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Opioid activity profiles indicate similarities between the nociceptin/orphanin FQ and opioid receptors

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Abstract

Nociceptin (orphanin FQ) is the recently discovered peptide agonist for the orphan receptor opioid receptor-like 1 (ORL1). Despite the high sequence homology between ORL1 and the opioid receptors, most opioids lack affinity for the nociceptin receptor. The affinity and functional profile of opioids possessing activity at the nociceptin receptor was determined using [^{3}H]nociceptin and nociceptin-stimulated [^{35}S]GTPγS binding. The μ-opioid receptor-selective agonist lofentanil potently and competitively displaced [^{3}H]nociceptin at rat brain receptors (IC_{50} 62 nM). Lofentanil exhibited full agonism for enhancement of [^{35}S]GTPγS binding to human recombinant ORL1 receptors (EC_{50} 50 nM). The related piperidines ohmefentanyl and sufentanil and the nonselective opioid receptor agonist etorphine were less potent nociceptin receptor agonists. The $\kappa_1 + \kappa_3$-opioid receptor agonist/μ-opioid receptor antagonist naloxone benzoylhydrazone was a pure antagonist at both rat brain and human ORL1 receptors. The nonselective opioid receptor partial agonist buprenorphine and the nonselective opioid receptor antagonist (−)-quadazocine exhibited pure antagonism at rat brain receptors, but displayed partial agonism at human ORL1 receptors. Thus, opioids displaying full agonism at the nociceptin receptor are also opioid receptor agonists, whereas opioids that are antagonists or partial agonists at the nociceptin receptor show antagonism or partial agonism at opioid receptors. In addition, the stereospecificity required at opioid receptors appears to be retained at the nociceptin receptor, since (+)-quadazocine is inactive at both receptors. These findings illustrate the structural and functional homology of the opioid recognition site on these two receptor classes and suggest that opioids may provide leads for the design of nonpeptide nociceptin receptor agonists and antagonists lacking affinity for the classical opioid receptors.

Keywords: Nociceptin; Orphanin FQ; ORL1; Opioid; Opioid receptor; GTPγS

1. Introduction

Following the cloning of the δ-opioid receptor (Evans et al., 1992; Kieffer et al., 1992), homology screening of brain cDNA libraries resulted in the cloning of other members of the opioid receptor family. In addition, a clone termed orphan receptor-like 1 (ORL1) having ~50% overall sequence homology with the opioid receptors was identified and given orphan receptor status since it was not activated by any known opioid peptide (Mollereau et al., 1994). Subsequently, a 17 amino acid peptide, named nociceptin (Meunier et al., 1995) or orphanin FQ (Reinscheid et al., 1995), was discovered and found to be a selective agonist for this receptor. Despite the structural similarities of both ORL1 with the opioid receptors and nociceptin with opioid peptides (particularly dynorphin), nociceptin and its receptor constitute a novel neurotransmitter system distinct from the opioid receptor systems (for review, see Henderson and McKnight, 1997; Meunier, 1997; Darland et al., 1998).

Nociceptin has been shown to regulate a number of behavioral and physiological processes. Although effects on pain systems are somewhat controversial, i.c.v. nociceptin appears to oppose opioid-mediated analgesia and has been argued to be part of an anti-opioid system in the brain (Tian et al., 1997). In contrast, i.t. nociceptin is generally analgesic (Tian et al., 1997). Nociceptin has also been shown to have anxiolytic-like activity (Jenck et al., 1997), impair spatial learning (Sandin et al., 1997), in-

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crease food intake (Pomonis et al., 1996), decrease heart rate and blood pressure (Kapusta et al., 1996), and produce diuresis (Kapusta et al., 1996).

Clarification of the pharmacology of the nociceptin system would be aided by the discovery of selective bioavailable agonists and antagonists. Recently, the discovery of the first selective ORL1 receptor antagonist has been reported (Kawamoto et al., 1999). Prior to this report, opioids provided the only known templates for small molecule ligands. Although most opioids are inactive, a few do possess affinity for the nociceptin receptor. The current study examines the structural requirements of opioids retaining affinity for the nociceptin receptor and characterizes their functional activity in relation to their known opioid receptor profiles.

2. Materials and methods

2.1. Materials

Buprenorphine HCl, guanosine 5′-diphosphate (GDP), and guanosine-5′-(3-thio)triphosphate (GTPγS) were purchased from Sigma (St. Louis, MO) and naloxone benzoylhydrazone was from RBI (Natick, MA). Lofentanil oxalate was obtained from Janssen Pharmaceutica (Beerse, Belgium); (−)-quadazocine mesylate (WIN 44,441-3), (+)-quadazocine mesylate (WIN 44,441-2), and zenazocine mesylate (WIN 42,964-4) were from Sterling-Winthrop (Rensselaer, NY); and sufentanil citrate was from NIDA (Rockville, MD). Ohmefentanyl was a generous gift of Dr. Wen-qiao Jin, Department of Pharmacology, Shanghai Institute of Materia Medica (Shanghai, China). Etorphine HCl was obtained from Reckitt and Coleman (Hull, UK). [^{3}H]Nociceptin (157–173 Ci/mmol) was purchased from Amersham (Arlington Heights, IL); [^{35}S]GTPγS (1100–1400 Ci/mM) was from NEN (Boston, MA), nociceptin was from Bachem (Torrance, CA); and human embryonic kidney (HEK) cells expressing human ORL1 receptors were from Receptor Biology (Beltsville, MD). The K_D and B_{max} values supplied by Receptor Biology for [^{3}H]nociceptin binding to these human ORL1/HEK cell membranes were 0.41 nM and 1.3 pmol/mg protein, respectively.

2.2. [^{3}H]Nociceptin competition

The rat brain membrane preparation and [^{3}H]nociceptin binding assay were modified from Dooley and Houghten, 1996. Whole frozen male Sprague–Dawley rat brains were obtained from Pel-Freez (Roger, AK). The cerebella were removed, and the brains minus cerebella were homogenized with a glass/Teflon homogenizer in 50 mM Tris–HCl, pH 7.4 (1 brain/30 ml buffer). The homogenate was centrifuged at $38{,}000 \times g$ for 10 min, resuspended (1 pellet/30 ml buffer), recentrifuged, and the pellet was frozen and stored at −80°C. On the day of the binding assay, the pellet was resuspended at 0.5 mg/ml in 50 mM Tris–HCl containing 250 μM phenylmethanesulfonyl fluoride. Protein was measured using a modified Lowry procedure (Peterson, 1977). Membranes (100 μg) were incubated with 80–130 pM [^{3}H]nociceptin in 2 mM EDTA/50 mM Tris–HCl, pH 7.4 buffer containing 0.2% bovine serum albumin and 100 μM phenylmethanesulfonyl fluoride in a final volume of 0.5 ml. Opioids were added in 5 μl dimethyl sulfoxide (DMSO; final 1%). Nonspecific binding was determined in the presence of 100 nM unlabeled nociceptin. Following a 30-min incubation at room temperature in 96-well plates (1.0 ml; Beckman, Fullerton, CA), the assays were terminated by filtration onto 96-well GF/B filter plates (Packard, Merdiden, CT) presoaked for 1 h and prerinsed with 0.05% polyethylenimine. The plates were rinsed three times with ~0.8 ml ice-cold rinse buffer (2 mM EDTA/50 mM Tris–HCl, pH 7.4). Microscint scintillation cocktail (50 μl; Packard) was added to each well of the dried filter plates, which were then sealed, shaken vigorously for 30 min, and counted for 5 min/well on a TopCount 12-detector scintillation counter (Packard). Competition assays using the recombinant human nociceptin receptor ORL1 were identical to the rat brain competition assay except that frozen membranes from HEK cells expressing human ORL1 were thawed, diluted, and homogenized and then 10 μg was added to each well.

2.3. [^{3}H]Nociceptin saturation

Rat brain membranes (150 μg) prepared as described above were incubated with increasing concentrations of [^{3}H]nociceptin (0.3–45 pM) in 2 mM EDTA/50 mM Tris–HCl, pH 7.4 buffer containing 0.2% bovine serum albumin and 100 μM phenylmethanesulfonyl fluoride in a final volume of 15 ml. Opioids were added in 15 μl DMSO (final 0.1%). Following a 60-min incubation at room temperature in 15-ml conical centrifuge tubes, the assays were terminated by filtration on glass fiber filters (Schleicher and Schuell No. 32) presoaked for 1 h in rinse buffer containing 0.05% polyethylenimine. The cell harvester (Brandel, Gaithersburg, MD) was prerinsed with 0.05% polyethylenimine in rinse buffer prior to filtration. The filters were then rinsed three times with ~5 ml ice-cold rinse buffer, shaken for 1 h in Ready Protein$^+$ scintillation cocktail (5 ml; Beckman), and counted for 10 min on an LS-6000C scintillation counter (Beckman).

2.4. [^{35}S]GTPγS modulation

The rat brain membrane preparation and [^{35}S]GTPγS binding assay were modified from Sim et al., 1996. Whole brains minus cerebellum from male Sprague–Dawley rats were homogenized with a Polytron homogenizer in 1 mM EGTA/3 mM $MgCl_2$/50 mM Tris–HCl, pH 7.4 (1 brain/25 ml buffer). The homogenate was centrifuged at $48{,}000 \times g$ for 10 min, resuspended (1 pellet/25 ml

buffer), recentrifuged, and the final pellet was resuspended at 5 mg/ml and stored at −80°C. On the day of the binding assay, the membrane suspension was diluted to 0.1 mg/ml. For standard basal binding, membranes (10 μg) were preincubated in 100 mM NaCl/3 mM $MgCl_2$/0.2 mM EGTA/50 mM Tris–HCl, pH 7.4 buffer containing 0.2% bovine serum albumin and 60 μM GDP for 30 min at 30°C in 96-well plates (1.0 ml; Beckman). When present, opioids were added to the preincubation in 5 μl DMSO (final 1%). Binding was initiated by the addition of 200 pM [^{35}S]GTPγS followed by a 60-min incubation at 30°C in a final volume of 0.5 ml. Nonspecific binding was determined in the presence of 10 μM unlabeled GTPγS. For standard nociceptin-stimulated binding, 100 nM unlabeled nociceptin was included in the preincubation. The assays were terminated by filtration onto GF/B 96-well filter plates and rinsed three times with ~0.8 ml ice-cold buffer (100 mM NaCl/3 mM $MgCl_2$/0.2 mM EGTA/50 mM Tris–HCl, pH 7.4). The sealed filter plates were shaken vigorously for 2 min prior to counting. Opioid modulation of [^{35}S]GTPγS binding using the recombinant human nociceptin receptor human ORL1 were identical to the rat brain [^{35}S]GTPγS assay except that 10 μg membranes from HEK cells expressing the human ORL1 receptor was incubated with 6 μM GDP.

2.5. Data analysis

For saturation experiments, nonlinear curve fitting of the specific binding as a function of free radioligand concentration was done using the hyperbolic function in Prism 2.0 (GraphPad, San Diego, CA). In no case did a two-site hyperbolic function fit the data significantly better than a one-site hyperbolic function by *F*-test (Prism). The one-site hyperbolic fit was transformed into Scatchard format and plotted with the transformed data. In all cases, dissociation constant (K_D) and receptor density (B_{max}) values were obtained from one-site hyperbolic fits and means ± S.E.M. values were calculated from the individual experiments. For concentration–effect curves, the concentration of test compound producing 50% inhibition (IC_{50}) or enhancement (EC_{50}) of specific binding, and the maximal extent of inhibition (I_{max}) or enhancement (E_{max}) were determined for the individual experiments using the sigmoidal equation in Prism.

3. Results

3.1. Affinity of nociceptin and displacement by opioids

[^{3}H]Nociceptin bound a single population of receptors present in rat brain membranes with very high affinity (K_D 1.75 pM) (Fig. 1A,B). Several opioids displaced [^{3}H]nociceptin binding in membranes from rat brain and HEK cells expressing human ORL1 (Table 1). Lofentanil

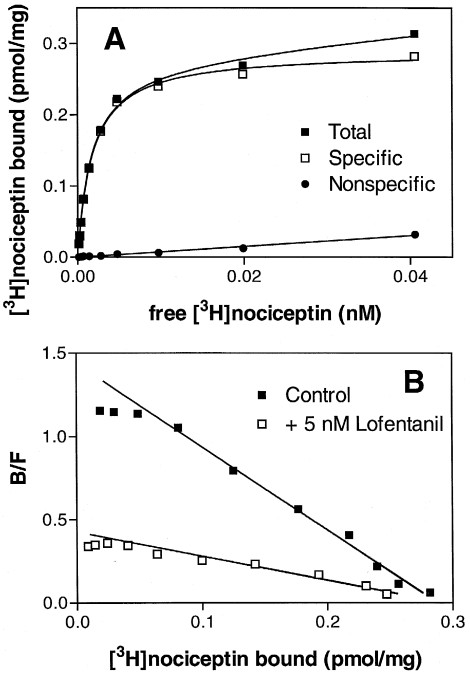

Fig. 1. Saturation analysis of [^{3}H]nociceptin binding to rat brain nociceptin receptors. (A) Representative control experiment. (B) Scatchard transformation of specific binding from the same experiment. Also included is the transformed saturation data in the presence of 5 nM lofentanil. Rat brain membranes (150 μg protein) were incubated for 60 min at room temperature in a final volume of 15 ml. Control K_D and B_{max} values were 1.75 ± 0.04 pM and 260 ± 40 fmol/mg protein, respectively. In the presence of 5 nM lofentanil or 200 nM buprenorphine, the K_D values were 8.8 ± 1.8 or 14 ± 2 pM, respectively, and B_{max} values were 300 ± 50 or 300 ± 60 fmol/mg protein, respectively. For this representative experiment, K_D and B_{max} values under control conditions were 1.8 pM and 290 fmol/mg protein and in the presence of 5 nM lofentanil were 6.1 pM and 290 fmol/mg protein.

was the most potent inhibitor (IC_{50} ~ 50 nM), whereas the other opioids displayed lower potency (IC_{50} ~ 0.5–4 μM). The potencies of these opioids were very similar at rat brain and human ORL1 nociceptin receptors. Lofentanil and buprenorphine were evaluated in saturation experiments and were found to increase the apparent K_D values for [^{3}H]nociceptin five- and eightfold, respectively, with little change in B_{max} (Fig. 1B) consistent with competitive displacement.

3.2. Optimization of the [^{35}S]GTPγS assay

Optimal conditions to obtain a high nociceptin enhancement of [^{35}S]GTPγS binding relative to the basal level were evaluated at rat brain receptors. A concentration of 100 mM NaCl provided the optimal ratio of nociceptin-stimulated to basal binding. Optimal nociceptin stimulation occurred between 1 and 3 mM $MgCl_2$. Nociceptin-stimulated [^{35}S]GTPγS binding was detected at GDP concentrations > ~1 μM and increased up to the highest GDP concentration examined (100 μM). Under standard conditions, nociceptin was more potent at human ORL1 recep-

Table 1
Potency of opioids as inhibitors of [^{3}H]nociceptin binding to rat brain nociceptin receptors and human ORL1
Rat brain membranes (100 μg) or HEK/human ORL1 membranes (10 μg) incubated with ~100 pM [^{3}H]nociceptin for 30 min at room temperature. Values are the means ± S.E.M. of at least three independent experiments.

Opioid	Rat brain		Human ORL1	
	IC_{50} (μM)	Slope	IC_{50} (μM)	Slope
Buprenorphine	1.8 ± 0.2	0.94 ± 0.04	1.0 ± 0.1	0.76 ± 0.03
Naloxone benzoylhydrazone	0.74 ± 0.05	0.82 ± 0.04	1.4 ± 0.4	0.72 ± 0.05
(−)-Quadazocine	2.0 ± 0.2	1.04 ± 0.09	2.2 ± 0.5	0.74 ± 0.06
(+)-Quadazocine	> 100[a]	–	> 100[a]	–
Zenazocine	4.0 ± 0.1	0.95 ± 0.09	3.1 ± 0.4	0.89 ± 0.03
Lofentanil	0.062 ± 0.002	0.90 ± 0.03	0.045 ± 0.015	0.79 ± 0.14
Ohmefentanyl	0.52 ± 0.11	0.83 ± 0.04	0.52 ± 0.16	0.71 ± 0.10
Sufentanil	3.8 ± 0.8	0.99 ± 0.05	5.0 ± 1.4	0.89 ± 0.09
Etorphine	1.3 ± 0.2	0.78 ± 0.06	2.0 ± 0.3	0.83 ± 0.04

[a]Two independent experiments.

tors (EC_{50} 4.5 ± 0.8 nM, E_{max} 97 ± 5%, slope 0.60 ± 0.06) than at rat brain nociceptin receptors (EC_{50} 19 ± 2 nM, E_{max} 134 ± 9%, slope 0.62 ± 0.17).

3.3. Profile of opioids in the [^{35}S]GTPγS assay

At rat brain receptors, buprenorphine, naloxone benzoylhydrazone, (−)-quadazocine, and zenazocine completely inhibited nociceptin-stimulated [^{35}S]GTPγS binding with IC_{50} values ranging from 1.5 to 6.4 μM (Fig. 2A–D; Table 2). At human ORL1 receptors, naloxone benzoylhydrazone also completely inhibited nociceptin-stimulated [^{35}S]GTPγS binding, although it was 3.4-fold less potent than at rat brain receptors (Fig. 2B; Table 2). In contrast, buprenorphine, (−)-quadazocine, and zenazocine stimulated basal [^{35}S]GTPγS binding at human ORL1 receptors (Fig. 2A,C,D; Table 3). Buprenorphine and (−)-quadazocine also inhibited nociceptin-stimulated [^{35}S]GTPγS binding at human ORL1 receptors (Fig. 2A,C),

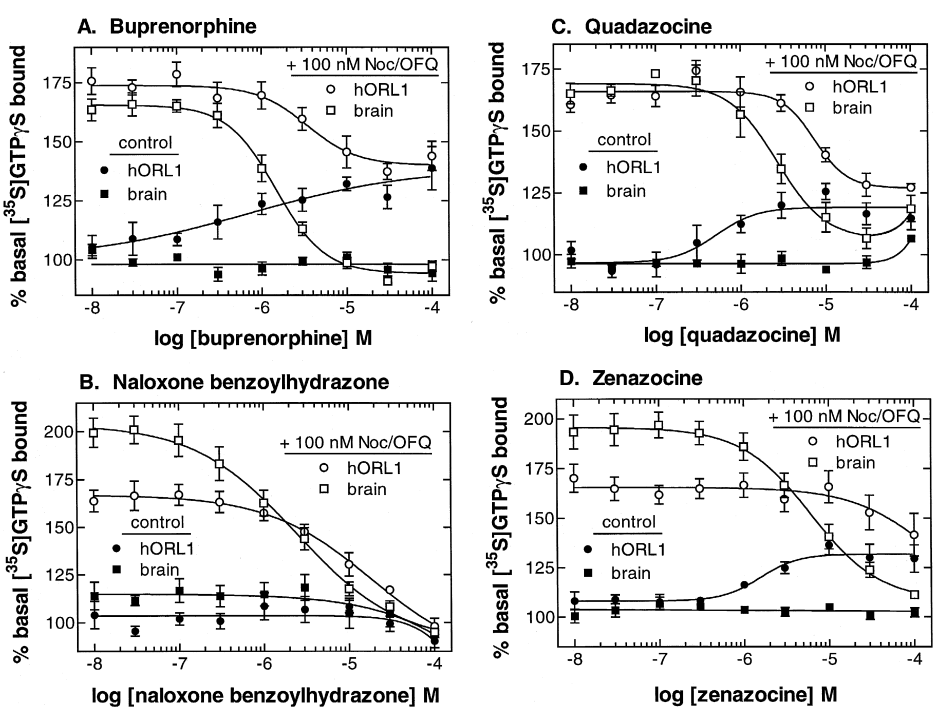

Fig. 2. Profile of opioids displaying antagonism of nociceptin-stimulated [^{35}S]GTPγS binding to rat brain nociceptin receptors and human ORL1. (A) Buprenorphine. (B) Naloxone benzoylhydrazone. (C) (−)-Quadazocine. (D) Zenazocine. Opioid modulation of [^{35}S]GTPγS binding in the absence (control) or presence of 100 nM nociceptin. Rat brain (or human ORL1) membranes (10 μg) incubated with 200 pM [^{35}S]GTPγS, 60 (or 6) μM GDP, 100 mM NaCl, and 3 mM $MgCl_2$ for 60 min at 30°C. See Table 2 for means ± S.E.M. IC_{50} values.

Table 2
Opioid antagonism of nociceptin-stimulated [^{35}S]GTPγS binding
Rat brain or HEK/human ORL1 membranes (10 μg) incubated with 200 pM [^{35}S]GTPγS and 60 or 6 μM GDP, respectively, for 60 min at 30°C in the presence of 100 nM nociceptin. Values are the means ± S.E.M. of at least three independent experiments.

Opioid	Rat brain		Human ORL1	
	IC_{50} (μM)	I_{max} (%)	IC_{50} (μM)	I_{max} (%)
Buprenorphine	1.5 ± 0.1	72 ± 3	3.0 ± 1.0	37 ± 2
Naloxone benzoylhydrazone	2.5 ± 0.5	118 ± 12	8.4 ± 2.1	67 ± 6
(−)-Quadazocine	2.6 ± 0.5	63 ± 3	8.2 ± 1.1	40 ± 2
Zenazocine	6.4 ± 1.2	88 ± 9	> 100	–

whereas zenazocine was not a potent inhibitor at the recombinant receptor (Fig. 2D).

Lofentanil was the most potent stimulator of [^{35}S]GTPγS binding (EC_{50} 50 nM) at human ORL1 receptors (Fig. 3A, Table 3). Ohmefentanyl, sufentanil and etorphine were less potent stimulators of [^{35}S]GTPγS binding (EC_{50} 0.63–1.8 μM). The rank order efficacy for stimulation of [^{35}S]GTPγS binding was etorphine ≥ lofentanil ~ ohmefentanyl > sufentanil. In addition, lofentanil, ohmefentanyl, and etorphine increased [^{35}S]GTPγS binding above that produced by a submaximal concentration of nociceptin (100 nM) (Fig. 3B). In contrast, sufentanil produced little increase, if any, in [^{35}S]GTPγS binding beyond that elicited by 100 nM nociceptin. These opioids were not evaluated as stimulators of [^{35}S]GTPγS binding at rat brain receptors because their activity at the nociceptin receptor was difficult to distinguish from their activity at opioid receptors in this tissue.

4. Discussion

Despite the high sequence homology (~ 50%) of the cloned nociceptin receptor ORL1 with μ-, δ-, and κ-opioid receptors (Mollereau et al., 1994) and also between the nociceptin peptide and the opioid peptides, particularly dynorphin A (Meunier et al., 1995; Reinscheid et al., 1995), there is little or no cross-talk between the nociceptin and opioid receptor systems (Reinscheid et al., 1998). Similarly, most opioids lack affinity for the nociceptin receptor. The present study characterizes the few opioids that do possess activity at the nociceptin receptor.

Table 3
Opioid-stimulated [^{35}S]GTPγS binding at human ORL1
HEK/human ORL1 membranes incubated with [^{35}S]GTPγS as described in Table 2 except in the absence of nociceptin. Values are the means ± S.E.M. of at least three independent experiments.

Opioid	EC_{50} (μM)	E_{max} (%)
Lofentanil	0.050 ± 0.010	102 ± 8
Ohmefentanyl	0.860 ± 0.15	96 ± 9
Sufentanil	1.8 ± 0.4	65 ± 6
Etorphine	0.63 ± 0.11	149 ± 13

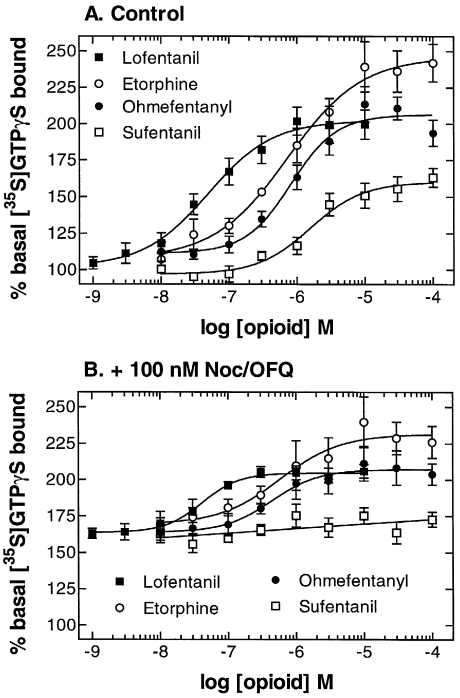

Fig. 3. Profile of opioids displaying agonism at human ORL1 in the [^{35}S]GTPγS assay. (A) Control. (B) Presence of 100 nM nociceptin. HEK/human ORL1 membranes incubated with [^{35}S]GTPγS as described in the legend to Fig. 2. See Table 3 for means ± S.E.M. EC_{50} values.

Saturation analysis indicates that [^{3}H]nociceptin interacts with a single population of nociceptin receptors present in rat brain membranes with very high affinity (K_D 1.75 pM), a value similar to that reported at human ORL1 using [^{125}I][Tyr14]nociceptin (Fawzi et al., 1997). Both one- and two-component saturation analyses with a wide range of K_D values (2 pM–5 nM) have been reported using brain membranes or recombinant receptors (Dooley and Houghten, 1996; Adapa and Toll, 1997; Ardati et al., 1997; Butour et al., 1997; Fawzi et al., 1997). Many of the reported differences may be due to high bound:free ratios. In the current study, saturation analyses were performed in a large incubation volume (15 ml) to obtain bound:free ratios of ~ ≤ 1 for all concentrations of [^{3}H]nociceptin used.

The structures of opioids active at the nociceptin receptor are shown in Fig. 4. The most potent inhibitor of [^{3}H]nociceptin binding is the phenylpiperidine lofentanil (IC_{50} ~ 50 nM), confirming an earlier report (Butour et al., 1997). The related fentanyl analogs ohmefentanyl and sufentanil also possess affinity for the nociceptin receptor. Fentanyl itself is inactive (Butour et al., 1997). Disubstitution at the 4-position of the piperidine ring and phenethyl substitution of the piperidine nitrogen as in lofentanil confer the highest known affinity of any opioid for the nociceptin receptor. In the morphinan series, key features for activity at the nociceptin receptor appear to be bulky

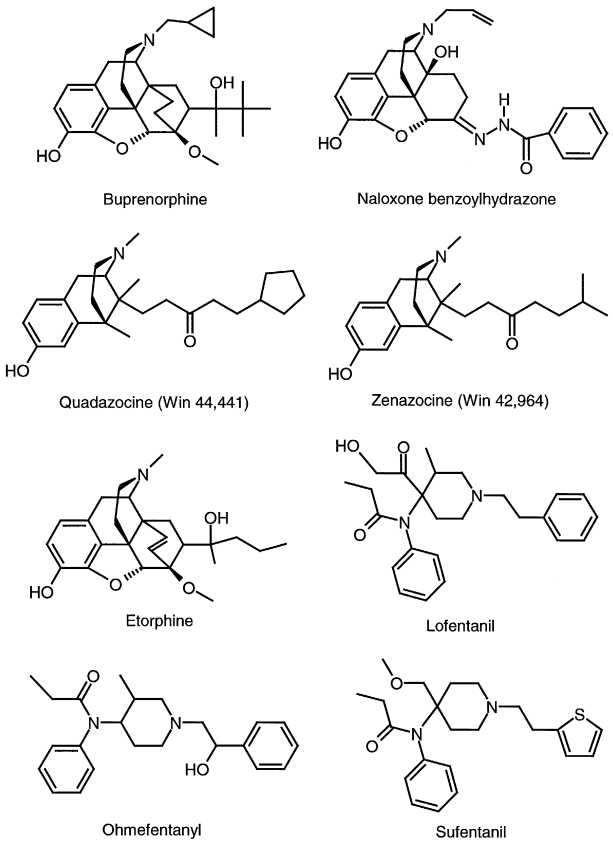

Fig. 4. Structures of opioids having activity at the nociceptin receptor.

oxygen-containing substituents extending from the 6- or 7-position of the C-ring, as in buprenorphine, naloxone benzoylhydrazone, and etorphine. This structure–activity relationship (SAR) is consistent with the recently reported activity of the 6-substituted morphinan TRK-820 at ORL1 (Seki et al., 1999). Similarly, bulky oxygen-containing substituents extending from the 11-position of the 6,7-benzomorphan nucleus confers affinity for the nociceptin receptor, as in (−)-quadazocine and zenazocine. Interestingly, the stereospecificity required at opioid receptors appears to be retained at the nociceptin receptor, since (+)-quadazocine is inactive at both the opioid (Wood et al., 1984) and nociceptin receptors. Lofentanil and buprenorphine increased the apparent K_D of [^{3}H]nociceptin with little change in B_{max}, consistent with competitive displacement.

The pharmacological profile of the opioids possessing affinity for the nociceptin receptor was determined by [^{35}S]GTPγS binding. Naloxone benzoylhydrazone did not affect basal binding, but completely inhibited nociceptin-stimulated [^{35}S]GTPγS binding at both rat brain and human ORL1 receptors indicating that it is a pure nociceptin receptor antagonist. This finding is consistent with competitive antagonism observed in the rat vas deferens (Dunnill et al., 1996), but contrasts with the agonist-like effect of naloxone benzoylhydrazone reported in a cAMP assay in cells expressing KOR-3, the mouse homologue of human ORL1 (Pan et al., 1996). Buprenorphine and (−)-quadazocine acted as pure antagonists at rat brain receptors. (−)-Quadazocine has been reported to be a nociceptin receptor antagonist in the rat vas deferens (Nicholson et al., 1996). However, at the human ORL1 receptor, buprenorphine and (−)-quadazocine stimulated basal binding and inhibited nociceptin-stimulated [^{35}S]GTPγS binding so that the stimulatory and inhibitory curves met at about the same level, indicative of partial agonism in this system. Agonism by buprenorphine has recently been demonstrated at ORL1 using a reporter gene assay (Wnendt et al., 1999). Zenazocine was also a pure antagonist at brain receptors and appeared to be a partial agonist at human ORL1. Human ORL1 receptors expressed in HEK cells may be more sensitive in detecting partial agonism as compared to nociceptin receptors in brain membranes because a higher receptor to G-protein ratio may exist in these recombinant cells. These differences in efficacy at native and cloned nociceptin receptors have also been observed with the peptide ligand acetyl-RYYRIK-NH_2, which is an antagonist at native nociceptin receptors and an agonist at ORL1 (Berger et al., 1999).

Lofentanil, ohmefentanyl, sufentanil, and etorphine stimulated [^{35}S]GTPγS binding at human ORL1 receptors indicating that they are nociceptin receptor agonists. Lofentanil, ohmefentanyl, and etorphine further increased the stimulation of [^{35}S]GTPγS binding induced by a submaximal concentration of nociceptin. The enhancement curves in the presence and absence of nociceptin met at about the same level indicating that the agonistic effect of these opioids was mediated by the human ORL1 receptor and that they are full agonists, as shown previously for lofentanil and etorphine as inhibitors of cAMP production (Butour et al., 1997). Sufentanil did not increase the stimulation of [^{35}S]GTPγS binding induced by nociceptin suggesting that it has less than full efficacy at the human ORL1 receptor.

In general, opioids that are agonists at the nociceptin receptor display only agonistic activity at opioid receptors, whereas opioids that are antagonists or partial agonists at the nociceptin receptor show antagonism or partial agonism at opioid receptors. Thus, the μ-opioid receptor-selective agonists lofentanil (Maguire et al., 1992), ohmefentanyl, and sufentanil (Goldstein and Naidu, 1989) as well as the nonselective opioid receptor agonist etorphine (Niwa et al., 1995) are all agonists at the nociceptin receptor. In contrast, the nonselective partial opioid receptor agonists buprenorphine (Kajiwara et al., 1986) and zenazocine (Ward et al., 1985), the nonselective opioid receptor antagonists (−)-quadazocine (Wood et al., 1984), and the $\kappa_1 + \kappa_3$-opioid receptor agonist/μ-opioid receptor antagonist naloxone benzoylhydrazone (Berzetei-Gurske et al., 1995) are antagonists or partial agonists at the nociceptin receptor. Considering the similarity between nociceptin and dynorphin and also the possible connection between ORL1 and the κ-opioid receptor (Pan et al.,

1996), it might be predicted that opioids selective for κ-opioid receptors would have a higher probability of binding to the nociceptin receptor. However, opioid receptor selectivity appears to have little to do with activity at the nociceptin receptor.

The similar actions of the opioids at the nociceptin receptor and at the opioid receptors indicate that there is structural and functional homology between the agonist binding sites on these two receptor classes. This conclusion is supported by the synthesis of a universal peptide agonist activating both nociceptin and κ-opioid receptors (Reinscheid et al., 1998). In addition, activation of the nociceptin receptor and opioid receptors have similar functional effects, including inhibition of cAMP production (Meunier et al., 1995; Reinscheid et al., 1995), inhibition of calcium channels (Knoflach et al., 1996), activation of potassium channels (Connor et al., 1996), and activation of mitogen-activated protein kinase (Fawzi et al., 1997). Further support for this conclusion comes from site-directed mutagenesis studies in which key residues of ORL1 are replaced with the corresponding opioid receptor residues. Opioids display greatly increased affinities at these mutant nociceptin receptors and have similar stereospecificity and agonist/antagonist profiles as at opioid receptors (Meng et al., 1998).

In conclusion, certain morphinan, 6,7-benzomorphan, and piperidine opioids possess affinity for the nociceptin receptor. Opioid affinity for the nociceptin receptor appears to be unrelated to opioid receptor selectivity, but rather is related to an agonist binding site on the nociceptin receptor displaying a unique SAR such that only a few available opioids have affinity. The mode of interaction of opioids with the nociceptin receptor is probably similar to that at opioid receptors since opioids displaying agonism at the nociceptin receptor are also agonists at opioid receptors. Similarly, opioids that are antagonists or partial agonists at the nociceptin receptor exhibit antagonism or partial agonism at opioid receptors. These opioid ligands may provide leads for the design of nonpeptide agonists and antagonists selective for the nociceptin receptor relative to opioid receptors.

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