-ARRESTIN-1 ARE INVOLVED IN HUMAN DELTA-OPIOID RECEPTOR DESENSITIZATION AND INTERNALIZATION.

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Recently we demonstrated that the human delta opioid receptor (hDOR), endogenously expressed by the neuroblastoma SK-N-BE cells, underwent an agonist-promoted and GRK-mediated phosphorylation, which was tightly correlated with the receptor desensitization (Hasbi et al., 1998). More recently, we demonstrated that this phosphorylation was also required for hDOR internalization.

We show here that the GRK2 is the unique protein kinase member of the GRK family expressed in the SK-N-BE cells. Furthermore, the etorphine treatment leads to a translocation of GRK2 to the plasma membrane. On the other hand, we show also that β-arrestin-1 is the unique arrestin expressed in our model and that it undergoes a similar translocation. Thus, our results strongly suggest that GRK2 and β-arrestin-1 are involved in hDOR desensitization and internalization.

Sun24

COUPLING OF ROD TRANSDUCIN TO THE HUMAN δ OPIOID RECEPTOR IN CHINESE HAMSTER OVARY (CHO) CELLS

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An RT-PCR method was used to characterize the pertussis toxin (Ptx) sensitive G-protein pool in CHO cells. The mRNAs for $G_{\alpha i2}$, $G_{\alpha i3}$, and $G_{\alpha o}$ are present in CHO cells, while $G_{\alpha i1}$ and $G_{\alpha z}$ were not detected. Unexpectedly we also found cDNA clones highly (91%) homologous to the appropriate fragment of the retinal rod transducin ($G_{t\alpha 1}$). The deduced amino acid sequence is 97.4% identical to that of the mouse $G_{\alpha t1.}$ The amino acid differences are conserved substitutions. The presence of the $G_{\alpha t1}$ protein in CHO cells was confirmed by immunoprecipitation. A Ptx insensitive (t1C347S) mutant of $G_{\alpha t1}$ was transfected into CHO cells stably expressing the human δ opioid receptor (hDOR/CHO). No agonist stimulated [^{35}S]GTPγS binding was detected after Ptx treatment in permeabilized hDOR/CHO cells. On the other hand SNC 80 stimulated [^{35}S]GTPγS binding 193% above basal level with an EC_{50} value of 54 nM after Ptx treatment in the co-transfected permeabilized hDOR/t1C347S/CHO cells. The stimulation was antagonized by naltrindole. (Supported by ADCRC and NIDA).

Sun25

IMMUNOLOGICAL CHARACTERIZATION OF DOR-1 IN NG108-15 SUBCELLULAR FRACTIONS.

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In NG108-15 neuroblastoma-glioma hybrid cells most delta opioid binding sites are found in plasma membrane-containing fractions, but some are located in preparations enriched in ER/Golgi, nuclear membranes and nuclear matrix. To determine the relative size of DOR-1 in these 4 subcellular fractions, a C-terminal (CTe)-directed anti-DOR-1 antibody (Ab) was generated and used to characterize them. Membrane proteins from NG108-15 cells were solubilized and purified by wheat germ agglutinin chromatography to isolate glycoproteins. Both the anti-CTe and an anti-NTe Ab detected a single band (68 kDa) in N-acetyl-D-glucosamine eluates indicating that this is intact, monomeric DOR-1. Deglycosylation of the purified proteins showed a decrease in the molecular size of DOR-1 to 38 kDa as detected by anti-CTe and anti-NTe Abs. The 38 kDa molecular size is in close agreement with the predicted mol. wt. based on amino acid sequence (40.5 kDa). DOR-1 was immunoprecipitated from the 4 subcellular fractions with anti-CTe Ab, immunoblotted with a biotinylated form of the same Ab and detected with Extravidin-HRP. In all subcellular fractions a major 68 kDa band was detected. The results suggest that all subcellular fractions contain the intact form of the receptor, but the presence of truncated forms of DOR-1 devoid of epitope remains a possibility.

Sun26

OPIOID RECEPTORS IN ZEBRAFISH BRAIN

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We have recently showed the existence of a gene from an anamniote vertebrate, the teleost zebrafish (Danio rerio), encoding a receptor with high homology to the mammalian delta opioid receptor. We are attempting to identify additional putative opioid receptors from this species. Towards this aim, we have characterized the presence of [3H]diprenorphine binding sites in membranes prepared from zebrafish brain. We observed specific, saturable binding sites for this non-specific opioid ligand, and saturation binding analysis showed the existence of at least two binding sites. Displacement of bound [3H]diprenorphine with various opioid ligands showed very low affinity of these sites for DPDPE and U69593, but moderate affinity for DAMGO and BW373U86. Striking differences in affinity were found between Leu- and Met-enkephalin, and between endomorphins 1 and 2. Thirty-percent of [3H]diprenorphine binding sites were not sensitive to naloxone. These results suggest that opioid receptors, and possibly their endogenous ligands, are different in teleost compared to mammals. Such information will help in elucidating mechanisms underlying opioid ligand-receptor interaction.

Sun27

EVIDENCE FOR CROSS-TALK BETWEEN OPIOID AND MUSCARINIC RECEPTORS IN SH-SY5Y CELLS.

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Pain is a major component of many disease states and its management is critical. Chronic use of opioids often necessitates that dosages be increased to produce equianalgesic effects, often resulting in an increased incidence of side effects. Convergence of opioid and other analgesic pathways (*e.g.* adrenergic, cholinergic) produces a synergistic effect; lower doses of analgesics thus produce fewer side effects. The cellular mechanism(s) underlying these synergistic interactions are not clear. Previous studies have reported that convergence at the second messenger level is responsible for an opioid-mediated permissive increase in bradykinin-induced PI hydrolysis in NG108-15 cells. SH-SY5Y human neuroblastoma cells contain mu, delta and $kappa_3$ opioid receptors coupled to inhibition of cAMP accumulation and muscarinic receptors coupled to stimulation of IP3 formation. While opioids alone have no effect on IP3 formation, the muscarinic agonist, carbachol, strongly stimulates IP3 formation. However, concomitant addition of the mu agonist, DAMGO (1 μM) or the $kappa_3$ agonist, NalBzoH (100 nM) significantly enhanced the PLC activation mediated by carbachol. This neuromodulatory role for opioids was dose-dependent, blocked by opioid antagonists, and insensitive to pertussis toxin pretreatment. The opioid mediated synergy with carbachol was sensitive to treatment with $G_z\alpha$ antisense (carbachol was not) and the PKC inhibitor, chelerythrine, suggesting that carbachol activation of PKC is necessary before opioid-mediated IP3 formation is measurable. We propose that one possible mechanism of this synergy is that PKC phosphorylation of the GAP protein RGSZ allows $G_z\alpha$ to mediate the opioid effect. Supported by grant DA 10738 to KMS.

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(+)PENTAZOCINE ((+)PTZ) AND PREGNENOLONE SULFATE (PS) MODULATE INTRACELLULAR CA^{2+} CONCENTRATION VIA SIGMA-1 RECEPTORS IN NG-108 CELLS.

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(+)PTZ and endogenous neurosteroid PS have been shown to interact with sigma-1 receptors in several behavioral paradigms involving learning and memory in animals. As Ca^{2+} plays an important role in learning and memory, we sought to examine the effects of (+)PTZ and PS on intracellular Ca^{2+} concentration in NG-108 cells using laser scanning confocal microscope. (+)PTZ and PS, at nanomolar concentrations which were of no effect by themselves, potentiated the bradykinin-induced increase in cytosolic free Ca^{2+} concentration ($[Ca^{2+}]$cyt). This effect of PS was antagonized by a selective sigma-1 antagonist NE-100, but was not affected by MK-801 or (+)bicuculline. A 21mer antisense oligodeoxynucleotide (AS ODN) directed against cloned sigma-1 receptors also blocked this action caused by (+)PTZ and PS. The NG-108 cells were depleted of the endoplasmic reticulum Ca^{2+} stores by treating the cells with thapsigargin, bradykinin, and caffeine. Cells were examined for the effects of (+)PTZ and PS on the depolarization (75 mM KCl)- induced changes in $[Ca^{2+}]$cyt. (+)PTZ and PS in nM caused an inhibition of the KCl-induced increase in $[Ca^{2+}]$cyt. These were blocked by the 21mer AS ODN and was abolished by a pretreatment of cells with pertussis toxin. Ca^{2+} signaling via sigma-1 receptors may therefore represent a new mechanism affecting signal transduction in cells. [Supported by IRP, NIDA/NIH]

Sun29

DIFFERENTIAL COUPLING BETWEEN CLONED δ-RECEPTORS AND CLONED OR NATIVE Ca^{2+} CHANNELS.

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Voltage-gated channels control Ca^{2+} entry into different cellular compartments. Regulation of these channels affects diverse processes including gene expression, action potential duration and neurotransmitter release. Opioids inhibit N- and P/Q-type Ca^{2+} channel activity leading to reduced neurotransmitter release. There is also evidence for L-type channel modulation by opioid receptors expressed in GH_3 cells. We compared the characteristics of cloned δ receptor coupling to native Ca^{2+} channels in GH_3 cells and cloned P/Q-type channels expressed in HEK cells. Ba^{2+} currents through Ca^{2+} channels were recorded from GH_3 cells stably expressing μ and δ receptors and HEK cells stably expressing δ receptors and transiently expressing rat Ca^{2+} channel α_{1A}, β_{2a}, and $\alpha_2\delta$ subunits. The threshold and peak activation of P/Q-type channels (-20 and 10 mV) occurred at more depolarized potentials than did activation of Ba^{2+} currents recorded from GH_3 cells (-50 and 0 mV). The activation of δ receptors reduced Ba^{2+} current amplitude which was reversed by depolarizing prepulses. The half maximal voltage for this effect was 0 mV for P/Q type channels and -13 mV for GH_3 Ca^{2+}channels. We also examined the rate of reinhibition of the two types of Ca^{2+} channels after voltage-dependent reversal of inhibition. Recovery of inhibition occurred with time constants of 72 and 67 ms for P/Q and GH_3 Ca^{2+} channels following a prepulse to +60 mV. These data suggest that similar processes are involved in coupling opioid receptors to P/Q-type channels and Ca^{2+} channels expressed by GH_3 cells.

Sun30

GENETIC MODIFICATION OF PHOSPHOLIPASE C BETA3 EXPRESSION ALTERS CELLULAR RESPONSES TO DAMGO

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Mu opioids regulate a number of signaling pathways, including voltage-sensitive Ca^{2+} channels and phospholipase C (PLC), the interaction of which may affect the behavioral response. Adult mice lacking the beta3 isoform of PLC were found to exhibit up to a 10-fold decrease in the antinociceptive ED_{50} values for morphine compared with their wildtype cohorts. In order to establish a possible cellular basis for this behavioral observation, opioid regulation of voltage-sensitive Ca^{2+} channels in primary sensory neurons (dorsal root ganglion, DRG) was tested using the whole-cell variation of the patch clamp technique. DAMGO (100, 300, 1000 nM) produced a concentration-dependent reduction in peak Ca^{2+} current magnitude in both wildtype and PLC beta3-deficient neurons. Consistent with the behavioral findings, the specific mu agonist DAMGO effected a greater whole-cell current inhibition in a larger proportion of neurons isolated from the PLC beta3-null mice than from the wildtype. Reconstitution of recombinant PLC protein back into PLC beta3-deficient DRG neurons reduced DAMGO responses to those of wildtype neurons. In neurons of both genotypes, activation of protein kinase C with phorbol esters markedly attenuated DAMGO-mediated Ca^{2+} current inhibition. These data demonstrate that PLC beta3 constitutes a significant pathway involved in negative modulation of mu-opioid responses, perhaps via protein kinase C, in neurons that constitute the first link in nociceptive pathways. *Supported in part by NIH grants DA07232 (GMS), DA03742 (JMB), GM53162 (DW), DA10514 (RAG).*

Sun31

THE INVOLVEMENT OF PROTEIN-TYROSINE PHOSPHORYLATION IN THE OPIOID RECEPTOR SIGNALING.

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We have previously shown that mu-opioid receptors (MOR) regulate the cytoskeleton of neurons via PKC and tyrosine kinase-dependent protein phosphorylation. In studying the signal transduction pathways of opioid receptor in neurons, we have provided evidence for a novel coupling of MOR to tyrosine kinase activation. We have identified two kinases that the specific mu-agonists activate as the cytosolic tyrosine kinases FAK and Src (JNR, 50: 1-11, 1997). To investigate whether this pathway functions for the delta-opioid receptors we employed NG108-15 cells. The delta-opioid agonist [D-Ala2,D-Leu5]-enkephalin (DADLE) elicited a time--dependent increase of tyrosine phosphorylation of several proteins, indicating that it is coupled to tyrosine kinases. The receptor is also known to couple to PKC with downstream activation of MAPK. Thus we used this paradigm and investigated whether MAPK activation was dependent on upstream Src activation. We now show in NG108 cells that DADLE activates MAPK in a time-dependent manner and that the activation is inhibited by the Src specific inhibitor PP1. PTX and the delta-antagonist naltrindole were also inhibitory. We also tested whether the coupling of the opioid receptors to Src crosstalks to the other major coupling of opioid receptors, namely the sensitization of adenylate cyclase activity after long-term treatment with opioids. Thus, using whole cell AC assays we found that 18 hr of DADLE or morphine treatment caused significant increases in forskolin-activated AC activity. The increase was inhibited by PTX, naloxone, and most importantly by the Src inhibitor PP1. Our data show for first time that Src is an important effector of both acute actions of the delta-opioid receptor and opiate withdrawal in NG108 (supported by BRF to DM; MM is a Fogarty Fellow).

Sun32

MORPHINE TRANSLOCATES CALMODULIN TO CELL NUCLEUS AND REGULATES THE PHOSPHORYLATION OF CYCLIC-AMP RESPONSE ELEMENT BINDING PROTEIN (CREB)

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In mu opioid receptor (OP_3) transfected HEK (HEK-OP_3) and SH-SY5Y cells, CaM content in plasma membranes was decreased, wherease in the nucleus was increased upon morphine stimulation. Morphine activated CaM translocation from cell membrane to nucleus was blocked by naloxone and pertussis toxin pretreatment. Morphine stimulation of OP_3 enhanced the nuclear CREB phosphorylation, which was inhibited by CaM inhibitor W7 and CaM kinase inhibitor KN62. Cyclosporin A, a CaM dependent phosphatase (PP-2B or calcineurin) inhibitor, had little effect on maximal pCREB formation, but prevented the time-dependent decline in pCREB formation after morphine stimulation. Moreover, Ca^{++} influx and PKC also play a role in morphine activated CREB phosphorylation. These results suggest that morphine translocates CaM to nucleus and activates both CaM dependent protein kinase and phosphatase, therefore, regulating the phosphorylation of CREB. Supported by grant DA04166.

Sun33

OPIOID-MEDIATED ACTIVATION OF MAPK DOES NOT REQUIRE RECEPTOR ENDOCYTOSIS

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Opioid receptors, members of the G-protein coupled receptor (GPCR) superfamily, are targets of endogenous opioid peptides and opiate drugs. Similar to other G-protein coupled receptors, opioid receptors are regulated by agonist-dependent rapid internalization and down-regulation. Recently, it has been shown that blocking an agonist-mediated internalization by overexpression of mutant beta-arrestin or dynamin suppresses the phosphorylation and subsequent activation of mitogen-activated protein kinases (MAPK). This has led to the proposal that agonist-induced internalization of receptors is required for the activation of MAPK. In order to test this we used ligands that are unable to induce receptor internalization and internalization-deficient mutants of opioid receptors (delta & mu). When examined for agonist-mediated phosphorylation of MAPK, we find that morphine that does not induce receptor internalization is able to stimulate MAPK phosphorylation. Moreover, receptors which fail to undergo agonist-dependent internalization also are able to efficiently induce agonist-dependent phosphorylation of MAPK. Taken together these data suggest that activation of MAPK is not a receptor internalization dependent phenomenon.

Sun34

TRANSLOCATION OF DYNAMIN FROM CYTOSOL TO PLASMA MEMBRANE IN BRAIN OF MORPHINE-TOLERANT RATS.

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The results in the literature reporting regulation of opioid receptors in morphine-treated rats are controversary. Using subcellular fractionation to prepare highly purified synaptic plasma membranes (SPM) and microsomes (MI) from morphine-dependent rat brains we have recently detected an up-regulation of the mu-opioid receptors (MOR) in the MI, representing intracellular binding sites (Bozo et al., 1996). Evidence exists that GPCRs internalize via the clathrin-coated vesicle-mediated endocytic pathway. Moreover, the involvement of dynamin (a major component and markers of the clathrin-mediated endocytic pathway) in GPCRs internalization has been demonstrated, while it has also been shown that GPCRs can utilize another endocytic dynamin-independent pathway. In the present study, we have studied the regulation of dynamin by chronic morphine treatment using subcellular fractionation by immunoblot. The level of dynamin decreased by about 20% in the MI, with a concomitant increase in SPM, without modifications of the total amount of this protein in the brain homogenates compared to control rats. These results, suggest that chronic morphine treatment induces translocation of dynamin from intracellular pools to plasma membranes in rat brain.

Bozo et al. (1996) Cell Biol. Int. 20 : 240.

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Sun35

REGULATORY CHANGES INDUCED BY SUSTAINED MU-OPIOID AGONIST TREATMENTS IN RAT BRAIN

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Repeated injection of the μ-opioid receptor specific peptides, DAMGO (Tyr-D-Ala-Gly-MePhe-Gly-ol) and its chloro-methyl ketone derivative DAMCK (Tyr-D-Ala-Gly-MePhe-CH_2Cl) at 10 μg for 8 days twice daily *icv* resulted in the development of analgesic tolerance measured in the tail-flick assay. The number of cell surface [^{3}H]DAMGO binding sites decreased by 20-25% compared to that in control rats. Desensitization was not observed by measuring DAMGO-stimulated [^{35}S]GTPγS binding. When analgesic tolerance was induced by morphine injection, the number of surface μ-opioid binding sites was not altered, however increased by 50% in a 'light membrane' fraction sedimented at 100,000 x g. These up-regulated intracellular sites displayed enhanced coupling to G-proteins measured with DAMGO-stimulated [^{35}S]GTPγS binding. Affinity labeling with [^{3}H]DAMCK followed by SDS-PAGE and fluorography indicated that it is the 50 kDa binding subunit and not a fragment of μ-opioid receptors that participated in receptor trafficking. Supported by TÉT-564 and OTKA T-016084 research grants.

Sun36

SUSTAINED MORPHINE EXPOSURE RESULTS IN ELEVATED DYNORPHIN CONTENT IN THE LUMBAR/SACRAL SPINAL CORD OF ICR, BUT NOT 129 SvEv, MICE: POSSIBLE ROLE IN OPIOID ANTINOCICEPTIVE TOLERANCE

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Opioid tolerance and neuropathic pain states share a number of pharmacological features including reduced opioid potency and efficacy. Based on our previous studies that demonstrated increased levels of dynorphin in the lumbar/sacral spinal cord of animals with experimental nerve injury, we hypothesized that an elevation of spinal dynorphin levels also contributes to the opioid tolerant state. ICR mice were implanted with a s.c. morphine pellet (75 mg). Seventy-two hours later, mice were either injected with i.th. morphine and tested for antinociception or were sacrificed and their lumbar/sacral spinal cord tissue assayed for dynorphin content. Morphine pellet treatment resulted in a significant rightward shift in the i.th. morphine antinociceptive dose-response curve (compared to naïve or placebo pelleted mice). Morphine pelleted mice also had significantly elevated levels of dynorphin in the lumbar/sacral spinal cord compared to controls (1612 pg/mg protein versus 1326 pg/mg protein, $t(25)=3.0$, $p<0.006$). In contrast, 129 SvEv mice did not display antinociceptive tolerance nor elevated dynorphin levels when implanted with a s.c. morphine pellet. These data suggest that elevated spinal dynorphin levels contribute to the development of morphine antinociceptive tolerance. The lack of elevated spinal dynorphin levels in the 129 SvEv mice may partially explain why these mice do not develop tolerance to sustained morphine exposure.

Sun37

PERIAQUEDUCTAL GREY NEURONS WITHDRAWN FROM OPIOIDS RESPOND DIFFERENTLY TO OPIOIDS.

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The periaqueductal grey (PAG) is involved in opioid withdrawal. Using perforated-patch voltage clamp recordings we examined the effect of opioids on PAG neurons in brain slices from opioid dependent Sprague Dawley rats and C57B16/J mice. In vehicle treated animals met-enkephalin (30μM) induced an outward current in opioid sensitive neurons which reversed polarity near the expected E_K (rats: -110mV, mice: -112mV). However in dependent rats or mice which were spontaneously withdrawn (slices washed for at least 1hr with morphine free ACSF) the met-enkephalin induced outward did not reverse polarity at membrane potentials positive of -140 mV. Similarly, as was previously shown with intracellular recordings, when slices from dependent rats were maintained in morphine (5μM) and then withdrawn with naloxone an inward current was induced which did not reverse polarity at membrane potentials positive of -140 mV. This implies that opioids activate a potassium conductance and couple to a novel conductance in dependent animals. We examined the possibility that increased adenylyl cyclase activation could contribute to this novel conductance. In vehicle treated mice forskolin (10μM) induced an inward current at all potentials examined below -60 mV. In the presence of forskolin, the met-enkephalin induced outward current no longer reversed polarity at membrane potentials positive of -140 mV supporting this possibility.

Sun38

CHRONIC MORPHINE MODULATES THE SYNAPTIC INPUTS TO INTERNEURONS, BUT NOT MEDIUM SPINY NEURONS IN THE NUCLEUS ACCUMBENS.

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The nucleus accumbens is a critical brain region for mediating the rewarding aspects of drugs of abuse. It may also be involved in drug craving, self-administration, and withdrawal. One of the consequences of repeated administration of opiates is an upregulation of cAMP signaling pathways, and one of the major functions of cAMP signaling pathways is the modulation of synaptic activity, primarily by promoting the release of neurotransmitter from nerve terminals. We investigated whether chronic morphine treatment in rats would induce an upregulation of adenylyl cyclase pathways in several different synapses of the nucleus accumbens. We found that activation of adenylyl cyclase increased, and activation of mu-opioid receptors decreased, neurotransmitter release in all synapses tested. However, chronic morphine treatment did not alter these responses in either the excitatory or inhibitory synapses onto nucleus accumbens medium spiny neurons. In contrast, chronic morphine treatments greatly increased the potentiating effects of adenylyl cyclase activation on inhibitory synapses to the large, aspiny interneurons. Chronic morphine also increased the inhibitory effects of mu-opioid receptor activation at these synapses. Hence, chronic morphine treatment selectively upregulates the cAMP cascade in the inhibitory synaptic inputs to aspiny interneurons, and does not affect the synaptic inputs to medium spiny neurons.

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Sun39

ANTAGONISM BY PYRROLIDINE DITHIOCARBAMATE, AN INHIBITOR OF NUCLEAR FACTOR-κB, OF MORPHINE DEPENDENCE. A HYPOTHESIS FOR THE DEVELOPMENT OF OPIATE DEPENDENCE.

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To investigate the role of nuclear factor-κB (NF-κB) in the development of opiate withdrawal, the effect of pyrrolidine dithiocarbamate (PDTC), an inhibitor of NF-κB activation, was studied on acute opiate withdrawal induced by morphine in vitro. After a 4 min in vitro exposure to morphine, a strong contracture of guinea pig isolated ileum was observed after the addition of naloxone. PDTC ($1x10^{-8}$-$5x10^{-8}$-$1x10^{-7}$ M) was able to reduce the naloxone-induced contracture after exposure to the opioid agonist in a concentration-dependent fashion. The results of the present study indicate that NF-κB is involved in the development of opiate withdrawal thus extending and explaining previous papers performed with dexamethasone and selective arachidonic acid metabolites inhibitors.

Sun40

EXPRESSION OF GDNF mRNA IN SPINAL CORD, STEM AND CORTEX DURING MORPHINE WITHDRAWAL IN RATS

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The recent studies have demonstrated that certain neurotrophic factors such as brain-derived neurotrophic factors and related neurotrophins can modify opiate action in the mesolimbic dopamine system. The present study initiated to observe the expression of GDNF gene in spinal cord, stem and cortex during morphine dependence and withdrawal in rats. The level of GDNF mRNA was assayed by reverse transcription polymerase chain reaction (RT- PCR) with the actin mRNA as an internal control. The basic expressions of GDBF mRNA in spinal cord, stem and cortex were lower in normal adult rats, the levels of GDNF mRNA in spinal cord and stem were decreased, and GDNF mRNA level in cortex was slightly increased during morphine dependence. While the GDNF mRNA levels in spinal cord, stem and cortex were increased significantly at 1 hour and at peak 2 hour after injection of naloxone during morphine withdrawal. The administration with L-N-nitric arginine methylester, an inhibitor of nitric oxide synthase, the GDNF mRNA levels in spinal cord and stem were not different from those of the morphine withdrawal animals, but the GDNF mRNA level in cortex decreased compared with those of morphine withdrawal animals. The beta-actin mRNA levels were not changed in each group. Therefore, the continuous expressions of GDNF mRNA induced by morphine withdrawal could contribute to the neuroadapatation associated with chronic morphine use.

Sun41

CHRONIC ADMINISTRATION OF MORPHINE ELEVATES SUBSTANCE P ENDOPEPTIDASE LIKE ACTIVITY IN RAT

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The activity of substance P endopeptidase (SPE) like enzyme was measured in CNS tissues of male rats after chronic administration of morphine. Four groups of rats (S/D 200g), seven rats in each, were treated with morphine (s.c., 10 mg/kg) saline respectively. When tolerant is completely developed, one of morphine group and one of saline were given naloxone (s.c., 2mg/kg). Withdrawal signs were observed. The enzyme activity was measured in extracts of CNS tissues by following the conversion of synthetic substance P using a radioimmunoassay specific for the product, SP_{1-7}. In ventral tegmental area (VTA), spinal cord, substantia nigra and central gray, a significant increase in enzyme activity was observed in the withdrawal group while the tolerant rats showed increased SPE like activity only in the VTA and substantia nigra. A negative correlation between the SPE like activity and the abstinence intensity was found in some of the withdrawal signs, e.g. wet dog shaking ($p<0.05$). The result suggests that the elevated SPE activity is responsible for enhansive release of the heptapeptide in morphine tolerant and withdrawal rats, affirming the modulatory or regulative role of SPE in these states of morphine dependence.

Sun42

INHIBITION OF CaMK II IN RAT HIPPOCAMPUS ATTENUATES MORPHINE TOLERANCE AND DEPENDENCE

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The involvement of learning and memory in the development of opiate addiction has been long speculated. We demonstrated that inhibition of CaMKII by intrahippocampal dentate gyrus administration of KN-62 and KN-93 to rats significantly attenuated the tolerance to the analgesic effect of morphine and the abstinence syndrome precipitated by opiate antagonist naloxone. In contrast, both KN-04 and KN-92, the inactive structural analogues of KN-62 and KN-93, failed to attenuate morphine tolerance and dependence, indicating that the observed effects of KN-62 and KN-93 are mediated through inhibition of CaMKII. Administration of CaMKII antisense oligonucleotide into rat hippocampal dentate gyrus, which decreased the expression of CaMKII specifically, also attenuated morphine tolerance and dependence, while the corresponding sense oligonucleotide of CaMKII did not exhibit such inhibitory effect. The KN-62 treatment abolished the rewarding properties of morphine as measured by the conditioned place preference. These results suggest that hippocampal CaMKII is critically involved in the development of morphine tolerance and dependence, and inhibition of this kinase may have therapeutic benefit in the treatment of opiate tolerance and dependence.

Sun43

DEXTROMETHORPHAN EFFECTIVELY PREVENTS ADVERSE EFFECTS OF CHRONIC MORPHINE TREATMENT IN MOTHER RATS TO THE NEXT GENERATION

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Children born from morphine or heroin addicted mothers have been shown to suffer acute withdrawal syndrome after birth and develop a long-term neurobiological sequels. Our previous study has shown that combined prenatal- and postnatal-exposure to morphine induced a down-regulation of brain NMDA receptors but with higher activity in hippocampal CA1 area on postnatal day (PND) 14. This implied that the NMDA receptor may be over-activated by chronic morphine treatment. In the present study, a NMDA receptor antagonist, dextromethorphan (DM, a safe clinical antitussive drug) was co-administered with morphine on mother rats. Adult female S.D. rats received morphine (M; 2 mg/kg, s.c., bid) or M+DM (same doses as M) for 7 days before conception. After conception the doses of M or M+DM were increased by 1 mg/kg per week. After delivery of the newborn rats, the doses were increased by 1 mg/kg every 2 weeks until 30 days. Antinociceptive activity was assessed by tail- flick test. Physical dependence was determined by the precipitated withdrawal signs induced by naloxone. We found chronic treatment of morphine in mother rats increased the death rate, decreased the body weight (PND30 & PND60) of postnatal rats. Both the mother and offspring rats developed tolerance and dependence to morphine. DM effectively prevented these adverse effects of chronic morphine in both mother and offspring rats.

Sun44

STRIATAL MODULATION OF OPIOID-INDUCED FEEDING: ANATOMICAL MAPPING STUDIES

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Given the evidence emerging from our previous studies that stimulation of mu opioid receptors within the nucleus accumbens (Acb) preferentially enhances feeding on palatable food containing sucrose and fat, it is proposed that this brain area may be associated with the rewarding characteristics of food by modulating taste and macronutrient preference. The present study was designed to further explore the nature of the involvement of intrastriatal opioids in feeding behavior, such as the location of effective feeding subregions of the striatum and the brain neural circuits involved in opioid-mediated hyperphagia. In the first part of the study, we conducted a microinfusion mapping study of feeding behavior in response to the stimulation of mu receptors within several striatal subregions. In the second part of the study, detection of the expression of the immediate early gene, c-Fos, was used to examine brain areas activated following intra-striatal microinfusion of mu opioid agonist-DAMGO. The microinjection mapping study demonstrated a broad anatomical gradient within striatum, with more sensitivity in the ventral and lateral striatum and much less sensitivity in dorsal striatum. The Fos mapping study revealed a similar profile of neural circuits involved in the hyperphagia induced by stimulation of the mu receptors in either medial or lateral shell. Activation of mu receptors in the dorsal striatum induced, to much less extent, Fos expression in several regions where Fos induction was also observed when mu receptors were stimulated in the ventral striatum. Together, these results suggest that the involvement of mu receptors within the striatum in palatable feeding is not region-specific, instead it may have some relevance to the patch-like distribution of mu receptors across this region.

Sun45

BRAIN AREAS INVOLVED IN LONG LASTING SENSITIZATION TOWARDS MORPHINE

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Certain brain areas respond to morphine application with an increased c-fos expression if the rats were repeatedly injected with the drug. We now examined the time course and the dose dependency of this process. The rats were pretreated with ascending morphine doses up to 100 mg/kg per day for ten days. Thereafter, they were left untreated for zero, one, two**,** four or eight weeks. A test dose of morphine (0, 10, 50 or 100 mg/kg) succeeding this period of abstinence was able to induce c-fos in the dorsal and lateral striatum and in several limbic areas as well as in regions closely associated with the limbic system. This expression pattern was detected by *in situ* hybridization for up to eight weeks after the end of the morphine pretreatment. Quantification of the results revealed that there was a slight but significant increase in signal intensity in response to a constant test dose for up to four weeks after cessation of the subchronic morphine application. The morphine test doses needed to obtain the maximal response differed widely between the different brain areas, ranging from 10 mg/kg in the dorsal striatum to 100 mg/kg in the lateral septum. Taken together, this study demonstrated that there is long lasting sensitization to morphine at the molecular level and that alterations in the responsiveness to the opiate seem to continue even after cessation of the subchronic application.

Sun46

THE EFFECT OF ANTISENSE TO PROTEIN KINASE A (PKA) ON OPIOID ANALGESIA, TOLERANCE AND μ-OPIOID RECEPTOR REGULATION.

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Studies suggest that PKA is involved in the chronic effects of opioids. In this study, the effect of an antisense oligodeoxnucleotide (ODN) to PKA on opioid analgesia, tolerance and receptor downregulation was determined. Mice were injected ICV and IT with saline, a mismatch ODN or an ODN to the catalytic α subunit of murine PKA. Mice were injected once a day (30μg/each site) for 4 days. On the 2nd day of treatment, an infusion of etorphine (250μg/kg/day,s.c.) was begun. Controls were implanted with a placebo pellet. The pumps and pellets were removed 48hr later, and 16hr later, mice were tested for s.c. etorphine-induced analgesia or whole brain μ-opioid receptor binding determined. Etorphine increased the ED_{50} by 7.7-fold and antisense increased the ED_{50} by 2-fold. In mice treated with both etorphine and antisense there was a 4.5-fold increase in the ED_{50}. Antisense had no effect on the 30% decrease in μ opioid receptor density produced by etorphine. Our results indicate that antisense knockdown of PKA decreases opioid potency and opioid tolerance without effecting μ opioid receptor downregulation. These results suggest that the cAMP-PKA pathway plays an important role in the functional potency of opioid analgesics, but does not impact on opioid receptor regulation *in vivo.*

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THE EFFICACY OF JIE-DU-LING ON OPIATE ADDICTION IN RAT

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Though there are now many ways to treat addiction, few of them are satisfactory. Hence it is necessary to proceed to develop more ideal drugs and therapies to facilitae the treatment of narcotic addiction. As a result of years' experimental study, we have prepared a mixture injection, temporarily named as Jie-Du-Ling. It has been tried to cure more than 100 patients addicted to heroin, dolantin etc. with definite and reliable results and welcomed by the recipiens.

The present method is acceptable and reliable for establishing the model of opiate addiction in rats. Continued use of morphine for 5 days followed by administration of M_{5050} for elicitation can successfully induce withdrawal symptoms. The experimental period is short, the amount of morphine consumed is relatively little, yet the rate of success is high. Besides, the withdrawal symptoms observed can be quantified to evaluate the effects of disintoxicating drugs.

The results revealed the Jie-Du-Ling produced significantly enhanced effects, in which the withdrawal symptoms were almost complely controlled, i.e. by 97 %. Two rats in the group were irritable and jumped in the 1st 15 min before they calmed down becoming symptomless. The other rats were all free from symtoms throughout the experiment.

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EFFECTS OF ROTUNDINE ON MORPHINE TOLERANCE AND DEPENDENCE

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Rotundine, is a l-optical isomer of tetrahydropalmatine, which is an active alkaloid isolated from the stem tubers of the chinese medicinal herb Corydalis ambigua. The effects of rotundine on morphine tolerance and dependence were investigated. We studied the analgesic effect of morphine in chronically morphine treated mice with a hot-plate test. Mice were given 10mg/kg of morphine every day for 9 days. The analgesic effect of morphine was decreased each day, until the 6th day morphine had no effect at all. 20mg/kg rotundine s.c. significantly inhibited the tolerance development. Physical dependence was evaluated in male rats, injected chronically with increasing dose of 10mg/kg morphine every day for 6 days. The behavioral signs of withdrawal were observed after injection of naloxone. Rotundine 20mg/kg s.c. suppressed the signs of physical dependence in rats. The psychic dependence was assessed by the conditioned place preference in male mice. 10mg/kg morphine administered i.p. induced a clear conditioned place preference. When 20mg/kg rotundine was coadministered s.c., the effect of morphine was significantly attenuated. The present study showed that rotundine suppressed morphine tolerance.

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INHIBITION OF AGMATINE ON OPIOID PHYSICAL DEPENDENCE.

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Agmatine is an endogenous ligand of imidazoline receptors (I-R). When given subcutaneously, agmatine has preventive and treatment effects on physical dependence of mice and rats *in vivo* as well as of guinea pig ileum longitudinal smooth muscle *in vitro* induced by pretreatment of morphine. Although inhibiting morphine physical dependence of rats, agmatine has no affinity to opioid receptors and has no influence on down-regulation and decrease in affinity of opioid receptors to ligands which occurs in the formative process of morphine physical dependence. Agmatine inhibits NOS activity in competitive manner and by activation of I-R, which are related to inhibitory effects of agmatine on physical dependence in mice. The pharmacological effects of agmatine as mentioned above could be blocked by idazoxan, a selective I-R antagonist.

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EFFECTS OF ACTH AND DEXAMETHASONE ON SECOND ORDER SCHEDULE OF INTRAVENOUS MORPHINE SELF-ADMINISTRATION BEHAVIOR BY RHESUS MONKEY

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There is a substantial body of work suggesting a positive relationship between plasma glucocorticoid levels and self-administration behavior of abused drugs (such as cocaine). This study investigated whether altering plasma glucocorticoid level by directly injecting ACTH and dexamethasone could affect morphine self-administration behavior. One rhesus monkey was trained to press lever for intravenous morphine self-administration on a daily 4 hr session, second order schedule FI10min (FR10:S), each 10 responses within 10 min was reinforced with 5s red stimulus light, after 10 min, the first 10 responses was reinforced with one morphine injection (0.25 mg/kg) which was paired with the same red stimulus light. ACTH and dexamethasone were given through the catheter 15min before test session. The results showed that ACTH (0.5, 1.0, 2.5 IU/kg) could significantly increase the total response rates, total morphine intake and spontaneous activity, dexamethasone only at high dose (1.5mg/kg) could increase total response rate. Dexamethasone also showed prolonged effect on morphine self-administration at doses of test range (0.1-1.5 mg/kg), total response rates were significantly decreased in the following day after dexamethasone treatment. The result suggested that ACTH-induced endogenous release of glucocorticoid could increase morphine self-administration behavior

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THE COMBINED USE OF SCOPOLAMINE, NALTREXONE AND NALOXONE AS A RAPID, SAFE AND EFFECTIVE DETOXIFICATION TREATMENT FOR HEROIN ADDICTS

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The efficacy of a rapid detoxification schedule for heroin addicts by scopolamine combined with opiate receptor antagonists was evaluated. The life signs such as temperature, blood pressure, ECG, heart rate, respiratory rate and blood oxygen saturation were monitored during process of rapid detoxification treatment. The naloxone challenge test, withdrawal symptoms scale and naltrexone maintenance were used to evaluate the efficacy of the rapid detoxification. Results showed that the withdrawal symptoms of 84 cases of heroin addicts induced by naltrexone and naloxone were controlled completely under scopolamine administration during the 6-8 hours treatment period, although the naloxone challenge test was able to only induce the slight and transient withdrawal symptoms in second day, and all patients were transferred rapidly to naltrexone maintenance. The temperature in 19 cases was arisen, and the heart rate, blood pressure and respiratory rate were increased, however, the blood oxygen saturation was still stable after oral-administration naltrexone and continuous injection with naloxone during the detoxification, the positive of blood test in gastric fluid were observed in 3 cases during the detoxification. The follow-up data showed that the average maintenance time of naltrexone was 2.6 months, and the percent of drug free was 21% in six months after the detoxification in 84 cases. The result suggests that combined use of scopolamine, naltrexone and naloxone be a rapid, safe and effective detoxification treatment protocol for heroin addicts.

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18-MC ALTERS THE LOCOMOTOR AND DOPAMIME RESPONSES TO ACUTE AND CHRONIC MORPHINE.

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18-methoxycoronaridine (18-MC), a synthetic *iboga* alkaloid congener, was developed with the goal of making available a safer ibogaine-like agent. To further investigate the basis for 18-MC's potential anti-addictive actions, the present study determined the effects of 18-MC (40 mg/kg, IP, 19 hr pretreatment) or vehicle (VEH) on the expression of locomotion induced by morphine (MOR) (0, 1.25, 5, 10, 20, 30 mg/kg, IP) in rats treated either acutely or chronically (5 daily injections of 20 mg/kg, IP) with MOR. In addition, the effects of 18-MC (40 mg/kg) were also assessed on the dopaminergic response in the nucleus accumbens to MOR (20 mg/kg) in chronic MOR-treated rats. Compared to VEH controls, 18-MC potentiated MOR-induced locomotion in both acute MOR rats and chronic MOR rats that did not sensitize during chronic MOR treatment. Interestingly, in rats that did sensitize during chronic treatment, 18-MC had little effect or attenuated MOR-induced locomotion. In addition, 18-MC attenuated MOR-induced dopamine release in the accumbens in chronic MOR-treated rats, an effect that may be related to the extent of sensitization induced by chronic MOR treatment. The present findings demonstrate that the effects of 18-MC on MOR-induced locomotion depend on the sensitized state of the animal following chronic MOR treatment. It is proposed that these behavioural effects are related to 18-MC's ability to block the expression of MOR-induced sensitization of accumbal dopamine release implicated in the development and maintenance of drug addiction. (Supported by NIDA grant DA 03817).

Sun53

WHERE DOES NALOXONE ACT TO BLOCK COCAINE-INDUCED CONDITIONED PLACE PREFERENCE?

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Evidence in the literature suggests opioid involvement in cocaine reward. There is an issue of naloxone itself producing aversion, however. We administered naloxone systemically, and found that both 1 mg/kg and 0.5 mg/kg naloxone (S.C.) block cocaine (10 mg/kg, I.P.) conditioned place preference. The high dose alone produced a robust place aversion, unlike the low dose. This led us to search for the locus of action of both naloxone aversion and non-aversive blockade of cocaine reward. There is evidence of ventral pallidum (VP) involvement in reward action. As such, we began our investigation with local administration in this region. Preliminary data suggest that neither 1 nor 10 micrograms naloxone intra-VP produce place aversion. (Supported in part by NIDA Grants DA05010 and DA09359; PS was supported by a NIDA training grant T32DA07272)

Sun54

MU OPIATE RECEPTOR KNOCKOUT MICE HAVE DECREASED RESPONSES TO ETHANOL.

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Responses to ethanol (ETOH) were examined in wildtype (WT) and both heterozygote (HET) and homozygote (HOM) MU opiate receptor knockout mice. Voluntary ETOH consumption, ETOH conditioned place preference (CPP) and ETOH-stimulated locomotion were examined. Voluntary ETOH consumption was examined in the home cage using a free-access paradigm in individually housed mice. Concentrations of ETOH were presented in ascending order for 2-3 days (2%, 4%, 8%, 12%, 16%, 24%, and 32%). Locomotor activity was determined after administration of 0.0, 0.5, 1.0 and 2.0 g/kg IP ETOH. CPP was determined after 4 pairings of 2.0 g/kg IP with the initially nonpreferred side. In all experiments, both HET and HOM MU opiate receptor knockout mice had diminished responses to ethanol. Thus, even a partial reduction of MU opiate receptor gene expression is sufficient to attenuate ETOH reward and locomotion.

Sun55

VOLUNTARY RUNNING MODULATES ETHANOL PREFERENCE.

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The genetically inbred Lewis rat strain is prone to self administer several addictive drugs including ethanol, and to run in running wheels in a compulsive manner. In this study we analyzed ethanol preference in Lewis rats with free access to running wheels.
Initially animals were given access to running wheels to habituate them to running during two weeks. After this, the animals were trained to self administer ethanol in a two-bottle choice paradigm in which they had free access to ethanol and water but not running wheels. In the next phase, animals were deprived from ethanol but given free access to running wheels during one, two or four weeks. This was followed by one week of free access to both running wheels and ethanol. In the control groups, the animals had the same access to ethanol but not to running wheels. During the last week of the experiment the impact of voluntary running on ethanol preference was analyzed. We compared ethanol preference in the groups of animals with access to both running wheels and ethanol to the groups of animals with access to ethanol but not running wheels. From our experiments, we conclude that voluntary running in running wheels can modulate ethanol preference.

Sun56

SUCROSE AND EXERCISE DIFFERENTIALLY ALTER THE BEHAVIORAL ACTIONS OF OPIOID DRUGS

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Previous research has shown that intake of a palatable sucrose solution increases, while exercise decreases the analgesic actions of opioid agonists in rats. The present studies explored whether sucrose intake and exercise would also alter the anorectic actions of opioid antagonists. In Experiment 1, Long-Evans rats were given either chow and water, or chow, water and a 32% sucrose solution for 3 wks. Sucrose was then removed, rats injected with naltrexone (0, 0.3, 1.0 or 3.0 mg/kg, sc) and chow intake measured. Rats which had consumed sucrose decreased food intake significantly more than rats which had not been given the sugar. In Experiment 2, drug-naive rats were housed in running wheels or standard cages for 3 wks. Animals then were given the same doses of naltrexone as in Experiment 1. Rats which had run were significantly less sensitive to the anorectic actions of naltrexone than inactive animals. Although previous studies suggest that both sucrose intake and exercise increase release of endogenous opioid peptides, the present data suggest that their effects on opioid-mediated behaviors are in opposite directions.

Sun57

THE INFLUENCE OF FORCED SWIMMING STRESS ON THE ANALGESIC EFFECT OF FINE CERAMIC SEMICONDUCTOR

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Fine ceramic (Fc) semiconductor is one of the high technological products which used clinically as a Chinese traditional acupuncture therapy. In our previous studies, it was suggested that embedding Fc semiconductors in the acupuncture point (Zusanli, S36) could produce obvious antinociception. We considered if a change of temperature would influence the analgesic effectiveness of Fc semiconductor. In this study, therefore, we embedded a Fc semiconductor in the acupuncture point (Zusanli, S36) to evaluate the analgesic activity in mice by the formalin tests. The analgesic effects of Fc semiconductor at different temperatures of 20, 30 and 40^{o}C were observed in the early and late phases. The antinociception of Fc semiconductor was enhanced by forced swimming stress in different temperatures at 20, 30 and 40^{o}C in the early phase and at 30 and 40^{o}C in the late phase. The potentiation was antagonized by naloxone at low temperature (20, 30^{o}C) and high dose (5 mg/kg i.p.) in early phase and was enhanced at low temperature (20^{o}C) in the late dose. However, it was not reversed by naltrindole (1 mg/kg, s.c.). The potentiation was enhanced by prazocin and yohimbine. It was suggested that the analgesic mechanism of Fc semiconductor was not related to adrenergic neurons and might be mainly via μ–opioid receptor.

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OPIATE RECEPTOR BLOCKADE INCREASES BAROREFLEX GAIN IN CONSCIOUS DOGS.

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Opioid peptides are well located to regulate cardiovascular function. Conscious, chronically instrumented dogs were used to investigate the role of opiate receptors in the baroreflex control of blood pressure. Phenylephrine and nitroprusside were used to elevate and decrease blood pressure by 15-20 mmHg, respectively, before and after opiate receptor blockade. Diprenorphine 100 μg/kg was injected to block all opiate receptors. Blood samples were collected from the coronary sinus and aortic catheters to measure catecholamine and enkephalin spillover. Diprenorphine significantly decreased heart rate (HR), left ventricular and mean arterial pressure (MAP) with little change in coronary blood flow, cardiac output, enkephalin and catecholamine spillover. The baroreflex gain (ΔHR/ΔMAP) increased in the presence of diprenorphine. The increased response to nitroprusside in the presence of diprenorphine was accompanied by decreased catecholamine spillover showing more arterial than coronary sinus catecholamines. There was no change in cardiac enkephalin spillover. These results indicate that opioid peptides are inhibiting catecholamine release during low but not high blood pressure stimulation. The mechanism for increased gain during opiate receptor blockade and high blood pressure is unclear.

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MYOCARDIAL PROTECTIVE EFFECTS OF MU OPIOID AGONISTS.

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Brief periods of myocardial ischemia (ischemic preconditioning; IP) protects the heart against prolonged ischemic challenge. Recent studies showed that pretreatment with delta-opioid agonists can mimic IP and reduce infarct size. We evaluated the cardioprotective actions of mu-agonists. Isolated, perfused guinea pig hearts were exposed to three 5-min intervals of either buffer, ischemia, DALDA or DALDA+naloxone, and followed by 30 min of global ischemia and 30 min of reperfusion. Our results show that pretreatment with ischemia or DALDA significantly improved myocardial contractile force after global ischemia, and the effect of DALDA was blocked by naloxone. The protective effects of ischemia and DALDA were associated with a significant reduction in norepinephrine overflow before and after ischemia. We propose that inhibition of the sympathetic nervous system by IP and DALDA and reduced energy demand takes part in the cardiac protection (PO1- DA08924-04).

Sun60

LOCAL MET - ENKEPHALIN - ARG - PHE ALTERS SINOATRIAL NODE RESPONSES DURING ISCHEMIA.

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The C-terminal proenkephalin sequence, Met-enkephalin-arg-phe (MEAP), is abundant in the myocardium and has significant vagolytic activity when delivered into the SA node by microdialysis. This study was conducted to determine if nodal MEAP increases, during ischemia and does it alter vagal function. Microdialysis probes were placed in the canine SA node and the SA node artery was occluded and released intermittently. The intermittent ischemia was then followed by periods of prolonged ischemia. Vagally mediated bradycardia was compared before, during, and after ischemia and a progressive step increase in MEAP (75 to 225nmol) was recorded during subsequent ischemic periods. MEAP returned to baseline during each 10-minute reperfusion. There was a sustained increase in MEAP during longer ischemias (100-150nmol). Surprisingly the increased MEAP during ischemia augments vagal bradycardia via an interaction between delta receptors and ATP-sensitive K^{+} channels since the augmentation is blocked when either naltrindole or glibenclamide are included in the microdialysis probe.

Sun61

ROLE OF DELTA RECEPTOR IN HYPERTENSION

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Our previous study has shown that the difference in distribution of opioid receptor was related to the hypertension in spontaneously hypertensive rat (SHR). However, which opioid receptor subtypes are related to the cardiovascular regulation in SHR is still obscure. In the present study, the different opioid receptor subtypes implicated in hypertension of SHR are further studied using autoradiographic technique. ^{3}H-ohmefentanyl (OMF), ^{3}H-U69593 and ^{3}H-etorphine after suppression of mu and kappa sites by unlabeled OMF and U-50488H were used to label the mu, kappa and delta receptors respectively. The results showed that more delta receptors were observed in hypothalamic nuclei and PAG etc., and no changes in mu and kappa receptor density was noted in such brain regions in SHJR vs. normotensive control Wistar-Kyoto rat (WKY). Using microdialysis and HPLC-EC system, the levels of monoamine and their metabolites in dialysates of anesthetized SHR and WKY were measured. The blood pressure (BP), heart rate and respiratory rate were also monitored simultaneously. Microinjecting 10 nmol DSLET, a delta agonist, to medial preoptic area in anesthetized SHR or WKY, decreased the BP in both rat (n=6 in each group), the decrease of BP in SHR was larger than that of WKY ($P<0.05$). The decrease of BP could be completely reversed by 20 nmol naloxone (n=6 in each group). HPLC results showed that compared with WKY rats, SHR had lower NE levels in dialysates after DSLET injection ($P<0.05$). These results suggested that delta opioid receptor may be more important in hypertension of SHR, and may regulate BP by influencing the NE level in certain brain regions.

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MODULATION OF LONG-TERM DEPRESSION BY ORPHANIN FQ IN HIPPOCAMPAL DENTATE GYRUS

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We previously reported that orphanin FQ (OFQ) inhibited long-term potentiation (LTP) in the dentate gyrus, and this effect was partially mediated via reduction of NMDA receptor-mediated synaptic currents in dentate granule cells. The present study examined the effect of OFQ on several other forms of NMDA receptor-dependent synaptic plasticity in mouse hippocampal slices. **a.** Long-term depression (LTD). A low frequency stimulation (LFS, 1 Hz, 15 min) to the lateral perforant path induced a lasting reduction in the filed EPSP slope (31±3%, n=12) in slices from 22-35 days old mice. Application of OFQ (1 μM) significantly reduced the magnitude of LTD. **b.** Primed LTD. Induction of LTD in slices from older mice (50-65 days old) required a priming procedure consisting of multiple high frequency stimulation trains (HFS) delivered in D-APV 40 min prior to the LFS. OFQ applied during the LFS, but not HFS, blocked induction of primed LTD. **c.** Depotentiation. Multiple HFS-induced LTP could be reversed by a subsequent LFS. This depotentiation was also attenuated by both OFQ and D-APV applied during LFS. Thus, OFQ is able to negatively modulate multiple forms of synaptic plasticity in the dentate, possibly via its inhibitory action on NMDA receptor currents. Supported by NIH grants DA05010 to CJE and DA08571 to CWX.

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ANALGESIC AND COGNITION-ENHANCING ACTIVITY OF RETRONOCICEPTIN METHYL-ESTER, A NOCICEPTIN ANTAGONIST

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Previously we found that methylesters of retro-opioid peptides such as retrodynorphin methylester had opioid antagonist activity. We synthesized retronociceptin methyl-ester (Ret-Noc-OMe). The peptide exhibited affinity for ORL_1(IC_{50} =10^{-5} M). In the guinea-pig ileum assay, Ret-Noc-OMe behaved as a nociceptin antagonist. Anti-nociceptive activity of the peptide was evaluated by tail pinch test after icv administration in mice. At a dose of 100 nmol/mouse, Ret-Noc-OMe exhibited analgesic activity which lasted for 30 min. The analgesic activity was blocked only partially by naloxone. Tolerance was not formed after repeated administration of Ret-Noc-OMe. Ret-Noc-OMe is expected to be a novel lead compound to design analgesic drug having no tolerance and physical dependence. Furthermore, Ret-Noc-OMe potentiated memory retention in passive avoidance test using step-through cage.

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OFQ/N AND THE ENDOGENOUS OPIOIDS ACT SYNERGISTICALLY IN PROTECTING FROM EXCESSIVE SENSATION OF FEAR AND PAIN.

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Orphanin FQ/nociceptin (OFQ/N) can alleviate some physical symptoms of stress. I.c.v. injection of OFQ/N in rats or mice produced anxiolytic-like effects and was able to reverse stress-induced analgesia. Genetically engineered OFQ/N-deficient mice display an increased susceptibility to acute and repeated stress. OFQ/N knockout mice behave more anxious in tests of fear and anxiety, show tonic induction of stress-induced analgesia and fail to adapt properly to repeated stress as compared to their wildtype littermates. These phenotypical changes are only evident in group housed animals. When male OFQ/N knockout mice were isolated for at least two weeks, their responsiveness to stressful stimuli as well as their basal pain threshold returned to control levels. These results indicate that social stress might contribute to the observed phenotype in mutant mice. The OFQ/N system seems to be functionally involved in the neuronal processing of stress responses. In nature, i.e. under physiological conditions, increased fear and the possibility of injury often coincide. Therefore, the opioids and OFQ/N appear to protect the organism synergistically from excessive fear and pain sensations, which could otherwise compromise its reactivity. *(Supported in part by DFG grant Re 1024/2-1, Fonds der Chemischen Industrie and by Hoffmann-La Roche)*

Sun65

THE EFFECTS OF ORPHANIN/FQ RECEPTORS LIGANDS IN THE FORMALINE ASSAY IN MICE

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Recently characterized ligands for the orphanin FQ/nociceptin receptors have various effects on pain and pain related procedures. Moreover they modify synaptic transmission and long term potentiation in CNS. Furthermore nociceptin induces hyperalgesia in some models of neuropathic pain. Therefore it was interesting to investigate the influence of the orphanin receptors ligands on two, different in the mechanisms phases of formaline induced pain (phase 1 - acute nociceptive pain, phase 2 - neuropathic, LTP related pain). The activity of nociceptin, the endogenous orphanin receptors agonist and its derivative [$Phe^1\Psi(CH_2\text{-}NH)Gly^2$]nociceptin-(1-13)-$NH_2$ was investigated. Formaline induced paw licking was determined. The duration of paw licking (an index of nociception) in two phases was measured. Nociceptin as well as its derivative was administered i.c.v. (at the doses of 1, 10 and 200 nmol) in adult Albino Swiss mice 20 min/before tests. Nociceptin (10, 200 nmol) produced an inhibition of both phases of formaline induced pain. Its tested derivative at the doses of 10 and 200 nmol, did not influenced the first phase of the test but similarly to nociceptin produced potent inhibition of the second (central sensitization reflected) phase.

Sun66

PERIPHERAL ORPHANIN FQ/NOCICEPTIN ANALGESIA IN THE MOUSE

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Orphanin FQ/Nociceptine (OFQ/N) administered peripherally was a effective analgesic in the tailflick test in mice with an ED_{50} of 16.3 μg. It had a peak effect at 5 min and its actions lasted 30 min. The $kappa_3$ analgesic naloxone benzoylhydrozone was also active peripherally (ED_{50} 3.8 μg). The analgesic actions of both agents were blocked by naloxone. Neither OFQ/N (1-11) nor OFQ (1-7) had appreciable peripheral activity. Antisense mapping both compounds against the murine orphan opioid receptors (KOR-3) confirmed the importance of this clone in their actions. Antisense probes targeting the second and third coding exons significantly lowered the analgesic effects of both compounds. However, the antisense targeting the first coding exon blocked only the actions of OFQ/N and not $kappa_3$ receptors.

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ORPHANIN FQ PRODUCES HYPERALGESIA IN THE PRESENCE, BUT NOT ABSENCE, OF NALOXONE IN THE RAT HOT PLATE TEST.

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The hyperalgesic effect of orphanin FQ (OFQ; also known as nociceptin) has been attributed to blockade of opioid receptor-mediated stress-induced antinociception. In the present study we used naloxone to block that stress-mediated antinociception and examine if OFQ would produce hyperalgesia in the rat hot plate test. Rats were injected with saline or naloxone (1 mg/kg, s.c.), 10 min later with OFQ (7.5-30 nmoles, i.c.v.) and tested on the hot plate apparatus 15 min later. OFQ dose-dependently produced hyperalgesia in the presence, but not absence, of naloxone. We also examined the effect of OFQ (15 nmoles) in the presence of various doses of naloxone (0.1-1.0 mg/kg, s.c.). Pretreatment with naloxone at 0.1 mg/kg had no effect, but at 0.5 and 1.0 mg/kg revealed the hyperalgesic effect of OFQ. Overall, our results indicate that OFQ can induce hyperalgesia in the absence of opioid receptor-mediated stress-induced antinociception. (Supported in part by NIDA Grants DA 05010 and DA09359; KL was supported by a NIDA training grant T32DA07272)

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ANTINOCICEPTIVE EFFECT OF ENDOMORPHIN-1 IN DIABETIC MICE IS MEDIATED BY δ-OPIOID RECEPTORS.

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We examined whether streptozotocin-induced diabetes can modulate endomorphin-1 (EDM1)-induced antinociception in mice. EDM1, at a dose of 10 μg, i.c.v., produced a marked inhibition of the tail-flick response in both diabetic and non-diabetic mice. There was no significant difference in the peak antinociceptive effect of EDM1 between diabetic and non-diabetic mice. In non-diabetic mice, the antinociceptive effect of EDM1 was significantly antagonized by treatment with β-funaltrexamine (β-FNA), μ-opioid receptor antagonist, or naloxonazine (NXZ), a selective μ_1-opioid receptor antagonist. However, either β-FNA or NXZ did not antagonize the antinociceptive effect of EDM1 in diabetic. On the other hand, the antinociceptive effect of EDM1 in diabetic mice was significantly reduced by pretreatment with naltrindole, a selective δ-opioid receptor antagonist. Furthermore, nor-binaltorphimine, a selective κ-opioid receptor antagonist, had no significant effect on the antinociceptive effects of EDM1 in both diabetic and non-diabetic mice. These results suggest that EDM1-induced antinociception in diabetic mice is mediated by activation of δ-opioid receptors, whereas EDM1-induced antinociception in non-diabetic mice is mediated by μ_1-opioid receptors.

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THE INFLUENCE OF [Phe$^1\psi$(CH$_2$-NH)Gly^2] NOCICEPTIN (1-13)-NH$_2$ ON SPONTANEOUS ALTERNATION IN MICE

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Newly discovered, widely distributed in the brain the orphanin receptors are supposed to have various pharmacological effects in CNS. The orphanin receptors ligand has been reported to modify not only pain related but also cognitive and memory processes (inhibition of excitatory synaptic transmission and LTP, impairment of spatial learning). In this study the Y maze was used to examine the influence of nociceptin upon spontaneous alternation (model of working memory) and horizontal locomotor activity in mice. Phe$^1\psi$(CH$_2$-NH)Gly^2] nociceptin(1-13)-NH$_2$ was investigated. The ligand at the doses of 1, 10 and 200 nmol were administered intracerebroventricularly in adult Albino Swiss mice 20 min before tests. Scopolamine was employed to provide a pharmacological model of memory dysfunction. Given i.p. 30 min before test (1mg/kg) produced a loss of spontaneous alteration behaviour and increased locomotor activity. The ligand alone, in all applied doses did not change either the spontaneous alternation or locomotor activity in mice. Given at the doses of 10 and 200 nmol reversed the amnestic effect of scopolamine. Besides, the ligand didn't prevent the increase of locomotor activity produced by scopolamine.

These results could indicate [Phe$^1\psi$(CH$_2$-NH)Gly^2]nociceptin-(1-13)-NH$_2$ alone doesn't disturb memory and locomotory processes but seem to improve impaired by scopolamine working memory in mice.

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ANTICONVULSANT EFFECTS OF ORPHANIN/FQ RECEPTORS LIGANDS

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The anticonvulsant activity of nociceptin, endogenous orphanin/FQ receptor agonist and its derivative [Phe$^1\psi$(CH$_2$-NH)Gly^2]nociceptin-(1-13)-NH$_2$ was investigated in two selected seizure models of experimental epilepsy. Nociceptin as well as its derivative were administered intracerebroventricularly (at the doses of 1, 10 and 200 nmol) in adult Albino Swiss mice 20 min. before tests. In the pentylenetetrazole induced seizures, anticonvulsant action of both ligands (at the doses of 10 and 200 nmol) was observed. On the other hand in the electroconvulsive model of generalized seizures both ligands influenced neither electroconvulsive threshold nor maximal electroshock induced seizures.

Our results demonstrated potential anticonvulsant properties of orphanin receptors ligands. It is suggested that inhibition of excitatory amino acid transmission, calcium synaptic currents and activation of potassium channels may contribute to the mechanisms of action.

Sun71

INTRA-AMYGDALA INJECTION OF ORPHANIN FQ INCREASES PLASMA CORTICOSTERONE CONCENTRATION.

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Orphanin FQ (OFQ or nociceptin) appears to interact with hormonal responses to stress. We have previously reported that intracerebroventricular (i.c.v.) injections of OFQ increase plasma adrenocorticotrophic hormone (ACTH) and corticosterone concentrations in unstressed rats. These injections also enhance hormonal responses to a mild stressor (Devine, Watson, Civelli & Akil, INRC 1997). We are currently investigating which brain sites mediate these effects. Unstressed rats were injected into the amygdala or medial prefrontal cortex (MPFC). Plasma was collected 30 minutes after injection, and the concentration of corticosterone was assayed by radioimmunoassay. Intraparenchymal injections of OFQ (1.0 nmole) into the central amygdala significantly elevated plasma corticosterone concentrations. Injections into the MPFC were ineffective. We are continuing to investigate the potential actions of OFQ in the amygdala and MPFC, as well as other sites that are known to be relevant to HPA functioning.

Sun72

ORPHANIN FQ/NOCICEPTIN MODULATION OF DOPAMINE RELEASE FROM MIDBRAIN PRIMARY CULTURES

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In order to determine the precise locus at which orphanin FQ/nociceptin (OFQ/N) suppresses mesolimbic dopamine (DA) neurons, we investigated OFQ/N's actions on DA release from primary midbrain cultures. OFQ/N was highly potent (IC50 approx. 1nM) in suppressing DA overflow. This action was unaltered in the presence of naloxone (10µM) though it was partially blocked by the putative ORL.1 antagonist naloxone benzoylhydrazone (1µM). Although the cultures contained large numbers of GABAergic neurons, no change in GABA release was observed during OFQ/N stimulation suggesting that in this system, GABA plays no role in mediating OFQ/N's suppression of DA release. This was confirmed by the inability of the GABA-A antagonist bicuculline (10µM) to effect OFQ/N's suppressive action. Finally, OFQ/N was unable to attenuate DA release evoked by high (15mM) K+. We conclude that OFQ/N is likely to directly affect DA neuronal activity by an action at the DA neuron cell body. This work was supported by NIDA #DA05010.

Sun73

THE ANATOMICAL RELATIONSHIP BETWEEN ORPHANIN FQ, THE OFQ RECEPTOR AND DOPAMINE NEURONS.

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Recent studies have shown that orphanin (OFQ) peptide alters accumbal and tegmental dopamine (DA) release, however it is unclear whether OFQ acts directly on DA neurons or indirectly through local circuitry. Two approaches were taken to determine whether OFQ and/or the OFQ receptor (ORL-1) are located within/on DA containing neurons. Firstly, dual in situ hybridization (ISH) studies determined whether tyrosine hydroxylase (TH), the rate limiting enzyme for DA synthesis, was co-localized with OFQ and/or ORL-1 in the VTA and SNc. Secondly, OFQ and ORL-1 mRNA content was measured subsequent to unilateral 6-hydroxydopamine (6-OHDA) lesions of the right medial forebrain bundle. In the dual ISH studies, a small population (~1%) of the TH positive neurons also expressed OFQ signal, whereas a majority of TH positive neurons (80-90%) contained ORL-1 signal within the VTA and SNc. These dual ISH studies suggest ORL-1 is found predominantly on DA neurons while OFQ is primarily located in non-dopaminergic neurons. In agreement with the colocalization studies, ORL-1 mRNA decreased by 60-70% on the 6-OHDA lesioned side as compared to vehicle-lesioned controls. In contrast, OFQ mRNA increased ~200-400% in DA cell body regions on the 6-OHDA lesioned side as compared to vehicle-lesioned controls, suggesting OFQ mRNA expression may be regulated by DA (or vice versa). OFQ is in a position to influence DA neuronal activity via the ORL-1 receptor located on DA neurons. Supported by NIDA (5T32DA07268).

Sun74

QUANTITATIVE AUTORADIOGRAPHY OF ORL1, MU, DELTA, AND KAPPA OPIOID RECEPTORS IN THE BRAINS OF ORL1 RECEPTOR OR NOCICEPTIN (OFQ) KNOCKOUT MICE.

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The structural homology between the ORL1 receptor and its endogenous peptide nociceptin with the classical opioid system has raised the question of interaction between the two systems. This has been further supported by the results of behavioural studies revealing anti-opioid properties mediated by the ORL1 receptor. To study potential compensatory plasticity between the two systems we have carried out detailed quantitative autoradiographic mapping of the ORL1, mu-, delta- and kappa- opioid receptors in the brains of mice lacking either the ORL1 receptor gene or nociceptin (orphanin FQ) peptide gene. [^{3}H] *leucyl*- nociceptin (0.4nM) binding was completely abolished in ORL1 receptor homozygote mice indicating that this receptor and any putative subtypes are encoded for by a single gene. In both the ORL1 and nociceptin knockout mice there were no major compensatory or distributional changes in the expression of the classical opioid receptors. Some upregulation of the ORL1 receptor was seen in the nociceptin knockout mice.

Sun75

ORL-1 RECEPTORS AND ORL-1-ACTIVATED G-PROTEINS IN RAT CINGULATE CORTEX

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ORL-1 receptors and ORL-1-activated G-proteins are found in high levels in the anterior cingulate cortex (area 24), an area involved in processing nociceptive stimuli. The localization of ORL-1 receptors in the rat anterior cingulate cortex was examined using two types of lesions: ibotenic acid injection into the anterior cingulate cortex or undercut lesions to remove afferent axons. Brain sections were processed for [^{3}H]nociceptin/orphanin FQ (N/OFQ) binding and N/OFQ-stimulated [^{35}S]GTPγS binding. [^{3}H]N/OFQ and N/OFQ-stimulated [^{35}S]GTPγS binding were found in all layers of the anterior cingulate cortex, with a peak in layers V and VI. Analysis of [^{3}H]N/OFQ and N/OFQ-stimulated [^{35}S]GTPγS binding was performed in layer V of areas 24a and 24b. Ibotenic acid lesions that destroyed neurons in this area reduced [^{3}H]N/OFQ binding by 75-80% and decreased N/OFQ-stimulated [^{35}S]GTPγS binding to basal levels. In contrast, deafferentation lesions resulted in an increase of 40-50% in [^{3}H]N/OFQ binding, whereas N/OFQ-stimulated [^{35}S]GTPγS binding was increased by only 5-20%. These data demonstrate that ORL-1 receptors in anterior cingulate cortex are located on neurons in layer V. Furthermore, deafferentation produced an increase in ORL-1 receptor binding, with a modest increase in ORL-1-activated G-proteins. Supported by NIDA DA-00287 (LJS) & DA-02904 (SRC).

Sun76

BINDING AND FUNCTIONAL PROFILE OF ORPHANIN FQ/NOCICEPTIN IN BE(2)-C, HUMAN NEUROBLASTOMA CELLS.

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Orphanin FQ/nociceptin (OFQ/N) is the endogenous ligand for the ORL-1/KOR-3 receptor and produces a wide variety of behavioral responses. In the present study, we characterized the binding of [^{125}I][Tyr^{14}]-OFQ/N in the human neuroblastoma cell line, BE(2)-C. The binding was linear with tissue and was saturable. In competition studies, the native OFQ/N peptide had an affinity similar to the Tyr substituted analog used in saturation studies. Dynorphin A 1-17, dynorphin A 1-11 and Met^5-enkephalin demonstrated modest affinity for this receptor. A series of mu, delta, and kappa$_1$ opioids did not compete binding, with K_i values above 1 μM. However, NalBzoH, a kappa$_3$ ligand, lowered binding but with poor affinity. OFQ/N did not have appreciably affinity for mu, delta or kappa opioid binding sites. OFQ/N inhibited forskolin stimulated cyclic-AMP accumulation in a dose dependent manner with an IC_{50} value below 10 nM. The opioid antagonists, naloxone and diprenorphine, were able to blocked the OFQ/N induced inhibition of forskolin stimulated cyclic-AMP accumulation. This work is supported by DA10738 (K.M.S.) and DA02615 (G.W.P.).

Sun77

MECHANISMS OF MOLECULAR SELECTIVITY FOR OPIOID AND ORPAHNIN FQ RECEPTORS

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The opioid and orphanin FQ (OFQ) systems are highly homologous yet subtle structural differences prevent cross-talk. Specific mutations of the OFQ receptor alter its binding profile to significantly improve affinity for dynorphin peptides. We now are probing mechanisms by which opioid receptors exclude OFQ but include their own ligands. Our initial efforts have focused on mutations in the kappa receptor that are complementary to those in the OFQ receptor (K-A [TM 5], IHI-VQV [TM 6], I-T [TM 7] and combinations). The individual mutations do not confer affinity for OFQ. While they have little effect on the affinity of alkaloid ligands, these mutations decrease the affinity of dynorphin peptides. Functionally, alkaloid agonists are less potent at the mutants than at wild-type (WT). The magnitude of response elicited by K-A and IHI-VQV is less than WT whereas that produced by I-T is greater than WT. The pharmacology of the combination mutants is being evaluated. Our preliminary results suggest that 1) these residues may represent a "dynorphin pocket" since they are important for kappa binding of dynorphins and they improve dynorphin affinity for the OFQ receptor, and 2) the "OFQ pocket" appears to be defined by different residues or a more complex combination of residues since the OFQ-like mutations still prevent OFQ from interacting with kappa receptors and complementary mutations in the OFQ receptor do not cause a loss of affinity for OFQ. These conclusions are consistent with recent reports that distinct evolutionary mechanisms arose to functionally isolate the opioid and OFQ systems. Supported by NIDA Grant 5T32DA07286.

Sun78

TRANSLATIONAL EFFICACY OF VARIOUS CLONED HUMAN OFQ/N SPLICE VARIANTS.

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Orphanin FQ or Nociceptin (OFQ/N), the heptadecapeptide ligand of the ORL-1 receptor, is derived from a larger precursor protein (preproOFQ/N) by proteolytic cleavage. The gene encoding ppOFQ/N is composed of four exons, of which, exons 2 and 3 comprise the open reading frame. Using reverse transcription polymerase chain reaction (RT-PCR) with primers spanning exons one and four, we have recently identified several alternatively spliced OFQ/N messages from the human cortex. Sequence analysis of these alternative transcripts revealed the excision of 57 bases from the 3' terminal of exon 3. In vitro translation experiments using this variant resulted in the synthesis of a larger precursor protein (approximately 27 kDa), relative to the 23 kDa native ppOFQ/N. An analogous splice variant has previously been reported in mice and murine neuroblastoma cell line N20K by Saito and colleagues. Additionally, we have isolated two novel OFQ/N splice variants both lacking exon 2. Since these transcripts lack the methionine start site located in exon 2, we are investigating these sequences for the possibility of translation starting at another internal methionine. Alternatively, these splicing events may be means for the regulation of protein translation. Sequence analysis has revealed an in-frame start site within a Kozac consensus sequence within exon 3. We are currently in the process of examining whether this alternative start site may also be used in the translation of truncated variants of ppOFQ/N.
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Sun79

CLONING & CHARACTERIZATION OF THE PROMOTER REGION OF THE HUMAN PREPRONOCICEPTIN GENE

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Nociceptin or Orphanin FQ, the endogenous ligand for the opiate-like receptor ORL 1, is localized in regions of brain and spinal cord involved in nociception. Although it was originally found to be hyperalgesic when administered intracerebroventricularly, subsequent studies have demonstrated a complex pharmacology including both antinociception and antiopiate activity depending on the site of administration and analgesia paradigm. To better understand the regulation of nociceptin production, we have undertaken the cloning, sequencing, and characterization of the promoter region of prepronociceptin. We have sequenced approximately 1.1 kb upstream of the initiation of the prepronociceptin sequence. Similar to the preproenkephalin promoter region, there are two cAMP response elements (CRE) with the consensus sequence CGTCA within approximately 200 base pairs (bp) of the transcription start site. In addition, a pyrimidine-rich initiator sequence (Inr), which is usually implicated in transcription initiation and has the consensus sequence CACTCCTC, is located just upstream of the CREs. A similar consensus Inr has been found in the prodynorphin promoter region, and has been implicated in the inhibition of prodynorphin production. We have inserted the 1.1-kb fragment, as well as a 230-bp insert into a pGL3 luciferase reporter vector. This reporter plasmid has been transiently transfected into CHO and Cos-7 cells. Inr appears to inhibit transcription in this promoter as well. The effects of various agents on the induction of transcription will be discussed.

Sun80

EXPRESSION OF Ag-NORs OF T-LYMPHOCYTES FROM HEROIN ADDICTS

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The nucleolar organizer regions(NORs) Associated protein(Ag-NORs) of lymphocytes in peripheral blood of 72 heroin addicts were 6.780.50%(ISSD%) which was significantly lower than those in normal subjects. Analysis showed that both of the expression rate and level of GST-p mRNA in these cells were significantly higher compared with those of normal subjects. There were, however, no relationship between the level of GST-p mRNA and sex or addictive time or the methods of drugtaking. In addition, the relationship between Ag-NORs and GST-p showed negative corelation. These results suggest that heroin dependence might induce immuno damage.

Sun81

ELECTROACUPUNCTURE STIMULATION CAN INDUCE THE RELEASE OF IL-1 BETA AND TNF-ALPHA IN THE BLOOD OF RATS.

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It is known that electroacupuncture (EA) stimulation of different frequencies induces the release of opioid peptides and produces analgesic effects, and that the effects are blocked by opioid antagonists. In the present study, the relationship between electroacupuncture stimulation and cytokines of the immune system was observed. One pair of stainless-steel needles of 0.25 mm diameter was inserted into the hind leg at the acupoints Zusanli and Sanyingjiao of S-D rats. Square waves of 0.3 ms duration were applied at frequencies of 2, 30 and 100 Hz. The intensity (constant current) of stimulation was set at 1.5 mA for 15 min, then shifted to 3.0 mA for an additional 15 min. Thirty minutes after termination of EA stimulation, blood samples were collected for serum. Serum was assayed by ELISA for the quantitative determination of concentrations of rat tumor necrosis factor alpha (TNF-alpha) and IL-1 beta in rat serum. Rats were divided into 2 groups: control (non-stimulation) and EA stimulation. The preliminary results of ELISA showed that (1) The release of IL-1 beta was increased more than 100% over control after both 2 Hz and 100 Hz EA stimulation; (2) The release of TNF-alpha was also increased more than 100% over the control group after 30 Hz EA stimulation. Although these preliminary results were only of borderline significance, further studies are under way to determine whether EA stimulation activates the immune system (Supported by NIH grants DA 00376 and DA 06650)

Sun82

MITOGEN - INDUCED ACTIVATION OF MOUSE T CELLS INCREASES κ OPIOID RECEPTOR EXPRESSION IN RELATION TO PHENOTYPIC MARKERS.

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The present study investigated whether mitogen activation of C57Bl/6 mouse splenocytes would alter the proportion of the κ opioid receptor (KOR) population in relation to the phenotypic markers CD4 and CD8. Mouse splenocytes were cultured for varying times with the mitogens PHA or Con A. KOR, CD4, and CD8 expression were measured using flow cytometry. In a time-dependent manner, Con A activated $CD8^+$ (T-cytotoxic) and $CD4^+$ (T-helper) cells, and increased their expression of the KOR in comparison to the phenotypic markers. Maximal expression was measured at 24 hr, where the ratio of KOR to CD8 and KOR to CD4 expression was 6- and 3-fold, respectively. For $CD8^+$ PHA-activated cells, the time course was similar to that of Con A activation, with the maximal increase of KOR:CD8 expression of 4-fold occurring at 24 hr. In PHA-treated $CD4^+$ lymphocytes, however, the greatest ratio of KOR:CD4 expression was 2-fold after 12 hr pretreatment. The percentage of double positive cells, defined as cells which express both the KOR and one of the phenotypic markers, was also measured. The percentage of double positive cells after treatment with either Con A or PHA remained constant at ~30% at all time points, suggesting that neither mitogen produced a notable effect on the percentage of double positive cells. The results show that the activation of T cells increases the ratio of KOR to the CD4 and CD8 phenotypic markers, which demonstrates a selective increase in KOR expression, and suggests a role for KOR in immune cell response. (Supported by NIDA grants DA04355 and K05-DA00360.)

Sun83

OPIOID RECEPTOR ACTIVITY IN THE DEVELOPING MOUSE.

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Approaches have been developed that can detect--biochemically and histologically--agonist induced GTP-gamma-^{35}S binding to G-proteins and thus allow receptor function to be measured (Sim, L.J., Selley, D.E., and Childers, S.J. (1997) *Meth in Mol Bio.)*. This current study utilizes these techniques to extend previous receptor binding and mRNA expression studies and gain a better understanding of the opioid receptor function during development. In initial studies, whole brain homogenates from pl mice were examined, and the results showed that both DAMGO and DPDPE induced significant mu and delta receptor coupling, respectively. In addition, it was found that basal G-protein coupling was considerably lower in the pl mouse as compared to the adult mouse, suggesting that G-proteins may function quite differently in the developing brain than in the adult brain. Histological analysis of pl mouse brains has shown that the anatomical distribution of mu receptor coupling induced by DAMGO closely mimics its mRNA expression, with prominent activity present in the caudate-putamen, hypothalamus, and habenula, with less abundant activity in the olfactory bulb and medulla. Consistent with the low abundance of delta receptor mRNA present at this stage, delta receptor coupling induced by DPDPE was found at low levels and only in the lateral caudate-putamen. Analysis of prenatal stages has thus far demonstrated mu and delta receptor coupling in el7.5, indicating that functional coupling of these receptors begins prior to birth. With the wild type pattern of receptor activation now determined, it will be possible to examine whether lines of mice bearing mutations of one of the opioid receptors exhibit altered developmental patterns of opioid receptor coupling.

Sun84

A ROLE FOR DIETARY CASEIN IN OPIOID RECEPTOR DEVELOPMENT.

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Weaning rats at postnatal day 21 stimulates a μ- to δ-opioid receptor transition in the mediation of swim-stress-induced analgesia (swim-SIA) by 25 days of age. Extended housing with their mother delays this weaning-induced transition by up to 10 days with pups retaining only μ-receptor mediated swim-SIA. To determine the precise stimulus for δ-receptor activation we have studied the effect of lactating (LS) or non-lactating surrogate mother (NLS) and casein-rich (CR) or casein-free (CF) milk substitutes after removal of the mother at day 21. Animals of 25 or 40 days of age were stressed by warm water (20°C) swimming for 3 min periods and analgesia assessed by the tail immersion test (50°C). Naltrindole (NTI) (1mg kg^{-1}) partially reversed swim-SIA in 25 day old weaned, NLS and CF groups but had no effect in non-weaned, LS or CR groups at this age. Provision of milk, whether from mother, LS or casein-rich milk formula between postnatal days 21 and 25, delays the onset of δ-mediated swim-SIA. Furthermore, absence of casein from milk formula results in loss of milks regulatory effect. Prolonging casein-rich milk feeding up to day 40 delays the μ- to δ-transition beyond that achieved by extended housing with the mother. Casein is a milk-derived protein containing peptide fragments shown to possess opioid activity, most notably the β-casomorphins. It therefore appears that a milk-derived opioid peptide exerts a regulatory effect on opioid receptor development.

Sun85

EFFECT OF MU AND DELTA$_2$ OPIOIDS ON DEVELOPING MOUSE GRANULE NEURON PRECURSORS *IN VITRO*

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The intrinsic role of the opioid system in neurogenesis was systematically explored in cerebellar external granular layer (EGL) neuronal precursors. EGL granule neuron precursors were isolated from postnatal day 5-6 mice by Percoll density-centrifugation and selective panning, and maintained *in vitro* in defined medium. Isolated neuronal precursors expressed specific mu and delta, but negligible kappa, opioid receptor immunoreactivity. The developmental effects of opioids were highly selective. Morphine-induced mu receptor activation inhibited neuroblast proliferation (assessed by reductions in bromodeoxyuridine and [^{3}H]-thymidine incorporation, and subsequent decreases in cell numbers). In contrast, the preferential delta$_2$ receptor agonist ([D-Ala^2]-deltorphin II), as well as Met-enkephalin, but neither mu (morphine) nor delta$_1$ ([D-Pen^2, D-Pen^5]-enkephalin) agonists, decreased the length of neurites in immature granule neurons following 48 h of opioid exposure. Importantly, neither mu nor delta agonists affected EGL precursor viability (assessed by ethidium homodimer exclusion and positive calcein fluorescence in living cells). If similar patterns occur in the developing cerebellum, spatiotemporal differences in endogenous mu and delta$_2$ opioid ligand-receptor interactions may serve to coordinate distinct aspects of granule neuron maturation (e.g., neurogenesis versus synaptogenesis). The findings additionally suggest that opiate drugs of abuse interfere with neurobehavioral development by disrupting normal opioid signaling and inhibiting neuroblast division. Supported by NIDA (DA 06204).

Sun86

ENKEPHALIN GENE EXPRESSION DURING NEOCORTICAL GLIAL PROLIFERATION

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Glial cells of the neocortex are generated in the subventricular zone (SVZ) of the neocortex during the last embryonic and the first postnatal days. The proenkephalin (PEnk) gene is expressed in cells of the SVZ (Olenik and Meyer, Mol. Brain Res. 44, 83-91,1997). We have studied the date of birth as well as the fate of the PEnk gene expressing cells. Pregnant rats or their offspring were injected between embryonic day 13 and postnatal day 7 with bromo-deoxyuridine (BrdU) which labels cells in the S-phase of the cell cycle. BrdU injections between embryonic days 13 and 17 did not label cells of the PEnk mRNA positive area of newborn rats. However, after an injection at embryonic day 18 nearly all cells were labelled which expressed the PEnk gene. In such newborn rats, about 6 layers of cells contained BrdU in the PEnk mRNA positive area, while at postnatal day 12 only a few BrdU positive cells were found close to the roof of the ventricle. Injections of BrdU between embryonic day 20 and postnatal day 7 did not label the PEnk mRNA positive cells when analysed 90 min later. Taken together, the PEnk mRNA containing cells were generated at embryonic day 18. Thereafter, they seemed to leave the SVZ without entering the mitotic cycle.

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Sun87

DELTA OPIOID DADLE BLOCKS NEURONAL DEATH CAUSED BY SERUM DEPRIVATION IN PC12 CELLS: AN OPIOID RECEPTOR-DEPENDENT ACTION.

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Delta opioid agonist DADLE blocks the methamphetamine-induced dopamine transporter loss and increases in *c-fos* MRNA in mice. DADLE also enhances the tissue survival in isolated lungs and hearts. These results suggest that one of the physiological roles of *delta* opioid peptide is to attenuate cell death. We examined in this study the effect of DADLE on the survival of PC12 cells in culture. Specifically, we examined the effect of DADLE on the cell death caused by deprivation of serum from culture medium. PC12 cells were cultured on collagen-coated dishes in RPMI 1640 medium containing 10% horse serum and 5% fetal calf serum. Serum was removed by 5 washings with serum-free medium and cells cultured with or without DADLE. PC12 cells were stained with nigrosin and living cells counted under microscope. Serum deprivation induced cell death in a time-dependent manner and two days after the deprivation, about 70% of cells died. DADLE inhibited cell death in a dose dependent manner with 1 μM DADLE significantly increasing the percentage of cell survival. This protective effect exerted by 100 μM DADLE was equivalent to that exerted by 100 ng/ml of nerve growth factor and the effect of DADLE was completed blocked by 10 nM naltrexone. Our results indicate that DADLE can attenuate neuronal cell death caused by serum or trophic factor deprivation and that this action of DADLE is via an opioid receptor-dependent mechanism. [Supported by IRP & DBR, NIDA/NIH

Sun88

NON-OPIOID CYTOTOXIC INTRACELLULAR EFFECTS OF DYNORPHINS IN TUMOR CELL LINES.

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Dynorphin A has been shown to have neurotoxic properties. We have found that both dynorphin A and big dynorphin consisting of dynorphin A and dynorphin B inhibited growth of and induced apoptosis in HeLa and Saos-2 cells when delivered into these cells by lipofection or electroporation. Big dynorphin was more effective than dynorphin A, whereas dynorphin B, dynorphin B-29, the proenkephalin fragment peptide E and the basic peptides pentalysine and polylysine failed to inhibit cell growth; thus effects were sequence specific**.** Naloxone did not prevent the effects of big dynorphin showing that they are not mediated through opioid receptors. Big dynorphin also potentiated apoptoses induced by the tumor suppressor p53 protein transfected into p53 negative Saos-2 cells. Capability to induce cytotoxic effects may be the basis of the neurotoxic activities of dynorphins in the brain and spinal cord.

Mon01

IDENTIFICATION OF SERINE 261 AND 266 AS SITES INVOLVED IN AGONIST-INDUCED PHOSPHORYLATION OF THE MU-OPIOID RECEPTOR IN HEK 293 CELLS

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Signaling of G protein-coupled receptors is often terminated by phosphorylation of intracellular serine and/or threonine residues. We have recently shown that mutation of two serine residues within the third intracellular loop, S261 and S266, as well as a threonine residue within the COOH-terminal tail, T394, of the mu-opioid receptor (MOR1) to alanine confers resistance to agonist-induced desensitization. In the present study, we have determined the relative contributions of these sites to agonist-induced phosphorylation after stable transfection of the T394A, S261A/S266A and S261A/S266A/T394A receptor mutants into HEK 293 cells. DAMGO stimulated a three-fold increase in phosphorylation of the wild-type MOR1 within 5 min which lasted for at least 45 min. While rate and extent of agonist-promoted phosphorylation of the T394A mutant receptor were virtually identical to the wild-type mu-receptor, agonist-stimulated phosphorylation was not detectable in the S261A/S266A mutant. Similar, the triple mutant S261A/S266A/T394A also did not undergo agonist-stimulated phosphorylation. However, confocal microscopy revealed that agonist-induced internalization of both the S261A/S266A as well S261A/S266A/T394A mutant receptors was not attenuated suggesting that phosphorylation of these sites was not required for receptor endocytosis. Taken together, our results suggests that the serine residues S261 and S266 with in the third intracellular loop are crucial for agonist-induced phosphorylation and desensitization of the mu-opioid receptor in HEK 293 cells.

Mon02

STUDY OF MU OPIOID RECEPTOR PHOSPHORYLATION BY C-TERMINUS DELETION AND POINT MUTATION OF THE RECEPTOR

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To understanding the mechanism of the mu opioid receptor phosphorylation it is critical to determine which domains and amino acid residues are phosphorylated within the receptor under specific stimulatory conditions. The role of the C-terminus in agonist-mediated receptor phosphorylation was determined by examining the C-terminal deleted or point-mutated mu opioid receptors in receptor phosphorylation. Both wild type and structurally altered mu opioid receptors were stably expressed in Chinese hamster ovary cells. The receptor expressions were confirmed by receptor radioligand binding and immunoblotting. Exposure to 1 μM of DAMGO causes phosphorylation in both wild type and mutated mu opioid receptors, but the level of receptor phosphorylation in the mutated receptor is less intense in comparison with that of the wild type. In correlating with receptor phosphorylation, the role of the mutated receptors in agonist-induced desensitization was also studied by adenylyl cyclase inhibition assay. The mutant receptors show a partial loss of DAMGO-induced desensitization. Taken together, these results suggest that C-terminal portion of the mu opioid receptor participates in agonist-mediated receptor phosphorylation and desensitization, but this domain is not the only one involved in mu opioid receptor phosphorylation and desensitization.

Mon03

THE C-TERMINUS PLAYS IMPORTANT ROLE IN THE INTERNALIZATION AND MEMBRANAL TARGETING OF THE RAT MU OPIOID RECEPTOR.

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Internalization and recycling of G-protein coupled receptors, such as the mu opioid receptor largely depends on agonist stimulation. In previous studies we have demonstrated that the C-terminal spliced isoforms (MOR1 and MOR1B) of the rat mu opioid receptor differ in their internalization and recycling rate *(Koch et al., J. Biol. Chem. 273, 13652-13657, 1998).* These results indicate an important role of the C-terminus in the regulation of the mu opioid receptor internalization and recycling. To investigate structural domains and putative phosphorylation sites involved in this mu opioid receptor internalization, we constructed three truncation mutants (Trunc 386, Trunc 360, and Trunc 344). To permit localization of the receptor by immunocytochemistry we added an epitope tag to the N-terminus of the wildtype and mutant receptors. The immunocytochemical analyses revealed that only wildtype and truncation mutant Trunc 386 showed membranal localization. Therefore, in addition to its importance for the receptor internalization, the C-terminus of the mu opioid receptor seems to contain information for receptor targeting to the plasma membrane.

Mon04

INVOLVEMENT OF MITOGEN-ACTIVATED PROTEIN KINASE IN AGONIST-INDUCED PHOSPHORYLATION OF THE MU-OPIOID RECEPTOR IN HEK 293 CELLS

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In the present work, we show that treatment of human embryonic kidney HEK 293 cells stably transfected to express the rat mu-opioid receptor (MOR1) with DAMGO stimulated a rapid and transient (3-5 min) activation and nuclear translocation of the mitogen-activated protein kinase (MAPK). Exposure of these cells to the mitogen-activated protein kinase kinase (MAPK kinase) 1 inhibitor, PD98059, not only prevented MAPK activation but also inhibited homologous desensitization of the mu-opioid receptor. We have therefore determined the effect of PD98059 on agonist-induced mu-receptor phosphorylation. DAMGO stimulated a three-fold increase in MOR1 phosphorylation within 20 min that could be reversed by the antagonist naloxone. PD98059 produced a dose-dependent inhibition of agonist-promoted mu-receptor phosphorylation with an IC50 of 20 μM. DAMGO also induced MOR1 internalization which reached a maximum at 30 min. Confocal microscopy revealed that DAMGO-induced MOR1 internalization was also largely inhibited in the presence of PD98059. U0126, another chemically-unrelated inhibitor of the MAPK cascade, mimicked the effect of PD98059 on mu-receptor phosphorylation and desensitization. MOR1 itself, however, appears to be a poor substrate for MAPK because mu-receptors immunoprecipitated from stably transfected HEK 293 cells were not phosphorylated by exogenous ERK 2 in vitro. The fact that morphine also triggered MAPK activation but did not induce MOR1 internalization and DAMGO-induced MAPK activation was not prevented by sucrose, an inhibitor of clathrin-mediated endocytosis, indicates that receptor internalization was not required for MOR1 mediated mitogenic signaling. We conclude that MOR1 stimulates a rapid and internalization-independent MAPK activation. Activation of the MAPK cascade in turn may not only relay mitogenic signals to the nucleus but also trigger initial events leading to phosphorylation and desensitization of the mu-opioid receptor.

Mon05

GRK2 AND β-ARRESTIN-1 ARE INVOLVED IN HUMAN DELTA-OPIOID RECEPTOR DESENSITIZATION AND INTERNALIZATION.

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Recently we demonstrated that the human delta opioid receptor (hDOR), endogenously expressed by the neuroblastoma SK-N-BE cells, underwent an agonist-promoted and GRK-mediated phosphorylation, which was tightly correlated with the receptor desensitization (Hasbi et al., 1998). More recently, we demonstrated that this phosphorylation was also required for hDOR internalization. We show here that the GRK2 is the unique protein kinase member of the GRK family expressed in the SK-N-BE cells. Furthermore, the etorphine treatment leads to a translocation of GRK2 to the plasma membrane. On the other hand, we show also that β-arrestin-1 is the unique arrestin expressed in our model and that it undergoes a similar translocation. Thus, our results strongly suggest that GRK2 and β-arrestin-1 are involved in hDOR desensitization and internalization.

Mon06

PHOSPHORYLATION OF ADENYLYL CYCLASE VI UPON CHRONIC SNC 80 TREATMENT IN CHO CELLS EXPRESSING THE HUMAN δ OPIOID RECEPTOR.

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We have previously shown that adenylyl cyclase (AC) VI and VII are the major AC isoenzymes present in Chinese Hamster Ovary (CHO) cells. An immunoprecipitation method was used to measure [^{32}P]phosphate incorporation into the AC VI protein in CHO cells stably expressing the human δ opioid receptor (hDOR/CHO). Chronic δ agonist (SNC 80) (1μM, 24 h) treatment increased [^{32}P] incorporation into a 200 kDa protein band 2.5-fold. The increase in AC VI phosphorylation was SNC 80 dose- and pretreatment time dependent and was antagonized by naltrindole (1μM). The immunoprecipitation was prevented by the saturation of the antibody with the blocking peptide. H-89 (2 μM) and the Ca-Calmodulin kinase inhibitors calmidazolium (0.5 μM) and KN-93 (5 μM) prevented the phosphorylation of AC VI. The specific PKA inhibitor KT 5720 (1μM) and the PKC inhibitors bisindolylmaleimide-I (2 μM) and chelerythrine (5 μM) had no effect. (Supported by grants from ADCRC and NIDA)

Mon07

CO-EXPRESSION OF THE HUMAN DELTA-OPIOID RECEPTOR AND ALPHA-TRANSDUCIN BLOCKS ADENYLYL CYCLASE SUPERACTIVATION IN RESPONSE TO CHRONIC AGONIST PRETREATMENT

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We have previously shown that CHO cells expressing the human delta-opioid receptor (2-209-2CHO) exhibit adenylyl cyclase superactivation and a cAMP overshoot in response to chronic SNC80. Here, co-expression of the alpha-subunit of transducin-1 and the human delta-opioid receptor (CHOhDOR/T1 #9) blocks adenylyl cyclase superactivation and the cAMP overshoot. Maximal forskolin-stimulated cAMP formation in 2-209-2CHO cells is increased by 75 ± 4% of basal as compared to INDM-treated cells following 4 hour 100 nM SNC80 pretreatment (n=4, p=0.05). In contrast, CHOhDOR/Tl #9 cells do not show any significant change in maximal forskolin-stimulated cAMP formation in response to chronic 100 nM SNC80 pretreatment (n=4, p=0.05). In addition, phospholipase C-beta (PLC-beta) activation in response to opioids has previously been shown to be mediated by free G-beta gamma subunits. Here we demonstrate that CHOhDOR/Tl #9 cells have a 111 ± 22% decrease in maximal SNC80-mediated IP_1 formation as compared to 2-209-2CHO cells (n=3, p=0.05), or that opioid-mediated PLC-beta activation is inhibited by the presence of alpha-transducin. These data indicate that the expression of alpha-transducin scavenges free G-beta gamma subunits, and furthermore, that G-beta gamma subunits play a role in opioid-mediated adenylyl cyclase superactivation in 2-209-2CHO cells. (Supported by ADCRC & NIDA).

Mon08

CELLULAR MODELS OF μOR TOLERANCE INDUCED DIFFERENTIALLY BY PROTEIN KINASE A AND MORPHINE

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Tolerance to morphine at the μ-opioid receptor (μOR) was examined in two cell lines: human neuroblastoma SH-SY5Y cells and μOR-transfected HEK293 (HEK-μ) cells. Morphine (MS) tolerance was determined as the diminished effect of MS to inhibit cAMP accumulation. In SH-SY5Y cells, treatment with MS (1 μM) for 4 h or longer significantly reduced both the efficacy and potency of MS. Treatment with forskolin (25μM), an AC activator, also desensitized μOR response to MS by 38% ($p<0.001$), which was reversed by H8 (100 μM), a PKA inhibitor, implying PKA activation in forskolin-induced μOR tolerance. Treatment with both MS and forskolin produced additive effect leading to μOR tolerance. In HEK-μ cells, MS treatment produced profound cAMP-upregulation, yet did not lead to μOR tolerance to subsequent MS. However, treatment with forskolin (25 μM) led to a 50% reduction of effect by MS, while HEK-μ cells treated with 8-bromo-cAMP (1mM), a cell permeable PKA activator, lost sensitivity to MS completely. Effects of both compounds were reversed by H8. *These results demonstrated that cAMP-upregulation did not necessary lead to mOR tolerance. Moreover, PKA activation led to profound tolerance to MS in both SH-SY5Y and HEK-m cells; however, MS-induced mOR tolerance in SH-SY5Y cells occurred by a mechanism different from PKA activation.* *Supported by NIDA grant DA04166*

Mon09

INDUCTION OF OPIOID DEPENDENCE IS NOT LINKED TO A SPECIFIC INHIBITORY G-PROTEIN

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Recent studies have shown that the development of opioid dependence relates to inhibition of specific adenylyl cyclase (AC) isoenzymes (types I, V, VI), whose common regulatory feature is their inhibition by Gα subunits. Because opioid receptors are known to interact with all types of inhibitory G proteins (Gil-3, Go, Gz), the question arises whether the development of opioid dependence is mediated by a specific G protein. We therefore examined the ability of the pertussis toxin (PTX)-insensitive G protein, Gz, to substitute for PTX-sensitive G proteins in inducing drug dependence. For this, COS-7 cells (endogenous G proteins: Gi2, Gi3, Gz) where transiently cotransfected with cDNA's coding for the μ-opioid receptor and either AC V (inhibited by Giα and Gzα) or AC VI (not regulated by Gzα). Subsequently, cells were chronically exposed to morphine (1 μM*;* 1d) in the presence or absence of PTX (100 ng/ml), and the state of opioid dependence was assessed by the ability of naloxone to precipitate a cAMP overshoot phenomenon (withdrawal sign). In the absence of PTX, acute μ-opioid receptor activation produced a strong inhibition of forskolin-stimulated AC activity. Similarily, chronic morphine treatment resulted in the development of drug dependence, and both effects were observed regardless of the AC type expressed. In contrast, inactivation of Gi proteins by PTX, which directs μ-opioid receptor coupling towards Gz, selectively blocked the induction of dependence in case of AC VI but not with AC V. Overexpression of Gzα produced the same effect. These results demonstrate that the development of drug dependence is not exclusively linked to a particular G protein. They rather suggest that the nature of AC isoenzymes present in a given cell system determines the type of G protein which provides the signal eventually leading to the development of opioid dependence.

Mon10

IRREVERSIBLE OPIOID ANTAGONISTS PREVENT OPIOID DESENSITIZATION IN SH-SY5Y NEUROBLASTOMA CELLS.

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Co-administration of irreversible μ opioid antagonists with i.c.v. morphine blocks the formation of acute morphine antinociceptive tolerance. To investigate this effect, SH-SY5Y human neuroblastoma cells were cultured up to 24 hr with the irreversible μ opioid antagonists ß-FNA, N-CPM-TAMO or N-CPM-MET-CAMO, with or without 1 μM DAMGO. Harvested membranes were used in [^{3}H]DAMGO saturation binding and adenylyl cyclase assays to measure changes in receptor number and efficacy. After a 24-hr incubation, low concentrations of all three irreversible antagonists demonstrated irreversible interactions with the μ receptor, producing a 70% reduction in maximal [^{3}H]DAMGO binding, but no significant changes in the IC_{50} values for DAMGO-induced inhibition of adenylyl cyclase activity. However, a 24-hr incubation of SH-SY5Y cells with DAMGO reduced maximal [^{3}H]DAMGO binding by 80%, while decreasing the efficacy of opioid-agonist inhibition of adenylyl cyclase activity four-fold, an example of desensitization. Co-incubation of the cells with the irreversible antagonists and DAMGO prevented this desensitization of the opioid response, demonstrating an IC_{50} value for DAMGO-induced inhibition of cyclic AMP production similar to control values. Naloxone, a reversible μ antagonist, was unable to prevent desensitization at similar concentrations, suggesting the irreversible antagonists were not acting through simple competitive inhibition. These results suggest that the behaviorally observed blockade of opioid antinociceptive tolerance produced by the irreversible μ opioid antagonists occurs on a receptor or signal-transduction level. (Supported by NIDA grants DA03742, DA07232 and K05-DA00360.)

Mon11

CHRONIC HEROIN TREATMENT PRODUCES DESENSITIZATION OF MU OPIOID-ACTIVATED G-PROTEINS IN SPECIFIC RAT BRAIN REGIONS.

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[^{35}S]GTPγS autoradiography has shown that chronic morphine treatment decreases mu-stimulated [^{35}S]GTPγS binding in specific brain regions. To extend these findings, DAMGO-stimulated [^{35}S]GTPγS binding was compared in membranes and coronal sections from rats non-contingently administered heroin. Rats were administered increasing doses of heroin i.v. hourly up to 288 mg/kg/day over 40 days. In sections, chronic heroin administration decreased DAMGO-stimulated [^{35}S]GTPγS binding in medial thalamus and amygdala, with no effect in cingulate cortex or nucleus accumbens. In membranes from amygdala and thalamus, chronic heroin treatment produced significant reductions in the maximal effect of DAMGO, while having little effect on DAMGO potency, in stimulating [^{35}S]GTPγS binding. [^{35}S]GTPγS saturation analysis showed that chronic heroin treatment increased the K_D of [^{35}S]GTPγS while having little effect on the B_{max}. These data reveal potential mechanisms by which chronic agonist treatment produce opioid receptor/G-protein desensitization in brain. Supported by DA-06634, DA-02904 and DA- 00247 (TJM) from NIDA.

Mon12

ACUTE MU-OPIOID DESENSITIZATION SELECTIVELY AFFECTS PATHWAYS TARGETING CALCIUM CHANNELS

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An important antinociceptive action of opioid peptides is the inhibition of neuronal voltage-sensitive Ca^{2+} channels. Attenuation of the functional response following short-term or prolonged exposure to opioid peptides is a major factor limiting the use of these compounds as analgesics. We compared the characteristics of acute- and long-term-desensitization at the cellular level to identify potentially distinct mechanisms of the attenuation of opioid responses. An acute application of the μ-selective opioid agonist DAMGO inhibited whole-cell Ca^{2+} currents in ~75% of neonatal rat dorsal root ganglion (DRG) neurons tested. Short-term (5-10 min) application of DAMGO (3 μM) resulted in an ~54% loss of agonist-mediated inhibition of the Ca^{2+} current magnitude. By contrast, prolonged exposure to DAMGO (5 μM, 24 hrs) resulted in a near complete loss of DAMGO-mediated inhibition of the Ca^{2+} current. In contrast to the acute attenuation observed over minutes, this required at least 12 hr to develop. Acute- and long-term desensitization also differed with respect to the time necessary to reverse these states. Finally, acute desensitization selectively affected that opioid-activated pathway targeting N-type Ca^{2+} channels. N- and non-N-type targeted channels were defined by voltage-dependent properties and pharmacological criteria. These data suggest that acute desensitization can 'distinguish' among opioid-activated pathways, providing a mechanism by which opioids effect cell-specific changes in activity or neurotransmitter release. *Supported in part by NIDA grants DA10514 (RAG) and DA07232 (GMS).*

Mon13

EFFECTS OF ENDOCYTOSIS INHIBITORS ON MU OPIOID RECEPTOR FUNCTION

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The mu opioid receptor has been shown to be internalized in response to various agonist treatments. One approach to studying the functional role of mu receptor internalization is to selectively block this process and determine the effects on signal transduction and adaptational processes such as desensitization and resensitization. We have studied the effects of the following treatments on agonist-induced receptor internalization in 293 cells transfected with a FLAG-tagged mu receptor: hyperosmolar sucrose treatment, K+ depletion, phenylarsine oxide treatment and concanavalin A treatment. Hyperosmolar sucrose pretreatment was found be the most efficent of the various treatments for blocking agonist-induced receptor internalization. Furthermore, the cells were able to recover the ability to internalize receptors within 5 minutes of sucrose removal. We are currently determining whether hyperosmolar sucrose treatment adversely effects acute signal transduction.

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Mon14

REGULATION OF OPIOID RECEPTOR FUNCTION BY DIMERIZATION

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The opioid system modulates several physiological processes including analgesia, stress response, immune response, and neuroendocrine function. Molecular cloning studies have identified three opioid receptor types (delta, kappa and mu) that mediate these diverse effects. We have examined the ability of these receptors to physically interact with each other to form dimers. Delta receptors exist as detergent sensitive homodimers whereas kappa receptor homodimers are detergent insensitive. Kappa receptors are able to dimerize with delta receptors and these heterodimers are also detergent insensitive. Moreover, kappa homodimers and kappa-delta heterodimers are sensitive to reducing agents. Ligand binding studies show that the heterodimers are unable to bind highly selective ligands although they are able to bind non-selective ligands with high affinity. These properties are similar to the properties of a previously described kappa2 opioid receptor subtype. Interestingly, the heterodimer co-operatively binds selective ligands and potentiates signal transduction as measured by the decrease in intracellular cAMP. These findings provide a molecular basis for kappa receptor subtypes and a novel mechanism that modulates their function.

Mon15

DIFFERENTIAL REGULATION OF KAPPA OPIOID RECEPTORS BY OPIATES AND OPIOID PEPTIDES

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Internalization and down-regulation are important steps in the modulation of receptor function. Recent work with the β2-adrenergic receptor and opioid receptors have implicated these processes in receptor mediated activation of mitogen activated protein kinase (MAP kinase). Here, we have used CHO cells expressing rat kappa opioid receptors and prodynorphin derived peptides to characterize the agonist mediated endocytosis of kappa receptors and activation of MAP kinase. We find that kappa receptor selective peptides induce receptor internalization and down-regulation whereas selective synthetic agonists do not. Surprisingly, all agonists induce robust phosphorylation of MAP kinase demonstrating that internalization is not required for MAP kinase activation. An examination of Dynorphin A-related peptides to promote kappa internalization, inhibition of adenylate cyclase and MAP kinase phosphorylation, reveals that the N-terminal seven residues play an important role in eliciting these responses. Taken together, these results point to a difference in the ability of synthetic compounds and peptides to promote kappa receptor internalization and supports the notion that receptor internalization is not required for MAP kinase activation.

Mon16

THE PROTEASOME AND OPIOID RECEPTOR DOWN-REGULATION

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This study was designed to characterize the proteases that are involved in mediating agonist-induced opioid receptor down-regulation. Incubation of 293 cells expressing FLAG-tagged delta opioid receptors with the peptide agonist, DADL, caused a time-dependent decrease in ^{3}H-bremazocine binding to washed membrane preparations. Western blot analysis indicated that the level of the 65 kDa delta receptor band was unchanged following DADL treatment for up to 2 h, but was decreased significantly at 3- 6 h after DADL, and was undetectable after overnight treatment with the agonist. Down-regulation of the 75 kDa FLAG-tagged mu receptor occurred with similar kinetics. Pretreatment of cells with E64d, a cell-permeable inhibitor of lysosomal cathepsins B, H, and L, and the cytosolic calcium-dependent protease, calpain, had little effect on agonist-dependent delta receptor down-regulation. However, pretreatment with the proteasomal inhibitors, MG 132, and lactacystin, significantly attenuated delta receptor down-regulation induced by overnight treatment with DADL. MG 132 also decreased the extent of agonist-induced down-regulation of delta receptors in NG108 cells, and mu receptors in 293 cells. Proteasome inhibitors attenuated the agonist-dependent decrease in the 65 kDa delta receptor band and 75 kDa mu receptor band, and induced the appearance of a spectrum of higher molecular mass receptor bands and a single lower M_r delta receptor band at 35 kDa and a lower M_r mu receptor band at 50 kDa. These results implicate the involvement of the proteasome complex in agonist-induced down-regulation of mu and delta opioid receptors.

Mon17

MECHANISM MEDIATING AGONIST-SPECIFICITY OF OPIOID RECEPTOR ENDOCYTOSIS

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Opioid receptors undergo rapid endocytosis by clathrin-coated pits following activation by a wide variety of peptide agonists. However, individual alkaloid agonists differ considerably in their ability to promote endocytosis of receptors in diverse cell types. We have identified a critical role of G protein-coupled receptor kinase (GRK)-mediated phosphorylation and receptor interaction with beta-arrestin in mediating agonist-specific differences in regulatory endocytosis of opioid receptors. Agonist-specific actions on this regulatory mechanism are associated with profound differences in the ability of individual agonists to promote functional uncoupling of receptors from heterotrimeric G proteins, suggesting that this regulatory mechanism plays a fundamental role in determining the physiological effects of opiate drugs. Recent studies focus on understanding the mechanism by which individual opiate drugs differentially affect the rapid regulation of opioid receptors. Pharmacological and mutational studies suggest the existence of a distinct functional parameter, in addition to agonist potency and efficacy, that distinguishes the physiological actions of individual opiate drugs on the rapid regulation of opioid receptors. These observations have interesting implications for the concept of partial agonism and suggest a new model for understanding effects of opiate drugs on the endogenous opioid signaling system, which may provide insight into agonist-specific differences in the development of physiological tolerance and/or dependence to opiate drugs.

Mon18

MECHANISMS OF AGONIST-INDUCED DOWN-REGULATION OF THE HUMAN KAPPA OPIOID RECEPTOR.

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Prolonged exposure of many GPCRs to agonists leads to down-regulation, in which a significant reduction in the number of receptors occurs with or without attenuated responsiveness. In the present study, we determined whether the human and rat kappa opioid receptors (hkor and rkor, respectively) stably expressed in CHO cells underwent down-regulation and, if so, the mechanisms involved. U50,488H caused a significant down-regulation of the hkor, while etorphine did not. Neither U50,488H nor etorphine caused down-regulation of the rkor. Transient expression of dominant negative mutants beta-arrestin (319-418) or dynamin I-K44A in CHO-hkor cells significantly reduced U50,488H-induced down-regulation of the hkor. In addition, co-expression of GRK2 and beta-arrestin significantly increased etorphine-promoted down-regulation of the hkor, while co-expression of GRK2, beta-arrestin or dynamin I did not. Thus, there are agonist and species differences in down-regulation of kappa opioid receptors and this process involves a beta-arrestin- and dynamin-dependent mechanism. (Supported by NIH grants DA 04745 and DA 11263 (to L.-Y. L.-C.) and GM44944 and GM47417 (to J.L.B.)).

Mon19

MU-OPIOID RECEPTOR DESENSITIZATION AND RESENSITIZATION IN VIVO.

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Prolonged exposure to mu-opioid agonists results in reduced receptor responsiveness. We recently observed rapid desensitization to the blood pressure (BP) response to mu-agonists. IV administration of highly selective mu-agonists (DAMGO, DALDA, and s-DALDA) resulted in elevation of BP for <15 min. Complete desensitization was observed when a second dose of the same or another mu-agonist was given 30 min later. Partial heterologous desensitization was found between mu- and kappa-receptors but not between mu- and delta-receptors. Recovery from desensitization was dependent on the agonist used, with complete resensitization to DAMGO after 4h while the response to s-DALDA was still completely desensitized. The rate of resensitization appears to be inversely related to the elimination half-life of the agonist and suggests that recovery from desensitization is dependent on the duration of agonist exposure. The short duration of action of DALDA and s-DALDA, despite their long elimination half-lives, may indicate immediate onset of desensitization with first exposure. (PO1-DA08924-04)

Mon20

ALTERATIONS OF MU OPIOID RECEPTOR GENE IN CXBK MICE

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CXBK mice are known to show insensitivity to morphine and have been used for various experiments as opioid receptor deficient mice. However, there was no molecular biological evidence indicating deficiency of opioid receptor gene in CXBK mice. In the present study, to understand molecular mechanisms underlying behavioral phenotypes of CXBK mice, we analyzed mu-opioid-receptor (MOR) mRNA in CXBK mice by Northern blotting, nucleotide sequencing and *in situ* hybridization. The CXBK MOR mRNA was abnormal in its size and contained a nucleotide sequence difference from that of C57BL/6 mice in the 5' region, although it coded normal MOR protein. The mRNA expression level was slightly decreased in the CXBK brain. Next, we analyzed morphine analgesia in the litter mates which were produced by mating of heterozygotes between CXBK and C57BL/6 mice. In hot-plate test, mice having only CXBK-type MOR gene showed significantly lowered analgesia after intraperitoneal administration of 10 mg/kg morphine when compared with their litter mates having only wild-type gene (n=10; P=0.037, Welch's *t*-test). These results suggest that reduced morphine analgesia in CXBK mice is caused by alterations of MOR gene.

Mon21

EXPRESSION OF MULTIPLE SPLICE VARIANTS OF THE MOUSE MU-OPIOID RECEPTOR (MOR-1) GENE DURING ONTOGENY. J. Xu, Y.-X. Pan, E. Bolan, L. Mahurter, S.R. Letchworth and G.W. Pasternak. Cotzias Laboratory of Neuro-Oncology, Department of Neurology, Memorial Sloan-Kettering Cancer Center, New York, NY 10021, U.S.A.

Seven distinct MOR-1 receptor isoforms have been identified. All the isoforms share same exons 1, 2 and 3 as MOR-1, but contain different exons 4, which are generated from alternative splicing of the MOR-1 gene. The expression of the splice variants mRNAs in adult mouse brain and several peripheral tissues as well as during embryo development was determined by using a multiple tissue cDNA panel derived from BALB/c mice (ClonTech). Our results indicated that mouse brain expressed MOR-1 and all the splice variants, while the expression of the MOR-1 and its variants was not found in most peripheral tissues examined. Yet, the testis showed high expression level of the MOR-1, but none of the variants. During mouse ontogeny MOR-1 expression was observed in 11-day embryos, which is similar to the results done by in situ hybridization. However, the expression of MOR-1C, MOR-1D and MOR-1E was not detected until 17-day embryos. Differential onsets of the MOR-1 and its variants during ontogeny, together with the previous observation of distinct distribution of the variants in brain regions, imply that alternative splicing of the MOR-1 gene is highly regulated. The physiological roles of this splicing need to be further explored. This work is supported by DA00296 (Y.X. P.) and DA02615 and DA00220 (G.W.P.).

Mon22

MU-OPIOID & MORPHINE-6-GLUCONURIDE ACTIONS IN MICE LACKING EXON1 OF MOR-1

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Mice with a targeted deletion of Exon 1 of MOR-1 have been reported to be insensitive to morphine but to retain analgesia to M-6-G in focussed heat/tail flick tests. In wild-type mice mu-opioid agonists and M-6-G inhibited GABAergic synaptic transmission in PAG and RVM, caused an outward current in LC and inhibited calcium channels in acutely isolated PAG, LC and trigeminal ganglion neurons. In mice with an exon-1 deletion the effects of mu-agonists and M-6-G were abolished in all regions examined. The exon-1 deletion mice were also completely insensitive to the analgesic actions of M-6-G (10 & 20 mg/kg i.p.) in a warm water (52 ^{o}C) tail flick assay, while wild type mice showed robust analgesia at 10 mg/kg M-6-G. This study demonstrates that the acute effects of mu-opioids on neurons known to be involved in nociception are similar in mice and rats. The results also suggest that analgesia mediated by the putative M-6-G receptor is mediated either via distinct brain regions to mu-opioids or by distinct and as yet unidentified effects on neurons in the PAG, RVM and sensory ganglia.

Mon23

RETINOIC ACID (RA) INDUCED EXPRESSION OF MU OPIOID RECEPTOR mRNA IN A HUMAN NEUROBLASTOMA CELL LINE, SH-SY5Y.

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SH-SY5Y cells, a subclone of SK-N-SH, have been shown to express μ, δ, and κ opioid receptors. Reports have suggested that RA-induced differentiation increased human μ opioid receptor (hMOR) density in SH-SY5Y (Zadina et al., 1993) and δ opioid receptor mRNA in NG108-15 cells (Beczkowska et. al. 1996). The purpose of this study was to determine if the RA-induced increase in hMOR receptors levels in SH-SY5Y was accompanied by increased steady-state levels of hMOR mRNA. SH-SY5Y cultures of 80-90% confluence were treated with 10μM all *trans* RA in DMSO every other day for a total of six days. Control cells received 10% FBS in DMEM or DMSO vehicle. Total cellular RNA (50 μg) was analyzed by a ribonuclease protection assay. There was no difference ($p>0.05$) in hMOR mRNA levels between control and DMSO-treated cells while RA-induced differentiation of SH-SY5Y resulted in a 4-fold increase ($p<0.05$) in steady state hMOR mRNA (rnean±sem=0.5±0.1, 0.5±0.1, 2.1±0.2 pg/μg total RNA; control, DMSO, RA; respectively). Thus, RA-induced differentiation of SH-SY5Y cells is accompanied by regulation of μ opioid receptor mRNA which may account for the established increases in hMOR protein. (Supported by NIH GM56821-02)

Mon24

GENDER DEPENDENT EXPRESSION AND BINDING OF DELTA OPIOID RECEPTOR IN MOUSE BRAIN

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Females of a number of mammalian species, including humans, have lower thresholds for and tolerance of, and higher sensitivity to noxious somatic stimuli than males. Delta opioid receptors are known to modulate the mu opioid receptors induced antinociception. In the present study we measured the number of delta opioid receptors (DOR) and the expression of DOR during postnatal development in Swiss CD-1 male and female mouse brains. The density and affinity of binding sites for the delta-selective opioid ligand, [^{3}H]naltrindole, were determined, by saturation curves, in whole brain homogenates from 23-day old and adult mice. Male delta-opioid receptor number was always higher than that of female (23-day old mice**:** males, 139± 8; females, 111± 9; adult mice: males, 123±10; females, 71±8 fmol/mg proteins). DOR expression was assessed by RT-PCR assay. The total RNA was extracted from 10-, 23-day old and adult mouse brains. RT-PCR was performed with a primer pair specific for DOR. At all ages, DOR expression was higher in males than in females. But female mDOR levels decreased during postnatal development, reaching 65% of male mDOR levels at 10 days, 20% at 23 days and 40% in adults.

Mon25

DIFFERENTIAL EXPRESSION OF DELTA-OPIOID RECEPTORS AND mRNA DURING THE CELL CYCLE

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Previous immunohistochemical and radioligand-binding studies have shown a cell cycle-dependent regulation of the delta-opioid receptor (DOR). The relationship between DOR expression and mitosis in primary astroglial cultures from rat cerebral cortex was investigated. The cultures were synchronized and thereafter arrested in the G1/S-transition or in the mitosis. The DOR protein increased 1.7-fold (P = 0.008) in mitosis while mRNA for the DOR was measured to decrease 1.8-fold (P = 0.008) compared to the G1/S-transition. DOR. Protein and DOR mRNA samples were measured from non-synchronized cultures which served as controls. DOR protein in mitosis increased 3-fold (P = 0.009) and DOR mRNA in G1/S-transition increased 1.6-fold (P = 0.002) respectively compared to control cultures. Increased levels of DOR mRNA prior to the mitotic phase of the cell cycle suggest that high DOR protein levels in mitosis are regulated at a pretranslational level.

Mon26

MOLECULAR MECHANISMS OF DELTA OPIOID RECEPTOR (DOR) GENE REGULATION BY PROTEIN KINASES A AND C.

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Earlier studies have shown that agents which modulate protein kinase A and protein kinase C signal transduction pathways may regulate DOR gene expression. In this study molecular mechanisms involved in such processes are presented. RNase protection assays revealed that treatment of NG108-15 cells with 100 nM O-tetradecanoylphorbol 13 acetate (TPA) for 48 h increased DOR mRNA levels gradually. Various concentrations of forskolin induced within the first 5 h a transient downregulation, followed by an induction of DOR mRNA with levels higher than controls at 48 h. Reporter gene assays with various 5' deletion constructs and mutation constructs in transiently transfected NG108-15 cells in combination with electrophoretic mobility shift assays indicate that the increase of DOR mRNA after stimulation with TPA is mediated by a AP-1 binding site 357 bp upstream of the translational start codon. The forskolin effects seem neither to be mediated via CRE dependent mechanisms involving transcription factors like CREB, CREM or ICERS, nor indirectly via AP-1. As suggested by our reporter gene assay data DOR mRNA may rather be upregulated by forskolin via an AP-2 binding site on the DOR gene promoter.

Mon27

MU AND KAPPA OPIOID RECEPTOR GENE EXPRESSION IN RAT CNS

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The aim of this study was to evaluate the competitive RT-PCR as a method for measuring opioid receptor levels in rat brain. Initially, nine brain regions from naive Sprague-Dawley rats were analysed for basal mRNA levels of mu and kappa opioid receptors. Highest levels of KOR mRNA were observed in the nucleus accumbens and the hypothalamus, whereas MOR mRNA levels were most abundant in the hypothalamus and the striatum. This was totally in agreement with earlier reports measuring mRNA levels in rat CNS, confirming the RT-PCR as a reliable method for opioid receptor measurement. Furtheron, the effects of "binge" cocaine treatment on mu and kappa opioid receptors in the nucleus accumbens were studied. Sprague-Dawley rats were administered with "binge" cocaine (3 consecutive doses of 15 mg/kg i.p. at hourly intervals) during two days. Competitive PCR was used to quantify mRNA levels for mu and kappa opioid receptors. "Binge" cocaine treatment for two days produced a significant decrease of kappa opioid receptor mRNA expression as compared to control treatment with saline. At the same time, mRNA levels for the mu opioid receptor remained unchanged. The role of both opioid receptors in cocaine abuse, as well as the competitive PCR as a highly sensitive method, will be discussed.

Mon28

EFFECT OF MORPHINE ON cAMP RESPONSE ELEMENT-BINDING PROTEIN AND CYCLIC AMP RESPONSE ELEMENT- DIRECTED GENE TRANSCRIPTION

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The cAMP/Ca^{2+}-responsive element (CRE) and its cognate transcription factor CRE-binding protein (CREB) regulates transcription of several genes. We investigated effect of morphine on CREB/CRE directed gene transcription in Neuro2a MOR1A cells expressing μ-opioid receptors. Morphine (0,5-10μM, 6h) inhibited CRE-directed gene transcription by about 40% and this effect was stronger following prolonged exposure (16h) to morphine. The acute effect of morphine was reversed by naloxone. After 48h tolerance to the morphine effect has been observed, although expression of the reporter gene remained below control level. Application of naloxone triggered increase in CREB/CRE directed gene transcription. Further, an analysis of nuclear protein binding to CRE using an electrophoretic mobility shift assay revealed that acute and chronic morphine increased binding to the CRE element and this effect was further potentiated during withdrawal.

Our results revealed that the CREB is likely to contribute to long-lasting consequences of the exposure to opioids.

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Mon29

METHAMPHETAMINE (METH) AND [D-ALA^2,D-LEU^5]ENKEPHALIN (DADLE) TREATMENTS AFFECT MOLECULAR PROPERTIES OF DOPAMINE TRANSPORTER IN CD-1 MICE.

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We demonstrated in an autoradiographic study that DADLE can block the dopamine transporter (DAT) loss caused by METH. To further investigate the properties of DAT affected by METH and DADLE, we examined here: (1) the mRNA levels of DAT, (2) the binding characteristics of DAT labeled by [^{125}I]RTI-121 by using the homogenate binding assay and (3) the functionality of DAT by using [^{3}H]DA uptake assay. Male CD-1 mice received 4 injections of METH (10 mg/kg, i.p.) at 2 h intervals. DADLE (4 mg/kg, i.p.) was given 30 min before each METH administration. Brains were removed at designated time points (post METH) and P2 fractions were prepared from striata for binding and functional assays. The DAT mRNA level was not affected by METH or DADLE at all time points. The Bmax of [^{125}I]RTI-121 binding to DAT was reduced by METH to about 25% of controls at 30 min, day (d) 1 and d 14. DADLE completely restored the Bmax of [^{125}I]RTI-121 binding to the control level at all time points. METH caused an about 70% reduction in [^{3}H]DA uptake capacity at 30 min, 1 h, 2.5 h, 5 h, d 1 and d 14. However, DADLE did not block the reduction of DAT functionality caused by METH. These results indicate that DADLE can restore the DAT level at the DA terminals but could not restore the DAT function reduced by METH. METH apparently can cause a long-term damage to the DA uptake site on the DAT that could not be blocked by DADLE. [Supported by IRP & DBR, NIDA/NIH]

Mon30

GENE SEQUENCE ANALYSIS OF FISCHER AND LEWIS RATS DIFFERING IN SENSITIVITY TO THERMAL STIMULI

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Analysis of gene polymorphism of Fischer and Lewis rats, two inbred strains known to differ in their responsiveness to stress and to drugs of abuse, may provide valuable insight into factors which may predispose to addictive diseases. In the present study, we sought to determine whether sequence differences in genes relevant to responsivity to drugs of abuse or stress could be identified in these strains, which we have characterized with respect to sensitivity to the hot plate test at 48, 51 and 54^{o}C. While there was no difference between strains in basal latency at 48^{o}C, Fischer rats had significantly lower paw lick latencies at both 51 and 54^{o}C (p<0.001). We have sequenced coding regions of the mu opioid receptor, 5HT1A and 5HT2A serotonin receptors, D1 dopamine receptor, corticotropin-releasing hormone and pro-opiomelanocortin genes from both strains. There were no nucleotide sequence differences in the genes studied, except a silent mutation in the mu opioid receptor at Ala-314. This suggests that the primary structure of these gene products does not underlie the observed difference in basal sensitivity to thermal stimuli in Fischer and Lewis rats.

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Mon31

RUNNING BEHAVIOR IN SIX INBRED MOUSE STRAINS

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The rodent running-wheel model offers possibilities as an experimental method to study natural reward. We compared voluntary running behavior of six genetically inbred mouse strains given access to running wheels. The distance and circadian rhythm of running was assessed using a computerized system which registered running distance every 20 minutes. There were substantial differences in both in distance and temporal distribution of running between strains allowing grouping into high, intermediate and low runners. CBA mice were high runners, BALB/c, C57 and C3H intermediate whereas DBA and 129 were low runners. The CBA mice ran constantly eight h/day and rested 16h/day. The other mouse strains did not have such strict running and resting periodicity. To study mechanisms of natural reward by running, opioid receptor antagonists were administered to the addiction-prone C57 mouse. We found differential effects on running after mu- and kappa- receptor antagonists, suggesting that endogenous opioid peptides (dynorphin and beta-endorphin) might exert different roles controlling running behavior. In this putative experimental model for natural reward, we thus demonstrate a differential pattern of running in six genetically inbred mouse strains and show running is modulated by opioid receptor antagonists.

Mon32

ANABOLIC STEROIDS, AMPHETAMINE - EFFECT ON SUBSTANCE P & DYNORPHIN B LEVEL IN RAT BRAIN

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In recent years an increase in the abuse of anabolic-androgenic steroids (AAS) has been seen among drug addicts and others not connected to sports. Clinical evidence suggest that profound personality changes such as psychosis, depressions and increased aggressiveness may result from the abuse of these steroids. In addition, it has also been suggested that the brain gets sensitised to central stimulantia such as amphetamine after being exposed to AAS. In this study we have used radioimmunoassay to examine the substance P (SP) and dynorphin B concentration in rats treated with nandrolone decanoate alone and in combination with amphetamine. The results show increased levels of dynorphin B and SP in the periaqueductal grey (PAG) after chronic treatment with AAS (15 mg/kg/day i.m. during 14 days) compared to control animals. When a single dose of amphetamine (5 mg/kg) was given several weeks after the AAS treatment period, the stimulating drug potentiated the effect on dynorphin B in the PAG. However, the SP concentration in PAG and striatum seems to decrease after a single dose amphetamine, and furthermore appears to inhibit the AAS induced enhancement of SP. We also observed an increase in defensive aggression when rats pretreated with AAS received a single dose of amphetamine compared to animals treated with vehicle and an amphetamine injection. We are currently studying other peptides as well as substance P and dynorphin B in other brain regions in these animals.

Mon33

IMPAIRED OPIOID PEPTIDE PROCESSING AND ALTERED LEVELS OF PROHORMONE CONVERASES (PCS) IN MICE LACKING ACTIVE CARBOXYPEPTIDASE E *(Cpe^{fat} / Cpe^{fat})*.

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The processing of precursor proteins via limited proteolysis is an important cellular mechanism for the generation of biologically active peptides. Processing of opioid peptide precursors is initiated by endoproteolytic cleavages mediated by PCs such as PC1 and PC2, followed by the carboxypeptidase E *(Cpe)* catalyzed removal of C-terminal basic residues. The effect of carboxypeptidase E deficiency on the processing of Proopiomelanocortin (POMC) and PC1 and PC2 levels were examined in the brains of *Cpe^{fat} / Cpe^{fat}* mice. These mice carry a mutation in the *Cpe* gene which completely abolishes enzyme activity and have multiple endocrine disorders including obesity, infertility, and hyperproinsulinemia-diabetes. We have previously shown that the processing of ProDyn is significantly altered in these mice. Recently we have found that the processing of POMC is also altered; the levels of POMC-derived peptides such as alpha-MSH and beta-endorphin are substantially decreased in these mice. This impaired processing could be due to changes in the level of PC1 and PC2 activities since the levels of both enzymes are altered in these mice. PC2 activity and protein levels are increased in all brain regions and the level of fully active form of PC1 (66-68 kDa) is decreased [with a corresponding increase in the level of lesser active form (87 kDa)]. These results imply that *Cpe* deficiency leads to impaired processing of opioid peptides and changes in the levels and activities of PC1 and PC2. The differences in the activation state of PC1 and PC2 may contribute to differentially altered opioid peptide processing in *Cpe^{fat} / Cpe^{fat}* mice.

Mon34

ANTINOCICEPTION AND ITS MODULATION BY MET-ENKEPHALIN AND FMRFa CHIMERIC PEPTIDE ANALOGS.

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Following our studies on synthetic chimeric peptide - YGGFMKKKFMRFa - (CP-I), for the possible role of such amphiactive sequences in antinociception and its modulation (Peptides, 1999-in Press), two analogs of CP-I were designed. [D-Ala^2]YAGFMKKKFMRFa - (CP-II), an enzymatically more stable analog, induced naloxone reversible dose-dependent antinociception in the tail-flick test in mice (1,2 and 5 µg/mouse), after i.c.v. administration. When coinjected with morphine, CP-II (1µg/mouse, i.c.v.) potentiated morphine (2 µg/mouse, i.c.v.) antinociception. NPFF (1µg/mouse, i.c.v.) and FMRFa (1µg/mouse, i.c.v.) attenuated CP-II (1µg/mouse, i.c.v.) induced antinociception. These data indicate that the antiopioid effect of the FMRFa portion of CP-II was not apparent. YGGFMKKKFMRa-(CP-III), designed to test the importance of C-terminal modification on the observed antinociceptive effects of CP-I, produced significantly lower antinociception in mice (60 and 80 mg/kg, i.p) compared to CP-I. CP-III (60 mg/kg, i.p.) coinjected with morphine (7 mg/kg,i.p.) potentiated morphine antinociception, however this effect was lower than that produced by CP-I. Taken together, these preliminary results suggest that Arg-Phe-amide motif may be important in the observed antinociceptive effects of CP-I.

Mon35

IDENTIFICATION OF HUMAN, RAT AND MOUSE NOCISTATIN IN BRAIN AND HUMAN CSF

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Nocistatin was recently isolated from bovine brain and shown to block hyperalgesia and allodynia induced by nociceptin and prostaglandin E_2 (Nature 392, 286-289, 1998). Although the counterparts of human, rat and mouse are deduced from their precursor prepronociceptin to be 30, 35, and 41 residue peptide respectively, the endogenous structure have not been identified. To identify these mature forms of nocistatin, three peptides were synthesized and a detection program for nocistatin was developed, using reverse-phase high pressure liquid chromatography (HPLC) along with specific radioimmunoassay (RIA). The RIA method enabled us to detect amounts between 50 fmol and 2 pmol of these three nocistatins.

Nocistatin extracted from human (temporal lobe and cerebellum), rat and mouse whole brain were subjected to HPLC to collect eluates with one min interval and nocistatin-like immunoreactivity (NST-IR) was determined in each fraction. All three species showed two NST-IR peaks, one of which coincided with that of the corresponding putative nocistatin. Because RIA was not sensitive enough to detect the presence of nocistatin in human cerebrospinal fluid (CSF), we developed the extraction method to concentrate nocistatin from CSF. The same NST-IR was also detected in the extracts of pooled CSF obtained from chronic pain patients.

Mon36

DISTRIBUTION OF ENDOMORPHIN-1 AND ENDOMORPHIN-2 IMMUNOREACTIVTIES IN THE CENTRAL NERVOUS SYSTEM.

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The endomorphins are endogenous peptides with high affinity and selectivity for the mu opiate receptor. Endomorphin-1 and -2-like immunoreactivity (EM1-LI and EM2-LI) were examined in the rodent CNS with antisera specific for each peptide. Cell bodies expressing both EM1- and EM2-LI were present in hypothalamus while only EMI-LI was visible in perikarya of the NTS. EM1-LI fibers were present in numerous forebrain, diencephalic, midbrain and limbic structures including the diagonal band, BNST, OVLT, nuc. accumbens, septum, PAG, locus coeruleus, hypothalamic, midline thalamic, and amygdaloid nuclei. EM2-LI was present in lower abundance than EM1-LI in these structures, but in greater abundance in the superficial layers of the dorsal horn of the spinal cord and medulla. Both EM1- and EM2-LI were present in the parabrachial nuclei and the NTS but with differences in distribution. EM1- and EM2-LI were present in several regions known to contain high densities of mu opiate receptors. The results support the role of endomorphins as endogenous ligands for the mu receptor and as modulators of effects regulated through the mu receptor, particularly nociceptive, autonomic, and homeostatic functions as well as responsiveness to stress. Supported by the VA, NIDA (DA11655 to JEZ, DA05743 to SMS), and PVA/SCRF (1865 to JEZ).

Mon37

STEREOCHEMICAL REQUIREMENTS FOR RECEPTOR RECOGNITION OF ENDOMORPHIN-1.

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D-amino substitutions at any of the four positions of the endomorphin-1 tetrapeptide (EM1) decreases its biological activity, as measured by the guinea pig ileum essay. Replacement of Phe^4 results in a 10 fold loss of potency, while potencies of [D-Tyr^1]-EM1 and [D-Trp^3]-EM1 are 50 and 80-fold lower, respectively. Complete loss of activity occurs upon inversion of chirality at Pro^2 in [D-Pro^2]-EM1 and [D-Pro^2-D-Trp^3]-EM1 peptides. NMR spectroscopy of [D-Pro^2]-EM1 in DMSO-d_6 shows this peptide to exist in a 13% to 87% *cis/trans* ratio with respect to the peptide bond preceding D-Pro. Conformational analysis simulations of *trans*-[D-Pro^2l-EM1 using NMR-derived constraints results in a set of conformations with an extended backbone structure in the Pro^2 -Trp^3 region and a *gauche*- conformation of Trp-χ^1 side chain. Molecular dynamics simulations in an explicit solvent system show that structures compatible with the NMR results are present in these simulation conditions. Inactivity of [D-Pro^2]-EM1 is explained based on a reversal of the relative orientation of Tyr^1 and Trp^3 compared to EM1 (Podlogar et.al., FEBS Lett. 1998, 439, 13). Comparison of the endomorphin analogs with previous structural data of DAMGO identifies similarities and differences between these two opioid peptides. [Supported by NIDA-NIH grants DA01533 and DA00377 (to MGP)].

Mon38

NOVEL ENDOMORPHIN AND DELTORPHIN ANALOGUES

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Several new analogues of endomorphins were designed and synthesized using the concept of conformational restriction. βMe-Phe residues were incorporated in position four of endomorphin 1 and 2 and in position three of endomorphin 2. The absolute configuration of βMe-Phe determined the biological properties of the modified endomorphins. Endomorphins with L-βMe-Phe (2S,3S or 2S,3R) exhibited higher affinity (measured by radioligand experiments in rat brain membranes) compared to D-βMe-Phe containing endomorphins. (2S,3S) βMe-Phe^4 endomorphins were four to five times more active than the parent peptides with a concomitant increase in selectivity. Tyr^1 residue of $Ile^{5,6}$deltorphin II was replaced by (S and R) Hat (6-OH-2-aminotetraline-2-carboxylic acid). The new ligands showed high affinity and selectivity toward the delta-sites without subtype specificity. It was found that L stereochemistry in position one is preferential but not an absolute requirement for high affinity and delta selectivity; the sidechain conformation of deltorphins was mainly trans. Supported by OTKA 022104, 030086, Flemish-Hungarian grant B-3/96,

Mon39

SOLID-PHASE EXTRACTION OF MORPHINE FOR AN IMPROVED SEMI-QUANTITATION IN HUMAN URINE

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TLC seems ideally suited for analyses outside laboratory as well as in situations where advanced instrumentation are unavailable. While a number of techniques have been used for quantification of TLC plates, the most common detection method is slit-scanning densitometry. The aim of this study was to compare the ability of a solid-phase extraction (SPE) system (Samchrom) with liquid-liquid extraction (LLE) for urinary detection of morphine by TLC. Forty urine samples were collected from opioid abusers and each sample was divided into four fractions; two fractions extracted by the SP system and the other two by LLE (chloroform/2-propanol, 9:1; 20min shaking & centrifugation). One fraction in each system was hydrolysed by overnight treatment with 0.1M HCI at room temperature. All samples were analysed by TLC and the plates were quantified using a CS-9000 dual wavelength flying-spot scanning densitometer. The results showed both systems are relatively well on the hydrolysed samples. However, the AUC for the non-hydrolysed samples extracted by the SP system was significantly higher than the liquid extraction. In addition, a marked increase was observed for the AUC of morphine bands in the hydrolysed samples over the non-hydrolysed samples.

Mon40

CHARACTERIZATION OF MORPHINE-6-SULFATE AND CODEINE-6-SULFATE.

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Morphine-6-sulfate (M6S) and codeine-6-sulfate (C6S) are mu-selective opiate receptor ligands, the former of which has been found in brain. Similar to morphine-6-glucuronide (M6G), M6S is an effective and potent (ED_{50} 10ng, i.c.v.) analgesic agent with 30-fold greater potency than morphine in the mouse tailflick test. M6S analgesia was antagonized by 3-methoxynaltrexone at doses that are inactive in blocking the effect of morphine but selectively block M6G analgesia. This suggests that both M6S and M6G are acting at a different receptor than morphine. The gene encoding a mu opiate receptor (MOR-1) has been cloned and its exons characterized. Consistent with the existence of a mu receptor subtype resulting from alternate splicing of MOR-1 transcripts, M6S analgesia was inhibited by antisense targeting of exon 2 but not by targeting exon 1. C6S also showed analgesic activity 10 fold less than M6S. However, characterization of C6S was impeded by the occurrence seizures at doses below full analgesic activity. Thus, M6S, one of the major metabolites of morphine, is a potent analgesic agent with pharmacological properties similar to that of M6G. C6S has a limited utility due to its high level of toxicity. Supported by DA06241 (G.W.P.)

Mon41

PHARMACOLOGICAL ACTIVITIES OF NALTREXONE-DERIVED PYRIDO- AND PYRIMIDOMORPHINANS.

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We have been evaluating a series of pyrido- and pyrimidomorphinans synthesized from the opioid antagonist naltrexone. SoRI 9409, which has an introduced chlorine atom at the *para* position of the pendant 5'-phenyl group of the 5'-phenylpyridine, possesses both opioid mu agonist and delta antagonist activity in vitro (Ananthan et al., J. Med. Chem., in press, 1999). It is hypothesized that this mixed opioid profile may produce analgesia with limited development of tolerance and physical dependence. Here, we report on some of the in vivo actions of SoRI 9409 in male ICR mice. When administered by the intracerebroventricular (i.c.v.) or intraperitoneal (i.p.) route, SoRI 9409 produced partial agonist activity in the 55°C tail-flick test. In contrast, i.c.v. and i.p. SoRI 9409 produced full agonist effects in the acetic acid abdominal stretching assay. I.p administration of SoRI 9409 blocked the antinociceptive actions of the delta agonist DPDPE (30 nmol, i.c.v.) in the 55°C tail-flick assay. Repeated administration of i.c.v. or i.p. SoRI 9409 (~A_{90} dose, x2 daily, 3 days), did not result in a significant shift in the respective dose-response curves. Interestingly, repeated injections of SoRI 9409 produced diarrhea and mild weight loss across the tolerance regimen. These effects were in contrast to animals receiving repeated injections of vehicle or morphine. These studies suggest that compounds with mixed mu-agonist/delta-antagonist properties may offer therapeutic advantages over currently available mu opioid analgesics.

Mon42

3-AMINOMORPHINES - SYNTHESIS & BIOLOGY

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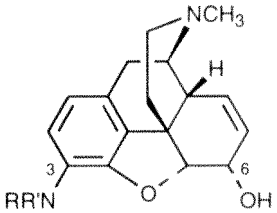
As part of an effort to identify novel opioid receptor interactive agents, we recently prepared a series of 8-(substituted)amino analogues of cyclazocine. We found the chiral 8-phenylamino (NHC_6H_5) cyclazocine derivative to have subnanomolar affinity for kappa opioid receptors; the compound was 2-fold less affinic for mu opioid receptors. To determine if the benefits of (substituted)amino groups could be extended to the morphine core structure, we have made five novel 3-aminomorphine derivatives of general structure **1** where RR'N = H_2N, CH_3NH, $(CH_3)_2N$, NHC_6H_5, and $NHCH_2C_6H_5$. Relative to morphine, these derivatives were 38- to 270-fold less affinic for mu opioid receptors and 10- to 140-fold less affinic for kappa. We prepared these compounds using a new method for the selective protection (via t-butyldiphenylsilylation) of the 6-OH group of morphine followed by Pd-catalyzed amination of the 6-OTBDPS-3-OTf-morphine. (Supported by DA01674 and DA03742).



1

Mon43

SAR OF 8-AMINOCYCLAZOCINE ANALOGUES

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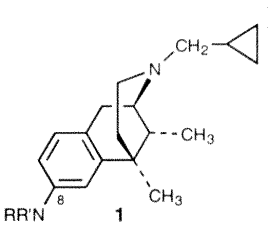
A series of novel 8-aminocyclazocine derivatives (1) were prepared and evaluated for binding to mu, delta, and kappa opioid receptors. Our goal was to develop an SAR in order to understand the role of the 8-substituent in opioid binding and to identify novel compounds with kappa agonist and mu antagonist properties having potential as anti-cocaine medications. Targets were synthesized from the triflate of cyclazocine using a Pd-catalyzed amination procedure. At least one H on N is required for reasonable binding affinity. Tertiary amine groups significantly decreased affinity for opioid receptors. For secondary amine derivatives, such as phenylamino, benzylamino, and methylamino, high affinity for opioid receptors was observed. For example, the optically active (2R, 6R, 11R)-8-benzylamino analogue had K_i = 0.67 nM vs. mu and 2.1 nM vs. kappa. Other derivatives had a modest preference for kappa binding. (Supported by DA01674 and DA03742).



1

Mon44

MOLECULAR MODELING AND AUTOMATED RECEPTOR DOCKING OF FENTANYL ANALOGS IN THE *MU*-OPIOID RECEPTOR

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The three-dimensional structure, dynamics, and docking modes of a series of highly selective *mu*-agonists based on the fentanyl prototype have been investigated using various molecular modeling techniques. A 2ns molecular dynamics simulation of solvated fentanyl, *cis*-methylfentanyl, *cis*-, *trans*-ohmefentanyl, and remifentanil revealed high conformational flexibility for the ligands in solution. Docking of the solution structures onto the transmembrane domain (TM) of the human *mu*-opioid receptor identified TM-2, -3, -6, and -7 to be directly involved in ligand binding. An ion-pair between D149 and the protonated nitrogen of the ligands anchor the receptor-ligand association. The proposed docking hypothesis corroborates available pharmacological data, explains the structure-activity relationship of several fentanyl derivatives, and rationalizes the enantiospecific binding of *cis*-ohmefentanyl. Comparison of the binding modes of fentanyls and opiate ligands will also be presented while highlighting the importance of H299 in the *mu*-receptor-binding pocket.

Mon45

AUTOMATED RECEPTOR DOCKING OF δ-OPIOID LIGANDS AS A BASIS FOR SIMULATION OF LIGAND-RECEPTOR INTERACTION

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A model of transmembrane domain of δ opioid receptor (δOR) has been established allowed automated docking of δ-selective and non-selective ligands. Most of a dozen of simulated ligands has been docked close to the commonly believed anchoring point in OR's, Asp128 (TM3), as "the best docking structure" using DOCK 3.5 suite of programs. Conformational flexibility of the ligands have been modeled both in gas phase and in explicit solvent with periodic boundary condition using AMBER 5.0 software suite. Torsional parameters have been adjusted or newly derived to fit torsion profiles to those calculated at MP2/6-31G* level of theory for related molecular fragments. The identified ligand binding mode is consistent with available site-directed mutagenesis and other experimental data and can be used for predictions of binding modes of other δ-opioid ligands and design of protein engineering experiments.

Mon46

CONSTRUCTION, ASSESSMENT, AND USE OF 3D MODELS OF THE THREE CLONED OPIOID RECEPTORS

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Refined models of the transmembrane domains of the cloned mouse μ, δ, and κ opioid receptors were constructed from a multiple sequence alignment using the recently reported alpha carbon template of rhodopsin. The thermal and structural stability of the three models was successfully validated by performing unconstrained molecular dynamics simulations of the energy optimized structures. The ligand-free delta receptor was then used to identify the most promising binding sites for the antagonist naltrexone and the agonist etorphine. These ligands were docked in all sites of the model delta-opioid receptor which were sterically accessible and to which the protonated amine of the ligands could be anchored to a complementary proton accepting residue. The lowest energy complex of each ligand most consistent with known experimental results was selected for further characterization. These ligand-receptor complexes were subjected to unconstrained molecular dynamics simulations to explore possible differences in properties between the agonist and the antagonist complex that could be determinants of activation. In addition, specific sites for mutation that could affect ligand recognition and activation of the delta opioid receptor were identified that can serve as a structure based guide to future experimental studies.

Mon47

PHARMACOLOGICAL STUDY OF A NEW MU SELECTIVE OPIOID DIPEPTIDE ANTAGONIST.

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We have recently designed potent delta selective opioid antagonist dipeptides on the basis of a simple conformational analysis. Following a similar procedure, we found a μ selective dipeptide antagonist 2,6-dimethyl-Tyr-D-Phe-NH_2. The μ opioid antagonist activity of this dipeptide was considered on the guinea-pig isolated ileum (GPI) electrically stimulated. The μ antagonism was measured in relation to the μ agonists DAMGO, morphine and dermorphin. Although this compound was inactive as an agonist in the stimulated GPI tissue up to concentration of 10^{-4}M, it showed a pA2 ranging from 6.5 to 7.3 depending on the μ agonist used. Owing to its simple chemical constitution, the 2,6-dimethyl-Tyr-D-Phe-NH_2 looks to be a very promising μ antagonist both as a lead structure and for practical applications.

Mon48

INCREASED ANTINOCICEPTIVE ACTIVITY AND BLOOD BRAIN BARRIER PERMEABILITY WITH SAM1095, A NOVEL GLYCOPEPTIDE ENKEPHALIN ANALOGUE.

H.D. Jones, S.A. Mitchell,* R.D. Egleton,* T.J. Gillespie,* T.P. Davis,* R. Polt* and E.J. Bilsky. University of Northern Colorado and *University of Arizona.

The pharmacological assessment of systemic opioid peptides has been limited by poor CNS bioavailability. Our previous work has suggested that a L-serinyl β-D-glucoside analogue of [Met^5]enkephalin penetrates the blood brain barrier (BBB) better than its unglycosolated parent peptide. Here we report on the pharmacological actions of a structurally less complex enkephalin analogue (SAM1095) and the unglycosolated parent molecule SAM995. Systemic administration (i.v. or i.p.) of SAM1095 to male ICR mice produced full agonist activity in the 55°C warm-water tail-flick test. On a μmol/kg basis, SAM1095 was 1.5 times less potent than morphine i.v. with an A_{50} value of 12.2 pmol/kg. SAM995 was significantly less potent when administered i.v. (A_{50} value > 50 μmol/kg). Site directed injections of naloxone methiodide confirmed a CNS mediated opioid effects. In a rat in situ BBB perfusion assay, SAM1095 had a significantly greater unidirectional transfer constant than SAM995 (1.32 μl/min/g brain versus 0.64 μl/min/g brain). SAM1095 displayed greater hydrophilicity (octanol saline distribution) and stability (15% mouse brain membranes and 100% mouse serum) compared to SAM995. Given that SAM1095 is (a) relatively potent when given systemically, (b) has moderate CNS bioavailability and (c) can be synthesized in gram quantities, it may be possible to begin assessing the development of antinociceptive tolerance, physical dependence and other side-effects of systemically administered SAM 1095.

Mon49

IN VIVO CHARACTERIZATION OF NALOXONE DERIVATIVES

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Recent studies revealed that the addition of a methoxy group at the third position of naltrexone, a traditional opioid antagonist, created an antagonist selective against morphine-6β-glucuronide (M6G) and heroin. *In vivo*, 3-methoxynaltrexone (3MeONtx) did not interfere with morphine (mu), [D-Pen^2,D- Pen^5]enkephalin (delta), U50,488H (kappa$_1$), and naloxone benzolhydrazone (kappa$_3$) analgesia at doses which blocked M6G and heroin (Brown *et. al*, FEBS Letters, **412,** 1997). We now have synthesized a series of analogous naloxone derivatives. The methyl, ethyl and propyl-3-ethers of naloxone were all antagonists. In these studies, 3-methoxynaloxone (3MeONlx) is a selective antagonist in blocking morphine analgesia. However, there is no significant difference 3-ethoxynaloxone's (3EtONlx) ability to antagonize either morphine or M6G analgesia. 3-Propoxynaloxone (3PrONlx), similar to 3MeONtx, has shown to be more selective against M6G analgesia. Thus, substitutions at the third hydroxy position of naloxone yields interesting compounds.

Mon50

ON THE BELL-SHAPED DOSE-RESPONSE CURVE OF BUPRENORPHINE

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Buprenorphine exhibits a characteristic bell-shaped dose-response curve in a number of *in vivo* assays. Using a cumulative dosing paradigm in the rat warm water tail withdrawal assay at 50^{o}C, it was confirmed that buprenorphine showed a biphasic dose-response curve with peak antinociceptive effect at 0.32 mg kg^{-1}. In contrast to previous findings, when rats were pre-treated with naltrexone a shift in the ascending, but not the descending, portion of the dose-response curve was observed. Investigation of the time-course of antinociceptive effect of various doses of buprenorphine revealed that the bell-shaped dose-response curve was time-dependent. Low doses of buprenorphine showed a slow onset and offset of antinociceptive effect. In contrast, high doses produced a rapid onset of antinociception within 15 min of administration. This was followed by rapid decline in antinociceptive effect; reaching a plateau of around 35% maximal antinociception 1.5 hr after administration. A high dose of the opioid antagonist naltrexone (10 mg kg^{-1}) fully reversed the initial peak of antinociception produced by buprenorphine but could only partially reverse the plateau phase when administered 1.5 or 3 hr following buprenorphine. It is suggested that buprenorphine produces an initial, rapid, reversible phase of antinociception followed by a longer lasting, weaker antinociceptive phase of an "irreversible" nature. Supported by DA 00254.

Mon51

NEW DELTORPHIN ANALOGS WITH DIFFERENT DELTA-OPIOID AFFINITY AND EFFICACY.

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Affinity and efficacy of the new *met*-deltorphin analogs, predicted from cloned cDNAs from skin of *Pachymedusa dacnicolor and Agalychnis annae* (*ile*-DELT, *a-ile*-DELT), were studied in comparison with deltorphin I (DELT-I), deltorphin II (DELT-II) and some synthetic analogs (*val*-DELT, *n-leu*-DELT). The ability of the drugs to inhibit [^{3}H]naltrindole binding and to stimulate [^{35}S]GTPγS binding, was evaluated in NG108-15 cells and CHO cells expressing the mouse delta- opioid receptor (DOR). In NG108-15 and in CHO cells DELT-I and –II maximally stimulated [^{35}S]GTPγS binding at 0.1 μM concentration; *met*-DELT and *val*-DELT produced no more than 70% stimulation at 1 μM and 10 μM concentration respectively; *ile*- and *a-ile*-DELT showed the lowest affinity for the delta-receptor and the lowest efficacy in [^{35}S]GTPγS binding stimulation (+36%). In NG108-15 cells, *n-leu*- DELT functioned as a partial agonist in [^{35}S]GTPγS binding stimulation, while, in CHO cells, it behaved as a full agonist, 100 times more potent than DELT-I.

Mon52

NON-PEPTIDE DELTA OPIOID ANTINOCICEPTION AND SIDE EFFECTS AFTER INTRAVENOUS ADMINISTRATION IN MICE.

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Delta opioid agonists exhibit potent analgesia through many routes of administration and in a number of models of: nociception. SNC80 and (±)TAN-67 are selective and specific non-peptide delta-opioid receptor agonists reported as analgesic agents that lack the typical mu and kappa opioid liabilities. However, convulsions have been noted following dosing with a number of delta-opioid agonists in mice and primates. SNC80 (i.p.) produced convulsive behavior in mice at antinociceptive doses. Lethality occurred only at very high doses, above the analgesic dose range. (±)TAN-67 has not been reported to cause the same convulsive behavior in mice at analgesic doses regardless of route of administration. The present studies evaluated the analgesic, convulsive and toxic properties of SNC80 and (±)TAN-67 after intravenous (i.v.) administration in the mouse abdominal irritant test. Antinociception was observed within a similar dose range as those that caused lethal convulsions and death for SNC80. (±)TAN-67 was not analgesic after i.v. administration, but did cause lethal convulsions. Compounds synthesized with similar delta-opioid profile were found to be analgesic without lethal convulsions and death after i.v. administration. However, oral administration of these compounds in the same dose range yielded reduced antinociception but an increased adverse event profile - convulsions and lethality. These studies underscore the complex issues surrounding the search for delta-opioid therapeutic agents.

Mon53

DYNORPHIN A ANALOGUES WITH ANTAGONISM FOR δ AND κ-RECEPTORS

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We report herein the pharmacological study of 3 dynorphin A analogues (Tic2-DYN-A(1-13)-NH_2 (1); Tic2-DYN-A(1-11)-NH_2 (2); Dmt1, Tic2-DYN-A(1-11)-NH_2 (3)) to verify their antagonism for δ and κ receptors. The δ and κ opioid antagonist activity of these dynorphin A analogues was considered on the guinea-pig isolated ileum (GPI) electrically stimulated for κ activity and rabbit jejunum for δ activity. The δ and κ antagonism were measured in relation to the δ-agonist deltorphin and κ-agonist U50-488H, respectively. In stimulated GPI, peptides 1-3 antagonized the κ-agonist U50-488H (pA2: 6.34, 8.33 and 7.04, respectively). In rabbit jejunum assay, peptides 1-3 antagonized the δ-agonist deltorphin with a pA2 ranging from 7.25 to 8.12.

Mon54

A NEW APPROACH TO THE DESIGN OF LONG- ACTING OPIOID ANTAGONISTS: AROMATIC ALDEHYDE DERIVATIVES OF BETA- NALTREXAMINE.

Bertrand Le Bourdonnec, Mary Lunzer, P.S. Portoghese. Department of Medicinal Chemistry, University of Minnesota, Minneapolis, MN 55455.

In an attempt to develop long lasting opioid antagonists, we have synthesized several *ortho*-phthalaldehyde (OPTA) and salicylaldehyde (SA) derivatives of beta-naltrexamine (series 1 and 2). The design of these compounds is based on the reactivity of OPTA and SA with primary amines. Hence, OPTA is known to react with nucleophilic amino acids (lysine and cysteine) to form an isoindole adduct. Moreover, SA reacts with primary amines to afford a hydrogen bond-stabilized Schiff base. Given that primary amines are present in proteins (eg, lysine) and in lipid membranes (eg, ethanolamine), such an approach has the potential for covalent bonding of the ligands to opioid receptors or to biological membranes. The OPTA and SA derivatives should exhibit a long duration of opioid antagonist action through these covalent reactions. The sustained *in vivo* opioid antagonist action observed for these compounds will be discussed in light of the covalent mechanisms described above.

Mon55

NALTRINDOLE ANALOGUES AS POTENT AND SELECTIVE κ-OPIOID RECEPTOR ANTAGONISTS.

W.C. Stevens Jr., R.M. Jones, G. Subramanian, D.M. Ferguson, and P.S. Portoghese. Department of Medicinal Chemistry, College of Pharmacy, University of Minnesota, Minneapolis, MN 55455.

Superposition of the conserved structural motif of the delta-opioid antagonist naltrindole (NTI), and the kappa-opioid antagonist norbinaltorphimine (norBNI), led to the design of 5'-alkylamidine substituted NTI analogues that proved to be potent and selective kappa-opioid antagonists. In the present study, substitutions at the 5'-position of NTI were systematically varied in an effort to optimize the receptor-ligand interactions and to determine how functionality, pK_a, size, and shape of the substituents affect antagonist properties. 5′-Guanidinyl-NTI (GNTI), and some closely related 5'-substituted NTI analogues, represent a new class of kappa-opioid antagonist that are more potent and selective than norBNI. Pharmacological results reveal GNTI to exhibit an approximate 10 fold increase in κ/μ-selectivity and 4-5 fold enhanced potency over norBNI. Molecular modeling studies have suggested that GNTI and its potent analogues derive their selectivity by ion-pairing with acidic residues in TM3(Asp138) and TM6(Glu297) helices of the kappa receptor.

Mon56

NEW KAPPA-SELECTIVE OPIOID AGONISTS

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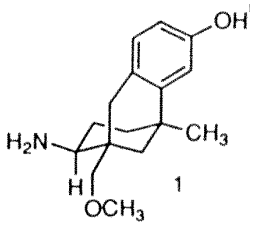
It has been reported (J. Med. Chem. 1996, 39 1956-1966) that the racemic 8-amino-5,9-methanobenzocyclooctene 1 has high affinity for both the mu-opioid receptor [K_i (nM) = 1.6] and the kappa-receptor (2.2) and less affinity for the delta-receptor (68). Antinociceptive studies demonstrated that 1 is a full kappa-agonist. In a new program to develop a kappa-selective analogue of 1, structural modifications at nitrogen and the methoxy group were investigated; replacing OCH_3 in 1 with OC_2H_5 gave Ki (nM) = 39 at mu, 450 at delta and 6.2 at kappa; with OC_3H_7 gave 140 at mu, 610 at delta and 4.9 at kappa; with $OC_{10}H_{21}$ shows complete loss of affinities for the opioid receptors. Opioid receptor affinities and selected antinociceptive studies for the full range of analogues will be presented.



1

Mon57

BINDING CHARACTERISTICS OF KAFFIRALIN A KAPPA SPECIFIC TETRAPEPTIDE- ffir-NH_2

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Kaffiralin is a tetrapetide composed of D amino acids, it was identified as a ligand for the opioid receptors using a positional scanning combinatorial library. Kaffiralin binds with high selectivity for the kappa receptor binding to mu and delta receptors in the micromolar range. Kaffiralin is a full agonist at the kappa receptor and has been shown to have peripheral analgesic effects in two collaborative studies by Dr. Bidlack and Dr. Riviére. Since the peptide has little resemblance to any of the known kappa ligands we have prepared a tritiated form of kaffiralin and (Nle^3)-kaffiralin (specific activities 28 and 50 Ci/mmole respectively) and examined their binding characteristics. The radiolabels were found to bind to guinea pig membranes in a saturable manner and the observed Kd value is in agreement with the reported K*i* values (2 nM). Kappa ligands were found to inhibit the binding of ^{3}H-kaffiralin in order of decreasing affinities (Bremazocine > U69,593 > U50,488 > Dynorphin A > Kaffiralin >> Naltrindole > DAGO > DPDPE). Binding characteristics including saturation, association/ dissociation kinetics, stability of the radioligands will be presented for guinea pig, rat and cloned human receptors.

Mon58

NOVEL, D-AMINO ACID TETRAPEPTIDES DEMONSTRATE UNPRECEDENTED κ-OPIOID RECEPTOR SELECTIVITY AND ANTINOCICEPTION.

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Selective peptidic kappa-ligands were recently identified by screening positional scanning format mixtures of a tetrapeptide combinatorial library using opioid receptor radioloigand binding assays (rat and guinea pig brain homogenate preparations). An all D-amino-acid tetrapeptide, ff(D-Nle)r-NH2 (FE 200041) with high affinity, selectivity and agonist activity at the kappa, vs. mu and delta, human opioid receptors was identified; FE 200041 showed approx. 30,000-fold kappa vs. mu selectivity. Other D-amino acid tetrapeptide analogs showed agonist activity and more than 90,000-fold kappa, vs. mu or delta, opioid receptor selectivity. FE 200041 and other tetrapeptides stimulated [^{35}S]GTPγS binding in hKOR-expressing cells with EC_{50}'s of approx. 1 nM. After i.v. administration, the tetrapeptides produce potent antinociception (mouse acetic acid writhing, both phases of the rat formalin flinch assay, incisional pain) while showing poor activity in the mouse tail immersion assay (52^{o}C). When compared with enadoline and asimadoline, non-peptidic kappa agonists with putative peripheral activity, the tetrapeptides showed equivalent antinociceptive potency but markedly decreased CNS penetration as shown by reduced sedation in the mouse rotarod assay. The ratios of A_{50}'s between mouse rotarod and writhing assays after i.v. administration were 2, 4, and up to 647 for enadoline, asimadoline, and the tetrapeptides, respectively. This D-amino acid tetrapeptide series shows unprecedented affinity and selectivity for kappa-opioid receptors, high antinociceptive potency and excellent peripheral selectivity.

Mon59

PHARMACOLOGICAL ACTIVITY OF THE DIASTEREOMERS OF THE KAPPA AGONIST GR 94839

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GR 94839 is a kappa agonist that has limited CNS penetration (Rogers et al., Br. J. Pharmacol. 106: 783, 1992). GR 94839 can exist as four diastereomers that may have distinct pharmacological activities. The four diastereomers of GR 94839 were prepared and tested in rats and mice in models of antinociception and pruritis. *R,S* inhibited acetic acid-induced writhing and compound 48/80-induced scratching in the mouse with ED_{50} values of 0.3 and 0.73 mg/kg, s.c., respectively. *R,S* also inhibited late phase formalin-induced flinching in the rat after both i.paw (87% I @ 300 ug) and s.c. administration (ED_{50} = 4 mg/kg). *S,R* inhibited acetic acid-induced writhing (ED_{50} = 2.2 mg/kg s.c.) and formalin-induced flinching after i.paw but not s.c. administration. Both *S,S* and *R,R* were weak in all of the assays. To evaluate the degree of sedation produced by GR 94839 that could be attributed to the *R,S* diastereomer, a comparison between the *R,S*, and *S,S* diastereomers was performed. The ED_{50} values for impairment of rotarod performance was 11 mg/kg s.c. for *R,S*. *S,S* (300 mg/kg, s.c.) failed to affect rotarod performance. The results from the rotarod experiment suggest that no improvement in the separation between antinociceptive activity and motor impairment was achieved by resolution of the diastereomers of GR 94839. Therefore, the profile of activity of GR 94839 was not improved by separation of the individual diastereomers.

Mon60

AGOUTI-RELATED PROTEIN: A UNIQUE SYSTEM FOR ENDORPHINS MEDIATED BEHAVIORS AMPLIFICATION.

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Agouti-related protein (AGRP) is a recently discovered neuropeptide that inhibits the binding and action of alpha-melanocyte stimulating hormone at both the melanocortin 3 (MC3R) and 4 receptors, and has been proposed to function primarily as an endogenous melanocortin antagonist. To evaluate if Agrp work as a melanocortin antagonist or might have other functions, and to better understand the interplay between the AGRP and melanocortin signaling systems; we compared their nerve fiber distributions to each other and to the distribution of messenger RNA for the MC3R and MC4R. Though deriving from distinct cell groups, AGRP and melanocortin terminals project to identical brain areas. Given the fact that melanocortins are coreleased with endorphins by POMC terminals and therefore can interact to mediate POMC behaviors, this study provide the neuroanatomical substrate for an indirect modulation of endorphin-mediated effects by AGRP. Thus endorphin and POMC mediated behaviors would be under the control of the amount of AGRP and POMC-derived peptides released at the terminals as well as the presence of melanocortin or opioid receptors in their vicinity. Such a mechanism is novel and may represent a new concept in neurotransmission. In addition, both AGRP and POMC neurons selectively express the MC3R, which provides a neuroanatomical basis for a dual-input circuit with biological amplification and reciprocal feedback inhibition. These studies highlight a broader complexity in POMC-mediated behavior in the brain.

Mon61

CHRONIC MORPHINE INCREASES NADPH-DIAPHORASE ACTIVITY IN MOUSE BRAIN

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Chronic morphine increases the NOS mRNA levels and NOS-positive cells in rat spinal cord (Machelska *et al* 1997 Neuroreport 8, 2743) and the NOS activity in brain (Kumar and Bhargava 1997 Gen. Pharmacol 29, 223). We have studied the expression of NOS activity in mouse forebrain by NADPH-diaphorase histochemical method. Groups of female CD-1 mice were daily administered, either saline or morphine (60 mg/kg body wt) or naloxone (5 mg/kg) or naloxone in combination with morphine, for a period of 70 days. They were then sacrificed and vibratome sections of their brains, fixed in 4% paraformaldehyde, were processed for NADPH histo-chemistry. In saline control group, the NOS-positive cells were high in number in the neocortex, striatum and other brain regions but they were scarce in the hippocampus. In morphine-treated group, the number of NOS-positive dendrites per cell increased remarkably although that of NOS-positive cells did not increase significantly. In the group exposed to morphine along with naloxone, such increase in the number of positive dendrites was not registered. These results indicate that the increase in NOS-activity could be a long-term adaptive change for opiate addiction.

Mon62

ALTERED OPIOID RECEPTOR BINDING IN BRAINS OF ALZHEIMER'S DISEASE (AD) PATIENTS

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A possible linkage between AD and the endogenous opioid system is suggested by evidence that endogenous opioids may have a function in memory and learning. Our studies compared opioid receptors in limbic structures from AD patients (n=l1) to those from age-matched control individuals(n=10). Criteria for control subjects included normal physiological and neurological testing, absence of more than occasional signs of focal degeneration in the cerebral cortex and absence of senile plaques and/or neurofibrillary tangles. μ, δ, and κ opioid receptors were labeled, respectively, with tritiated DAMGO, DPDPE or bremazocine (mu/delta blocked). Nonspecific binding was determined with μM naloxone. Data from autoradiographs showed statistically significant reductions in μ-opioid receptor binding in the subiculum and hippocampus of AD brains. Binding of δ-opioid receptors was also decreased in the amygdaloid complex of AD brains. Large increases in κ-opioid receptor binding were found in the dorsal and ventral putamen and cerebellum of AD subjects. μ, δ, and κ opioid receptor binding was unaltered in caudate, parahippocampal gyrus and occipito-temporal gyrus. These results confirm and expand results published by us previously on binding studies in homogenates of brain areas from control and AD individuals and suggests a role for the endogenous opioid system either in the etiology of this neurodegenerative process or in the multitude of effects that accompany this disease.

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ADAPTIVE CHANGES IN THE EXPRESSION OF CENTRAL MU-, DELTA- AND KAPPA-OPIOID RECEPTORS IN D2 KNOCKOUT MICE.

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Behavioural as well as biochemical and molecular interactions have been described between opioidergic and dopaminergic systems. We have therefore investigated if the deletion of the D2-dopamine receptor gene could induce any compensatory changes in the expression of opioid receptors. For this purpose, detailed quantitative autoradiographic mapping of the mu-, delta- and kappa- opioid receptors has been carried out in the brains of wild type (+/+), heterozygous (+/-) and homozygous (-/-) D2 knockout mice, using [^{3}H] DAMGO, [^{3}H] Deltorphin I and [^{3}H] CI-977, respectively. D2 dopamine receptors were labelled with [^{3}H] raclopride. No D2 receptors could be detected in the brains of (-/-) mice, with the exception of residual [^{3}H] raclopride binding observed in the islands of Calleja and in the rostral part of the nucleus accumbens, where D3 receptors are localized. A decrease in the density of mu-opioid receptors was observed in the nucleus accumbens and the caudate-putamen (with some rostro-caudal differences) of the mutant mice, and in their respective projection areas, the ventral pallidum and the lateral globus pallidus. In contrast, an increase in the density of kappa receptors was found in the nucleus accumbens, the caudate-putamen and the substantia nigra. The opposing changes observed in these specific dopaminergic areas are likely to be related to the opposite effects induced by the selective stimulation of mu or kappa receptors on motor behaviour and motivational components of opiate dependence. We are currently studying the expression of delta receptors in D2 knockout mice and that of D2 and D1 receptors in mu knockout mice.

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MEDULLARY PROJECTING CELLS IN THE PERIAQUEDUCTAL GRAY CONTAIN MU OPIOID RECEPTOR.

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In the ventrolateral periaqueductal gray (PAG) opioids are thought to act primarily on inhibitory interneurons but not on neurons that descend to the ventromedial medulla. In addition, some data has suggested that medullary projecting cells from the lateral PAG, rather then the ventrolateral PAG are inhibited by MOR ligands. Here we examined the ultrastructural distribution of MOR on neurons in the lateral and ventrolateral PAG that were retrogradely labeled from the ventromedial medulla. To compare between the lateral and ventrolateral areas, matched pairs of tissue blocks originating from the same section of tissue were sampled. MOR labeling was often found in retrogradely labeled neuronal dendrites (128/275) and soma (21/73). No difference in the distribution of MOR within projecting neurons was detected between the lateral and ventrolateral PAG regions (p=0.55 paired t-test). In addition, MOR-labeled axon terminals were observed synapsing on medullary projecting PAG neurons. These data suggest that opioid ligands may directly as well as indirectly modulate descending neurons in both the lateral and ventrolateral PAG.

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REGIONAL COMPARISON OF NMDA AND MU OPIOID RECEPTOR LOCALIZATION IN THE ROSTRAL AND INTERMEDIATE NUCLEI OF THE SOLITARY TRACT

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NMDA and Mu opioid receptors (MOR) have been implicated in the regulation of gustatory and cardiorespiratory functions, which involve visceral reflex neurons located in the rostral and intermediate nuclei of the medial solitary tract (mNTS), respectively. We examined the ultrastructural immunocytochemical localization of NMDAR1 and MOR in these regions of rat brainstem to determine the functional sites for receptor activation. NMDAR1 were detected in dendritic and axonal profiles throughout the mNTS containing MOR. The dendrites composed 64% (n = 261) and 35% (n = 390) of the dually labeled structures in the rostral and intermediate mNTS, respectively. In dendrites as well as dendritic spines, NMDAR1 labeling was located on postsynaptic densities and membrane of smooth endoplasmic reticulum, while MOR gold particles were seen mainly at nonsynaptic sites on the plasma membrane. In contrast to dendrites, only 11% of the dually labeled profiles (n = 261) in the rostral, but 46% of those (n = 390) in the intermediate mNTS were axon terminals. The dually labeled terminals in the intermediate mNTS showed the characteristic morphology of visceral afferents. These were large and formed multiple asymmetric excitatory-type synapses with dendrites containing MOR or NMDAR1, or lacking detectable immunoreactivity. Our results suggest that NMDAR and MOR agonists target many common neurons throughout the mNTS, but have a more significant postsynaptic action in the rostral, and a more significant presynaptic role involving viscera afferents in the intermediate region. (Supported by grants from NIH: HL18974 and from NIMH: MH48776, MH00078).

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PRESENCE OF MU OPIATE RECEPTOR IN MANY NON-DOPAMINERGIC AND A SUBPOPULATION OF DOPAMINERGIC NEURONS IN THE VENTRAL TEGMENTAL AREA

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Mu opioid receptor (MOR) agonists are thought to have indirect actions on dopaminergic neurons in the ventral tegmental area (VTA) that are critical to opiate reward and reinforcement. To determine the functional sites for MOR activation in this region, we examined the electron microscopic immunocytochemical localization of MOR and the association with dopaminergic neurons containing the catecholamine-synthetizing enzyme tyrosine hydroxylase (TH) in rat VTA. MOR immunoreactivity was mainly localized in postsynaptic dendrites, but significative labeling was also present in presynaptic axon terminals and small unmyelinated axons. In medium-small dendrites, the MOR was localized mainly to perisynaptic plasma membrane, whereas in large dendrites the receptor was seen primarily on membranes of the smooth endoplasmic reticulum and tubulo-vesicular organelles. Axonal labeling was mainly seen on plasma membranes and membranes of small synaptic vesicles. In sections processed for dual labeling, over 75% of the MOR-containing dendrites were without detectable TH immunoreactivity. Other dendrites contained MOR and low density labeling for TH as compared with nearby dendrites that were intensely TH-immunoreactive. These results provide ultrastructural evidence that, in VTA, MOR agonists exert their primary actions on non-dopaminergic neurons, but also have direct postsynaptic actions on a subpopulation of dopaminergic neurons and presynaptic effects on release of other neurotransmitters.

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CELLULAR BASIS FOR MODULATION OF DOPAMINERGIC TRANSMISSION THROUGH KAPPA-OPIOID RECEPTORS IN THE RAT NUCLEUS ACCUMBENS SHELL

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The nucleus accumbens shell (AcbSh) is a brain region critically involved in kappa-opioid receptor (KOR) mediated inhibition of dopaminergic transmission. Using electron microscopic immunocytochemistry for localization of antipeptide antibodies directed against KOR and the dopamine transporter (DAT), we investigated the specific neuronal sites for modulation of dopamine function through activation of KOR in the AcbSh. Immunoreactivity for KOR and DAT primarily was observed in axon terminals and intervaricosities, but also was detected within small axons. Many of these morphologically heterogenous presynaptic profiles contained both antigens. Within these dually-labeled profiles, immunogold-silver particles for DAT were randomly distributed along the plasma membranes, while the KOR peroxidase reaction product was restricted to membranes of small synaptic vesicles and segments of plasma membranes that sometimes overlapped DAT labeling. A few axons and axon terminals that were apposed to one another were differentially labeled for KOR or DAT. In addition, KOR-immunoreactivity was seen in selective dendritic spines receiving convergent input from DAT-containing terminals and unlabeled terminals forming asymmetric excitatory-type synapses. Our results suggest that in the AcbSh, KOR agonists modulate dopaminergic transmission primarily through direct presynaptic actions on dopaminergic and non-dopaminergic terminals. In addition, these data suggest a more minor role for KOR in the dual modulation of postsynaptic responses to dopamine and excitatory amino acids within spiny striatal neurons. Supported by NIDA grants DA11768 to A.L.S. and DA04600 to V.M.P.

Mon68

COLOCALIZATION OF MU- AND DELTA-OPIOID RECEPTORS IN SPINY NEURONS AND SELECTIVE AXON TERMINALS IN RAT STRIATAL PATCHES

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The mu- and delta-opioid receptors (MOR, DOR) are abundant in the caudate-putamen nucleus (CPN), where agonists for both receptors inhibit dopamine-sensitive adenylate cyclase activity. In contrast to MORs, which have a patchy distribution, DORs are more evenly located in the CPN and play roles in enhancing stimulated dopamine release. We examined the ultrastructual immunocytochemical distribution of two antipeptide antisera differentially recognizing MOR and DOR in the rat striatal MOR-enriched patches to determine the cellular basis for their common and diverse actions. Dual immunoperoxidase and immunogold labeling showed that MOR and DOR were often colocalized in dendrites and spines of spiny neurons. In these profiles, immunogold particles for MOR were associated with the plasma membrane, whereas DOR labeling was more frequently distributed in the cytoplasm attaching to membranes of smooth endoplasmic reticulum. MOR- and/or DOR-labeled neuronal profiles were also associated through asymmetric axospinous synapses. Both labelings were colocalized in selective axons, whereas DOR-labeling was more frequently detected than MOR. Our results suggest that in patches of the CPN, the output of single spiny neurons can be dually modulated by activation of MOR and DOR. In addition, DOR, but more rarely MOR, agonists may act presynaptically to regulate neurotransmitter release. (Supported by grant from NIDA DA04600)

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AUTORADIOGRAPHIC CHARACTERISATION OF G-PROTEIN COUPLING OF DELTA-OPIOID RECEPTORS IN THE BRAINS OF MU-RECEPTOR KNOCKOUT MICE.

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Mice lacking the mu-opioid receptor gene (muKO) have partially reduced functional activity of the delta-opioid receptor. In addition there is some down regulation of delta binding in discrete brains regions of muKO mice. To determine if this down regulation is due to uncoupling of the δ-receptor, quantitative autoradiography in the presence of the GTP analogue, GMPPNP has been studied in muKO brain sections. Binding of [^{3}H]Deltorphin I ([^{3}H]DELT I), [^{3}H]-R-Atc-Ile^{5,6} deltorphin II ([^{3}H]RATGLU), [^{3}H]SNC-121 or [^{3}H]naltrindole ([^{3}H]NTI) was investigated in the presence or absence of GMPPNP. GMPPNP (50μM) significantly decreased [^{3}H]DELT I, [^{3}H]SNC-121 and [^{3}H]RATGLU binding in all genotypes. Lower delta receptor binding for [^{3}H]DELT I and [^{3}H]SNC-121 was detected in some brain regions of muKO mice compared to wild type, whereas no difference in binding of either [^{3}H]NTI or [^{3}H]RATGLU was observed. In the brain regions that showed a decrease in [^{3}H]DELT I and [^{3}H]SNC-121 binding in muKO brains, the GMPPNP shift was greater in wildtypes than muKO mice. This study suggests that the reduced receptor binding sites recognised by [^{3}H]DELT I and [^{3}H]SNC-121 in muKO brains is due to some delta-receptors being uncoupled from G-proteins in the absence of the mu-receptor. This finding may provide an explanation for the partial loss of activity of delta-agonists in muKO mice.

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AUTORADIOGRAPHY IN MICE LACKING BOTH MU AND DELTA OPIOID RECEPTOR GENES PROVIDES NO EVIDENCE FOR KAPPA$_2$ SUBTYPES.

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Autoradiography of mu, delta and kappa opioid receptors has been carried out in the brain of wild type and homozygous mu/delta double knockout (double KO) mice. [^{3}H]Deltorphin I and [^{3}H]DAMGO were used to label delta and mu opioid receptors. Kappa opioid receptors were labelled with [^{3}H]CI-977 alone and with bremazocine in the presence of cold DPDPE and cold DAMGO or in the presence of cold CI-977. NSB was determined in the presence of naloxone. In the double KO mice there was a complete absence of mu and delta ligand receptor binding throughout the whole brain confirming the disruption of the MOR and DOR genes. At any one level there was the same density of [^{3}H]CI-977 binding in both wild type and double KO brains corresponding to the level of [^{3}H]bremazocine binding in the double KO brains. In the presence of cold DPDPE and DAMGO, in regions of high mu and delta receptor density in wild type brains, there were increased levels of [^{3}H]bremazocine binding suggesting that the cold competing ligands could not completely supress [^{3}H]bremazocine binding to mu or delta receptors. CI-977 abolished [^{3}H]bremazocine binding in double KO brains. In some cortical areas of the double KO brains increased [^{3}H]CI- 977 binding indicated up-regulation of the kappa receptor. These results suggest there is only one kappa receptor gene and that labelling of the mu and delta receptors has led to the hypothesis of the kappa$_2$ subtype.

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DISTRIBUTION OF KOR-3/ORL-1 SPLICE VARIANT TRANSCRIPTS IN MOUSE BRAIN.

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Five splice variants of the KOR-3/ORL-1 gene have recently been identified in mouse (Pan et al., 1998). Three of these have insertions between the first and second coding exons. The insertions are 34 bp (KOR-3a), 98 bp (KOR-3b and KOR-3c) and 139 bp (KOR-3c) in length. In this study, 48-mer oligonucleotide probes were created against the unique insertions of the KOR-3a, KOR-3b, and KOR-3c mRNA transcripts. Probes were 3' end-labeled with [^{35}S]dATP and used for *in situ* hybridization in brain sections from CD-1 mice. KOR-3a was the most prevalent of the variants examined, with high levels of expression in the cortex, striatum, hippocampus, cerebellum and spinal cord. Hybridization to KOR-3b mRNA was visible in the hypothalamus and hippocampus. KOR-3c transcript levels were the lowest of the three variants examined. These results support the concept of a complex KOR-3 receptor system. This work is funded by CA09461 (SRL), DA00296 (YXP), DA02615, DA06241 and DA00220 (GWP).

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SPINAL KAPPA OPIOID RECEPTOR DENSITY VARIES ACROSS THE ESTRUS CYCLE IN RATS

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Animal and human studies suggest that kappa opioid receptor (KOR) agonists may be differentially effective analgesics in females and males, however, the anatomical basis for such differences is not understood. As a first step in examining the influence of hormonal status on KOR function, KOR levels were examined in female rats across the estrus cycle. A previously characterized polyclonal antibody to the KOR was immunocytochemically localized in the lumbosacral spinal cord (spinal segments L6-S2) of female rats in proestrus, estrus, or diestrus. To enable quantitative comparisons, all tissue in each experiment was labeled simultaneously using the same solutions. By light microscopy (LM), KOR labeling was most abundant in the superficial layers (laminae I-II) of the dorsal horn, in area X and in the dorsolateral funiculous in all rats. Quantitative LM densitometry was used to examine the superficial dorsal horn. KOR density was highest during estrus, lower during proestrus, and lowest during diestrus. Rats in diestrous had significantly lower KOR densities than those in estrus. These results suggest that female reproductive hormones regulate spinal KOR levels, which in turn may contribute to the variations in analgesic effectiveness of KOR agonists. Supported by DE12738-01 (CTD).

Mon73

EFFECT OF ETHANOL AND STRESS ON THE HPA-AXIS AND BETA-ENDORPHIN RELEASE.

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Stress has been associated with high alcohol consumption. Both ethanol and stress increase the activity of the pituitary Beta-endorphin (B-EP) and hypothalamic-pituitary-adrenal (HPA)-axis. It has been proposed that ethanol affects differently non-stressed than stressed subjects. Thus, it was the objective of the present studies to test this hypothesis by investigating the effect of ethanol and stress, alone or in combination, on the plasma adrenal corticotropin (ACTH), cortisol and B-EP contents of young male subjects at high (HR) and low (LR) risk of alcoholism, who were social drinkers at the time of testing. Exposure to stress, but not to alcohol induced an increase in the plasma content of ACTH, cortisol and B-EP of both LR and HR subjects. An alcohol drink 30 min prior to stress, prevented or blunted the stress induced increase in the plasma ACTH, cortisol and B-EP in LR and HR subjects. An alcohol drink 30 min post-stress induced a faster recovery of plasma ACTH, cortisol and B-EP contents only in the HR subjects. These results support the hypothesis that ethanol affects differently stressed than non stressed subject.

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Mon74

HPA TOLERANCE TO CHRONIC ALCOHOL BLUNTS ACUTE ALCOHOL-INDUCED POMC AND CRF_1R mRNA CHANGES

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Endogenous corticotropin-releasing factor (CRF), its pituitary CRF_1 receptor (CRF_1R) and proopiomelanocortin (POMC) may be involved in the hypothalamic-pituitary-adrenal (HPA) responses to alcohol. In the present study, mRNA levels of CRF, CRF_1R and POMC in the hypothalamic- pituitary axis, and plasma ACTH and corticosterone (B) levels were measured following acute or chronic regimens of oral "binge" pattern alcohol administration. Male Fischer rats were given alcohol (18% in water) by oral gavage three times daily at hourly intervals (total daily dose 4.5 g/kg). After 1 day of acute alcohol, both plasma ACTH and B levels were dramatically elevated. After 14 days of chronic alcohol, the ACTH response to alcohol was blunted, and alcohol-elevated plasma B levels were significantly attenuated. Hypothalamic CRF mRNA levels were not altered after either acute or chronic alcohol. Hypothalamic POMC mRNA levels were significantly decreased after acute alcohol. However, after chronic alcohol, this difference was not observed. Anterior pituitary CRF_1R mRNA levels were also significantly decreased after acute alcohol, with no change after chronic alcohol. Pituitary POMC mRNA levels were not altered by either acute or chronic alcohol. Taken together, these results suggest that: (1) rats exposed to chronic "binge" alcohol develop tolerance in HPA activity; (2) the observed decrease in anterior pituitary CRF_1R mRNA levels may be caused by glucocorticoid inhibition, and (3) the altered hypothalamic POMC mRNA levels may help us to understand neuroendocrine responses to alcohol at the molecular level.

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SEX DIFFERENCES IN ANTAGONISM OF OPIOID ANTINOCICEPTION

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Previous studies have shown that there are sex differences in opioid antinociception. The goal of this study was to determine if a possible explanation for these sex differences is a difference in opioid receptors. The opioid receptor selective antagonists, beta-funaltrexamine (beta-FNA), nor-binaltorphimine (nor-BNI), and naltrindole (NTI), acting at mu, kappa, and delta opioid receptor subtypes, respectively, were administered into the cerebral ventricles (i.c.v.) at various doses. Following antagonist administration, rats of each sex were injected systemically with morphine, U-50,488H, butorphanol, or (-)-pentazocine, using a cumulative dosing procedure, and then tested on a 52°C hotplate. Nor-BNI shifted the U-50,488H dose-effect curve to the right to a similar degree in both sexes. Beta-FNA shifted the U-50,488H dose-effect curve approximately a ½-log unit to the right in both sexes. Nor-BNI did not shift the morphine dose-effect curve in either sex. However, beta-FNA shifted the morphine dose-effect curve to the right to a significantly greater degree in females compared to males. This result suggests that receptor differences could explain why males are more sensitive than females to the antinociceptive effects of morphine and other mu opioids. Receptor mediated actions of (-)-pentazocine and butorphanol will also be characterized.

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MORPHINE MICROINJECTED TO THE vlPAG PRODUCES GREATER ANTINOCICEPTION IN MALE VS. FEMALE RATS.

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Sex differences in antinociception have been shown after morphine was injected to the rostral ventromedial medulla (Boyer et al., Brain Res 796 (1998) 315-318). The present study further characterized brain areas important in sex differences in morphine-induced antinociception. Male (N=9) and female (N=10) Sprague-Dawley rats were implanted with guide cannulae aimed at the vlPAG. Saline and morphine (1.0, 3.0 and 10.0 micrograms) were infused in a repeated measures design, and rats were tested in a 52°C tail withdrawal assay at various times post-injection. After rats had received all agonist infusions, 1.0 microgram beta-funaltrexamine (a mu-opioid receptor antagonist) was infused to the vlPAG, and systemic morphine was administered to obtain a morphine dose-effect curve. At all doses, morphine microinjected to the vlPAG produced greater antinociception in male compared to female rats. Beta-funaltrexamine shifted the male dose-effect curve approximately one-quarter log unit to the right, whereas the female dose-effect curve was shifted approximately one-half log unit to the right. This study demonstrates that sex differences in mu-opioid induced antinociception are centrally mediated and can be elicited from the vlPAG. Additional antagonist experiments are underway to determine whether sex differences in morphine-induced antinociception may be explained by differential opioid receptor activation in vlPAG.

Mon77

APPARENT PA2 ANALYSIS OF NALTREXONE AND 3-METHOXYNALTREXONE ANTAGONISM OF THE ANTINOCICEPTIVE EFFECTS OF HEROIN, 6-ACETYLMORPHINE AND MORPHINE IN RATS

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Although heroin's effects traditionally have been ascribed to the actions of its metabolite morphine at mu-opioid receptors, recent research suggests that heroin may also act via other opioid receptor subtypes. For example, 3-methoxynaltrexone (3-MNTX) was reported to be a relatively selective ligand for a novel opioid binding site labeled by morphine-6-glucuronide. In previous studies, 3-MNTX blocked the antinociceptive properties of heroin, but was relatively ineffective as an antagonist of morphine in mice. Thus, it was proposed that this site may mediate some effects of heroin. In this study, the ability of naltrexone (NTX) and 3-MNTX to antagonize the antinociceptive effects of heroin, 6-acetylmorphine (6-ACM) and morphine was compared in 54 adult male Sprague-Dawley rats. Nociception was determined with a warm-water tail-withdrawal assay (52^{o}C). Complete dose-effect curves for each agonist were determined alone and following pretreatment with NTX (0.01-0.32 mg/kg) and 3-MNTX (0.1-10.0 mg/kg). Heroin (0.032-1.0 mg/kg), 6-ACM (0.032-1.0 mg/kg) and morphine (0.32-10.0) produced dose-dependent increases in tail-withdrawal latency. NTX and 3-MNTX produced dose-dependent rightward shifts in the dose-effect curves for all three agonists, but 3-MNTX was more potent as an antagonist of heroin (pA2=6.73) than of 6-ACM (5.86) or morphine (5.73). The potency of NTX was comparable across all three agonists and apparent pA2 values for NTX antagonism of heroin (7.82), 6-ACM (7.72), and morphine (7.74) were similar. These data suggest that the antinociceptive effects of heroin may be mediated by a different receptor subtype than those of morphine and 6-ACM in rats. (Supported by NIDA grants P50-DA04059, K05-DA00101, and T32-DA07252).

Mon78

INVOLVEMENT OF THE SPINAL GABA AND GLUTAMATE SYSTEM IN THE (+)TAN67-INDUCED NOCICEPTION

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TAN67 is a highly selective nonpeptide delta receptor agonist. However, it has been reported that TAN67 produces no or little antinociception. TAN67 is a racemate, and (+) and (-) forms given intrathecally (i.t.) induce nociception and antinociception, respectively. We already found that the (+)TAN67-induced nociception was modulated by $GABA_A$ and $GABA_B$ receptors in the spinal cord. To investigate further mechanism of the (+)TAN67-induced nociception, we examined the involvement of $GABA_B$ and NMDA receptors in the (+)TAN67-, picrotoxin (PIC)- and bicuculline (BIC)-induced nociception, because (+)TAN67 has an affinity to $GABA_A$ receptors. I.t. (+)TAN67, PIC and BIC produced a dose-dependent nociception; the responses were significantly suppressed by $GABA_B$ receptor agonist baclofen. Moreover, these nociceptions were significantly suppressed by MK-801, a noncompetitive NMDA receptor antagonist. These results suggest that (+)TAN67-, PIC- and BIC-induced nociceptions may be modulated by $GABA_B$ and NMDA receptors in the spinal cord. Therefore, (+)TAN67 may induce nociception by release of glutamate which activates NMDA receptors, via antagonism of $GABA_A$ receptors in the spinal cord, and $GABA_B$ receptors may play an important role in the (+)TAN67- induced nociception in the spinal cord.

Mon79

A PRELIMINARY STUDY ON ANTINOCICEPTIVE EFFECT OF PAEONIFLORIN IN MICE LACKING THE MU-OPIOID RECEPTOR

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Paeoniflorin, a principal component from paeony roots, has been used as an antispasmodic and analgesic agent. From our previous study, we found that paeoniflorin showed antinociceptive effect on both the writhing response test and formalin test performed in mice. Antinociceptive effect of paeoniflorin is dose-related. In order to postulate the effect of paeoniflorin on mu-opioid receptor, we used the formalin test performed in wild-type, heterozygous mutant and homozygous mice. The result showed paeoniflorin had antinociceptive effect on wild-type, heterozygous mutant and homozygous mice. However, there's no significant difference on paeoniflorin-induced antinociception among these three groups. Besides, antinociceptive effect of paeoniflorin was not potentiated on morphine-treated naive mice. From our preliminary data, it suggested that antinociceptive effect of paeoniflorin might not have a close relationship with mu-opioid receptor.

Mon80

POSSIBLE MECHANISMS FOR INSULIN-INDUCED ATTENUATION OF THE ANTINOCICEPTIVE EFFECT OF DAMGO.

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The effects of pretreatment with protein kinase C and protein kinase A inhibitors on the i.c.v. insulin-induced attenuation of the antinociceptive effect of DAMGO were studied in mice. I.c.v. pretreatment with insulin dose- and time-dependently attenuated the antinociceptive effect of i.c.v. DAMGO (5.6 ng). I.c.v. pretreatment with a highly selective tyrosine kinase inhibitor, herbimycin A, at doses of 200 and 600 ng for 70 min, dose-dependently reversed the attenuation of the antinociceptive effect of DAMGO caused by insulin. I.c.v. pretreatment with serine/threonin kinase inhibitor, H-7, at doses of 3-30 nmol for 60 min, dose-dependently reversed the attenuation of the antinociceptive effect of DAMGO caused by insulin. I.c.v. pretreatment with selective protein kinase C inhibitor, calphostin C, at doses of 1 and 3 pmol for 60 min, but not with a highly protein kinase A inhibitor, KT5720, at dose of 10 pmol for 60 min, reversed the attenuation of the antinociceptive effect of caused by insulin. These results suggest that the reduction of DAMGO-induced antinociception by insulin in mice may be, in part, due to the activation of protein kinase C followed by the activation of tyrosine kinase.

Mon81

THE ANALGESIC EFFECT OF VENLAFAXINE

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The antinociceptive effects of the novel phentylethylamine antidepressant drug venlafaxine and its interaction with various opioid noradrenaline and serotonin system receptor subtypes were evaluated. Mice were tested with a hotplate analgesia meter. Venlafaxine induced a dose-dependent analgesic effect. The ED_{50} for mice in the hotplate assay was 46.7 mg/kg. The analgesia induced by venlafaxine was significantly inhibited by naloxone and nor-BNI but not by β-FNA, naloxonazine and naltrindol (implying κ1 mechanisms of action but less μ and δ mechanisms). On the other hand yohimbine but not phentolamine and metergoline, decreased analgesia elicited by venlafaxine, implying a α_2 adrenergic mechanism of action and to lesser extent α_1 adrenergic and serotonergic mechanisms. Venlafaxine was administered together with various agonists of the opioid subtypes and α_2 adrenergic receptor. We found that the antinociceptive effect of venlafaxine is influence by the κ opioid receptor and by α_2 adrenergic receptor. These results suggest a potential use of venlafaxine in the management of some pain syndromes.

Mon82

A PAIN FACILITORY ROLE FOR OPIOID RECEPTORS.

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Five experiments studied the contribution of opioid receptors to the hyperalgesia produced by intraperitoneal (i.p.) injection of the illness-inducing drug lithium chloride (LiCl). Intraperitoneal injection of 127.2 mg/kg of LiCl produced hyperalgesia when rats were tested for pain sensitivity using the tailflick test which was apparent within 5-min of injection and persisted for the 30-min test period. Hyperalgesia was prevented by s.c. injection of 2.5 mg/kg of naloxone, and neither hyperalgesia nor its reversal by naloxone could be attributed to variations in tailskin temperature. Hyperalgesia was also prevented when opioid receptor antagonism was restricted to: 1) the periphery through i.p. administration of the quaternary opioid receptor antagonist naloxone methiodide (4mg/kg); 2) the brain by i.c.v. microinjection of naloxone (5 microgram); and 3) the spinal cord by i.t. microinjection of naloxone (5 microgram). These results document evidence for a pain facilitory role of opioid receptors throughout the neuraxis. Supported by grants from the Australian Research Council, The Government Employee's Medical Research Fund, and an Australian Postgraduate Award.

Mon83

(D-MET^{2}PRO^5) ENKEPHALINAMIDE, MORPHINE, INFLAMMATION AND HYPERSENSIVITY

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The effects of (D-Met^2,Pro^5)-enkephalinamide, a highly potent opioid peptide, and those of morphine were examined several in vivo models of inflammation and hypersensitivity. In doses about 3-10 fold above their analgesic ED_{50} values both opioids inhibited the edema induced by carrageenan and zymosan in rats and mice, resp. They also suppressed both phases of formalin-induced paw lick reaction. However, these opioids dose-dependently aggravated the oxazolone-induced ear edema in previously sensitized mice, an effect reversible by naloxone. In adjuvant arthritis assay a week inhibition was seen on the site of primary inflammation but the secondary (allergic) edema on the opposite side was moderately aggravated. Naloxone suppressed the opioid-induced potentiation of the late allergic swelling. Thus morphine and (D-Met^2,Pro^5)-enkephalinamide seem to have an acute antiinflammatory activity but they apparently augment the secondary hypersensitivity phenomena.

Mon84

ACTIONS OF CANNABINOIDS AND OPIOIDS IN BRAIN REGIONS MEDIATING ANALGESIA

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The midbrain periaqueductal grey (PAG) and the rostral ventromedial medulla (RVM) play a critical role in the supraspinal antinociceptive actions of both mu-opioids and cannabinoids. Unlike mu-opioids, the cellular actions of cannabinoids within these brain regions are unknown. The cannabinoid agonist WIN55,212 (1) reduced the amplitude of evoked IPSCs (inhibitory postsynaptic currents), and (2) reduced the rate but not the amplitude of spontaneous miniature IPSCs, in PAG and RVM neurons. The endogenous cannabinoid anandamide inhibited evoked IPSCs in the presence, but not in the absence of the anandamide transport inhibitor AM404. A stable analog R1-methanandamide inhibited evoked IPSCs. The effects of cannabinoids were reversed by the CB1 cannabinoid antagonist SR141716. WIN55,212 had no effect on postsynaptic K^+ and Ca^{2+} conductances in PAG and RVM neurons. Thus, cannabinoids produce antinociception in PAG and RVM by inhibition of GABA release from nerve terminals, while mu-opioids also have postsynaptic inhibitory effects. The potency of endogenous cannabinoids is limited by uptake and breakdown.

Mon85

DECREASED MORPHINE ANALGESIA BY ANTISENSE OLIGONUCLEOTIDE TO $G_s\alpha$.

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In tissue culture, chronic opiate treatment produces a compensatory increase of adenylyl cyclase activity that may be associated with the development of tolerance. We have performed antisense experiments to study the effect of $G_s\alpha$ downregulation on morphine analgesia over time. Antisense oligodeoxynucleotides (ODN's) approaches have been successfully utilized to downregulate G-proteins and have been correlated with opioid receptor mediated effects (Rossi et at., 1997). Antisense ODN's against different G-protein α subunits differentially affect opioid supraspinal and spinal analgesia. An antisense ODN to $G_s\alpha$ has no effect on spinal analgesia (Standifer et al. 1996). CD-1 mice were injected intrathecally (i.t) on days 1, 3 and 5 with antisense ODN to $G_s\alpha$ and daily with morphine (5 or 7.5 mg/kg). A control group received morphine with a nonsense ODN control or saline. $G_s\alpha$ antisense ODN treatment prevented the decline in the analgesic action of 5 mg/kg injection of morphine ($p< 0.001$) and 7.5 mg/kg morphine ($p <0.005$) over 7 days. This effect was not seen with the nonsense or saline control. These results suggest a role for $G_s\alpha$ in maintaining the action of chronic morphine. (Supported by NIH grants DA07242, DA00220, DA06241, DA00310).

Mon86

ANTI-ANALGESIC AND ANTI-AMNESIC EFFECT OF COMPLEMENT C3a

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Casoxin C (YIPIQYVLSR) was isolated from the trypsin digest of bovine kappa-casein in the wake of screening its anti-opioid activity in GPI assay. The peptide proved to be a complement C3a agonist having as essential sequence to exert C3a activity, hydrophobic residue-X_1-Leu-X_2-Arg, at the carboxyl terminal. We found that casoxin C exerted central effects after icv. administration in mice. At doses of 1 to 100 nmol/mouse the peptide showed antagonistic effect on analgesia induced by morphine and U-50488H, known to be a mu- and kappa-opioid receptor agonist, respectively, whereas it had no effect on analgesia induced by DTLET, a delta-opioid receptor agonist. Casoxin C improved scopolamine- and ischemia-induced amnesia at doses of 10 and 100 nmol/mouse. Given the fact that casoxin C is a complement C3a agonist, we injected complement C3a by icv. in mice and found that C3a exhibited antagonistic effect at a dose of 10 pmol/mouse on analgesia induced by morphine and U-50488H. Moreover, complement C3a improved scopolamine- and ischemia-induced amnesia at a dose of 10 pmol/mouse. These results suggest that complement C3a is not only a well-documented immunopeptide, but also a neuropeptide having anti-opioid activity.

Mon87

TRANSIENT DECREASE IN MORPHINE-INDUCED RELEASE OF CHOLECYSTOKININ IN THE RAT DORSAL HORN AFTER PERIPHERAL AXOTOMY

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The neuropeptide cholecystokinin (CCK) is regarded to counteract opioid analgesia at the spinal level and morphine has recently been reported to induce the release of CCK in the dorsal horn of the spinal cord. An axotomy of the sciatic nerve, an animal model for deafferentiation or phantom limb pain, induces an upregulation of CCK and CCK-B receptor mRNA in dorsal root ganglia of the rat. The mu-opioid receptor like immunoreactivity (MOR-LI) in the dorsal horn is transiently reduced at 14 days after axotomy of the sciatic nerve and returns to normal 31 days after the lesion. The aim of this study was to investigate if the effect of morphine on the release of CCK-LI in the dorsal horn follows the same time course as the density of the mu-opioid receptor after axotomy of the sciatic nerve. In vivo microdialysis in halothane anesthestised rats: Control (n=13), 2-3 weeks (n=18) and 5-7 weeks (n=12) after axotomy. Morphine was administered either subcutaneously (5 mg/kg) or locally (via perfusion of the dialysis probe, 100 µM). Systemic and local administration of morphine induced a significant release of CCK-LI in intact rats ($p<0.05$; Friedmans and Dunn's test). This effect of morphine was transiently inhibited 2-3 weeks after axotomy, but returned to normal 5-7 weeks after axotomy. The present data indicate that morphine-induced CCK release in the dorsal horn of rats is transiently, but not permanently affected by a complete transaction of the sciatic nerve. This time course mirrors the density of mu-opioids receptors in the dorsal horn after the same lesion.

Mon88

ENDOMORPHIN-1 MODULATES THE PERIPHERAL INFLAMMATORY RESPONSE IN THE RAT HIND PAW

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Using a blister model of inflammation, we have previously shown that the peripheral inflammatory response can be inhibited by endogenous opioids acting at a post-terminal level under chronic injury conditions or by hemorphin-7 acting at a pre-terminal level in acute injury conditions. In the present study we have examined the effect of endomorphin-1 (EM-1), a putative endogenous µ-opioid receptor agonist, on the inflammatory response under different injury conditions. We have used a vacuum induced blister model in anaesthetised rats (Nembutal 60mg/kg i.p.) to examine the effect of EM-1 on the acute inflammatory response induced by; (1) electrical stimulation (ES) of the distal portion of the exposed/cut sciatic nerve or; (2) superfusion of Substance P (SP) over the blister base. The results showed that EM-1 significantly reduced the vascular response to substance P in a naloxone reversible manner under acute injury condition as well as under recurrent (repeated blister induction) and chronic (chronic sciatic nerve lesion) injury conditions. The results demonstrated a greater inhibitory effect of EM-1 on the vascular response to electrical nerve stimulation (58% inhibition) compared to a 34% inhibition of the vascular response to exogenously perfused SP, suggesting the involvement of both pre-and post-terminal mechanisms in the inhibitory actions of EM-1. The results also indicated that EM-1 is equipotent in inhibiting the inflammatory response under acute (34%), recurrent (39%) and chronic (42%) injury conditions. The significance of this study in conjunction with our previous work is that it supports the proposition that different endogenous inhibitory mechanisms operate under different injury conditions.

Mon89

NEUROTENSIN NEURONS REDUCE BETA-ENDORPHIN-ANTINOCICEPTION.

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Antinociception induced by opioids is determined by their net effect on inhibitory and facilitory pain modulatory neuronal processes. Neurotensin neurons extending from the periaqueductal (PAG) to the rostroventral medial medulla (RVM) function in a pronociceptive manner when activated by opioids. Microinjection into the RVM of a neurotensin receptor antagonist (SR 48692), in a dose (30 pmol) which selectively antagonizes the pronociceptive action of neurotensin, causes a marked enhancement of the antinociceptive response to morphine injected systemically or into the PAG (JPET 282: 899, 1997). Herein, using the tail flick test of antinociception, a similar response is observed injecting 1-3 nmol of Beta-endorphin in the PAG with 30 pmol of SR 48692 in the RVM of conscious rats. Beta-endorphin's maximum response was enhanced nearly three-fold, and its duration of action was prolonged. Physiologically, Beta-endorphin neurons in the PAG may be functionally linked to this pronociceptive mechanism, suggesting a mechanism for alterations in pain sensitivity induced by behavioral challenge (Analgesia, 3: 101, 1997).

Mon90

FOOT-SHOCK STRESS ACTIVATES A SPINAL ANTI-OPIOID SYSTEM IN RATS

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We examined the effects of foot-shock (FS; 1.0 mA, 0.2 Hz, 1 sec duration for 15 min) stress on morphine analgesia in SD male rats. The antinociceptive effects were estimated by a hind- paw pressure test (cut off pressure; 1500g) and were evaluated by maximum increase in pain thresholds (MAX) and area under the analgesic curve (AUC). For i.c.v. and i.t. injections, guide cannula and polyethylene tube (PE10) were implanted on a surface of the skull and in the spinal canal at least 7 days before experiments, respectively. The plasma morphine concentration was measured by the HPLC-ECD method. The FS stress- induced increase in pain thresholds was restored to control level at 15 min after the end of FS stress. The morphine analgesia (10 mg/kg, s.c.) in both MAX and AUC was significantly reduced by the FS stress, when morphine was injected 15 min after the end of FS stress. This suppressive effects were not observed when morphine was injected 90 min after the FS stress. The plasma morphine concentration was not affected by the FS stress, indicating that the suppressive effects of the FS stress on morphine analgesia were not due to the reduction of plasma morphine concentration. The morphine analgesia induced by i.t. (10 μg/rat), but not i.c.v. (30 μg/rat), was suppressed by the FS stress. These results suggest that an anti-opioid system, which is activated by the FS stress, is located in the rat spinal cord.

Mon91

OPIOID MODULATION OF NOCICEPTIVE THRESHOLDS IN FISH.

L. Chervova and D. Lapshin*. Department of Ichthyology, Moscow State University, and *Institute for Infomation Transmission Problems, Moscow, Russia. Teleost fishes possess the capacity to react to pain stimuli (Chervova, 1997). Newly improved optico-mechanical system was used to determine behavioral nociceptive thresholds (NT) in common carp, *Cyprinus carpio*. Peripheral nociceptive electrical stimulation of the caudal fin (bursts of short pulses 0.5 ms of current 0.5-2.0 mA, with repetition rates 300/s) induced the undulations of the caudal peduncle. The stimulation and registration of the locomotor reaction were made by computer control. Amplitude and latency of response visualized on display. The setup allowed to measure the NT to an approximation of 10%. It was found that NT of fish under this condition was comparable with human's one. I.m. administration of drugs produced dose-dependent and lasting for at least 1 h increase of NT in 1.5-3 times. Drugs used were mu agonist tramadol (10-100 nmol/g), partial mu agonist buprenorphin (30-100 nmol/g), kappa agonist U-50488 (30-80nmol/g), delta agonist DADLE (10-50 nmol/g). Antinociceptive effects were blocked or significantly reduced by pretreatment with naloxone.