Convegno Monotematico SIF

La Nocicettina/Orfanina FQ ed il suo recettore: recenti acquisizioni fisiofarmacologiche e prospettive farmacoterapeutiche

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ABSTRACTS

KEYNOTE

LECTURE

NOCICEPTIN AND ITS RECEPTOR: UTILIZING FUNCTIONAL GENOMICS TO IDENTIFY NEW PAIN AND OTHER TREATMENTS

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Nociceptin, a neuropeptide also known as orphanin FQ, is the first novel bioactive substance to have been discovered by the implementation of a functional genomics/reverse pharmacology approach. Nociceptin was indeed identified as the natural ligand of a previously cloned orphan G protein-coupled receptor, the opioid receptor-like 1 (ORLI) receptor. Nociceptin (FGGFTGARKSARKLANQ) bears a structural resemblance to opioid peptides, notably dynorphin A, and is synthesised as a larger precursor polypeptide, pre-pronociceptin, which is widely distributed in the nervous system. Nociceptin is primarily an inhibitory neuropeptide that acts on neurons to depress synaptic transmission, either pre-synaptically by inhibiting transmitter release or post-synaptically by decreasing neuronal excitability.

Since the publication of its structure late 1995, nociceptin has been the subject of intensive study to establish its role in normal brain function and its possible involvement in neurophysiopathology. Thus, the neuropeptide has been variously shown to modulate nociception, locomotion, stress and anxiety, food intake, neuroendocrine secretion, learning and memory, drug addiction, smooth muscle tone in the cardiovascular system, and respiratory, gastro-intestinal and urogenital tracts. Unlike opioids, nociceptin appears to be free of abuse potential, and may indeed possess anti-opioid/anti-addictive properties. The broad spectrum of pharmacological effects of nociceptin suggests multiple therapeutical applications for ORLI receptor ligands, e.g. for the treatment of chronic pain states, stress-related abnormal feeding behavior (anorexia and bulimia), etc. However, validation of the ORLI receptor as therapeutical target will require the use of receptor agonists and/or antagonists that are superior to nociceptin in terms both of metabolic stability and bioavailability. A handful such ligands have recently become available whose pharmacological profiling in appropriate animal models will clarify the clinical value of the ORL1 receptor.

ORAL

COMMUNICATIONS

NON PEPTIDE/PEPTIDE CHIMERIC LIGANDS FOR THE NOCICEPTIN/ORPHANIN FQ RECEPTORS

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Nociceptin/orphanin FQ (N/OFQ, H-Phe-Gly-Gly-Phe-Thr-Gly-Ala-Arg-Lys-Ser-Ala-Arg-Lys-Leu-Ala-Asn-Gln-OH) is the endogenous ligand for the G-protein coupled receptor referred to as NOP receptor. NOP receptor activation by N/OFQ modulates several biological functions both at central and peripheral level. SAR studies demonstrated that the N/OFQ sequence can be divided into a N-terminal tetrapeptide "message" crucial for receptor activation and a C-terminal "address" important for receptor binding. On the basis of this message / address concept we synthesized some chimeric compounds in which we substituted the natural message domain with the non selective non peptide NOP ligand NNC 63-0532 and used as address domain the peptide sequences Thr-NH_2, N/OFQ(5-9)-NH_2, N/OFQ(5-13)-NH_2 and N/OFQ(5-17)-NH_2. All the compounds were pharmacologically evaluated in the electrically stimulated guinea pig ileum. NNC 63-0532 produced a concentration dependent inhibition of the electrically induced twitches showing in comparison with N/OFQ lower potency and higher maximal effects. In addition, contrary to N/OFQ the effects of NNC 63-0532 were insensitive to the NOP selective antagonist UFP-101 while prevented by naloxone. Similar results were obtained with NNC 63-0532/Thr-NH_2 and NNC 63-0532/N/OFQ(1-9)-NH_2. On the other hand, the inhibitory effects of NNC 63-0532/N/OFQ(5-13)-NH_2 and NNC 63-0532/N/OFQ(5-17)-NH_2 were slightly antagonized by UFP-101 while naloxone prevented the effects of the high but not of the low concentrations of the ligands. These data indicate that it is possible to functionalize with the N/OFQ address sequence a non peptide NOP ligand for increasing its binding to the NOP receptor. Moreover, these results corroborate the idea that the 5-13 sequence represents the crucial core of the N/OFQ address domain.

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UFP-102, A NOVEL HIGLY POTENT AGONIST FOR THE NOCICEPTIN/ORPHANIN FQ RECEPTOR – IN VIVO STUDIES

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UFP-102 ([(pF)Phe^4,Arg^{14},Lys^{15}]N/OFQ-NH_2) is a novel ligand for the nociceptin/orphanin FQ (N/OFQ) peptide receptor (NOP). Data obtained in vitro in isolated tissues from various species demonstrate that UFP-102 behaves as a highly potent and selective full agonist for the NOP receptor. Aim of this study was to investigate the pharmacological profile of UFP-102 in vivo in the mouse tail withdrawal and locomotor activity assays where N/OFQ produces after i.c.v. administration pronociceptive and locomotion inhibitory effects, respectively. UFP-102 (0.01- 0.3 nmol/mouse, i.c.v.) mimicked the effects of N/OFQ. However, in both the assays, UFP-102 was at least 30 fold more potent than N/OFQ and produced longer lasting effects; in fact, the effects of UFP-102 lasted more than 90 min while those evoked by the natural peptide were no longer evident after 30 min. UFP-101 (30 nmol/mouse, i.c.v.) a potent and selective NOP receptor antagonist, reduced the pronociceptive and locomotion inhibitory effects elicited by 0.1 nmol UFP-102.
Similar experiments were performed in wild type mice ($NOP^{+/+}$) and in animals lacking the NOP receptor gene ($NOP^{-/-}$). I.c.v. injections of 10 nmol N/OFQ or 0.3 nmol UFP-102 produced profound pronociceptive and locomotion inhibitory effects in $NOP^{+/+}$, while the two peptides were completely inactive in $NOP^{-/-}$ mice.
Collectively, these data demonstrate that UFP-102 behaves in vivo as a potent and selective NOP receptor agonist able to produce long lasting effects.

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UFP-101, A NOVEL PEPTIDE SELECTIVE ANTAGONIST FOR THE NOP RECEPTOR – SUMMARY OF IN VITRO AND IN VIVO STUDIES

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UFP-101 is a novel peptide ligand for the nociceptin/orphanin FQ (N/OFQ) peptide receptor (NOP). UFP-101 has been generated by combining into the sequence N/OFQ-NH_2 the chemical modification which confers antagonist properties ($Nphe^1$) with that which increases agonist potency (Arg^{14},Lys^{15}). UFP-101 binds with high affinity (pKi 10.1) to recombinant human NOP receptor expressed on CHO cells and competitively antagonises N/OFQ stimulation of GTPγ^{35}S binding (pA_2 9.1) and inhibition of cAMP formation (pA_2 7.1). The pure antagonist action of UFP-101 was recently confirmed at native human NOP receptors which inhibit neurogenic contractions of the bronchus and stimulate monocyte chemotaxis. NOP selective antagonism (pA_2 7.1-7.8) has been confirmed for UFP-101 in vitro in several central and peripheral preparations of animal origin with bioassay, neurochemical and electrophysiological techniques. In vivo studies demonstrated that UFP-101 antagonizes the following N/OFQ actions: hyperalgesia, reversal of stress-induced analgesia, inhibition of locomotor activity, stimulation of diuresis in mice, bradycardia, hypotension and reduction of plasma NE levels in guinea pigs, stimulation of food intake and spinal analgesia in rats. Moreover UFP-101 (like other selective NOP antagonists) produces antidepressant-like effects in normal mice in the forced swimming or the tail suspension test. In mice lacking the NOP receptor gene these actions are absent.
Collectively these results demonstrate the pure antagonist properties of UFP-101 in a wide range of preparations/assays and the usefulness of this peptide as a pharmacological tool for investigating in vitro and in vivo the pathophysiological roles played by the N/OFQ – NOP receptor system.

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USING THE ECDYSONE INDUCIBLE EXPRESSION SYSTEM TO PROBE PARTIAL AGONISM AT HUMAN NOP. STUDIES WITH [PHE^{1}ψ(CH$_2$-NH)GLY^2]N/OFQ(1-13)NH$_2$.

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In order to quantify the intrinsic efficacy of the presumed partial agonist [Phe^{1}ψ(CH$_2$-NH)Gly^2]N/OFQ(1-13)NH$_2$ (F/G), the ecdysone inducible expression system was used to vary NOP receptor density whilst maintaining a consistent cellular background. To induce expression, CHO cells with the ecdysone system for the human NOP receptor (CHO$_{INDhNOP}$) were incubated in the presence of ponasterone A (P) at 1, 2, 5, 10μM for 20hours. Saturation binding using [*leucyl*-^{3}H]N/OFQ and GTPγ[^{35}S]/cAMP assays were used to determine B_{max} and agonist efficacy respectively. Binding of [*leucyl*-^{3}H]N/OFQ increased from 24±4 fmol/mg with 1μM P to 1101±145 at 10μM. At 20μM P there was an apparent decrease in expression. pEC$_{50}$ values for GTPγ^{35}S binding ranged from 7.23±0.38 to 7.72±0.06 (2μM-10μM P) for [F/G] and 8.12±0.32 to 8.60±0.07 (1μM-10μM P) for N/OFQ(1-13)NH$_2$ and E_{max} values (stimulation factor relative to basal) ranged from 1.51±0.15 to 3.21±0.38 (2μM-10μM P) for [F/G] and 1.28±0.03 to 6.95±1.05 (1μM-10μM) for N/OFQ(1-13)NH$_2$. [F/G] alone did not stimulate GTPγ^{35}S binding at 1μM P but competitively antagonised the effects of N/OFQ(1-13)NH$_2$ with a pK_B of 7.62±0.08. pEC$_{50}$ values for inhibition of cAMP formation ranged from 8.26±0.87 to 8.32±0.13 (2μM-10μM P) for [F/G] and 9.42±0.49 to 10.35±0.22 (2μM-10μM P) for N/OFQ(1-13)NH$_2$ and E_{max} values ranged from 19.6±4.8 to 83.2±4.0 (2μM-10μM P) for [F/G] and 40.9±2.2 to 86.0±3.7 (2μM-10μM) for N/OFQ(1-13)NH$_2$. At 1μM P there was no consistent inhibition with either peptide. In the same cellular environment with the only variable being the number of receptors we show that the pharmacological profile of [F/G] can be manipulated to encompass full and partial agonism along with pure antagonism.

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BLOCKADE OF NOCICEPTIN/ORPHANIN FQ – NOP RECEPTOR SIGNALLING PRODUCES ANTIDEPRESSANT-LIKE EFFECTS

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Nociceptin/orphanin FQ (N/OFQ) produces several biological actions by selectively activating the N/OFQ peptide receptor (NOP). It has been recently reported that NOP receptor antagonists induce antidepressant-like effects in the mouse forced swimming test, i.e. reduce immobility time (1). The present study was aimed to further investigate the role of N/OFQ signalling in animal models of depression. In male Swiss mice, intracerebroventricular (i.c.v.) injection of the novel NOP receptor antagonist, UFP-101 (1-10 nmol), 5 min prior the test, dose-dependently reduced the immobility time (control 192 ± 14 s, UFP-101 10 nmol 91 ± 15 s; $p<0.05$). The effect of 3 or 10 nmol UFP-101 was fully or partially reversed, respectively, by the co-administration of 1 nmol N/OFQ, which was *per se* inactive. NOP receptor knockout mice ($NOP^{-/-}$) showed a reduced immobility time compared to their wild-type ($NOP^{+/+}$) littermates (143 ± 12 s and 215 ± 10 s, respectively; $p<0.05$). Moreover, i.c.v. injected UFP-101 (10 nmol) significantly reduced immobility time in $NOP^{+/+}$, but not in $NOP^{-/-}$ mice. Similar results were obtained in the tail suspension test in mice acutely treated with UFP-101 (control 179 ± 11 s, UFP-101 10 nmol 111 ± 10 s; $p<0.05$) and comparing the behaviour of $NOP^{+/+}$ and $NOP^{-/-}$ mice (133 ± 23 s and 75 ± 14 s, respectively; $p<0.05$). Moreover, in rats acutely treated with 10 nmol i.c.v., UFP-101 decreased the immobility time (control 113 ± 11 s; UFP-101 40 ± 11 s; $p<0.05$) and increased the climbing behaviour (control 122 ± 11 s; UFP-101 204 ± 15 s; $p<0.05$) in the forced swimming test.
In conclusion, these results indicate that blockade of the N/OFQ-NOP receptor signalling in the brain produces antidepressant-like effects. These effects appear to be robust across species (mouse and rat) and tests (forced swimming and tail suspension) and thus candidate the NOP receptor as a target for the development of innovative antidepressant drugs.

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THE BED NUCLEUS OF THE STRIA TERMINALIS: SITE FOR CRF-INDUCED ANOREXIA AND ITS REVERSAL BY NOCICEPTIN/ORPHANIN FQ

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Nociceptin/orphanin FQ (N/OFQ), possesses anti-stress properties (1). In our laboratory we have shown that intracerebroventricular (ICV) injection of N/OFQ reverses the anorectic effect induced by stress or by the ICV administration of corticotropin releasing factor (CRF) (2). Since N/OFQ shows no affinity for CRF receptors, this effect may be expression of functional antagonism.
To shred new light on the mechanisms involved in the anti-stress action of N/OFQ we investigated the ability of N/OFQ to prevent CRF-induced anorexia following microinjection of both peptides into the bed nucleus of the stria terminalis (BNST), the central amygdala (CeA) and the locus coeruleus (LC).
The results of the present study show that microinjection of 0.05-0.1 μg/site of CRF into the BNST, but not the CeA or the LC induces pronounced anorexia in 20-h food deprived rats. This effect of CRF was completely reversed by 0.025-0.5 μg/site of N/OFQ given into the BNST, but not into the CeA or the LC. Pretreatment with 0.025-0.5 μg/site of N/OFQ into the BNST also blocked the anorectic action of 0.1 μg/site of CRF given into the same site. Lastly, intra-BNST microinjection of 0.025-0.5 μg/site of N/OFQ did not modify basal food intake in rats.
The present results demonstrated that the BNST is a site of action for the anorectic effect of CRF and that NOP receptors in the BNST, but not in the CeA or the LC, are critical for reversal of CRF-induced anorexia by N/OFQ.

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ACUTE AND CHRONIC STRESS INDUCE AN UP-REGULATION OF ORL1 MRNA EXPRESSION IN THE RAT HIPPOCAMPUS.

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Nociceptin receptor, the opioid-like receptor 1 (ORL1), is widely distributed in the central nervous system, being more abundant in the cortex, septum, hippocampus, thalamic and hypothalamic nuclei, dorsal raphe and locus coeruleus. The role of nociceptin in the modulation of emotional behavior and in the regulation of stress response has been investigated demonstrating that, when injected centrally, it has anxiolytic-like properties in a variety of behavioral tests. In addition, nociceptin knock-out mice are characterized by an increased anxiety. However, a pharmacological or genetic blockade of the receptor produces antidepressant-like effects. In the present study, the possible alteration of ORL1 mRNA expression in limbic regions was investigated in rats exposed to an acute stress (restraint) or to a chronic social stress (social defeat) to further investigate the role of ORL1 in stress regulation. Sprague-Dawley rats (6/group) were exposed to a 1 hour restraint stress or to 21 days social defeat (30 min./session). *In situ* hybridization was then performed using a riboprobe specific for the ORL1 receptor, generated from a cDNA fragment that spans 600 base pairs (101-700) of the rat sequence (accession number NM_031569). The distribution of ORL1 mRNA expression was in line with that previously described. In the restrained rats, a significant up-regulation in ORL1 mRNA expression was found in the hippocampal CA1 region (15%; $p = 0.029$), while no differences were seen in other regions analyzed, such as dentate gyrus, paraventricular and arcuate hypothalamic nuclei, septum, medial and central amygdala. In the rats exposed chronically to social defeat, an increase in ORL1 mRNA expression was detected in the CA2 region of the hippocampus (26%; $p = 0.027$). No differences were evident in the other regions analyzed, such as dentate gyrus, paraventricular and arcuate hypothalamic nuclei, septum, medial and central amygdala.
The present results show the alteration of ORL1 mRNA expression levels after exposure to acute and chronic stress in the hippocampus, a limbic area often associated to higher brain functions and mood regulation, giving further support for a role of the nociceptin system in emotional behavior.

BLOCKADE OF STRESS-, CRF- AND UROCORTIN II-INDUCED ANOREXIA BY THE NOP RECEPTOR AGONIST RO 64-6198.

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(1S,3aS)-8-(2,3,3°,4,5,6-hexahydro-1H-phenalen-1-yl)-1-phenyl-1,3,8- triazaspiro[4.5]decan-4-one (Ro 64-6198), a nonpeptidic agonist for the NOP receptor, exhibits anxiolytic properties in stressful conditions. The present study was aimed at evaluating whether activation of NOP receptors by Ro 64-6198 may reverse the anorectic effect of restraint stress, of intracerebroventricular (ICV) injection of corticotropin releasing factor (CRF, which is not selective for CRF1 and CRF2 receptors) or of the selective CRF2 receptor agonist Urocortin II (UcnII).

In body restraint experiments, 20-h food deprived rats were treated with intraperitoneal (IP) injection of Ro 64-6198 or vehicle. Ten min later they were confined in cylindrical Plexiglas tubes for 60 min and then returned to their cage with food. In microinjection experiments, 20-h food deprived rats were IP injected with Ro 64-6198 or vehicle. Ten min later, they received ICV 200 ng/rat of CRF or 6000 ng/rat of Ucn II or their vehicles. Food was offered 20 min after ICV injections.

Pretreatment with Ro 64-6198 reverted the hypophagic effect induced by both restraint or CRF [$F(4,44)= 15.37$ $p<0.01$ and $F(3,37)=16.44$ $p<0.01$, respectively]; the effect was statistically significant at the 3 doses tested (0.3, 1.0 or 2.5 mg/kg). ICV administration of the selective NOP receptor antagonist [Nphe^1]N/OFQ(1-13)NH_2 (2 injections of 33 or 66 µg/rat) abolished the effect of Ro 64-6198 on CRF-induced anorexia. Pretreament with Ro 64-6198 (0.3 or 1.0 mg/kg, IP) significantly inhibited the anorectic effect elicited by 6000 ng/rat of UcnII [$F(3,30)= 3.46$ $p < 0.05$]. Post-hoc comparisons revealed an effect of Ro 64-6198 at both 0.3 and 1.0 mg/kg ($p<0.05$). In freely feeding rats, Ro 64-6198 significantly increased feeding at 2.5, but not at 0.3 or 1.0 mg/kg; thus reversal of stress-, CRF- and UcnII-induced anorexia by Ro 64-6198 can be evoked at doses lower than those that are hyperphagic.

Present results demonstrate that the nonpeptidic NOP receptor agonist Ro 64-6198 markedly and selectively inhibits the anorectic effect of stress, CRF and related peptides.

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INVOLVEMENT OF THE NEUROPEPTIDE NOCICEPTIN/ORPHANIN FQ IN KAINATE SEIZURES

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The neuropeptide nociceptin/orphanin FQ (N/OFQ) modulates neuronal excitability and neurotransmitter release. Furthermore, mRNA levels for the N/OFQ precursor (proN/OFQ) are increased following seizures. However, it is unclear if N/OFQ plays a role in seizure expression. Therefore, 1) we analyzed proN/OFQ mRNA levels, NOP (the N/OFQ receptor) mRNA levels and receptor density and N/OFQ release in the kainate model of epilepsy, using Northern blot analysis, *in situ* hybridization, receptor binding and microdialysis; 2) we examined susceptibility to kainate seizures in mice treated with J-113397, a selective NOP receptor antagonist, and in proN/OFQ knock-out mice. After kainate administration, increased proN/OFQ gene expression was observed in the reticular nucleus of the thalamus and in the medial nucleus of the amygdala. In contrast, NOP mRNA levels and receptor density decreased in the amygdala, hippocampus, thalamus and cortex. Furthermore, N/OFQ release increased during status epilepticus in hippocampus and thalamus: basal N/OFQ release was 4.59±0.26 fmol/tube in the hippocampus and 3.30±0.75 fmol/tube in the thalamus. Kainate seizure-induced N/OFQ release in the rat hippocampus and thalamus reached its maximum (2.2-fold increase over basal values) once the status epilepticus was established (i.e. 90 min after treatment). On the following day, the release of N/OFQ was not significantly different compared to the basal levels preceding kainate administration. Mice treated with the NOP receptor antagonist J-113397 displayed reduced susceptibility to kainate-induced seizures (i.e significant reduction of behavioral seizure scores). N/OFQ knock-out mice were less susceptible to kainate seizures compared with their wild-type littermates, in that lethality was reduced, latency to generalized seizure onset prolonged, behavioral seizure scores decreased. Intracerebroventricular administration of N/OFQ prevented reduced susceptibility to kainate seizures in N/OFQ knock-out mice. These data indicate that acute limbic seizures are associated with increased N/OFQ release in selected areas, causing down-regulation of NOP receptors and activation of N/OFQ biosynthesis, and support the notion that the N/OFQ-NOP system plays a facilitatory role in kainate seizure expression.

THE N/OFQ-NOP RECEPTOR SYSTEM IN THE SUBSTANTIA NIGRA MODULATES STRIATAL DOPAMINE RELEASE AND MOTOR BEHAVIOR IN RATS.

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Nociceptin (N/OFQ) and its receptor (NOP receptor) are moderately expressed in the substantia nigra pars reticulata (SNr) and pars compacta (SNc) two of the major mesencephalic structures that are involved in the control of motor behavior. Until now, the inhibitory effect of central N/OFQ administration on locomotor activity is mainly attributed to its inhibitory action on the meso-accumbal rather than nigro-striatal dopaminergic pathways. The present study was undertaken to investigate the role of the N/OFQ-NOP receptor system in SNr in the control of nigro-striatal dopaminergic transmission and motor behavior in awake freely moving rats. A dual probe microdialysis technique, coupled with a microinjection method, was employed to investigate the effects of NOP receptor ligands given into the SNr on striatal dopamine (DA) release. The accelerating rotarod test was performed to study the effects of NOP receptor ligands on the physiologically-stimulated motor activity. Stimulation and blockade of nigral NOP receptors oppositely modulated both striatal DA release and motor behavior. In fact, striatal DA efflux was reduced by nigral perfusion with N/OFQ (100 µM) while it was increased by UFP-101 (10 µM). Likewise, striatal DA release was inhibited by intranigral injection of N/OFQ (10 nmol/0.5µl) and increased by the NOP receptor antagonists UFP-101 (30 nmol). The non peptide NOP receptor antagonist J-113397, either locally injected in the SNr (1 nmol) or administered systemically (3 mg/Kg i.p.), facilitated striatal DA release. N/OFQ microinjected into the SNr (0.01-10 nmol) promptly reduced rat motor performance at 0.1 and 1 nmol (40 % and 70 %, respectively) and abolished it at 10 nmol. On the contrary, both UFP-101 (0.1-10 nmol) and J-113397 (0.1-10 nmol) facilitated motor activity. The present data suggest that the N/OFQ-NOP receptor system in the SNr exerts a tonic inhibitory control both on the nigro-striatal dopaminergic pathway and motor behavior in rats. In view of the up-regulation of the N/OFQ-NOP receptor system observed in the SNc of DA-depleted rats, NOP receptor antagonists may represent a new therapeutic tool for the treatment of hypokinetic motor disorders such as Parkinson's disease.

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EFFECT OF NOCICEPTIN ON OPIOID AND NON-OPIOID ANTINOCICEPTIVE ACTIVITY

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The role of nociceptin in modulating pain processing remains controversial. Some studies have demonstrated an antinociceptive effect of intrathecal administration of nociceptin, others showed that the antinociceptive effect is present only in hyperalgesia induced by inflammatory tissue damage. Nociceptin antagonizes supraspinal opioid analgesia, on the other hand the antinociceptive effect of nociceptin is sensitive to the opioid antagonist naloxone. These contradictory results could depend on the different experimental procedure and/or route of administration. In the present study we examined the effects of central (i.c.v) nociceptin injection in two different noxious tests involving spinal and supraspinal pain processing: Randall& Selitto (RS) and hot-plate. The role of central nociceptin was studied in opioid and non-opioid antinociception induced by morphine (M) or calcitonin (CT). Rats were injected with carragenin in the hind paw and, 120 min after, paw pressure responses were examined. Then nociceptin was administered i.c.v. (10 μg/rat), 5 min before M (1 mg/Kg, s.c.) or before CT (2.5 μg/rat, i.c.v.) and 60 min thereafter. Pain thresholds were measured at 30, 60 and 120 min from M or CT. In the hot-plate test, nociceptin (10 μg/rat, i.c.v.) was injected 5 min before M (10 mg/Kg, s.c.) or CT (2.5 μg/rat, i.c.v.) and hot-plate latencies were detected at 15, 30, 60 and 120 min. In the RS nociceptin did not modify the hyperalgesia induced by carragenin but significantly decreased M induced antinociceptive activity at 30 min. On the contrary nociceptin did not modify CT induced antinociception. The same results were obtained in the hot-plate test. The present results show that central nociceptin is able to remove opioid induced antinociception whatever the nociceptive test used. The involvement of nociceptin in the pathways subserving non-opioid analgesia can be ruled out since it did not modify CT-induced antinociceptive activity.

POSSIBLE INVOLVEMENT OF NOCICEPTIN/NOP ENDOGENOUS SYSTEM IN MORPHINE TOLERANCE IN THE RAT HIPPOCAMPUS

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An interplay between nociceptin/orphanin FQ (N/OFQ) and the opioid system (1, 2) has been widely shown and an "anti-opioid" role has been suggested for this neuropeptide, at supraspinal level (1). In this context, we previously reported (3) changes in proN/OFQ biosynthesis in the rat mesocorticolimbic system, after chronic morphine administration.
With the aim to investigate the involvement of N/OFQ and of its receptor (NOP) in the mechanisms underlying the development of tolerance to the antinociceptive effect of opiates, here we studied the possible alterations of the N/OFQ system in the hippocampus of morphine-tolerant rats. For this purpose, different groups of rats were i.p. administered with morphine (10 mg/kg) or saline (controls, C) twice daily, for five days. Two hours after the last administration, animals were killed and proN/OFQ and proNOP mRNA levels (Northern analysis) as well as N/OFQ peptide levels (radioimmunoassay) were measured. In addition, the condition of the NOP receptor was also studied in this area, by radioreceptor binding.
Results showed that, in the hippocampus, chronic morphine administration caused an increase in proN/OFQ mRNA (265 ± 46 % vs C=100; $P < 0.01$) and in proNOP mRNA (167 ± 17 % vs C; $P < 0.01$) levels. N/OFQ measurement (by RIA) showed a decrease of the peptide levels in the same brain area (1.45 ± 0.06 vs 1.68 ± 0.07 pmol/g tissue in morphine-tolerant and C, respectively; $P < 0.05$), and, finally, an increase of Bmax values for NOP was ascertained by binding studies (236.9 ± 22 vs 145.6 ± 15 fmol/mg protein; $P < 0.01$).
These data suggest a possible role for the N/OFQ-NOP system in mechanisms underlying the devolopment of morphine tolerance. While the increase of proNOP is also reflected by the increase of NOP Bmax values, the observed decrease of N/OFQ levels, showed by RIA, might correspond to an increase of the utilization and turnover of the neuropeptide.
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NOCICEPTIN/ORPHANIN FQ INHIBITS ETHANOL SELF-ADMINISTRATION AND CONDITIONED REINSTATEMENT OF ALCOHOL-SEEKING BEHAVIOUR IN ALCOHOL-PREFERRING RATS

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Nociceptin/orphanin FQ (N/OFQ) has been shown to reduce voluntary 10% ethanol consumption and to abolish ethanol-induced conditioned place preference in alcohol-preferring rats (1). Moreover, N/OFQ inhibited stress-induced reinstatement of alcohol-seeking behaviour (2).

The present study was aimed at evaluating the effect of N/OFQ on the self-administration of 10% ethanol or 10% sucrose, on a fixed-ratio 1 (FR1) and on a progressive-ratio (PR) schedule of reinforcement in genetically selected Marchigian Sardinian alcohol-preferring (msP) rats. Furthermore, using an experimental model of "relapse", in which rats were trained to lever press for ethanol in the presence of the discriminative stimulus of orange odor (S^+) and 1 s cue light (CS^+) or for water in the presence of anice odor (S^-) and 1 s white noise (CS^-), the effect of N/OFQ on cues-induced reinstatement of extinguished ethanol-responding was investigated.

Chronic (6 days) intracerebroventricular (ICV) injection of 0.5 or 1.0 µg/rat of N/OFQ significantly reduced alcohol self-administration under both FR1 and PR contingency, but did not modify sucrose self-administration. Acute ICV administration of N/OFQ, 1.0 or 2.0 µg/rat, significantly ($p < 0.05$) inhibited reinstatement of extinguished ethanol responding under S^+/CS^+ conditions, whereas lever pressing under S^-/CS^- was not modified.

The present study shows that the reinforcing effects of ethanol are markedly blunted by N/OFQ. Moreover, N/OFQ is able to prevent reinstatement of ethanol-seeking behaviour elicited not only by stress (2), but also by environmental conditioning stimuli.

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ALCOHOL AND NEUROPEPTIDES IN GABAERGIC TRANSMISSION IN THE CENTRAL NUCLEUS OF AMYGDALA

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Behavioral studies indicate that GABAergic systems in the central nucleus of the amygdala (CeA) play a major role in the reinforcing effects of ethanol and in the anxiogenic response to ethanol withdrawal. The corticotrophin releasing factor (CRF) system within the CeA is also implicated in regulating voluntary ethanol intake. Recent behavioral studies have focused attention on brain nociceptin/orphanin FQ (N/OFQ), the endogenous ligand of the NOP receptor, in relation to the rewarding effects of ethanol. N/OFQ participates in neuronal regulation of hormonal responses to acute stress exposure and exerts marked antagonist effects on endogenous opioid and CRF systems. In particular, N/OFQ reduces ethanol self-administration and prevents the reinstatement of ethanol-seeking behavior elicited by alcohol-related cues. N/OFQ also has anxiolytic and anti-stress effects. We recently reported that (using electrophysiological techniques) in most CeA neurons low ethanol concentrations augmented GABAergic neurotransmission at both pre-and postsynaptic sites. We also found that CRF enhances GABAergic inhibitory responses, suggesting that these interactions may relate to the reinforcing effect of ethanol. Therefore, we investigated the acute ethanol interactions with CRF and N/OFQ and with GABA receptor-mediated inhibitory responses (GABA-IPSP/Cs). Our preliminary results show that N/OFQ (500 nM) slightly decreased the amplitude of GABAA-IPSP/Cs and blocked the increase of GABAA-IPSP/Cs induced by 44 mM ethanol, as well as the ethanol-induced increase in frequency of spontaneous GABAergic responses. N/OFQ also increased the paired-pulse facilitation (PPF) ratio, suggesting a decrease in GABA release that would oppose the ethanol effect. In another group of cells, we found that CRF (100 nM) like ethanol increased the amplitude of GABAA-IPSP/Cs. The addition of N/OFQ blocked the effect of CRF, suggesting that N/OFQ antagonizes the effect of CRF on GABAergic transmission. However, the effect of N/OFQ on CRF appeared to take a longer perfusion time (about 8 min) compared to the effect of N/OFQ versus ethanol (about 3 min), suggesting different mechanisms. Our findings are consistent with an overall reduction in the activity and output of the CeA and may account in part for the anxiolytic or "tension-reducing" effect of ethanol consumption. Our hypothesis is that the anxiolytic and anti-stress properties of N/OFQ may be mediated by interactions within the CRF-GABAergic systems.

INTRAVESICAL NOCICEPTIN/ORPHANIN FQ IN PATIENTS WITH NEUROGENIC DETRUSOR OVERACTIVITY: TWO YEARS OF EXPERIENCE

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The aim of this study is to review our experience with intravesical nociceptin/orphanin FQ (N/OFQ) in patients with a neurogenic detrusor overactivity.
From January 2001 to April 2003 we assessed the clinical and urodynamic effects of intravesical N/OFQ, in patients with a neurogenic detrusor overactivity, by three different protocols: i) Monocentric, pilot, non randomised, non controlled study (9 pts); ii) Double blind randomised controlled study (14 pts); iii) Dose/effect curves study (5 pts). In the first two studies we used a dose of 1 M, while in the third one this dose was matched with 100nM. We measured the following urodynamic parameters: bladder capacity (BC), volume threshold for the appearance of detrusor overactivity (VT-DO), and maximum bladder pressure (MBP), measured during the involuntary bladder contraction. Finally a voiding chart before and 15 days after the treatment was recorded in all the patients. All the studies were statistically tested and $p < 0.05$ was considered significant.
In the pilot non controlled, non randomized study we demonstrated that intravesical instillation of N/OFQ at 1 µM inhibits, in a statistically significant assessment, the micturition reflex in spinally lesioned patients but not in normal subjects. In particular we recorded an increase of BC and VT-DO ($p < 0.05$), but not of MBP ($p > 0.05$). In the controlled study the 7 patients who received N/OFQ displayed a clear inhibition of the micturition reflex: BC (ml) and VT-DO (ml) significantly ($p < 0.05$) increased from 139 ± 48 to 240 ± 61, and from 84 ± 32 to 201 ± 68, respectively. Finally the third study showed that the efficacy of N/OFQ is dose dependent: BC (ml) and VT-DO (ml) significantly increased from 154 ± 73 to 276 ± 94, and from 103 ± 41 to 198 ± 54, respectively, when 1 M of N/OFQ was used. 100 nM of NC-FQ did not produced a statistically significant modification of BC and VT-DO ($p > 0.5$). No clinical effect was recorded after 15 days. No significant side effect was recorded during the intravesical instillation of N/OFQ.
In conclusion, these studies demonstrateded that N/OFQ inhibited the micturition reflex by activating NOP receptors in patients with neurogenic bladder. The effects are acute and a chronic treatment is requested for long lasting effects. In order to determine if the inhibitory effect of N/OFQ undergoes tolerance or not, further studies remain mandatory.

POSTERS

1

MESSAGE/ADDRESS SEQUENCE MODIFICATIONS OF NOCICEPTIN/ORPHANIN FQ GENERATE NEW HIGLY POTENT LIGANDS FOR THE NOP RECEPTOR

[1]Trapella C., [1]Zucchini M., [1]Guerrini R., [2]Calò G., [1]Marzola E., [1]Arduin M., , [2]Carra' G., [2]Rizzi D., [2]Regoli D. and [1]Salvadori S.
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Structure-activity studies on nociceptin/orphanin FQ (N/OFQ: H-Phe-Gly-Gly-Phe-Thr-Gly-Ala-Arg-Lys-Ser-Ala-Arg-Lys-Leu-Ala-Asn-Gln-OH) identified Phe^{1} and Phe^{4} as the critical residues of the peptide message domain (Phe^{1}-Gly^{2}-Gly^{3}-Phe^{4}), while the two couples of positively charged residues Arg^{8}-Lys^{9} and Arg^{12}-Lys^{13} are the most important of the peptide address domain. Modifications of Phe^{1} by reducing the Phe^{1}-Gly^{2} peptide bond or by shifting the Phe^{1} side chain on the N-terminal nitrogen led to the discovery of the NOP partial agonist [$Phe^{1}\Psi(CH_2NH)Gly^{2}$]N/OFQ(1-13)-$NH_2$ and the pure antagonist [$Nphe^{1}$]N/OFQ(1-13)-NH_2, respectively. The addition of F to the Phe^{4} residue generates the highly potent agonist [(pF)Phe^{4}]N/OFQ(1-13)-NH_2. Moreover, even a larger increase of potency can be obtained by insertion of the extra couple of basic amino acids Arg^{14}-Lys^{15} in the address domain of N/OFQ. Based on these findings we combine in the N/OFQ-NH_2 template, the chemical modifications Arg^{14}-Lys^{15} and (pF)Phe^{4} which increase the agonist potency with those conferring partial agonist ($Phe^{1}\Psi(CH_2NH)Gly^{2}$) or pure antagonist ($Nphe^{1}$) properties. 12 peptides were synthesized using standard solid phase peptide synthesis methods, purified by preparative HPLC and their purity assessed by analytical HPLC and mass spectrometry. The 12 peptides were pharmacologically evaluated using the electrically stimulated mouse vas deferens and guinea pig ileum assays. All peptides behaved as NOP ligands; those with the normal Phe^{1}-Gly^{2} peptide bond as full agonists, those with the $Phe^{1}\Psi(CH_2NH)Gly^{2}$ modification as partial agonists with different degree of efficacy, while those with the $Nphe^{1}$ modification as partial agonists or pure antagonists depending on the presence or absence of the (pF)Phe^{4} modification, respectively. The most interesting compounds were the full agonist [(pF)$Phe^{4}Arg^{14}Lys^{15}$]N/OFQ-NH_2 (UFP-102), the partial agonist [$Phe^{1}\Psi(CH_2NH)Gly^{2}$ (pF)$Phe^{4}Arg^{14}Lys^{15}$]N/OFQ-NH_2 (UFP-103), and the pure antagonist [$Nphe^{1}Arg^{14}Lys^{15}$] N/OFQ-NH_2 (UFP-101): they represent up to now the most potent peptide ligands for the NOP receptor.

2

ANTAGONIST HEXAPEPTIDES FOR THE NOCICEPTIN RECEPTOR: STRUCTURAL MODIFICATIONS, RECEPTOR BINDING AND FUNCTIONAL BIOCHEMICAL CHARACTERISATION

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Acetyl-hexapeptides with Ac-Arg-Tyr-Tyr-Arg-Ile-Lys-NH_2, Ac-Arg-Tyr-Tyr-Arg-Trp-Arg-NH_2 or similar structure, originally isolated by combinatorial peptide chemistry are potent, positively charged ligands of the nociceptin receptor (1). We have previously shown that reducing the C-terminal lisine-amide to lisine-ol structure (Ac-RYYRIK-NH_2 ® **Ac-RYYRIK-ol**) results in a compound with high affinity and competitive antagonist property (2). Further chemical modifications on the structure were carried out including N-terminal changes and amino-acid replacements within the hexapeptide backbone. Ligand binding experiments were performed with p[^{3}H]Phe^1-nociceptin-NH_2, p[^{3}H]Phe^1-NC-1-13-NH_2 or [^{3}H]Tyr^1-nociceptin-OH radioprobes developed in our lab. Receptor-mediated G-protein activation in the presence of the nociceptin analogs was determined in [^{35}S]GTPγS binding assays. Among the structures examined, Ac-RYYRIK-ol was found to be the best in terms of binding affinity and its ability to antagonise nociceptin-induced stimulation of [^{35}S]GTPγS binding in rat brain membranes and in cell lines expressing cloned nociceptin receptors (CHO-ORL1). Because Ac-RYYRIK-ol was potent and antagonistically effective in both assays the ligand could be considered as high affinity peptide antagonist for the nociceptin (ORL1, NOP) receptor. Structure-affinity relationship of the acetyl-hexapeptide ligands will also be presented and discussed.

This study was supported by grants of the Hungarian Scientific Research Fund OTKA T-035211, T-033078, T-030841 and from the Ministry of Education, NKFP 1/027 Budapest, Hungary.

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3

UFP-102, A NOVEL HIGLY POTENT AGONIST FOR THE NOCICEPTIN/ORPHANIN FQ RECEPTOR – IN VITRO STUDIES

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UFP-102 ([(pF)Phe^4,Arg^{14},Lys^{15}]N/OFQ-NH_2) is a novel ligand for the nociceptin/orphanin FQ (N/OFQ) peptide receptor (NOP). UFP-102 has been generated by combining in the full agonist template N/OFQ-NH_2 two chemical modifications ([Arg^{14},Lys^{15}] and [(pF)Phe^4]) which increase N/OFQ potency. UFP-102 has been recently demonstrated to behave as a potent agonist at the human recombinant NOP receptor. In the present study the effects of UFP-102 were evaluated in vitro on the native NOP receptor, in the electrically stimulated mouse and rat vas deferens and in guinea pig ileum according to protocols and experimental conditions described by Calo' et al. (1). UFP-102 mimicked the effects of N/OFQ showing similar maximal effects but higher potencies (more than 10 fold: pEC_{50} in the range 8.57 – 9.36 in the three preparation). In all the preparations the effects of UFP-102 were not modified by 1 μM naloxone, although antagonized by the NOP receptor antagonists UFP-101 and (±)trans-1-[1-cyclooctylmetil-3-hydroxymethyl-4-piperidyl-3-ethyl-1,3-dihydro-2H-benzimidazol-2 one (J-113397), with pA_2 values similar to those found against N/OFQ (pA_2 in the range 6.91 – 7.41 for UFP-101 and 7.75 – 8.12 for J-113397). The effects of UFP-102 were investigated also using wild type ($NOP^{+/+}$) and NOP receptor knockout ($NOP^{-/-}$) mice. In the electrically stimulated mouse vas deferens from $NOP^{+/+}$ mice UFP-102 mimicked the inhibitory effects of N/OFQ (E_{max} 91 ± 1%; pEC_{50} 7.62), showing similar maximal effects (86 ± 2%) but higher potencies (pEC_{50} 9.40). In tissues taken from $NOP^{-/-}$ mice, N/OFQ was inactive and UFP-102 produced a slight inhibition of the electrically induced contraction only at the higher concentration tested, i.e. 1 μM. In the same series of experiments, the DOP receptor selective agonist deltorphin-I displayed similar high potency and efficacy in tissues from $NOP^{+/+}$ and $NOP^{-/-}$ animals. Taken together, the present data demonstrate that UFP-102 behaves as a highly potent and selective agonist for the NOP receptor.

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4

GTPg[^{35}S] BINDING AFFINITY IS INDEPENDENT OF CELL TYPE AND RECEPTOR ACTIVATION. STUDIES USING NATIVE AND RECOMBINANT NOP RECEPTORS.

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The purpose of this study was to measure GTPγS affinity and capacity in different tissues and at varying NOP receptor densities. Membranes prepared from rat cerebral cortex or CHO cells stably expressing the human NOP (CHO_{hNOP}) or expressing the ecdysone inducible expression system encoding human NOP ($CHO_{INDhNOP}$) were incubated for 4 hours at 30°C in GDP free assay buffer [McDonald 2003 367:183-7,N-S.Arch.Pharm]. To determine GTPγS binding affinity ~70-80pM GTPγ[^{35}S] was diluted with unlabelled GTPγS. Agonist stimulated binding was measured with GDP (5μM), peptidase inhibitors (10μM) and N/OFQ (1μM) and incubated for 1 hour. Affinity (IC_{50}) of GTPγS varied between 8.20-8.41, Table (mean±SEM, n=3). Competition curves had slopes less than unity, which could be modelled to high (8.58-9.05) and low affinity (7.42-7.70) components. Using $CHO_{INDhNOP}$ and in the presence of GDP, 1μM N/OFQ stimulated GTPγS binding increased from 1116±51 DPM to 4147±556 DPM as NOP density increased from ~23.6fmol/mg protein to ~1101fmol/mg protein. However, GTPγS affinity for the G-protein was essentially unchanged (8.51-9.07).

Membrane preparation	B_{max} (fmol[^{3}H]-N/OFQ/mg)	1-Site analysis		2-Site analysis		
		IC_{50}	Max DPM bound	% (High)	IC_{50}(High)	IC_{50}(Low)
$CHO_{INDhNOP}$ (non-induced)	Non-measurable	8.26±0.05	24648±2253	47.47%	8.80±0.14	7.74±0.16
$CHO_{INDhNOP}$ (induced)	1101±145.3	8.20±0.04	23342±3131	58%	8.67±0.11	7.48±0.06
CHOhNOP	1348±44	8.24±0.01	28719±1912	63.31%	8.58±0.13	7.42±0.30
Rat cerebral cortex	157±4	8.41±0.01	44082±986	77%	8.62±0.06	7.46±0.14

Whilst relative differences in GTPγS binding capacity are apparent in membranes from native and recombinant cells the affinity for GTPγS is independent of receptor density or activation.

5

INTERNALIZATION AND RECYCLING OF THE HUMAN NOP RECEPTOR EXPOSED TO NOCIEPTIN-RELATED PEPTIDES IN CHO CELLS

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Desensitization and internalization of G protein-coupled receptors observed after agonist activation are considered two important regulatory processes of receptor transduction. Internalization is generally envisioned as a rapid, agonist-induced, movement of the receptor into a cell compartment distinct from the plasma membrane. Desensitization and internalization processes of the nociceptin receptor (NOP) in the presence of nociceptin have been the object of our recent research. In this study we examined agonist-induced internalization, recycling and signalling of the cloned human nociceptin receptor (hNOP) expressed in CHO-K1 cells. Exposure of CHO-hNOP cells to NC, NC(1-13)NH_2 and [Arg^{14}, Lys^{15}]NC (1 μM; 10 min at 37°C) promotes a rapid internalization of the hNOP receptor, as measured by loss of cell surface receptors for the hydrophilic ligand [^{3}H]-NC. The purported antagonists JTC-801, [$Nphe^1$]nociceptin(1-13)-NH_2 (10 μM) and UFP-101 (1 μM) induced only modest internalization of hNOP receptors and significantly reduced internalization caused by NC-related peptides. To assess whether the internalized receptors could return to the cell surface, the cells were initially exposed to peptides for 10 min; then they were extensively washed with acid ice-cold phosphate-buffered saline to remove the added peptides and allowed to recover at 37°C without the agonist for 30 and 60 min. About 30% of internalized hNOP receptors gradually returned to the cell surface in 60 min following exposure to NC and NC(1-13)NH_2. However, this event was not observed in cells exposed to the potent agonist [Arg^{14}, Lys^{15}]NC. NOP receptors undergo rapid desensitization upon agonist challenge: efficacy of NC-related peptides to inhibit forskolin-stimulated cAMP production was significantly reduced 10 min after exposure and it correlated with the rate of receptor internalization. Moreover, we observed that the lack of hNOP receptor recycling by 1μM [Arg^{14}, Lys^{15}]NC would cause a more prolonged desensitization of this receptor. These results showed complex relationships between hNOP internalization and desensitization and it was observed that NOP receptor could undergo a distinct sorting by different peptide agonists.

6

ORPHANIN FQ PREVENTED EEG LIMBIC SEIZURES INDUCED BY DELTORPHIN II IN RABBITS.

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Since opioid and OP_4 receptor/orphanin FQ (OP_4r /OFQ) systems may be involved in epilepsy [1-2] and OFQ exhibits anti-opioid properties, we investigated the effects of intracerebroventricular (i.c.v.) administration of OFQ (10 nmol/30 µl, i.c.v.) on the electroencephalographic (EEG) limbic seizures induced by the OP_1/δ receptor agonist deltorphin II (Del II 20 nmol /30 µl, i.c.v.) in rabbits. The influence of the presumed OP_4r antagonist [$Phe^1(CH_2\text{-}NH)Gly_2$]nociceptin(1-13)$NH_2$ (*Phe^1*, 12 nmol/30 µl, i.c.v) on OFQ effects was also verified. Cannulae, cortical and hippocampal electrodes were surgically implanted for drug administration and EEG studies in animals, according with European Community and national regulations; CEE Council 86/609; D.Lgs.116/92. Like agonists for OP_3/μ opioid receptors, OFQ increased the synchronized EEG activity (high-voltage slow waves EEG pattern). This opioid-like OFQ effect was prevented by pre-treatment with *Phe^1*. When OFQ was administered 30 min before opioid, it prevented the appearance of hippocampal EEG seizures induced by Del II. *Phe^1* did not change EEG pattern, but (injected 40 min before OFQ+Del II) it blocked the antiepileptiform activity of OFQ, restoring Del II-induced seizures (animal with EEG seizures: saline 0/5, Del II 5/6 * $P<.01$ *vs* saline; OFQ+Del II 0/6° $P<.01$ *vs* Del II, *Phe^1*+OFQ+Del II 6/6$^{\#}$ $P<.01$ *vs* OFQ+Del II; Fisher's exact test). An increase of OFQ release and a down-regulation of OP4r, have been shown in kainate-induced seizures, suggesting that OP4r/OFQ system may participate in epileptogenic phenomena [1]. According with the inhibitory effect of OFQ on hippocampal excitability and kindling epileptogenesis [1, 3], we reported that OFQ exerted antiepileptiform activity on deltorphin II-induced seizures through an activation of OP_4r.

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ANTIEPILEPTIFORM ACTIVITY OF ORPHANIN FQ ON EEG SEIZURES INDUCED BY ß-ENDORPHIN IN RABBITS.

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Opioids and the endogenous ligand for the opioid-like OP_4 receptor (OP_4r) named orphanin FQ (OFQ) may play a role in stress, anxiety and epilepsy. Since OFQ exerts anti-opioid activity we studied the effects of intracerebroventricular (i.c.v.) administration of OFQ (10 nmol/30 µl, i.c.v.) on the electroencephalographic (EEG) limbic seizures induced by the OP_1/OP_3 receptor agonist ß-endorphin (ßE, 30 nmol /30 µl, i.c.v.) in rabbits. The influence of the presumed OP_4r antagonist [$Phe^1(CH_2\text{-}NH)Gly_2$]nociceptin(1-13) NH_2 (*Phe^1*,12 nmol/30 µl, i.c.v) on OFQ effects was also investigated. Cannulae, cortical and hippocampal electrodes were surgically implanted for drug administration and EEG studies in animals, according with European Community and national regulations; CEE Council 86/609; D.Lgs.116/92. Like opioids, OFQ increased the synchronized EEG activity (high-voltage slow waves pattern). This OFQ effect was antagonized by pretreatment with *Phe^1* (mean ± s.e. of % of min spent in synchronized EEG pattern: saline 41.2 ± 3.3, OFQ 63.5 ± 2.7 * P<.01 *vs* saline; *Phe^1*+OFQ 40.9 ± 4.3° P<.01 *vs* OFQ; ANOVA). When OFQ was administered 30 min before opioid, it prevented the appearance of hippocampal EEG seizures but not miosis and respiratory depression induced by ßE. *Phe^1* did not modify EEG pattern but blocked (when injected 40 min before OFQ+ßE) the antiepileptiform activity of OFQ, then restoring ßE-induced seizures (animal with EEG seizures: saline 0/5, ßE 5/5 * P<.01 *vs* saline; OFQ+ßE 0/5° P<.01 *vs* ßE; *Phe^1*+OFQ+ßE 5/5 # P<.01 *vs* OFQ+ßE; Fisher's exact test). It has been shown that kainate-induced seizures are associated with increased OFQ release and down-regulation of OP4r, suggesting that OP4r/OFQ system plays a facilitatory role in epileptogenesis [1]. According with the inhibitory effect of OFQ on hippocampal excitability [2], we reported now that OFQ exerted antiepileptiform effect on opioid seizures through an activation of OP_4r .

[1] Bergola G. et al., (2002) J. Neurosci: 22 (22): 10030-10038.
[2] Gutierrez R. et al., (2001) Neuroscience 105: 325-333.

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BEHAVIOURAL EVIDENCE FOR FUNCTIONAL HETEROGENEITY OF ORL-1 RECEPTORS.

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The aims of the present work were twofold. First the ability of a broad dose-range of nociceptin (NC) and Ro 64-6198 to modulate locomotor activity in mice not habituated to the test environment was analyzed. Secondly, it was investigated whether the locomotor effects by NC and Ro 64-6198 are blocked by the putative ORL1 antagonists [NPhe1]nociceptin(1-13)NH2 (NNN), naloxone benzoylhydrazone (NBZ) and UFP-101 and the non-selective opioid antagonist naloxone.

Higher doses of NC (>5nmol i.c.v.) reduced whereas lower doses (<1 nmol i.c.v.) stimulated locomotor activity. Both effects were blocked by the putative ORL1 antagonists NNN (10 nmol i.c.v) and UFP-101 (10 nmol, i.c.v.). The effects were also blocked by NBZ (1 mg/kg s.c.) but not by the non-selective opioid antagonist naloxone (1 mg/kg s.c.).

In contrast to NC, the synthetic ORL1 agonist Ro 64-6198 (0.01-1.0 mg/kg i.p.) produced monophasic inhibition of locomotor activity, which was insensitive to the treatment with NNN or NBZ. Treatment with UFP-101 abolished the locomotor inhibition induced by Ro 64-6198 (1.0 mg/kg) whereas naloxone (1.0 mg/kg, s.c.) further increased the locomotor-inhibitory effects.

NBZ (3 mg/kg) significantly stimulated locomotor activity whereas NNN tended to do so. The present findings suggest that brain NC systems may be tonically involved in the control of spontaneous locomotor behavior in mice.

In conclusion, the present results support the view for the existence of several functional subtypes of ORL-1 receptors. The biphasic effects of NC may be due to different subsets of receptors with different functions on subpopulations of dopaminergic and non-dopaminergic neurons. The synthetic agent, Ro 64-6198 seems to interact also with an opioid system. The further exploration of the NC/ORL-1 system and its functional/therapeutic potential would greatly benefit by the access to more (and more specific) molecular probes.

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DIFFERENT PHARMACOLOGICAL PROPERTIES OF PRESYNAPTIC NOCICEPTIN/ORPHANIN FQ RECEPTORS MODULATING 5-HYDROXY-TRYPTAMINE AND NORADRENALINE EFFLUX IN THE RAT NEOCORTEX.

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Nociceptin/orphanin FQ (N/OFQ) and its receptor (NOP) modulate the activity of brain monoaminergic systems via different mechanisms and at different neuroanatomical levels. To investigate the pharmacology of presynaptic NOP receptors regulating monoamine release, we monitored [^{3}H]5-hydroxytryptamine ([^{3}H]5-HT) and [^{3}H]noradrenaline ([^{3}H]NE) release from superfused rat neocortex synaptosomes challenged with KCl 10 mM.
N/OFQ (0.001-3 μM) inhibited in a concentration-dependent manner the K^+-evoked [^{3}H]5-HT and [^{3}H]NE overflow with similar potency (pEC_{50} ~7.9 and ~7.7 respectively) and efficacy (maximal inhibition ~ 40 %). The inhibitory effect of N/OFQ (0.1 μM) on [^{3}H]5-HT and [^{3}H]NE release was antagonized by the selective NOP receptor peptide ([Nphe^1]N/OFQ(1-13)NH_2 and UFP-101) and non peptide (J-113397 and JTC-801) antagonists. Antagonists were routinely applied 3 min before N/OFQ. However, J-113397 and JTC-801 prevented N/OFQ inhibition of [^{3}H]NE overflow only when applied 21 min prior to N/OFQ.
The NOP receptor partial agonist [Phe^{1}ψ (CH_2-NH)Gly^2]N/OFQ(1-13)NH_2 ([F/G]) (3 μM) did not affect the K^+-evoked [^{3}H]NE release, but partially inhibited the K^+-evoked [^{3}H]5-HT overflow in a UFP-101 sensitive manner. [F/G] (3 μM) also antagonized the effect of N/OFQ (0.1 μM) on [^{3}H]5-HT and [^{3}H]NE release.
In conclusion, pharmacological analysis of presynaptic NOP receptors localized on serotonergic and noradrenergic terminals in the rat neocortex reveals a different time-dependent sensitivity towards non peptide receptor antagonists and responsiveness to the partial agonist [F/G].

Acknowledgements
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PHARMACOLOGICAL CHARACTERIZATION OF PRESYNAPTIC NOCICEPTIN/ORPHANIN FQ RECEPTORS MODULATING 5-HYDROXY-TRYPTAMINE EFFLUX FROM MOUSE CORTICAL SYNAPTOSOMES.

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In order to pharmacologically characterize those nociceptin/orphanin FQ receptors (NOP) regulating 5-HT release in the mouse cerebral cortex, a preparation of superfused mouse neocortex synaptosomes challenged with 10 mM KCl was employed. N/OFQ (added 9 minutes before KCl) inhibited in a concentration-dependent manner the K^+-evoked [^{3}H]5-HT release with a pEC_{50} value of 8.56 ± 0.07 and maximal inhibition at 1 µM (~33 %). Higher N/OFQ concentrations (3-10 µM) were ineffective. [Arg^{14}, Lys^{15}]N/OFQ also inhibited [^{3}H]5-HT release, showing maximal efficacy at 300 nM (~50 %). Nevertheless, the concentration-response curve was biphasic, being compatible with two separate sites of interaction (pEC_{50} of 9.29 ± 1.04 and 7.12 ± 1.3, respectively). At 10 µM [Arg^{14}, Lys^{15}]N/OFQ was ineffective. The K^+-evoked [^{3}H]5-HT overflow was also inhibited by the NOP receptor partial agonists [Phe^{1}ψ(CH_2-NH)Gly^2]N/OFQ(1-13)NH_2 ([F/G]) and [Ac-RYYRWKNH_2] (pEC_{50} 7.23 ± 0.061 and 8.34 ± 0.034 respectively), which displayed maximal efficacy at 3 µM (~30 %) and 0.1 µM (~36 %), respectively. N/OFQ, [F/G] and [Arg^{14}, Lys^{15}]N/OFQ were ineffective in NOP receptor knockout mice. Experiments were then carried out to investigate whether NOP receptors undergo desensitization. N/OFQ, added 3 minutes before KCl, gave a concentration response-curve with a pEC_{50} of 6.3 ± 0.079 and maximal effect at 10 µM (~35 %). Moreover, pre-treating synaptosomes with the PKC inhibitor BIM (1 µM), unmasked an inhibitory effect (~30 %) of N/OFQ (10 µM; 9 min). In conclusion, these data bring direct evidence that presynaptic NOP receptors regulate 5-HT release in the mouse neocortex. Although these receptors respond to classical NOP receptor ligands, they bear pharmacological properties distinct from the homologous population of NOP receptors previously described in the rat neocortex, in terms of sensitivity to N/OFQ and [F/G] as well as tendency to desensitize. Moreover they appear to undergo desensitization via PKC dependent mechanisms.

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IS NOCICEPTIN/ORPHANIN FQ, AT LOW DOSES, ANXIOGENIC RATHER THAN ANXIOLYTIC?

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Nociceptin/orphanin FQ (N/OFQ), the endogenous ligand of the NOP receptor, has been reported to induce, in rodents, anti-stress and anxiolytic effects after intracerebroventricular (i.c.v.) administration (1). The present study was designed to examine, in rats, the efficacy of N/OFQ on an elevated plus maze (EPM), a test usually sensitive to anxiolytic/anxiogenic drugs.

Male Wistar rats (180-200 g, body weight), divided in groups of 8 animals each, were i.c.v. injected with increasing doses of N/OFQ (0.3, 0.5, 0.75, 1, 1.5 nmol per rat). Five minutes after i.c.v. treatment, rats were placed on the EPM for 5 min and entries into and time spent on open and closed arms were recorded alongside other parameters. Data were expressed as mean±S.E.M. and submitted to ANOVA followed by Student-Newman- Keuls test.

N/OFQ, at the doses of 0.3 and 0.5 nmol, shows no influence on the parameters assessed. From the dose of 0.75 nmol, in a dose dependent manner, N/OFQ is able to reduce the time spent in open arms (controls: 86.9±15.6; NO/FQ 0.75: 9.2±3.4*; N/OFQ 1: 5.1±1.9*; N/OFQ 1.5: 1.7±0.9*; * $P<0.05$ vs controls), to increase the time spent in closed arms (controls: 207.2±12.9; NO/FQ 0.75: 270.2±15.3*; N/OFQ 1: 279.7±16.1*; N/OFQ 1.5: 297.5±21.3*) and to decrease the number of closed arm entries (controls: 5.8±0.5; NO/FQ 0.75: 2.7±1.0*; N/OFQ 1: 1.6±0.5*; N/OFQ 1.5: 1.3±1.1*), that is an index of reduced locomotor activity.

Conflicting results of behavioural effects of N/OFQ are available in literature. In this context, our results seem to be contradictory with respect to the anxiolytic-like properties shown by NOP receptor agonists (1) indicating that N/OFQ, like other peptides, can exhibit opposite effects in dependence to different dose ranges.

1) Le-Cudennec C., Naudin B., Do-Rego J.C. and Costentin J. (2002) Life Sci. 72:163-171.

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MAPPING OF BRAIN SITES SENSITIVE TO THE INHIBITORY EFFECT OF NOCICEPTIN/ORPHANIN FQ ON CRF-INDUCED ANOREXIA

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Central injections of nociceptin/orphanin FQ (N/OFQ) or peripheral administration of the NOP receptor agonist Ro 64-6198 inhibit stress- and CRF-induced anorexia in rats (1). Since these NOP receptor agonists show no affinity for CRF receptors, the effect may be expression of functional antagonism. The present study evaluated the sensitivity of several forebrain sites to the effect of N/OFQ on CRF-induced anorexia in male Wistar rats.

Under anaesthesia, rats were implanted with bilateral cannulae (for injection in the bed nucleus of the stria terminalis (BNST), the paraventricular nucleus (PVN), the ventro-medial hypothalamus (VHM), the central nucleus of the amygdala (CeA), or with unilateral cannula for injection into the dorsal raphe nucleus (DR) or the lateral cerebroventricle (LV). Rats were food deprived for 20 h, injected in the LV with 0.2 µg/rat of CRF or its vehicle, and given access to food 20 min after LV injection. N/OFQ, 0.05-1 µg/rat, was injected 10 min before CRF injection.

In the BNST N/OFQ significantly reduced CRF-induced anorexia at 0.05 µg/rat, while it was ineffective following injection in the PVN, VMH, CeA or DR at doses up to 1 µg/rat. In the LV a significant effect was detected at 1 µg/rat. On the otehr hand, the injection of N/OFQ, 0.05-1 µg/rat, into the BNST did not modify food intake in food deprived rats (not injected with CRF), or in freely feeding rats.

The present findings show that the BNST, which exhibits high density of NOP receptors, is the most sensitive brain area to the anti-anorectic effect of N/OFQ, suggesting that it may represent the site of action for this effect of the peptide.

1) Ciccocioppo R., Biondini M., Antonelli L., Wichmann J., Jenck F., Massi M. (2002) Psychopharmacology 161: 113-119.

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INCREASED INFLAMMATORY HYPERALGESIA IN MICE LACKING THE NOCICEPTIN PRECURSOR POLYPEPTIDE OR THE NOCICEPTIN RECEPTOR

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Nociceptin/orphanin FQ (N/OFQ) is the endogenous agonist of the N/OFQ peptide receptor (NOP receptor). It is released from a larger precursor polypeptide, called pre-pro-nociceptin (ppN/OFQ) from which, in addition to N/OFQ, other biologically active neuropeptides may be derived. Increasing evidence indicates that exogenous application of N/OFQ to the CNS of mice and rats induces pro- and anti-nociceptive effects depending on the dose and site of administration. Much less is known about a potential contribution of endogenous N/OFQ to pain control.

Here, we have used a genetic approach to address this topic. Mice deficient in either the NOP receptor (NOP-$R^{-/-}$ mice) or the N/OFQ precursor polypeptide (ppN/$OFQ^{-/-}$ mice) or both (double knock-out mice) were compared with wild type littermates in animal models of acute and tonic pain.

Nociceptive responses to acute noxious heat of all three types of mutant mice were indistinguishable from those of wild type mice. Accordingly, nociceptive behavior was very similar in the early phase of the formalin test. However, NOP-$R^{-/-}$, ppN/$OFQ^{-/-}$ and double knock-out mice showed markedly stronger nociceptive responses during prolonged nociceptive stimulation in the second phase of the formalin test and significantly lower thermal pain thresholds in inflamed tissue after zymosan A injection.

These results indicate that N/OFQ significantly contributes to endogenous pain control during prolonged nociceptive stimulation, but does not affect acute pain sensitivity. Among the three types of mutant mice nociceptive behavior was nearly identical, indicating that the lack of other potential ppN/OFQ products in the ppN/$OFQ^{-/-}$ mice was apparently without effect on the nociceptive phenotype.

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SUPRASPINAL INJECTION OF NOCISTATIN PREVENTS NOCICEPTIN ANTAGONISTIC EFFECT ON OPIOID ANALGESIA IN THE RAT

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Nocistatin is a recently isolated peptide derived from the prepro-neuropeptide containing Nociceptin/Orphanin FQ. The role of Nociceptin in pain transmission is very controversial. In fact intracerebroventricular (i.c.v) administration of Nociceptin induces hyperalgesia and allodynia, whereas intrathecal (i.t) injection of the peptide causes hyperalgesia or analgesia depending on the doses used. Nocistatin, that doesn't bind to NOP receptor, blocks the effects produced by i.t. Nociceptin. The aim of the present study was to evaluate whether Nocistatin, i.c.v. injected, was capable to reverse the antagonistic effect of supraspinal Nociceptin on opioid analgesia in the rat. Groups of 8-10 Sprague-Dawley rats weighing 180±20g were used and implantation of i.c.v. cannulae was stereotaxically performed under pentobarbital anaesthesia. Nociception was assessed by tail-flick latency (TFL). Opioid analgesia was induced by central administration of selective agonists for MOR, DOR-1, DOR-2 and KOR. TFLs were expressed as values of MAUC over 60 min testing section. The results evidentiated that Nocistatin (0.5ng i.c.v./rat), exhibited no significant changes in the basal TFL and didn't affect the analgesia induced by single opioid agonists. Administration of Nocistatin (0.5ng i.c.v./rat), immediately prior to Nociceptin (18µg i.c.v./rat), prevented significantly the hyperalgesic action of Nociceptin. Analgesia of DAMGO, DPDPE, Deltorphin II, U50488H (respectively at the dose of 1, 20, 16, 100µg i.c.v./rat) was inhibited by i.c.v. Nociceptin, while it was maintained when followed by Nocistatin injection. In addition to prevent the antiopioid effect of Nociceptin, Nocistatin prolonged the analgesia induced by opioids, especially that by MOR agonist. These data show that i.c.v. injection of Nocistatin may reverse the inhibitory effect of Nociceptin on analgesia induced by selective opioid agonists. Our study is in agreement with Zhao et al (1) who have reported that Nocistatin antagonized the effect of Nociceptin on morphine analgesia. Complexively these results could suggest that Nocistatin may act on nociceptive transmission as "functional antagonist of Nociceptin".

1) Zhao C. et al (1999) Neuroreport 10: 297-9.

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EFFECTS OF NOCICEPTIN/ORPHANIN FQ CREAM ON CAPSAICIN CREAM-INDUCED PAIN IN HUMAN VOLUNTEERS

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The actions of nociceptin/orphanin FQ (N/OFQ) on human nociception are poorly understood. In the present study we have investigated whether N/OFQ cream inhibits capsaicin cream-induced spontaneous pain in human volunteers.
Eight volunteers received 0.1g capsaicin cream (0.075%) with either 0.2 g N/OFQ (0.1%) or 0.2 g control paraffin cream topically on the right or left volar forearm at random and at the same time. Capsaicin-induced pain was assessed using a visual analog scale (VAS) from 0-100 mm (where 0 = "no pain" and 100 = "worst pain imaginable") before and at 10 min intervals following administration for 90 min. We also measured MAP (mmHg), HR (b.p.m) and plasma N/OFQ concentrations ([N/OFQ])(pg/ml) before and after the experiment. [N/OFQ] was determined using an Enzyme Immunoassay Kit (Phoenix Pharmaceuticals, Inc. U.S.A.). N/OFQ and paraffin cream were prepared at one of our institutes and capsaicin cream was purchased from Chattem, Inc. (Tennessee, U.S.A.) Statistical analyses were performed by two way repeated measures ANOVA and paired t-test as appropriate. $P<0.05$ was considered significant.
There were no adverse events following N/OFQ administration. N/OFQ cream did not affect either the onset of capsaicin-induced pain or the maximum effect. MAP, HR and [N/OFQ] did not change before and after the experiment with the values of 85.4±2.7mmHg, 74.5±2.8bpm and 57.1±16.6pg/ml and 85.5±2.9mmHg, 68.6 ± 4.3bpm and 36.5±7.5 pg/ml, respectively.
N/OFQ cream did not prevent capsaicin cream-induced pain in human volunteers. This negative result may depend on the poor pharmacokinetic properties of the peptide N/OFQ.

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POSSIBLE INTERACTIONS BETWEEN OPIOID SYSTEM AND ALCOHOL INTAKE: BEHAVIORAL EVIDENCES.

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Several clinical and experimental evidences show a strong involvement of opioid system in alcoholism. Recently, the non-selective opioid receptor antagonist naltrexone has been approved as a treatment for alcohol dependence. One possible mechanism of action is that opiod receptors are involved in hedonic aspects of alcohol consumption. On the other hand, other results show that the reduction in alcohol intake is related to blockade of the endogenous opioid receptor and activation of the stress-responsive axis. Unluckily, while clinical effects are significant, the effect size is rather small and unpleasant side effects may limit the benefits of the compounds. A possible pharmacological strategy to avoid such a problem could be the use of partial opioid receptor agonist. Buprenorphine (Bup), a mixed agonist-antagonist acting as partial agonist at the μ receptor and antagonist at the κ receptor, has been used for a long time for the management of pain in both cancer and non-cancer patients. Several evidences have shown the efficacy of this drug in combination with the non-selective opioid antagonist naloxone in the treatment of opioid and alcohol dependence. On the other hand, the mechanism underlining the action of these drugs is still unclear. Using oral operant ethanol self-administration (10% v/v) in presence and in absence of saccharin (0.2 %, v/w), we have tested the effects of systemic injection of Bup either in basal conditions (alone) or following non-selective opioid receptor blockade by i.p. naloxone, or following intracerebroventricular administration of the "anti-opioid peptide" nociceptin (NC). Alcohol intake, either in the presence of saccharin or not, was reduced in dose-related manner by systemic administration either with Bup (1.5-0.03 mg/kg, i.p.) or naloxone (Nal, 0.3-0.003 mg/kg i.p.) or NC (250, 1000 ng/rat). An increase in intake reduction was observed when Bup was injected in association with Nal and NC. Saccharin intake was decreased only by the highest dose of these treatments. Interestingly, an inactive dose of Bup (0.3 mg/kg) on saccharin intake reduced water intake. These data could indicate that Bup might act on opioid receptors in connection with an unknown system in unspecific manner.

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NOCICEPTIN/ORPHANIN FQ REDUCES ETHANOL SELF-ADMINISTRATION IN GENETICALLY SELECTED MARCHIGIAN SARDINIAN ALCOHOL-PREFERRING RATS, BUT NOT IN HETEROGENEOUS WISTAR RATS

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Intracerebroventricular (ICV) treatment with nociceptin/orphanin FQ (N/OFQ), the endogenous ligand of the NOP receptor, reduces voluntary 10% ethanol intake and ethanol-induced conditioned place preference in Marchigian Sardinian alcohol-preferring (msP) rats (1); moreover it blocks stress-induced reinstatement of ethanol-seeking behavior in Wistar rats (2).

The present study evaluated the effect of N/OFQ on oral ethanol self-administration, both in msP and heterogeneous Wistar rats. Animals were trained to operantly self-administer 10% ethanol in 30 min daily session on an FR-1 schedule (0.1 ml/response) of reinforcement. Following acquisition of a stable baseline of responding 3 groups (n=9 group) of msP rats were ICV treated with 0.5, 1.0 µg/rat of N/OFQ or its vehicle, before each self-administration session. The first day of drug treatment controls responded for alcohol with 51.2±5.4 bar pressing; treatment with 1.0 µg/rat of N/OFQ significantly reduced it to 28.4±6,4; $p<0.05$. Throughout the subchronic (6 days) treatment with N/OFQ ethanol self-administration remained significantly lower in rats treated with both 0.5 or 1.0 µg/rat [$F(2,24)=7.30$; $p<0.01$)].

Wistar rats received N/OFQ, 1.0 or 2.0 µg/rat or its vehicle. The first day of drug treatment controls responded for alcohol with 36.3 ±9.1 bar pressing, whereas rats treated with 1.0 or 2.0 µg/rat of N/OFQ lever pressed 23.3±6.0 or 26.0±4.2 times, respectively. Statistical analysis revealed absence of significant drug effects. The treatment was continued for other 5 days; however, difference between groups never reached significancy [$F(2,27)=0.06$; NS].

Present results demonstrate that N/OFQ inhibits ethanol self-administration in msP, but not in heterogeneous Wistar rats.

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ROLE OF NOCICEPTIN IN THE BRONCHOCONSTRICTION AND AIRWAY INFLAMMATION INDUCED BY HCL INTRAESOPHAGEAL INSTILLATION IN THE RABBIT

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Nociceptin, a novel neuropeptide of the opioid peptide family, has the ability to influence airway physiology by modulating tachykinergic neurotransmission (1). This neurotransmission is involved in the gastroesophageal acid reflux (GER) induced by intraoesophageal HCl instillation in guinea-pigs (2).
We investigated in rabbit airways the effects of nociceptin on lung function and plasma extravasation induced by HCl intraoesophageal instillation.
Anaesthetized New Zealand rabbits were intubated with an endotracheal tube, using a polyethylene catheter with an attached latex balloon in the midoesophagus for the pulmonary function measurement. Airway microvascular leakage was quantified by extravasation of Evans blue dye (30 mg/kg i.v.), 10 min. after HCl (1N) instillation in the oesophagus, with or without nociceptin (0.003-0.03 mg/kg, i.v.) pretreatment.
HCl intraoesophageal instillation significantly increased the lung resistance (from 48 ± 1.5 to 73 ± 2.1 cm H_2O/l/sec; $p<0.01$), that was inhibited by nociceptin (0.016 and 0.03 mg/kg) (61 ± 1.5; 56 ± 1.2 cm H_2O/l/sec; $p<0.05$ $p<0.01$ respectively) but not by nociceptin at dose of 0.003 mg/kg. Moreover, our results showed that HCl intraoesophageal instillation significantly increased plasma extravasation in trachea (from 10.5 ± 4 to 49.7 ± 4 ng/mg tissue; $p<0.01$) and in main bronchi (from 9.7 ± 3 to 32.1 ± 4.3 ng/mg tissue; $p<0.01$). Both trachea and bronchi effects were significantly inhibited by nociceptin (0.016 and 0.03 mg/kg $p<0.05$ $p<0.01$ respectively) but not by 0.003 mg/kg of nociceptin.
These findings suggest that bronchoconstriction and protein extravasation in the airway induced by HCl intraoesophageal instillation may be inhibited by nociceptin, probably through an inhibition of tachykinins release. However, further experiments are needed to clarify the exact mechanism of nociceptin effect on GER.

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NOCICEPTIN/ORPHANIN FQ INHIBITS ELECTRICALLY INDUCED CONTRACTIONS OF THE HUMAN BRONCHUS VIA NOP RECEPTOR ACTIVATION AND STIMULATION OF POTASSIUM CURRENTS

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Nociceptin/orphanin FQ (N/OFQ) via selective activation of the N/OFQ peptide receptor (NOP) has been reported to inhibit neurogenic contractions in various animal tissues, including airways. In the present study we investigated the ability of N/OFQ to affect electrical-field stimulated (EFS) contractions of human bronchi. Tissues were obtained from 23 patients undergoing surgery for lung cancer. EFS (20Hz, 320 mA, 1.5 ms, 10 s) were applied every 20 min for 5 times. EFS responses were stable over the time course of the experiment and amounted to 83 ± 13% of the contraction induced by 1 mM Ach. Contractions induced by EFS were fully abolished by either tetrodotoxin (1 μM) or atropine (1 μM). N/OFQ (10 nM – 1 μM) concentration dependently inhibited EFS induced contractions showing however very low maximal effects (about 10% inhibition). The inhibitory effect of N/OFQ was mimicked by the NOP ligand [Arg^{14},Lys^{15}]N/OFQ which displayed however higher maximal effects (15-20% inhibition). The actions of N/OFQ and [Arg^{14},Lys^{15}]N/OFQ were not affected by naloxone (1 μM) while fully prevented by the selective NOP receptor antagonist UFP-101 (10 μM). Moreover the inhibitory effects of NOP agonists were no longer evident in tissues treated with tertiapin (10 μM), an inhibitor of inward-rectifier potassium channels.
In conclusion these data, which are in line with results obtained in animal airways, demonstrated that N/OFQ inhibited Ach release in the human bronchi via NOP receptor activation and subsequent stimulation of potassium currents.

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CENTRAL NOCICEPTIN PREVENTS THE DEVELOPMENT OF ETHANOL-INDUCED GASTRIC LESIONS IN THE RAT

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Nociceptin (N/OFQ) has been previously demonstrated to stimulate, following intracerebroventricular injection, gastric acid secretion in the rat (1) and colonic and small bowel transit in the mouse (2). The present study evaluated the ability of N/OFQ to affect the gastric mucosa protective mechanisms in the rat.
N/OFQ was injected into the lateral cerebroventricle (LV) 30 and 90 min before the intragastric administration of saline or 50% ethanol solution (1 ml/rat). Five min after intragastric ethanol administration (IEA) rats were sacrificed and their stomachs were removed to evaluate the presence of macroscopically visible lesions. LV injections of the selective N/OFQ antagonist, UFP-101 (10, 20 and 40 µg/kg), were given 30 min before IEA either alone or in combination with N/OFQ. Individual hemorragic lesions were measured along their greatest length and the overall total lesion was designated as the "lesion index".
IEA caused hemorrhagic lesions in the glandular portion of rat stomach. N/OFQ did not modify the appearance of the mucosa while it produced a dose-dependent reduction of ethanol-induced gastric damage. N/OFQ (4 µg/rat) produced a significant reduction of ethanol-induced gastric lesions only when injected 30 min before IEA. UFP-101 alone did not modify total ethanol-induced gastric lesions up to the 20 µg/rat dose, while the 40 µg/rat dose produced a significant reduction of ethanol-induced gastric lesions. UFP-101 (10 and 20 µg/rat) reversed the N/OFQ (4 µg/rat) protective effect.
The results reported in the present study provide evidence that N/OFQ is endowed with the ability to activate the mechanisms responsible for the protection of gastric mucosa. In line with its kinetics features, the protective effect appears to be short-lasting. The reversal effect of UFP-101 suggests the involvement of NOP receptors.

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DIFFERENTIAL CARDIOVASCULAR AND RENAL RESPONSES PRODUCED BY THE CENTRAL VERSUS PERIPHERAL ADMINISTRATION OF NOP RECEPTOR PARTIAL AGONISTS.

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Partial agonists of the nociceptin/orphanin FQ (N/OFQ) peptide (NOP) receptor produce variable antagonist, partial agonist or full agonist behavior in different *in vitro* / *in vivo* assays, indicating multiple sites and/or mechanisms of action (1). Accordingly, we examined the cardiovascular and renal responses produced by the central versus peripheral administration of NOP receptor partial agonists ([F/G]N/OFQ(1-13)-NH_2, Ac-RYYRIK-NH_2, Ac-RYYRWK-NH_2) in Sprague-Dawley rats. In conscious rats, intracerebroventricular (i.c.v.) administration of each NOP receptor partial agonist produced profound cardiovascular (depressor), renal excretory (water diuresis), renal sympathetic nerve activity (inhibitory) and feeding (hyperphagia) responses similar to those produced by i.c.v. injection of the native ligand, N/OFQ. In contrast, intravenous (i.v.) bolus injection of these NOP receptor partial agonists produced responses unlike N/OFQ; i.v. bolus N/OFQ evoked profound bradycardia and hypotension with no change in urine output whereas i.v. bolus NOP receptor partial agonists elicited water diuresis without altering cardiovascular function. Unlike their central effects, i.v. bolus NOP receptor ligands produced renal sympathoexcitation and did not evoke hyperphagia. In other studies, i.v. bolus pretreatment of rats with NOP receptor partial agonists prevented/attenuated the hypotension and bradycardia produced by an i.v. bolus N/OFQ challenge, however in these same animals the cardiovascular depressor responses to i.c.v. N/OFQ remained intact. Thus, in conscious rats, central and peripheral administration of NOP receptor partial agonists produced different response profiles. Together, these findings are evidence for separate central versus peripheral NOP receptor pathways involved in the control of cardiovascular and renal function. The selective activation of peripheral NOP receptor pathways with partial agonists may provide an approach to elicit water diuresis for the clinical management of water retention and/or hyponatremia without producing adverse cardiovascular/CNS effects.

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RENAL EFFECTS PRODUCED BY CENTRAL ACTIVATION OF THE N/OFQ-NOP RECEPTOR SYSTEM: AN IN VIVO STUDY IN MICE

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Synthetic or endogenous opioid agonists evoke profound changes in renal function; since the N/OFQ-NOP receptor system shares a number of structural, functional and anatomic features with endogenous opioid peptides, it may be speculated that this new system may also participate in the regulation of renal function. Previous studies have shown that intracerebroventricular (i.c.v.) administration of N/OFQ produces a marked diuresis in rats. In the present studies, we examined whether N/OFQ affects the renal excretion of water and/or electrolytes following central administration in conscious mice (ICR-CD1, 23-28 grams).
As compared to urine output in saline-treated mice (0.134±0.01 cc/120-min), i.c.v. N/OFQ (0.1, 1, 3 and 10 nmol) produced a significant diuresis that was evident at a dose of 1 nmol (0.216±0.01 cc/120-min) and maximal at 3 and 10 nmol (0.327±0.03 cc/120-min and 0.317±0.01 cc/120-min, respectively). For the following studies, we chose to use the 3 nmol i.c.v. dose and separate the experimental collections into first and second hour periods, in order to evaluate the time course of the N/OFQ-NOP receptor response. N/OFQ (3 nmol) induced a significant increase in urine flow rate during only the first hour (N/OFQ: 0.303±0.03 cc/60-min vs Saline: 0.072±0.03 cc/60-min). I.c.v. N/OFQ did not alter urinary potassium excretion, but urinary sodium excretion tended to decrease in both hours. However, the antinatriuresis was not statistically significant. In other studies, UFP-101 (30 nmol i.c.v.), a pure, selective and potent NOP receptor antagonist, when administrated alone did not alter urine flow rate or the urinary excretion of sodium/potassium during the first or second hours. When co-administered i.c.v., UFP-101, completely blocked the diuretic effect produced by N/OFQ normally elicited during the first hour.
These findings indicate that in conscious mice, activation of the N/OFQ-NOP receptor system produces a diuretic effect similar to that observed in rats. Further, when administered centrally, N/OFQ is a sodium- and potassium-sparing aquaretic that increases free water clearance via a NOP receptor pathway that is prevented by the NOP receptor antagonist, UFP-101.

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CHEMOTACTIC EFFECTS OF NOCICEPTIN/ORPHANIN FQ ON HUMAN MONOCYTES ARE MEDIATED BY NOP RECEPTOR ACTIVATION

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Nociceptin/orphanin FQ (N/OFQ) produces several biological actions by selectively activating the N/OFQ peptide receptor (NOP). It has been reported that N/OFQ is able to stimulate leukocyte chemotaxis both in vitro and in vivo (1). In the present study we investigated the ability of N/OFQ to stimulate human neutrophil and monocyte chemotaxis and the release of lysozyme and superoxide anion (O_2^-) production from neutrophils. In the same experiments the effects of N/OFQ were compared to those evoked by the proinflammatory tripeptide for-Met-Leu-Phe (fMLP). Experimental techniques and protocols were as previously described (2). Confirming previous findings (2), fMLP stimulated all the leukocyte functions examined. N/OFQ stimulated monocyte but not neutrophil chemotaxis. The production of O_2^- from neutrophils was not affected by N/OFQ while the release of lysozyme was increased in a concentration dependent manner although the maximal effects evoked by N/OFQ were about half of those of fMLP. The NOP ligands [Arg^{14},Lys^{15}]N/OFQ, N/OFQ(1-13)NH_2, Ro 64-6198, UFP-101 and the opioid antagonist naloxone were used for pharmacologically characterizing the receptor involved in the monocyte chemoattractant action of N/OFQ. [Arg^{14},Lys^{15}]N/OFQ, N/OFQ(1-13)NH_2, and Ro 64-6198 mimicked the action of N/OFQ showing similar maximal effects and the following order of potency: [Arg^{14},Lys^{15}]N/OFQ > Ro 64-6198 > N/OFQ(1-13)NH_2 > N/OFQ. Moreover the monocyte chemoattractant action of N/OFQ was not modify by naloxone 1 μM while antagonized by UFP-101 10 μM (pA_2 7.84). Thus, the order of potency of agonists and the antagonist sensitivity demonstrated that N/OFQ stimulates monocyte chemotaxis via NOP receptor activation.

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GASTROINTESTINAL EFFECTS OF CENTRALLY INJECTED ORPHANIN FQ/NOCICEPTIN IN RATS

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Orphanin FQ/nociceptin (OFQ/N) is the endogenous ligand for the G-protein coupled receptor referred as NOP receptor. OFQ/N and its receptor are found in peripheral tissues but also in brain and spinal cord of several species where they regulate important functions. Although this new regulatory system differs from opioid system, it has similar pharmacological and physiological roles in gastrointestinal functions. However, in contrast to the many reports describing the peripheral actions of OFQ/N on gastrointestinal functions, few studies have investigated its possible central nervous system role in gastrointestinal physiology (1,2). Thus, in this study we examined the effects of intracerebroventricularly (icv) injected OFQ/N on gastric emptying, small intestinal transit, colonic propulsion and gastric acid secretion in rats. OFQ/N (0.2-20 g/rat) significantly and dose-dependently delayed gastric emptying of a phenol red meal, inhibited transit of a nonabsorbable charcoal marker through the small intestine and significantly but not in a dose-related manner increased the mean colonic bead expulsion time. The OFQ/N-evoked gastrointestinal antitransit effects were abolished by the NOP receptor antagonist, [N Phe^1] N (1-13)NH_2, but left unchanged by the classic opioid receptor antagonist, naloxone. Icv injected OFQ/N (20 g/rat) decreased gastri c acid secretion in 2-hr pylorus ligated rats in a naloxone dependent manner. [N Phe^1] N (1-13)NH_2 icv administered alone stimulated gastric acid secretion. The intrinsic activity of this antagonist precluded the assessment of any antagonistic effect and the characterization of the exact effect of OFQ/N on this function.

In conclusion, the opioid-like peptide OFQ/N, icv administered in the rat, inhibits gastrointestinal transit and colonic propulsion in a naloxone insensitive manner, but decreases gastric acid secretion in a partially naloxone sensitive way. OFQ/N and its receptors therefore represent a novel central peptidergic pathway regulating gastrointestinal motor and secretory functions.

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