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Different domains of the ORL1 and κ-opioid receptors are involved in recognition of nociceptin and dynorphin A

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Abstract In order to gain further insight into the functional architecture of structurally related G protein-coupled receptors, the ORL1 (nociceptin) and opioid receptors, we have constructed chimeras of ORL1 and μ-, δ- and κ-opioid receptors, and compared their binding and functional properties with those of the parent receptors. We find in particular that a ORL1–κ-opioid (O-K) hybrid construct has retained high affinity for non-type-selective opiate ligands, and has acquired the ability to bind and respond to enkephalins and μ- and/or δ-opioid receptor-selective enkephalins analogs, thus behaving like a 'universal' opioid receptor. Most significantly however, whilst the ORL1 and κ-opioid receptors display high binding preference (K_D 0.1 vs. 100 nM) for their respective endogenous ligands, nociceptin and dynorphin A, the O-K chimeric receptor binds both nociceptin and dynorphin A, with high affinity ($K_D < 1$ nM). Together, these data (i) add weight to the hypothesis that the extracellular loops of opioid receptors act as a filter for ligand selection, and (ii) demonstrate that different domains of the ORL1 and κ-opioid receptors are involved in recognition of their endogenous peptide ligands.

Key words: Non-opioid–opioid hybrid receptor; Gain-of-function protein engineering; Opioid peptide; Structure-function relationship

1. Introduction

The ORL1 (opioid receptor-like 1) receptor [1–8] is the non-opioid, G protein-coupled receptor whose primary structure is most closely related to those of the μ (MOR1), δ (DOR1) and κ (KOR1) types of opioid receptor. In terms of percent homology, based on sequence alignment, the ORL1 receptor is nearly equidistant (63–65%) to the three types of opioid receptor, amino acid conservation being prominent in the putative transmembrane domains and cytoplasmic loops. However, the ORL1 receptor possesses a common structural feature with the κ-opioid receptor, but not present in either the μ- or the δ-opioid receptors, i.e. a highly acidic second exofacial loop. On the basis of these physical characteristics, the ORL1 receptor appears to be structurally closer to the κ than to the μ and δ types of opioid receptor. The endogenous agonist of the ORL1 receptor, nociceptin [9] or orphanin FQ [10], was recently identified in nerve tissue extracts as a highly basic neuropeptide of 17 amino acids. Nociceptin is similar in composition to the naturally occurring opioid heptadecapeptide dynorphin A, the endogenous agonist of the κ-opioid receptor. It has been suggested that nociceptin might interact with the ORL1 receptor through the same mechanism(s) as dynorphin A does with the κ-opioid receptor [9]. In vitro, the two peptides display high selectivity toward their respective receptors: nociceptin binds the ORL1 receptor with 500–1000-fold the affinity it does the κ-opioid receptor, while, conversely, dynorphin A binds the κ-opioid receptor with 500–1000-fold the affinity it does the ORL1 receptor.

The dual homology of receptors and of peptide ligands provides a unique opportunity to compare the functional architectures of the ORL1 and opioid receptors. With this objective in mind, we have constructed ORL1–MOR1 (O-M), -DOR1 (O-D) and -KOR1 (O-K) receptor cDNA chimeras, and stably expressed them in CHO cells. The binding and functional properties of the chimeric receptors have been compared with those of the parent ORL1 and opioid receptors. We find in particular that the O-K hybrid receptor not only binds nociceptin and dynorphin A with high affinities, but also opioids lacking affinity for the two wild-type receptors. In marked contrast, the reciprocal (K-O) chimeric receptor is inactive. These results are discussed in terms of different nociceptin and dynorphin A binding domains in the parent ORL1 and κ-opioid receptors. Notwithstanding the apparent structural similarity of the two peptide-receptor systems, the mechanisms of peptide recognition and activation are clearly different.

2. Materials and methods

2.1. Construction of the chimeric cDNAs

The human cDNAs encoding the ORL1 [1], MOR1 (a gift from Dr L. Emorine), DOR1 and KOR1 (gifts from Dr B. Kieffer) receptors, inserted in Stratagene's Bluescript SK$^+$ plasmid, were used. The O-M, O-D, O-K and reciprocal M-O, D-O and K-O chimeric cDNAs were constructed at a common *Afl*III restriction site present in the cDNA region encoding the third transmembrane helix of the four receptors. Since the DOR1 cDNA contains an additional *Afl*III site, partial digestion was used to obtain the appropriate fragment for construction of the O-D and D-O chimeras. The O-M, O-D and O-K chimeric cDNAs encoded ORL1 residues 1–133, followed by MOR1 residues 152–400, DOR1 residues 131–372 and KOR1 residues 142–380, re-

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Abbreviations: hORL1, human opioid receptor-like 1 receptor; hMOR1, hDOR1 and hKOR1, human μ-, δ- and κ-opioid receptor, respectively; O-M, O-D and O-K, hORL1-(1–133)–hMOR1-(152–400), -hDOR1-(131–372) and -hKOR1-(142–380) chimeric receptors, respectively; M-O, D-O and K-O, hMOR1-(1–151)–hORL1-(134–370), hDOR1-(1–130)- and -hKOR1-(1–141)- chimeric receptors, respectively; DAGO, [D-Ala^2,*N*-Me-Phe^4,Gly-ol^5]enkephalin; DTLET, [D-Thr^2,Leu^5,Thr^6]enkephalin; U-50488, *trans*-(1*S*,2*S*)-3,4-dichloro-*N*-methyl-*N*-[2-(1-pyrrolidinyl)-cyclohexyl]-benzeneacetamide; EKC, ethylketocyclazocine; nor-BNI, *nor*-binaltorphimine

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spectively. The reciprocal M-O, D-O and K-O chimeric cDNAs encoded MOR1 residues 1–151, DOR1 residues 1–130 and KOR1 residues 1–141, followed by ORL1 residues 134–370, respectively. The cDNAs were transferred into the eukaryotic expression vector pRC/CMV (Invitrogen). All constructs were verified by restriction endonuclease analysis and sequencing.

2.2. Expression of the chimeric receptors

Chinese hamster ovary cells (CHO-K1) were transfected with 5 μg of Qiagen-purified plasmid DNA using the calcium phosphate precipitation method [11]. Colonies with stable expression were selected in culture medium containing 400 μg/ml geneticin (Gibco BRL), on the basis of etorphine (10 μM) inhibition of forskolin-induced accumulation of cAMP, and specific binding of [^{3}H]diprenorphine and/or [^{3}H]nociceptin.

2.3. Membrane preparation

The transfected cells were harvested by centrifugation (2500×*g* for 15 min) in ice-cold phosphate-buffered saline. The pellet was homogenized using a Polytron homogenizer in 0.05 M Tris-HCl (pH 7.6) and 1 mM EDTA, and the suspension was centrifuged (1000×*g* for 15 min) at 4°C in order to remove unbroken cells and nuclei. The resulting supernatant was then centrifuged at 100 000×*g* for 35 min at 4°C. The pellet was resuspended in the 50 mM Tris, EDTA 1 mM, pH 7.6 buffer and used in the radioligand binding assay. Protein concentration was determined according to Lowry et al. [12] with bovine serum albumin as the standard.

2.4. Binding and competition studies

[^{3}H]Diprenorphine (1.52 TBq/mmol, Amersham) or [^{3}H]ethylketocyclazocine (0.67 TBq/mmol, New England Nuclear) were used to probe the O-K and κ-opioid receptor sites, and [^{3}H]nociceptin (0.85 TBq/mmol, Amersham) to probe the O-K and ORL1 binding sites. To minimize loss of material, particularly of nociceptin and dynorphin A, due to non-specific adsorption, the following precautions were taken: (i) stock solutions and intermediate dilutions of peptides were in 0.1 mg/ml protease-free BSA (fraction V, Sigma) in polypropylene tubes, (ii) binding and competition studies were carried out in polypropylene tubes in the presence of 0.1 mg/ml protease-free bovine serum albumin (BSA), and (iii) glass fiber disks (Whatman GF/B) pre-soaked in polyethyleneimine (0.33%, v:v) were used. For equilibrium binding studies, each incubation mixture (0.5 ml, in triplicate) contained about 30 μg membrane protein and [^{3}H]diprenorphine or [^{3}H]nociceptin at the desired concentration (in the range 0.02–2 nM) in 50 mM Tris-HCl, pH 7.5. Non-specific binding was determined in the presence of 1 μM unlabelled diprenorphine or nociceptin. Following a 1 h incubation at 25°C, unbound radioligand was removed by rapid suction through glass fiber filters (see above) and rinsed three times with 4 ml 10 mM Tris-HCl buffer (pH 7.5). Filter-bound radioactivity was counted in 3 ml of Beckman Ready Protein$^+$ cocktail using a Kontron MR 300 liquid scintillation counter. In the competition studies, each incubation mixture (0.5 ml, in triplicate) consisted of 30 μg membrane protein, [^{3}H]diprenorphine, [^{3}H]ethylketocyclazocine or [^{3}H]nociceptin at a fixed concentration of 1 nM, together with the unlabelled ligand at the desired concentration, in 50 mM Tris-HCl, pH 7.5. Total (no unlabelled ligand) and non-specific (+1 μM unlabelled diprenorphine or nociceptin) binding was determined in triplicate. Bound radioactivity was isolated and measured as described above.

2.5. Assay for intracellular cyclic AMP

Sterile hemolysis tubes were seeded with ~200 000 recombinant CHO cells in culture medium and incubated overnight at 37°C. The culture medium was then removed and replaced by 200 μl of HEPES-buffered Krebs-Ringer saline (KRH: 124 mM NaCl, 5 mM KCl, 1.25 mM $MgSO_4$, 1.5 mM $CaCl_2$, 1.25 mM KH_2PO_4, 25 mM HEPES, 8 mM glucose, 0.5 mg/ml BSA; pH 7.4) containing 0.1 μM adenine and 0.6 μCi [^{3}H]adenine (0.85 TBq/mmol, Amersham). After 1 h at 37°C, the cells were rinsed with 400 μl of KRH and 180 μl of fresh KRH was added to each tube. Intracellular accumulation of cAMP was initiated by addition of 20 μl of KRH containing 100 μM forskolin (Sigma), 1 mM 3-isobutyl-1-methylxanthine (Sigma), 1 mM Ro20-1724 (Biomol. Res.), and the ligand(s) to be tested at 10-fold the desired final concentration. After exactly 10 min at 37°C, the reaction was stopped by addition of 20 μl HCl 2.2 N and rapid mixing (Vortex), and the [^{3}H]cAMP content of each tube was determined by selective batch elution on columns of 0.65 g alumina (activity grade 1, type WA-1: acid, Sigma) essentially as described by Alvarez and Daniels [13].

2.6. Unlabelled ligands

Dynorphin A and nociceptin were solid phase synthesized in the laboratory by Dr H. Mazarguil. [Leu^5]Enkephalin, [Met^5]enkephalin, DAGO and DTLET were from Bachem, etorphine and diprenorphine from Reckitt and Colman, EKC from Sterling Winthrop, nor-BNI and U-50488 from Research Biochemicals. Lofentanil was kindly provided by Dr. J. Leysen (Janssen Pharmaceuticals).

2.7. Analysis of the data

Non-linear regression analysis of the data was performed using Prism version 2.0 from GraphPad Software, San Diego, CA, USA.

3. Results

In saturation binding experiments, the O-K hybrid receptor (Fig. 1) was found to bind [^{3}H]diprenorphine, a non-selective opiate receptor antagonist, and [^{3}H]ethylketocyclazocine, a non-selective opiate, with affinities comparable to those of the κ-opioid receptor (K_D values at 0.1 and 0.4 nM, respectively), as well as [^{3}H]nociceptin with an affinity hardly lower (K_D = 0.4 nM) than the ORL1 receptor, already indicating the mixed ORL1 and KOR1 nature of the O-K chimera (Fig. 2). In marked contrast, the O-M and O-D chimeric receptors, although they bound [^{3}H]diprenorphine with high affinity (K_D values of 0.5 and 0.3 nM, respectively), failed to bind nociceptin (K_I > 10 μM). Moreover, the reciprocal M-O, D-O and K-O hybrid receptors, whose expression in CHO was assessed qualitatively both at the mRNA level by Northern blotting, and at the protein level by using a polyclonal antibody to the ORL1 receptor (data not shown), could not be shown to bind any of the three radioligands.

The binding properties of the ORL1, O-K hybrid and κ-opioid receptors were further investigated, in competition experiments with various unlabelled ligands, using [^{3}H]nociceptin as a probe of the ORL1 receptor.

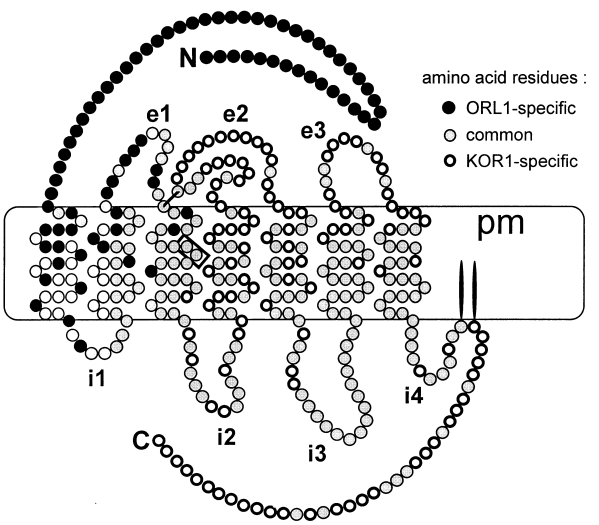


Fig. 1. Putative secondary structure and transmembrane organization of the ORL1(1–133)–KOR1-(142–380) (O-K) chimeric receptor. The amino acid residues at the junction of the two receptor sequences are boxed. e: extracellular loop; i: intracellular loop; pm: plasma membrane; N: amino-end (extracellular); C: carboxyl-end (cytoplasmic).

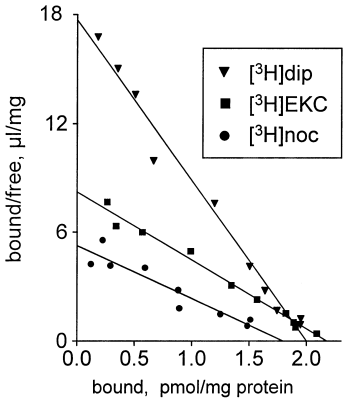


Fig. 2. Saturation equilibrium binding of [^{3}H]diprenorphine (dip), [^{3}H]ethylketocyclazocine (EKC), and [^{3}H]nociceptin (noc) in membranes from CHO cells expressing the O-K hybrid receptor. Scatchard transform of the data. In this particular, representative experiment, K_D (nM) and B_{max} (pmol/mg protein) values were 0.11 and 2.3 for [^{3}H]dip, 0.26 and 2.2 for [^{3}H]EKC, and 0.34 and 1.9 for [^{3}H]noc.

[^{3}H]Diprenorphine and [^{3}H]ethylketocyclazocine were used to probe the κ-opioid receptors, and any one of the three radioligands to probe the O-K chimeric receptor. The results are presented in Table 1. The κ-opioid receptor bound a number of ligands with high affinity, particularly dynorphin A ($K_I = 0.17$ nM), the κ-selective agonist U-50488H ($K_I =$ 3 nM) and nor-BNI ($K_I = 0.3$ nM), a κ-selective antagonist. The κ-opioid receptor showed only low affinity for nociceptin ($K_I \approx 100$ nM), and bound neither Met- and Leu-enkephalin, nor DAGO and DTLET, the respective μ- and δ-opioid receptor-selective enkephalin analogs. Amongst the opioid ligands tested, only a few displayed a measurable potency in binding competition for [^{3}H]nociceptin at the ORL1 receptor. These included lofentanil ($K_I = 24$ nM), the opioid peptide dynorphin A ($K_I = 110$ nM) and the non-selective opiate etorphine ($K_I = 530$ nM). In particular, the ORL1 receptor did not bind diprenorphine ($K_I > 10$ μM), the κ agonists EKC and U-50488H, the κ antagonist nor-BNI, enkephalins and enkephalin analogues. The O-K hybrid receptor displayed a very different pharmacological profile. Unlike the parent receptors, the O-K receptor bound all the ligands tested with high to moderate affinities, and had therefore acquired new binding capabilities. In addition to exhibiting high affinity both for diprenorphine and nociceptin (see above), and dynorphin A ($K_I \approx 0.1$ nM), the O-K receptor recognized ligands that the ORL1 and κ-opioid receptors normally do not, particularly the enkephalins and their respective μ- and δ-selective analogs DAGO and DTLET. Table 1 also shows that the hybrid receptor binding profile was essentially the same, irrespective of the probe used ([^{3}H]diprenorphine, [^{3}H]ethylketocyclazocine or [^{3}H]nociceptin), suggesting that the ligands, although not structurally related, interact with overlapping binding sites in the chimera.

The O-K hybrid receptor was functional, mediating inhibition of forskolin-induced accumulation of cyclic AMP in intact recombinant CHO cells, not only by nociceptin and dynorphin A but also and most noteworthy, by Met- and Leu-enkephalin, DAGO and DTLET (Table 2). Indeed, these peptides and analogs were several hundred fold more potent agonists of the O-K receptor than of the ORL1 and κ-opioid receptors. Ligands exhibiting very little affinity (K_I values ≥1 μM) did not stimulate any of the three receptors, otherwise they showed full agonist activity, producing maximal inhibition (approx. 80%) of forskolin-induced accumulation of cAMP. Dynorphin A and nociceptin, while displaying appreciable affinity for the ORL1 and κ-opioid receptor, respectively, failed to stimulate these receptors. Poor correlation between receptor binding affinity and biological activity was observed for the majority of the ligands acting at the O-K hybrid receptor, consistent with the presence of distinct binding and transduction sites. The O-M and O-D chimeric receptors were insensitive, or nearly so, to dynorphin A ($ED_{50} \approx 0.5$ and > 1 μM, respectively), and nociceptin ($ED_{50} > 10$ μM) (Table 3). They were, however, responsive to the non-selective opiate etorphine (ED_{50} values of 1.3 and 0.9 nM, respectively).

4. Discussion

The fact that the O-K hybrid and κ-opioid receptors have high affinity for the non-selective opioid receptor ligands diprenorphine, etorphine and ethylketocyclazocine indicates that the two receptors contain a similar opioid binding region. Since there is evidence that this region lies within the receptor bundle of transmembrane helices [14], it may be concluded

Table 1
Affinity (K_I, nM) of nociceptin, opioids and antagonists for the ORL1, O-K hybrid and KOR1 (κ-opioid) receptors expressed in CHO cells

	ORL1	OK			KOR1
	[^{3}H]nociceptin	[^{3}H]diprenorphine	[^{3}H]nociceptin	[^{3}H]EKC	[^{3}H]diprenorphine
Etorphine	530 ± 40	0.56 ± 0.05	0.64 ± 0.02	0.13 ± 0.00	1.6 ± 0.2
Diprenorphine	> 10 000	0.09 ± 0.01	0.15 ± 0.03	0.2 ± 0.03	0.33 ± 0.06
Nociceptin	0.13 ± 0.01	0.49 ± 0.02	0.3 ± 0.01	1.2 ± 0.1	104 ± 2
Dynorphin A	111 ± 9	0.06 ± 0.01	0.22 ± 0.01	0.4 ± 0.05	0.17 ± 0.01
U-50488	> 10 000	98 ± 8	33.5 ± 2.5	27.5 ± 2.5	3 ± 0.4
Lofentanil	724.2 ± 0.3	0.14 ± 0.01	0.16 ± 0.03	0.06 ± 0.01	5.5 ± 0.6
EKC	> 10 000	1.7 ± 0.1	2.1 ± 0.2	0.15 ± 0.05	1.86 ± 0.04
Nor-BNI	> 10 000	0.02 ± 0.02	0.23 ± 0.01	0.13 ± 0.01	0.34 ± 0
DAGO	> 10 000	68 ± 9	19.4 ± 0.8	29.5 ± 2.5	> 1 000
DTLET	> 10 000	165 ± 5	25.5 ± 0.9	50 ± 5	> 1 000
[Met^5]Enkephalin	> 1 000	24 ± 2	40.4 ± 1.7	6.9 ± 0.9	> 1 000
[Leu^5]Enkephalin	> 1 000	52 ± 6	19.9 ± 1.6	21.6 ± 3	> 1 000

K_I values were calculated from the concentration (IC_{50}) of unlabelled ligand that halves binding of the indicated radioligand in competition experiments, using the Cheng and Prussof equation [26], $K_I = IC_{50}/(1+L/K_D)$, where L and K_D are the concentration of free radioligand, and the equilibrium dissociation constant of the radioligand, respectively. Each value is the mean ± S.E.M. of values obtained from at least three separate experiments.

Table 2
Potency (ED_{50}, nM) of nociceptin and opioids to inhibit forskolin-induced accumulation of cAMP via the ORL1, O-K hybrid and KOR1 (κ-opioid) receptors expressed in recombinant CHO cells

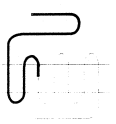
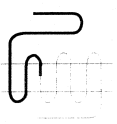
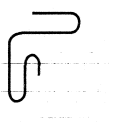
	ORL1	OK	KOR1
Etorphine	400 ± 48	0.09 ± 0.01	4.3 ± 0.8
Nociceptin	0.84 ± 0.02	115 ± 6	> 10 000
Dynorphin A	> 10 000	0.13 ± 0.02	0.5 ± 0.06
U-50488	> 10 000	460 ± 53	2.95 ± 0.04
Lofentanil	6.7 ± 0.2	0.07 ± 0.01	0.32 ± 0.02
EKC	> 10 000	2.9 ± 0.1	0.63 ± 0.02
DAGO	> 10 000	25 ± 3	> 10 000
DTLET	> 10 000	8 ± 1	> 10 000
[Met^5]Enkephalin	> 1 000	6.40 ± 1	> 1 000
[Leu^5]Enkephalin	> 1 000	5.30 ± 0.01	> 1 000

Each value is the mean ± S.E.M. of values obtained from at least three separate experiments.

that the ORL1 transmembrane amino acid residues highlighted in Fig. 1, which are different in ORL1 and the κ-opioid receptors, are not directly involved in opioid recognition. Most significantly, the O-K hybrid receptor displays novel binding and functional capabilities not expressed by the κ-opioid receptor, in particular a dramatically increased ability to bind and respond to the μ-selective agonists lofentanil and DAGO, the δ-selective agonist DTLET, and the enkephalins, whose strong aversion for the κ-opioid receptor is well documented (see [15] for a recent review). Indeed, the O-K hybrid receptor behaves as a non-selective, 'universal' opioid receptor, i.e. an opioid receptor which has lost, to a large extent, the ability to discriminate between type-selective opioid receptor ligands. The likely explanation is that the O-K hybrid receptor extracellular domain, which is different from the κ-opioid receptor extracellular domain, is less efficacious than the latter in preventing access of μ- and δ-opioid receptor ligands to the opioid binding pocket. This is in keeping with the recently proposed hypothesis that the extracellular loops play a major role in ligand selection by opioid receptors, by acting as a gate or filter [16]. The κ-selective agonist U-50488H appears to be an exception to the rule since it shows a somewhat lower affinity for the O-K hybrid than for the κ-opioid receptor. This particular ligand, however, appears to require the entire κ-opioid receptor apart from the second extracellular loop for high affinity binding [17]. Replacement of the κ-opioid receptor sequence (1–141) by the corresponding ORL1 receptor sequence (1–133) in the κ-opioid receptor may therefore have removed a structural determinant necessary for high affinity binding of U-50488H.

Of further note is that the O-K chimeric receptor, unlike the ORL1 and κ-opioid receptors, binds *both* nociceptin and dynorphin A with high affinity. Thus, exchange of the N-terminal (1–141) sequence of the κ-opioid receptor for the corresponding sequence (1–133) of ORL1 has conferred on the modified opioid receptor (the O-K chimeric receptor) the ability to bind nociceptin without impairing binding of dynorphin A. In other words, this change has not suppressed essential determinants for dynorphin recognition, yet has introduced crucial elements for nociceptin recognition. The identity of the determinants involved is difficult to pinpoint at present since the two exchanged sequences have many amino acids in common (those in gray in Fig. 1), especially in the putative transmembrane domains and the C-terminal half of the first exofacial loop. However, the N-terminal domain and N-terminal half of exofacial loop 1 are highly divergent, suggesting that these sequences are not critical for dynorphin A recognition by the κ-opioid receptor. A similar observation has been reported for the MOR–KOR chimeric receptor equivalent of O-K [17]. Conversely, the acquired ability of the O-K hybrid receptor to bind nociceptin with very high affinity can be accounted for by the introduction of the ORL1-specific amino acid highlighted in black in Fig. 1. These are predominantly located in the putative N-terminal, first and second

Table 3
Potency (ED_{50}, nM) of etorphine, nociceptin, and dynorphin A to inhibit forskolin-induced accumulation of cAMP via the O-M, O-D and O-K hybrid receptors expressed in recombinant CHO cells

	ED_{50}, nM		
	O-M	O-D	O-K
			
Etorphine	1.6 ± 0.1	0.9 ± 0.1	0.1 ± 0.01
Nociceptin	>10,000	>10,000	115 ± 6
Dynorphin A	501 ± 36	>1,000	0.13 ± 0.02

Each value is the mean ± S.E.M. of values obtained from at least three separate experiments.

transmembrane domains, and first extracellular loop. However, high affinity binding of nociceptin by the O-K chimera is also expected to be contributed by residues that are common to ORL1 and KOR1, and different in MOR1 and DOR1, since the M-O and D-O hybrid receptors neither bind nor respond to nociceptin. Since the three opioid receptor sequences diverge most in the exofacial loops, it is likely that these, and in particular the second (see Section 1) provide the O-K chimera with important determinants to nociceptin recognition.

Finally, the high affinity of nociceptin for the O-K hybrid receptor is not complemented by a high biological potency. This is difficult to explain without assuming that there are (at least) two distinct nociceptin binding subsites on the ORL1 receptor, one (the 'address' site) responsible for affinity and the other (the 'message' site), for biological activity, as has been shown for C5a [18] and calcitonin [19] for their receptors. Accordingly, the O-K hybrid receptor lacks the ORL1 receptor 'message' site, but contains the opioid receptor 'message' site. This indicates that these sites are not the same in the parent receptors, and that nociceptin and opioids use different receptor pathways for signal transduction.

In summary, the present study has identified a non-opioid–opioid hybrid receptor (O-K) whose properties replicate, to a large extent, and even enhance those of the parent non-opioid (ORL1) and opioid (KOR1) receptors. The binding and activity profiles of the O-K receptor (i) lend considerable support to the hypothesis of a ligand gating function for opioid receptor extracellular domains [16] and, most importantly, (ii) demonstrate that nociceptin and dynorphin A bind and activate their cognate receptors in different ways, in keeping with growing evidence that nociceptin and dynorphin A have a different functional architecture [20–26]. The O-K chimera is, however, not a perfect bifunctional receptor since it lacks elements supporting full responsiveness to nociceptin. A genuinely 'universal' receptor chimera will afford invaluable assistance in the identification and differentiation, by site-directed mutagenesis, of residues that are important for the recognition and activation of the ORL1 and κ-opioid receptors by their endogenous peptide ligands. Obviously, the O-K hybrid receptor is a significant step toward this goal.

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