

29th International Narcotics Research Conference

INRC 1998 Programme

Plenary lectures, symposia and oral presentations will be held in the „Festsaal Werdenfels“ of the Congress Center of Garmisch-Partenkirchen. Posters should be mounted in the Foyer of the Congress Center before the plenary lecture on Tuesday morning (July 21, 1998). They should be left throughout the whole Conference until Saturday noon (July 25, 1998). The presenting authors should man the posters during the poster viewing time. (Figures in parenthesis indicate the number of symposia talks, oral communications or abstracts).

Monday, July 20, 1998

13:00 - 19:00 **Registration** Foyer of the Congress Center

19:00 - 22:30 **Reception** Foyer of the Congress Center

Tuesday, July 21, 1998

08:30 - 8:35 Opening remarks

08:35 - 9:20 **Plenary lecture Molecular genetic analysis of glucocorticoid and cAMP signaling by gene targeting**

Günter Schütz (Heidelberg) Chair: Walter Zieglgänsberger

09:30 - 12:30 **Symposium 1 Gene deletions in vivo and its use to analyse opioid receptor and peptide function**

Chairpersons: Brigitte Kieffer and Gavril Pasternak

09:30 - 10:00 John Pintar (S1-1) Genetic analysis of opioid receptor function

10:00 - 10:30 Andreas Zimmer (S1-2) Genetic analysis of nociceptive behaviors in mice

10:30 - 11:00 Malcolm Low (S1-3) Analgesia and neuroendocrine homeostasis in β -endorphin-deficient mice

11:00 - 11:30 Coffee Break

11:30 - 11:45 Michael King (O1-1) Enkephalin/delta receptor systems involvement in opioid tolerance

11:45 - 12:00 Josephine Lai (O1-2) Effects of opioid delta agonists in mu „knock-out“ mice

12:00 - 12:15 Yermiliya Berman (O1-3) Defective prodynorphin processing in transgenic micelacking carboxypeptidase E and prohormone convertase 2

12:15 - 12:30 Hanspeter Fischer (O1-4) Neutral endopeptidase knockout induces nociceptive overcompensation

12:30 -14:00 **INRC Executive Meeting**

13:30 - 15:30 **Posters 1**

15:30 - 19:00 **Symposium 2 Inter and intra species variation of opioid receptor genes: expression and regulation**

Chairpersons: Horace Loh and Volker Höllt

15:30 -16:00 Marc Darlison (S2-1) Evolutionary conservation of structure and function of opioid receptors

16:00 - 16:30 George Uhl (S2-2) Knockout mice and drug responses variants: Opiate, DAT and VMAT2

16:30 - 17:00 Margarete Höhe (S2-3) Genetic variability of the human μ opioid receptor gene and its implication for substance abuse

17:00 - 17:30 Coffee break

17:30 - 17:45 Mary J. Kreek (O2-1) Single nucleotide polymorphism in the human μ opioid receptor gene in methadone-maintained and control studysubjects

17:45 - 18:00 Lei Yu (O2-2) Studies of receptor function in a single nucleotide poly- morphism of the human μ opioid receptor gene

18:00 -18:15 Peter Mayer (O2-3) Allelic variations of the delta and kappa opioid receptor in the general population

18:15 - 18:30 Li-Na Wei (O2-4) Regulation of the mouse kappa opioid receptor gene activity

18:30 - 18:45 Raquel Rodriguez (O2-5) Molecular characterization of μ and d opioid receptor from zebrafish

18:45 - 19:00 Craig Stevens (O2-6) The uniceptor hypothesis of opioid antinociception in amphibians: implications for evolution of opioid receptors

Wednesday, July 22, 1998

08:30 - 9:15 **Plenary lecture Functions of potassium channels in the central nervous system**

Olaf Pongs (Hamburg) Chair: George R. Siggins

09:30 - 13:00 **Symposium 3 Opioid receptor signaling**

Chairpersons: Huda Akil and Ping Y Law

09:30 - 10:00 George R. Siggins (S3-1) Signaling mechanisms in limbic neurons with acute and chronic opiate administration

10:00 - 10:30 MacDonald Christie (S3-2) Opioid receptor signaling via arachidonic acid metabolites in single brain neurons

10:30 - 11:00 Charles Chavkin (S3-3) Homologous desensitization of opioid receptor mediated by G protein receptor kinases

11:00 - 11:30 Coffee Break

11:30 - 12:00 Hiroshi Ueda (S3-4) In vitro and in vivo signaling of nociceptin (NOCI-R) receptor using NOCI-R K/O mice

- 12:00 - 12:15 Zafiroula Georgoussi (O3-1) Mechanisms of adenylyl cyclase inhibition of the δ - and μ -opioid receptors using series of receptor derived peptides
- 12:15 - 12:30 Kenneth Kramer (O3-2) Role of protein kinase (PKC) in opioid receptor plasticity
- 12:30 - 12:45 Jay P. McLaughlin (O3-3) Enhanced antinociceptive effect of morphine in mice lacking the enzyme PLC β 3.
- 12:45 - 13:00 Donna Gruol (O3-4) Involvement of NMDA receptors in opiate regulation of Ca^{2+} oscillations in cultured hippocampal neurons
- 13:45 - 15:35 **Workshop Novel opioids - endomorphins** Chair: James Zadina
- 13:45 - 13:55 James Zadina (W-1) Mu-selective endomorphins in regions of high mu opiate receptor density
- 13:55 - 14:05 Tracie Pierce (W-2) Relationship between endomorphin-2-immunoreactivity and the μ -opioid receptor in rat CNS
- 14:05 - 14:15 Stefan Schulz (W-3) Immunofluorescent identification of endomorphin-2-containing nerve fibers and terminals in the rat brain and spinal cord
- 14:15 - 14:25 Gordon Barr (W-4) Maturation of endomorphin-2-like immunoreactivity in the rat
- 14:25 - 14:35 Anna Borsodi (W-5) In vitro binding and signaling profile of the novel μ opioid agonists, endomorphins, in different systems
- 14:35 - 14:45 Mariana Spetea (W-6) Binding of μ -opioid receptor agonist 3H -endomorphin 2 in rodent brain membranes
- 14:45 - 14:55 Geza Toth (W-7) Synthesis of 3H -endomorphin II and new endomorphin I and II analogues with high affinity and selectivity for the μ -opiate receptor
- 14:55 - 15:05 R. Przewlocki (W-8) The spinal antinociceptive effects of endogenous μ opioid receptor agonists, endomorphin-1 and -2 in rats
- 15:05 - 15:15 Ji-Sheng Han (W-9) Brain endomorphin and electroacupuncture analgesia
- 15:15 - 15:25 John A Russel (W10) Endomorphin 1 inhibits oxytocin but not vasopressin neurones in vivo and in vitro
- 15:25 - 15:35 Suli L. Chang (W-11) Lipopolysaccharide (LPS) increases the expression of mesenteric and plasma levels of endomorphin-1
- 15:35 - 16:00 Coffee Break
- 16:00 - 17:00 **INRC Business Meeting**
- 17:00 - 19:00 **Symposium 4 Targeting of opioid receptors: from ultrastructural localisation to in vivo imaging**
Chairpersons: Robert Elde and Eric Simon
- 17:00 - 17:30 Stan Watson (S4-1) Opioid and orphanin receptor anatomy (and function?)
- 17:30 - 18:00 Virginia Pickel (S4-2) Ultrastructural localization of μ opioid receptors: Sites for functional interactions involving amino acid transmitters
- 18:00 - 18:30 James Frost (S4-3) Brain opioid receptors by PET and SPECT
- 18:30 - 18:45 T. Tölle (O4-1) Central post-stroke pain is associated with changes in opioid receptor binding : a PET study with ^{11}C -diprenorphine
- 18:45 - 19:00 Rüdiger Schulz (O4-2) Interference of phosducin with opioid receptor signaling

Thursday, July 23, 1998

- 08:30 - 09:15 **Plenary Lecture Toxic gains of function in the course of Alzheimer and related diseases**
Konrad Beyreuther (Heidelberg) Chair: Rüdiger Schulz
- 09:30 - 13:00 **Symposium 5 Role of opioids and related neuropeptides in pain mechanisms**
Chairpersons: Ji-Sheng Han and Richard Przewlocki
- 09:30 - 09:55 Christoph Stein (S5-1) Pain inhibition by immune-derived opioids
- 09:55 - 10:20 Eberhard Weihe (S5-2) The opioid tachykinin connection in pain
- 10:20 - 10:35 J.J. Carmody (O5-1) κ -opioids, gender, arthritis and substance P
- 10:35 - 10:50 Frank Porreca (O5-2) Pharmacological and pathological actions of dynorphin in pain
- 10:50 - 11:05 Michael Schäfer (O5-3) Cholecystokinin inhibits local opioid analgesia by activation of peripheral CCKB-receptors
- 11:05 - 11:25 Coffee Break
- 11:25 - 11:50 Jean-Claude Meunier (S5-3) Is nociceptin hyperalgesic, allodynic, antiopioid or analgesic?
- 11:50 - 12:10 Rainer Reinscheid (S5-4) Pain modulating effects of OFQ in brain and spinal cord
- 12:10 - 12:30 Emiko Okuda-Ashitaka (S5-5) Nocistatin, a peptide that blocks nociceptin action in pain transmission
- 12:30 - 12:45 Makato Inoue (O5-4) In vivo roles of nociceptin (NOCI) in pain transmission using mutant mice lacking NOCI-receptor and substance P
- 12:45 - 13:00 Beata Buzas (O5-5) Regulation of orphanin FQ gene expression by LPS and TNF α : Role of MAP-kinases and NF κ B
- 14:00 - 19:00 **Excursions / free afternoon**

Friday, July 24, 1998

08:30 - 09:15 **Plenary Lecture Glycine neurotransmission and nociception: a receptorologist's view**

Heinrich Betz (Frankfurt) Chair: Volker Höllt

09:30 - 13:00 **Symposium 6 Cellular mechanisms of opioid tolerance and dependence**

Chairpersons: Brian Cox and Rüdiger Schulz

09:30 - 10:00 Lakshmi Devi (S6-1) Dimerization of opioid receptors: Implications for a role in receptor regulation

10:00 - 10:30 Wolfgang Sadee (S6-2) Basal μ receptor activity and Ca^{++} signaling: Relevance to tolerance and dependence

10:30 - 11:00 Zvi Vogel (S6-3) Regulation of adenylyl cyclase by acute and chronic exposure to opiates and agonists of other inhibitory receptors

11:00 - 11:15 Hermann Ammer (O6-1) Requirement of functional stimulatory receptors for the development of opioid dependence

11:15 - 11:30 Coffee Break

11:30 - 11:45 Javier Garzon (O6-2) Icv-injected Myr⁺ai2 subunits of G proteins attenuate morphine tolerance and withdrawal

11:45 - 12:00 Ping Y Law (O6-3) Identification of the putative phosphorylation sites in the μ -opioid receptor that are involved in the agonist-induced down-regulation of the receptor

12:00 - 12:15 Thomas Koch (O6-4) Carboxyl-terminal splicing of the rat μ opioid receptor modulates agonist-mediated internalisation and receptor resensitization

12:15 - 12:30 Gang Pei (O6-5) Differential efficacies of kappa agonists to induce homologous desensitization of human kappa opioid receptor

12:30 - 12:45 Lee-Yuan Liu-Chen (O6-6) U50,488H-induced internalization of the human κ opioid receptor: involvement of GRK2, β -arrestin and dynamin

12:45 - 13:00 Sue Ann Smith (O6-7) A μ opioid receptor associated molecule (μ RAM-I): morphine induced tolerance

14:00 - 16:00 **Posters 2**

16:00 - 18:30 **Symposium 7 Sensitization to opioids**

Chairpersons: Fred Nyberg and Walter Zieglgänsberger

16:00 - 16:30 Rafael Maldonado (S7-1) Behavioral expression of opiate-induced dependence and rewarding effects in genetically modified mice

16:30 - 17:00 Rainer Spanagel (S7-2) Manipulation of HPA system activity upon morphine-induced sensitization processes

17:00 - 17:30 Jan van Ree (S7-3) Endogenous opioids, reward and addiction

17:30 - 17:50 Luc Vanderschuren (S7-4) Behavioral sensitization evoked by dopamine agonists predicts their ability to reinstate heroin- and cocaine-seeking behavior in rat

17:50 - 18:05 C.L. van den Berg (O7-1) The effects of isolation and morphine treatment during the play period on social behaviors and distinct opioid receptors in adulthood of rats

18:05 - 18:20 Martina Erdtmann- (O7-2) Long lasting sensitization of morphine-induced c-fos expression

18:20 - 18:35 Guy Simmonet (O7-3) Opiate tolerance: an apparent phenomenon associated with a pain sensitization process

20:00 - 23:00 **Conference Dinner**

After dinner speaker: Albert Herz

Saturday, July 25, 1998

09:00 - 11:45 **Symposium 8 Role of opioids in development and apoptosis** Chairpersons: Anna Borsodi and Ian Kitchen

09:00 - 09:30 Patricia McLaughlin (S8-1) Opioid growth factor regulation of prenatal and postnatal development

09:30 - 10:00 Kurt Hauser (S8-2) Opioids as messengers of life and death: opioid system diversity and impact on the genesis of neurons and glia

10:00 - 10:15 Carmine Coscia (O8-1) Mechanism of opioid modulation of cell proliferation

10:15 - 10:30 Georgy Bakalkin (O8-2) Receptor-mediated and nuclear effects of opioids on the cell cycle and apoptosis

10:30 - 11:00 Coffee Break

11:00 - 11:15 Tsung-Ping Su (O8-3) Delta opioid DADLE blocks dopamine transporter loss and p53 mRNA increase induced by methamphetamine by acting as a free radical scavenger?

11:15 - 11:30 Jean M. Bidlack (O8-4) Activation of mouse T cells increases the expression of the kappa opioid receptor

11:30 - 11:45 Veeramani Maharajan (O8-5) Altered patterns of calbindin D-28K immunoreactivity in adult and developing mouse brains exposed to morphine

12:00 **End of the Meeting**

Poster 1 (Tuesday, July 21, 13:30 - 15:30)

Pain and Inflammation

- Tu01: Spinal antisense oligodeoxynucleotide (ODN) to Goa subunit blocks both morphine (MS) and clonidine (Cl) antinociception, S Roerig and F Karim
- Tu02: Differential effects of antisense oligodeoxynucleotides to G protein alpha-subunits on endomorphin-1 and morphine analgesia, J Garzon, I Deantonio, M Rodriguez-Diaz and P Sanchez-Blazquez
- Tu03: Endomorphin-1 and morphine analgesia are affected differentially by mu-receptor „knock-down" in mice, P Sanchez-Blazquez, M Rodriguez-Diaz, I DeAntonio and J Garzon
- Tu04: Multireceptor ligands for spinal analgesia - antinociceptive effects of AA501, I Maszczynska, DB Carr, RM Kream, A Misicka, VJ Hruby and AW Lipkowski
- Tu05: Involvement of ATP-sensitive K⁺ channels in opioid, adrenergic, and muscarinic agonist antinociception, KD Wild, DJ Stone and SJ Hipp
- Tu06: An antinociceptive interaction between risperidone and opioids, CG Pick, MM Backer, R Weizman and S Schreiber
- Tu07: Analgesic interaction of mu opioid, alpha-2 adrenoceptor and 5-HT receptor agonists: three-dimensional isobolographic analysis, D Paul
- Tu08: In vivo signaling of peripheral opioid analgesia, M Inoue and H Ueda
- Tu09: Topical morphine analgesia and tolerance, YA Kolesnikov and GW Pasternak
- Tu10: Opioid analgesia in fishes, L Chervova
- Tu11: Studies of action mechanisms of electroacupuncture in different frequencies on spinal serotonergic and opioid receptors, Y-F Chen, C-C Kuo, J-G Lin and H-Y Tsai
- Tu12: Studies of mechanisms in the different frequencies of EAc analgesia on central monoaminergic and opioid receptors, H-J Tsai, Y-F Chen, M-C Yu, J-G Lin and C-H Tsai
- Tu13: Comparison of κ 1, κ 2, m and δ opioid agonists on hyperalgesia and allodynia in rat models of persistent pain, RM Caudle, E Eliav and MJ Iadarola
- Tu14: An antisense oligonucleotide targeting mGluR1 restores opioid sensitivity in neuropathic rats, ME Fundytus, A Dray, JL Henry and TJ Coderre
- Tu15: The effect of opioid receptor agonists and antagonists on the development of neuropathic pain after sciatic nerve injury in rats, J Mika, G Toth and B Przewlocka
- Tu16: Lack of cross tolerance between systemic morphine and asimadoline, a peripherally selective kappa-agonist, in neuropathic rats, JS Walker, G Catheline, V Kayser and G Guilbaud
- Tu17: Increased levels of heat-shock protein 47 following formalin induced tonic pain, RY Yukhananov, Z Guan and GJ Crosby
- Tu18: Comparison of dynorphin A and dynorphin A-(2-17) in murine models of pain, itch and CNS depression, A Cowan, GB Kehner and SJ Rittenhouse
- Tu19: p-Hydroxymercuribenzoate-induced antinociception through the dynorphin system, K Tan-No, K Kisara and L Terenius
- Tu20: Morphine-induced GABA, glycine and glutamate release in the dorsal horn of the spinal cord in anaesthetised rats, C-O Stiller, H Gustafson and J Bergquist
- Tu21: Peripheral effects of the kappa agonist EMD 61753 on nociception and inflammation, H Machelska, AL Maycock and C Stein
- Tu22: κ -Opioids affect cell trafficking in arthritis, JL Wilson, W Binder and JS Walker
- Tu23: Human gastrointestinal opioid binding sites in inflammatory bowel disease and idiopathic chronic constipation, JRW Menzies, SJ Paterson, R McKee and AD Corbett

Effect of opioids on circulation and endocrine systems

- Tu24: Renovascular hypertension increases canine cardiac met-enkephalin-arg-phe and proenkephalin, BA Barron, DA Yoshishige, GP Kline and PA Gwartz
- Tu25: Opioid receptors do not mediate canine ischemic preconditioning, BA Barron, A Heymann, A Williams, X Bian and HF Downey
- Tu26: Inotropic effects of μ -opioid agonists in normal and stressed hearts, AT Omoniyi, D Wu, Y Soong, Y Holsey, JF Clapp III and HH Szeto
- Tu27: The negative inotropic effects in cardiomyocytes mediated via κ -opioid receptors are sensitive to pertussis toxin, H Wenzlaff, Weil, Stein and H-J Teschemacher
- Tu28: Phospholipase C mediates κ -receptor stimulation induced arrhythmias in the isolated rat heart, JS Bian, WM Zhang Q Xia and TM Wong
- Tu29: Dynorphin A1-17 stimulates insulin secretion from a pancreatic β -cell line (MIN6), PY Cheng

Receptor Desensitization and Down-regulation

- Tu30: G protein receptor kinase (GRK3) and β -arrestin 2 mediated desensitization of the kappa opioid receptor in *Xenopus* oocytes, SM Appleyard, W Jin, V Pineda, A Kovoov, J Celver and C Chavkin
- Tu31: Agonist-induced desensitization of the μ opioid receptor is affected by serine 266 mutation to proline in the third intracellular loop, T Krosiak, T Koch, M Averbeck, E Raulf and V Höllt
- Tu32: Desensitization of mu (OP-3) receptor-stimulated [³⁵S]GTP γ S binding by agonists of different efficacies in transfected mMOR-CHO cells, DE Selley, C-C Cao and SR Childers
- Tu33: μ Opioid receptor: Role of the C-terminus in agonist-mediated phosphorylation, Y Yu, HB Deng, GR Uhl and JB Wang
- Tu34: The serine residues S261 and S266 are required for agonist-induced phosphorylation of the μ opioid receptor, H Schmidt, S Schulz, M Händel, I Brüggemann, T Koch and V Höllt
- Tu35: Internalization and dimerization of kappa opioid receptors, B Jordan, S Cvejic and L Devi
- Tu36: Replacement of threonine 394 by alanine accelerates internalization of the rat μ opioid receptor, M Klutzny, T Koch, S Schulz, R Wolf, E Raulf and V Höllt
- Tu37: Desensitization of the δ -opioid receptor correlates with its phosphorylation in SK-N-BE cells: Involvement of a GRK, A Hasbi, J Polastron, S Allouche, L Stanasila, D Massotte and P Jauzac
- Tu38: δ receptor down-regulation: G protein coupling and proteases, RD Howells, N Chinen, G Wu, P Bandari and K Chaturvedi
- Tu39: Regulation of mu opioid receptors by chronic opioid treatments in rat brain, M Szücs, G Fabian, B Bozo, E Kicsi, M Macsai, G Szabo and M Szikszay
- Tu40: Different regulation of dynamin by chronic treatment with mu and delta agonists, F Noble and BP Roques

Tolerance/Dependence

- Tu41: PKA maintains tolerance in hypothalamic neurons with chronic morphine: convergence with estrogen, MJ Kelly, EJ Wagner and OK Ronnekleiv
- Tu42: Morphine tolerance up-regulates nitric oxide synthase expression in the rat spinal cord, CS Wong
- Tu43: Abatement of acute spinal tolerance to morphine, remifentanyl and endomorphin-2 by inducible nitric oxide synthase inhibitors, CA Fairbanks, HO Nguyen and GL Wilcox
- Tu44: The mechanism of dextromethorphan on blocking morphine tolerance in rats, PL Tao, SL Chen, EYK Huang and CC Wu
- Tu45: Availability of the non-competitive NMDA receptor antagonist ifenprodil as adjunct of morphine, T Suzuki, H Kato, M Tsuda and M Misawa
- Tu46: Chronic morphine treatment alters NMDA receptor-mediated transmission, G Martin, S Ahmed and GR Siggins
- Tu47: Effects of baclofen on antinociception tolerance to and physical dependence on morphine in mice, Y Yajima, A Takimoto, M Tsuda, T Suzuki and M Misawa
- Tu48: Restoration of morphine action in tolerant animals by a neuropeptide FF analog, K Jhamandas and M Sutak
- Tu50: The mechanism of 100 Hz electroacupuncture tolerance, Y Wang, XM Wang, YN Dong, HW Dong and JS Han
- Tu51: The development of tolerance to and physical dependence on mu subtype opioid agonists, S Fürst, T Friedmann and S Hosztafi
- Tu52: Genetic differences in morphine sensitivity, tolerance and withdrawal in rats, O Hofmann and Z Wiesenfeld-Hallin
- Tu53: Arachidonic acid cascade involvement in the acute dependence produced by μ , κ and δ opioid agonists, A Capasso
- Tu54: Enhanced opioid efficacy in opioid dependence, SL Ingram, CW Vaughan, EE Bagley, MA Connor and MJ Christie
- Tu55: A possible mechanism of inhibitory effect of PGE receptor EP3-subtype selective agonists on naloxone-precipitated withdrawal on morphine-dependent rats, T Nakagawa, T Masuda, M Minami and M Satoh
- Tu56: A molecular mechanism for supersensitization of adenylyl cyclase system following sustained stimulation of opioid receptor, T Nakagawa, M Minami, T Watanabe, T Ozawa and M Satoh
- Tu57: SR142801, a tachykinin NK-3 receptor antagonist, inhibits the atropine-resistant component of the opioid withdrawal response of guinea-pig isolated ileum, LA Chahl
- Tu58 Alterations in brain levels of substance P(1-7) during opioid withdrawal, Q Zhou, Z Liu, A Ray, K Karlsson and F Nyberg

Addiction

- Tu59: Opiate abuse and dependence over a decade in South India, Eranti V Savithasri and S K Chaturvedi
- Tu60: Naltrexone use in China, B-Y Qin
- Tu61: Long-term changes in the behavior of rats after treatment with morphine: evidence of the „addiction" memory, G Grecksch, A Becker and V Höllt
- Tu62: Decreased mu (OP-3) opioid receptor-activated G-Proteins following chronic heroin self-administration rats, LJ Sim, DE Selley, L Vogt, TJ Martin and SR Childers

- Tu63: Chronic heroin abuse produces marked alterations in synthesis and storage of dopamine in human brain, S Izenwasser, BJ Ciliax, AI Levey and DC Mash
- Tu64: Craving to morphine may be related with the level of anxiety in two inbred strains of rats, OF Medvedeva, SK Sudakov, YV Lyupina, DY Rusakov and IV Rusakova
- Tu65: Inhibition of heroin-induced place preference by aODN targeting the rat delta opioid receptor, R Lattanzi; L Negri, P Melchiorri and A Borsodi
- Tu66: Role of opioid receptor types in pentazocine-induced place preference under inflammation, M Sato, T Suzuki and M Misawa
- Tu67: Involvement of kappa opioid receptors in experimental drug addiction, MAFM Gerrits, AV Kuzmin, EE Zvartau and JM van Ree
- Tu68: Role of σ receptor in discriminative stimulus effect of κ receptor agonist U-50-488H, M Nomura, T Kazama, Y Fukuoka, T Suzuki, M Misawa and H Nagase
- Tu69: Immunohistochemical localization of second messengers in the rat extended amygdala, KN Gracy and GF Koob
- Tu70: c-Fos expression in the bed nucleus-amygdala complex of the rat following acute administration of opioid agonist or antagonist, J Schadrack, C Kirschke, B Schuller and W Zieglgänsberger
- Tu71: Effects of morphine and cocaine on the biosynthesis of opioid peptides and CRF in rat amygdala, J Turchan, M Maj, B Przewlocka and R Przewlocki
- Tu72: Mu-opioid receptor-stimulated [³⁵S]GTPγS autoradiography in fetal monkey brain: effect of prenatal cocaine exposure, Y Fang, NZ Lu and OK Ronnekleiv
- Tu73: Chronic interactions between opioid and cannabinoid agonists in N18TG2 cells, Y Sarne, M Gafni and M Shapira
- Tu74: Effects of concurrent chronic ethanol and binge cocaine administration on brain kappa mRNA levels and stereotypic behavior in rats, A Rosin, S Lindholm, J Franck and J Georgieva
- Tu75: Alcohol consumption is elevated in mas-deficient mice, B Maul, G Heder, T Walther, M Bader and W-E Siems
- Tu76: Alcohol consumption in transgenic mice lacking or overexpressing angiotensinogen, W-E Siems, B Maul, G Heder, A Winkler, T Walther and M Bader
- Tu77: Ethanol consumption and its influence on the δ -opioid receptor gene expression in the GI-tract of the rat, J Fickel and B Joest

Development and Apoptosis

- Tu78: Glutamatergic afferents from neocortex maintain the proenkephalin gene expression in organotypic slices of rat neostriatum, L Just and DK Meyer
- Tu79: A novel uridine diphosphate glucuronosyltransferase (UGT) in guinea pig: up regulation with in utero morphine exposure, SA Smith, SR Nagalla, DP Andrews and GD Olsen
- Tu80: The effect of neonatal handling on opioid peptides, K Ploj, L Bergström, E Roman and I Nylander
- Tu81: Changes in opioid receptors in the brain of preweanling male and female rats following chronic treatment with naltrindole, R Goody, MT Antelo, B Fernandez, MP Viveros and I Kitchen
- Tu82: Differential effects of sodium ions and guanine nucleotides on δ -ligand receptor recognition in weaned and nonweaned rat brain, M Kelly, RG Hill, A Borsodi, G Toth and I Kitchen
- Tu83: Chronic neonatal naltrindole administration and manipulation affect adrenocortical reactivity in young rats, B Fernandez, MT Antelo, C Guaza, I Alberti, ML Pinillos and MP Viveros
- Tu84: Developmental changes in cross-talk between κ -opioid and β -adrenergic receptors in the cardiomyocytes of hypertensive rats, XC Yu, HX Wang, WM Zhang and TM Wong
- Tu85: The effect of naloxone on the differentiation of PC12 cell induced by NGF, S-F Chang, T-H Hsieh, C-L Cheng, G-C Yeh
- Tu86: Bombesin inhibits methadone-induced apoptosis of human lung cancer cells, R Maneckjee and L Heusch

Posters 2 (Friday, July 24 14:00 - 16:00)

Opioids (Peptides and Alkaloids)

- Fr01: Endomorphin 1 and Endomorphin 2: Effects on the signal transduction pathways, K Monory, E Tzavara, M-C Bourin, G Toth, HW Matthes, B Kieffer, J Hanoune and A Borsodi
- Fr02: Binding properties of new endomorphin 1 and 2 derivatives, D Biyashev, S Benyhe, CS Tomboly, ZS Laszlo, G Toth and A Borsodi
- Fr03: Conformational analysis of endomorphin-1 using NMR and molecular modeling, MG Paterlini, BL Podlogar, DM Ferguson, GC Leo, EE Codd, DA Demeter, FK Brown and AB Reitz
- Fr04: Isolation and sequence of cDNAs coding for precursors of endogenous opioid peptides from the guinea pig brain, KS LaForge and MJ Kreek

- Fr05: The atypical antidepressant bupropion affects prodynorphin gene expression in rat brain, P Romualdi, A Capobianco, A Donatini and S Ferri
- Fr06: Dynorphin A(2-17) stimulates an increase in $[Ca^{2+}]_i$ in cultured rat cortical neurons by a non-opioid, non-NMDA mechanism, J Lai, Q Tang, RM Lynch and F Porreca
- Fr07: Soluble protein factor with non-opioid dynorphin A-binding and convertase activities, T Yakovleva, M Melzig, I Nylander, J Silberring, G Bakalkin and L Terenius
- Fr08: Regulation of proenkephalin gene expression in rat and mouse striatum by NMDA receptor antagonists, B Ziolkowska and R Przewlocki
- Fr09: G-protein activation and receptor binding of met-enkephalin-arg6-phe7 derived peptides, B Bozo, S Benyhe, J Farkas, G Toth, M Wollemann and M Szücs
- Fr10: Ligand binding and pharmacological properties of DAMGO-chloromethyl ketone, E Kicsi, B Bozo, J Farkas, G Toth, M Macsai, G Szabo and M Szücs
- Fr10a Chimeric peptide of metenkephalin & FMRF, unlike FMRF, does not attenuate acute as well as chronic morphine antinociception in vivo, S Pasha, S Gupta and YK Gupta
- Fr11: Is LEF553 a substrate of P-glycoprotein in vivo?, L Alari, L Sögren and L Anderson
- Fr12: New deltorphin analogs predicted from cloned cDNAs from skin of phyllomedusinae frog, L Negri, P Melchiori, C Wechselberger and G Kreil
- Fr13: Enkephalin-analog maleoyl peptide hydrazides: synthesis and binding studies, A Magyar, G Orosz, AZ Ronai and K Medzihradzsky
- Fr14: Maleoyl peptide derivatives - novel affinity labels for opioid receptors, I Szatmari, G Orosz, K Medzihradzsky and A Borsodi
- Fr15: Synthesis and biological activity of enkephalin-related alkylating peptides, A Blahunka, A Magyar, G Orosz, AZ Ronai, H-M Schweiger, K Medzihradzsky, J Szikra, S Benyhe and A Borsodi
- Fr16: Novel TIPP-derived δ opioid antagonists and δ agonists with high potency and unprecedented δ selectivity, PW Schiller, G Weltrowska, I Berezowska, C Lemieux and NN Chung
- Fr17: MALDI-MS quantitative analysis of synthetic opioid peptide analogs, DM Desiderio, U Wirth, J-L Tseng, HH Szeto, J Clapp and PW Schiller
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- Fr23: Synthesis and biochemical evaluation of 14-alkoxy substituted indolo- and benzofuormorphinans, E Greiner, H Schmidhammer, K Monory, D Biyashev, R Meditz, R Kraßnig and A Borsodi
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- Fr33: Anaesthetic activity of ohmefentanyl in animals, Z-Q Chi, X-L Jin, G-M Zhao, W-Q Jin, Y-U Zhu and D-H Zhou

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- Fr35: Identification and characterization of a novel splice variant of the mouse mu-opioid receptor (MOR1) gene, Y-X Pan, J Xu, GC Rossi, L Leventhal, B-L Wan, AB Zuckerman and GW Pasternak
- Fr36: Mutation of human μ opioid receptor extracellular „disulphide bond" cysteine residues alters ligand binding but not receptor targeting to the cell plasma membrane, P Zhang, PS Johnson, ZJ Wang, AE Montes, KB Seidleck, CJ Blaschak and CK Surrat
- Fr37: Interaction between Asp114 in TMH2 and Asn332 in TMH7 of the μ opioid receptor, L-Y Liu-Chen, W Xu, F Ozdener, J-G Li and C Chen
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- Fr55: Differential G protein activation by alkaloid and peptide opioid agonists in the human neuroblastoma cell line SK-N-BE, S Allouche, J Polastron, A Hasbi and P Jauzac
- Fr56: Coincident signalling between δ -opioid and Gq coupled receptors, A Yeo, FJ Gunn-Moore, JM Tavare and G Henderson
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- Fr58: An altered opioid signal transduction as induced by an anxiolytic homophthalazine, girisopam, I Berzetei-Gurske, L Toll, K Horvath, JI Szekely and MIK Fekete
- Fr59: Further study on the mechanism responsible for inhibition of cAMP production in CNE-2 and H9c2 cells upon k-opioid receptor stimulation, Diao Tiemei, TM Wong and NS Wong
- Fr60: Identification of L-type calcium channels associated with kappa opioid receptors in human placenta, B Cemerikic, R Zamah, and MS Ahmed
- Fr61: Opioid-stimulated GTP[γ - ^{35}S]-binding in different brain regions of C57BL/6 and DBA/2 mice, E Albrecht and H Berger
- Fr62: In vitro autoradiography of opioid receptor/G-protein coupling in human brain, A Winkler, S Platzer, D Dworzak, J Schadrack, TR Tölle, W Zieglgänsberger and R Spanagel
- Fr63: Distribution of a new splice variant of the μ -opioid receptor (MOR1) in the rat central nervous system, C Abbadie, Y-X Pan and GW Pasternak

- Fr64: Mu opioid receptors are present in distinct hippocampal interneuron subpopulations, CT Drake and TA Milner
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- Fr66: Localization of μ - and δ -opioid receptors to dopaminergic (DA) neurons projecting from the ventral tegmental area (VTA) to nucleus accumbens (Nac) in rats, AE Kalyuzhny and MW Wessendorf
- Fr67: Pre- and postsynaptic localization of κ -opioid receptors in contact with dynorphin-immunoreactive neurons in the rat nucleus accumbens shell, AL Svingos, EEO Colago, C Chavkin and VM Pickel
- Fr68: κ -opioid receptor gene expression in rat posterior pituitary, MK-H Schäfer
- Fr69: The study of distal promoter in the mu opioid receptor (MOR) gene expression, CY Choe, HJ Im, JL Ko and HH Loh
- Fr70: Kappa and delta opioid receptor gene expression is regulated by AP-1, J Kraus, M Wöltje and V Höllt

Nociceptin/Orphanin FQ and ORL1

- Fr71: Autoradiographic localization of ^{125}I [Tyr¹⁴] OFQ/N and ^{125}I [Tyr¹⁰] OFQ/N(1-11) in rat brain, SR Letchworth, JP Mathis, GC Rossi, RJ Bodnar and GW Pasternak
- Fr72: Alterations in neuronal orphanin FQ content following exposure to stress, DP Devine, M Hoversten and H Akil
- Fr73: Orphanin FQ produces distinct effect on pain response and endomorphin-induced analgesia in brain and spinal cord of rats, C-B Zhu, Y-Q Wang, J-L Wang, X-D Cao and C-C Wu
- Fr74: The pharmacological actions of nociceptin in the isolated colon of rat, mouse and man, JRW Menzies, AD Corbett, MRP Davies and SJ Paterson
- Fr75: Orphanin FQ increases heart rate and blood pressure in the conscious sheep via sympathetic activation, M Arndt, Y Soong, D Wu and H Szeto
- Fr76: Bidirectional effect of nociceptin on food intake, S Candaletti and S Ferri
- Fr77: In vivo role of nociceptin in the development of morphine tolerance, H Ueda, M Inoue, S Tokuyama, A Yoshida and H Takeshima
- Fr78: Orphanin FQ blocks development of morphine place preference, NP Murphy and NT Maidment
- Fr79: Effects of the nociceptin antagonist [Phe¹-y(CH₂NH)Gly²]NC(1-13)NH₂ on nociception in rats, S Candeletti, R Guerrini, G Calo and S Ferri
- Fr80: The effect of the agonist Ac-RYYRWK¹NH₂ and the antagonist Phe¹-y (CH₂-NH)Gly²]nociceptin (1-13)NH₂ at the ORL1 receptor of central and peripheral sites, J Nicholson, SL Mason, TL Lee and AT McKnight
- Fr81: Agonist activity of ORL1 antagonists is dependent upon receptor number, L Toll, J Burnside and I Berzetei-Gurske
- Fr82: Analgesic activity of murine preproorphanin¹⁶⁰⁻¹⁸⁷ in mouse brain, JP Mathis, GC Rossi, AB Zuckerman, L Leventhal, RG Allen and GW Pasternak
- Fr83: Cloning and expression of multiple alternative splice variants of the mouse KOR-3/ORL-1 receptor gene, Y-X Pan, J Xu, B-L Wan, AB Zuckerman and GW Pasternak
- Fr84: Structure and alternative splice variants of the rat KOR-3/ORL-1 receptor gene, J Xu, Y-X Pan, B-L Wan, AB Zuckerman and GW Pasternak
- Fr85: The second extracellular loop is required for ORL1 receptor activation by nociceptin, C Mollerau, L Mouledous, S Lapalu, C Moisand, J-L, J-L Butour and J-C Meunier
- Fr86: Molecular modelling of the ORL1 receptor and its complex with nociceptin, C Topham, L Mouledous, G Poda, B Maigret and J-C Meunier
- Fr87: ORL1 receptor expression in the brains of μ - or κ -receptor knockout mice, SJ Slowe, S Clarke, R Lattanzi, L Negri, F Simonin, HWD Mathes, B Kieffer and I Kitchen

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S1-1

GENETIC ANALYSIS OF OPIOID RECEPTOR FUNCTION.

John E. Pintar, Jiwen Zhang, Michael King, Alwin Schuller, Yanxin Zhu, Zhen-Ping Chen, and Gavril Pasternak. Dept. Neuro, UMDNJ-RWJMS, Piscataway, NJ and Dept. Oncology, Mem.Sloan-Kettering, NY.

We have produced targeted mutations in the mu, delta, and kappa opioid receptors as well as in the ORL-1 receptor now thought to mediate the effects of orphanin FQ. In the absence of major changes in prenatal development, we have primarily examined analgesic responses in these mutant strains following agonist challenge but have also begun to investigate other parameters, including the development of tolerance, as well. Our previous work had shown that M-6-G can still elicit analgesia in mice containing an ablation of mu exon 1; we now find that GI transit, as measured by charcoal lavage, is increased in homozygous mutant MOR-1 mice (compared to +/-), but that GI transit in these mice is still inhibited by M-6-G but not by morphine. We also find that, while DPDPE and morphine analgesia are essentially unaffected in delta KO mice, the development of tolerance to these agents is abolished as it is in a novel line of mice from which enkephalin encoding sequences of the enkephalin gene have been deleted. In addition to analysis of individual KOs, production of combinatorial mutants lacking multiple opioid receptors has also begun and will be discussed.

S1-3

ANALGESIA AND NEUROENDOCRINE HOMEOSTASIS IN β -ENDORPHIN-DEFICIENT MICE

MJ Low, JS Mogil, JE Grisel, MD Hayward, JL Smart, J Young, and M Rubinstein. Vollum Institute, Oregon Health Sci. Univ., Portland OR, USA.

We produced a mutant strain of mice (C57BL/6-Pomc2^{tmLow}) by gene-targeting that are unable to synthesize beta-endorphin. These mice have been studied to gain new insights into the physiological role played by endogenous opioids in analgesia and modulation of hypothalamic circuits controlling the stress axis, reproduction, growth and metabolism. The mutant mice exhibited a loss of opioid stress-induced analgesia (SIA) and a potentiation of non-opioid SIA. To further investigate the mechanisms for these phenotypes we analyzed analgesic responses to μ -receptor agonists given i.p., i.c.v., or i.t. beta-endorphin-deficient and wild-type control mice had identical dose-response curves to morphine administered i.p., however they exhibited an unpredicted disassociation in analgesia to agonists administered by the two central routes. The mutant mice were 10-fold more sensitive to the analgesic actions of morphine and DAMGO injected i.c.v., but insensitive to the same drugs administered i.t., compared to controls. There were no genotype differences in μ , δ , or κ -receptor binding sites in brain or spinal cord to explain the observed responses. β -endorphin deficient mice had normal fertility and normal HPA axis responses to stress. However, male mutants were moderately obese, without hyperphagia or decreased metabolic rate. In summary, the total lack of beta-endorphin alters several analgesic pathways in mice, likely by mechanisms independent of opioid receptor levels. The studies of hypothalamic function in these mutant mice suggest an important modulatory role of beta-endorphin in determining the set-point for fat mass, which may be due primarily to the genotype of the nursing dams, and not the offspring.

S1-2

GENETIC ANALYSIS OF NOCICEPTIVE BEHAVIORS IN MICE.

Anne Zimmer, Monika König, Eva Mezey, Miklos Palkovits, Tom I. Bonner, and Andreas Zimmer, Section on Genetics, NIMH, Bethesda, MD 20892.

Morphine has been used for its analgesic and euphoric properties since millennia and it continues to be used to treat severe pain. We are studying the role of the opioid system in pain signaling with genetic methods. Knockout mice with deletions in the enkephalin and substance P genes were generated. These mouse strains are healthy and fertile, but display significant abnormalities in nociceptive responses. Substance P and enkephalin often have antagonistic effects in nociception, with substance P facilitating and enkephalin inhibiting the transmission of nociceptive signals. Mice that can not produce enkephalin exhibited shorter response latencies in the hotplate assay and highly abnormal responses in the formalin test. In contrast, Tac 1 knockout mice display no significant pain responses following formalin injection and have an increased pain threshold in the hotplate test. Animals from both strains exhibited normal response latencies in the tail flick test and normal stress induced analgesia. Substantial differences in nociceptive behaviors and neuropeptide levels were also observed among inbred strains of mice. Strains with high levels of enkephalin had low levels of substance P and vice versa. Together, our data suggest that strain differences in opioid and tachykinin peptide production may contribute to their differential responses to pain.

S2-1

EVOLUTIONARY CONSERVATION OF STRUCTURE AND FUNCTION OF OPIOID RECEPTORS.

M. G. Darlison, F. R. Greten, H.-J. Kreienkamp, T. Stuehmer and D. Richter, Institut fuer Zellbiochemie und klinische Neurobiologie, Universitaet Hamburg, 20246 Hamburg, Germany.

Opioid peptides are found in a variety of species ranging from molluscs to man. This suggests the early evolutionary emergence of receptors for these neuropeptides. To begin to investigate the evolution of this class of G-protein-coupled receptors, we have isolated complementary DNAs (cDNAs) from a lower vertebrate, namely the teleost fish *Catostomus commersoni*. In this way, we have identified six different opioid-like receptors in this species, which is in contrast to the situation in mammals where only four such receptors (the m, d, k and ORL1 types) have been reported. When expressed in human embryonic kidney 293 cells, one of the piscine cDNAs (which encodes a μ -like opioid receptor), binds the μ -receptor selective agonist DAMGO and the non-selective antagonist naloxone with nanomolar affinities. In addition, we have demonstrated the functional coupling of this receptor, to a mammalian G-protein-gated inward-rectifying potassium channel (GIRK1), by co-expression in *Xenopus laevis* oocytes. Taken together, we conclude that the structure and function of the μ -opioid receptor have been highly conserved over a period of ~400 million years.

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S2-2

KNOCKOUT MICE AND DRUG RESPONSES VARIANTS: OPIATE, DAT and VMAT2.
Uhl, G., Sora, I., Takahashi, N. NIDA-IRP, NIH & JHUSM, Baltimore, MD. 21224.
Mice with deletions of genes encoding the vesicular monoamine transporter gene (VMAT2), the mu opiate receptor (MOR) and the dopamine transporter gene (DAT) can reveal baseline differences and variant drug responses. Baseline differences support roles for MOR in normal mechanisms of pain perception and of DAT in exploratory locomotion. Virtual ablation of morphine and heroin analgesic and rewarding responses in MORKO and cocaine locomotor stimulation in DATKO indicate near-total MOR and DAT dependence of these drug effects. Reduced amphetamine reward in VMAT2KO heterozygotes point to a role for VMAT2 expression in amphetamine reward. Failure of DAT deletion to reduce cocaine reward and reductions in psychostimulant reward in MUKOs reveal unexpected roles for these sites in actions of these drug classes. Knockout mice can enhance understanding of drug actions. They provide models for human individual differences at these loci.

S3-1

SIGNALLING MECHANISMS IN LIMBIC NEURONS WITH ACUTE AND CHRONIC OPIATE ADMINISTRATION.
G.R. Siggins, G. Martin, S. Madamba and P. Schweitzer, Department of Neuropharmacology, The Scripps Research Institute, La Jolla, CA, USA.
Neurochemical studies have stressed opiate receptor linkages to the adenylyl cyclase system, yet electrophysiological data point to other mediators. In our intracellular recordings of hippocampal pyramidal neurons in a slice preparation, both dynorphin-A and nociceptin augment the M-current (I_M), a voltage-dependent K^+ conductance, with reversal by a k receptor antagonist. This IM effect is mimicked by arachidonic acid but not cyclic AMP, suggesting k receptor linkage to phospholipase A2. In neurons of nucleus accumbens (NAcc) slices, superfusion of a μ receptor agonist had no apparent direct effect on membrane conductances, decreased glutamatergic transmission, but enhanced currents evoked by exogenous NMDA. NMDA currents and NMDA-EPSCs were also enhanced by PDAc, a protein kinase C (PKC) activator, and sphingosine prevented this effect, suggesting a PKC linkage for μ receptors in Nacc neurons, as reported for spinal neurons (Chen and Huang, Neuron 7:319, 1991). Chronic morphine treatment significantly reduced the PDAc facilitation of NMDA-EPSCs. These data suggest that k and μ opiate receptors in two different brain regions are functionally linked to different second messenger systems. Supported by NIH grants DA-03665 and AA-06420.

S3-3

HOMOLOGOUS DESENSITIZATION OF OPIOID RECEPTORS MEDIATED BY G PROTEIN RECEPTOR KINASES.
C. Chavkin, A. Kovoov, S.M. Appleyard, J.P. Celver and W.Z. Jin, Dept of Pharmacol, Univ of Washington, Seattle, WA 98195 USA.
Xenopus oocytes expressing mammalian opioid, β -adrenergic or cannabinoid (CB1) receptors along with G protein coupled inwardly rectifying potassium channel subunits (Kir3.1 & 3.2) reconstituted an established receptor - ion channel signaling process. Homologous desensitization was only observed in oocytes injected with both cRNA for G protein receptor kinase 3 (GRK3) and beta arrestin 2. Site-directed mutagenesis of the mu and kappa opioid receptors or CB1 receptor has recently been done to identify the specific phosphorylation sites on the receptors required for GRK3/ β arr-mediated desensitization. GRK5 was found to effectively substitute for GRK3. (β arr1 substituted for β arr2, and dominant positive β arr's work produce agonist-dependent desensitization in the absence of GRK. Mu opioid agonists of higher efficacy produced a faster rate of homologous desensitization. These results suggest that the *Xenopus* oocyte expression system can be used as an effective model of the homologous desensitization processes likely to be important in opioid tolerance. Supported by DA04123.

S2-3

GENETIC VARIABILITY OF THE HUMAN MU OPIOID RECEPTOR (hMOR) GENE AND ITS IMPLICATION FOR SUBSTANCE ABUSE (SA). M.R. Hoehe, Max-Delbrück-Center, Berlin, Germany
Genetic variation in the hMOR gene affecting structure, regulation, or expression of the receptor protein may confer genetic vulnerability to SA. To test this hypothesis, we first extended the previously known cDNA sequence information (2162 bp) up to a total of 6968 bp by cloning 2412 bp of 5' regulatory region, crucial 5' and 3' sequences of introns 1 and 3, as well as the complete intron 2. Applying a novel multiplexing approach, we comparatively sequenced the entire gene in about 250 individuals, including controls and addicts with a family history (cocaine, methadone abusers, and other forms of SA). A substantial number of variants in the 5' regulatory, coding, and intronic regions was identified, resulting in a large number of individual hMOR gene profiles (haplotypes). These were then classified by means of similarity analyses into two allelic categories. Evidence that a specific constellation of polymorphisms potentially affecting gene expression may be involved in the genetic disposition to SA in African Americans was obtained. Moreover, the functional characterization of coding variants provided insight into naturally selected structure function-relationships (collaboration B. Kieffer, Illkirch).

S3-2

OPIOID RECEPTOR SIGNALLING VIA ARACHIDONIC ACID METABOLITES IN SINGLE BRAIN NEURONS
M.J. Christie, C.W. Vaughan, S.L. Ingram, M.A. Connor, Department of Pharmacology, University of Sydney, NSW 2006, Australia.
Actions of μ -opioids on GABAergic neurotransmission in rat periaqueductal grey (PAG) were examined using whole-cell patch clamp recordings in brain slices. Met-enkephalin decreased the amplitude of evoked GABAergic synaptic currents (eIPSCs) as well as frequency of miniature IPSCs by 65% in all PAG neurons. Inhibition was abolished by 4-AP (100 μ M) and a-dendrotoxin (100 nM), suggesting involvement of a voltage-dependent K-channel. Inhibition was abolished by PLA₂ inhibitors, general lipoxigenase and 12-lipoxigenase inhibitors. Cyclooxygenase (COX1) and 5-lipoxigenase inhibitors did not block inhibition. Opioid actions were mimicked by arachidonic acid and 12-lipoxigenase metabolites, but not by cyclooxygenase or 5-lipoxigenase metabolites, indicating that presynaptic inhibition is mediated by 12-lipoxigenase metabolites, CQX1 and 5-lipoxigenase inhibitors enhanced the efficacy of opioids presumably by shunting metabolism via 12-lipoxigenase.

S3-4

IN VITRO AND IN VIVO SIGNALING OF NOCICEPTIN RECEPTOR (NOCI-R) USING NOCI-R K/O MICE.
Hiroshi Ueda¹, Makoto Inoue¹, Hiroshi Takeshima² and Andreas Zimmer³
Dept. Mol. Pharmacol. & Neurosci., Nagasaki Univ. Sch. Pharm. Sci., Nagasaki 852-8521, Japan, ²Univ.Tokyo, Tokyo 113-0033, Japan. ³NIMH, Bethesda MD 20892, U.S.A.
Noci-stimulated *in vitro* and *in situ* [³⁵S]GTP γ S binding was abolished by pertussis toxin-treatments, and recovered by G_i1 or G_o1-reconstitution. Similar selective coupling was confirmed in *in vivo* reconstitution experiments using baculovirus-infected Sf21 cells. On the other hand, we characterized the Noci (i.pl.)-induced peripheral nociceptive responses through sequential signaling mechanisms in peripheral nerve endings followed by a local substance P release in experiments using Noci-receptor K/O mice and mice with a targeted disruption of the tachykinin 1 gene.

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S4-1

OPIOID AND ORPHANIN RECEPTOR ANATOMY (AND FUNCTION?)

S.J. Watson, C. Neal, E. Curran, C. Norton, and H. Akil.

University of Michigan/Mental Health Research Institute, USA.

This presentation focuses on light microscopic analysis of endorphin and orphanin systems, both pre- and post-synaptic, using immunocytochemistry, autoradiography and *in situ* hybridization. The first emphasis is on the endorphin systems as they relate to reinforcement circuits, especially the nucleus accumbens core and shell. Of particular interest are the relationships between opioid peptide and receptor systems, tachykinins and dopamine receptor containing cells in the shell. The second focus is on the OFQ systems, as it is found throughout CNS. This is a uniquely broad system with potential impact on a very large number of special circuits and systems. This complexity may well be of value in appreciating this systems' confusing physiology. NIDA, R01 DA08920; NIDA; T32 DA07268.

S4-3

Brain Opioid Receptors by PET and SPECT

J.J. Frost, John Hopkins University, Baltimore, Maryland 21287, USA

In 1984, brain opioid receptors were first imaged by positron emission tomography (PET) using C11 carfentanil, a μ selective ligand. This advance subsequently led to the development of ligands for other opioid receptor subtypes and to studies aimed at elucidating the role of the endogenous opioid system in human disease. Disease applications have included epilepsy, depression, drug abuse and dementia. Some of the results of these studies will be reviewed in this presentation. Most recently, opioid receptor imaging has begun to elucidate the role of the endogenous opioid system in primary affective disorder and cocaine abuse. Cocaine abuse is associated with increased μ opioid receptors which, in certain regions, correlate with the degree of subjectively reported craving by cocaine addicts. Furthermore, the extent to which opioid receptor binding normalized during monitored abstinence is correlated with increased time to relapse following discharge. In primary affective disorder, increased opioid receptors are also observed, but in a different pattern compared to cocaine addiction. Correlations between the severity of depressive symptoms and opioid receptor changes suggest not only a relation to the trait of depression, but also the state of being depressed. The effect of antidepressant treatment can also be monitored by PET imaging of opioid receptors. The application of statistical parametric mapping (SPM), a technique long used in functional blood flow mapping studies, to PET images of brain opioid receptors results in significant improvement in the extent to which regional abnormalities can be identified. Another new advance in brain opioid receptor imaging is the measurement of acute changes in *in vivo* binding that occur as the result of release of endogenous opioid peptides, a change in affinity state, receptor internalization, etc. For example, capsaicin-induced pain, acute administration of cocaine or acute induction of seizures has been shown to modulate regional opioid binding, providing insight into regional brain function, as in blood flow studies, but with much greater chemical specificity. There remain many new opportunities for study of the endogenous brain opioid system, but the examples available to date provide a strong basis for elaborating and expanding this area of neuroimaging.

S4-2

ULTRASTRUCTURAL LOCALIZATION OF μ -OPIOID RECEPTORS: SITES FOR FUNCTIONAL INTERACTIONS INVOLVING AMINO ACID TRANSMITTERS.

V.M. Pickel, Department of Neurology and Neuroscience, Cornell University Medical College, New York, NY. 10021.

The potential functional sites for μ -opioid receptor (MOR) activation have been examined by electron microscopic immunocytochemistry in regions known to be critically involved in the analgesic, motor, and motivational effects of opiates. In these regions, antipeptide antiserum against MOR was mainly distributed along portions of dendritic plasma membranes near, but not within asymmetric, excitatory-type synapses. In striatal regions that were examined most extensively, these dendrites belong primarily to spiny GABAergic neurons. Excitatory afferents that project to these regions are known to be glutamatergic, producing their physiological effects, in part, through activation of NMDA receptors. We combined immunogold-silver and immunoperoxidase labeling to show the presence of MOR along plasma membranes of many GABAergic dendrites, as well as several GABAergic axon terminals in the nucleus accumbens. Similar methods were used to demonstrate co-distribution, as well as differential pre- and postsynaptic labeling for MOR and NMDAR1 in the dorsal and ventral striatum. These results, together with our single labeling studies in the brainstem and spinal cord suggest that at several different levels within the central nervous system there is a common mechanism for functional interactions involving MOR agonists and neurons containing amino acid neurotransmitters. (Supported by NIH grants DA04600 and L18974).

S5-1

PAIN INHIBITION BY IMMUNE-DERIVED OPIOIDS

C. Stein, M. Schäfer, S.A. Mousa and H. Machelska. Klinik f. Anästhesiologie, Freie Universität Berlin, Germany

Locally applied opioids have antinociceptive and antiinflammatory actions (1-3). These effects are mediated by opioid receptors on peripheral sensory nerve terminals in rats (4, 5) and in humans (6). Opioid receptors are synthesized in the dorsal root ganglia, they are axonally transported towards the nerve terminals and they can be activated by exogenous agonists as well as by endogenous opioid peptides expressed in inflammatory cells (7). This presentation will review mechanisms of release of opioid peptides from immune cells, the migration of these cells to peripheral inflamed tissue and interactions of opioid peptides with their corresponding receptors resulting in pain inhibition.

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S5-2

THE OPIOID TACHYKININ CONNECTION IN PAIN.

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The present study investigates the involvement of opioids and tachykinins (TK) and their receptors (rec) in inflammatory pain along the entire pain neuraxis: Proenkephalin (PENK) is expressed in small and large diameter sensory neurons, in spinal interneurons and projection neurons, and in many supraspinal pain centers. NK-1 and NK-3 receptors are abundantly expressed in primary sensory afferents exhibiting complex co-existence/non-coexistence patterns with substance P, opioids and opioid rec. The most dramatic response to peripheral inflammatory pain is the upregulation of prodynorphin expression in the dorsal horn, which highly correlates with the arthritic index and is substantially antagonized by corticosteroids, NK-1 antagonists and k agonists. Peripheral inflammation cause moderate increase in PENK expression in the dorsal horn, downregulation of PENK in primary afferents and upregulation of μ -receptors both in primary afferents and in the dorsal horn with only minor changes of k- and d-receptors. Distinct alterations in supraspinal opioid and opioid receptor expression are paralleled by changes in TK and TK-rec expression. Thus, depending on the level of the pain neuraxis specific combinations of opioids and TKs and their receptors are involved in the organism's response to peripheral inflammation suggesting hierarchical control of nociceptor activation by level-specific pre- and postsynaptic opioid and TK receptors. Supported by the DFG

S5-4

PAIN MODULATING EFFECTS OF OFQ IN BRAIN AND SPINAL CORD.

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The recently discovered endogenous neuropeptide orphanin FQ (OFQ, also known as nociceptin) has been reported to modulate pain perception and reverse stress-induced as well as morphine-induced analgesia in experimental animals. However, some controversy exists about hyperalgesic vs. analgesic effects in the spinal cord. Since no selective OFQ antagonist is currently available to study the function of the OFQ system in nociception and other brain functions we have generated OFQ-knock out mice. Using homologous recombination in ES cells we have disrupted the murine OFQ gene. After injection of the ES cells into blastocysts these were reimplanted into foster female mice to generate chimeric offspring. Several chimeric mice showed germline transmission of the mutated allele. Heterozygous and homozygous offspring was fertile and appeared anatomically and behaviorally normal under home cage conditions. Preliminary studies indicate that OFQ-deficient homozygous mice appear to differ from their wildtype littermates in basal pain thresholds and expression of stress-induced analgesia. This could result from enhanced activity of the endogenous opiate system. We are currently investigating pain responsiveness in the OFQ-deficient mice under various experimental conditions. The OFQ-knock out mice will also be valuable to define the interactions of the OFQ system with the endogenous opioid system.

S5-3

IS NOCICEPTIN HYPERALGESIC, ALLODYNIC, ANTI-OPIOID OR ANALGESIC?

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Electrophysiological and pharmacological studies have shown that nociceptin (orphanin FQ), the endogenous ligand of the ORL1 (LC132) receptor, regulates nociception, however in complex manner. In the CNS, nociceptin has been consistently found to depress neuronal activity. Yet, in intact animals, the peptide has been reported to variously affect nociceptive responsiveness, ranging from hyperalgesia to analgesia, and spinal allodynia (refs 1-3 for recent reviews). The conflicting nature of these data may however only be apparent, reflecting our limited understanding of pain neuronal circuitry. It may also reflect differences in experimental conditions, particularly those affecting idiosyncrasy and/or physiological status of the animals, as cogently discussed in a recent review (3).

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S5-5

NOCISTATIN, A PEPTIDE THAT BLOCKS NOCICEPTIN ACTION IN PAIN TRANSMISSION.

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Nociceptin (orphanin FQ) is an endogenous ligand for the orphan opioid-like receptor which induces both touch-evoked pain (allodynia) and thermal hyperalgesia when administered intrathecally. The nociceptin precursor comprises another bioactive peptide designated as nocistatin. Nocistatin blocked the nociceptin-induced allodynia and hyperalgesia. It also attenuated pain responses evoked by prostaglandin E2. The carboxyl-terminal hexapeptide of nocistatin Glu-Gln-Lys-Gln-Leu-Gln, which is conserved in bovine, human, and murine species, possessed the allodynia-blocking activity. In addition, we isolated endogenous nocistatin from bovine brain. Furthermore, intrathecal pretreatment with anti-nocistatin antibody decreased the threshold for the nociceptin-induced allodynia. While nocistatin did not bind to the nociceptin receptor, it bound to the membrane of mouse brain and spinal cord with a high affinity. These results demonstrate that nocistatin is a novel bioactive peptide produced from the same precursor as nociceptin and that these two peptides may play opposite roles in pain transmission.

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S6-1

DIMERIZATION OF OPIOID RECEPTORS: IMPLICATIONS FOR A ROLE IN RECEPTOR REGULATION.

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Dimerization of G-protein coupled receptors has been increasingly noted in the regulation of their biological activity. We have examined the ability of opioid receptors to dimerize, and the role of receptor dimerization in agonist induced internalization. Using differential (flag and c-Myc epitope) tagged receptors we show that the δ opioid receptors exist as dimers. The level of dimers is agonist dependent; increasing concentrations of agonists reduce the levels of dimer with corresponding increases in the levels of monomer. Interestingly, morphine does not affect the levels of either form. It has been shown that morphine, unlike other opioid agonists, does not induce δ opioid receptor internalization. This suggests a relationship between modulation of the levels of dimer and receptor internalization. The rate of the agonist induced decrease in δ opioid receptor dimers is faster than the rate of internalization, suggesting that monomerization precedes the agonist induced internalization of the receptor. Recently, we have found that κ opioid receptors also exist as dimers. However, agonist treatment does not induce significant monomerization of these receptors. Interestingly, high efficacy agonists are not able to induce substantial internalization of κ opioid receptors. Taken together, these results suggest that internalization and dimerization of the opioid receptors are interrelated and exhibit agonist selectivity as well as subtype specificity. The differential agonist-specific and type-specific endocytosis and dimerization of opioid receptor types provide additional modes of regulation of opioid receptor function.

This work is supported by grants DA08863 and NS01788 (to L.A.D).

S6-3

REGULATION OF ADENYLYL CYCLASE BY ACUTE AND CHRONIC EXPOSURE TO OPIATES AND AGONISTS OF OTHER INHIBITORY RECEPTORS.

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Acute stimulation of opiate receptors leads to adenylyl cyclase (AC) inhibition, while chronic activation leads to a progressive increase in AC activity. This phenomenon is referred to as AC superactivation and has been proposed to play a role in the mechanism underlying opiate addiction. We reconstituted the ability of opiates to inhibit AC and to induce superactivation using CHO and COS cells transfected with μ -, δ - or κ -opioid receptors. We found that AC superactivation is mediated via G_i/o proteins and that b g dimers have a role in this process. Nine AC isozymes have recently been cloned. Transfecting these isozymes into COS cells, we found that acute activation of the μ receptor inhibited AC-I, V, VI and VIII, while AC-II, IV and VII were stimulated and AC-III was not significantly affected. Chronic opioid receptor activation led to superactivation of AC-I, V, VI and VIII, but not of AC-II, III, IV, or VII, demonstrating that superactivation is isozyme-specific. Interestingly, AC-V, which is predominantly localized in brain areas involved in addiction and reward processes, yielded the largest superactivation. These results suggest that tolerance and withdrawal may involve specific AC isozymes. A similar pattern of AC isozyme regulation was observed with other G_i/o -coupled receptors (m2- and m4-muscarinic, D2-dopaminergic, CB1-cannabinoid), demonstrating the generality of the phenomenon of AC superactivation.

S6-2

BASAL RECEPTOR ACTIVITY AND Ca^{++} SIGNALING: RELEVANCE TO TOLERANCE AND DEPENDENCE.

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We have previously postulated that the μ opioid receptor (MOR) displays basal signaling activity. By measuring ^{35}S -GTP γ S binding to membranes of HEK293 cells transfected with MOR (HEK- μ), we now demonstrate a basal level of G protein coupling, in particular to Gai3. Moreover, basal MOR signaling is enhanced after morphine pretreatment and removal of the agonist. Naloxone appears to act as a partial agonist with low efficacy in untreated HEK- μ cells, whereas it behaves like an inverse agonist in morphine pretreated cells. This could account in part for its high potency in precipitating withdrawal in dependent subjects. To address the mechanisms underlying these observations, we have studied Ca^{++} signaling induced by MOR activation, and a possible role for Ca^{++} /calmodulin in the chain of events leading to the dependent state. We find that both Ca^{++} signaling by MOR and Ca^{++} /calmodulin contribute to biochemical changes during prolonged MOR activation. Calmodulin appears to bind directly to MOR, and thus, could serve as a second messenger that is detached from the membrane upon MOR activation. Supported by PHS grants DA04166 and GM43102.

S7-1

BEHAVIOURAL EXPRESSION OF OPIATE-INDUCED DEPENDENCE AND REWARDING EFFECTS IN GENETICALLY MODIFIED MICE.

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The neurobiological mechanisms involved in the development and expression of opiate dependence have been recently investigated by using mice with a genetic disruption of genes related to the opioid responses. The pharmacological effects induced by morphine were first evaluated in mice lacking the cAMP-responsive element-binding protein (CREB) gene. No change in morphine-induced analgesia was observed in these mice. In contrast, the expression of the somatic symptoms of morphine withdrawal was strongly attenuated in CREB deficient mice. The involvement of dopaminergic system was investigated by using mice deficient in D2 dopaminergic receptors. Morphine-induced analgesia and the somatic symptoms of withdrawal were not modified in these animals. However, the rewarding properties of morphine in the place preference paradigm were completely abolished in D2 receptor deficient mice. Morphine responses were also evaluated in mutant mice lacking opioid receptor genes. μ deficient mice did not show any analgesic or rewarding response after morphine administration. Besides, chronic morphine did not develop any behavioral or biochemical manifestation of dependence in these mice. Analgesic and rewarding effects of morphine were not modified in κ deficient mice, whereas the development of morphine-dependence was only slightly attenuated in these animals.

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S7-2

MANIPULATIONS OF HPA-SYSTEM ACTIVITY UPON MORPHINE-INDUCED SENSITIZATION PROCESSES.

R. Spanagel and I. Sillaber. Max Planck Institute of Psychiatry, Munich, Germany. Stress alters the sensitivity to drugs of abuse and is, therefore, considered to be an important contributory factor to drug-seeking behavior. Thus, enhanced morphine-induced locomotor activity was observed in response to repeated application of restraint, handling and social defeat stress. Those stressors caused stimulation of the hypothalamus-pituitary-adrenal (HPA) system and release of corticosterone. Repeated intermittent injections of corticosteroids, in particular of DEX, also increased morphine-induced locomotor activity whereas adrenalectomy decreased it. Enhanced morphine-induced behavioural effects and dopamine release in the nucleus accumbens were also observed in a transgenic mouse model of impaired glucocorticoid (type II) receptor function. Furthermore, corticotropin-releasing hormone (CRH) receptors are essential in mediating stress responses. The effects of morphine in CRH receptor 1 deficient mice will be also reported.

S7-4

EXPRESSION OF BEHAVIORAL SENSITIZATION EVOKED BY DOPAMINE AGONISTS PREDICTS THEIR ABILITY TO REINSTATE HEROIN- AND COCAINE-SEEKING BEHAVIOR IN RATS.

L.J.M.J. Vanderschuren, A.N.M. Schoffelmeer, A.H. Mulder and T.J. De Vries, Research Institute Neurosciences Vrije Universiteit, Department of Pharmacology, Medical Faculty, Free University, Amsterdam, The Netherlands. Long-term sensitization to drugs of abuse has been proposed to play an important role in the persistence of drug craving. To test this hypothesis, we evaluated the effects of priming injections with direct and indirect dopamine agonists on drug-seeking behavior following long-term extinction of cocaine and heroin self-administration, an animal model of drug craving. In parallel experiments, the ability of these drugs to elicit expression of long-term sensitization in amphetamine- and morphine-pretreated rats was investigated. Heroin-seeking behavior was reinstated by heroin, amphetamine, cocaine, and the selective dopamine reuptake inhibitor GBR-12909, while the dopamine D1 receptor agonist SKF-82958, the D2 agonist quinpirole and the D1/D2 agonist apomorphine were ineffective. Likewise, in morphine-pretreated rats, the locomotor responses to morphine, amphetamine, cocaine and GBR-12909, but not to SKF-82958, quinpirole and apomorphine were sensitized. Cocaine-seeking behavior was reinstated by amphetamine, cocaine, GBR-12909 and quinpirole, but not heroin, SKF-82958 or apomorphine. Locomotor sensitization in amphetamine-preexposed rats could be evoked with amphetamine, cocaine, GBR-12909 and quinpirole, but not with morphine, SKF-82958 or apomorphine. Moreover, rats with a history of heroin- but not cocaine self-administration displayed a sensitized locomotor response to heroin, while both heroin and cocaine rats showed locomotor sensitization to amphetamine. The differences between direct and indirect dopamine agonists indicate a clear, but complex, involvement of dopamine in the persistence of drug-seeking behavior. Moreover, the exact match observed between the drugs that induced sensitized locomotor responses in morphine- or amphetamine- pretreated rats and those that reinstated heroin- or cocaine-seeking behavior, respectively, indicates a strong relationship between drug-induced craving and drug hyperresponsiveness in long-term abstinent rats.

S7-3

ENDOGENOUS OPIOIDS, REWARD AND ADDICTION.

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Different stages in the addiction course can be delineated: the maintenance phase, the withdrawal phase and the relapse phase. Various psychological and biological mechanisms seem to be important for the drug use in these stages. The psychological mechanisms include liking the drug, which has been linked to reinforcement and euphoria, and wanting the drug, playing a role in the daily pattern of drug intake during maintenance, but also in the phenomenon of craving which may be important for relapse. The significance of endogenous opioids for the different stages of addiction and the psychological mechanisms is still a matter of debate. There are indications that these peptides play a role in modulating drug reinforcement, which may be pertinent to the individual susceptibility with respect to development of (psychic) dependence, in the dynamics of drug taking behaviour during the maintenance phase of drug dependence and in certain motivation effects induced by repeated drug (self) administration, which may be involved in craving and relapse.

S8-1

OPIOID GROWTH FACTOR REGULATION OF PRENATAL AND POSTNATAL DEVELOPMENT.

Patricia J. McLaughlin, Penn State University.

The native pentapeptide, [Met⁵]-enkephalin (termed opioid growth factor, OGF), interacts with the newly cloned and sequenced zeta (ζ) opioid receptor to modulate growth related to development, neoplasia, cell renewal, and wound healing. An important question is whether OGF is present and functions during prenatal life, and if OGF-z interactions in fetal life have ramifications on postnatal well-being. Immunocytochemical studies reveal that both OGF and the ζ receptor are present in a wide variety of tissues of the fetal rat. OGF is an autocrine growth factor as demonstrated by the finding of preproenkephalin mRNA in these tissues by Northern blot analysis and *in situ* hybridization. Furthermore, each organ has its specific ontological sequence as to transcription and translation of the growth regulatory peptide. The cellular function and mechanism of OGF is related to DNA synthesis, as evidenced by studies using [³H]-thymidine and autoradiography and measurement of organ weight. Using the strategy of blocking the tonic action of OGF throughout gestation with the potent and long-acting opioid antagonist, naltrexone (NTX), we found pups to have enhanced brain and organ weights, increased body weights, early acquisition of physical characteristics, precocious development of spontaneous motor, sensorimotor, and reflexive behaviors. Investigations with reversed phase-high performance liquid chromatography showed that NTX crossed the placenta and entered the fetus; and organ culture studies implied the direct action of OGF in fetal tissues. These data suggest that an endogenous opioid molecule is vital to the course of embryogenesis, and that interactions of peptide with receptor in prenatal life are determinants of somatic, physical, and behavioral development in postnatal life.

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S8-2

OPIOIDS AS MESSENGERS OF LIFE AND DEATH: OPIOID SYSTEM DIVERSITY AND IMPACT ON THE GENESIS OF NEURONS AND GLIA.

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Opioids affect brain development by altering neural cell numbers. To determine how opioids affect neural cell numbers, the effects of μ , δ , and κ opioid ligands and receptor activation on the proliferation and survival of immature murine neurons, astroglia, and oligodendroglia were assessed in vitro. Opioids affect each cell type uniquely. Astroglia can express μ , δ and/or κ receptors and the activation of these receptors typically inhibit cell division without affecting cell viability. In contrast, oligodendroglia uniformly express μ and κ receptors, and stimulating μ receptors increases DNA synthesis. Neurons display responses that are more complicated. Developing cerebellar granule neurons uniformly express μ and δ receptors, and the activation of these receptors inhibits cell division without affecting cell viability. In contrast, some subpopulations of developing dorsal spinal cord neurons express κ receptors, and in these neurons, activating κ receptors enhances survival in the presence of excitotoxic insults. Collectively, this suggests that the opioid system can modify neural development by altering cell proliferation, and to a lesser extent, cell survival. The diversity of opioid ligand and receptor types among cells strategically positions the opioid system to be able to regulate neuronal and glial numbers in different regions of the CNS during development. Supported by NIDA (DA 06204).

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O1-1

ENKEPHALIN/DELTA RECEPTOR SYSTEMS INVOLVEMENT IN OPIOID TOLERANCE.

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Tolerance remains a prominent problem in the treatment of patients. Yet, studies in recent years have uncovered several steps in opioid tolerance which can be blocked pharmacologically. In addition to analgesic systems, opioids induce tolerance by activating NMDA receptors which in turn generate nitric oxide (NO) through neuronal nitric oxide synthase. Blockade of these systems prevents opioid tolerance without interfering with analgesia. Other studies have reported that low naltrindole doses also prevent tolerance, presumably by blocking delta receptors. This concept was supported by antisense studies targeting DOR-1, which encodes the delta receptor. In the current studies, we examined opioid tolerance in knockout mice lacking either the delta receptor (DOR-1 knockout) or enkephalins (ENK knockout). Tolerance to morphine did not develop in the DOR-1 or ENK knockout. However, the development of tolerance with the kappa₁ drug U50,488H, implies that kappa tolerance involves a different mechanism.

O1-3

DEFECTIVE PRODYNORPHIN PROCESSING IN TRANSGENIC MICE LACKING CARBOXYPEPTIDASE E AND PROHORMONE CONVERTASE 2.

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The generation of dynorphin (Dyn) A-17, Dyn A-8, Dyn B-13 and a -neo-Endorphin from prodynorphin (proDyn) requires the action of prohormone convertases (PCs) and carboxypeptidases (CPs). PC1 and PC2 cleave proDyn at single and paired basic residue processing sites. PC1 cleaves proDyn to high molecular weight intermediates and further processing to the final opioid peptides is thought to be due to PC2. In vitro studies have demonstrated that PC2 is able to generate Dyn A-9 from Dyn A-17. The C-terminal trimming of Dyn A-9 to generate Dyn A-8 is carried out by CPE. We characterized proDyn processing in the brain of mice that lack CPE activity (CPE^{fat}) and PC2 activity (PC2 knock-out (K/O)) with specific antibodies against Dyn A-17, Dyn A-8, Dyn B-13, ProDyn carboxy terminal peptide and Leu-Enkephalin. We find substantially decreased levels of Dyn A-8 in the brain of PC2 K/O as well as in the brain of CPE^{fat} mice. This decrease corresponds to a concomitant increase in Dyn A-17. The fact that the absence of either CPE or PC2 leads to a similar decrease in monobasic processing suggests a complex regulation of endopeptidase activity. The impaired proDyn processing in CPE^{fat} mice could partially be due to abnormalities in processing by PC2. This is supported by our finding that PC2 activity is altered in discrete brain regions of CPE fat mice. Taken together, these data suggest that multiple enzymes are involved in proDyn processing and that the relative levels of various enzymes and mutual regulation of their activities could govern the processing of prodynorphin and thus modulate the level of opioid peptides in vivo.

O1-2

EFFECTS OF OPIOID d AGONISTS IN μ KNOCK-OUT MICE.

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Transgenic mice lacking μ opioid receptors have been used to delineate the role of selective agonists at opioid d receptors in intracellular coupling and in antinociception. Studies were conducted using homozygous μ-receptor knock-out (KO) and in wild-type (WT) mice with the same 129Sv/C57 genetic backgrounds. Mouse whole brain membranes were prepared for [³⁵S]GTPγS binding assays and antinociception was assessed using the 55°C hot-water tail-flick test with a 15 sec cut-off time. The potency or efficacy of GTPγS stimulation by SNC 80 and [D-Ala²,Glu⁴]deltorphin (DELTA) were not significantly different in WT or KO membranes; the potency and efficacy of DPDPE was significantly reduced in KO membranes. Intracerebroventricular (i.c.v.) morphine produced dose-dependent antinociception in WT but not KO mice. The antinociceptive potency and efficacy of i.c.v. DELTA in WT or KO mice did not differ significantly. In contrast, the antinociceptive dose-response curve for i.c.v. and i.th. DPDPE was displaced to the right by 4- and 9-fold in KO compared to WT mice. Systemic SNC80 (60 mg/kg, i.p.) produced significant and equivalent antinociception in both WT and KO mice. The loss of potency and efficacy of DPDPE in KO mice indicates that this agonist has a significant μ component of action. However, no changes in d-receptor coupling or antinociception were observed in μ KO mice with highly d-selective agonists, suggesting that μ receptor genetic deletion does not alter d receptor function.

O1-4

NEUTRAL ENDOPEPTIDASE KNOCKOUT IN-DUCES NOCICEPTIVE OVERCOMPENSATION.

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Neutral endopeptidase (NEP, EC 3.4.24.11) is thought to play a key role in the inactivation of the synaptically released endogenous opioids leu-enkephalin and met-enkephalin. As enkephalins decrease pain perception, it was expected that genetic elimination of NEP would result in a decreased sensitivity to noxious stimuli. However, in our rodent model NEP-knockout resulted in an increased sensitivity to noxious stimuli of both thermal (hot plate and warm water tail withdrawal) and chemical (acetic acid-induced writhing) nature. As expected, NEP-knockout mice became unresponsive to the enkephalinase inhibitor thiorphan. Thus, target disruption of the NEP gene resulted in an overcompensation of the tested nociceptive responses.

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O2-1

SINGLE NUCLEOTIDE POLYMORPHISM IN THE HUMAN MU OPIOID RECEPTOR GENE IN METHADONE MAINTAINED AND CONTROL STUDY SUBJECTS.

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To identify single nucleotide polymorphism (SNP) in the human μ opioid receptor, we used a PCR-based strategy to amplify the coding regions of the mu opioid receptor gene. We then determined the DNA sequence of the amplified exons. Using this method, we sequenced DNA samples from 152 subjects including both former long-term heroin addicts in methadone maintenance treatment and individuals with no history of opiate or non-opiate drug or alcohol dependence. Addictive disease patients and normal control subjects were extensively characterized with respect to the addictive and other medical diseases, psychological and psychiatric profiles, as well as ethnic background and family history. Several SNPs were identified at varying frequencies within the study groups. Allele frequencies with respect to gender, ethnicity and disease status were determined. The findings have possible implications for both opioid addiction and other diseases involving the μ opioid receptor. This work was supported by NIH grants DA09444 (LY and MJK), DA05130 and DA00049 (MJK), HGO0008 (SML), DA09116 and DA1 1891 (LY), and M01 - RROO 1 02 (RUH).

O2-3

ALLELIC VARIATIONS OF THE δ AND κ OPIOID RECEPTOR IN THE GENERAL POPULATION.

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The coding sequences of the δ and the κ opioid receptor was searched for allelic variations in humans. In the kappa receptor, four silent mutations were detected with allele frequencies ranging between 2% and 8%. The delta receptor revealed a coding mutation in exon 1 changing a phenylalanine residue to a cysteine residue. The site of this exchange was close to the N terminus in the extracellular part of the receptor molecule. The allele frequency was 10%. Furthermore, a frequent base variation (T+C) was identified in the third exon of the delta receptor. Although this base exchange did not affect amino acid sequence, it displayed a strong correlation with heroin addiction. In 103 drug dependent subjects, the allele frequency of C was 53% as compared with 39% in unaffected controls ($p < 0.01$). In particular, a high proportion of CC homozygotes was found among the heroin addicts (26% versus 10%) The other polymorphic site of the delta receptor was not correlated with heroin intake. These findings indicate that mutations in the delta opioid receptor gene might contribute to a predisposition for heroin abuse.

O2-2

STUDIES OF RECEPTOR FUNCTION IN A SINGLE NUCLEOTIDE POLYMORPHISM OF THE HUMAN MU OPIOID RECEPTOR GENE.

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We have identified several single nucleotide polymorphisms (SNPs) in the human opioid receptor gene. The most prevalent genetic polymorphism we identified is in the N-terminal region of the mu opioid receptor and predicts an Asn to Asp change in its encoded amino acid residue. This is a putative site for N-glycosylation; thus, the variant allele would result in the loss of a putative N-glycosylation site. To explore any potential effects of this polymorphism on the μ opioid receptor, we mutated the variant position of the prototype receptor cDNA by site-directed mutagenesis to generate a cDNA clone for the human μ opioid receptor containing the variant. Binding affinities of diverse opioid drugs and endogenous opioid ligands were assessed in AV-12 cells stably expressing either the prototype or variant receptors. We also used expression in *Xenopus* oocytes to compare the variant receptor with the more common allelic form with respect to agonist-induced activation of G protein-coupled potassium channels. Significant findings were made. This work was supported by NIH grants DA09444 (LY and MJK), DA05130 and DA00049 (MJK), HGO0008 (SML), DA09116 and DA1 1891 (LY), and M01-RROO102 (RUH).

O2-4

REGULATION OF THE MOUSE KAPPA OPIOID RECEPTOR GENE ACTIVITY

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The mouse kappa opioid receptor (KOR) gene transcripts are examined in mouse embryonic cells and their differentiated neurons. Two alternatively spliced transcripts are produced from the distal promoter identified previously, by using two different splicing acceptors for intron 1. Both of the two alternatively spliced KOR transcripts are detected in undifferentiated embryonal carcinoma cell line P19. However, none of the KOR transcripts are detected in embryonic stem cells HM1. Upon retinoic acid (RA) induction of P19 and HM1 neuronal differentiation, the two transcripts display very different expression profiles along the differentiation process. One of the splicing acceptor is preferentially used in RA induced neurons. The implication of differential splicing of KOR gene in neuronal differentiation is discussed.

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O2-5

MOLECULAR CHARACTERIZATION OF MU AND DELTA OPIOID RECEPTORS FROM ZEBRAFISH.

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Opioids and their receptors are not exclusive of mammals. Opioid peptides and opioid binding sites have been described in amphibian, fish and invertebrates. Here we report the cloning, molecular characterization, expression and distribution of two opioid receptor homologous to the delta and mu types from a teleost organism, the zebrafish (*Danio rerio*). The delta opioid receptor have been isolated as described previously. From the sequence of a genomic clone isolated with a rat mu opioid receptor probe, we designed oligonucleotides to perform RT-PCR on brain RNA; the resulting product was used to screen a brain cDNA library constructed in l-ExCell vector. Several clones were isolated and showed 77% homology to mammalian mu opioid receptor and conserved exon-intron boundaries in exons 1 to 3. In order to study expression of our clones, we designed oligonucleotide probes and used them for in situ hybridization studies for both putative opioid receptors. Characteristic and differentiated patterns of expression for both genes were obtained. This work was supported by Fondo de Investigacion Sanitaria (grant 96/2090), Junta de Castilla y Lesn (grant SA/1095) and Fundacisn Ramsn Areces.

O3-1

MECHANISMS OF ADENYLYL CYCLASE INHIBITION OF THE DELTA- AND MU-OPIOID RECEPTORS USING A SERIES OF RECEPTOR DERIVED PEPTIDES.

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Recent studies from our laboratory have shown the significance of the proximal and distal portions of the third intracellular loop and part of the carboxyl-terminal as major contact sites with the G proteins using a series of opioid receptor derived peptides (Merkouris et al. *Mol. Pharmacol.* 50:985, 1996, and Georgoussi et al. *Biochem. Biophys. Acta* 1359:263, 1997). Based on these observations we also examined the ability of these peptides to interfere in adenylyl cyclase activity. Our results have shown that the amino-terminal portion of the third intracellular loop (peptide i3.1), as well as a peptide derived from the entire third intracellular (i3) strongly enhanced in a dose dependent manner agonist stimulated adenylyl cyclase inhibition, in membranes from Rat-1 fibroblasts and murine neuroblastoma Neuro2A cells stably transfected to express the delta- and mu- opioid receptors respectively. Peptides i3 and i3.1 were less effective when adenylyl cyclase was stimulated by AIF-4 and were inactive when adenylyl cyclase was uncoupled from G protein regulation by MnCl₂. DAMGO-induced adenylyl cyclase inhibition appeared to be enhanced by 25(5 (n=7) when membranes from mouse neuroblastoma Neuro2A cells were treated with peptide i2.2 which corresponds to the c-terminal part of the second intracellular loop. Peptide induced adenylyl cyclase inhibition was sensitive to pertussis toxin, and was markedly reduced when both opioid receptors were uncoupled from the G protein population present in these cell lines.

Collectively, these data provide novel information about various domains of the delta- and mu- opioid receptors involved in adenylyl cyclase inhibition. This work was supported by the European Union (CHRX- CT94-0689) to Z.G. and G.M.

O2-6

THE UNIRECEPTOR HYPOTHESIS OF OPIOID ANTINOCICEPTION IN AMPHIBIANS: IMPLICATIONS FOR EVOLUTION OF OPIOID RECEPTORS.

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The type of opioid receptor(s) mediating antinociception in amphibians was investigated using type-selective opioid antagonists and spinal administration of mu, delta- and kappa-opioid receptor agonists. The antagonist actions of the m antagonist, beta-FNA (beta-funaltrexamine), the delta antagonist, naltrindole (NTI), and the kappa antagonist, nor-BNI (nor-binaltorphimine) were assessed in the Northern grass frog, *Rana pipiens*. Antinociceptive thresholds were measured using the acetic acid test. beta-FNA did not produce any agonist effects after spinal administration. 24h pretreatment of beta-FNA significantly blocked the antinociceptive effects of morphine, fentanyl, DAMGO, DADLE, DPDPE, and CI977. NTI blocked DPDPE, DSLET, and morphine effects and nor-BNI blocked the antinociceptive effects of U50488 and GR89696, but also morphine, fentanyl, DPDPE, and deltorphin. Binding studies from our lab and others show predominantly one type of opioid binding site, unlike any opioid binding site characterized in mammals. [3H]-diprenorphine bound to a single high affinity site in brain and spinal cord homogenates with a K_d of 0.65 nM and B_{max} of 288 fmol/mg. These data suggest that mu, delta, or kappa opioids produce antinociception in amphibians by interaction at one type of opioid receptor, termed the "unireceptor". Implications for the functional evolution of opioid receptors will be discussed. Supported in part by NIDA (07326)

O3-2

ROLE OF PROTEIN KINASE (PKC) IN OPIOID RECEPTOR PLASTICITY.

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Repeated exposure to opioid agonists leads to a progressive loss of opioid receptor function. Chronic opiate abuse is believed to produce biochemical and cellular changes, which contribute to the development of tolerance and addictive behaviors. Two separate phenomena - receptor desensitization (receptor uncoupling) and downregulation (receptor loss) - are under investigation to determine their contribution to opioid tolerance, withdrawal and addiction. Both processes appear dependent on phosphorylation of the opioid receptor complex. However, the identity of the specific kinases remains uncertain. One kinase, protein kinase C (PKC), has been the subject of debate as to its specific role in opioid receptor tolerance. Recent studies in our laboratory have dealt with developing an understanding of which aspect(s) of opioid receptor plasticity - desensitization, downregulation, or both - require or involve PKC. We will demonstrate that μ -specific opioid agonists, DAMGO and morphine, induce the physiological translocation/activation of multiple PKC isozymes (alpha, epsilon, and zeta) in SH-SY5Y neuroblastoma cells. Time-course and pharmacokinetic analysis of this effect will suggest that opioid-mediated PKC activation is important for the development of receptor downregulation rather than acute desensitization. Finally, we shall describe a putative mechanism for opioid-mediated PKC activation and present evidence for the involvement of diacylglycerol and calcium-dependent and independent PKC isoforms in homologous, agonist-dependent mu-opioid receptor down-regulation.

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O3-3

ENHANCED ANTINOCICEPTIVE EFFECT OF MORPHINE IN MICE LACKING THE ENZYME PLC β 3

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The second messenger enzyme phospholipase C (PLC) β 3 has been implicated in mu opioid signaling, but remains difficult to study in vivo. The antinociceptive effects and receptor selectivity of morphine were characterized in wild-type and transgenic mice lacking PLC β 3, using the mouse warm-water tail-flick test. Baseline tail flick latencies were similar in PLC β 3 mutant and wild-type mice. Morphine suppressed the tail-flick response in a dose-dependent manner after i.p. administration in both wild-type and mutant mice, with ED50 values of 12.8 mg/kg and 2.6 mg/kg, respectively, demonstrating greater morphine efficacy in the mutant mice. The μ -selective irreversible antagonist, β -FNA, significantly reduced the antinociceptive effect of morphine in both sets of mice, demonstrating that morphine acted through the μ receptor. Moreover, saturation binding assays performed with brain membranes prepared from each set of mice demonstrated no differences in ligand affinity or the number of m, d or k opioid receptors between the mutant and wild-type mice. These results suggest that PLC β 3 modulates the sensitivity of the opioid receptor to morphine, and plays a significant role in the opioid signaling pathway. (Supported by grants DA03742, DA07232, GM53162, the Tobacco Res. Council and AHA.)

O4-1

CENTRAL POST-STROKE PAIN IS ASSOCIATED WITH CHANGES IN OPIOID RECEPTOR BINDING: A PET STUDY WITH ^{11}C -DIPRENORPHINE.

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PET activation studies have identified some principal cerebral structures of a central network activated by pain. An analysis correlating the increases of regional cerebral blood-flow (rCBF) with multi-dimensional psychophysical assessment considered a region-specific encoding of pain intensity and pain unpleasantness in the brain. The cerebral structures included the cingulate gyrus in anterior-posterior extension, the frontal brain and midbrain and displayed a particular high-level opioid receptor binding, measured with the non-selective ligand ^{11}C -diprenorphine. In patients with central post-stroke pain (CPSP), spectral analysis was performed for absolute quantification of diprenorphine binding, and a pixelwise Z-score statistic was used to analyze regional changes of the tracer. CPSP was associated with a reduction in opioid receptor binding and glucose metabolism in pain-related central structures including the orbito-frontal cortex, cingulate gyrus, thalamus and insula. The findings suggest a displacement of diprenorphine by enhanced concentration of endogenous opioid peptides and/or a secondary down-regulation of opioid receptors during chronic pain states.

O3-4

INVOLVEMENT OF NMDA RECEPTORS IN OPIATE REGULATION OF Ca^{2+} OSCILLATIONS IN CULTURED HIPPOCAMPAL NEURONS.

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Opiate receptor agonists are known to alter neuronal activity through effects on membrane ionic conductances and receptor-activated channels. However, little is known about their effects on intracellular Ca^{2+} signaling pathways. We showed previously that opiates enhance and synchronize TTX-sensitive Ca^{2+} oscillations seen in cultured hippocampal neurons recorded in low Mg^{2+} saline. We now show using electrophysiological recordings that spontaneous TTX-sensitive burst events (depolarizations with spiking) are also evident under these recording conditions and are enhanced by bath application of DAMGO (1 mM). The spontaneous Ca^{2+} signals and burst events were blocked by the NMDA receptor (NMDAR) antagonist AP-5, indicating an involvement of NMDARs. Exogenous application of NMDA produced a Ca^{2+} signal and membrane depolarization that were also enhanced by DAMGO. These data indicate that opiates can synchronize intracellular Ca^{2+} signals in hippocampal neurons via synaptic interactions involving the NMDARs. Modulation of NMDAR-induced Ca^{2+} oscillations and membrane events could be an important pathway for opiate regulation of a variety of neuronal functions. (Supported by ARC 06420)

O4-2

INTERFERENCE OF PHOSDUCIN WITH OPIOID RECEPTOR SIGNALLING

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The cytosolic phosducin (Phd) may affect G protein coupled receptor signalling both by complexing G protein b/g subunits and by inhibition of GTPase. We have recently demonstrated that the protein is translocated to the membrane upon activation of excitatory PGE_1 receptors. The present study reports the effect of the Phd on opioid receptor function of NG 108-15 hybrid cells. Cells stably expressing Phd exhibit an increased sensitivity to d -receptor agonists that inhibit forskolin-stimulated cAMP generation. The opioid stimulated GTPase of the cells is inhibited, as is the case in membranes of nontransfected cells exposed to exogenous Phd. In contrast, in Phd-expressing cells the opioid stimulated incorporation of [^{35}S]GTP γ S is reduced. These biochemical investigations are supplemented with confocal microscopy in real time videos indicating both in non-transfected and in Phd-expressing NG cells differences with respect to the trafficking of μ -receptors, which have been fused to green fluorescence protein, as well as their distribution between the cell membrane and cytosol. Particular emphasis is given to the function of various μ receptor agonists. In conclusion, Phd functions as a regulating protein on inhibitory G protein signaling. It affects μ receptor trafficking and enhances inhibitory opioid activity on adenylate cyclase.

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O5-1

IN VIVO ROLES OF NOCICEPTIN (NOCI) IN PAIN TRANSMISSION USING MUTANT MICE LACKING NOCI-RECEPTOR AND SUBSTANCE P.

Makoto Inoue¹, Andreas Zimmer², Hiroshi Takeshima³ and Hiroshi Ueda¹. ¹Dept. Mol. Pharmacol. & Neurosci., Na aki Univ. Sch. Pharmac. Sci., Nagasaki 852-8521, Japan, ³NIMH, Bethesda, MD 20892, USA, ²Univ. Tokyo, Tokyo 113-0033, Japan.

Intrathecal injection of Noci produced nociceptive responses by stimulating the release of SP through a presynaptic mechanism, while higher doses inhibited SP-induced nociceptive responses in mice with a targeted disruption of tachykinin 1 gene or in capsaicin-pretreated mice through a postsynaptic mechanism. Mice lacking the gene for the Noci-receptor were hypersensitive to peripheral application of BK or SP, as well as intrathecal application of SP. However, there was no significant change in the NK1-receptor expression in the dorsal horn of spinal cord. These findings suggest that Noci has both presynaptic nociceptive and postsynaptic antinociceptive actions by modulating SP signalling.

O5-3

PHYSIOLOGICAL AND PATHOLOGICAL ACTIONS OF DYNORPHIN IN PAIN.

F. Porreca, M. H. Ossipov, V.J. Hruby, T.P. Malan, Jr. & J. Lai. Departments of Pharmacology, Chemistry and Anesthesiology, University of Arizona, Tucson, AZ 85724. Dynorphin A (1-17) (DYN) is an endogenous k opioid with high affinity and selectivity. Naloxone, nor-BNI or DYN antiserum produces a significant increase in the nociceptive response to formalin (2%, i.paw), suggesting tonic DYN antinociceptive activity mediated through kappa receptors. However, chronic pain resulting from peripheral nerve injury (i.e., neuropathic pain) produce elevated DYN levels across multiple rostrocaudal spinal cord segments. Spinal injection of DYN, or its des-Tyr fragments which do not bind to opioid receptors, results in enhanced responses to nociceptive stimuli similar to nerve-injury-induced pain. These pathological actions of exogenous dynorphins are blocked by MK801, but not naloxone, suggesting NMDA-receptor mechanisms. Similarly, nerve-injury induced pain can be blocked by DYN antiserum, or MK801. In order to test the possibility that DYN, or its fragments, may bind directly to NMDA receptors, DYN (2-17) was radioiodinated and found to exhibit high affinity (Kd of 10 nM) saturable, specific binding in rat brain membranes; this binding was modulated by a number of NMDA receptor ligands. Transfected HEK 293 cells expressing NMDAR1/NMDAR2A complexes (based on [³H]MK801 and [³H]CGP39653 binding) also showed specific binding of [¹²⁵I] DYN (2-17). These data suggest that DYN, and its fragments, have both physiological opioid receptor mediated antinociceptive actions, as well as pathological actions which may be mediated through a novel binding site on the NMDA receptor. Supported by DA 11823.

O5-2

REGULATION OF ORPHANIN FQ GENE EXPRESSION BY LPS AND TNF α : THE ROLE OF MAP-KINASES AND NF κ B.

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Regulation of orphanin FQ (OFQ, also known as nociceptin) by inflammatory mediators was investigated in primary astrocyte cultures. Proinflammatory cytokines and the bacterial endotoxin, LPS, activate several signal transduction pathways and regulate the expression of genes involved in the inflammatory response in astrocytes. LPS treatment lead to a 40-fold increase in OFQ mRNA expression while tumor necrosis factor α and interleukin 1 β induced OFQ three-fold. Interferon γ and IL-6 did not alter OFQ mRNA levels. LPS and TNF α appear to induce OFQ gene expression through activation of the p38 MAP kinase pathway, since a selective inhibitor of this kinase completely antagonized OFQ induction. Activation of NF κ B transcription factor by LPS and TNF α also seems to be involved in the regulation of OFQ transcription, since pyrrolidinedithiocarbamate pretreatment prevented OFQ induction. Interestingly, other stimuli, such as osmotic shock and UV light, which also activate the p38 MAP kinase pathway did not regulate OFQ expression. Regulation of OFQ expression by inflammatory mediators may point to a role of OFQ in CNS inflammatory responses and neuroimmune interaction.

O5-4

KAPPA-OPIOIDS, GENDER, ARTHRITIS AND SUBSTANCE P.

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Kappa (k) opioids are therapeutic in rat adjuvant arthritis, in part by local action on release of substance P (SP; [1]). As they also produce significantly greater analgesia in human females than males [2], we have examined possible gender differences in the efficacy of k-opioids in arthritic inflammation and nociception, especially in the possible involvement of SP. The incidence of arthritis was uniform in males and females. The centrally-acting k-agonist PNU-50488H and the peripherally-selective agent, asimadoline (Merck, KGaA) produced comparable reductions in three measures of inflammation (oedema, histology, radiology). In untreated animals, nociception (mechanical and thermal tests) was equal in males and females but there were gender differences in the drugs' analgesic efficacy. Measurements of SP in the joints showed striking differences: in both treated and untreated animals, levels were 75% higher in females and both drugs elevated these SP levels by 30 to 100%, raising the question about a role for SP in the gender differences.

[1] Walker, JS et al. (1997) Rheumatoid Arthritis: ID Research Alert 1, 291-299.

[2] Gear RW et al. (1996) Nature Medicine 2, 1248-1250.

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O5-5

CHOLECYSTOKININ INHIBITS LOCAL OPIOID ANALGESIA BY ACTIVATION OF PERIPHERAL CCKB-RECEPTORS.

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Both opioid and cholecystokinin (CCK)-receptors are present on peripheral sensory neurons. The present study investigated whether peripheral opioid analgesia is modulated by CCK in inflamed tissue. In Wistar rats, a painful hindpaw inflammation was induced by Freund's adjuvant and nociceptive thresholds were assessed by a paw pressure test. Intraplantar (i.pl.), but not intravenous administration of CCK (0.001-1.0 µg) dose-dependently inhibited analgesic effects of [D-Ala²,N-Me-Phe⁴, Gly-ol⁵]-enkephalin (DAMGO, 10 mg i.pl.) and fentanyl (1.5 mg i.pl.). Desulfated CCK was more effective than sulfated CCK. The peripheral anti-opioid effect of CCK was dose-dependently antagonized by the CCK_B receptor-selective antagonist L-365,260, but not by the CCK_A receptor-selective antagonist L-364,718. Local pretreatment with the protein kinase C-specific inhibitor calphostin C abolished CCK's anti-opioid effect. Peripheral analgesic effects of DAMGO and fentanyl were not influenced by i.pl. L-365,260 alone in either inflamed or noninflamed tissue. These results indicate that activation of peripheral CCK_B but not CCK_A receptors inhibits local analgesic effects of mu-opioid receptor agonists in inflamed tissue. This anti-opioid effect of CCK may be mediated by protein kinase C in sensory nerve terminals. Endogenous CCK does not seem to influence the efficacy of peripheral opioids.

O6-2

ICV-INJECTED Myr⁺-α₂ SUBUNITS OF G PROTEINS ATTENUATE MORPHINE TOLERANCE AND WITHDRAWAL.

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Tolerance to opioids is associated with Gbg-dependent phosphorylation of the cytoplasmic receptor domains. It was reported that icv-injected myristoylated Gi/oa subunits (myr⁺-Ga) were taken up and functionally incorporated into the receptor-initiated signaling cascade (J. Garzon, SENC 1998). In the present work, the dynamics of the loss of response associated with opioid exposure were analyzed using myr⁺-Ga subunits. The analgesic effects of beta-endorphin (0,6 nmol/mouse) were 66 +/- 4 and 41 +/- 3 % (n=12) of MPE respectively in control mice and in animals pretreated (-24 h) with an acute dose (ED₈₀) of morphine (tolerance to a single dose). Microinjection of myr⁺-Ga₁₂ (12 pmol/mouse) at different intervals before b-endorphin restored the potency of the endogenous opioid (up to 68 +/- 4% MPE). Over the first 24 h of chronic opioid exposure, but not longer, acute administration of myr⁺-Ga₁₂ decreased the rate at which tolerance developed and also reduced the incidence of the withdrawal syndrome precipitated by naloxone. Thus, subsensitivity promoted by a single dose of opioids, or the initial intervals of chronic opioid administration, is predominately due to depletion of G proteins in the receptor environment since it is myr⁺-Ga₁₂-sensitive. Later, receptor uncoupling by phosphorylation processes is predominant (myr⁺-Ga-resistant). (FIS97/0506 and CICYT SAF98-0057)

O6-1

REQUIREMENT OF FUNCTIONAL STIMULATORY RECEPTORS FOR THE DEVELOPMENT OF OPIOID DEPENDENCE.

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Chronic activation of opioid receptors produces complex adaptational changes in the stimulatory control of adenylyl cyclase (AC). These changes in transmembrane signalling become detectable during the state of opioid withdrawal by the expression of AC supersensitivity. The present study demonstrates that in cultured cells both the induction of opioid dependence as well as the expression of drug withdrawal requires the presence of functionally intact stimulatory receptor systems. Chronic morphine treatment of transiently µ-opioid receptor transfected S49unc lymphoma cells, which are defective of intact β₂-adrenoceptor / Gsa coupling, failed to develop cellular correlates of opioid dependence. In NG108-15 cells, the expression of cellular withdrawal is also controlled by stimulatory receptor systems, since both (i) inactivation of Gsa as well as (ii) discontinuation of PGE₁ receptor / Gs signalling in membranes from opioid dependent cells abolish the expression of AC supersensitivity, a sign of withdrawal. In addition, termination of basal β₂-adrenoceptor signalling by an inverse agonist in stably transfected NG108-15 cells suppressed the expression of a cellular withdrawal response. These data suggest that functional stimulatory signal transduction pathways represent an essential prerequisite for the development of drug dependence and the expression of withdrawal.

O6-3

IDENTIFICATION OF THE PUTATIVE PHOSPHORYLATION SITES IN THE MU-OPIOID RECEPTOR THAT ARE INVOLVED IN THE AGONIST-INDUCED DOWN-REGULATION OF THE RECEPTOR.

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Chronic agonist treatment has resulted in the initial desensitization, followed by the down-regulation of the mu-opioid receptor. Since the down-regulation of the GPCRs is closely related to their phosphorylation, the role of the mu-opioid receptor phosphorylation on the agonist-induced down-regulation of the receptor is investigated. We can demonstrate that agonist-induced phosphorylation of the mu-opioid receptor occurs at the carboxyl tail domain of the receptor by substituting all the Ser/Thr in this domain with Ala. Similarly, etorphine-induced receptor down-regulation was attenuated in this Ala receptor mutant. The exact Ser/Thr involved were then determined by receptor truncation, receptor deletion and multiple site mutation analysis. The ability of etorphine to induce down-regulation in these mutants were analyzed. The exact sites that play a part in the agonist-induced receptor down-regulation were identified and will be discussed. (Supported by NIDA grants DA05695 and DA07339).

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O6-4

CARBOXYL-TERMINAL SPLICING OF THE RAT μ OPIOID RECEPTOR MODULATES AGONIST-MEDIATED INTERNALIZATION AND RECEPTOR RESENSITIZATION.

T. Koch, S. Schulz, H. Schröder, R. Wolf, E. Raulf, and V. Höllt. Department of Pharmacology and Toxicology, Otto von Guericke University, Magdeburg, Germany. The rat μ opioid receptor is alternatively spliced into two C-terminal isoforms (MOR1 and MOR1B). When stably expressed in HEK 293 cells, the MOR1B isoform desensitized at a slower rate during prolonged DAMGO exposure but resensitized at a faster rate during agonist withdrawal than MOR1. Immunocytochemical analysis revealed that DAMGO-induced internalization of MOR1B proceeded much faster than that of MOR1 followed by rapid recycling of the receptor to the cell surface. The fast MOR1B resensitization and slower desensitization was abolished when receptor recycling was blocked with monensin. It is concluded that the sequence at the cytoplasmic tail of MOR1B facilitates clathrin-coated vesicle mediated endocytosis which, in turn, promotes accelerated receptor reactivation.

O6-6

U50,488H-INDUCED INTERNALIZATION OF THE HUMAN κ OPIOID RECEPTOR: INVOLVEMENT OF GRK2, β -ARRESTIN AND DYNAMIN

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Agonist-promoted internalization of some G protein-coupled receptors has been shown to mediate receptor desensitization, resensitization, and down-regulation. In this study we investigated whether opioids induce internalization of the human kappa opioid receptor stably expressed in CHO cells and the potential mechanisms involved in this process. Exposure to the agonists U50,488H or ethylketocyclazocine, but not etorphine, promoted an increase in intracellular kappa receptors. U50,488H-induced kappa receptor internalization was time- and concentration-dependent, with 30-40% of the receptors internalized following a 30-min exposure to 1 μ M U50,488H. Agonist removal resulted in the receptors gradually returning to the cell surface over a 60-min period. The antagonist naloxone blocked U50,488H-induced internalization without affecting internalization itself, while pretreatment with pertussis toxin had no effect on U50,488H-induced internalization. In contrast, incubation with sucrose (0.4-0.8 M) significantly reduced U50,488H-induced internalization of the kappa receptor. While co-expression of wild type GRK2, β -arrestin, or dynamin I had no effect on kappa receptor internalization, co-expression of the dominant negative mutants GRK2-K220R, β -arrestin (319-418) or dynamin I-K44A significantly inhibited receptor internalization. These results demonstrate that U50,488H-induced internalization of the human kappa opioid receptor in CHO cells is mediated by a GRK2-, β -arrestin- and dynamin I-dependent process that likely involves clathrin-coated pits. (Supported by NIH grants DA 04745 and DA 10702 and Adolor Corp.)

O6-5

DIFFERENTIAL EFFICACIES OF KAPPA AGONISTS TO INDUCE HOMOLOGOUS DESENSITIZATION OF HUMAN KAPPA OPIOID RECEPTOR.

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The efficacies of different Kappa agonists to activate kappa opioid receptor (kOR) and to induce acute homologous desensitization of kOR-mediated extracellular acidification response (ECAR) have been investigated using micro-physiometry in Chinese hamster ovary (CHO) cells stably expressing kOR. Although efficacy of dynorphin A (DA) to stimulate kOR-mediated ECAR was comparable to that of U69593, DA displayed a significant higher efficacy to induce desensitization of kOR. Moreover, the half life for the recovery of kOR responsiveness after DA prechallenge was four times longer than those observed after U69593 or etorphine pretreatment. In contrast, the efficacy of DA to stimulate and desensitize *mu* opioid receptors (μ OR) stably expressed in CHO cells were comparable to those of other mu agonists. The kOR desensitization was significantly blocked by pretreatment with PKC specific inhibitors.

O6-7

A MU-OPIOID RECEPTOR ASSOCIATED MOLECULE (μ RAM-I) : MORPHINE INDUCED TOLERANCE.

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Opioid receptors are known to mediate morphine induced effects (tolerance). To better understand the molecular basis for tolerance, we looked for opioid receptor (μ) associated molecules that may play a role in tolerance. To identify molecules that interact with carboxy-terminus (CT) of the opioid receptor, we generated a His-tagged CT of μ receptor in baculovirus and screened an expression cDNA library prepared from chronically morphine treated mouse brain mRNA. A majority of the positive clones corresponded to one particular cDNA (μ RAM-I). Full length μ RAM-I cDNA showed identity with a region of human ubiquitin specific protease. RT-PCR analysis indicated brainstem mRNA from chronically morphine treated mice had higher levels of μ RAM-I than either control or acutely morphine treated mice. Molecules associated with the carboxy-terminus of opioid receptors may potentially act as a regulatory mechanism in opioid induced tolerance. Supported by NIDA-DA11318.

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O7-1

THE EFFECTS OF ISOLATION AND MORPHINE TREATMENT DURING THE PLAY PERIOD ON SOCIAL BEHAVIORS AND DISTINCT OPIOID RECEPTORS IN ADULTHOOD OF RATS.

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Rats were either isolated or non-isolated during weeks 4 and 5 of age and treated daily with saline or morphine during these two weeks. After the isolation and treatment period, all rats were rehoused in matched pairs for isolation and treatment conditions. In week 10 of age a social interaction test was conducted and subsequently the brains were prepared for in vitro autoradiography. Juvenile isolation reduced social exploration and enhanced self-grooming in adulthood. Morphine treatment during the isolation period counteracted the reduced social exploration in isolated rats, but decreased social exploration in non-isolated rats. Furthermore, morphine treatment did not affect self-grooming, but suppressed other non-social behaviors in isolated rats, whereas it had no effect in non-isolated rats. In adulthood, regionspecific increased number of [³H]DAMGO (μ)-binding sites in the basolateral amygdala (58 %) and bed nucleus of the stria terminalis (33%) was observed upon juvenile isolation. Morphine treatment in isolated rats reversed this upregulation in both areas and increased binding in the basolateral amygdala in non-isolated rats. A decreased number of binding sites upon morphine treatment was found in the zona incerta. The number of [³H]DELT1 (δ)-binding sites did not differ between the experimental groups. A general upregulation of [³H]CI-977 (k_1)-binding sites was observed upon juvenile isolation, predominantly in the cortical regions, the hippocampus and the substantia nigra. Morphine treatment did not affect the level of k_1 -receptors. The results show that juvenile isolation causes long-term effects on social behaviors, μ - and k_1 -opioid receptors. There seems to be a correlation between the number of μ -receptors in the basolateral amygdala and the amount of social exploration.

O7-3

OPIATE TOLERANCE: AN APPARENT PHENOMENON ASSOCIATED WITH A PAIN SENSITIZATION PROCESS.

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Despite much research, it has not been conclusively demonstrated in vivo that the functional changes observed at cellular level in the responsiveness to opiates may account for the development of tolerance. An alternative hypothesis is that opioid receptor systems remain operative but that the analgesic effect is gradually masked by the sustained activation of opposing processes to analgesia associated with a pro-nociceptive system leading to a persistent increased pain sensitivity. In this study, we observed that analgesia induced by a single heroin injection (0.3-2.5 mg/kg, s.c.) was followed by an increased pain sensitivity in rat (paw-pressure vocalization test) over a period of several days in a dose-dependent manner. Moreover, repeated once daily heroin injections (0.3 or 2.5 mg/kg, s.c.) induced a progressive lowering of the nociceptive threshold which gradually masked a maintained heroin analgesic functional effect. The non competitive NMDA receptor antagonist MK-801 prevented the development of opiate-induced allodynia and consequently prevented the development of tolerance. We suggest that tolerance is an apparent phenomenon which appears in the absence of detectable changes in the actual analgesic functional effect of opiates and is associated with an NMDA-mediated pain sensitization process which gradually masks analgesic effect.

O7-2

LONG LASTING SENSITIZATION OF MORPHINE-INDUCED C-FOS EXPRESSION. M. Erdtmann-Vourliotis, U. Riechert, P. Mayer, G. Grecksch, and V. Höllt, Institute for Pharmacology and Toxicology, Otto von Guericke University, Magdeburg, Germany.

The induction of c-fos mRNA in rat brain due to morphine treatment was analyzed by in situ hybridization. A single morphine application elicited a relatively weak c-fos expression in the lateral septal area. In response to chronic morphine treatment with ascending doses, we observed that a sensitization towards morphine took place at the molecular level in certain circumscribed brain areas as revealed by a marked c-fos expression in response to a test dose of morphine. The respective brain areas included the dorsal striatum, the shell of the nucleus accumbens and parts of the cortex. The sensitization was long lasting, at least eight weeks after cessation of morphine pretreatment. Notably, sensitization became obvious in further brain areas within two weeks after finishing chronic morphine application. The latter regions included the lateral septum.

O8-1

MECHANISM OF OPIOID MODULATION OF CELL PROLIFERATION.

C.J. Coscia, E.G. Ignatova, L.M. Bohn and M.M. Belcheva. Dept. Biochem. & Mol. Biol., St. Louis Univ. Sch. of Med., St. Louis, MO, USA.

Opioids influence glia proliferation in developing brain. Previously, we implicated the opioid receptor (OR), G_{βγ} subunits and Ras in opioid activation of ERK, a member of the MAP kinase family that mediates cell proliferation and differentiation. We now show that OR endocytosis plays a role in opioid modulation of ERK activity. COS-7 or HEK 293 cells were co-transfected with cDNA of d, μ or k OR, wild type or mutant dynamin (K44A), which blocks clathrin-mediated endocytosis. ERK was activated by agonists in the presence of dynamin, whereas expression of K44A resulted in a reduction of ERK activity. Immunofluorescence confocal microscopy confirmed that d agonist-induced OR internalization and nuclear import of ERK was ablated by K44A. Similar implication of G_{βγ} subunits, Ras and OR endocytosis in opioid modulation of ERK were observed in transfected C6 glioma, an astrocytic cell line. Opioid regulation of ERK was correlated with thymidine incorporation into DNA in C6 cells. MEK inhibitor, PD98059, reduced opioid modulation of DNA synthesis.

INRC'98 Oral Presentation Abstracts

O8-2

RECEPTOR-MEDIATED AND NUCLEAR EFFECTS OF OPIOIDS ON THE CELL CYCLE AND APOPTOSIS.

G. Bakalkin, T. Yakovleva, K. Reznikov, I. Gileva, K. Tan No and L. Terenius, Department of Clinical Neuroscience, Karolinska Institute, Stockholm, Sweden.

Acute exposure of mouse embryos to opioid agonists and naloxone differentially affected cell cycle parameters in the ventricular, germinal zone which generates practically all neurons in the neocortex. Both μ - and κ -agonists exerted an inhibitory influence on cell cycle progression, whereas a δ -agonist seemed to stimulate this process. Naloxone induced an initial shortening of the S-phase, followed by a lengthening of the S- and M-phases. This suggests that dividing cells are under tonic control of the endogenous opioid systems. Preliminary experiments indicated that prodynorphin peptides are particularly relevant. Unprocessed full-size prodynorphin was found at high levels in the embryonic brain, where surprisingly, it was associated with cell nuclei. To explore the functions of nuclear prodynorphin, interactions of prodynorphin and its fragments with nuclear oncogenes and tumor suppressor proteins were investigated. Big dynorphin stimulated in vitro DNA-binding activity of Yin-Yang 1 (YY1) and the p53 tumor suppressor protein, activated YY1- and p53-dependent transcription and reduced the number of viable cells in cell transfection experiments. Two distinct mechanisms could be envisaged for opioid associated regulation of cell proliferation and/or death, one mediated by opioid receptors, the second, by a novel function at the level of the cell nucleus, where prodynorphin, and perhaps other opioid peptide precursors modulate the activity of nuclear proteins including p53 which affect the cellular fate.

O8-4

ACTIVATION OF MOUSE T CELLS INCREASES THE EXPRESSION OF THE KAPPA OPIOID RECEPTOR.

J. M. Bidlack, M. A. Abraham, and M. A. Thomas. University of Rochester, Rochester, NY 14642 U.S.A.

The κ opioid receptor is expressed on mouse thymocytes, peritoneal macrophages, and at a lower density on T- cytotoxic and helper lymphocytes. The present study investigated whether mitogen activation of T cells would alter the κ opioid receptor density and/or the number of either CD8+ (cytotoxic) or CD4+ (helper) lymphocytes, expressing the κ receptor. Mouse splenocytes were cultured for varying times with either Con A or PHA, and kappa receptor expression was measured by flow cytometry. In a dose- and time- dependent manner, the mitogens, Con A and PHA, increased the density of κ opioid receptors expressed on CD8+ cells, without changing the percentage of CD8+ cells expressing the receptor (~30%). In contrast, Con A, but not PHA, increased the density of κ opioid receptors on CD4+ cells, without having an effect on the number of CD4+ cells that expressed the receptor. These results demonstrate that activation of T cells increases the expression of the κ opioid receptor, suggesting a direct role for κ opioid receptor in immune cell responses. (Supported by DA00360 and DA04355.)

O8-3

DELTA OPIOID DADLE BLOCKS DOPAMINE TRANSPORTER (DAT) LOSS AND p53 mRNA INCREASE INDUCED BY METHAMPHETAMINE (METH): BY ACTING AS A FREE RADICAL SCAVENGER?

T-P Su¹, L-I Tsao¹, T. Hayashi¹, A.M. Andrews², C.C. Chiueh², B. Ladenheim¹, M. Asanuma¹, H. Hirata¹ and J.L. Cadet¹. Intramural Res. Programs, ¹NIDA, Baltimore and ²NIMH, Bethesda, Maryland.

METH (4x10 mg/kg, i.p., every 2 hr) caused a loss of DAT in the striatum (ST; 60%) and nucleus accumbens (NA; 40%) in mice (Tsao et al., Neurosci. Abstr. 23:273, 1997). A single injection of METH (25 mg/kg, i.p.) increased mRNA of apoptotic (tumor suppressor) gene p53 in ST, cortex (CRT), and hippocampus (HPC) (Hayashi et al. CPDD, 1998). As DADLE can protect myocardium against ischemic insult (Bolling et al., Ann. Thorac. Surg. 64:623, 1997), we examined if DADLE might affect METH-induced DAT loss and p53 mRNA increase. DADLE (4 mg/kg, i.p. before each of 4x10 mg/kg of METH or 20 mg/kg, i.p. before 25 mg/kg of METH, respectively) blocked the DAT loss in ST and NA and the p53 mRNA increase in ST and HPC but not in the CRT. In vitro, DADLE is equipotent to glutathione as a free radical scavenger sequestering the formation of superoxide anions, hydroxyl radicals, and brain lipid peroxidation. These results suggest that DADLE and, by extension, endogenous delta opioids, are neuroprotective in nature and support the view that free radicals and cell-death related genes are involved in METH-induced neurotoxicity.

O8-5

ALTERED PATTERNS OF CALBINDIN D-28K IMMUNOREACTIVITY IN ADULT AND DEVELOPING MOUSE BRAINS EXPOSED TO MORPHINE.

V. Maharajan, P. Maharajan, R. Prencipe and G. Ravagnan*. CNR Institute of Cybernetics, Arco Felice, Naples, Italy; *CNR Institute of Experimental Medicine, Tor Vergata, Rome, Italy.

The calcium-binding proteins like calbindin and parvalbumin in the mammalian CNS seem to have a role of neuroprotection against calcium-toxicity associated to ischemia or excitation. We have studied the effect of morphine on calbindin D-28k (CB) expression in adult and neonatal mouse brains to understand the role of this protein in the cellular actions of opioids. Female albino Swiss mice were administered physiological saline or morphine daily, for a period comprising 7 days before mating, through gestation and until 21 days post-partum, when they were sacrificed. The neonates allowed to suckle on their natural (treated) mothers and hence exposed to maternal morphine, were utilized on postnatal day 18. The brains of mothers and neonates were perfusion-fixed and processed for histology or immunohistology. No significant changes were evident histologically but a remarkable reduction of CB-immunoreactive neurons occurred in morphine-treated adult brain, particularly in the cingulate cortex (ACC) and parietal cortex I (ParI), as compared to controls. Also the neonates exposed to maternal morphine revealed altered CB immunoreactivity-patterns in ACC, ParI and the hippocampus. Thus alterations in the expression-patterns of the calcium-binding proteins in specific brain-areas might be one of the mechanisms by which opioids modify the functional aspects of adult and developing CNS.

INRC'98 workshop abstracts

W-1

MU-SELECTIVE ENDOMORPHINS IN REGIONS OF HIGH MU OPIATE RECEPTOR DENSITY

J.E. Zadina, S.Martin-Schild, A.J.Kastin, & A.A.Gerall. VA Medical Center and Tulane Univ. Sch. of Medicine. New Orleans, LA USA.

The recently discovered endomorphins (Nature 386:499, 1997) have high affinity and selectivity for the mu opiate receptor. They have been isolated from both bovine and human brain and are potent analgesic compounds, particularly after intrathecal injection.

Immunocytochemistry showed dense bands of endomorphin-2-like immunoreactivity (EM-2-LI) in the superficial layers of the spinal cord dorsal horn, co-localized with a subset of substance P-LI fibers. EM-2-LI fibers were also present in the homologous spinal trigeminal nucleus, and the n. tractus solitarius and n. ambiguus. These staining patterns match the known profile of high densities of mu opiate receptors in terminal regions of primary afferents. Dorsal rhizotomy dramatically reduced staining in the dorsal horn and EM-2-LI fibers were present in the dorsal root ganglion, where mu receptors are synthesized. Thus, endomorphin-2 is strategically located in primary afferents where its release can modify pain perception both postsynaptically and by presynaptic inhibition of the release of excitatory transmitters carrying pain signals to the CNS. The results support the role of endomorphins as natural modulators of pain perception and as endogenous ligands for the mu opiate receptor. Supported by the VA and NIDA.

W-3

IMMUNOFLUORESCENT IDENTIFICATION OF ENDOMORPHIN-2-CONTAINING NERVE FIBERS AND TERMINALS IN THE RAT BRAIN AND SPINAL CORD.

Stefan Schulz, Matthias Schreff, Dana Wiborny and Volker Hoell. Department of Pharmacology and Toxicology, Otto-von-Guericke-University, 39120 Magdeburg, Germany. Endomorphin-1 and -2 - potent and selective agonists for the mu-opioid receptor - have recently been isolated from bovine brain extracts. In the present study we used polyclonal antibodies specific for endomorphin-2 to determine its immunocytochemical distribution in the rat brain and spinal cord. Endomorphin-2-like immunoreactivity was confined to varicose fibers with an overall discrete distribution within the central nervous system. The immunostaining was completely abolished by preincubation of the antiserum with endomorphin-2 but not with endomorphin-1. Endomorphin-2-like immunoreactivity was enriched in some but not all brain regions known to contain dense mu-opioid receptors including nucleus accumbens, septum, midline thalamic nuclei, hypothalamic and amygdala nuclei, locus coeruleus, periaqueductal gray and spinal cord dorsal horn. In contrast, endomorphin-2 was absent from the cortex, striatum, hippocampus, nucleus of the solitary tract and dorsal root ganglia. Dual labeling experiments revealed that endomorphin-2-immunoreactive nerve fibers did not co-contain any other opioid peptide. Thus, the present findings strongly suggest that endomorphin-2 may be a natural ligand of the mu-opioid receptor likely to be involved in the modulation of nociceptive transmission and reward-seeking behavior.

W-2

RELATIONSHIP BETWEEN ENDOMORPHIN-2-IMMUNOREACTIVITY AND THE MU-OPIOID RECEPTOR IN RAT CNS.

T.L. Pierce and M.W. Wessendorf. Dept. Cell Biology & Neuroanatomy, Univ. Minnesota, Minneapolis, USA.

It has been proposed that the endomorphins are specific endogenous ligands for the mu-opioid receptor. In this study we examined the relationship between endomorphin-2-immunoreactivity (ir) and the cloned mu-opioid receptor (MOR1). Tenum cryostat sections of rat brain and spinal cord were immunofluorescently double-stained for MOR1 and endomorphin-2; these sections were microscopically examined to determine the relationship between endomorphin-2-ir terminals and MOR1-ir somata or processes. Overlapping distributions of endomorphin-2-ir and MOR1-ir were observed in the spinal dorsal horn and in locus coeruleus. However, in some areas classically associated with the mu-opioid receptor (e.g., striatum), negligible endomorphin-2-ir was observed. It therefore appears that endomorphin-2 and MOR1 are distributed together in some regions; however, there may be regions of mismatch. Supported by PHS grant DA 09642 from NIDA.

W-4

MATURATION OF ENDOMORPHIN-2-LIKE IMMUNOREACTIVITY IN THE RAT.

Gordon A. Barr* and James Zadina**. *Hunter College, *NY State Psychiatric Institute, NY, NY 10032 and the **VA Medical Center, New Orleans, LA 70146.

Endomorphins are recently discovered endogenous ligands for the mu opioid receptor (Nature 386:499, 1997). Endomorphin-2 (Tyr-Pro-Phe-Phe-NH₂) is localized in the superficial lamina of the dorsal horn of the spinal cord and in the trigeminal tract and trigeminal nucleus of the brainstem (Peptides 18:1641, 1997). Other endogenous opioid peptides and their receptors show differential maturation that might account for changes during development in responsiveness to drugs, stress and pain. To determine changes in the anatomy of endomorphin-2, we used the previously characterized antibody to the opioid peptide and examined its distribution in the spinal cord and hindbrain of infant rats using standard immunocytochemical methods. No immunoreactive staining was found in newborns or 3 day old pups in either brain or spinal cord. At 11 days of age, staining was present at both sites, but not in its full adult distribution. For example, in the spinal cord, the immunoreactivity was limited to the medial aspects of the dorsal horn, and the density of the stain was generally lighter. By 21 days of age, the distribution and density of endomorphin-2 staining was at adult levels. The appearance of the peptide is somewhat delayed compared to other opioid peptides and the mu opioid receptor. Characterization of the development of the endomorphins will be valuable for understanding the functional changes in mu opioid systems during ontogeny. Supported by NS36130, DA00325, the VA and DA11655.

INRC'98 workshop abstracts

W-5

IN VITRO BINDING AND SIGNALLING PROFILE OF THE NOVEL μ OPIOID RECEPTOR AGONISTS, ENDOMORPHINS IN DIFFERENT SYSTEMS.

A. Borsodi [1], M. Spetea, K. Monory, D. Biyashev, C. Toemboely, E. Tzavara, M.-C. Bourin, H. W. Matthes, B. L. Kieffer, S. Benyhe, J. Hanoune, G. Toth, Institute of Biochemistry and Isotope Laboratory, Biological Research Center, Szeged, Hungary, INSERM U-99 Hopital Henri Mondor Creteil, France UPR 9050 CNRS, ESBS Universite Louis Pasteur, Illkirch, Strassbourg, France.

The binding and signalling properties of endomorphin 1 (Tyr-Pro-Trp-Phe-NH₂) and 2 (Tyr-Pro-Phe-Phe-NH₂) were studied in both cellular and animal models. Endomorphin 2 was treated with a specific radioactivity of 1.98 TBq/mmol (53.4 Ci/mmol), and used for in vitro labelling of brain membranes of rats, wild type mice and mice lacking the μ receptor gene. The binding was saturable, stereospecific and of high affinity in membranes containing μ opioid receptor, but no detectable binding was observed in preparations of CHO cells or mice lacking the μ receptor gene. [³H]Endomorphin 2 was displaced by m -receptor selective specific peptides and heterocyclic compounds with high affinity, whereas κ - and δ -receptor specific ligands were much less potent. The K_i values of endomorphin 1 and 2 in inhibiting [³H]naloxone binding increased by 15-fold in the presence of 100 mM NaCl which indicates the agonist property of these peptides. The obtained data from functional assays - enhancement of [³⁵S]GTP γ S binding and decrease of adenyl cyclase activity - provided further evidence for the agonist character of endomorphins. These peptide ligands and especially [³H]endomorphin 2 can be essential tools for identification and characterisation studies of opioid receptors.

W-7

SYNTHESIS OF [³H]ENDOMORPHIN II AND NEW ENDOMORPHIN I AND II ANALOGUES WITH HIGH AFFINITY AND SELECTIVITY FOR THE μ -OPIATE RECEPTOR.

G. Toth, Cs. Tomboly, Zs. Laszlo, D. Byashev, A. Peter*, D. Tourw**, A. Borsodi, Institute of Biochemistry, Biological Research Center, Hungarian Academy of Sciences, Szeged, *Department of Inorganic and Analytical Chemistry, Attila Jzsef University, Szeged, Hungary, **Vrije Universiteit Brussel, ORGC VUB Belgium.

Endomorphin I (Tyr-Pro-Trp-Phe-NH₂) and endomorphin II (Tyr-Pro-Phe-Phe-NH₂) high specificity and affinity for the μ -opiate receptors were with isolated recently from mammalian brain. Several new analogs were synthesized using unnatural aromatic amino acids for substitution and characterized by radioligand binding assays in rat brain membrane preparation using tritiated endomorphin II and other δ and κ -opioid tritiated ligands. [³H]endomorphin II was synthesized from endomorphin II containing 3,5-diiodo-Tyr or pIle as precursors by catalytic dehalogenation using tritium gas. The stability of tritiated endomorphin II toward enzymatic degradation in membrane preparation was examined by incubation of the radioligand in membrane suspension in the presence and absent peptidase inhibitors and was determined by HPLC using a flow radiochromatography detector. In the presence of inhibitors the radioligand did not show any degradation. Supported by Biomed. II No BMH4-96-0510-001. J.E. Zadina, L. Hackler, L.-J. Ge, J. Kastin: Nature, 386, 1997, 499-502

W-6

BINDING OF μ -OPIOID RECEPTOR AGONIST [³H]ENDOMORPHIN 2 IN RODENT BRAIN MEMBRANES

M. Spetea, Cs. Tomboly*, G. Toth*, H. W. Matthes#, B. Kieffer# and A. Borsodi. Institute of Biochemistry and *Isotope Laboratory, Biological Research Center, Hungarian Academy of Sciences, Szeged, Hungary, #ESBS, ULP, Illkirch, Strassbourg, France.

Opioid receptor binding properties of [³H]endomorphin 2 μ -specific activity: 53.4 Ci/mmol) were characterized in membrane preparations from brains of rat, wild type and mutant mouse lacking the μ -opioid receptor gene. The binding was saturable, specific and of high affinity in rat brain and wild type mouse brain membranes (K_d: 1.12 and 1.77 nM, respectively). The maximal number of binding sites (B_{max}) was found to be 115 and 66 fmol/mg protein, respectively. In membranes from mouse lacking the μ -receptor, no binding was detected. In rat brain membranes, [³H]endomorphin 2 was displaced by μ -opioid receptor selective specific compounds with high affinity, whereas κ - and δ -receptor specific ligands were much less potent in inhibiting the binding. The use of [³H]endomorphin 2 can promote a further understanding of the opioid system at the molecular level.

W-8

SPINAL ANTINOCICEPTIVE EFFECTS OF THE ENDOGENOUS μ OPIOID RECEPTOR AGONISTS ENDOMORPHIN-1 AND -2 IN RATS.

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Institute of Pharmacology, Krakow, Poland. *Institute of Biochemistry, Biology Research Center, Szeged, Hungary.

Recently endomorphin-1 and endomorphin-2 two highly-selective μ -opioid receptor agonists, were discovered. The aim of our study was to evaluate the spinal antinociceptive activity of those peptides after their intrathecal administration to rats in acute, inflammatory and neuropathic pain. Male Wistar rats with chronically implanted intrathecal (i.th) catheters were used. Acute pain was induced by application of thermal (tail flick) and mechanical stimuli (paw pressure). Formalin (12%) injected into the dorsal surface of the hind paw was used as a model of inflammation. Neuropathic pain was induced by crush lesioning the sciatic nerve. Endomorphin-1 and endomorphin-2 increased dose-dependently the pain threshold in the tail flick and paw pressure tests. We also observed an enhanced antinociceptive action in the formalin-induced inflammation. All animals with crush injury of the sciatic nerve developed allodynia 2 days following the surgery. Endomorphin-1 and -2 administered i.th. (2.5-5mg) inhibited allodynia in a dose-dependent manner. The effect of endomorphin-1 was stronger than that of endomorphin-2 in all the tests used. In acute pain, the antinociceptive potency of both those endomorphins was comparable with that of the exogenous ligands of the μ opioid receptor, DAMGO and morphine, and was stronger in inflammatory pain. The antiallodynic effect of endomorphins was observed after doses 20 times lower than those of morphine. Our study suggests that the newly discovered endogenous opioid peptides appear to be powerful analgesics at spinal level. The potency of endomorphins in neuropathic pain is much stronger than that of morphine. The endogenous ligands with high affinity and specificity for the μ -opioid receptor may be considered in future as effective drugs in neuropathic pain. Supported by grant from EC CIPACT 94-0226 and Institute's statutory funds.

INRC'98 workshop abstracts

W-9

BRAIN ENDOMORPHIN AND ELECTRO-ACUPUNCTURE (EA) ANALGESIA.

JS Han, WZ Jiang, Y Wan, M Gu, JB Tian, JK Chang*
Neuroscience Research Institute, Beijing Med Univ, China, *Phenix Pharmaceuticals Inc, USA.
Endomorphins (EM) are the newly characterized endogenous mu-opioid receptor agonists. The aim of this work was to study its possible role in EA-induced analgesia of the rat. The content of EM as assessed by radioimmunoassay was measured in thalamus, hypothalamus, periaqueductal gray (PAG) and lumbar dorsal spinal cord. EA of 2 Hz or 100 Hz for 30 min produced an increase of EM-1-IR in the PAG, but not in the rest 3 central areas. A positive correlation was found between the analgesic effect of EA of both frequencies and the increase of EM-1-IR content in PAG. In addition, an increase of EM-1-IR in the cerebral ventricular perfusate (rather than spinal perfusate) was found in responder rats showing an increase in tail flick latency (rather than non-responders) toward 2 Hz (rather than 100 Hz) EA. Similar results were obtained for EM-2-IR. In conclusion, EA stimulation accelerates the production and release of EM in the brain, which may play a role in mediating 2 Hz EA-induced analgesia (Supported by a grant DA 03983 from NIDA, USA).

W-11

LIPOPOLYSA CCHARIDE (LPS) INCREASES THE EXPRESSION OF MESENTERIC MU OPIOID RECEPTOR AND PLASMA LEVELS OF ENDOMORPHIN-1.

*S.L Chang, *R. Kong, *N.A. Patel, *B. Felix, and *J.E. Zadina, DE of Biology, Seton Hall University, South Orange, NJ and *VA Medical Center and Department of Medicine, Tulane University School of Medicine, New Orleans, LA, USA

Interactions between opioid and cytokine (interleukin-1, IL-1) systems have been implicated in many recent studies. Mu opioid receptors in neural microvascular endothelial cells have been shown to be induced by co-treatment with IL-1 (α and IL-1 β (Vidal et al., 1998)). We hypothesized that conditions that increase IL-1 may modulate the expression of opioid receptors, and alter the levels of endomorphin-1, the endogenous ligand of the mu opioid receptor. Adult male rats were injected (i.p.) with 5 or 30 mg/kg LPS, or saline, or no treatment. Twelve h after treatment, the mesentery and the caudate putamen (CPU) of the brain were collected from each rat for RT-PCR analysis. In untreated rats, there was no detectable mu opioid receptor mRNA in the mesentery, while it was readily detectable in the CPU, as expected. However, after 30 mg/kg LPS, mu opioid receptor expression was clearly increased in the mesentery. In parallel studies, rats received either 15 or 30 mg/kg of LPS, or saline. Thirteen hours after injection, the rats receiving either 15 or 30 mg/kg of LPS had significantly higher levels of both plasma endomorphin-1 and IL-1β compared to animals receiving saline. These results show that LPS shock may alter opioid dependent pathways by inducing expression of opioid receptors of mesentery and increasing plasma levels of endomorphin-1.

W-10

ENDOMORPHIN 1 INHIBITS OXYTOCIN BUT NOT VASOPRESSIN NEURONES IN VIVO AND IN VITRO.

J. A. Russell, C. H. Brown and N. Doi. Department of Physiology, University Medical School, Edinburgh EH8 9AG, UK.

Previous studies have indicated that supraoptic nucleus (SON) oxytocin (OT) neurones, are more sensitive to morphine than vasopressin (VP) neurones, whereas both types of neurone are inhibited by kappa agonist or nociceptin. We have now studied actions of the highly mu-selective endogenous opioid tetrapeptide endomorphin 1 (EM1) in urethane-anaesthetised female rats, and in coronal hypothalamic brain slices. In vivo, extracellular recordings were made of action potentials of antidromically-identified SON neurones, characterised as OT or VP neurones by their activity patterns, and the excitatory response of OT neurones to i.v. CCK; in vitro whole cell current-clamp recording was used, with OT and VP neurones distinguished by different responses to hyperpolarisation. Intracerebroventricular (i.c.v.) EM1 (5-50 pmole) inhibited the firing of OT, but not VP neurones, with naloxone reversal; subsequently i.c.v. nociceptin (0.5-1 pmole) inhibited both OT and VP neurones. In vitro, EM1 dose-dependently (10-100 nM) inhibited OT, but not VP neurones, with naloxone reversal; both OT and VP neurones were inhibited by U50,488 (10 μM). Thus, SON OT, but not VP, neurones express functional mu opioid receptors, but both have ORL1 and kappa receptors.

INRC'98 Tuesday Poster Abstracts

Tu01

SPINAL ANTISENSE OLIGODEOXYNUCLEOTIDE (ODN) TO G α SUBUNIT BLOCKS BOTH MORPHINE (MS) AND CLONIDINE (Cl) ANTINOCICEPTION.

Sandra C. Roerig and Farzana Karim, Department of Pharmacology, Louisiana State University Medical Center, Shreveport, LA USA 71130 USA.

When administered spinally (intrathecally, i.t.) both morphine sulfate (MS; opioid agonist) and clonidine (Cl; a 2-adrenergic agonist) produce analgesia that is likely mediated by G proteins. Our previous studies showed that i.t. pretreatment of mice with 10 μ g antisense ODN to G $\alpha_{12/13}$ subunit almost completely blocked i.t. Cl-induced anti-nociception, but did not affect MS-induced antinociception. In the present studies, mice were injected i.t. with various doses of either missense or antisense ODN to G α_a . After 48 hr, antinociceptive responses to i.t. MS or Cl were measured using the tail flick test. A dose-related decrease in MS (300 pmol)-induced antinociception occurred after antisense ODN treatment, with the two highest doses used, 20 and 40 μ g, producing decreases of 43 and 42%, respectively. In further studies, pretreatment with 20 μ g antisense ODN produced equal (about 4-fold) increases in ED50 value for both MS and clonidine compared to missense ODN treatment. Taken together, these results suggest that some similar and some different G proteins mediate antinociception produced by spinal opioid and a 2 adrenergic receptors. (Supported by DA07972).

Tu03

ENDOMORPHIN-1 AND MORPHINE ANALGESIA ARE AFFECTED DIFFERENTIALLY BY MU-RECEPTOR ANTISENSE "KNOCK-DOWN" IN MICE.

P. Sanchez-Blazquez, M. Rodriguez-Diaz, I. DeAntonio and J. Garzon.

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Endomorphin-1 (Tyr-Pro-Trp-Phe-NH₂) is an endogenous peptide which shows high binding affinity and selectivity for mu-opioid receptors (*Zadina et al., Nature 386: 499, 1997*). In the present work the antisense oligodeoxynucleotide (ODN) strategy was used to ascertain whether the murine cloned mu-opioid receptor is the only one involved in the supraspinal antinociceptive effects of this peptide. Five characterized ODNs targeting distinct exons of the cloned mu receptor (*Rossi et al., JPET 281: 109, 1997; Sanchez-Blazquez et al., JPET 280: 1423, 1997*) were subchronically injected into mice. The analgesia induced by endomorphin-1 (15 nmol/mouse, icv) was highly reduced in mice receiving the ODN directed to nucleotides 677 to 697 of the receptor gene. ODNs targeting exons 1, 2 or 4 were totally inactive. In contrast, the five antisense ODNs blocked the antinociception induced by morphine (6 nmol/mouse, icv) or b-casomorphin (Tyr-Pro-Phe-Pro-Gly; 15nmol/mouse, icv). Naloxonazine, a selective antagonist at mu₁-receptors, reduced the analgesia evoked by morphine and b-casomorphin, but not that of endomorphin-1. Thus, different receptors mediate the antinociceptive effects of morphine and endomorphin-1. (*Supported by FIS97/0506 and CICYT SAF98-0057*).

Tu02

DIFFERENTIAL EFFECTS OF ANTISENSE OLIGO-DEOXYNUCLEOTIDES TO G PROTEIN α -SUBUNITS ON ENDOMORPHIN-1 AND MORPHINE ANALGESIA.

J. Garzon, I. DeAntonio, M. Rodriguez-Diaz and P. Sanchez-Blazquez. Neurofarmacologia. Instituto Cajal, CSIC. Madrid 28002, Spain.

Endomorphin-1 has been described as an endogenous ligand at the mu-opioid receptor. Opioid-receptor genes encode G-protein-coupled receptors with seven transmembrane domains. The antisense oligodeoxynucleotide (ODN) strategy was used to characterize the specific classes of G proteins regulated by mu-agonists in the production of supraspinal antinociception (e.g. morphine and DAMGO analgesia is sensitive to ODNs directed to G α_{12a} and G α_{2a} subunits). In the present study, ODNs to diverse G-protein α subunits were subchronically icv-injected into mice. The antinociception evoked by mu-agonists and endomorphin-1 was then evaluated by the warm water tail-flick test. In vivo "knock-down" of the α subunits of G α_{11a} , G α_{13a} and G α_{2a} proteins inhibited the analgesia induced by this peptide. Conversely, oligos targeting G α_{2a} , G α_{1a} , and G α_{2a} subunits were inactive. These results demonstrate the participation of multiple G protein classes in the antinociception induced by endomorphin-1 and suggest that as well as acting at mu-receptors, endomorphin-1 has effects at others in the production of analgesia. (*Supported by FIS97/0506 and CICYT SAF98-0057*).

Tu04

MULTIRECEPTOR LIGANDS FOR SPINAL ANALGESIA - ANTINOCICEPTIVE EFFECTS OF AA501.

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The tetrapeptide dimer, biphalin possesses high affinity for all three opioid receptor types and is an extremely potent analgesic in *in vivo* tests. Recent SAR studies indicate that the fragment containing one tetrapeptide connected via a hydrazide bridge with an additional phenylalanine is a minimal necessary structural element responsible for biphalin's high biological activities.

Therefore, we predicted that the second "arm" of biphalin could be replaced by other, non-opioid type of pharmacophore which may extend affinity, of analogue to non-opioid receptors. Following such idea, AA501 has been synthesized which in one molecule combines neurokinin antagonist with opioid agonist.

Tyr-D-Ala-Gly-Phe-NHNH<-Trp<-Cbz

AA501

AA501 given intrathecally produces potent antinociceptive effects in rats, suggesting its value as a potential therapeutic agent.

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Tu05

INVOLVEMENT OF ATP-SENSITIVE K⁺ CHANNELS IN OPIOID, ADRENERGIC, AND MUSCARINIC AGONIST ANTINOCICEPTION.

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ATP-sensitive K⁺ channels (K_{ATP}) are composed of four dimers of a sulfonyleurea binding subunit (either SUR1, SUR2A, or SUR2B) and a pore forming subunit (either Kir6.1 or Kir6.2). Here, an antisense oligonucleotide to Kir6.2 was used to "knockdown" levels of the channel and determine its role in antinociception induced by i.c.v. administration of the opioid delta agonist DPDPE, the b₂-adrenoceptor agonist clonidine, and the muscarinic agonist pilocarpine to mice. We have previously shown that 24-hr i.c.v. pretreatment with this oligo antagonizes i.c.v. morphine antinociception as does glyburide, the K_{ATP} blocker. In contrast, pretreatment with Kir6.2 antisense enhances the antinociception of DPDPE and pilocarpine, while having no effect on clonidine. Because DPDPE, clonidine, and pilocarpine antinociception is blocked by glyburide, the results of the present study suggest that these agonists interact with a K_{ATP} not composed of Kir6.2 subunits; perhaps Kir6.1. Further, we have found that diazoxide, a K_{ATP} opener, enhances DPDPE and pilocarpine but not clonidine antinociception, suggesting that clonidine may interact with a K_{ATP} composed of SUR2A (insensitive to diazoxide) as well as Kir6.1 subunits.

Tu07

ANALGESIC INTERACTION OF MU OPIOID, ALPHA-2 ADRENOCEPTOR AND 5-HT RECEPTOR AGONISTS: THREE-DIMENSIONAL ISOBOLOGRAPHIC ANALYSIS.

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Stimulation of α and 5-HT receptors produce analgesia that is additive or supra-additive to morphine-induced analgesia. When injected spinally, morphine produces analgesia primarily through μ -2 receptors, NE through α -2 receptors and 5-HT through 5HT₃ receptors. Accordingly, we examined the three-dimensional interaction of analgesia produced by stimulation of these systems. Morphine, NE and 5-HT, injected i.t. each produced tail-flick analgesia dose-dependently. NE was supra-additive with morphine, whereas 5-HT was additive. The combination of NE and 5-HT tended toward supra-additivity. The analgesia produced by isobolic combinations of i.t. morphine, NE and 5-HT showed no further potentiation of analgesia beyond that seen with 2-dimensional interactions. Similarly, the μ -2 agonist Tyr-W-MIF-1, the α -2 agonist clonidine and the 5-HT agonist 2-methyl-5-HT each produced tail-flick analgesia dose-dependently. Analgesia produced by i.t. Tyr-W-MIF-1 was additive with i.t. 2-methyl-5-HT. However, Tyr-W-MIF-1 and clonidine were additive, and 2-methyl-5-HT and clonidine were additive. Likewise, the simultaneous i.t. administration of these three drugs produced analgesia that could be predicted by an additive model. Thus, the interactions observed with the nonselective agonists morphine, NE and 5-HT could not be accounted for by selective stimulation of spinal μ -2, α -2 and 5-HT₃ receptors.

Tu06

AN ANTINOCICEPTIVE INTERACTION BETWEEN RISPERIDONE AND OPIOIDS.

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Risperidone is a novel atypical neuroleptic with a favorable profile of side effects due to its unique pharmacological activity: it exhibits both potent dopamine D₂ and 5-HT₂ α -2-receptor blocking activity, as well as high affinity for adrenergic receptors and H₁ receptor. We found that risperidone has a potent antinociceptive effect in the tailflick assay with an ED₅₀ of 26.4 mg/kg. This effect of risperidone was antagonized by naloxone. This sensitivity to naloxone indicates a possible opioid mechanism of action. We found that the sensitivity of risperidone antinociceptive effect to selective antagonists implies involvement of μ ₁-, μ ₂- and κ ₁-opioid and to a lesser extent δ -opioid mechanisms. These results suggest a possible role for risperidone both in the management of pain and in the management of opiate withdrawal and detoxification.

Tu08

IN VIVO SIGNALING OF PERIPHERAL OPIOID ANALGESIA.

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In a newly developed peripheral analgesic test, various pain-producing substances, such as bradykinin (BK), substance P, histamine, nociceptin, prostaglandin E and I₂ showed potent nociceptive flexor responses, when given into the plantar of the hind paw. Morphine given to the ipsilateral side of hindpaw (i.pl.) produced a marked inhibition of BK-induced nociceptive responses in a naloxone-reversible manner. However, the morphine injection into the contralateral side did not show it, suggesting the peripheral morphine analgesia. The type of opioid receptors involved in this mechanism was μ and κ , but not δ . The intrathecal injection of antisense oligonucleotide for each receptor selectively blocked these analgesic effects. The in vivo sequential signaling mechanisms in peripheral nerve endings following BK or opioids will be discussed.

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Tu09

TOPICAL MORPHINE ANALGESIA AND TOLERANCE.

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Morphine is a potent μ analgesic with many central and peripheral sites of actions. In our initial studies we showed that systemic administration of morphine produced profound tolerance locally. In current studies we explored the development of peripheral morphine tolerance using tail immersion approach. Topical administration of drugs has been performed by immersion of mouse tail in DMSO containing different concentrations of opioids. Morphine, DAMGO and M6G in this paradigm produced dose and naloxone reversible analgesia. Daily topical morphine produced profound tolerance by day 3, shifting morphine's ED₅₀ value over 9-fold. Co-administration of MK-801 topically with morphine prevents the development of tolerance to the analgesic effect of opioid. Thus, peripheral mechanisms may be useful therapeutic target to attack opioid tolerance.

Tu11

Studies of Action Mechanisms of Electroacupuncture in Different Frequencies on Spinal Serotonergic and Opioid Receptors

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The possible analgesic mechanisms of electroacupuncture (EAc) in different frequencies were studied by using modified intrathecal catheterization method. The efficacy of electroacupuncture analgesia (EAA) in low current (1 mA) are more potent than in high current (2, 3 mA). Thus, the electric current of 1 mA EAc was chosen to study the mechanism of EAc in different frequencies (2 Hz, 10 Hz and 100 Hz). The present study was designed to access what subtypes of opioid and serotonergic receptors were involved in the different frequencies of EAA by the tail-flick test in rats after EAc stimulation at Zu-San-Li (S36). Opioid and serotonergic receptor ligands were administered intrathecally. The results showed that naloxone (0.05 μ g) and naltrindole (0.05 μ g) blocked both low and high frequencies of EAA. The former was more susceptible in low frequency and the latter was susceptible in high frequency of EAc. The effects of EAA in three frequencies were attenuated by pretreatment with 5,7-DHT (100 μ g), implying the serotonergic neuron may participate in the EAA. Furthermore, pindobind-5-HT_{1A} (0.5, 5 μ g) could reverse low and high frequencies of EAA; 8-OH-DPAT (0.5 μ g) inhibited the high frequency EAA but facilitated the low frequency of EAA at doses of 1 and 2 μ g. Low frequency EAA was attenuated by ketanserin (0.5 μ g); DOI (10 μ g) did not affect the EAA. LY-278584 (0.5 μ g) attenuated the high frequency EAA and inhibited the EAA at dose of 5 μ g. In addition, 2-methyl-5-HT (50 μ g) could enhance the EAA. These data suggest that the μ -, d -opioid receptors and 5-HT_{1A}, 5-HT₂, 5-HT₃ receptors in rat spinal cord are involved in the EAA. The μ - and d -opioid receptors may play a role mainly in low and high frequency of EAc. However, the exact relationships between EAc and 5-HT subtypes on spinal pre- or postsynapse need further studies.

Tu10

OPIOID ANALGESIA IN FISHES.

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In fishes, the system of pain sensitivity is almost never investigated (Klinger, 1988).

Experiments were performed with rainbow trout and White Sea cod. An originally registration electromechanical system was used. In response to pain stimulation, undulation of the caudal peduncle deviated the moveable "fork" from the zero point. The range and duration of these undulations characterizing the impulse of force (N*s) were recorded by an electronic integrator with digital indication. The pain stimuli were discharges during 0.1 s of 30 pulses by 0.5 ms each, the stimulus amplitude 1.5- 5 mA. Drugs used were mu-opioids dermorphin and beta-casomorphin. Intranasal administration of dermorphin 0.20-0.75 mg/kg produced in trout a dose- dependent decrease pain responses by 12-55%. The analgetic effect lasted for up to 2-3 h in some fish. Intranasal administration of beta-casomorphin 2.5-12.5 mg/kg, and peritoneal one 10-30 mg/kg caused analgesia in cod by 15-37 and 14-35%, respectively. The obtained data suggest that fishes, like higher vertebrates, possess the pain sensitivity and system of opioid antinociception.

Tu12

Studies of Mechanisms in the Different Frequencies of EAc Analgesia on Central Monoaminergic and Opioid Receptors.

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In the past decades, it has been proved that the mechanism of antinociception was related to the concentration of neuropeptides and monoamines in CNS. In order to understand the relationship of electro-acupuncture (EAc) analgesia and monoaminergic neurons and opioid receptors, this study was accomplished by the formalin test in ICR mice. The evidences suggest that (1) Exogenous 5-HT and NE enhanced the analgesic effect of the different frequencies (2, 10 and 100 Hz) of Eac, especially at 100 Hz. (2) The antinociception of EAc at different frequencies stimulation were attenuated by PCPA, and were potentiated by 5-HTP. (3) Naloxone could reverse the EAc analgesia of 2Hz and 10Hz but not 100 Hz. However, naltrindole could reverse the EAc analgesia of 10 Hz and 100 Hz but not 2 Hz. (4) Prazosin and clonidine could potentiate the antinociception of different frequencies of Eac, whereas yohimbine could reverse 2 Hz and 10 Hz EAc analgesia and potentiate 100 Hz EAc analgesia. (5) Pindobind-5-HT_{1A} and LY278584 could reverse the three different frequencies of Eac analgesia and ketanserin potentiate 100 Hz EAc analgesia. From the above results, we suggest that the analgesic effect of EAc is related to serotonergic and noradrenergic neurons at different frequency stimulation. In serotonergic pathway, EAc analgesia may be mediated via 5-HT_{1A} and 5-HT₃ receptors. Besides, 5-HT₂ may be involved in high frequency EAc analgesia. In noradrenergic pathway, both a 1 and a 2 receptors were involved in EAc analgesia and may be play in the opposite function. Moreover, the Eac analgesia may be mediated via μ -opioid receptor at low frequency stimulation and d -opioid receptor at high frequency stimulation.

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Tu13

COMPARISON OF κ_1 , κ_2 , μ , AND δ OPIOID AGONISTS ON HYPERALGESIA AND ALLODYNIA IN RAT MODELS OF PERSISTENT PAIN.

R. M. Caudle, E. Eliav and M. J. Iadarola. Pain and Neurosensory Mechanisms Branch, National Institute of Dental Research, National Institutes of Health, Bethesda, MD, USA. Opioids are the mainstay of pain therapy. However, hyperalgesia and allodynia, two symptoms of chronic pain that most influence the quality of life, are often poorly controlled by currently used opioids. We compared the effects of intrathecally administered κ_1 , κ_2 , μ and δ agonists on three models of persistent pain. In rats with complete Freund's adjuvant (CFA) induced hind paw inflammation μ and δ agonists effectively blocked hyperalgesia to heat. These agonists also decreased heat sensitivity in the non-injured limbs indicating an analgesic action. The κ agonist U69,593 had no effect in this model. The less selective κ agonist GR89,696 effectively blocked heat hyperalgesia without producing analgesia. In contrast to the inflammation model, μ and δ agonists were less analgesic in peripheral neuritis and neuropathy models. U69,593 also had no effect in these models while GR89,696 effectively blocked both hyperalgesia and allodynia. These findings are consistent with binding studies and electrophysiological studies demonstrating two pharmacologically distinct κ opioid receptors. The κ_1 site is defined as the site to which U69,593 binds selectively, whereas the κ_2 site is the κ like binding that remains in the presence of μ , δ and κ_1 receptor blockade. By using a process of elimination, these same criteria are used to assign pharmacological actions to κ_1 and κ_2 receptors. These data also demonstrate that selective agonists for the GR89,696 receptor, or κ_2 receptor, may be useful agents for the treatment of hyperalgesia and allodynia in chronic pain patients.

Tu15

THE EFFECTS OF RECEPTOR AGONISTS AND ANTAGONISTS ON THE DEVELOPMENT OF NEUROPATHIC PAIN AFTER SCIATIC NERVE INJURY IN RATS

J. Mika, G. Toth*, B. Przewlocka. Institute of Pharmacology, Krakow, Poland. *Institute of Biochemistry, Biology Research Center, Szeged, Hungary. Neuropathic pain that develops after injury of central or peripheral nerve systems is an important clinical problem. Mechanisms underlying the neuropathic pain have not been thoroughly understood as yet. The aim of our present study was to evaluate the role of agonists and antagonists of opioid receptors in the development of neuropathic pain. Male Wistar rats under pentobarbital anaesthesia were chronically implanted with intrathecal (i.th.) catheters. The crush lesioning of the sciatic nerve was performed 10 days after i.th. implantation. The lesioning procedure has been described in detail by De Koning et al., 1986. The drugs were injected 24 h before crushing, and chronically, during the following 12 days, once a day. All animals with crush injury of the sciatic nerve developed allodynia which lasted up to 12 days. Behavioral tests were used at 2, 4, 6, 8, 10 days after nerve crushing to evaluate antinociceptive and antiallodynic effects. Endomorphin-1, a new highly selective ligand of μ -opioid receptor, administered i.th. (5 mg) inhibited allodynia in a time-dependent manner. Behavioral tests also demonstrated an antiallodynic effect of δ_1 (DPDPE), δ_2 (DEL II) and κ (U50,488H) opioid receptor agonists, that antiallodynic effect persisted up to 10 days. The κ opioid receptor antagonist norBNI slightly potentiated the allodynic effect caused by nerve injury. Summing up, our study shows that endomorphin-1, a novel endogenous peptide described as an endogenous ligand for the μ -opioid receptor, as well as other agonists of δ_1 , δ_2 and κ opioid receptors can significantly inhibit nociception and allodynia in the model of neuropathic pain. Our results also show that kappa opioid receptors are tonically involved in the development of processes induced by nerve injury. *Supported by a grant from EC CIPACT 94-0226 and by the Institute's statutory funds.*

Tu14

AN ANTISENSE OLIGONUCLEOTIDE TARGETING mGluR₁ RESTORES OPIOID SENSITIVITY IN NEUROPATHIC RATS.

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Pain in humans and animals due to nerve injury is relatively refractory to treatment, due largely to insensitivity to morphine. It has been suggested that opioid tolerance/dependence and neuropathic pain have similar etiology, and we have shown that mGluRs are involved in the development of opioid dependence, as well as neuropathic pain. We tested the hypothesis that activity at mGluRs may contribute to opioid insensitivity in neuropathic subjects. Neuropathy was induced by placing a 2mm length of PE90 polyethylene tubing around one sciatic nerve. A 7 day intrathecal (i.t.) infusion of either vehicle (artificial CSF), or 50 mg/day antisense (AS) or missense (MS) oligonucleotides began 3 days before nerve injury. Thermal sensitivity was measured, using tail flick from 55°C hot water, prior to i.t. infusion and again 4 days after nerve injury, both before, and every 15 min for 60 min after i.t. morphine (0, 10 or 30 mg/20 ml via lumbar puncture). Neither CSF- nor MS-treated neuropathic rats were analgesic following injection of even 30 μ g of morphine. In contrast, AS-treated neuropathic rats were analgesic at 10 μ g of morphine, suggesting that AS treatment restored opioid sensitivity in these rats. *(Supported by ASTRA Research Centre Montreal & MRC Canada grants to TJC & JLH).*

Tu16

LACK OF CROSS TOLERANCE BETWEEN SYSTEMIC MORPHINE AND ASIMADOLINE, A PERIPHERALLY-SELECTIVE KAPPA-AGONIST, IN NEUROPATHIC RATS.

J.S. Walker [1], G. Catheline, V. Kayser and G. Guilbaud. Inserm U161, Paris, France and Physiology & Pharmacology, University of NSW, Sydney, Australia [1] The development of tolerance following repeated doses of morphine hinders the treatment of clinical pain. We have previously shown that morphine tolerance developed in neuropathic rats without cross-tolerance to a systemic κ -opioid agonist (1); in this work we studied the antinociceptive effect of a new peripherally-selective κ -opioid agonist, asimadoline (Merck KGaA) in morphine-tolerant rats (paw-pressure vocalisation thresholds) 2 weeks after sciatic nerve injury. In opioid-naive rats, intraplantar (ipl) injection of asimadoline into the nerve-injured paw, at doses of 10, 15 and 20 but not 30 μ g, relieved the mechanical allodynia; norbinaltorphine (30 μ g, ipl) reversed this effect. When injected into the contralateral paw (ipl) or iv, asimadoline (15 μ g) had no effect, however. In morphine-tolerant rats (10 mg/kg sc, twice daily for 4 days) and naive rats, asimadoline (15 μ g ipl) relieved the hyperalgesia to the same extent, indicating no cross-tolerance between morphine and the peripherally-selective drug. These findings show promise for the treatment of neuropathic pain with peripherally-selective κ opioids.

(1) Catheline et al., *Europ.J.Pharmacol.* [1996] 318, 273.

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Tu17

INCREASED LEVELS OF HEAT - SHOCK PROTEIN 47 FOLLOWING FORMALIN INDUCED TONIC PAIN.

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Release of endogenous opioids following pain-induced stress triggers the endogenous mechanism of neuronal protection such as an activation of heat-shock proteins (HSP). HSP47, a 47-kD heat shock protein, was isolated as a collagen-binding stress protein participating in cell differentiation and stress protection. In this study we determined the level of HSP47 in the spinal cord following formalin-induced tonic pain. Formalin, injected in the hind of the rats, causes neuronal sensitization in the ipsilateral but not contralateral half of the spinal cord. Therefore the lumbar part of the spinal cord was divided in four segments: dorsal ipsilateral (DIL), dorsal contralateral (DCL), ventral ipsilateral (VIL) and ventral contralateral (VCL). Using RNase protection assay we have found that HSP47 level was increased more than two times in DIL as compared to DCL part of the spinal cord. HSP47 levels, however, were not different between DCL and brain. We suggest the increased level of HSP47 in DIL indicates the involvement of this protein in neuronal synaptogenesis and sprouting following chronic pain.

Tu19

p-HYDROXYMERCURIBENZOATE-INDUCED ANTINOCICEPTION THROUGH THE DYNORPHIN SYSTEM.

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The antinociceptive effect of intrathecally (i.t.) administered p-hydroxymercuribenzoate (PHMB), a cysteine protease inhibitor, was tested in the mouse capsaicin test. When administered i.t. 60 min prior to the injection of capsaicin solution (800 ng) into the dorsal surface of a hindpaw, PHMB (2-8 nmol) produced a dose-dependent and significant reduction of the capsaicin-induced paw licking response. The antinociceptive effect of PHMB was significant from 5 min to 90 min after administration. PHMB (4 nmol)-induced antinociception was completely antagonized by i.t. nor-binaltorphimine, a selective kappa-opioid receptor antagonist, but not by naltrindole, a selective delta-opioid receptor antagonist. The results indicate that the antinociceptive effect of PHMB may be due to the inhibition of a cysteine protease degrading endogenous dynorphins.

Tu18

COMPARISON OF DYNORPHIN A AND DYNORPHIN A-(2-17) IN MURINE MODELS OF PAIN, ITCH AND CNS DEPRESSION.

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Kappa agonists acting centrally and/or peripherally are very effective in attenuating scratching induced by s.c. compound 48/80 in male Swiss mice and show promise as antipruritics. We now report that i.v. dynorphin A is active in this model whereas Dyn A-(2-17), which does not bind to opioid receptors, gives an unimpressive dose-response curve of low maximum. Dynorphin A is essentially equipotent against (presumed) itch and pain (mouse formalin test) but active doses also antagonize spontaneous vertical rearing by mice placed in a novel environment. Antinociception obtained with Dyn A-(2-17) and morphine is also associated with CNS depression in this test.

A50 (umol/kg, i.v.)

Formalin Test Rearing Test 48/80 Test

Dyn A 0.70 0.50 1.01
(0.27-1.13) (0.23-0.77) (0.65-1.37)
Dyn A-(2-17) 0.83 0.65 5.0
(0.37-1.29) (0.34-0.96) (flat DRC)
Morphine 0.93 1.01
(0.48-1.38) (0.45-1.57)

(Grant DA 07237)

Tu20

MORPHINE INDUCED GABA, GLYCINE AND GLUTAMATE RELEASE IN THE DORSAL HORN OF THE SPINAL CORD IN ANESTHETISED RATS.

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Objective: To study the effect of spinal morphine on GABA, glycine, glutamate and aspartate release in the dorsal horn of the spinal cord at L3-L4.

Methods: In vivo microdialysis in halothane anaesthetised rats (n = 8-11) with quantification of amino acids by capillary electrophoresis with laser induced fluorescence detection.

Results: Perfusion of the dialysis probe with Morphine [100 µM] induced a significant 4 fold increase of GABA, 2 fold increase of glycine, 13 fold increase of glutamate and an almost 2 fold increase of aspartate (not significant).

Conclusion: An increased release of GABA and glycine in the dorsal horn may contribute to the analgesic effect of spinally administered morphine, whereas a spinal release of glutamate is likely to counteract opioid analgesia.

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Tu21

PERIPHERAL EFFECTS OF THE KAPPA AGONIST EMD 61753 ON NOCICEPTION AND INFLAMMATION.

H. Machelska¹, A. L. Maycock² and C. Stein¹. Klinik f. Anaesthesiologie und operative Intensivmedizin, Freie Universität Berlin, Germany¹; Adolor Corporation, Malvern, PA, USA². The objective of the present study was to evaluate the effects of the peripherally selective kappa opioid receptor agonist EMD 61753 on nociception and inflammation in rats with Freund's complete adjuvant-induced inflammation. Paw pressure threshold (PPT), paw volume (PV) and paw temperature (PT) were measured. Bilateral, intraplantar (i.pl.) injection of EMD 61753 (100-3200 ug) resulted in dose-dependent antinociception in both inflamed and noninflamed paws, with a peak at 5 min. However, at later time points (1 h-4 days) a significant decrease in PPT was observed. This was accompanied by an increase in PV and PT in both paws, with a peak at 6 h. EMD 61753 (1600 ug)-induced antinociception was blocked by the peripheral opioid receptor antagonist naloxone methiodide (NLXM; 2.5-10 mg/kg s.c.) and by the kappa opioid receptor antagonist NorBNI (100 µg; i.pl.). In contrast, EMD 61753 (1600 micrograms)-induced hyperalgesia and increase in PV and PT were not blocked by NLXM (10-40 mg/kg s.c.). This data show differently mediated peripheral actions of EMD 61753: kappa opioid receptor-induced antinociception and non-opioid proinflammatory and hyperalgesic effects.

Tu23

HUMAN GASTROINTESTINAL OPIOID BINDING SITES IN INFLAMMATORY BOWEL DISEASE AND IDIOPATHIC CHRONIC CONSTIPATION.

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Opioid binding sites are known to be present in the gastrointestinal tract of many species including man. Differences in the amounts of opioid peptides in the colon of patients with inflammatory bowel disease (IBD; Crohn's disease or ulcerative colitis) have been reported (Corbett et al., Analgesia 1: 379, 1995). In this study, we have used radioligand binding assays to characterise opioid and ORL1-binding sites in the mucosa+submucosa and muscularis externa of human colon and ileum in health and disease. Specimens of human gut were obtained from patients undergoing surgical resection for a non-stenotic tumour ('healthy'), IBD or idiopathic chronic constipation (ICC). Radioligand binding assays were carried out using the method of Kosterlitz et al. (Br. J. Pharmacol. 73: 939, 1981). Using the non-selective opioid receptor ligand [3H]-bremazocine, opioid binding sites were found to be present in both the muscularis and, to a lesser extent, the mucosa+submucosa of human colon, and ileum. These sites were exclusively d -opioid binding sites. No specific binding was seen with µ- or k -opioid receptor ligands. Furthermore, no specific binding was seen using [3H]-nociceptin, the endogenous ligand of the ORL1-receptor. Opioid binding sites were also present in tissue from patients with ICC and both forms of IBD. Again, these sites were exclusively of the d -type and there was no significant difference in B_{max} values between healthy tissue and any of the disease forms. In conclusion, these data show that only d -opioid binding sites are present in the human colon and ileum and that the number of sites appear to be unaffected as a result of IBD or ICC.

Tu22

k -OPIOIDS AFFECT CELL TRAFFICKING IN ARTHRITIS.

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Rheumatoid arthritis is characterised by infiltration of inflammatory cells in the synovium. Our work shows that k -opioids have anti-arthritic and anti-inflammatory properties [1]. So here we investigated the effect of k -opioids on recruitment of mast cells and macrophages in adjuvant arthritic joints by immunohistochemistry. Paraffin sections of ankle joints from adjuvant arthritic rats were stained for mast cells (toluidine blue) or macrophages (ED1). In untreated arthritic rats, macrophage cell numbers in the joint increased with progressive disease. In contrast, mast cell numbers peaked during the active stage of the disease. Treatment with the k -opioid PD 117302, produced a dose-dependent decrease in mast cell (11-48%) and macrophage (43-85%) numbers in the joint. Furthermore, after treatment with a peripherally selective k -opioid, asimadoline (Merck KGaA), cell numbers were 65-80% lower in the joint compared to untreated arthritic rats. These results suggest that in adjuvant arthritis, k -opioids may act by decreasing cell trafficking to the joint.

[1] Walker, JS et al. (1997) Rheumatoid Arthritis: ID research alert 1, 291-299.

Tu24

RENOVASCULAR HYPERTENSION INCREASES CANINE CARDIAC MET-ENKEPHALIN-ARG-PHE AND PROENKEPHALIN.

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Rodent studies have shown hypertension increased cardiac enkephalins (enk). We investigated the distribution and processing of proenk A in the hearts from sham and hypertensive instrumented dogs. Tissue was collected and extracted after 4-6 weeks of renovascular hypertension or sham treatment. Extracts were processed by gel filtration chromatography to separate enk by molecular weight. Fractions corresponding to large (proenk), intermediate (peptide B) and small (met-enk-arg-phe) peptides were quantitated by RIA. Proenk increased by 25% in the ventricles and 8% in the atrium. Peptide B was unchanged. Met-enk-arg-phe increased by more than 50% in all heart sections (right atrium, right and left ventricle and interventricular septum). The possibility that the cardiac enkephalins are involved in the hypertension-induced diminished cardiac responsiveness to autonomic stimuli is being investigated.

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Tu25

OPIOID RECEPTORS DO NOT MEDIATE CANINE ISCHEMIC PRECONDITIONING. B.A. Barron, A. Heymann, A. Williams, X. Bian, H.F. Downey. Department of Integrative Physiology, Cardiovascular Research Institute, UNT Health Science Center, Fort Worth, Texas, USA.

A short period of coronary ischemia prior to a longer ischemic event results in a reduced infarction size. Ischemic preconditioning (IPC) in rodents may involve a delta-opioid receptor mechanism. IPC in rodents has been blocked by infusion of naloxone and corticosterone. Naloxone may have mechanisms unrelated to opioid receptor function. We decided to investigate the involvement of opioid receptors in IPC in a model allowing more invasive cardiovascular measurements. In the dog model, IPC reduces infarct size/area at risk from 22.4 ± 4.6 to $4.6 \pm 1.7\%$ (Cardiovasc Res 26:534, 1992). The role of opioid receptors in ischemic preconditioning was investigated by injecting diprenorphine ($100 \mu\text{g}/\text{kg}$) prior to coronary artery occlusion. Dogs were anesthetized and instrumented to monitor cardiac function. The left anterior descending (LAD) artery was isolated and a snare positioned. Collateral flow was decreased by tying surface arteries not occluded by the snare in the LAD territory. Any animal having collateral flow greater than 20% was excluded from data collection. Infarct size was determined by tetrazolium staining and the area at risk by Evans blue dye. Collateral flow was determined by infusion of SN microspheres during LAD occlusion. The infarct size/area at risk was 4.7% in the presence of diprenorphine. Diprenorphine did not reverse the IPC as had been seen with naloxone in rodents.

Tu27

THE NEGATIVE INOTROPIC EFFECTS IN CARDIOMYOCYTES MEDIATED VIA κ -OPIOID RECEPTORS ARE SENSITIVE TO PERTUSSIS TOXIN.

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In isolated, electrically driven rat ventricular cardiomyocytes the selective κ opioid receptor (OR) agonist U-50,488 concentration-dependently reduced contractile response (CR) within 15 min, while the selective μ and δ OR agonist DADLE had no significant effect on CR. The selective κ OR antagonist nor-binaltorphimine antagonized the effects of U-50,488 on CR. Preincubation with pertussis toxin (PTX, 200 ng/ml; 4.5-5 h) completely abolished the effects of U-50,488 on CR. In the presence of isoprenaline (5 nM) U-50,488 concentration-dependently reduced CR within 15 min to predrug values, DADLE exerted no change in CR. These results imply that the effects of OR agonists on CR in ventricular cardiomyocytes are mediated via κ ORs coupling to PTX-sensitive signal transducing Gi/o proteins. The observed mechanisms may contribute to the modulation of positive inotropic effects exerted by adrenergic stimuli.

Tu26

INOTROPIC EFFECTS OF μ -OPIOID AGONISTS IN NORMAL AND STRESSED HEARTS.

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In the awake sheep, DAMGO and DALDA (μ -selective opioid agonists) increased blood pressure (BP). This increase in BP may be due to changes in peripheral resistance, cardiac output, heart rate and/or stroke volume (SV). The present study showed an increase in SV, suggesting that DAMGO and DALDA increase SV by a direct inotropic action. However, using the healthy isolated guinea pig heart, we found a dose-dependent negative inotropic effect (10^{-11} - 10^{-7} M) suggesting that the increase in SV may be due to an increase in venous return. In isolated guinea pig hearts stressed by ischemia or rapid pacing, DAMGO and DALDA (10^{-9} - 10^{-7} M) induced a positive inotropic effect. Recent data also revealed multiple exposures to DAMGO and DALDA (3, 5 min cycles of 10^{-9} M) prior to a 30-min ischemic challenge significantly reduced post-ischemic injury to the heart. These data suggest that the inotropic action of DAMGO and DALDA depend on the pre-existing condition of the heart and that stimulation of the μ -opioid receptor elicits a cardioprotective effect.

Tu28

PHOSPHOLIPASE C MEDIATES κ -RECEPTOR STIMULATION INDUCED ARRHYTHMIAS IN THE ISOLATED RAT HEART.

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To determine whether the phospholipase C (PLC)/inositol 1,4,5 trisphosphate (IP_3)/ Ca^{2+} pathway mediates cardiac arrhythmias induced by κ -opioid receptor stimulation, the effects of U50,488H, a selective κ -opioid receptor agonist, on cardiac rhythm in a isolated perfused rat heart, intracellular calcium ($[\text{Ca}^{2+}]_i$) in a single ventricular myocyte and IP_3 production in myocytes were studied in the presence and absence of PLC inhibitors. U50,488H, the effects of which had been shown to be abolished by a selective κ -receptor antagonist nor-binaltorphimine, induced arrhythmias dose-dependently and increased both $[\text{Ca}^{2+}]_i$ and IP_3 production in the heart. More importantly, the effects of U50,488H were blocked by selective PLC inhibitors, neomycin and streptomycin. To further confirm the selectivity of action of the PLC inhibitor, the effects of another PLC inhibitor U73122 and its inactive structural analog, U73343 on cardiac rhythm in the isolated perfused rat heart were compared. The former did, while the latter did not, block the arrhythmogenic effect of U50,488H. We also determined whether the effects of κ -receptor stimulation involves a pertussis toxin (PTX) sensitive G-protein. We found that pretreatment with PTX at $4 \mu\text{g}/\text{L}$ for 10 min, a treatment shown to affect PTX sensitive G-protein mediated functions, attenuated significantly the U50,488H-induced arrhythmias. The present study provides evidence that κ -receptor stimulation-induced cardiac arrhythmias involves the PLC/ IP_3 / Ca^{2+} pathway as well as a PTX sensitive G-protein.

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DYNORPHIN A₁₋₁₇ STIMULATES INSULIN SECRETION FROM A PANCREATIC b - CELL LINE (MIN6).

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Evidence is accumulating that opioid peptides are involved in regulation of pancreatic hormone secretion. Effects of opioid peptides on pancreatic b -cells to stimulate insulin secretion have been shown using isolated rat islets of Langerhans. However, the mechanism of this action of dynorphin and whether it represents a direct effect on b -cells remains unclear, although it does not appear to involve opioid receptors. We report that dynorphin A₁₋₁₇ directly stimulates insulin secretion from a non-neuronal cell line, MIN6, which was derived from mouse b -cells. It has been suggested that dynorphin binds to N- methyl-D-aspartate (NMDA) receptors. We have confirmed previous findings that MIN6 cells naturally express ionotropic glutamate receptors. We are currently testing the hypothesis that dynorphin A₁₋₁₇ stimulation of insulin secretion is mediated via the NMDA receptor.

Tu31

AGONIST-INDUCED DESENSITIZATION OF THE μ OPIOID RECEPTOR IS AFFECTED BY SERINE 266 MUTATION TO PROLINE IN THE THIRD INTRACELLULAR LOOP.

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As previously shown the desensitization of the rat μ opioid receptor is affected by two CAM Kinase II phosphorylation site mutations (S261A and S266A) in the third intracellular loop. A comparable allelic variation of the μ opioid receptor (S266P) is known in human. In this study we mutated only Serine 266 to Proline to investigate the role of this phosphorylation site mutation in agonist induced μ opioid receptor desensitization. When stably expressed in HEK293 cells as well as after expression in *Xenopus* oocytes S266P receptor mutant is slower desensitized after opioid administration than the wild type receptor. These results suggest that S266 seems to be more important for receptor desensitization than the S261 phosphorylation site. Another explanation might be that a single site mutation of serine 266 to proline probably affects phosphorylation or coupling of the μ opioid receptor by changing receptor conformation in the third intracellular loop.

Tu30

G PROTEIN RECEPTOR KINASE (GRK3) AND b -ARRESTIN 2 MEDIATED DESENSITIZATION OF THE KAPPA OPIOID RECEPTOR IN XENOPUS OOCYTES.

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The kappa agonist U69,583 increased the conductance of G protein activated inwardly rectifying potassium channels in *Xenopus* oocytes co-expressing the rat kappa opioid receptor (KOR) with Kir 3.1 and Kir 3.4. Only a small desensitization of the KOR activated response was seen in the absence of GRK and arrestin. Co-expression of GRK3 and b -arrestin 2 significantly increased the homologous desensitization of the k -opioid response. Truncation of the COOH terminal tail of the KOR reduced the desensitization induced by GRK3 and b -arrestin 2, although the truncated KOR coupled effectively to Kir 3. In contrast, mutation of the three Ser residues in the 3rd intracellular loop to Ala did not effect the agonist induced desensitization. To determine whether this agonist induced phosphorylation occurs in vivo we examined kappa agonist desensitization in hippocampal slices. The KOR is phosphorylated in an agonist dependent manner in hippocampal slices and remains elevated in tolerant animals. Supported by DA04123.

Tu32

DESENSITIZATION OF MU (OP-3) RECEPTOR-STIMULATED [³⁵S]GTPg S BINDING BY AGONISTS OF DIFFERENT EFFICACIES IN TRANSFECTED mMOR-CHO CELLS.

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We have previously reported on the mechanisms of opioid agonist efficacy for stimulation of [³⁵S]GTPg S binding in μ -transfected CHO cells and rat brain (Mol. Pharmacol. [1997] 51, 87). In the present study, mMOR-CHO cells pretreated overnight with agonists of different efficacies were assayed for stimulation of [³⁵S]GTPg S binding by DAMGO, methadone, morphine, sufentanil, buprenorphine and nalbuphine. Results showed that desensitization correlated with efficacy of the pretreatment drug. Cross-desensitization of lower efficacy agonists by pretreatment with higher efficacy agonists tended to be more profound. However, two exceptions were noted: 1) some low efficacy partial agonists produced sensitization similar to the antagonist naloxone, and 2) sufentanil, although of lower efficacy than DAMGO for stimulation of [³⁵S]GTPg S binding, was highly efficacious in desensitizing the receptor. These results suggest that both the efficacy and affinity of agonists contribute to desensitization. Supported by NIDA grants DA10770 and DA02904.

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Tu33

μ OPIOID RECEPTOR: ROLE OF THE C-TERMINUS IN AGONIST-MEDIATED PHOSPHORYLATION

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To examine a role of the C-terminus of μ opioid receptor in agonist-mediated receptor phosphorylation, we studied function of C-terminal deleted μ opioid receptors. Both wild type and truncated μ opioid receptors were epitope-tagged and stably expressed in CHO cells. The receptor expressions were confirmed by receptor radioligand binding and immunoblotting with epitope specific antibody. Exposure to 1 μ M of DAMGO induced phosphorylation on both wild and truncated μ opioid receptors, however, the level of receptor phosphorylation in the truncated receptor is reduced in comparison with that of the wild type. The mutant also shows a partial loss of DAMGO-induced desensitization measured by adenylyl cyclase inhibition assay. Taken together, these data indicate that multiple domains of the μ opioid receptor are involved in agonist-mediated receptor phosphorylation and desensitization.

This work was supported by PhRMA Faculty Development Award to J.B.W. and NIDA intramural research program.

Tu35

INTERNALIZATION AND DIMERIZATION OF KAPPA OPIOID RECEPTORS

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Receptor regulation is the key process in the development of tolerance to specific stimuli. In addition to the attenuation of response, which may involve every step in the signalling pathway, internalization and down-regulation are crucial to the modulation of receptor function. Although a number of studies have characterized the agonist mediated endocytosis of δ and μ opioid receptors, few studies have explored these processes in κ opioid receptors (KORs). We have used CHO cells expressing KORs and prodynorphin derived peptides to examine the properties of KOR endocytosis. We find that dynorphin peptides induce receptor internalization. In contrast, KOR selective alkaloid agonists do not. Furthermore, only peptide agonists are able to induce receptor down-regulation. Interestingly, amongst all peptides tested, only Dynorphin B-14 and Dynorphin A-17 were able to cause significant internalization and down-regulation. Our results also show that receptor number and cell density markedly affect the extent of down-regulation. Previously we have shown that delta opioid receptors (DORs) exist as dimers that monomerize upon agonist stimulation. Moreover, monomerization precedes receptor internalization, which suggests that this process may be a prerequisite for receptor endocytosis. Here we show that κ opioid receptors exist as dimers and the properties of KOR dimers are distinct from DOR dimers. In naive cells, about 90% of KORs exist as dimers compared to about 50-60% of DORs. Also, KOR dimers are stable in 2% SDS whereas DOR dimers are not. Furthermore, KOR dimers do not exhibit significant monomerization in response to κ selective agonists while DOR dimers monomerize in response to δ selective agonists. It is possible that the high stability of KOR dimers contributes to the lack of significant monomerization and decrease in extent of internalization in response to agonists.

Tu34

THE SERINE RESIDUES S261 and S266 ARE REQUIRED FOR AGONIST-INDUCED PHOSPHORYLATION OF THE μ -OPIOID RECEPTOR.

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Recently, the serine residues S261/S266 within the third intracellular loop which are putative CaM kinase II sites (Koch et al., J. Neurochem. 69, 1767-1770, 1997) as well as the threonine residue T394 which is a putative GRK2 site (Pak et al., J. Biol. Chem. 272, 24961-24965, 1997) have been shown to be involved in agonist-induced desensitization of the μ -opioid receptor. In the present study, we have mutated these sites to alanine and investigated agonist-induced phosphorylation of these mutants after stable expression in HEK-293 cells. DAMGO stimulated a five fold increase in phosphorylation of the wild-type receptor within 15 min. While the T394A mutant showed DAMGO-induced phosphorylation to a similar extent as the wild-type, the S261A/S266A mutant showed lower levels of phosphorylation. In the triple mutant S261A/S266A/T394A agonist-induced phosphorylation was not detectable. Similar, the S261A/S266A mutant of MOR1B which naturally lacks T394 did not undergo agonist-induced phosphorylation. Thus, these findings suggest that CaM kinase II largely contributes to agonist-induced phosphorylation of the μ opioid receptor in HEK-293 cells. In addition, CaM kinase II also appears to be in a position to phosphorylate the μ -opioid receptor in vivo as CaM kinase II but not GRK2 is frequently colocalized with the μ -receptor in the superficial layers of the spinal cord.

Tu36

REPLACEMENT OF THREONINE 394 BY ALANINE ACCELERATES INTERNALIZATION OF THE RAT μ OPIOID RECEPTOR.

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We have previously shown that the sequence at the cytoplasmic tail of the alternatively spliced isoform (MOR1B) of the rat μ -opioid receptor (MOR1), facilitates clathrin-coated vesicle mediated endocytosis which, in turn, promotes accelerated receptor reactivation. This fast receptor reactivation results in a slower receptor desensitization of MOR1B compared to MOR1. MOR1B lacks only one putative phosphorylation site (T394) compared to MOR1. Our study revealed that replacement of this T394 by an alanine accelerates internalization and recycling of the μ opioid receptor in stably transfected neuroblastoma cells (Neuro 2a). These results suggest that agonist-induced desensitization and resensitization of the μ opioid receptor is determined by threonine 394.

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DESENSITIZATION OF THE δ -OPIOID RECEPTOR CORRELATES WITH ITS PHOSPHORYLATION IN SK-N-BE CELLS : INVOLVEMENT OF A GRK.

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Phosphorylation of G protein coupled receptors is considered as an important step during their desensitization. In SK-N-BE cells, recently presented as a pertinent model for the studies of the human δ -opioid receptor, pretreatment with the opioid agonist, etorphine, increased time-dependently the rate of phosphorylation of a 51 kDa membrane protein. Immunological characterization of this protein with an antibody, raised against the amino-terminal region of the cloned human δ -opioid receptor, revealed that it corresponded to the δ -opioid receptor. During prolonged treatment with etorphine, phosphorylation increased as early as 15 min. to reach a maximum within 1h. Phosphorylation and desensitization of adenylyl cyclase inhibition paralleled closely and okadaic acid inhibited the resensitization, a result strongly suggesting that phosphorylation of the δ -opioid receptor plays a prominent role in its rapid desensitization. The increase in phosphorylation of the delta-opioid receptor, as well as its desensitization, were not affected by H7, an inhibitor of PKA and PKC, but was drastically reduced by heparin or Zn⁺⁺, known to act as guanine nucleotide coupled receptor kinases inhibitors. These results are the first to show, on endogenously expressed human δ -opioid receptor, that a close link exists between receptor phosphorylation and agonist-promoted desensitization and that desensitization involves a GRK.

Tu39

REGULATION OF MU OPIOID RECEPTORS BY CHRONIC OPIOID TREATMENTS IN RAT BRAIN.

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We have studied the effect of mu opioid receptor (MOR) specific ligands on receptor trafficking in rat brains. Control and treated rat brains were subjected to subcellular fractionation to prepare synaptic plasma membranes (SPM) and microsomes (MI), the latter representing intracellular binding sites. Repeated icv injection of the MOR specific peptide, DAMGO-chloromethyl ketone at 100 ng for 5 days resulted in the development of analgesic tolerance. In parallel, the B_{max} of [³H]DAMGO binding sites decreased by about 22% in the SPM fraction with a concomitant increase in MI. When analgesic tolerance was induced by morphine injecting subcutaneously at increasing doses twice daily for 5 days, the number of surface MOR binding sites was not changed, however nearly doubled in MI. It is concluded that chronic treatment resulted in ligand specific regulation of the endogenously expressed MOR in rat brain. Supported by TET-564 and OTKA T-016084 research grants.

Tu38

δ OPIOID RECEPTOR DOWN-REGULATION: G PROTEIN COUPLING AND PROTEASES

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In these experiments we tested the effect of pertussis toxin and protease inhibitors on delta opioid receptor binding and down-regulation. Pretreatment of HEK 293 cells stably expressing the δ receptor with 10 or 100 ng/ml pertussis toxin for 3 h had no effect on 3H-DSLET binding (1 nM) to membrane preparations. This result suggested that high affinity agonist binding to the δ receptor is independent of G protein coupling. This is consistent with our observation that 3H-DSLET binding to membranes from these cells is not inhibited by 100 mM GPP(NH)P. Overnight treatment of δ -expressing HEK 293 cells with 1 μ M DADL caused a 80-90% decrease in the B_{max} with minimal effect on the K_D. Pretreatment of cells with 100 ng/ml pertussis toxin for 3 h had very little effect on the extent of agonist-dependent down-regulation, suggesting that coupling to G_i and/or G_o heterotrimers is not required for down-regulation of the δ receptor. Prolonged treatment of cells with pertussis toxin alone resulted in a 2-fold increase in the δ receptor B_{max}, which may be due to regulation of the CMV promoter that directs expression of the receptor in these cells, since the effect was not observed in NG108 cells. Pretreatment of cells with a proteasome inhibitor, but not a lysosomal protease inhibitor, attenuated the extent of DADL-induced delta receptor down-regulation.

Tu40

DIFFERENT REGULATION OF DYNAMIN BY CHRONIC TREATMENT WITH μ AND δ AGONISTS.

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Mechanisms of opioid receptor desensitization following chronic opioid treatments are not well understood. Two-different changes occurred: rapid desensitization, with a reduced ability of the agonist to inhibit adenylyl cyclase, and a longer desensitization, due to down-regulation of various components of the opioid system. The cellular mechanisms involved in this desensitization process have not been well established. Evidence exists that GPCRs internalize via the clathrin-coated vesicle-mediated endocytic pathway. Recently, the involvement of dynamin (a major component and markers of the clathrin-mediated endocytic pathway) in GPCRs internalization has been established. In good agreement with these results, we have shown that chronic opioid receptor activation with morphine led to down-regulation of μ and δ opioid receptors which was correlated with an up-regulation of dynamin in SHSY5Y cells. Using selective opioid agonists, it appears that this effect is restricted to stimulation of μ -opioid receptors. The physiological relevance of this result has been established in the caudate putamen of mice following chronic morphine treatment, while it did not occur in mice lacking the μ -opioid receptor gene.

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PKA MAINTAINS TOLERANCE IN HYPO-THALAMIC NEURONS WITH CHRONIC MORPHINE: CONVERGENCE WITH ESTROGEN.

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We examined protein kinase (PKA and PKC) involvement in tolerance to μ -opioid receptor agonists caused by chronic opiate in neurons of the arcuate nucleus (ARC). The common pathway mediating the diminution of μ -opioid receptor coupling produced by acute 17 β -estradiol (E₂) or chronic opiate was also investigated. Intracellular recordings were made in hypothalamic slices from ovariectomized guinea pigs. Chronic morphine treatment for 4 days reduced DAMGO potency 2.5-fold, as did the PKA activator Sp-cAMP. The protein kinase inhibitor staurosporine abolished the reduced DAMGO potency, as did the PKA inhibitor Rp-cAMP. E₂ did not further reduce DAMGO potency. Thus, increased PKA activity maintains tolerance in ARC neurons. Moreover, acute E₂ and chronic opiate treatments attenuate μ -opioid responses via a common PKA pathway. (Supported by DA05158 & DA00192.)

Tu43

ABATEMENT OF ACUTE SPINAL TOLERANCE TO MORPHINE, REMIFENTANIL, AND ENDOMORPHIN-2 BY INDUCIBLE NITRIC OXIDE SYNTHASE INHIBITORS.

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Non-selective (Bhargava, 1995, Elliott, et al., 1994, Kolesnikov, et al., 1993, Kolesnikov, et al., 1992, Majeed, et al., 1994) and selective neuronal (Fairbanks and Wilcox, 1997) nitric oxide synthase (NOS) inhibitors have proven effective in preventing the development of opioid tolerance. The participation of inducible NOS (iNOS) in the development of morphine antinociceptive tolerance has yet to be examined. The present experiments tested the ability of iNOS inhibitors (agmatine, L-N⁶-(1-iminoethyl)lysine (L-NIL)) to modulate the development of acute tolerance to morphine (MOR), remifentanil (REMI) and endomorphin-2 (ENDO-2). Antinociception was detected via the hot water (52.5 °C) tail flick test. Acute tolerance to morphine, remifentanil, or endomorphin-2 was induced by a single supramaximal dose of opioid (30-40 nmol, i.t.). A significant six- to ten-fold rightward shift in the probe dose-response curve of toleragen-pretreated animals confirmed the development of tolerance. Copretreatment of all toleragens with agmatine (4, 40 nmol) demonstrated a probe toleragen potency comparable to that of saline-pretreated controls. Therefore, intrathecally-administered agmatine attenuated the induction of acute spinal MOR, REMI, or ENDO-2 tolerance. Copretreatment of MOR with L-NIL (40 nmol) yielded a probe morphine potency comparable to that of saline-pretreated controls. Therefore, intrathecally-administered L-NIL attenuated the induction of acute spinal MOR tolerance. The evidence presented here supports a role for iNOS in the development of acute tolerance to spinally administered opioids. (Supported by NIH/R01-DA-04274 to GLW and ADAMHA/NIDA training grant T32A07234 supports CAF)

Tu42

MORPHINE TOLERANCE UP-REGULATES NITRIC OXIDE SYNTHASE EXPRESSION IN THE RAT SPINAL CORD.

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Long-term morphine administration may develop tolerance. Cumulative evidence shows that N-methyl-D-aspartate (NMDA) receptor antagonists inhibit morphine tolerance development. Nitric oxide synthase (NOS) inhibitor has also been demonstrated to prevent opioid tolerance. In the present study, male rats were intrathecally (i.t.) infused with various drugs and assigned into 4 groups. In control animals, saline (1 ml/hr) or MK-801 (10 μ g/hr) was infused for 5 days. Tolerance was induced by morphine (10 μ g/hr, i.t.) infusion for 5 days. Co-administered MK-801 (10 μ g/hr) with morphine (10 μ g/hr) for 5 days to prevent morphine tolerance development. Tail-flick test was used for the antinociception evaluation. On day 6, all rats were sacrificed and the dorsal lumbar spinal cord segments were removed for western blot analysis. The specific antibodies against constitutive and inducible NOS were used to specific antibodies against constitutive and inducible NOS were used to detect constitutive or inducible NOS expression. The results show up-regulation of the constitutive NOS, but not the inducible NOS, expression in morphine tolerant spinal cord while MK-801 prevents the change. The results suggest that increases constitutive NOS expression may play a role in morphine tolerance development.

Tu44

THE MECHANISM OF DEXTROMETHORPHAN ON BLOCKING MORPHINE TOLERANCE IN RATS

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Dextromethorphan (DM), a widely used oral antitussive, has been shown to have antagonized effect on NMDA receptor. In this study, adult male S.D. rats were treated with escalating doses of morphine (5-65 mg/kg, s.c., bid) for 6 days. We found the AD₅₀ of morphine was 1.23 +/- 0.49 mg/kg before treatment and 9.03 +/- 1.41 mg/kg after 6 days of morphine treatment. When DM (20 mg/kg) was given 20 min. prior to each morphine administration, the AD₅₀ was 0.84 +/- 0.18 mg/kg before treatment and 4.22 +/- 0.62 mg/kg after 6 days treatment. Rats were then killed by decapitation, and whole spinal cord was quickly dissected and lumbar tissue was sliced to 30 pieces (thickness: 450 μ m). Every 10 pieces were put in a petri dish with 2 ml of aCSF and were bubbled with O₂/CO₂ (95%/5%). The effects of morphine (100 μ M) or morphine + MK-801 (100 μ M) on nitric oxide (NO) release were determined. We found there was a significant increase of basal NO release after chronic morphine treatment. Pretreatment with DM blocked this effect of morphine. Acutely adding morphine (100 μ M) in the medium also increased NO release in each group. This effect was suppressed by MK-801 indicating NMDA receptor activation may be involved in this process. Our binding studies showed that the affinity of NMDA displaced [³H] glutamate binding increased but the B_{max} decreased after 6 days of morphine treatment. DM did not reverse these binding changes. Our conclusion is that the effect of DM on morphine tolerance is through blocking the NMDA channel and decreasing NO release induced by morphine.

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AVAILABILITY OF THE NON-COMPETITIVE NMDA RECEPTOR ANTAGONIST IFENPRODIL AS ADJUNCT OF MORPHINE.

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The present study focused on the effects of the non-competitive NMDA receptor antagonist ifenprodil on morphine-induced several pharmacological actions, and the availability of ifenprodil as adjunct of morphine was examined. We examined the effects of ifenprodil on the morphine-induced antinociception, rewarding effect and physical dependence using tail-flick, conditioned place preference and drug-admixed food methods, respectively. Morphine (3 mg/kg, s.c.)-induced antinociception was significantly potentiated by co-injection of ifenprodil (10-30 mg/kg, i.p.) with morphine, and development of tolerance to the morphine-induced antinociception was inhibited by ifenprodil. Morphine (5 mg/kg, s.c.)-induced place preference was suppressed by pretreatment with ifenprodil (5-20 mg/kg, i. p.). Total withdrawal score and body weight loss were significantly suppressed by co-treatment with ifenprodil. In conclusion, the present study found that ifenprodil potentiates morphine-induced antinociception, and suppresses developments of morphine-induced antinociceptive tolerance, rewarding effect and physical dependence. Therefore, ifenprodil may be useful for adjunct of morphine, because ifenprodil may suppress the side effects, with potentiating antinociceptive activity of morphine.

Tu47

EFFECTS OF BACLOFEN ON ANTINOCICEPTIVE TOLERANCE TO AND PHYSICAL DEPENDENCE ON MORPHINE IN MICE.

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To investigate the interaction between morphine and baclofen (GABA_B receptor agonist), the effects of baclofen on development of antinociceptive tolerance to and physical dependence on morphine in mice, and expression of naloxone-precipitated withdrawal signs in morphine dependent mice were examined. Morphine-induced antinociception was evaluated using the tail-flick method. On days 3 and 5, morphine-induced antinociception was significantly reduced compared to that on day 1, suggesting that chronic treatment with morphine (3 mg/kg, s.c.) for more than 2 days produces tolerance to morphine antinociception. The development of tolerance to morphine antinociception was significantly suppressed by pretreatment with baclofen. Mice were treated with morphine (8-45 mg/kg, s.c.) twice a day for 5 days and withdrawal signs were then precipitated by injecting naloxone (3 mg/kg, s.c.). Naloxone-precipitated jumping, rearing, forepaw tremor and weight loss, but not body shakes, were suppressed by pretreatment with baclofen 30 min prior to naloxone injection on day 5. Moreover, naloxone-precipitated jumping and weight loss were also significantly attenuated by co-administration of baclofen with morphine for 4 days, suggesting that baclofen may suppress both the expression of naloxone-precipitated withdrawal signs in morphine-dependent mice and the development of physical dependence on morphine. These findings suggest that baclofen combined with morphine may be useful for the treatment of pain, because baclofen suppresses several side effects of morphine such as tolerance and physical dependence with keeping the potent antinociceptive activity of morphine.

Tu46

CHRONIC MORPHINE TREATMENT ALTERS NMDA RECEPTOR-MEDIATED TRANSMISSION.

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Many studies have shown that the NMDA receptor, an ionotropic glutamate receptor subtype, plays a key role in morphine addiction. However, the cellular mechanisms underlying its role remains unknown. A possible explanation is that morphine treatment would alter NMDA receptor properties by modifying their subunit composition. To assess this hypothesis, we recorded NMDA-EPSCs in naive and morphine-treated rats using intracellular recording in nucleus accumbens slices. We first evaluated a change of glutamate affinity for NMDA receptors by measuring NMDA-EPSC amplitudes evoked by local stimulation at -70 mV. Although morphine treatment decreased NMDA-EPSC amplitudes, the difference was not statistically significant. We tested the ability of 1.5 mM Mg²⁺ to block NMDA-EPSCs in a voltage-dependent manner. We showed that the Mg²⁺ block was significantly smaller in morphine-treated rats compared to naive animals. Interestingly, this effect persisted one week after withdrawal. The enhancement of NMDA-EPSC amplitudes by PDAc, a protein kinase C activator, was smaller in morphine-treated rats. These data suggest that the expression of NMDA receptor subunits could be altered by chronic morphine treatment.

Tu48

RESTORATION OF MORPHINE ACTION IN TOLERANT ANIMALS BY A NEUROPEP-TIDE FF ANALOG.

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Neuropeptide SF (SLAAPQRF-NH₂), an analog of the morphine modulatory peptide neuropeptide FF, has been identified in the spinal cord. We examined whether neuropeptide SF (NPSF) influences spinal opioid analgesia in drug naive and opioid tolerant animals. In naive animals, intrathecal NPSF, at doses lacking intrinsic activity, significantly augmented morphine analgesia in the tail-flick and paw pressure test. In animals rendered tolerant to spinal morphine and showing reduced response to the opioid, injection of NPSF with morphine produced analgesic effects comparable to those in the drug naive animals. The analgesic response showed full recovery and was reproducible on the following day. The results show that NPSF has the potential to restore morphine effect in opioid tolerant animals. (Supported by MRC Canada)

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Tu49
withdrawn

Tu51
THE DEVELOPMENT OF TOLERANCE TO AND PHYSICAL DEPENDENCE ON MU
SUBTYPE OPIOID AGONISTS.

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We have examined receptor mechanisms of development of tolerance to and dependence on selective opioid ligands acting on mu-receptor. The appearance of physical dependence, and acute tolerance to antinociceptive, respiratory depressant and gastrointestinal transit inhibitory actions in animals treated chronically with increasing sc. doses of 6-substituted oxycodone derivative were different in time. In contrast, to mu-1 mediated analgesia, the constipation and the inhibitory influence on the respiratory depression induced by fentanyl, showed no significant tolerance after chronic treatment. Our results demonstrate that tolerance and physical dependence develops to the naloxonazine insensitive actions faster, than to analgesia. This work was supported, in part by grants: OTKA-TO- 25424, ETT 568 (1997-1999) and OMF 96-97-48-1370

Tu50

THE MECHANISM OF 100 Hz ELECTROACUPUNCTURE(EA) TOLERANCE.

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Previous study showed that electroacupuncture (EA) could produce analgesic effect by the release of endogenous opioid peptides. However, a chronic tolerance to analgesia could be observed when EA in 100 Hz was administered once a day for several times. It can be postulated that the possible mechanisms that cause EA tolerance include: (1) the decreased activity of endogenous opioid system which reduced analgesic effect of EA directly. (2) the increased activity of endogenous opioid which possibly stimulated negative feedback reduced the analgesic effect of EA indirectly. In the present study, we observe the role of endogenous opioid and CCK and the changes of the number, affinity and the mRNA transcription level of opioid receptor using RIA, In situ hybridization, RRA and RT-PCR etc.. The main results are as following: (1) The release and content of dynorphin increased in spinal cord during multiple 100 Hz EA stimulation. (2) CCK receptor antagonist L365,260 could partly reverse the tolerance of chronic 100 Hz EA. (3) The number, affinity and mRNA transcription of opioid receptor in the spinal cord was decreased during the development of 100 Hz EA tolerance. It is concluded that the increased activity of endogenous dynorphinergic system could down-regulate opioid receptor which may serve as one of the mechanisms for the development of 100 Hz EA tolerance. Endogenous CCK is involved in the development of 100 Hz EA tolerance.

Tu52

GENETIC DIFFERENCES IN MORPHINE SENSITIVITY, TOLERANCE AND
WITHDRAWAL IN RATS.

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Significant genetic differences in the endogenous opioid system and in responses to a variety of noxious stimuli are present in rodents. We now compared the response to noxious heat with the hot plate test, morphine-induced antinociception and the development of tolerance and dependence to morphine in Spontaneously Hypertensive (SHR), Wistar-Kyoto (WK) and Sprague-Dawley (SD) rats. Significant differences were observed in basal nociception among the three strains, where SHRs were hypoalgesic compared to WK and SD. The antinociceptive effect of morphine (10 mg/kg) varied among strains (SD>SHR>WK) as did the rate of tolerance development to this dose of the opiate administered 2/day for 4 days (WK>SD=SHR). SHR rats developed hyperalgesia following morphine administration during the course of tolerance development. Tolerance was partially reversed by the cholecystokinin-B receptor antagonist CI 988 only in SDs. Furthermore, although naloxone (2 mg/kg) precipitated withdrawal symptoms in all rats, the panorama of symptoms varied among the three strains. Thus, there are significant genetic differences in many opiate effects.

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ARACHIDONIC ACID CASCADE INVOLVEMENT IN THE ACUTE DEPENDENCE PRODUCED BY μ , κ AND δ OPIOID AGONISTS.

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The effect of mepacrine (a phospholipase A2 inhibitor), tolmetin (selective cyclooxygenase-1 inhibitor), meloxicam (selective cyclooxygenase-2 inhibitor) and nordihydroguaiaretic acid (5-lipoxygenase inhibitor) on acute opiate withdrawal induced by selective μ , κ and δ receptor agonists was investigated in vitro. After a 4 min in vitro exposure to DAMGO (highly selective μ agonist) and U50-488H (highly selective κ agonist) a strong contracture of guinea pig isolated ileum was observed after the addition of naloxone. This effect was also observed when rabbit isolated jejunum was pretreated with deltorphin (highly selective δ agonist). Mepacrine, tolmetin, meloxicam and nordihydroguaiaretic acid treatment before or after DAMGO or U50-488H were able of both preventing and reversing the naloxone-induced contracture after exposure to the opioid agonists in a concentration-dependent fashion. By contrast, the above inhibitors did not affect the naloxone contracture after exposure to deltorphin. The results of the present study confirm and extend our previous studies performed with morphine (less selective μ agonist) indicating that arachidonic acid and its metabolites (prostaglandins and leukotrienes) are involved in the development of opioid withdrawal induced by selective μ and κ opioid agonists whereas no effects were observed on the withdrawal by selective δ opioid agonist deltorphin.

Tu55

A POSSIBLE MECHANISM OF INHIBITORY EFFECT OF PGE RECEPTOR EP₃-SUBTYPE SELECTIVE AGONISTS ON NALOXONE-PRECIPIATED WITHDRAWAL IN MORPHINE DEPENDENT RATS.

T. Nakagawa, T. Masuda, M. Minami and M. Satoh. Department of Molecular Pharmacology, Faculty of Pharmaceutical Sciences, Kyoto University, Kyoto, Japan.

We explored for the mechanism of the inhibitory effect of EP₃ agonists on naloxone-precipitated withdrawal in morphine-dependent rats. Rats were rendered morphine-dependent by subcutaneous implantation of two pellets containing 75 mg morphine for 5 days. Morphine withdrawal was precipitated by intraperitoneal injection of naloxone (3 mg/kg). Intracerebroventricular (i.c.v.) administration of M&B28,767 (EP₃ agonist) at 30 min before naloxone suppressed significantly various withdrawal signs. Northern blot and in situ hybridization studies revealed that expression of c-fos mRNA was elevated by naloxone-precipitated withdrawal in many brain regions, including the locus coeruleus (LC) and amygdala. I.c.v. administration of M&B28,767 (1 pg/rat) attenuated the elevation of c-fos mRNA, especially in the LC. Double in situ hybridization study revealed that, in the LC, most of the tyrosine hydroxylase mRNA-positive neurons expressed μ -opioid receptor mRNA and more than half of those neurons were positive to EP₃ receptor mRNA. These results suggest that the EP₃ agonist possibly acts on the LC noradrenergic neurons expressing μ -opioid receptors to suppress the naloxone-precipitated withdrawal.

Tu54

ENHANCED OPIOID EFFICACY IN OPIOID DEPENDENCE.

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The periaqueductal grey (PAG) is involved in expression of many signs of opioid withdrawal. Using whole cell patch clamp recording in brain slices, opioids acutely inhibit GABAergic electrically evoked and spontaneous miniature synaptic currents in the PAG through production of metabolites of arachidonic acid which in turn activate dendrotoxin sensitive K⁺-channels. (Vaughan et al., Nature 390, 611-614, 1997). This study describes increased efficacy of μ -opioid agonists on GABAergic nerve terminals in PAG following chronic morphine, resulting in enhanced GABAergic synaptic transmission during withdrawal. Actions of opioids during withdrawal are not prevented by dendrotoxin, which abolishes acute opioid presynaptic inhibition. However, the enhancement is blocked by protein kinase A inhibitors. Clonidine, which attenuates opioid withdrawal signs, efficaciously inhibits the withdrawal-induced increase in GABAergic neurotransmission. The induction of protein kinase A dependent GABAergic neurotransmission and enhanced efficacy of μ -receptor agonists in presynaptic GABAergic terminals is likely to be important for the expression of opioid withdrawal behaviours mediated by the PAG.

Tu56

A MOLECULAR MECHANISM FOR SUPERSENSITIZATION OF ADENYLYL CYCLASE SYSTEM FOLLOWING SUSTAINED STIMULATION OF OPIOID RECEPTOR.

T. Nakagawa, M. Minami, T. Watanabe, T. Ozawa and M. Satoh. Department of Molecular Pharmacology, Faculty of Pharmaceutical Sciences, Kyoto University, Kyoto, Japan.

Chronic opioid treatment has been shown to develop supersensitization of adenylyl cyclase (AC) system or cAMP overshoot. In this study, we investigated the molecular mechanism of this phenomenon using CHO cells expressing the cloned opioid receptors. In the cells sustainly (4 hr) treated with agonist, the challenge by antagonist induced the supersensitization of AC system over the naive level, but had no effect on GTPase activity. To examine whether the interaction between G protein and AC is involved, we investigated the development of supersensitization of AC system in CHO cells coexpressing the opioid receptor and some chimeric Ga proteins between Ga i2 and Ga q. The results revealed that a specific region of Ga i2, which is responsible for the interaction with AC, is closely related to the supersensitization. Further, preliminary data showed that the supersensitization was developed also through Ga z. These findings suggest that the development of supersensitization is attributed to a continuous inhibition of AC by Ga i or Ga z, but not to a continuous activation of the opioid receptor or G protein itself.

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SR142801, A TACHYKININ NK-3 RECEPTOR ANTAGONIST, INHIBITS THE ATROPINE-RESISTANT COMPONENT OF THE OPIOID WITHDRAWAL RESPONSE OF GUINEA-PIG ISOLATED ILEUM

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The opioid withdrawal response of guinea-pig ileum comprises atropine-sensitive and atropine-resistant components. The aim of the present experiments was to determine whether the atropine-resistant response to opioid withdrawal was mediated by tachykinin NK-3 receptors. Guinea-pigs were killed by a blow to the head, isolated ileal segments were set up in organ baths in oxygenated Tyrode solution at 37°C, and isometric responses were recorded. Responses to withdrawal from [Met⁵]enkephalin (1 µM) were precipitated by addition of naloxone, 1 µM, to the bath 2 min after addition of [Met⁵]enkephalin. In all experiments atropine, 5 µM, was added to the bath 5 min before the addition of [Met⁵]enkephalin. Responses were obtained in the presence and absence of the NK-3 receptor antagonist, SR142801 (1 µM), its less active enantiomer, SR142806 (1 µM), or vehicle, added to the bath 5 min before addition of [Met⁵]enkephalin. SR142801, but not SR142806 or vehicle, abolished the naloxone-precipitated [Met⁵]enkephalin withdrawal response in 8 out of 9 preparations. It is concluded that tachykinin NK-3 receptors are involved in the atropine-resistant response of the guinea-pig isolated ileum to opioid withdrawal.

Tu59

OPIATE ABUSE AND DEPENDENCE OVER A DECADE IN SOUTH INDIA: A TREND ANALYSIS.

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Objective: To study the trends of opiate abuse and dependence over a period of 10 years at a major psychiatric centre in South India. Method: This study was conducted at NIMHANS, India. Data of all patients with a diagnosis of opiate abuse or dependence (ICD-9 and 10) during the period 1987-96 was collected from the medical records department. Annual rate per 1000 of psychiatric patients for Opiate abusers/dependents were computed with corresponding trends of psychiatric attendance.

Results: Trend analysis over a decade showed a gradual decline in opiate abuse/dependence except for a peak in 1993. The opiate inpatients were 10-14/1000 in the late 80's, 6/1000 by 92, 12/1000 in 93 and 4/1000 in the mid 90's. In the late 80's, majority were in a higher age group as compared to the mid 90's (20-35 yrs). The type of opiate used has been heroin in the late 80's, buprenorphine and pentazocine in the early 90's and codeine and dextropropoxyphene in the mid 90's.

Conclusions: The trend analysis reveals a gradual decline of opiate abuse over the decade and a switch from stronger to weaker opiates.

Tu58

ALTERATIONS IN BRAIN LEVELS OF SUBSTANCE P(1-7) DURING OPIOID WITHDRAWAL.

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Studies have shown that substance P (SP) may modulate the abstinence reaction to opioid withdrawal. Further, its N-terminal fragment SP1-7 may inhibit the intensity of the withdrawal reactions in morphine dependent mice. The present study was designed to determine whether the endogenous concentration of the SP1-7 fragment in the brain are affected during naloxone-precipitated withdrawal in the male rat. The amount of the peptide was assessed by a specific radioimmunoassay in extracts of discrete brain regions (including cerebral cortex, hippocampus, hypothalamus, nucleus accumbens, striatum, substantia nigra, ventral tegmental area and spinal cord) during morphine tolerance and withdrawal. The results indicated that the concentrations of SP1-7 were significantly elevated in the ventral tegmental area (VTA) both in morphine tolerant rats and during naloxone-precipitated withdrawal. During morphine withdrawal significant increases in the peptide concentration were also observed in the hypothalamus, and the spinal cord. It was concluded that the enhanced content of SP1-7 may indicate the involvement of the substance P system during opioid withdrawal also in the rat. The enhanced production of SP1-7 may reflect a increased release and/or metabolism of SP, which, in turn, counteracts the withdrawal.

Tu60

NALTREXONE USE IN CHINA.

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Since methadone maintenance regimen is not used in China, naltrexone becomes the main adjuvant for prevention of relapse after detoxification in China. The plasma concentration-time curve after oral administration of naltrexone 50 and 100 mg in healthy volunteers conforms to first-order absorption and two-compartment model. The pharmacokinetics of naltrexone in Chinese is not different statistically from that in Westerns. Naltrexone has been used successively in more than 1000 cases in 40 hospitals for voluntary detoxification since 1992. The rate of accepting naltrexone in different hospitals was about 20%-30%. The rate of maintaining medication for half a year was about 30% and duration of keeping drug free after discontinuation of medication was usually 3 months to 1 year. All these patients had history of relapse in the past and the time to relapse was less than 1 duration of keeping drug free after discontinuation of medication month on average. The rate of maintaining medication for half a year in patients taking placebo was only 7%. During the period of taking naltrexone no clear adverse reaction was seen except mild increase in GPT in a few patients, the patients who took placebo also had other similar protracted withdrawal syndromes. For convenience of adjusting dosage and development of new indications, the naltrexone marketed in China is in 5mg tablet form. The long-lasting injection form is under development at present.

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LONG TERM CHANGES IN THE BEHAVIOR OF RATS AFTER TREATMENT WITH MORPHINE: EVIDENCE OF THE "ADDICTION MEMORY" ?

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To address the question of a putative addiction memory, rats were rendered tolerant to morphine by injection of increasing doses of morphine from 20 to 120 mg/kg per day during a period of 21 days. After this treatment the antinociceptive effect of a test dose of 10 mg/kg morphine was no more evident.

Four weeks after completion of the morphine treatment the self-administration behavior of the animals was investigated using a computer-controlled apparatus. Rats could trigger an intraventricular injection of 2 µg morphine via a microcanula by a nose poking reaction. The self-administration behavior was registered for 1 hour per day for 17 days. Whereas rats previously tolerant to morphine and control animals started with the same morphine injection rate, the self-administration increased more quickly in the morphine group. After changing the injection solution to saline the injection rate of all rats decreased. In a conditioned place preference task with morphine as stimulus, rats previously tolerant to morphine learned the place preference with 1 mg/kg morphine as stimulus, whereas control rats needed the higher dose of 5 mg/kg. In addition, a stronger locomotor hyperactivity after dopaminergic stimulation by 0.7 mg/kg apomorphine was seen in rats 8 weeks after morphine treatment as compared to controls.

These findings that a former experience of morphine accelerates the self-administration of the opiate, reinforces place preference learning and causes a dopaminergic hyperresponsiveness, support the hypothesis of the existence of an addiction memory.

Tu63

CHRONIC HEROIN ABUSE PRODUCES MARKED ALTERATIONS IN SYNTHESIS AND STORAGE OF DOPAMINE IN HUMAN BRAIN.

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Chronic abuse of heroin produces alterations in brain neurochemistry that underlie the tolerance and addiction of this drug. Since opioid agonists such as heroin regulate dopamine release in terminal regions of the brain, it is likely that continued use of heroin will produce alterations in dopaminergic markers. Immunautoradiographic and radioligand binding techniques were used to measure levels of the dopamine and vesicular monoamine (VMAT) transporters, and tyrosine hydroxylase in the striatum and nucleus accumbens of human brain from heroin overdose and drug-free and age-matched control cases. Antibodies against the dopamine transporter, tyrosine hydroxylase or VMAT were used to visualize the distributions of these synaptic proteins. Chronic heroin abuse and subsequent overdose produced a significant decrease in VMAT in the anterior caudate and putamen, and in the nucleus accumbens of human brain. Tyrosine hydroxylase immunoreactivity also was reduced in these regions in heroin overdose, compared to control cases. In contrast, there were no significant alterations in the dopamine transporter in these brain regions. Thus, chronic heroin use interferes with both synthesis and storage of dopamine. Supported by DA-09484

Tu62

DECREASED MU (OP-3) OPIOID RECEPTOR-ACTIVATED G-PROTEINS FOLLOWING CHRONIC HEROIN SELF-ADMINISTRATION IN RATS.

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We have previously shown that chronic morphine treatment decreased mu opioid-stimulated [³⁵S]GTPγS binding in specific brainstem nuclei (J. Neurosci. [1996] 16, 2684). In this study, [³⁵S]GTPγS autoradiography was performed using DAMGO and DPDPE in brain sections from rats trained to self-administer i.v. heroin for 6-10 weeks with up to 300 mg/kg/day of heroin. Analysis of autoradiograms showed significant decreases in DAMGO-stimulated [³⁵S]GTPγS binding in brainstem nuclei including locus coeruleus, parabrachial n., dorsal raphe n. and PAG. In contrast to chronic morphine treatment, chronic heroin self-administration also significantly decreased DAMGO-stimulated [³⁵S]GTPγS binding in forebrain regions including nucleus accumbens, caudate-putamen, thalamus and amygdala. No changes were observed in delta opioid-stimulated [³⁵S]GTPγS binding. These data indicate that regional differences exist in desensitization of mu opioid-activated G-proteins following chronic non-contingent morphine administration versus heroin self-administration. Supported by NIDA (DA06634, DA00287 and DA00247).

Tu64

CRAVING TO MORPHINE MAY BE RELATED WITH THE LEVEL OF ANXIETY IN TWO INBRED STRAINS OF RATS.

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The associations of genetic differences in the morphine-related behavior, level of anxiety and characteristics of central benzodiazepine receptors were studied in two inbred rat strains - WAG/G and Fischer-344. The level of intravenous morphine self-administration in F-344 rats was 4-10 times greater than in WAG/G rats.

The study of anxiety level in different experimental paradigms show, that Fischer-344 rats demonstrated higher level of anxiety, than WAG/G rats. Density of benzodiazepine receptors in brains of WAG/G rats were much higher, than in Fischer-344 rats. Affinity of benzodiazepine receptors had no strain differences. These finding let us to propose that the level of anxiety and activity of endogenous benzodiazepine system may be connected with the sensitivity to morphine reinforcement in rats.

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INHIBITION OF HEROIN INDUCED PLACE PREFERENCE BY aODN TARGETING THE RAT DELTA OPIOID RECEPTOR.

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When infused into the rat brain for 21 days, four different aODN targeting each of the exons of the δ -opioid receptor gene (DOR), were able to reduce the δ -opioid receptor binding of four different δ -opioid ligands (DELTA-I, DELTA-II, DPDPE and NLT) in rat brain homogenates. Only the aODN₂₈₀₋₂₉₉ (exon 2) appeared to distinguish between the two putative δ -opioid receptor subtypes since it depressed the binding of the δ_2 preferring ligands DELTA-I (-42%) and DELTA-II (-51%) more intensely than the binding of the δ_1 ligand DPDPE (-28%); moreover, the infusion of aODN₂₈₀₋₂₉₉ inhibited DELTA-I induced locomotor behaviour and place preference, without affecting DPDPE-induced locomotor responses. aODN₂₈₀₋₂₉₉ treatment attenuated heroin-induced place preference without affecting the place aversion produced by heroin withdrawal.

Tu67

INVOLVEMENT OF KAPPA OPIOID RECEPTORS IN EXPERIMENTAL DRUG ADDICTION.

M.A.F.M. Gerrits¹, A.V. Kuzmin², E.E. Zvartau² and J.M. van Ree¹. ¹Department of Pharmacology, Rudolf Magnus Institute for Neurosciences, Utrecht University, Utrecht, The Netherlands. ²Laboratory of Pharmacology, Pavlov Medical Institute, St. Petersburg, Russia. Modulation of the reinforcing effects of cocaine and morphine by κ -opioid receptor ligands was studied using the method of intravenous self-administration in drug-naive rats and DBA/2 mice. Self-administration of cocaine (by rats) and morphine (by mice) was readily initiated and showed an inverted U-shaped curve. Treatment with the κ -opioid receptor agonist U50,488H dose dependently decreased the intake of both cocaine and morphine when offered in doses that readily initiated and sustained self-administration behavior. Interestingly, treatment with U50,488H induced self-administration behavior with lower sub-threshold doses of cocaine and morphine. With regard to the inverted U-shaped relation between dose of the drug and the number of self-infusions, activation of the κ -opioid receptor with U50,488H produced an almost parallel shift to the left, indicating an increased sensitivity for the reinforcing effects of cocaine and morphine, while blockade of the κ -opioid receptor with nor-BNI produced a shift of the dose response curve to the right, suggesting a decreased sensitivity for the reinforcing effects of cocaine. These data demonstrate an involvement of κ -opioid receptors in the neurobiological mechanisms underlying drug addiction in general, and the sensitivity for drug reward in particular.

Tu66

ROLE OF OPIOID RECEPTOR TYPES IN PENTAZOCINE-INDUCED PLACE PREFERENCE UNDER INFLAMMATION.

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Pentazocine (PET) frequently used as an analgesic has a weak dependence-producing potential. Recently, we reported that morphine-induced place preference was suppressed under formalin-induced inflammation. In the present study, we examined the effect of inflammatory nociception on PET-induced place preference and the role of opioid receptor types in the preference, using conditioned place preference paradigm. 2.5 % formalin (50 μ l) was injected into the plantar surface of the right hind paw of rat 24 hr before the conditioning. Rats injected s.c. with PET or saline were confined to respective compartment once daily. On day 5, conditioning scores were measured. In non-inflamed group, PET (10, 17 mg/kg) produced a significant place preference, while, in inflamed group, higher doses of PET (25, 29 mg/kg) did. In inflamed group, PET-induced place preference was significantly suppressed by δ receptor antagonist NTI and selective δ_2 receptor antagonist NTB, but not by κ -receptor antagonists. These results suggest that the dose-response curve for PET-induced place preference was shifted to the right under inflammation, and that δ receptor system, especially δ_2 receptor, may be involved in this shift.

Tu68

Role of σ receptor in discriminative stimulus effect of κ receptor agonist U-50,488H in rats. M. Nomura, T. Kazama, Y. Fukuoka, T. Suzuki, M. Misawa and H. Nagase*
Department of Pharmacology, School of Pharmacy, Hoshi University, Tokyo and *Basic Research Laboratories, Toray Industries, Inc., Kamakura, Japan.

It is known that κ receptor agonists have aversive and psychotomimetic effects, and σ receptors may be involved in the expression of psychotomimetic effects. In the present study, the effects of σ receptor antagonist NE-100 on the aversive effect produced by κ receptor agonist U-50,488H and the effects of non-competitive NMDA receptor antagonist phencyclidine (PCP) and NE-100 on the discriminative stimulus effect of U-50,488H in rats were examined. Eight male Fischer 344 rats were trained to discriminate between U-50,488H (3 mg/kg) and saline under a fixed ratio (FR) 10 schedule. After the animals attained the criterion, tests were initiated. NE-100 attenuated the U-50,488H aversion and discrimination. PCP generalized to the U-50,488H discrimination, and the generalization of PCP was attenuated by NE-100. These results indicate that the U-50,488H aversion and discrimination may be related to the activation of σ receptor, and may reflect PCP-like psychotomimetic effects via σ receptor. Furthermore, these results suggest that the aversive effect of U-50,488H may result from the psychotomimetic effects.

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Tu69

IMMUNOCYTOCHEMICAL LOCALIZATION OF SECOND MESSENGERS IN THE RAT EXTENDED AMYGDALA.

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Chronic opiate administration is known to cause long-lasting changes in second messenger systems associated with the μ -opiate receptor. Many of these changes have been shown to occur in the extended amygdala, a region strongly associated with the rewarding properties of opiate use. In order to elucidate the anatomical and cellular localization of some of these changes in this region, we examined the immunocytochemistry of several antibodies made against proteins active at various points in the second messenger cascade. Antibody labeling against the G protein α subunit and protein kinase A was distributed in cell bodies throughout the extended amygdala, particularly in the central nucleus. Protein kinase C labeling was also seen in cells in the central nucleus and in both cell bodies and processes in the anterior bed nucleus of the stria terminalis. CREB labeling was localized to the nucleus of cells in the accumbens, anterior and lateral bed nucleus of the stria terminalis and was found in many cells in the basolateral amygdala. Results suggest that the extended amygdala is a rich source of second messenger system associated with μ -opioid receptor activation.

Tu71

EFFECTS OF MORPHINE AND COCAINE ON THE BIOSYNTHESIS OF OPIOID PEPTIDES AND CRF IN RAT AMYGDALA.

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The mesolimbic system, especially the extended amygdala plays an important role in the mechanism of drug addiction. The extended amygdala is comprised of the central nucleus of amygdala and the shell of the nucleus accumbens. The amygdala is rich in numerous of the neuropeptides implicated in the reinforcing action of drugs of abuse, such as proenkephalin (PENK), prodynorphin (PDYN) and corticotropin-releasing factor (CRF). We studied changes in the level of PENK, PDYN and CRF mRNA in the central nucleus of amygdala after acute and chronic morphine and cocaine administration. Morphine was injected i.p. in a single dose of 20 mg/kg, or twice daily for 10 days in increasing doses of 20-100 mg/kg. Cocaine was administered acutely and repeatedly (20 mg/kg i.p., every hour, for 1 or 5 days). Rats were decapitated 3 h after a single and last dose of chronic morphine or cocaine and, additionally, 24 and 48 h after chronic administration of either drug. Single morphine and cocaine administration showed a tendency to increase the levels of PDYN and PENK mRNA in the central nucleus of amygdala. Chronic administration of morphine decreased, whereas chronic cocaine did not change the level of PDYN mRNA; and, on the other hand, neither drug changed the PENK mRNA level. Withdrawal of the drugs increased the level of PDYN mRNA in that structure. An increase in the CRF mRNA level was observed 3h after single morphine and at the time of cocaine withdrawal. Such specific adaptive changes in peptide systems may play some role in the neurochemical mechanism of morphine and cocaine addiction. *Supported by Institute's statutory funds.*

Tu70

c-FOS EXPRESSION IN THE BED NUCLEUS-AMYGDALA COMPLEX OF THE RAT FOLLOWING ACUTE ADMINISTRATION OF OPIOID AGONIST OR ANTAGONIST.

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Objectives: Acute morphine increases c-Fos expression in diverse forebrain regions, including the bed nucleus of the stria terminalis (BST) and amygdala. The present study tested for differential effects of opioid agonists or antagonists [morphine (MOR), tilidine (TIL; partial μ -opioid agonist), naloxone (NAL), or co-administration of μ -opioid agonist and antagonist] on c-Fos expression in these areas. *Methods:* Experiments were performed in drug-naive normal male rats (200-250g). Following administration (s.c.) of the various compounds (mg/kg: MOR 10; NLX 10; TIL 200; NLX 10 plus MOR 10; NLX 10 plus TIL 200), rats were fixation-perfused. Saline-treated rats served as controls. Brains were immunohistochemically processed for c-Fos-positive neurons (c-Fos-pn) in cryostat sections and quantified in regions of interest. *Results:* Compared to controls (35-40 c-Fos-pn/50 μ m section), the number of c-Fos-pn was increased in the lateral BST (BSTL) after MOR (ca. 180% of control levels) and TIL (ca. 300%), but also after NLX (ca. 220%). Interestingly, co-application of NLX did not reduce TIL-induced increase of c-Fos expression, but showed the highest rise (NLX+TIL: ca. 560%); animals treated with NLX plus MOR displayed an increase amounting to ca. 235%. A similar pattern of differential c-Fos expression for the various groups was observed in the central amygdala, a region known to form a functional unit with the BSTL. *Conclusions:* The data show that besides opioid agonists, also an antagonist (NLX) may activate c-Fos expression in the areas investigated. Interestingly, in combination with an agonist an additive/synergistic effect is seen. This may indicate intrinsic effects of NLX or an interference with endogenous opioidergic circuits. Further analysis investigates the dose-dependency and region-specificity of these observations.

Tu72

MU-OPIOID RECEPTOR-STIMULATED [³⁵S]GTP-g S AUTORADIOGRAPHY IN FETAL RHESUS MONKEY BRAIN: EFFECT OF PRENATAL COCAINE EXPOSURE.

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Prenatal cocaine treatment decreases μ -opioid receptor mRNA expression in the day 70 fetal diencephalon, although μ -opioid receptor binding density is not altered. Therefore, we studied the effect of cocaine exposure on the functional coupling of μ -opioid receptors and G-proteins in fetal brain with DAMGO-stimulated [³⁵S]GTPg S (0.05 nM, 1250 Ci/mmol, NEN) binding. Pregnant monkeys were treated with (-)-cocaine (3 mg/kg, i.m., 4 times/day) or saline (n=4, each) from day 22 of gestation until day 70. DAMGO (1 μ M) specifically stimulated [³⁵S]GTPg S binding in the thalamus, medial habenula, fasciculus retroflexus, interpeduncular nucleus, substantia nigra, parabrachial nucleus and nucleus of the solitary tract (up to 400 %), which was blocked by naloxone (0.1 μ M). DAMGO-stimulated [³⁵S]GTPg S binding was found only in areas with dense [³H]DAMGO binding, and not in areas with less dense DAMGO binding such as the striatum and prefrontal cortex. Fetal cocaine exposure had no significant effect on basal or DAMGO-stimulated [³⁵S]GTPg S binding at this stage in gestation. In conclusion, μ -opioid receptors are functionally coupled to G-proteins in the day 70 fetal monkey brain, but this coupling appears not to be influenced by cocaine exposure. (Supported by NIH Grants DA07165 and P51-00163).

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Tu73

CHRONIC INTERACTIONS BETWEEN OPIOID AND CANNABINOID AGONISTS IN N18TG2 CELLS.

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Etorphine and desacetyllevonantradol (DALN) stimulated [³⁵S]GTPγS binding to isolated membranes of N18TG2 cells which co-express d opioid and CB1-cannabinoid receptors. Long-term exposure of the cells to either etorphine or DALN induced desensitization to the respective drug. In addition, asymmetric cross-tolerance was found: DALN induced homologous desensitization and did not reduce the effect of opioids, while etorphine induced heterologous desensitization and partially reduced the effect of cannabinoids. Chronic treatment of N18TG2 cells with either drug not only induced desensitization, but also elevated basal activity of G-proteins in the exposed cells. The combination of etorphine and DALN failed to yield an additive elevation, and a significant interference between the two drugs was found, suggesting that chronic exposure to opioid and cannabinoid agonists modified a common responding element within the cells. This work presents N18TG2 cells as a suitable experimental model to study the molecular mechanism(s) underlying chronic interactions between opioid and cannabinoid drugs.

Tu75

ALCOHOL CONSUMPTION IS ELEVATED IN *Mas*-DEFICIENT MICE.

B. Maul¹, G.Heder¹, T. Walther², M. Bader² and W.-E. Siems¹

¹Dept. Mol. Neurobiol., FMP, Berlin and ²Dept. Hypert. Res., MDC, Berlin-Buch, Germany. The multiple relations between the renin-angiotensin system and the consumption of alcohol in animals have been controversially discussed during recent years. The evidence that the *Mas* oncogene is involved in angiotensin signalling has focussed our interest on alcohol consumption and craving in mice with a deficient *Mas* gene (*Mas*^{-/-}) compared to the corresponding wildtypes (*Mas*^{+/+}). *Mas*^{-/-} mice drank highly significantly ($p < 0.001$, 72%) more ethanol in free choice experiments with water and 10% ethanol than did *Mas*^{+/+} mice. Oral intake of the ACE inhibitor spirapril (10mg/kg) led to only slight reductions of voluntary alcohol consumption in both groups of animals (*Mas*^{+/+}: 13%, *Mas*^{-/-}: 19%). Biochemical and molecular assays confirmed that the chronic ethanol consumption elevated the activity and expression of several peptidolytic enzymes involved in neuropeptide degradation in different regions of the CNS.

Tu77

ETHANOL CONSUMPTION AND ITS INFLUENCE ON THE d-OPIOID RECEPTOR GENE EXPRESSION IN THE GI-TRACT OF THE RAT.

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Ethanol consumption influences the opioid/ opiod receptor system but little is known about those relations within the digestive system. Previously we described the μ- and κ-receptor expression in the gut, here we present the d-receptor expression via RNase protection assay (with GAPDH-expression used for data correction). When comparing the consumption of ethanol, rats preferred 5% (2.79g Eth/day and kg body weight) and 20% Eth solutions (3.68g Eth/ day and kg body weight). The d-receptor expression in control rats was very similar in stomach (St), duodenum (D), proximal and distal colon (Pc, Dc), but was about 2 times higher in the ileum (Il) compared to the other regions. This pattern changed after 10 weeks of ethanol consumption. d-receptor expression in St, D and Dc were reduced by 50-60%, in Il by about 80%, but increased in Pc by about 100%.

Tu74

EFFECTS OF CONCURRENT CHRONIC ETHANOL AND BINGE COCAINE ADMINISTRATION ON BRAIN KAPPA mRNA LEVELS AND STEREOTYPIC BEHAVIOUR IN RATS.

E. Rosin, S. Lindholm, J. Franck and J. Georgieva. Department of Clinical Neuroscience, Experimental and Clinical Drug Addiction, Karolinska Institute, Stockholm, Sweden. The concurrent abuse of cocaine and alcohol is a common phenomenon among human addicts. The reinforcing effects of cocaine as well as ethanol are partly mediated via the endogenous dynorphin-kappa opioid system. We have studied the effects of forced chronic ethanol exposure combined with cocaine treatment, looking for changes in KOR mRNA levels. In addition, we studied stereotypic responses (sniffing, rearing, repetitive head movements, etc.). Rats were treated with either ethanol (2g/kg*day, i.p.) or saline, twice daily for 14 days. On days 13 and 14, cocaine was administered in a binge pattern (3 i.p injections separated by 1 h; 15 mg/kg*binge) to rats in both the ethanol and saline group. On day 14, rats were decapitated 30 minutes after the last cocaine/saline binge and 11 different brain regions were dissected out. Quantitative measurements of KOR mRNA levels were performed by competitive RT-PCR. Behavioural stereotypies were studied by video taping all animals, and analysed according to the rating scale of Creese and Iversen. The effects on the kappa opioid mRNA levels in different brain regions following coadministration of cocaine and ethanol as well as the behavioural changes associated with it will be discussed.

Tu76

ALCOHOL CONSUMPTION IN TRANS-GENIC MICE LACKING OR OVEREX- PRESSING ANGIO-TENSINOGEN.

W.-E. Siems¹, B. Maul¹, G. Heder¹, A. Winkler¹, T. Walther² and M. Bader².

¹Dept. Mol. Neurobiol., FMP, Berlin and ²Dept. Hypert. Res., MDC, Berlin-Buch, Germany. The relationship between the renin-angiotensin system and consumption of alcohol in animals has been controversially discussed in recent years. Transgenic mice overexpressing angiotensinogen (123+) and mice lacking angiotensinogen (TLM-) should be excellent models to clarify this matter. 123+ mice drank 53% more and TLM-mice drank 33% less ethanol in free choice experiments with water and 10% ethanol compared to the corresponding wildtype ($p < 0.001$ in each case). Oral administration of the ACE inhibitor spirapril (10mg/kg) significantly reduced the alcohol consumption of 123+ mice (minus 14%, $p < 0.05$), but there was no significant reduction of these values for either wildtype or TLM-mice. The influence of chronic ethanol consumption on the expression and activity of several peptidolytic enzymes in the brain also varied between the strains.

Tu78

GLUTAMATERGIC AFFERENTS FROM NEO-CORTEX MAINTAIN THE PROENKEPHALIN GENE EXPRESSION IN ORGANOTYPIC SLICES OF RAT NEOSTRIATUM

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We have investigated, whether neocortical projection fibers cause the strong expression of the PENk gene in the neostriatum. Slice cultures were used which contained the neostriatum with or without the adjacent neocortex. In neocortical-neostriatal slices, in situ hybridization showed a large number of neurons in the neostriatum which strongly expressed the PENk gene. After removal of the neocortex, neurons which expressed the gene in the neostriatum gradually disappeared during the subsequent 4 days. When neocortical-neostriatal slices were treated with the NMDA receptor antagonists MK801 or APV PENk gene expression disappeared within 2 days. In contrast, NMDA induced the expression of the gene within 4 days in isolated striatal slices. The protein kinase inhibitor H7 as well as the Ca²⁺/calmodulin kinase inhibitor KN-62 strongly reduced the effect of NMDA. In summary, these experiment showed that the neostriatal Penk gene expression was under the control of glutamatergic neocortical afferents which acted via NMDA receptors. (Acknowledgement: The study was supported by Deutsche Forschungsgemeinschaft Me 541/3-2).

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Tu79

A NOVEL URIDINE DIPHOSPHATE GLUCURONOSYLTRANSFERASE (UGT) IN GUINEA PIG: UP REGULATION WITH IN UTERO MORPHINE EXPOSURE.

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Morphine is metabolized to 2 glucuronides by uridine diphosphate glucuronosyltransferases (UGTs). To identify UGTs that are regulated during in utero morphine exposure and may play a role in morphine metabolism, a morphine treated guinea pig liver cDNA library was prepared and screened. Analysis of cDNAs isolated revealed a novel type 2 UGT (UGT-2GP). Northern blot analysis revealed 3 isoforms of mRNA for UGT-2GP. Sequence analysis revealed the splicing occurs in the 3' untranslated region of the mRNA. UGT-2GP is expressed in guinea pig liver, kidney and small intestine. RT-PCR analysis of control and chronic morphine exposed 7 day pups showed significant up regulation of UGT-2GP in liver following morphine exposure. Fetal developmental effects induced by chronic morphine exposure may involve regulation of specific UGTs by morphine. Supported by NIDA, KO8DA00295

Tu81

CHANGES IN OPIOID RECEPTORS IN THE BRAIN OF PREWEANLING MALE AND FEMALE RATS FOLLOWING CHRONIC TREATMENT WITH NALTRINDOLE.

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¹University of Surrey, UK. ²Universidad Complutense, Madrid, Spain.

Antinociceptive studies of 20 day old rats following chronic administration of naltrindole have demonstrated loss of μ receptor mediated analgesia in males but not females. We have performed quantitative autoradiography of μ , δ and κ opioid receptors in the brain using [³H]-DAMGO, [³H]-deltorphin I and [3H]-CI-977 following chronic administration of naltrindole (1mg/kg s.c.) or saline solution (from day of birth to day 19). Quantitative comparisons were made across cortical and non-cortical regions for both sex and naltrindole treatment differences. In saline treated 20 day old males [³H]-DAMGO binding was higher in both cortical and non-cortical areas and [³H]-CI-977 binding higher in cortical areas than in corresponding females. These sex differences were lost by 25 days of age. [³H]-deltorphin I binding did not demonstrate sex differences at either age studied. Naltrindole treatment produced isolated regional differences in μ and δ receptor density in both age and sex groups. [³H]-CI-977 binding was not affected by naltrindole treatment. The results support the existence of μ - δ receptor interactions during development and also indicate sexual differences in μ and κ receptor ontogeny.

Tu80

THE EFFECT OF NEONATAL HANDLING ON OPIOID PEPTIDES.

Karolina Ploj, Lena Bergström, Erika Roman and Ingrid Nylander, Department of Pharmaceutical Biosciences, Division of Pharmacology, Uppsala University, Uppsala, Sweden. Early postnatal handling during the critical period of development, i.e. the first 21 days of life in the rats, has shown attenuation of neuroendocrine responses to stress both at the behavioral and the biochemical levels in adult life. The purpose for this study was to investigate if postnatal handling can induce long-term biochemical changes in brain opioid levels in female Sprague-Dawley rats. The rat pups were removed from their cages and placed together in small containers. Fifteen minutes later they were returned to their home cages and mothers. This procedure was performed daily for 21 days. Two months after this period, the rats were decapitated and the opioid peptides dynorphin B, Met-enkephalin Arg6Phe7 (MEAP) and nociceptin were analysed with specific radioimmunoassay. In this study we found that handled rats had increased levels of dynorphin B, MEAP and nociceptin in the periaqueductal grey compared to controls. Further, handled rats had decreased levels of dynorphin B in the frontal cortex and decreased levels of MEAP in the striatum compared to controls. All changes were statistically significant. The results indicate that a manipulation early in life induce permanent biochemical changes in the opioid peptide system.

Tu82

DIFFERENTIAL EFFECTS OF SODIUM IONS AND GUANINE NUCLEOTIDES ON δ LIGAND RECEPTOR RECOGNITION IN WEANED AND NONWEANED RAT BRAIN.

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We have previously shown that there are differing antinociceptive responses to δ agonists in weaned and non-weaned rats and that weaning increases δ receptor binding in homogenates of whole rat brain at day 25. This has been confirmed using two new δ receptor selective deltorphin analogues. Weaning induced activation of δ receptor might be due to stimulation of a δ receptor subtype or activation of G-protein coupling processes. To address the latter possibility we have carried out saturation studies with four [³H] δ receptor agonists and one antagonist in the presence of Na⁺ and a GTP analogue. The binding of three agonists is sensitive to the presence of the GTP analogue in weaned but not non-weaned rat brain and the effects of Na⁺ are ligand and treatment dependent. The results show ligand dependent sensitivity to membrane environment and suggest the weaning process may influence δ receptor/G-protein coupling.

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Tu83

CHRONIC NEONATAL NALTRINDOLE ADMINISTRATION AND MANIPULATION AFFECT ADRE-NOCORTICAL REACTIVITY IN YOUNG RATS.

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We have recently shown that the functional blockade of the δ -opioid receptor during the preweaning period by a chronic naltrindole (NTI) treatment markedly reduced a δ -mediated form of stress-induced antinociception (SIA) in 25-day old female rats. We have studied the effects of the same NTI treatment (1 mg/Kg s.c. daily injections from birth to day 19) on the corticosterone response to the same stress-procedure (a 3 min swimming session in water at 20°C) in 25-day old Wistar rats of both sexes. The effects of manipulation were studied by including two control groups, one group received daily injections of saline and a second control group was not manipulated. Animals were sacrificed by decapitation and blood samples were collected from the trunk for serum corticosterone determinations by Radioimmunoassay. Basal and post-stress (5 min following swim-stress) corticosterone levels were obtained in all groups. Control non-manipulated animals showed a significant increase in corticosterone levels in response to stress ($p < 0.05$). Manipulation decreased basal hormone levels in females and prevented the rise in corticosterone in response to stress in males ($p < 0.05$). Neonatal NTI treatment significantly reduced the corticosterone response to stress in females ($p < 0.05$) and these animals also showed a trend towards increased basal hormone levels. The results indicate the involvement of the δ -opioid receptor in the modulation of the adrenocortical response to stress in young female rats and the existence of sex activity during the postnatal period. Taken into account our previous findings in studies on SIA, the results also support the utility of the corticosterone response to stress as a functional correlate of SIA. Supported by the European Commission BMH4-CT96-0510 (DG 12-SSMA).

Tu85

THE EFFECT OF NALOXONE ON THE DIFFERENTIATION OF PC12 CELL INDUCED BY NGF.

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We determined the effect of naloxone and its antagonist, morphine, on the differentiation of pheochromocytoma cell, the PC12 cells, induced by nerve growth factor (NGF). PC12 cells were grown in medium containing NGF (50 ng/ml) with or without morphine (10 μ M), and/or naloxone (10 μ M) for 10 days. NGF induced morphological differentiation of PC 12 cells manifested by the increase in percentage of differentiated cells and the average length of neurites per cell. Co-addition of morphine with NGF did not affect both parameters as compared to that of NGF alone. On the contrary, co-addition of naloxone with NGF significantly increased the percentage of differentiated cells, but did not affect the neurites outgrowth. This effect of naloxone was reversed by the addition of morphine, suggesting that naloxone produced its effect by inhibiting the endogenous activity of opioid receptor. The present study indicates a significant functional role of opioid receptor in NGF-induced differentiation of PC12 cells.

Tu84

DEVELOPMENTAL CHANGES IN CROSS-TALK BETWEEN κ -OPIOID AND β -ADRENERGIC RECEPTORS IN THE CARDIOMYOCYTE OF HYPERTENSIVE RATS

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κ -receptor exerts a negative modulatory action on β -adrenoceptor and the action is blunted in adult spontaneously hypertensive rats (SHRs). In order to determine whether the blunted negative interaction of κ -opioid receptor with β -adrenoceptor contributes to the development of hypertension, the electrically-stimulated intracellular calcium [Ca^{2+}] transient was measured in the single ventricular myocyte of SHRs at 4, 6, 8 and 13 weeks old and the age-matched WKY rats. Both the basal and augmented [Ca^{2+}] transients by norepinephrine (NE) a β -adrenoceptor agonist, were attenuated by U50,488H and the attenuation was blunted in SHRs of 13 weeks old. Interestingly, the altered responses were not changed in the SHRs with blood pressure (BP) restored to normal. In conclusion, the blunted inhibitory effects of κ -opioid receptor stimulation occurred in SHR after hypertension was fully developed, indicating that this attenuated inhibitory modulation does not contribute to the initiation of hypertension. The blunted response occurred in hypertensive rats with or without normalization of BP, indicating that the response is not secondary to hypertension. Both NE and forskolin at 0.5 μ M significantly increased electrically-induced basal [Ca^{2+}] transient. The effects were attenuated in SHRs at 8 weeks of age when the BP increases rapidly. The diminished responses of β -adrenoceptor and adenylate cyclase may not contribute to the blunted inhibitory modulator of κ -opioid receptor on β -adrenoceptor. (Supported by Research Grants Council, Hong Kong)

Tu86

BOMBESIN INHIBITS METHADONE-INDUCED APOPTOSIS OF HUMAN LUNG CANCER CELLS

Rhoda Maneckjee and Wendy L. Heusch. Oregon Health Sciences University of Surgical Oncology, Portland, OR 97201-3098 USA.

The therapeutic opioid drug methadone, generally used to treat cancer pain and opioid addiction, is also a potent inducer of apoptosis in human lung cancer cells, thereby inhibiting their growth. However, in contrast to its CNS actions, its apoptosis-inducing effect appears to be mediated through a non-opioid mechanism involving bombesin, an autocrine growth-stimulatory factor for lung cancer cells that plays a central role in pulmonary carcinogenesis. Exposure of lung cancer cells (NCI-N417 and -H157) to methadone resulted in an increase in mitogen-activated protein (MAP) kinase phosphatase levels and inactivation of MAP (ERK) kinase, suppression of bcl-2 protein and induction of apoptosis. These effects of methadone could not be blocked by pertussis toxin, and methadone binding to lung cancer cell membranes was not inhibited by GTP. However, methadone's effects were reversed by bombesin in a dose-dependent manner. "Variant" small cell lung carcinoma (SCLC) and non-SCLC cells, which secrete trace amounts (< 0.1 pmol/mg protein) of bombesin-like peptides compared to "classic" SCLC cells, are more responsive to methadone. Since these histologic types respond less to conventional therapy, these findings suggest a novel therapeutic approach for their treatment (supported by USPHS grant CA 59037).

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Fr01

ENDOMORPHIN 1 AND ENDOMORPHIN 2: EFFECTS ON THE SIGNAL TRANSDUCTION PATHWAYS.

Krisztina Monory¹, E. Tzavara³, M.-C. Bourin³, G. Toth², H. W. Matthes⁴, B. L. Kieffer⁴, J. Hanoune³ and A. Borsodi¹, ¹Inst. of Biochemistry and ²Isotope Laboratory, Biological Research Center, Szeged, Hungary, ³INSERM U-99, Hopital Henri Mondor, Creteil, France, ⁴UPR 9050 CNRS, ESBS Universite Louis Pasteur, Illkirch, Strassbourg, France. Signalling properties of endomorphin 1 (Tyr-Pro-Trp-Phe-NH₂) and 2 (Tyr-Pro-Phe-Phe-NH₂) were studied in both cellular and animal models. Experiments were carried out on rat striata, CHO cells transfected either with μ or δ opioid receptors, and on brains of wild type or mutant mice lacking the μ opioid receptor gene. Both tetrapeptides stimulated [³⁵S]GTP γ S binding and inhibited adenylyl cyclase activity in membranes from rat striata or CHO cells stably expressing the μ -receptor. These effects were reversed by the μ receptor specific antagonist CTAP (D-Phe-Cys-Tyr-D-Trp-Arg-Thr-Pen-Thr-NH₂). Endomorphins were unable to produce such changes in cells stably expressing the delta receptor. In membranes of mice lacking the receptor, neither the stimulation of the [³⁵S]GTP γ S binding, nor the inhibition of adenylyl cyclase were detected upon endomorphin exposure. These data strongly support the hypothesis that endomorphin 1 and 2 act through the μ opioid receptor exclusively.

FR03

CONFORMATIONAL ANALYSIS OF ENDOMORPHIN-1 USING NMR AND MOLECULAR MODELLING.

Paterlini MG, Podlogar BL, Ferguson DM, Leo GC, Codd EE, Demeter DA, Brown, FK, Reitz AB. Dpt. of Medicinal Chemistry and Supercomputer Institute, University of Minnesota, Minneapolis, MN 55455. The W. Johnson Pharmaceutical Institute, Raritan, NJ 08869. The structural attributes of endomorphin-1 (EM1) were studied using 2D-NMR and molecular modeling. NMR data in DMSO and water indicate that EM1 exists in the cis- and trans-isomers in a 25%/75% ratio. In DMSO, cis- EM1 adopts a compact form in which the Tyr and Trp aromatic rings pack against the Pro side chain, whereas the trans-isomer adopts an extended conformation. Condensed-phase molecular dynamics simulations of cis-EM1 do not show the compact conformation observed in DMSO, while simulations of trans-EM1 are in agreement with the NMR data. A structural comparison with the μ -ligand PL017 rule out the compact cis-conformation and show that the trans isomer is likely to be the bioactive conformer. Structural comparisons of trans- EM1 with the μ -ligand D-TIPP, and the delta-ligands TIPP-NH₂ and DPDPE were also performed. A hypothesis is proposed to explain the structural basis for μ and δ selectivity among these ligands.

Fr02

BINDING PROPERTIES OF NEW ENDO-MORPHIN 1 AND 2 DERIVATIVES

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Following the discovery of new endogenous μ -opioid receptor agonists, endomorphin 1 (Tyr-Pro-Trp-Phe-NH₂) and endomorphin 2 (Tyr-Pro-Phe-Phe-NH₂) several derivatives of these compounds were synthesised and tested in radioligand binding assay using μ - (endomorphin 2, DAMGO), δ - (Ile^{5,6} deltorphin 2) and κ - (ethylketocyclazocine) selective tritiated ligands. Dehydration of Pro at position 2 in endomorphin 2 resulted in higher selectivity for μ versus κ sites, but also increased the affinity of the compound towards the δ sites. The K_i values for μ sites were not significantly changed. b -methylation of Phe at position 4 in endomorphins 1 and 2 resulted in enhanced affinity of the ligands towards the μ sites and increased μ/κ selectivity for both compounds and also μ/δ selectivity in case of endomorphin 2. These results suggest an important role of b -methylation of Phe-4 for μ sites affinity and selectivity. The new ligands can be used as tools in different biological assays.

Compound	K _i μ (nM)*	K _i δ /K _i μ	K _i κ /K _i μ
Tyr-Pro-Trp-Phe-NH ₂	2.07	3454	50
Tyr-Pro-Trp-(2S,3S)b MePhe-NH ₂	0.6	1097	247
Tyr-Pro-Phe-Phe-NH ₂	4.97	678	380
Tyr-D Pro-Phe-Phe-NH ₂	4.32	46	1435
Tyr-Pro-Phe-(2S,3S)b MePhe-NH ₂	0.97	9310	904

*measured by [³H]-endomorphin 2

Fr04

ISOLATION AND SEQUENCE OF cDNAs CODING FOR PRECURSORS OF ENDOGENOUS OPIOID PEPTIDES FROM THE GUINEA PIG BRAIN.

K. S. LaForge and M. J. Kreek, Laboratory on the Biology of the Addictive Diseases, The Rockefeller University, New York, U.S.A.
A cDNA lambda bacteriophage library was constructed from RNA isolated from the guinea pig brain and pituitary. The library was screened using radiolabeled probes corresponding to precursors of the endogenous opioid peptides. Positively hybridizing clones were sequenced to confirm the primary structure of the mRNAs which had been previously predicted from the sequences of the corresponding genes. These cDNAs will serve as templates for the generation of cRNA probes for use in ribonuclease protection assays in studies of the neurobiological changes caused by drugs of abuse. Supported by NIH-NIDA DA05130 and DA00049 (MJK)

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Fr05

THE ATYPICAL ANTIDEPRESSANT BUPROPION AFFECTS PRODYNORPHIN GENE EXPRESSION IN RAT BRAIN.

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Functional alterations in neurotransmitter systems occur after chronic exposure to addictive drugs. We reported that opiates, cocaine and methamphetamine differently modulate the gene expression of the opioid precursor prodynorphin (PDYN) in the rat brain. Different drugs of abuse might exert their chronic effects through common neuronal pathways; the possibility exists that, beside addictive drugs, chronic CNS drugs affecting specific neurotransmitter systems might be able to affect opioid gene expression. The aim of this study was to investigate the effect of chronic treatment with the antidepressant (AD) bupropion (BUP, 20 mg/kg ip) for 15 days on the PDYN gene expression in the rat striatum, hypothalamus, hippocampus, and in mesocorticolimbic areas (nucleus accumbens and prefrontal cortex). BUP elicited a significant decrease of PDYN mRNA levels in the hypothalamus (60 +/- 5 % of controls, $p < 0.05$ by Newman-Keuls), in the prefrontal cortex (73 +/- 4 % of controls, $p < 0.05$) and an increase in the striatum (149 +/- 17 % of controls, $p < 0.01$). No changes were observed in the accumbens (112 +/- 14 % of controls) and in the hippocampus (116 +/- 13 % of controls). Our results indicate that PDYN gene expression might be affected tissue-specifically by BUP, as reported for the previously investigated drugs of abuse. Since BUP is a dopamine uptake inhibitor, these findings confirm that dopaminergic transmission participates in the mechanisms regulating opioid gene expression in rat brain, namely in prefrontal cortex, striatum and hypothalamus.

Fr07

SOLUBLE PROTEIN FACTOR WITH NON-OPIOID DYNORPHIN A-BINDING AND CONVERTASE ACTIVITIES.

T. Yakovleva, M. Melzig, I. Nylander, J. Silberring, G. Bakalkin and L. Terenius, Department of Clinical Neuroscience, Karolinska Institute, Stockholm, Sweden.

A novel protein factor with non-opioid dynorphin A-binding sites was characterized in the brain, spinal cord and tumor cell lines. The factor binds dynorphin A(1-13), A(1-17), dynorphin A(2-17) and the proenkephalin fragment peptide E, but not other opioid and nonopioid peptides, opiates and benzomorphans. The IC_{50} for dynorphin A(1-17) and dynorphin A(2-17) was about 50 and 5 nM respectively. SH groups of the factor were apparently involved in the binding of dynorphin A, since PHMB inhibited this process. Bound labelled dynorphin A cannot be easily displaced by a nonlabelled peptide and seemed to undergo conversion to the shorter fragment Leu-enkephalin which remained bound to the protein. The dynorphin A-binding factor had an apparent molecular mass of about 70 kDa and was found in a nuclear extract and in cytosol. These data suggest that the factor functions as a dynorphin A convertase interfering with the activity of this peptide.

Fr06

DYNORPHIN A₍₂₋₁₇₎ STIMULATES AN INCREASE IN $[Ca^{2+}]_i$ IN CULTURED RAT CORTICAL NEURONS BY A NON-OPIOID, NON-NMDA MECHANISM.

Lai, Q. Tang, *R.M. Lynch and F. Porreca, Departments of Pharmacology and *Physiology, The University of Arizona, Tucson, AZ 85724, U.S.A.

Dynorphin A is an endogenous opioid which has a moderate antinociceptive effect when administered in vivo. Numerous studies have also implicated a pathological role of dynorphin A, where exogenous dynorphin A can cause paralysis, neuronal excitotoxicity, long-lasting allodynia and anti-opioid effects. These effects may be blocked by antagonists to the NMDA receptors but not by naloxone, suggesting dynorphin A may directly or indirectly activate NMDA receptors via a non-opioid mechanism. In vitro analyses have shown that dynorphin may have a concentration-dependent, dual action on neuronal excitability. We have examined the non-opioid effect of dynorphin A on cultured cortical neurons from neonatal rat brain by using the non-opioid peptide, (des-Tyrosyl)dynorphin A₍₂₋₁₇₎ (DYN₂₋₁₇). This peptide stimulates an increase in intracellular calcium ($[Ca^{2+}]_i$) in individual neurons in a dose-dependent manner. This excitatory effect of DYN₂₋₁₇ is not blocked by naloxone (10 μ M) or MK-801 (30 μ M), suggesting that this effect of DYN₂₋₁₇ on $[Ca^{2+}]_i$ is mediated by a novel, non-opioid, non-NMDA mechanism. Unlike dynorphin A(1-17), DYN₂₋₁₇ does not stimulate [³⁵S]GTP-g-S binding in cortical brain membranes, arguing against a G-protein mediated pathway. Further experiments are being conducted to further characterize this novel mechanism, which may be important in the excitotoxicity associated with dynorphin.

Fr08

REGULATION OF PROENKEPHALIN GENE EXPRESSION IN RAT AND MOUSE STRIATUM BY NMDA RECEPTOR ANTAGONISTS.

Barbara Ziolkowska and Ryszard Przewlocki; Institute of Pharmacology, Polish Academy of Sciences, Krakow, Poland.

The role of the NMDA receptor in regulation of striatal proenkephalin (PENK) gene expression was investigated in the present study. Mice and rats were injected acutely or chronically with a non-competitive or competitive NMDA receptor antagonist (MK-801 and CGP 40116, respectively), and levels of PENK mRNA in the striatum were measured by in situ hybridization and Northern blot methods. MK-801 (0.2 and 1 mg/kg, i.p.) injected acutely to rats decreased dose-dependently striatal PENK mRNA levels by up to 30% at 12h after injection. Such an effect was not observed after single injection of CGP 40116 (2 or 10 mg/kg, i.p.), nor was it produced by repeated administration of MK-801 (5 injections of 1 to 3 mg/kg, i.p., 12h apart). Moreover, no decrease in the level of striatal PENK mRNA was observed when MK-801 was administered acutely directly into the striatum in a dose of 0.1 or 1 μ g. In the mouse, single injections of MK-801 (0.2 and 1 mg/kg, i.p.) and CGP 40116 (2 and 10 mg/kg, i.p.) did not influence striatal PENK mRNA levels, while chronic administration of either drug (twice daily for 10 days) decreased those levels by 15-30% in the striatum and nucleus accumbens. The above results indicate that glutamate acting via the NMDA receptor stimulates tonically the expression of PENK gene in the mouse striatum and nucleus accumbens. This may be due to the action of glutamate released within the striatum by cortical afferents. On the other hand, the down-regulation of the rat striatal PENK gene expression produced selectively by MK-801, but not CGP 40116, does not seem to involve striatal NMDA receptors and may result from a stimulatory influence of MK-801 on dopaminergic neurotransmission in the nigrostriatal pathway. *Supported by grant No. 4 P05A 006 09 from KBN (Warsaw).*

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Fr09

G-PROTEIN ACTIVATION AND RECEPTOR BINDING OF MET-ENKEPHALIN-ARG6-PHE7-DERIVED-PEPTIDES.

B. Bozo, S. Benyhe, J. Farkas, G. Toth, M. Wollemann, M. Szücs. Institute of Biochemistry, Biological Research Center, Szeged, Hungary
Met-enkephalin-Arg-Phe (MERF) is a naturally occurring heptapeptide that binds to opioid and non-opioid binding sites in the central nervous system. Tyr-D-Ala-Gly-Phe-Met-Arg-Phe (I) and Tyr-D-Ala-Gly-Phe-D-Nle-Arg-Phe (II) were synthesized to achieve proteolitically more stable structures. The effect of these peptides at concentrations of 10^{-9} - 10^{-5} M on GTP-binding regulatory proteins was studied by using agonist-stimulated [35 S]GTPgS binding in rat and frog brain membranes, the latter representing a rich source of k-opioid receptors. The most potent peptide was MERF that displayed about 130% stimulation over basal activities in both systems. Analog (I) was as potent as MERF in rats, but showed a decreased potency in frogs. Analog (II) stimulated [35 S]GTPgS binding by only about 30% even at 10 microM in both systems. These effects were further evaluated by using μ , d or k -selective antagonists. The rank order of the potencies was MERF > (I) >> (II) against [3 H]MERF and [3 H]naloxone binding in rat brain membranes. We concluded that MERF and its derivatives are able to activate G-proteins via k and d opioid receptors. Supported by OTKA T-16084 grant.

Fr10a

CHIMERIC PEPTIDE OF METENKEPHALIN & FMRF, UNLIKE FMRF, DOES NOT ATTENUATE ACUTE AS WELL AS CHRONIC MORPHINE ANTINOCICEPTION *in vivo*.

S. Pasha, S. Gupta & Y.K. Gupta*. Centre for Biochemical Technology (CSIR), *Dept. of Pharmacology, AIIMS, Delhi, India.
Chimeric amphipathic peptide of met-enkephalin & FMRFA (CP-1) was designed to address both opioid and 'antioioid' systems simultaneously to provide further insight into their role in nociceptive mechanisms. I.p. administration of CP-1 in mice produced dose-dependent, naloxone reversible antinociception in the tail flick test. I.c.v. naloxone attenuated the antinociceptive effect of i.p. CP-1. Coinjection of CP-1 potentiated morphine antinociception. These results indicate that the masking of the analgesic action (met-enkephalin portion) by the antioioid FMRFA is not evident. Further, tolerance to the antinociceptive action of morphine was attenuated by CP-1, indicating that the antinociceptive effects may be due to opiate receptor(s) stimulation only and/or by interacting/blocking antioioid receptor(s).

Fr12

NEW DELTORPHIN ANALOGS PREDICTED FROM CLONED cDNAs FROM SKIN OF PHYLLLO-MEDUSINAE FROG.

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By cloning of cDNA from skin of Phyllomedusinae frogs, the existence of a new deltorphin with the sequence of Tyr-Ile-Phe-His-Leu-Met-Asp-NH₂ could be predicted. This peptide was synthesized with either D-Ile (1) or D-Ala (2) as the second amino acid and compared with a number of related peptides. Peptide (1) exhibited d -opioid receptor affinity and biological potency comparable to that of already known deltorphins and three to ten times higher than that of peptide (2). They displayed higher affinity for the d -receptor of NG108-15 cells than for the cloned d -receptor. When given i.c.v. in rats, peptide (1) induced a dose-dependent analgesia (ED₅₀ = 7.5 μ g/rat) antagonized by naloxone (0.1 mg/kg, s.c.) but unaffected by naltrindole (3 mg/kg, s.c.). Grooming and scratching were observed but scarce locomotor activity. High doses always induced spontaneous contralateral barrel rotation.

Fr10

LIGAND BINDING AND PHARMACOLOGICAL PROPERTIES OF DAMGO-CHLOROMETHYL KETONE.

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When DAMGO was equipped with a chloromethyl ketone group at the C-terminal, the resulting compound Tyr-D-Ala-Gly-MePhe-CH₂Cl (DAMCK) retained the μ opioid receptor specificity of the parent compound. [3 H]DAMCK specifically and covalently labelled proteins of about 54, 40 and 32 kDa in synaptic plasma membranes of rat brains. DAMCK administered at 0.01-100 ng/kg either centrally (icv), or systemically (iv) produced a dose-dependent, opioid receptor mediated, profound antinociception measured with the tail-flick assay in male rats. While its analgesic effect persisted up to 240 min, the longest time tested, the effect of identical dose of DAMGO peaked at 30 min. The effect of DAMCK *in vivo* was reversed by naloxone given 45 min after the peptide only by about 50%, suggesting that the rest was due to irreversible binding. Supported by TIT-564 and OTKA T-016084 research grants.

Fr11

IS LEF553 A SUBSTRATE OF P-GLYCOPROTEIN *IN VIVO*?

L. Alari, L. Sjögren and L. Andersson. Concept Div., Astra Pain Control AB, S-151 85 Södertälje, Sweden.

LEF553 is a highly μ -selective opioid tetrapeptide with restricted passage into the CNS. Lower doses of systemic LEF553 in mice do not induce any centrally mediated antinociceptive effect in the tail-flick test while a minor increase in the dose does. These findings might be explained by LEF553 being a substrate of P-glycoprotein, which transports the tetrapeptide out of the CNS. Higher doses of LEF553 might saturate this transporter, thus decreasing its efficacy. Male NMRI mice were given 1, 3, 6 or 10 μ mol/kg verapamil s.c. to inhibit P-glycoprotein prior to 30 μ mol/kg of s.c. LEF553. In other mice treated using the same protocol, CSF was drawn 30 min after drug and analysed for concentration of LEF553 using an ELISA method. A clear dose-related increase in the tail-flick latencies was seen for the different doses of verapamil given together with LEF553. This is different to the minor effect induced by LEF553 alone. Verapamil by itself was without effect in this test. The amount of LEF553 in the CSF was clearly higher when LEF553 was given together with verapamil than with saline. It is concluded that LEF 553 is a substrate of a saturable efflux system, which can be inhibited by verapamil, most likely P-glycoprotein.

Fr13

ENKEPHALIN-ANALOG MALEOYL PEPTIDE HYDRAZIDES: SYNTHESIS AND BINDING STUDIES.

A. Magyar, G. Orosz, A.Z. Ronai, K. Medzihradzsky, Research Group of Peptide Chemistry, Budapest, Hungary.

There are indications that in the binding of opioid peptides to their receptors a mercapto group near to the binding site is involved. A specific ligand bearing a functional group in a proper position is capable of reacting with this mercapto group. Maleoyl (Mal) amino acids are known to react with thiol groups thus their incorporation into opioid peptide derivatives gives the possibility to label opiate receptors. In these studies the maleoyl amino acid is attached to the peptide through a hydrazide function, i.e., the opioid peptide hydrazide is acylated by the maleoyl amino acid. Among the several methionine and leucine-enkephalin derivatives studied the derivatives containing an extended peptide chain (Met-enkephalin-Arg-Phe-NH-NH-Gly-Mal and DSLET-NH-NH-Gly-Mal) were found to show good affinity to opioid receptors. Substitution of Gly₂ with D-Ala₂ increased the affinity of the compounds with a slight change in selectivity shifting to a better d affinity. The synthesis and *in vitro* pharmacological data (mouse vas deferens, guinea pig ileum) will be presented.

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Fr14

MALEOYL PEPTIDE DERIVATIVES - NOVEL AFFINITY LABELS FOR OPIOID RECEPTORS.

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¹Institute of Biochemistry, Biological Research Center, ²Research Group of Peptide Chemistry, Hungarian Academy of Sciences, Hungary.

Opioid receptor binding properties of peptides containing maleoyl function were determined in order to develop affinity labels. Maleoyl aminoacids are known to react with thiol groups, thus their incorporation into opioid peptide derivatives gives the possibility to label opioid receptors. Based on the enkephalin structure peptide ligands were synthesized and tested. The ability of the compounds to compete with the binding of known type-specific radioligands were determined over a wide concentration range of the compounds. All ligands showed agonist character with high affinity (K_d in nM range) and good to moderate selectivity. Replacement of Gly² in the enkephalin frame with D-Ala led to better delta affinities with a small decrease in selectivity. The longer peptide chains (Met-enkephalin-Arg-Phe-NH-NH-Gly-Mal and DSLET-NH-NH-Gly-Mal) resulted in compounds with very high percentage (up to 86%) of irreversible binding. The newly synthesized peptides could be used in further studies to determine more detailed characteristics of the ligand-receptor interaction.

Fr16

NOVEL TIPP-DERIVED δ OPIOID ANTAGONISTS AND δ AGONISTS WITH HIGH POTENCY AND UNPRE-CEDENTED δ SELECTIVITY.

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The δ opioid antagonist TIPP (H-Tyr-Tic-Phe-Phe-OH) was structurally modified by substitution of artificial amino acids at the 3-position of the peptide sequence and/or replacement of the peptide bond between the Tic² and Phe³ residues with a reduced or reduced and alkylated amide bond. Among the compounds prepared, the pseudopeptide H-Tyr-TicY [CH₂NH]Cha-Phe-OH (TICP[Y]; Cha = cyclohexylalanine) showed subnanomolar δ antagonist δ selectivity ($K_i \mu / K_i \delta = 4050$) in the rat brain membrane receptor binding assays, being 200 times more δ -selective than naltrindole, and 30 times more potent and 6 times more δ -selective than the dipeptide delta antagonist H-Dmt-Tic-OH. Structural modification of the prototype dipeptidated agonist H-Tyr-Tic-NH-(CH₂)₂-Ph (Ph = phenyl) produced a new class of potent and highly δ -selective dipeptide agonists with δ receptor affinities ($K_i \delta$) as high as 0.5 nM and with δ selectivities ($K_i \mu / K_i \delta$) up to 2000. These compounds, which include H-Tyr-Tic-NH-CH₂-CH(Ph)COOEt (isomer I) and H-Tyr-Tic-NH-CH₂-CH(Ph[pNH₂])₂, look promising as analgesics because they may be small and lipophilic enough to cross the blood-brain barrier. Subtleties in structure - agonist vs. antagonist relationships observed with these compounds are interpreted in terms of a receptor model implicating multiple active and inactive states.

Fr15

SYNTHESIS AND BIOLOGICAL ACTIVITY OF ENKEPHALIN-RELATED ALKYLATING PEP-TIDES.

A. Blahunka, A. Magyar, G. Orosz, A.Z. Ronai, H. M.-Schweiger, K. Medzihradzsky, J. Szikra, S. Benyhe and A. Borsodi, Research Group of Peptide Chemistry and Biological Center of Hungarian Academy of Sciences, Hungary.

Enkephalin chloromethyl ketones has been known as potential opioid receptor affinity labels. There is some interest to modify the selectivity and reactivity of these derivatives to achieve more selective reaction between the ligand, carrying the reactive group and the functional group on the receptor surface. To study these interactions, the following modified enkephalin derivatives were prepared and compared in biological studies: bromomethyl ketones, chlorohydrins, glycidol esters and epoxysuccinyl derivatives. There are similarities and sharp differences in the biological activity as determined in the binding studies on rat brain membranes. Under the conditions employed in the experiments, (no mercapto containing peptidase inhibitor is allowed in the buffer because of the possible reaction with the reactive peptide derivative) only the D-Ala²-substituted derivatives showed good affinity in the displacement studies. Detailed biological data for the binding and the selectivity of the compound will be presented.

Fr17

MALDI-MS QUANTITATIVE ANALYSIS OF SYNTHETIC OPIOID PEPTIDE ANALOGS.

D.M. Desiderio, U. Wirth, J.-L. Tseng, H.H. Szeto, J. Clapp, and P. Schiller. Department of Neurology, University of Tennessee-Memphis, Memphis, TN, USA

Matrix-assisted laser desorption/ionization (MALDI) time-of-flight (TOF) MS has had a revolutionary impact on the analysis of high-MW and involatile, biologically important compounds. Due to its detection sensitivity, speed of analysis, and ease-of-use, we are developing MALDI for the quantitation of peptides with a MW < ca. 1000 Da. We demonstrate MALDI-TOF quantitative data that are useful in pharmacokinetic (PK) studies in ovine plasma of metabolically stable, synthetic opioid peptide analogs. The working curve obtained for the quantification of synthetic Tyr-D-Arg-Phe-Lys-NH₂ (DALDA) has a dynamic range from 0 to 100 ng. Excellent MALDI reproducibility (5-6%; 4 sets, n=10 in each set) is routinely obtained in spot-to-spot and well-to-well laser shots. The use of a stable isotope-incorporated (δ_s) synthetic peptide internal standard optimized the reproducibility. PK data of DALDA in ovine plasma were obtained. Similarly, a reproducibility of <10% (3 sets, n=5 in each set) was attained for Tyr-D-Ala-G-MePhe-NH(CH₂)₂OH (DAMGO) in ovine plasma. The limit of detection for DAMGO in ovine plasma is <1pmol. Supported by NIDA 08924.

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SYNTHESIS AND EVALUATION OF AFFINITY LABELS BASED ON THE DELTA OPIOID ANTAGONIST, TIPP.

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Affinity labels are important pharmacological tools used in the study of receptors. In an effort to synthesize affinity labels based on δ -selective peptide antagonists, our laboratory prepared derivatives of the tetrapeptide Tyr-Tic-Phe-Phe (TIPP) containing an isothiocyanate (SCN) moiety as potential irreversible ligands. The SCN-substituents were positioned at the para position of either Phe³ or Phe⁴ of the peptide. Once synthesized, the peptides were then tested for inhibition of radioligand binding in δ and μ opioid receptors expressed in Chinese hamster ovary (CHO) cells. All of the δ opioid receptor. Both SCN-derivatives were able to inhibit [³H]DPDPE binding at the delta receptor in a wash-resistant fashion. Therefore, these two peptides represent two potential affinity labels that may prove useful in the pharmacological study of δ opioid receptors.

Fr20

SYNTHESIS OF 7-ARYLMORPHINANS AS δ -SE-LECTIVE LIGANDS.

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As an approach to designing δ -selective ligands using the 'message-address' concept, a series of 7-aryl opiates have been synthesized and tested for opioid agonist and antagonist activity in the guinea pig ileum (GPI) and mouse vas deferens (MVD). The 7-phenylnaltrexone derivative was found to be 20 times less potent than naltrindole (NTI) as a δ antagonist and was 5 times less selective. Molecular modeling studies revealed that the conformationally mobile phenyl group prefers an orthogonal-like orientation with respect to the plane of ring C of the morphinan system. The lower potency and selectivity of 7-phenyl naltrexone is consistent with the idea that NTI exhibits high δ potency and selectivity owing to the coplanarity of its conformationally constrained indole benzene moiety with ring C of the morphinan structure. This molecular feature affords NTI an enhanced affinity for δ receptors and produces steric interference at μ and κ receptors. The greater antagonist potency of 7-phenylnaltrexone at μ and κ receptors may be attributed to the conformationally mobile phenyl group being more easily accommodated by these receptors. Other members of this series similarly exhibited lower potency and selectivity for δ receptors. In summary, attachment of a phenyl group at the C-7 position of naltrexone changes antagonist selectivity from μ to δ , presumably due to the aromatic group functioning as a δ "address" in a fashion similar to that of NTI. The substantially lower δ -selectivity found for those compounds having a more spatially adaptable phenyl at this position may be attributed to a lack of coplanarity of the two linked ring systems when compared to the rigidly held benzene moiety in NTI.

Fr19

DISCOVERY OF CAPPED DIPEPTIDES AS SELECTIVE AGONISTS OF THE DELTA RECEPTOR.

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Combinatorial chemistry and solid phase synthesis has provided the pharmaceutical industry with a powerful tool for the rapid synthesis of libraries of compounds for lead identification and optimization. High throughput screening of a library of capped dipeptides led to the identification of RWJ-105319 as a potent (24 nanomolar) and selective agonist of the delta opioid receptor. Several targeted libraries were prepared and tested for affinity to the delta receptor. The synthesis and biological data for these interesting compounds will be presented.

Fr21

IN VITRO CHARACTERIZATION OF NEW POTENT AND δ SELECTIVE PYRROLOMORPHINANS.

P. Petrillo, M. Artico, G. Dondio, P.A. Gatti, D. Graziani, C. Manzoni, S. Ronzoni. SmithKline Beecham S.p.A., via Zambelletti, 20021, Baranzate, Milan, Italy.

To characterize new delta ligands belonging to the pyrrolomorphinans class (SAR studies presented in an accompanying presentation), binding studies have been carried out using cloned human δ , μ and κ receptors stably expressed in CHO cells. The activity on cAMP accumulation in CHO cells/ δ receptors has also been evaluated. SB 227122 and SB 244525 emerged as interesting compounds, displaying nanomolar δ affinity and good μ and κ selectivity ratios. SB 227122, in the cAMP assays, displayed good agonist activity and its effects were completely antagonized by naltrindole (100 nM). SB 244525 behaved as a potent antagonist, and lacked any significant effect per se on cAMP levels up to 1 μ M. These two novel peptide derivatives might be valuable tools for further study δ receptors role and function.

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Fr22

SAR STUDIES ON A NEW CLASS OF POTENT AND SELECTIVE δ LIGANDS, THE PYRROLO-MORPHINANS.

S. Ronzoni, M. Artico, A. Artino, P.A. Gatti, D. Graziani, C. Parini, P. Petrillo and G. Dondio. SmithKline Beecham S.p.A., via Zambelletti, 20021, Baranzate, Milan, Italy.

Following our studies related to the extension of the "message-address" concept (J. Med. Chem. 1997, 40, 3192), a new class of highly potent and selective δ ligands, the pyrrolomorphinans (I), was synthesised. Extensive SAR studies, based on opioid binding affinity and selectivity, were aimed at the identification of the best substitution pattern for this novel class of derivatives. Detailed in vitro pharmacology of the most interesting compounds will be discussed in an accompanying presentation.

Fr24

ANALOGS OF THE DELTA OPIOID RECEPTOR ANTAGONIST HS 378 WITH HIGH AFFINITY AND SELECTIVITY FOR DELTA RECEPTORS

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Introduction of a 14 β -ethoxy and a 5 β -methyl group onto the delta opioid receptor antagonist naltrindole resulted in a pure opioid antagonist (HS 378) with somewhat lower delta potency but much higher delta selectivity in bioassays [1]. In an attempt to improve on the delta affinity and/or selectivity of HS 378 we prepared 5-Me substituted and 5-unsubstituted indolomorphinans with different 14-alkoxy substituents. The indolomorphinans unsubstituted in position 5 exhibited considerably higher antagonist potency at delta opioid receptors than HS 378 in bioassays while the delta selectivity was retained.

[1] H. Schmidhammer, D. Daurer, M. Wieser, K. Monory, A. Borsodi, J. Elliott, J. R. Traynor, *Bioorg. Med. Chem. Lett.* 1997, 7,151.

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Fr23

SYNTHESIS AND BIOCHEMICAL EVALUATION OF 14-ALKOXY SUBSTITUTED INDOLO- AND BENZOFUROMORPHINANS

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A recent study suggests that a 14-alkoxy group is essential for high δ opioid receptor antagonism and selectivity in analogues of naltrindole and naltriben, while a 5-methyl group is not necessary [1]. Thus, we synthesized further analogues of naltrindole and naltriben differently substituted in position 14 and/or 5. Opioid receptor selectivity of the novel compounds has been determined by binding assays, while agonist/antagonist properties were studied using the [³⁵S]GTP γ S assay and the sodium indices. Several highly potent and δ -selective agonists and antagonists were found.

[1] H. Schmidhammer, R. Krassnig, E. Greiner, J. Schütz, A. White, I. P. Bezetei-Gurske, *Helv. Chim. Acta*, in press.

Fr25

PHARMACOLOGICAL PROFILE OF NON-PEPTIDE δ OPIOID RECEPTOR LIGANDS

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Several nonpeptidic opioids were developed with the aim of obtaining δ -selective agonists to be used as analgesic agents. Three of the compounds are based on the octahydroquinoline structure and one has the morphinan structure. Affinities of these compounds for δ and μ receptors expressed in HEK 293 cell lines were determined by competition analysis of ³H-bremazocine binding to membrane preparations. All compounds exhibited high affinity and selectivity, with affinity constants in the range of 0.4-1.7 nM for the δ opioid receptor and 240-1165 nM for the μ opioid receptor. We next sought to determine which domain of the δ receptor was critical for mediating the highly selective binding by analysis of affinities for μ/δ receptor chimeras. Two chimeras were studied, M2D, consisting of μ receptor sequence from the amino terminus to the beginning of transmembrane domain (TM) 2 followed by δ receptor sequence to the carboxyl terminus, and D5M, which has δ receptor sequence from the amino terminus to the middle of TM 5 followed by μ -receptor sequence to the carboxyl terminus. All compounds displayed δ -like affinities for the M2D receptor, and μ -like affinities for the D5M receptor, suggesting that a critical site of receptor/ligand interaction was located between TM5 and the carboxyl terminus of the δ receptor.

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Fr26

PERSISTANT HIGH AFFINITY ³H-MORPHINE-6-GLUCURONIDE BINDING IN MOR-1 KNOCKOUT MICE.

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Previous antisense studies have revealed that morphine analgesia is sensitive to oligodeoxynucleotides (ODNs) targeting the first exon of MOR-1 but not exons two and three. The reverse profile has been demonstrated for morphine-6-glucuronide (M6G) analgesia. These data suggest that different receptor mechanisms may be responsible for morphine and M6G analgesia. A knockout strain of mice deficient in MOR-1, the gene encoding a μ -opioid receptor, has been developed by replacing the first coding exon of the gene with a neomycin-resistant cassette. These μ -opioid receptor deficient mice lack analgesia with the traditional μ -opioid drugs DAMGO and morphine but are analgesic with M6G and heroin. We here report that binding studies indicate that the potent morphine metabolite M6G labels an additional site in mouse brain distinct from the traditional μ -opioid receptor. Although the μ -knockout mice lack demonstrable ³H-DAMGO binding, these mice retain high affinity ³H-M6G binding in brain homogenates with a K_D in the low nanomolar range based on saturation and competition studies. This high affinity ³H-M6G binding in brain of the MOR-1 deficient mice lends further support for the proposal that a novel receptor mechanism mediates at least some M6G and heroin action. Supported by DA09040 (J.P.) and DA06241 (G.W.P.)

Fr28

DOES MORPHINE-6-GLUCURONIDE CONTRIBUTE TO THE ANALGESIC EFFECTS OF MORPHINE?

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The opioid agonist activity of morphine-6-glucuronide (M6G) is well recognized. The present study attempted to quantify the relative contribution of M6G to analgesia observed after systemic administration of morphine. In a placebo-controlled, sixfold crossover study in 20 healthy men, the effects of M6G were assessed at steady-state plasma concentrations of M6G identical to and two and three times higher than those measured after administration of morphine. Morphine and M6G were administered as an intravenous bolus followed by infusion over 4 h at doses which were reported to be active in the clinical situation. The analgesic effects of M6G and morphine were measured before administration and after 3.5 h using an experimental pain model based on pain-related cortical potentials and pain ratings after specific stimulation of the nasal nociceptor with gaseous CO₂. Morphine significantly reduced subjective and objective pain correlates compared with placebo. In contrast, M6G produced no statistically significant effects. It is concluded that distribution of M6G into the central nervous system is slow and, therefore, M6G does not contribute to the effects of morphine after short term intravenous administration. As it has been estimated that the ke₀ of M6G is in the range of 20 h (ke₀ of morphine ca. 15 min) the formation of M6G may become relevant under chronic dosing. Moreover, pharmacokinetics of M6G are of clinical importance in patients with renal insufficiency.

Fr27

MORPHINE AND MORPHINE-6-GLUCURONIDE (M6G) STIMULATED [³⁵S]-GTP γ S BINDING IN NEONATAL GUINEA PIG BRAINSTEM (BS).

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The major side effect of morphine and M6G is respiratory depression, which is postulated to be mediated through mu opioid receptors in the BS. Therefore we examined morphine and M6G induced [³⁵S]-GTP γ S binding and their relationship to mu opioid receptor mediated [³⁵S]-GTP γ S changes caused by DAMGO. Stably transfected CHO cells expressing mu receptor and BS membranes prepared from 7 day old guinea pigs were used to measure agonist stimulated binding. Maximum DAMGO stimulation of binding compared to basal was 266% for CHO cells and 59% for BS membranes. Efficacy for morphine and M6G, relative to DAMGO (100%), was 88% and 83%, respectively, for cells, and 68% and 55% for BS. Stimulation of binding by agonists was antagonized by naloxone. These results suggest that other regulatory mechanisms may be involved in the increased potency of M6G in vivo.

Fr29

EFFECTS OF MORPHINE-6-GLUCURONIDE ON ANTINOCICEPTION AND RESPIRATORY DEPRESSION IN THE RHESUS MONKEY.

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Morphine-6-glucuronide (M6G) has increased potency over morphine in rodent assays of antinociception. It is analgesic in man but its contribution to the analgesic activity of morphine is inconclusive. The aim of the present study was to determine the relative effectiveness of the morphine and M6G in a non-human primate. Morphine or M6G were administered iv to rhesus monkeys. Latencies to remove tails from 50 °C water (20s cut-off) and respiration in 5% CO₂ were measured. At a dose of 3.2 mg/kg tail-withdrawal latencies were increased by both morphine (MPE 54.3 +/- 6.7%, n=4) and M6G (MPE 34.2 +/- 9.0%, n=3), 30 min after administration. The compounds were equiactive on a molar basis and had a similar time-course of action (90 min). At the same dose both compounds decreased respiratory frequency, minute and tidal volume. The effects of M6G were less severe than those of morphine. The results show that when given by the iv route M6G does not have a increased antinociceptive potency over morphine in the non-human primate, but does show a reduced degree of inhibition of respiration, suggesting an added margin of safety. Supported by DA00254.

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Fr30

IN VIVO CHARACTERIZATION OF MORPHINE 3,6-DINICOTINATE AND MORPHINE 6-NICOTINATE.

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A prominent problem with morphine treatment is its side effects, respiratory depression and constipation. Structure activity relationship (SAR) studies have attempted to develop morphine analogues, which retain their analgesic potency but display decreased unwanted effects. Hosztafi et al. synthesized morphine 3,6-dinicotinate (MDN) showing decreased dependence and respiratory depression as compared to morphine. We pharmacologically characterized MDN and morphine 6-nicotinate (M6N) utilizing selective antagonists as well as antisense oligodeoxynucleotides targeting MOR-1. The two drugs are 28 times more potent than morphine. 3-methoxynaltrexone, a M6G receptor antagonist, blocked MDN analgesia. Antisense targeting MOR-1 exon 1 partially blocked M6N analgesia but had no effect on MDN analgesia. Thus, MDN displays similar mechanism of action as M6G, while M6N appears to act at both the M6G and mu opioid receptor.

Fr32

COMPARED TO SUFENTANIL, 14-METHOXY-ME-TOPON INDUCES NO RESPIRATORY DEPRESSION, LESS SEDATION AND LESS BRADYCARDIA

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A derivative of the 14-alkoxymorphinans, 14-methoxymetopon (HS-198), reportedly is 20,000 times more potent than morphine in the AcOH-writhing test (1). It differs in regard to its low dependence liability and degree of tolerance in rats and mice (2). High affinity has been demonstrated for the μ -binding site, but little is known on its respiratory, cardiovascular and sedative effects. Graded doses of HS-198 (3-6-12 g/kg) were tested on respiration (oxygen saturation), heart rate (lead II ECG) and on delta-power of the EEG in awake and trained canines. Effects were compared to similar doses of another potent μ -ligand sufentanil (SUF). Compared to control, max. bradycardic effect was 19% after HS-198 and 42% after SUF. Delta power increased by 288% after HS-198 and by 439% after SUF. PaO₂ dropped by 41% after SUF and only by 4% after HS-198. The effects of both ligands could be reversed by the potent antagonist naltrexone (20 μ g/kg). HS-198 inherits potentials of clinical interest. References: (1) Schmidhammer: Helv Chim Acta 73, 1784, 1990 (2) Fürst: Eur J Pharmacol 236, 209 1993(1993).

Fr31

AFFINITY, POTENCY AND EFFICACY OF TRAMADOL AND ITS METABOLITES AT THE CLONED HUMAN μ -OPIOID RECEPTOR.

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Tramadol is an analgesic drug working on the μ -opioid receptor and by inhibiting noradrenalin-reuptake transporter. In order to assess the relative contribution of the different metabolites of tramadol to therapeutic efficacy, in vitro studies with the cloned human μ -opioid receptor were performed. [³H]-naloxone binding experiments yielded K_i-values that were consistent with the K_i-values of rat brain membranes. G protein activation was analysed using agonist stimulated [³⁵S]GTP γ S binding to membranes in order to determine the potency and relative efficacy of tramadol and its metabolites. In-vitro binding was performed under conditions of the GTP γ S-assay in order to calculate the intrinsic efficacy of the compounds. In comparison with the in vivo-activity, the in vitro data confirmed that the (+) enantiomer of the metabolite M1 is the prominent metabolite responsible for the μ -opioid activity. The metabolites M2 and M3 had almost no affinity for the μ -receptor, while M5 is apparently 6 fold lower in naloxone binding that corresponds with a 5 fold lower potency of GTP γ S-binding and therefore contributes to the analgesic activity of tramadol to a less extent.

Fr33

ANESTHETIC ACTIVITY OF OHMEFENTANYL IN ANIMALS.

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Ohmefentanyl (OMF) is a potent analgesic and high selective agonist for μ -opioid receptor. Anesthetic activity of OMF was studied. The results showed that ED₅₀ of loss of righting reflex for OMF in mice and rats were 0.27 and 0.75 μ g/kg respectively. The potency of OMF was 19 and 94 times as potent of fentanyl. The anesthetic ED₅₀ of OMF for no response to bone crush injury in rat was 2.32 μ g and 5.78 μ g/kg in mice, 1.41 μ g/kg in rabbits. The anesthetic potency of OMF were 32 to 76 times as that of fentanyl. The effects induced by OMF were effectively and rapidly abolished or antagonized by a single dose of naloxone. The change in EEG from low voltage rapid waves to high voltage and slow delta waves were observed with 1 min post injection in rabbits and dogs. The EEG effect was completely reversed by naloxone. The results indicated that OMF display anesthetic properties and may be used as an anesthetic agent.

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Fr34

MULTIPLE OPIOID ALKALOID BINDING SITES IN THE OPIOID RECEPTORS?

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In order to analyze the structural elements in the "common opioid binding pocket", we first adopted a "gain of function" approach by mutating residues in the OFQ receptor to those conserved in the opioid receptors at the corresponding positions. Our experiments revealed that: 1) Several residues in the OFQ receptor are critical to its selectivity against the opioid alkaloids. Most strikingly, an Ala to Lys mutation at the top of TM5 increases the affinities of several opioid alkaloids by almost two orders of magnitude. 2) Effects of these mutations are largely additive. The combination of these mutations leads to a receptor that binds naltrexone, naltrindole and nor-BNI with nanomolar affinities. 3) This mutant receptor preserves both the stereospecificity and the agonist/antagonist nature of opioid alkaloids. These data strongly supported the hypothesis that the corresponding residues in the opioid receptors may form a functional common binding pocket for opioid alkaloids. To verify this hypothesis, we made the reciprocal Lys to Ala mutation at the top of TM5 in both the d and the k receptors. Surprisingly, while this mutation has differential effects on the binding affinity of several d₁ and d₂ peptide ligands in the d receptor, it does not reduce the binding affinities of the opioid alkaloids such as bremazocine and EKC in either the d or the k receptors. This unexpected result raises the intriguing possibility that the wild type opioid receptors may use more than one mechanism to bind opioid alkaloids with high affinity. Such a possibility also points to new ways to understand partial agonism and differential trafficking induced by various classes of agonists.

Fr36

MUTATION OF HUMAN μ OPIOID RECEPTOR EXTRACELLULAR "DISULFIDE BOND" CYSTEINE RESIDUES ALTERS LIGAND BINDING BUT NOT RECEPTOR TARGETING TO THE CELL PLASMA MEMBRANE.

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Two μ OR extracellular cysteine residues are highly conserved among G protein-coupled receptors (GPCR); the residues have been confirmed to form a disulfide bond in some GPCR. Such a bond would dramatically govern the topology of the extracellular loops (and possibly that of the TM domains). Alanine or serine substitution mutants at each position tested the importance of both cysteines for ligand binding and receptor function, expression and targeting. Little or no specific binding of representative alkaloid (naloxone) and peptide (DAMGO) radioligands was detected at the mutant receptors, which were nevertheless expressed in both transiently and stably transfected cells, some at wild type receptor levels. Tested mutants successfully reached the cell membrane, as evidenced by electron microscopy. As with other GPCR, the μ opioid receptor apparently also employs the extracellular disulfide bond, information that permits more accurate molecular modeling of extracellular aspects of the receptor with ligands known to require specific extracellular loop features for high affinity binding.

Fr35

IDENTIFICATION AND CHARACTERIZATION OF A NOVEL SPLICE VARIANT OF THE MOUSE MU-OPIOID RECEPTOR (MOR1) GENE.

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Using a 3' RACE and RT-PCR approach, we isolated a novel splice variant of MOR1 gene from mouse brain. Sequence analysis of the variant (MOR1C) indicates that it contains the same coding exons 1, 2 and 3 as MOR1, but a different exon 4. The new exon 4 is predicted to encode 52 amino acids in the C-terminal of the MOR1C, which is much longer than that of MOR1 (12 aa) or MOR1A (4 aa), the exon 4 variants from rat and human. Northern blot analysis showed that a single mild transcript with a size 9 kb was detected with the new exon probe, compared to a single strong transcript with a size 12 kb with the MOR1 exon 4 probe. The MOR1C mRNA was only expressed in brain and not in the peripheral tissues examined. Immunolocalization of MOR1C with a polyclonal Ab raised against the MOR1C exon 4 peptide differed from that of MOR1. Two antisense oligodeoxynucleotides directed against the MOR1C exon 4 selectively blocked morphine analgesia in vivo. Binding studies in CHO cells stably transfected with the MOR1C/pcDNA3 construct indicate that MOR1C encodes a mu opioid receptor. ³H-DAMGO labeled the site with high affinity (K_d, 1nM) and its binding is readily competed by traditional mu ligands. This work is supported by DA00296(Y.-X.P.) and DA02615 and DA00220 (G.W.P.).

Fr37

INTERACTION BETWEEN ASP114 IN TMH2 AND ASN332 IN TMH7 OF THE μ OPIOID RECEPTOR

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Opioid receptors are G protein-coupled receptors (GPCRs). Asp114 in TMH2 and Asn332 in TMH7 in the μ opioid receptors are two highly conserved residues in GPCRs. These two loci have been shown to interact with each other in the 5HT_{2A} and GnRH receptors. In the present study, we examined whether these two residues are important for receptor functions and whether the two residues interact with each other. D114N, N332D and D114N/N332D mutants of the rat μ opioid receptor were generated and effects of these mutations on ligand binding and receptor activation by agonists were examined. Mutation of the Asp in TMH2 to Asn greatly reduced binding affinities of morphine, DAMGO and CTAP, but did not affect those of naloxone and [³H]diprenorphine, and virtually abolished morphine- or DAMGO-induced increase in [³⁵S] GTPγS binding. Replacing Asn in the TMH7 by Asp eliminated detectable binding with either [³H]diprenorphine or [³H]DAMGO. In contrast, the combination of both mutations (D114N/N332D) restored high affinity binding for [3H]diprenorphine, CTAP and naloxone, with K_i values approaching to those of the wildtype μ receptor. Binding affinities of morphine and DAMGO were restored to a less extent. In addition, morphine and DAMGO stimulated the D114N/N332D mutant to increase [³⁵S] GTPγS binding. The results indicate that D114 and D332 of the μ receptor are close in space and interact with each other and this interaction is important for ligand binding and receptor activation. (Supported by NIH Grants DA 04745 and DA 10702 and Adolor Corp.)

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Fr38

OPTIMIZATION OF HUMAN MU OPIOID RECEPTOR EXPRESSION IN BACULOVIRUS-INFECTED CELLS.

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Different recombinant baculoviruses were constructed for human mu opioid receptor overexpression in insect cells. Fusion proteins with either an amino- or a carboxy-terminal hexahistidine tag were generated. The coding sequence (with or without the carboxy-terminal hexahistidine tag) was also fused to the cleavable gp67 signal sequence. Two different insect cell lines (Sf9 and T.ni) in combination with different growth media were tested for maximal protein production. Scale up in shaker and biofermentor was also performed. Up to 6,000,000 receptors were detected at the cell surface and all recombinant constructs bound antagonists with nanomolar affinities.

Fr40

μ -OPIATE RECEPTOR-DEFICIENT MICE - A NEUROCHEMICAL STUDY OF TRANSMITTER RECEPTORS.

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Endogenous opioids modulate processes of central excitability such as long-term potentiation (LTP) and kindling. Alterations of dopamine and glutamate receptor subtypes, necessary components for kindling, LTP and other plastic-adaptive processes, were studied in different brain regions of mice brain using μ -opiate receptor(MOR)-deficient mice.

In receptor binding and autoradiographic studies as well as Western blot analysis no changes of the D1 and the glutamate receptor subtypes were detected. As a possible compensatory mechanism the D2 binding sites of hippocampal membranes of MOR deficient mice were decreased by about 30%.

The data are discussed in the light of an altered neuronal excitability observed after kindling or LTP.

Fr39

DEVELOPMENT OF ANTIBODIES THAT ARE CAPABLE OF IMMUNOPRECIPITATING SOLUBILIZED RAT MU OPIOID RECEPTORS AND OF RECOGNIZING MU OPIOID RECEPTORS ON WESTERN BLOTS.

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Two fusion proteins were made: one was composed of GST and the 50 C-terminal amino acids of the mu receptor (GST-C50) and the other was composed of GST and 61 amino acids near the N-terminal of the mu receptor (GST-N61). Antibodies directed against both of these fusion proteins were capable of immunoprecipitating ~70% of mu receptors solubilized from rat brain as determined by saturation [³H]DAMGO binding. [³H]DAMGO binding to the immunoprecipitate was completely abolished by 10 nM GTP γ S. Immunoprecipitation of the mu receptor by GST-C50 antibodies was blocked by 1 μ M GST-C50 but not by 1 μ M GST-N61 while immunoprecipitation by GST-N61 antibodies was blocked by 1 μ M GST-N61 but not by 1 μ M GST-C50. Antibodies against GST-C50 also identified two bands, MW 49.2 kDa and 51.4 kDa, on a Western blot of rat brain. The labelling of the bands was blocked by 1 μ M GST-C50, but not by 1 μ M GST. These same two bands were also identified in the pellet of solubilized brain membranes that had been immunoprecipitated with the antibodies raised against GST-N61.

Fr41

BINDING AND SIGNALLING PROPERTIES OF POLYMORPHIC HUMAN MU-OPIOID RECEPTOR VARIANTS.

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Large-scale sequencing of the human MOR gene revealed polymorphic variants (Hoehle M. et al.). Some of the mutations occur within coding regions, at locations that may severely affect receptor function: N40D in the N-terminal portion, N152D in the third transmembrane domain, R265H and S268P in the third intracellular loop. In order to determine whether these mutations have any incidence on receptor activity, we have constructed the hMOR variants by site-directed mutagenesis and expressed them in COS cells. The N152D receptor was expressed at lower levels compared to wild-type and other mutants receptors. Affinities of peptide agonist (DAMGO) and antagonist (CTOP), as well as alkaloid agonist (morphine) and antagonist (diprenorphine) were not markedly modified for any mutant receptor. Morphine and DAMGO-promoted activation was measured by [³⁵S]GTP γ S binding and preliminary results suggest changes in signalling properties of receptors mutated in the third intracellular loop.

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Fr42

HUMAN MU OPIOID RECEPTOR GENE POLY-MORPHISMS IN DRUG ABUSERS AND CONTROLS

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Twin and adoption studies suggest genetic influences in abuse of drugs including opioids. Individuals display varying mu opioid receptor (MOR) levels and different responses to opioid drugs. MOR is a strong candidate gene for genetic contribution to these individual differences. MOR mediates the analgesic and rewarding effects of morphine-like opioids. MOR maps to a major locus contributing to strain differences in morphine consumption in mouse QTL studies. We have examined a number of potential polymorphic markers in the MOR genes obtained from BAC and lambda genomic clones. Di- and trinucleotide repeats have been mapped by direct sequencing and Southern blotting. One dinucleotide tandem repeat, several hundred bp 5' to the 4th coding exon, displays at least 5 different genotypes with the predominant genotype being 20 GT repeats. Several single nucleotide polymorphisms (SNP) were also genotyped in drug abusers and controls. A SNP located between second and third coding exons was examined in 124 control Caucasian subjects and 185 drug abusers. Allele frequencies were 44% for G and 56% for C. No association to either opioid or polysubstance abuse behaviors was found. Identifying MOR genetic variability could help to understand the individual differences in response to opioid analgesics and drug abuse vulnerability, and lead to individualized pain control regimens. Supported by NIDA Intramural Research Program.

Fr44

THE RATE OF G PROTEIN ACTIVATION IS INDEPENDENT OF OPIOID RECEPTOR DENSITY.

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We were interested in determining if the rate of agonist-stimulated [³⁵S]GTPγS binding was dependent upon opioid receptor (OR) expression level. If receptors activate G proteins as described by a collision-coupling model, a higher receptor density would be paralleled by a faster rate of receptor-mediated G protein activation assuming that receptor-G protein interaction is rate-limiting. Membranes were prepared from C6 glioma cells stably expressing either the rat μ (C6μ) or δ (C6δ) opioid receptor. Some cells were pretreated with either a -funaltrexamine (C6μ) or naltrindole 5'-isothiocyanate (C6δ) to decrease the receptor number. The agonist-stimulated rate of [³⁵S]GTPγS binding did not vary in membranes containing 5 - 25 pmol/mg μ OR or 1.4 - 4.3 pmol/mg δ OR. Thus, these data do not support the collision coupling model of G protein activation. (Support: NIH DA04087).

Fr46

A MOLECULAR MECHANISM OF THE CLEAVAGE OF A DISULPHIDE BOND AS THE PRIMARY FUNCTION OF AGONIST BINDING TO G-PROTEIN COUPLED RECEPTORS.

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A model of a binding site of δ-opioids in the extracellular region is presented. A close distance between Asp288 and the disulphide bridge (Cys121-Cys198) was detected. Semiempirical quantum chemical calculations as well as ab initio studies showed that the interaction of the carboxylic acid side chain of aspartic acid leads to the polarisation of the disulphide bond to allow an acceptance of a proton from the protonated nitrogen of the agonists. The accompanied cleavage of the disulphide bond may cause a conformational change of the extracellular loops in a way that the pore formed by the seven helix bundle opens for penetrating the ligand, water and ions from the extracellular into the intracellular regions of a cell. This mechanism is assumed to be common for a multitude of G-protein coupled receptors.

Fr43

APPLICATION OF THE MESSAGE-ADDRESS CONCEPT TO THE DOCKING OF NALTREXONE AND SELECTIVE NALTREXONE-DERIVED OPIOID ANTAGONISTS INTO OPIOID RECEPTOR MODELS.

T.G. Metzger, M.G. Paterlini, P.S. Portoghese, and D.M. Ferguson. Department of Medicinal Chemistry, University of Minnesota, Minneapolis, Minnesota.

A binding site model for the opioid antagonist naltrexone and selective, naltrexone-derived antagonists naltrindole (NTI) and norbinaltorphimine (norBNI) is proposed based on the message-address concept of ligand recognition. Ligand docking studies of the universal antagonist naltrexone provide the structural basis for 'message' recognition across all three receptor types, μ, δ and κ. The binding mode proposed for naltrexone serves as the basis for orienting the naltrexone moiety of the selective ligands into the receptor model. This orientation places key 'address' elements of NTI and norBNI in proximity to amino acid residues critical to selectivity among receptor types. Selectivity is rationalized by sequence differences in the μ, δ, and κ receptors at these recognition points. Support for the model is derived from site directed mutagenesis studies and ligand binding data for the opioid receptors.

Fr45

DEMONSTRATION OF BASAL SIGNALING ACTIVITY WITH AN INDUCIBLE MAMMALIAN EXPRESSION SYSTEM: DOPAMINE D2L RECEPTOR.

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Basal receptor signaling activity (occurring in the absence of any agonist) has been demonstrated for the D1, D3, and D5 receptor types, but not for the D2 receptors. To test for basal signaling of the D2L receptor, we have expressed this dopamine receptor subtype under the control of an ecdysone-inducible vector system, with RXR as the promoter, in human embryonic kidney cells, HEK293. Thus, receptor expression can be controlled by varying the concentrations of inducer (muriesterone A or ponasterone A). In the absence of the inducer, D2L receptor expression was minimal, whereas high inducer levels yielded maximally 2 pmol D2L/mg protein. With this approach, we have demonstrated the presence of substantial basal D2L receptor activity, by comparing basal signaling after induction with increasing concentrations of inducer. Basal signaling, determined with cAMP and [³⁵S]-GTPγS binding assays, correlated directly with the level of receptor expression. This approach provides a sensitive measure of basal receptor signaling. Fully induced cells were then used to screen D2L ligands as agonists, neutral antagonists, and inverse agonists. These distinctions between ligands may be relevant to the treatment of disease involving D2 receptors. Supported by PHS grants DA04166 and GM43102.

Fr47

A UNIFORM MOLECULAR MODEL OF δ-OPIOID AGONISTS AND ANTAGONISTS PHARMA-COPHORE CONFORMATIONS.

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On the basis of a model of the pharmacophore conformation of agonists of the δ-opioid receptor [1] the corresponding δ-antagonists conformations were determined by means of force field calculations. The results explain the unusual behavior of several cyclic b-casomorphin analogues on molecular level. Thus, for instance, the model helps to understand why Tyr-c[D-Orn-2-Nal-D-Pro-Gly] is a mixed μ-agonist and δ-antagonist. Furthermore, the model is consistent with low energy conformations of other delta-antagonists such as Tyr-Tic-Phe, Tyr-Tic-Phe-Phe, naltrindol and BNTX. The occupation of a special spatial area by bulky groups close to the protonated N-terminus of opioid peptides is assumed to be highly critical for the switch from agonist to antagonist behavior.

[1] W. Brandt, M. Stoldt, H. Schinke, J. Comp.-Aided Mol. Des., 10, (1996), 201-212.

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THERMODYNAMIC ANALYSIS OF THE HUMAN DELTA OPIOID RECEPTOR (h-DOR) EXPRESSED IN COS CELLS

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The thermodynamic parameters of the binding of five ligands binding to h-DOR expressed in COS cells were determined. The K_d value of the tritiated selective (d antagonist naltrindole ($[^3\text{H}]\text{NTI}$) was measured by saturation analysis at four temperatures (5, 15, 25, and 35°C). K_i values were then determined by competition studies against $[^3\text{H}]\text{NTI}$ at each of these temperatures for the (d -antagonist naltriben (NTB), the (d -agonist SNC-80, the (k -agonist bremazocine, and the (μ -antagonist naloxone (NLX). The K_d and K_i values were used to construct van't Hoff plots for each ligand. The binding of the ligands was found to be entropy-driven and nearly isoenthalpic. NTB was the only one that was exothermic ($\Delta H = -0.9$ kcal/mol). The largest unfavorable enthalpy change was for NLX ($\Delta H = 2.68$ kcal/mol). These results are consistent with a recent study of the cloned mouse DOR (m-DOR) (Maguire and Loew, EJP 318: 505, 1996) and a previous study of m-DOR in NG 108-15 cells and brain (Wild et al., PNAS 91: 12018, 1994), where ligand binding primarily was entropy-driven. The close agreement of the thermodynamic parameters suggest that the binding of opioid ligands to h-DOR and m-DOR (in NG 108-15 or brain) occurs with similar energetics and within a homologous region(s) of the receptors.

Fr50

STRUCTURE, DYNAMICS, AND BINDING MODES OF SELECTIVE k -AGONISTS ON THE k -OPIOID RECEPTOR.

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The 3D-structure, dynamics, and binding modes of k -agonists (U69,593; U62,066; CI-977; ICI199,441; ICI199,067; and BRL52,656) based on the U50,488 structural prototype have been investigated using molecular modelling techniques. The torsional flexibility of the ligands explored using 2ns molecular dynamics (MD) simulations in solution reveal consistent conformational trends. Docking of selected conformers onto the transmembrane (TM) domain of the k -receptor suggest all these k -agonists to align along the receptor helices. A salt-bridge formed between the protonated nitrogen of the ligand and the aspartate (D138) in TM-3 is identified, and provide a key anchoring point for receptor-agonist association in this ligand series. The contribution of H291, I294, E297, and G319 residues towards the receptor-agonist binding, and the enantiomeric preference of the ligands are presented. Finally, ligand substitutions that could enhance k -binding affinity, and mutational targets vindicating our binding hypotheses are also proposed.

Fr49

d₁- AND d₂-OPIOID RECEPTOR SUBTYPES IN THE HUMAN NEUROBLASTOMA CELL LINE SK-N-BE : NO EVIDENCE FOR DISTINCT MOLECULAR ENTITIES.

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The existence of two pharmacological d opioid receptor subtypes, d₁ and d₂, have been suggested but they remain to be characterized at the molecular level, since a single cDNA have been cloned. The present study aimed to investigate the pharmacological properties of d₁- and d₂-opioid subtypes expressed in the human neuroblastoma cell line SK-N-BE and to study the presence of their putative different mRNAs. Binding experiments confirmed the presence of high- and low-affinity sites for both d₁ (DPDPE) and d₂ (deltorphin I) selective opioid agonists previously identified (Polastron et al., 1994). The activation of these subtypes by DPDPE and deltorphin I induced the incorporation of [α - ^{32}P]azidoanilide-GTP into G α_{i2} /G α_o subunits with the same efficiency and potency. Functional studies revealed that both agonists displayed similar efficiency to inhibit the cAMP accumulation and that a sustained activation of d -opioid subtypes for 15 min. by the peptide agonists induced a cross-desensitization between both subtypes. The absence of additivity on adenylyl cyclase regulation and the different antagonistic effects of naloxone suggest that DPDPE and deltorphin I would bind the same receptor but at different sites. The possibility that d -opioid subtypes could arise from alternative splicing was ruled out by RT-PCR experiments, revealing the presence of a single transcript encoding for the d-opioid receptor. Taken together, our results strongly suggest that the distinct binding properties of DPDPE and deltorphin I could reflect their binding to various regions on the same d -opioid receptor.

Fr51

A TRUNCATED KAPPA OPIOID RECEPTOR SHOWS A LOSS OF AFFINITY FOR HIGHLY KAPPA-SELECTIVE, BUT NOT NON-SELECTIVE, OPIOID LIGANDS.

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A truncated human kappa opioid receptor (hKOR) which lacks part of the putative third extracellular loop (o3), the seventh transmembrane domain (TM7) and the C-terminal region retains high affinity for the opioid antagonist [^3H]diprenorphine ($K_D = 226$ pM) when stably expressed in HN9.10 cells. Ligand competition analysis of [^3H]diprenorphine binding shows that the mutant receptor exhibits high affinity for the agonists bremazocine (0.75 nM), dynorphin A₍₁₋₁₇₎ (5.2 nM) and morphine (95 nM), similar to that for the wild type hKOR (0.19 nM, 2.0 nM and 243 nM, respectively). However, its affinity for the highly k -selective agonists, CI977 (440 nM), U69,593 (9 μM), and the kappa antagonist, nor-BNI (46 nM), was substantially lower than that exhibited by the wild type receptor (0.42 nM, 5.5 nM and 0.22 nM, respectively). The mutant's differential effect on the affinity of these ligands suggests that the truncated region, presumably the o3 and/or the TM7 domain, is crucial for the binding of the highly kappa-selective compounds. The mutant receptors also retain the ability to couple to G-proteins based on the [^3H]GTP-g -S binding activated by bremazocine and dynorphin A₍₁₋₁₇₎ ($\text{EC}_{50} = 2.3$ nM and 230 nM, respectively). This finding, consistent with previous observations with a C-terminal truncated mu opioid receptor, suggests that the truncated region is not critical for the coupling of kappa receptors to G-proteins.

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THE EVIDENCE OF PALMITOYLATION OF THE RAT κ -OPIOID RECEPTOR IN COS-1 CELLS.

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Palmitoylation is an important posttranslational modification of membrane signalling proteins including the dopamine and adrenergic receptors. As the cloned κ -opioid receptor has potential site for the palmitoylation, we determined whether this opioid receptor has this modification. COS-1 cells were permanently transfected with the cloned KOR1 gene and denoted as the KCOS cells. The ligand binding assay showed a significant increase of the U-50488 specific displacement of the [³H]-diprenorphine binding in this KCOS cell membrane. Results from Western blotting and immunofluorescence staining demonstrated the presence of the κ -opioid receptor-specific immunoreactivity in the KCOS cells but not in the COS-1 cells. Metabolic labeling KCOS cells with [³H]-palmitate showed that the rat κ -opioid receptor is palmitoylated. Further determination of the exact palmitoylation site on the KOR-1 is under investigation.

Fr54

POSSIBLE DIRECT INTERACTION BETWEEN CALMODULIN AND THE MU OPIOID RECEPTOR.

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Calmodulin (CaM) may affect signaling of the μ opioid receptor (MOR). This is supported by previous findings that morphine treatment alters the subcellular CaM distribution in rat brain (Nehmad et al., Mol. Pharmacol. 22:389 (1982)), and CaM binds to the b subunit of G proteins (Liu et al., J.Biol.Chem. 272 18801 (1997)). A sequence alignment search (BLAST) revealed a possible CaM binding motif in the third intracellular loop (i3) of the μ opioid receptor (MOR), and of related receptors. Biotinylated CaM can be co-immunoprecipitated with the MOR, and MOR binds to a CaM affinity gel. Binding is suppressed by peptides derived from the i3 loop of MOR that include the putative CaM binding motif. Moreover, the same i3 loop peptides bind to CaM in a gel shift assay, and CaM prevents G protein activation by an i3 loop peptide. In membranes from HEK293 cells transfected with MOR (HEK- μ), CaM modulates G protein activation, measured with ³⁵S-GTP γ S incorporation. Further, morphine treatment of HEK- μ affects the subcellular distribution of CaM. CaM appears to be involved in biochemical changes occurring during long-term MOR stimulation. Supported by DA 04166 and GM43102.

Fr53

G α TRANSDUCES μ OPIOID MODULATION OF ERK ACTIVITY.

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Previously, we reported that chronic treatment with μ or κ agonists inhibited EGF-induced ERK activation via b g subunits akin to the mechanism of acute opioid stimulatory action on ERK in this system. A unique PTX insensitivity of the chronic μ opioid (but not κ) action was observed. Since G $\alpha_{i/o}$ have been shown to modulate PLC via b g subunits, we tested the possibility that G α_x or G α_{12} , members of the G protein family that are not ADP-ribosylated by PTX, may serve as transducers of the opioid inhibitory actions. G α_x cotransfected with μ or κ OR in COS-7 cells was capable of transducing acute μ but not κ activation of ERK in the presence of PTX. Data from PTX- and chronic μ or κ agonist-treated COS-7 cells that overexpressed G α_x implicated this G protein in the μ (but not κ) opioid inhibition of EGF activated ERK. A fatty acylation-deficient double mutant of G α_x abolished both acute and chronic μ effects. Overexpression of G α_{12} in COS-7 cells did not facilitate acute μ or κ activation of ERK.

Fr55

DIFFERENTIAL G PROTEIN ACTIVATION BY ALKALOID AND PEPTIDE OPIOID AGONISTS IN THE HUMAN NEURO-BLASTOMA CELL LINE SK-N-BE.

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Interactions between δ 1- and δ 2-opioid receptor subtypes with Gi/Go proteins were determined in the human neuroblastoma cell line SK-N-BE using non-selective, etorphine, and selective agonists, DPDPE and deltorphin I, in the presence of a photoreactive GTP analogue, [α -³²P]azidoanilide-GTP. Identification of G $\alpha_{i/o}$ subunits by western-blot revealed that SK-N-BE cell membranes contained G $\alpha_{i1,2,3}$ and G α_{o2} subunits. Using one- and two-dimensional electrophoresis, we observed that while both peptide agonists activated G α_{i2} and G α_{o2} subunits, etorphine stimulated the incorporation of [α -³²P]azidoanilide-GTP into G α_{i1} , G α_{i2} , G α_{i3} and a PTX-insensitive G α_x subunits. These data suggest that the nature of the agonist, peptide or alkaloid, is critical in determining the interaction between human δ -opioid receptors and G α_x subunits.

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COINCIDENT SIGNALLING BETWEEN δ OPIOID AND Gq COUPLED RECEPTORS. A. Yeo, F.J. Gunn-Moore, J.M. Tavare and G. Henderson, Departments of Pharmacology and Biochemistry, University of Bristol, Bristol, UK.

In monolayers of either SH-SY5Y cells or CHO cells expressing the δ -opioid receptor, DPDPE (1nM - 1 μ M) alone did not elevate $[Ca^{2+}]_i$. However, if there was concomitant activation of Gq-coupled receptors by either oxotremorine-M (SH-SY5Y cells) or UTP (CHO-d cells) then DPDPE elevated $[Ca^{2+}]_i$. To determine if the opioid elevation of $[Ca^{2+}]_i$ was mediated by the release of free b g subunits from Gi/Go we transiently transfected SH-SY5Y cells with the b g binding domain of GRK2 to sequester free b g subunits. In these transfected cells the ability of DPDPE to elevate $[Ca^{2+}]_i$ in the presence of oxo-M was b g subunits. In CHO-d cells the opioid elevation of $[Ca^{2+}]_i$ appears to arise from b g facilitation of Gq stimulation of phospholipase C β because DPDPE (1 μ M) enhanced the production of inositol-1-phosphate evoked by UTP. However, in SH-SY5Y there was no enhancement by DPDPE of inositol-1-phosphate production evoked by oxo-M. We are currently investigating the possibility that in SH-SY5Y cells the b g subunits released upon δ receptor stimulation enhance the binding of inositol trisphosphate (IP3) to its receptor and thus elevate $[Ca^{2+}]_i$.

Fr58

AN ALTERED OPIOID SIGNAL TRANSDUCTION AS INDUCED BY AN ANXIOLYTIC HOMOPHTAL-AZINE, GIRISOPAM.

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Girisopam in a dose of 10 mg/kg increased the potency of morphine to induce catalepsy and blocked the inhibitory action of naloxone in the same test situation. Naloxone, 1 mg/kg in rats treated with girisopam, was ineffective in suppressing the analgesic action of morphine in the tail flick test. In addition, the morphine analgesia was increased by girisopam. Employing CHO cells expressing μ -opioid receptors, and using DAMGO as agonist, measurement of the inhibition of cAMP formation indicated that the inhibitory potency of naloxone disappeared in the presence of 10⁻⁷M girisopam. The results show that girisopam induces a characteristic alteration of signal transduction via opioid receptors, possibly through a biochemical action linked to a specific binding site.

Fr57

THE Ca⁺⁺/CALMODULIN- AND PKC-DEPENDENT ACTIVATION OF CREB INDUCED BY MORPHINE IN NG108-15 CELLS.

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Regulation of gene transcription is proposed to be an important mediator of cellular responses to opioids. In the present study we investigated the effect of morphine on the level of transcription factors CREB and c-FOS as well as the activation of CREB in NG108-15 cells. The level of the non-phosphorylated CREB appeared to be regulated by neither morphine nor δ -opioid receptor agonist DPDPE. On the other hand, morphine as well as DPDPE produced dose-dependent increase in phosphorylation of CREB. The effect was reversed by naloxone or naltrindole, respectively. Preincubation of cells with cycloheximide or anisomycin had no effect on the morphine- or DPDPE- induced CREB phosphorylation. Calmodulin antagonist W7, protein kinases inhibitor staurosporine and inhibitor of protein kinase C and cAMP-dependent protein kinase - H7 but not inhibitor of cAMP- and cGMP dependent protein kinase H8 blocked the opioid-induced CREB phosphorylation. Morphine and DPDPE also caused an increase in c-FOS protein level in the cell line studied. The above effect was inhibited by either protein kinases or protein synthesis inhibitors and was reversed by naloxone or naltrindole. It is concluded that opioids - via δ -opioid receptors - exert stimulatory effect on CREB phosphorylation in NG108-15 cells. The stimulation is not dependent on newly synthesized proteins but requires Ca⁺⁺/calmodulin and activation of protein kinase C. Morphine-induced increase in c-FOS appears to be regulated by CREB which contributes to chronic effects of opioids such as tolerance and dependence. Supported by grant No. 4 P05A 017 13 KBN - Warsaw.

Fr59

Further study on the mechanism responsible for inhibition of cAMP production in CNE-2 and H9c2 cells upon κ -opioid receptor stimulation

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A previous study showed that U50,488H at micromolar and nanomolar concentrations inhibits cAMP production in CNE-2 and H9c2 cells, respectively, which were blocked by naltorphine a κ -opioid receptor antagonist (1). In another study, we found that U50,488H at micromolar concentrations inhibits cAMP production via activating the phospho-inositol/Ca²⁺ pathway(2). The purpose of the present study was to delineate the role of the phospho-inositol/Ca²⁺ pathway in the inhibition of cAMP production in these two cell lines. In the present study we first determined the inhibitory effects of U50,488H on forskolin-stimulated production of cAMP in these two cell lines pretreated either with neomycin, a phospholipase C inhibitor, or BAPTA, a Ca²⁺ chelator. We found that in CNE-2 cells U50,488H at a concentration range of 20-100 μ M inhibited the forskolin-stimulated cAMP production and the effects were completely abolished following pretreatment with either 1mM neomycin or 20 μ M BAPTA. On the other hand in H9c2 cells, only the effects of U50,488H at a concentration higher than 30 μ M were attenuated by either neomycin or BAPTA although U50,488H at a concentration as low as 10 nM inhibited cAMP production. The results indicate that in CNE-2 cells the inhibitory effects of U50,488H at 20-100 μ M were resulted from activating the phospho-inositol/Ca²⁺ pathway in these two cell lines while only the effects of U50,488H at a concentration as high as 30 μ M were mediated via the phospho-inositol/Ca²⁺ pathway in the H9c2 cells.

In another series of experiments, the inhibitory effects of U50,488H on cAMP production were determined in both cell lines pretreated with 1 μ M chelerythrin choline a protein kinase C (PKC) inhibitor. We found that the effects of U50,488H were exactly the same as without the PKC inhibitor. The results indicate that PKC, another product of PLC activation, was not involved in mediating the action of U50,488H in the suppression of cAMP production. (Supported by The Research Grant Council, Hong Kong).

1. Lau, S.Y., T.M. Wong and N.S. Wong. /Abstract) 27th meeting of the

International Narcotics Research Conference, Long Beach, CA. 1996, p. 89.

2. Wie-Min Zhang and Tak-Ming Wong. Am. J. Physiol. 274: C82-C87, 1998

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IDENTIFICATION OF L-TYPE CALCIUM CHANNELS ASSOCIATED WITH KAPPA OPIOID RECEPTORS IN HUMAN PLACENTA.

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Human placenta at term expresses only Kappa opioid receptors thus offering a unique opportunity to study its transduction pathways. The receptors mediate the release of acetylcholine and placental lactogen and require the influx of extracellular calcium into the cells, possibly via voltage-dependent channels. We report here the identification of L-type calcium channels in human placenta which are involved in the influx of the cation and that the latter is associated with kappa receptor-mediated release of human chorionic gonadotropin (hCG). Data presented demonstrate that the stimulation of hCG secretion by the kappa agonist U-69,593 was abolished in presence of either EGTA or the calcium channel blocker nifedipine. The combined effect of opioids and dihydropyridines indicated that the receptors could be coupled to L-type calcium channels. The identification of the latter in villus membrane preparations, reported here for the first time, further contributes to the hypothesis that, in human placenta, kappa receptors-linked transduction mechanisms involve calcium and its conductance across villus membranes.

Fr62

IN VITRO AUTORADIOGRAPHY OF OPIOID RECEPTOR/G-PROTEIN COUPLING IN HUMAN BRAIN.

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The selective μ , δ and κ opioid agonists DAMGO, DPDPE and CI-977 were used in the present study to stimulate GTP[g - ^{35}S] binding in human brain sections of frontal cortex and cerebellum. The specific activation of particular opioid receptor subtypes was confirmed by blocking the agonist stimulated GTP[g - ^{35}S] binding with appropriate antagonists. In human frontal cortex μ and δ opioid stimulated GTP[g - ^{35}S] binding was evenly distributed throughout the grey matter, while κ opioid stimulated GTP[g - ^{35}S] binding was detected predominantly in laminae V and VI. These findings are in good agreement with the opioid receptor distributions as determined by receptor binding autoradiography. In cerebellum stimulated GTP[g - ^{35}S] binding revealed the existence of functional μ and κ opioid receptors in this tissue. The use of agonist-stimulated in situ GTP[g - ^{35}S] binding in human tissue can give new insights in possible alterations in the functional coupling of opioid receptors caused by neurological and psychiatric diseases.

Fr61

OPIOID-STIMULATED GTP[g - ^{35}S]-BINDING IN DIFFERENT BRAIN REGIONS OF C57BL/6 AND DBA/2 MICE.

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The C57BL/6 and DBA/2 inbred mouse strains exhibit many behavioural differences. C57/BL6 shows a preference for ethanol and morphine and DBA/2 an aversion to them. The aim of the study was to compare, without any drug intake, the functional activities of opioid receptors, including ORL1, in different brain regions of these strains. The method used was quantitative autoradiography of GTP[g - ^{35}S]-binding stimulated by DAMGO (μ), DPDPE or Ile5,6-deltorphin (δ), U-50,488 (κ), and nociceptin (ORL1). Autoradiograms of specific brain areas are presented. The distribution of GTP[g - ^{35}S] binding stimulated by nociceptin was found in various brain regions to be different from that stimulated by μ -, δ - and κ -opioid agonist. Remarkable is the higher capacity of nociceptin-stimulated binding in all regions of cerebral cortex. But there are only small quantitative or no differences in the stimulated GTP[g - ^{35}S] binding between the two mice strains, from which it may be concluded that there are no major differences in the functional activities of all four opioid receptors between the strains.

Fr63

DISTRIBUTION OF A NEW SPLICE VARIANT OF THE MU-OPIOID RECEPTOR (MOR1) IN THE RAT CENTRAL NERVOUS SYSTEM.

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We have identified a new splice variant of the mouse μ -opioid receptor (MOR1) gene, whose fourth exon undergoes alternative splicing. The aim of the present study was to compare the distribution of MOR1 (Incstar, Chemicon) with the distribution of the splice variant, MOR1C using immunohistochemistry. An anti-peptide anti-sera to the C-terminal peptide sequence from the new fourth exon was raised in rabbit (Multiple Peptide Systems). The specificity of the MOR-1C antibody was determined using transfected CHO cells, Western blots and immunoisolation studies. Prominent MOR1C-like immunoreactivity (MOR1C-LI) was found in the spinal cord (greater intensity was observed in laminae I-II, but also to a lesser extent in III-VI and X), lateral spinal nucleus (nu), arcuate nu, suprachiasmatic nu, periventricular nu and medial eminence. Moderate MOR1C-LI was observed in the spinal trigeminal tract, Gracilis nu, raphe magnus, septal nu, supra-chiasmatic nu and cerebellum. Faint MOR1C-LI was found in the nu of the solitary tract, nu ambiguus, locus coeruleus, parabrachial, lateral tegmental nucleus, periaqueducal gray, thalamus (lateroposterior and posterior), ventromedial hypothalamus and cerebral cortex. No detectable MOR1C-LI was found in the vagus nerve, interpeduncular nu, hippocampus and the striatum where MOR1-LI is present. Using double labeling, we are presently looking for colocalization of MOR1 and MOR1C. These results indicate that the splice variant of the MOR1, MOR1C, has a discrete distribution, different from MOR1, suggesting that each may play a different role.

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MU OPIOID RECEPTORS ARE PRESENT IN DISTINCT HIPPOCAMPAL INTERNEURON SUBPOPULATIONS

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We have previously localized hippocampal mu opioid receptor -immunoreactivity (MOR-I) to some GABAergic neurons, where its presence in dendrites and axon terminals suggests both postsynaptic and presynaptic sites of action. The goal of the present study was to further elucidate the identity of MOR-I neurons, to better understand the specific cellular targets of MOR agonists. By light microscopy, MOR-I was in scattered perikarya and processes in all CA1 laminae. MOR-I cells (n=1882) were nonhomogeneously distributed: stratum (s.) pyramidale contained 49%, s. oriens/alveus 29%, s. radiatum 13%, and s. lacunosum-moleculare 8%. This distribution and the diverse somatic morphology suggest that more than one population of interneurons may contain MORs. To determine which neurochemically-identified interneuron populations contain MOR-I, sections were dual-labeled for MOR-I and cholecystokinin (CCK)-, calretinin (CR)-, somatostatin (SS)-, or parvalbumin (PV)-I and examined by electron microscopy. MOR-I often was colocalized with PV-I in axons, terminals, and dendrites. The distribution and synaptic targets of many PV+MOR labeled profiles resembled those of the PV-containing basket cells. In contrast, MOR-I was not observed in CCK-I profiles, suggesting that the CCK-positive basket cells are not directly inhibited by MOR agonists. A few SOM-I dendrites and terminals contained MOR-I, suggesting that a subset of SOM neurons are modulated by MOR activation. However, no colocalization of MOR-I and CR-I was observed, indicating that the CR-containing nonprincipal cells, which inhibit other nonprincipal neuron populations, are not directly modulated by MOR activation. These findings suggest that MORs modulate specific populations of nonprincipal neurons, and furthermore that MOR agonists may directly affect both feedforward and feedback inhibition of pyramidal cells.

Support: DA08259, Aaron Diamond Postdoctoral Fellowship.

Fr66

LOCALIZATION OF μ -AND δ -OPIOID RECEPTORS TO DOPAMINESYNTHESIZING (DA) NEURONS PROJECTING FROM VENTRAL TEGMENTAL AREA (VTA) TO NUCLEUS ACCUMBENS (NAc) IN RATS.

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In previous studies we have reported that dopaminergic neurons in VTA express cloned μ -opioid receptors (MOR1) and are apposed by varicosities expressing δ -opioid receptors (DOR1). Since opiates are thought to affect central reward pathways by modulating release of dopamine by neurons projecting from VTA to NAc, we studied the distribution of MOR1 - and DOR1-ir to DA neurons projecting from VTA to NAc. Projection neurons were labeled by iontophoretic injection of retrograde tract tracer FluoroGold into NAc. For double-labeling immunocyto-chemistry, we combined MOR1 and DOR1 antisera with antisera to tyrosine hydroxylase (TH). Secondary antibodies were conjugated with cyanine 3.18, FITC or cyanine 5.18. We observed apposition of DOR1-ir varicosities to neurons either double-labeled for FG and TH, or single-labeled for FG or TH. In addition, we observed that neurons single-labeled for MOR1 were colocalized in VTA with neurons double-labeled for MOR and TH and neurons triple-labeled for MOR1/TH/FG. We conclude that δ -opioid receptors control DA release from VTA- NAc indirectly, whereas μ -opioid receptors control it directly.

Fr65

ULTRASTRUCTURAL ANALYSIS OF THE CELLULAR SITES MEDIATING MOR ACTION IN THE PERIAQUEDUCTAL GRAY.

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Opioids activate antinociceptive pathways in the ventrolateral periaqueductal gray (vlPAG), and are thought to do so through disinhibition, namely, by inhibiting inhibitory (GABAergic) neurons. Glutamate or NMDA receptor agonists also produce antinociception in the vlPAG, but according to the disinhibition model would act on distinct populations of neurons, i.e. directly on PAG output neurons. This model therefore has two anatomical predictions: (1) that MOR should be selectively localized to GABAergic neurons, and, (2) separate neuronal populations would be sensitive to opioid ligands versus NMDA receptor ligands. Here, we tested these anatomical predictions using electron microscopic analysis of tissue dually immunolabeled for MOR and GABA, and, separately, for MOR and the NMDA receptor subunit NR1. GABA-labeling was found in a large fraction of MOR-labeled profiles: 41% (45/111) of MOR-labeled dendrites and 32% (14/44) of MOR-labeled axon terminals also contained GABA labeling. However, there was an even more robust colocalization between MOR and NR1, especially within neuronal dendrites: 72% (176/245) of MOR-labeled dendrites contained NR1 labeling and 72% (176/242) of NR1-labeled dendrites contained MOR labeling. Thus, MOR and NMDA-receptor ligands may act on common cell populations. These observations support the hypothesis that MOR ligands can produce antinociception within the vlPAG through mechanisms which include, but extend beyond, disinhibition. Supported by NIDA DA-05833.

Fr67

PRE- AND POSTSYNAPTIC LOCALIZATION OF κ -OPIOID RECEPTORS IN CONTACT WITH DYNORPHIN-IMMUNOREACTIVE NEURONS IN THE RAT NUCLEUS ACCUMBENS SHELL.

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The nucleus accumbens shell (AcbSh) is a region uniquely involved in mediating aversive states induced by κ -opioid receptor (KOR) activation. To investigate the cellular targets of KOR activation, we examined the electron microscopic localization of an antipeptide antibody against KOR in the AcbSh of rat. In addition, we dually labeled tissue for KOR and dynorphin (Dyn) to examine their spatial relationship. KOR immunoreactivity was distributed mainly along discrete segments of the plasma membrane, and in select clusters of small synaptic vesicles in axon terminals. These terminals either lacked recognizable synapses, or formed asymmetric, excitatory-type synapses. KOR labeling was more infrequently associated with postsynaptic densities and extrasynaptic plasma membranes of dendritic spines receiving input from unlabeled terminals. KOR labeling also was occasionally localized to glial processes, where the immunoreactivity was restricted to the plasma membrane. In dually-labeled tissue, Dyn immunoreactivity was seen in axon terminals that contained large dense core vesicles (DCVs), and apposed terminals, spines, and glial processes labeling for KOR. Our results suggest that in the AcbSh, Dyn is released from DCVs to activate KOR, having a primary role in the regulation of neurotransmitter release, but also in the postsynaptic responses of spiny neurons, and modulation of non-neuronal sites.

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k -OPIOID RECEPTOR GENE EXPRESSION IN RAT POSTERIOR PITUITARY.

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In addition to functional k -opioid receptors (KOR) on neurosecretory terminals of the posterior pituitary (PP), k -binding sites on pituitary cells have also been reported, which exhibit profound plasticity under conditions of altered neurosecretory activity. While the expression of KOR in neurosecretory neurons could be confirmed by molecular means, there has been no evidence for KOR synthesis in resident pituitary cells as yet. The aim of this study was to investigate the expression of the KOR gene in rat PP at the mRNA and protein level using in situ hybridization (ISH) and immunohistochemistry (ICC). ISH with ³⁵S-labelled cRNA probes yielded weak signals over posterior lobe perikarya. ICC with affinity-purified antisera revealed - in addition to numerous immunoreactive (ir) neurosecretory fibers - KOR-ir cells throughout the neural lobe, which were identified as pituitary cells in co-staining experiments. In the intermediate lobe (IL), KOR expression was absent from melanotrophs, but the IL surrounding cell layer, consisting of pituitary cells and microglia-like cells, exhibited both positive ISH and ir signals further confirming the local synthesis of KOR in non-neuronal cells of the PP. These results support the role of KOR-mediated communication between neuronal and non-neuronal elements in the regulation of neurosecretion at the level of the PP. Supported in part by DFG.

Fr70

KAPPA AND DELTA OPIOID RECEPTOR GENE EXPRESSION IS REGULATED BY AP-1.

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We have investigated the regulation of transcription of the kappa and delta opioid receptor genes (KOR and DOR, respectively) employing transient transfection experiments to define regulatory promoter elements and gel mobility shift assays (EMSAs) to characterize protein interactions with these elements. In transfection experiments with reporter gene constructs both promoters are responsive to TPA. The region conferring this effect on the KOR promoter was located to sequences spanning from nt's -801 to -327, with respect to the translation start site. It contains a sequence with one mismatch compared to the classical TPA responsive element/AP-1 site. Competitive EMSAs indicate that nuclear AP-1 proteins bind to the KOR element. On the DOR gene promoter the region conferring TPA responsiveness is located between nt's -600 and -262. Here, two elements, a proximal and a distal element with one and two mismatches compared to the classical AP-1 site, respectively, are present. EMSAs show that both elements bind AP-1 proteins, the proximal element, however, with a higher affinity than the distal.

Fr69

THE STUDY OF DISTAL PROMOTER IN THE MU OPIOID RECEPTOR (MOR) GENE EXPRESSION.

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MOR is the principal receptor responsible for the analgesic actions of endogenous opioid peptides and the narcotic alkaloid derivatives of morphine. Mouse MOR gene has two promoters, the activities of which are different in the brain. Previous studies showed that the distal promoter has strong inhibitory sequences in the 5' untranslated region. In the present study, the exact size and location which are responsible for the inhibition of the distal promoter will be reported. These sequences identified by 3' deletion and transfection analysis are functional in neuronal and non-neuronal cells. Linker scanning analysis confirmed that these sequences, which are highly evolutionarily conserved are critical for the negative effect on the distal promoter. In addition, full characterization of these sequences obtained by changing their orientation and position in the MOR distal and heterogeneous SV40 promoter will be presented. (supported by NIH grants DA-00546, DA-01583 and DA-05695).

Fr71

AUTORADIOGRAPHIC LOCALIZATION OF ¹²⁵I[TYR¹⁴]OFQ/N AND ¹²⁵I[TYR¹⁰]OFQ/N(1-11) IN RAT BRAIN.

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The endogenous ligand for the ORL1/KOR-3 receptor, orphanin FQ/nociceptin (OFQ/N), has recently been characterized. The OFQ/N peptide sequence contains paired basic amino acids that indicate a truncated peptide, OFQ/N(1-11), may be processed from OFQ/N. Our laboratory has recently synthesized ¹²⁵I[Tyr¹⁴]OFQ/N and ¹²⁵I[Tyr¹⁰]OFQ/N(1-11) and characterized these ligands in mouse brain homogenate studies. While ¹²⁵I[Tyr¹⁴]OFQ/N labeled both a high and low affinity site, ¹²⁵I[Tyr¹⁰]OFQ/N(1-11) only labeled a single high affinity binding site. In the present study, we examined the autoradiographic distribution of ¹²⁵I[Tyr¹⁴]OFQ/N and ¹²⁵I[Tyr¹⁰]OFQ/N(1-11) in coronal rat brain sections. Non-specific binding was defined with cold OFQ/N or OFQ/N(1-11). Both ligands demonstrated high levels of specific binding (<95% of total). ¹²⁵I[Tyr¹⁴]OFQ/N exhibited heavy binding throughout the cortex, thalamus, and hippocampus. In contrast, ¹²⁵I[Tyr¹⁰]OFQ/N(1-11) exhibited lower binding levels than OFQ/N with a more restricted pattern of labeling. ¹²⁵I[Tyr¹⁰]OFQ/N(1-11) binding was evident in distinct cortical regions, midline thalamic nuclei, and hippocampal layers. Both ligands exhibited lower binding levels in the caudate nucleus and the cerebellum. The distinct binding patterns of these two ligands indicate differential functional activities of OFQ/N and OFQ/N(1-11) peptides.

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ALTERATIONS IN NEURONAL ORPHANIN FQ CONTENT FOLLOWING EXPOSURE TO STRESS

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We examined the effects of acute and chronic stress on the regional content of orphanin FQ (OFQ) in rat brains. Thirty-two male Sprague-Dawley rats were exposed to a regimen of chronic non-habituating stress, or a single acute stress before decapitation. The rats' brains were dissected, and OFQ was measured in a variety of brain regions. In the rats that were exposed to chronic stress, OFQ content did not differ from the content in unstressed controls in any of the brain regions. In the rats that were stressed acutely just before decapitation, OFQ content was decreased by 25-30% in the striatum (including caudate nucleus, accumbens, and bed nucleus). This finding concurs with our previous report that OFQ activates the HPA axis, and appears to be implicated in the initiation of physiological responses to acute stressors. This research was funded by RO1-DA08920 and RO1-DA02265.

Fr74

THE PHARMACOLOGICAL ACTIONS OF NOCICEPTIN IN THE ISOLATED COLON OF RAT, MOUSE AND MAN.

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As a direct result of attempts to clone opioid receptors, the ORL₁-receptor (an "orphan opioid receptor") was identified which has a high degree of homology to μ -, δ - and κ -opioid receptors (Mollereau et al., FEBS Lett. 341: 33, 1994). The endogenous opioid peptides show little or no affinity for this receptor but recently the endogenous ligand was isolated and named nociceptin (Meunier et al., Nature 377:532, 1995) or orphanin FQ (Reinscheid et al., Science 207: 792, 1995). In this study, we have investigated the pharmacological actions of nociceptin in isolated segments of colon from mouse, rat and man. Nociceptin, but not [des-Phe¹]-nociceptin, caused concentration-dependent contractions of the descending colon from the mouse and the rat; these contractions were not antagonised by naloxone. In the mouse colon, the addition of peptidase inhibitors did not affect the sensitivity of the tissue to nociceptin and the contractions were unaffected by either atropine or TTX; interestingly, TTX alone caused a contracture indicative of tonic release of an inhibitory mediator. In the rat preparation, the nociceptin-induced contractions were unaltered by addition of atropine, mepyramine or methysergide. [Met⁵]enkephalin and the selective μ -opioid receptor agonist [D-Ala², MePhe⁴, Gly-ol³]enkephalin, but not selective κ -opioid receptor agonists, also caused contractures in the rat and mouse colon. Nociceptin had no contractile activity in either ascending or descending human colon. Thus in some species, nociceptin causes contractions of descending colon by a direct action at non-opioid ORL₁-receptors on smooth muscle.

Fr73

ORPHANIN FQ PRODUCES DISTINCT EFFECT ON PAIN RESPONSE AND ENDORMORPHIN-INDUCED ANAL-GESIA IN BRAIN AND SPINAL CORD OF RATS.

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Since the discovery of orphanin FQ (OFQ) in 1995, much attention has been paid on the role of OFQ in pain modulation. However, the results are conflicting. Hence, the present study was undertaken to further elucidate the question of this issue. Tail-flick test and formalin test were used as pain measurement at physiological and pathological states respectively. OFQ or endomorphin (EM, a newly-discovered endogenous μ -opioid receptor agonist) was delivered either intracerebroventricularly (icv) or intrathecally (ith). It was found that icv OFQ at lower dose (< 0.5 μ g) did not affect rat pain response, while at higher dose (>1 μ g) enhanced pain response at both physiological and pathological states, indicated by the shortening of tail flick latency (TFL) and prolonged lifting, licking, biting or shaking of the formalin-injected paw with increased pain intensity scoring (PIS). At any state, OFQ dose-dependently antagonized icv EM-induced analgesia. At spinal level, OFQ at lower doses had no effect on TFL, but mildly prolonged TFL at higher doses; and it showed an obvious synergistic effect on EM (ith)-induced analgesia. In pathological state, OFQ (ith) significantly inhibited formalin pain in dose-dependent manner. The results in present study suggest that OFQ enhances pain response of rat and antagonizes EM-induced analgesia in brain, while has analgesic effect and synergistic effect with EM-induced analgesia at spinal level. *Supported by NSFC and STCS.

Fr75

ORPHANIN FQ INCREASES HEART RATE AND BLOOD PRESSURE IN THE CONSCIOUS SHEEP VIA SYMPATHETIC ACTIVATION.

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Orphanin FQ (OFQ) is a seventeen amino acid peptide which is the endogenous agonist for a novel G-protein coupled receptor with high homology to opioid receptors. OFQ-like immunoreactivity has been found in spinal autonomic nuclei, supporting a role for OFQ in cardiovascular (CV) function. This study was undertaken to examine the CV effects of systemically-administered OFQ. OFQ dose-dependently (10-300 nmol/kg, i.v.) stimulates an increase in mean blood pressure and heart rate (HR) in chronically catheterized sheep. This effect was not blocked by naloxone. Pretreatment with phenoxybenzamine attenuated the immediate pressor response, consistent with sympathetically-mediated vasoconstriction. Furthermore, the lack of a reflex bradycardia suggests either blunting of the baroreflex by OFQ or direct β -adrenergic activation. The bradycardic response to norepinephrine remained intact after OFQ administration, demonstrating that OFQ does not blunt the baroreflex. Additionally, the increase in HR was completely reversed by propranolol pretreatment. These data suggest that OFQ plays a role in CV regulation via sympathetic activation.

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BIDIRECTIONAL EFFECT OF NOCICEPTIN ON FOOD INTAKE

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Several studies indicated that nociceptin (NOC, or orphanin FQ), the endogenous ligand for the ORL1 receptor, is able to affect different central functions including feeding. At this regard, an increase of food intake has been observed either after intracerebroventricular (i.c.v.) or intrahypothalamic administration of NOC in satiated rats (1). In the present study, we investigated the NOC effect on food intake in fasted rats. NOC (1-10 nmol/rat in 5 μ l) was i.c.v. administered in 22 h-fasted male rats and food intake (corrected for spillage) was measured at 30 and 120 min after drug injections. During the first interval NOC caused a highly significant reduction of food ingestion (4.9 \pm 0.2 and 4.7 \pm 0.6 for 1 and 10 nmol/rat vs 7.1 \pm 0.5 g in controls; $p < 0.01$). Data from the entire period of observation, though showing a trend toward reduction, did not reach statistical significance (8.7 \pm 0.6 and 9.1 \pm 0.8 for 1 and 10 nmol/rat vs 11.1 \pm 0.7 g in controls; $p > 0.05$). These results, together with literature data, indicate that NOC exerts a bidirectional effect on food intake in satiated or fasted animals. It is known that fasting increases β -endorphin in the hypothalamus, and a higher morphine orexigen effect occurs in fasted than in fed rats. On these bases, an involvement of the hypothesized anti-opioid role of NOC is suggested for the anorectic action of the neuropeptide in fasted rats.

(1) - Meunier J.-C., Eur. J. Pharmacol., 340, 1-15, 1997.

Fr78

ORPHANIN FQ BLOCKS DEVELOPMENT OF MORPHINE PLACE PREFERENCE.

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Previous study in our laboratory has demonstrated that i.c.v. administration of orphanin FQ (OFQ) suppresses dopamine release in the mesolimbic reward pathway. However, OFQ has neither aversive nor rewarding properties when tested alone in a conditioned place preference paradigm. In order to assess the ability of OFQ to affect the rewarding properties of abused drugs we administered OFQ intracerebroventricularly (i.c.v., 3 to 30 nmol) to male Sprague Dawley rats during the induction of morphine place preference (3mg/kg sub cutaneous, 3 x 40 min sessions). Animals that received orphanin FQ (3 or 10 nmol) in conjunction with morphine during conditioning failed to show a significant morphine place preference. Those receiving vehicle or 30nmol OFQ i.c.v. did exhibit morphine preference. Furthermore, the rate of extinction of morphine place preference was notably accelerated in animals that had received mid to high doses of OFQ. Thus, OFQ blocks the reinforcing properties of morphine. This work was supported by NIDA #DA05010.

Fr77

IN VIVO ROLE OF NOCICEPTIN IN THE DEVELOPMENT OF MORPHINE TOLERANCE.

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We have shown that the peripheral morphine analgesia was not observed in mice chronically treated with morphine (Life Sci. 62, 1667-1681, 1988). This suggests that the analgesic mechanisms located in the central nervous system, but not in the periphery could be regulated through synaptic plasticity using anti-opioid neurotransmitters during the chronic treatment with morphine. Indeed such a morphine tolerance was markedly attenuated in mutant mice lacking receptor for Noci possessing an anti-opioid action (Neurosci. Lett. 237, 136-138, 1997). We further examined the change in the Noci-receptor gene expression by chronic morphine in mice. We will report the significant increase in the gene expression measured by quantitative RT-PCR in specific regions.

Fr79

EFFECTS OF THE NOCICEPTIN ANTAGONIST [Phe¹-Y (CH₂NH)Gly²]NC(1-13)NH₂ ON NOCICEPTION IN RATS.

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It has been ascertained that nociceptin (NOC) is involved in the regulation of several functions, both in periphery and in the CNS. At this regard, NOC exerts both pronociceptive and antinociceptive effects when administered at different CNS sites. Very recently, a new molecule [Phe¹-Y (CH₂NH)Gly²]NC(1-13)NH₂ (NOC-Ant) that behaves as a NOC antagonist in the mouse was defferens in vitro preparation, has been proposed. In this study, we investigated NOC-Ant effects on nociception after intracerebroventricular (i.c.v.) or intrathecal (i.t.) administration in rats, alone or in combination with NOC. The i.c.v. administration of NOC-Ant (10 nmol/rat) caused a brief and significant decrease of tail-flick thresholds (5 min: 3.7 \pm 0.3 vs 5.2 \pm 0.3 in controls; $p < 0.05$). The i.c.v. coadministration of NOC-Ant (10 nmol/rat) did not significantly modify the tail-flick threshold decreasing effect of NOC (1 nmol/rat i.c.v.). The i.t. administration of NOC-Ant (0.2-10 nmol) caused a dose-related increase of tail-flick latencies (15 min: 8.2 \pm 0.6, 12.1 \pm 1.2 and 14.6 \pm 0.5 for 0.2, 2 and 10 nmol/rat vs 5.9 \pm 0.2 in controls; $p < 0.05$). The i.t. coadministration of NOC-Ant (10 nmol) did not reduce the antinociceptive effect of NOC (10 nmol/rat). These data indicate that, as regards NOC effects on nociceptive transmission, NOC-Ant does not seem to reply the NOC antagonism proven in peripheral in vitro preparations. This evidence could suggest the existence of multiple NOC receptors.

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THE EFFECT OF THE AGONIST Ac-RYYRWKNH₂ AND THE ANTAGONIST PHE¹ y (CH₂-NH)GLY²NOICEPTIN(1-13)NH₂ AT THE ORL1 RECEPTOR OF CENTRAL AND PERIPHERAL SITES.

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Using orphanin FQ (OFQ), the hexapeptide agonist Ac-RYYRWK-NH₂ (Dooley et al., 1997, JPET, 283 735) and the antagonist [Phe¹Y (CH₂-NH)Gly²]nociceptin(1-13)NH₂ (Guerrini et al., 1998 Br.J.Pharmac., 123 163) we have conducted radioligand binding studies at ORL1 receptors in rat brain membranes with [³H]OFQ and GTPg³⁵S and have studied the effects of these ligands in the rat vas deferens. In vivo we have compared the stimulatory effects of OFQ and the hexapeptide on feeding and tested the effect of the antagonist. In frontal cortex Ac-RYYRWK-NH₂ and OFQ had equal affinities (pK_i 10.4) and both were potent in stimulating GTPg³⁵S binding (EC₅₀~6nM), although the hexapeptide had lower efficacy (53% of OFQ E_{max}). In the hypothalamus the affinity for the hexapeptide was equally high (pK_i=10.1) but the efficacy was lower still (23% of OFQ E_{max}) even though the B_{max} was similar to that of the cortex. Despite some efficacy in the cortex the antagonist would block the response to OFQ in the GTPg³⁵S assay (pA₂ 8.6). In the vas, the hexapeptide was more potent than OFQ although not as efficacious (EC₅₀ 0.86nM, E_{max} 0.71). The antagonist blocked the response to OFQ and the hexapeptide, although with lower affinity than in cortex (pA₂ values 7.29 and 7.20). In vivo, administration of 50pmoles OFQ (+ peptidase inhibitors) increased food intake in satiated rats (2.61 +/- 0.16g, n=7 cf vehicle 0.90 +/- 0.24g, n=10). Alone, the antagonist (1nmol) also increased food intake (1.68 +/- 0.08g cf vehicle: 0.95 +/- 0.1g, n=6), however no increase was observed after co-administration of this dose of antagonist with 50pmol OFQ (1.05 +/- 0.26g). Similarly, the hexapeptide (30pmol) increased food intake in satiated animals although here, in contrast, the amount of food eaten (1.98 +/- 0.26g, n=12) was not affected when 1nmol [Phe¹Y (CH₂-NH)Gly²]nociceptin(1-13)NH₂ was co-administered (2.60 +/- 0.38g, n=6). Thus, in vitro the interactions of the agonists and antagonist at the ORL1 receptor seem straightforward, whereas in vivo the results are more complex.

Fr82

ANALGESIC ACTIVITY OF MURINE PREPRO-ORPHANIN 160-187 IN MOUSE BRAIN.

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Orphanin FQ/nociceptin (OFQ/N) is the endogenous ligand for the ORL-1/KOR-3 receptor (Meunier et al., 1995; Reinscheid et al., 1995) and produces a wide variety of behavioral responses. Prepro-OFQ/N (ppOFQ/N) contains several paired basic amino acids, raising the possibility that posttranslational processing could lead to the production of other peptides. Several of these processing products have been shown to have pharmacological activity. Murine ppOFQ/N₁₆₀₋₁₈₇ has potent analgesic activity supraspinally (ED₅₀ 5 µg, i.c.v.). This analgesic activity was reversed by naloxone (5 mg/kg, s.c.) indicating involvement of opioid systems. However, murine ppOFQ/N₁₆₀₋₁₈₇ has no affinity (K_i > 1 µM) in Mu, Delta, Kappa1, Kappa3, OFQ or OFQ 1-11 binding assays. These findings suggest that murine ppOFQ/N₁₆₀₋₁₈₇ may be a relevant neuropeptide with physiological actions. This work is supported by DA00310 (G.C.R.) and DA02615 (G.W.P.).

Fr81

AGONIST ACTIVITY OF ORL1 ANTAGONISTS IS DEPENDENT UPON RECEPTOR NUMBER.

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[Phe¹Y (CH₂-NH)Gly²]nociceptin(1-13)NH₂ has recently been demonstrated to possess antagonist activity, attenuating ORL1-mediated inhibition of contractions in both guinea pig ileum and mouse vas deferens preparations. The affinity of [Phe¹Y (CH₂-NH)Gly²]nociceptin(1-13)NH₂ is similar to nociceptin and that of nociceptin(1-13)NH₂ at ORL1 receptors. However, in both CHO and SH-SY5Y cells transfected with the human ORL1, this compound showed clear partial agonist activity, relative to nociceptin, for both the stimulation of [³⁵S]GTPg S binding and inhibition of forskolin-stimulated cAMP accumulation. As with the partial agonist Ac-RYYRIK-NH₂, the extent of the agonist activity is dependent upon the receptor number in the transfected cells, with each partial agonist showing full antagonist activity in low expressing cells. Based upon the functional assays conducted, [Phe¹Y (CH₂-NH)Gly²]nociceptinNH₂ has slightly higher efficacy than Ac-RYYRIK-NH₂ at ORL1. These studies indicate that various assays are required to properly characterize the functional activity of ligands at ORL1, and that assays conducted in cells transfected with a high receptor number will not necessarily produce results similar to those found in isolated tissues.

Fr83

CLONING AND EXPRESSION OF MULTIPLE ALTERNATIVE SPLICE VARIANTS OF THE MOUSE KOR-3/ORL-1 RECEPTOR GENE.

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Using an RT-PCR approach we identified at least five alternatively spliced variants of the mouse KOR-3/ORL-1 receptor gene. The first clone (KOR-3a) had a 34 bp insertion between the first and the second coding exons. The second clone (KOR-3b) had a 98 bp insertion and the third (KOR-3c), a 139 bp insertion which includes the 98 bp in the KOR-3b within the same location as the KOR-1a. The fourth one (KOR-3d) was a 15 bp deletion in the first coding exon. The fifth clone (KOR-3e) has a 81 bp insertion between the second and the third exons. All the cDNA sequences were identical to the KOR-3 cDNA's except for the insertion sequences, while all the insertion sequences were aligned to the intron 2 or the intron 3 of the KOR-3 gene with 100% identity. All of the splice sites used in the alternative splicing are in agreement with GT/AG rule. Northern blot analysis using mRNA from whole brain revealed different sizes and abundances of the variant transcripts. Regional expression of the variants in brain examined using an RT-PCR and Southern Blotting approach showed different distributions and abundance of the variants. Pharmacological characterization of the variants was investigated in CHO cells stably transfected with pcDNA3 constructs containing the full length cDNAs of the variants. Although these variants bound OFQ/N, they also bound the kappa3 ligand, naloxone benzoylhydrazone. This work is supported by DA00296 (Y.-X.P.) and DA02615 (G.W.P.).

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STRUCTURE AND ALTERNATIVE SPLICE VARIANTS OF THE RAT KOR-3/ORL-1 RECEPTOR GENE.

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A genomic fragment containing the cDNA sequence encoding a rat KOR-3/ORL-1 receptor was obtained by a PCR-based approach. Sequence analysis of the genomic fragments indicates a similarity to the mouse KOR-3 gene. The rat KOR-3 gene contains five exons separated by four introns with the intron 2 being the largest. Using an RT-PCR strategy, we isolated eight alternatively spliced variants of the rat KOR-3 gene. Five had insertions with different mini-exons between the first and the second coding exons while one was between the second and the third coding exons. All of the insertion sequences have been mapped to the rat KOR-3 gene with 100% identity and are highly homologous with those identified in the mouse variants. Another two clones are exon-skipping variants. Expression of the variants in rat brain was examined by Northern blot and RT-PCR approaches. Binding studies in CHO cells stably transfected with pcDNA3 constructs containing the full length cDNAs of the variants confirm that they are members of the KOR-3/ORL-1 receptor family. This work is supported by DA00296 (Y.-X.P.) and DA02615 and DA00220 (G.W.P.).

Fr86

MOLECULAR MODELLING OF THE ORL1 RECEPTOR AND ITS COMPLEX WITH NOCICEPTIN.

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A molecular model of the ORL1 receptor has been built, including the TM domain and the extra- and intra-cellular loops which have been structurally validated using an environmental amino acid propensity table. An extended binding site able to accommodate nociceptin-(1-13), the shortest fully active analogue of nociceptin, has been characterised. The N-terminal FGGF tetrapeptide is proposed to bind in a highly conserved region, comprising two distinct hydrophobic pockets in a channel formed by TM helices 3, 5, 6 and 7, capped by the acidic second extracellular loop (EL2) controlling access to the TM elements of the peptide binding site. The nociceptin conformation provides for the selective preference of the ORL1 receptor for nociceptin over dynorphin A, conferred by residue positions 5 and 6 (TG vs LR) (Lapalu, S. et al., 1997, FEBS Lett., 417:333), and the favourable interaction of its highly positively charged core (residues 8-13) with the EL2 loop, thought to mediate receptor activation.

Fr85

THE SECOND EXTRACELLULAR LOOP (EL2) IS REQUIRED FOR ORL1 RECEPTOR ACTIVATION BY NOCICEPTIN.

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Replacing discrete domains located between the N-terminus and top of TM3 of the k-opioid receptor (KOR1) by the homologous domains of the nociceptin (ORL1) receptor yields hybrid receptors which, in comparison with KOR1, display up to 300-fold increased affinity yet still low sensitivity towards nociceptin (noc), and unchanged (high) affinity and sensitivity towards dynorphin A (dynA). Substituting further in these chimeras the second extracellular loop (EL2) for that of the ORL1 receptor fully restores responsiveness to noc, while still not impairing sensitivity to dynA. Thus, a bi-functional hybrid receptor is generated which binds and responds to both noc and dynA as efficiently as ORL1 does to noc and KOR1 to dynA. Together, these results suggest that EL2 is required for activation of the ORL1 receptor by nociceptin, but not or clearly less so, for activation of the k-opioid receptor by dynorphin A. This finding indicates pathways to the design of new ORL1 receptor ligands.

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ORL₁ RECEPTOR EXPRESSION IN THE BRAINS OF MU OR k RECEPTOR KNOCKOUT MICE.

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Since the cloning of the ORL₁ receptor and its endogenous peptide nociceptin (orphanin FQ) much evidence has accumulated to suggest an important interaction between this system and that of the classical opioid receptors. To further study these putative interactions we have carried out a detailed autoradiographic mapping study of the ORL1 receptor in the brains of mice deficient in either the μ or k-opioid receptor gene. Receptor binding was carried out as previously described (Kitchen et al., 1997 Brain Res., 778, 73-88). Adjacent sections (20μm) were cut from wild-type, heterozygous and homozygous μ or k knockout brains for the determination of total and non-specific [³H]leucyl nociceptin (0.4 nM) binding to the ORL₁ receptor. Non-specific binding was defined in the presence of 10 μM unlabelled nociceptin and an incubation time of three hours was employed. There were no major differences in number and distribution of the ORL₁ receptor throughout the brain of both μ and k-knockout animals.