OPIOID RECEPTORS

Alistair Corbett, Sandy McKnight and Graeme Henderson
Dr Alistair Corbett is Lecturer in the School of Biological and Biomedical Sciences, Glasgow Caledonian University, Cowcaddens Road, Glasgow G4 0BA, UK.
Dr Sandy McKnight is Associate Director, Parke-Davis Neuroscience Research Centre, Cambridge University Forvie Site, Robinson Way, Cambridge CB2 2QB, UK.
Professor Graeme Henderson is Professor of Pharmacology and Head of Department, Department of Pharmacology, School of Medical Sciences, University of Bristol, University Walk, Bristol BS8 1TD, UK.

Introduction

Preparations of the opium poppy *papaver somniferum* have been used for many hundreds of years to relieve pain. In 1803, Sertürner isolated a crystalline sample of the main constituent alkaloid, morphine, which was later shown to be almost entirely responsible for the analgesic activity of crude opium. The rigid structural and stereochemical requirements essential for the analgesic actions of morphine and related opioids led to the theory that they produce their effects by interacting with a specific receptor.[1] The concept that there is more than one type of opioid receptor arose to explain the dual actions of the synthetic opioid nalorphine, which antagonises the analgesic effect of morphine in man but also acts as an analgesic in its own right. Martin (1967) concluded that the analgesic action of nalorphine is mediated by a receptor, later called the κ-opioid receptor, that is different from the morphine receptor.[2] Evidence for multiple receptors, μ, κ and σ, came from the demonstration of different profiles of pharmacological activity in the chronic spinal dog with the prototype agonists morphine, ketazocine and N-allylnormetazocine (SKF 10047).[3] The existence of the δ-receptor was subsequently proposed to explain the profile of activity *in vitro* of the enkephalins (the first endogenous opioid peptides), and on the basis of the relative potency of the non-selective opioid antagonist naloxone to reverse endogenous opioid peptide inhibition of the nerve-evoked contractions of the mouse vas deferens.[4] Its existence was further confirmed by radioligand binding studies using rat brain homogenates.

It is now clear from work carried out in many laboratories over the last 20 years that there are 3 well-defined or "classical" types of opioid receptor μ, δ and κ. Genes encoding for these receptors have been cloned.[5,6,7,8] More recently, cDNA encoding an "orphan" receptor was identified which has a high degree of homology to the "classical" opioid receptors; on structural grounds this receptor is an opioid receptor and has been named ORL_1 (opioid receptor-like).[9] As would be predicted from their known abilities to couple through pertussis toxin-sensitive G-proteins, all of the cloned opioid receptors possess the same general structure of an extracellular N-terminal region, seven transmembrane domains and intracellular C-terminal tail structure. There is pharmacological evidence for subtypes of each receptor and other types of novel, less well-characterised opioid receptors, ε, λ, ι, ζ, have also been postulated. The σ-receptor, however, is no longer regarded as an opioid receptor.

Receptor Subtypes

μ-Receptor subtypes

The MOR-1 gene, encoding for one form of the μ-receptor, shows approximately 50-70% homology to the genes encoding for the δ-(DOR-1), κ-(KOR-1) and orphan (ORL_1) receptors. Two splice variants of the MOR-1 gene have been cloned, differing only in the presence or absence of 8 amino acids in the C-terminal tail. The splice variants exhibit differences in their rate of onset and recovery from agonist-induced internalization but their pharmacology does not appear to differ in ligand binding assays.[10] Furthermore, in the MOR-1 knockout mouse, morphine does not induce antinociception demonstrating that at least in this species morphine's analgesia is not mediated through δ- or κ-receptors.[11] Similarly morphine did not exhibit positive reinforcing properties or an ability to induce physical dependence in the absence of the MOR-1 gene.

μ_1 and μ_2: The μ_1/μ_2 subdivision was proposed by Pasternak and colleagues to explain their observations, made in radioligand binding studies, that [^{3}H]-labelled-μ, -δ and -κ ligands displayed biphasic binding characteristics.[12] Each radioligand appeared to bind to the same very high affinity site (μ_1) as well as to the appropriate high affinity site (μ, δ or κ) depending on the radioligand used. Naloxazone and naloxonazine were reported to abolish the binding of each radioligand to the μ_1-site. Furthermore, in *in vivo* studies it was observed that naloxazone selectively blocked morphine-induced antinociception but did not block morphine-induced respiratory depression or the induction of morphine dependence.[13,14] Subsequent work

Is there another, novel form of the μ-opioid receptor?

Several related observations suggest the existence of a novel form of μ-receptor at which analogues of morphine with substitutions at the 6 position (e.g. morphine-6β-glucuronide, heroin and 6-acetyl morphine) are agonists, but with which morphine itself does not interact.[14] In antinociception tests on mice it has been reported that morphine does not exhibit cross tolerance with morphine-6β-glucuronide, heroin or 6-acetyl morphine. Furthermore, in mice of the CXBX strain morphine is a poor antinociceptive agent whereas morphine-6β-glucuronide, heroin and 6-acetyl morphine are all potently antinociceptive. The 6-substituted morphine analogues do not appear to be acting through δ- or κ-receptors because the antinociception they induce is not blocked by selective δ- or κ–receptor antagonists, whereas 3-methoxynaltrexone has been reported to antagonise morphine-6β-glucuronide- and heroin-induced antinociception without affecting that induced by morphine, [D-Pen^2, D-Pen^5]enkephalin (DPDPE , δ-selective) or U50488 (κ-selective).[16]

Recently it has been reported that heroin and morphine-6-glucuronide, but not morphine, still produce antinociception in MOR-1 knockout mice in which the disruption in the MOR-1 gene was engineered in exon-1.[17] The same authors observed that in other MOR-1 knockout mice in which exon-2, not exon-1, had been disrupted, all three agonists were ineffective as antinociceptive agents. They conclude that the antinociceptive actions of heroin and morphine-6-glucuronide in the exon-1 MOR-1 mutant mice are mediated through a receptor produced from an alternative transcript of the MOR-1 gene differing from the MOR-1 gene product, the μ-opioid receptor, in the exon-1 region. To substantiate this conclusion they report that in RT-PCR experiments using primers spanning exons 2 and 3, a MOR-1 gene product was still detected in MOR-1 knockout mice.

δ-Receptor subtypes

The DOR-1 gene is the only δ-receptor gene cloned to date. However, two, overlapping subdivisions of δ-receptor have been proposed (δ_1/δ_2 and δ_{ox}/δ_{non}) on the basis of *in vivo* and *in vitro* pharmacological experiments.

δ_1 and δ_2: The subdivision of the δ-receptor into δ_1 and δ_2 subtypes was proposed primarily on the basis of *in vivo* pharmacological studies (Table 1). In rodents *in vivo,* the supraspinal antinociceptive activity of DPDPE can be selectively antagonised by 7-benzylidenenaltrexone (BNTX) or [D-Ala^2, D-Leu^5]enkephalyl-Cys (DALCE)[18,19] whereas the antinociceptive activity of [D-Ala^2]-deltorphin II (deltorphin II) and [D-Ser^2, Leu^5]enkephalyl–Thr (DSLET) can be reversed by naltriben or naltrindole 5′-isothiocyanate (5′-NTII).[18,19,20] Furthermore, while mice develop tolerance to the antinociceptive effects of repeated injections of either DPDPE or deltorphin II, this tolerance appears to be homologous in that there is no cross tolerance between these ligands.[21] *In vivo,* δ_1- and δ_2-receptor-induced antinociception can be differentially antagonised by blockers of different types of potassium channels.[22]

The best evidence from *in vitro* experiments to support the δ_1 and δ_2 subdivision of δ-receptors comes from inhibition of adenylyl cyclase activity in membranes from rat brain[24,25] and from the δ-receptor-mediated elevations of intracellular Ca^{2+} in the ND8-47 cell line[26] where BNTX selectively antagonised DPDPE, and naltriben selectively antagonised deltorphin II. Surprisingly, little selectivity was seen in radioligand displacement studies.[24] The converse has been observed in studies on neuronal cell lines. Two distinct δ-receptor binding sites were observed in radioligand binding experiments on SK-N-BE cells.[27] Studies on NG108-15 cells[28] or the human neuroblastoma cell line, SH-SY5Y,[29,30] have failed to find any functional evidence for δ-receptor subtypes.

The pharmacological properties of the cloned DOR-1 receptor are somewhere between those predicted for either the δ_1 or δ_2 subtypes. DPDPE and deltorphin II are both potent displacers of [^{3}H]-diprenorphine binding to mouse and human recombinant receptors, which is not consistent with either the δ_1 or δ_2 classifications.[31] In contrast, [^{3}H]-diprenorphine binding to the mouse recombinant receptor is more potently displaced by naltriben than BNTX, suggesting that the cloned receptor is of the δ_2 subtype. It will be of importance to determine in the DOR-1 knockout mouse if analgesia can still be induced by either δ_1- or δ_2-receptor selective agonists.

δ_{ox} and δ_{non}: The δ_{ox} and δ_{non} subdivision of δ-receptors was based on the hypothesis that one type of δ-receptor (δ_{ox}) was complexed with μ-receptors (and perhaps also κ-receptors) whereas the other type of δ-receptor (δ_{non}) was not associated with an opioid receptor complex.[32] It was originally observed that sub-antinociceptive doses of agonists at the δ_{ox} receptor (e.g. low doses of DPDPE), potentiated μ-receptor-mediated analgesia, an effect which could be antagonised by 5′-NTII. On the other hand, at higher doses, DPDPE then acted as an agonist at the δ_{non}-receptor and itself induced analgesia which was reversed by DALCE. Data obtained from subsequent radioligand binding studies have been interpreted as demonstrating the existence of further subtypes of the δ_{non} receptor i.e. $\delta_{(non\,1)}$ and $\delta_{(non\,2)}$. More recently it has been suggested that the $\delta_{(non\,1)}$ receptor is in fact synonymous with the δ_1-receptor and the δ_{ox}-receptor synonymous with the δ_2-receptor of the previous classification.[33]

Table 1. Putative ligands for δ-receptor subtypes

Receptor subtype	Agonists	Antagonists	
		Competitive	Nonequilibrium
δ_1	DPDPE / DADLE	**BNTX**	DALCE
δ_2	**Deltorphin II / DSLET**	**Naltriben**	5′-NTII

(bold text denotes compounds available from Tocris)

N.B. DPDPE may not in fact be a selective δ_1 agonist but may also be a partial agonist at δ_2-sites.[23]

κ-Receptor subtypes

The situation regarding the proposals for subtypes of the κ-receptor is rather more complex than for the μ- and δ-receptors, perhaps because of the continuing use of non-selective ligands to define the putative sites. The evidence for the need for sub-division of the κ-receptor comes almost entirely from radioligand binding assays.

The first characterisation of a κ-receptor binding site in brain came from work using [^{3}H]-ethylketocyclazocine (EKC).[3,4] Crucial to this success was the use of the guinea-pig brain where κ-sites are present in relative abundance, and of "suppression", or quenching of the binding of this non-selective ligand to μ- and δ-sites, by incubation with non-radioactive ligands that bound selectively at these other sites.

Studies of [^{3}H]-EKC binding in guinea-pig spinal cord pointed to the existence of a non-homogeneous population of high-affinity binding sites, and led to the first proposal for κ_1- and κ_2-sites distinguished by their sensitivity to DADLE.[3,5] The DADLE-sensitive κ_2 site bound β-endorphin with high affinity, and was later identified with the recognition site of the ε-receptor in brain.[3,6] Another study using [^{3}H]-EKC identified a κ-site in bovine adrenal medulla, with a pharmacology similar to that of the κ_1-site in guinea-pig cord[3,7] but labelling with [^{3}H]-etorphine revealed two additional sites, one resembling κ_2 that bound [Met]enkephalyl-Arg-Gly-Leu with high affinity and another termed "κ_3" or "MRF" that bound [Met^5]enkephalyl-Arg-Phe with high affinity.

The κ_1/κ_2 terminology has more recently been applied by other groups to the putative subtypes defined in other tissues in their hands, but it is not always clear how closely the common nomenclature reflects a common pharmacology. The introduction of the first selective κ-agonist U-50,488 and its congeners (U-69,593, PDÊ117302, CI 977, ICI 197067) led to a refinement of the definition of the putative subtypes, but pointed to the need for careful considerations of the effect of technical differences in assays and of species as a possible explanation for discrepancies. Thus a direct comparison of the binding of [^{3}H]-EKC in guinea-pig and rat (with suppression of binding to μ- and δ-sites) pointed to the existence of a high affinity κ_1-site that predominated in guinea-pig brain and was selectively sensitive to U-69,593, and a low affinity, U-69,593-insensitive κ_2-site that predominated in rat brain.[3,8] Others resorted to the binding of [^{3}H]-bremazocine to reveal U-69,593-insensitive κ_3-binding sites; in contrast to the κ_2-site originally defined in guinea-pig spinal cord, the κ_2-site in brain after suppression of κ_1 was insensitive to DADLE.[3,9]

Subdivision of the κ_1-site in guinea-pig brain into κ_{1a} and κ_{1b} was proposed to resolve the complex displacement of either [^{3}H]-EKC or [^{3}H]-U-69,593 with dynorphin B and α-neo-endorphin which both preferentially bound to the proposed κ_{1b} sub-subtype.[4,0] The same study proposed the existence of a κ_3 subtype, insensitive to U-50,488, that was identified from the binding of [^{3}H]-naloxone benzoylhydrazone. The pharmacology of this later "κ_3-site" is rather different from the κ_3/MRF site of bovine adrenal medulla, and has been proposed to be the receptor mediating the antinociceptive effect of nalorphine, Martin's "N"-receptor.[4,1]

Nomenclature differences appear to have arisen in the context of subtyping of the κ_1-subtype. Using binding surface analyses to allow highly accurate estimation of binding parameters, the binding of [^{3}H]-U-69,593 resolved two binding sites termed κ_{1a} and κ_{1b}. The ligand demonstrating the highest affinity, and around 30-fold preference, for the "κ_{1a} binding site" was α-neo-endorphin.[4,2] More recently putative κ_{1a}- and κ_{1b}-sites in mouse brain were identified from complex displacement curves against the binding of [^{3}H]-U-69,593, in an attempt to compare the pharmacology of the mouse κ_1-sites, with that at the cloned rat KOR stably expressed in a host neuroblastoma cell line.[4,3] Based on the high affinity of bremazocine and α-neo-endorphin, it was deemed "consistent to term the cloned KOR a κ_{1b} subtype".

Rothman (1990) also reported subdivision of the κ_2-binding of [^{3}H]-bremazocine into 2a- and 2b-sub-subtypes.[4,2] The κ_{2b}-site had high affinity for β-endorphin and DADLE, reminiscent of the original κ_2-binding site of guinea-pig spinal cord. The κ_{2a}- and κ_{2b}-sites in guinea-pig brain have undergone a further subdivision (sub-sub-subtypes?) on the basis of investigations using a combination of depletion (of μ- and δ-sites) and suppression, against the binding of 6β-[^{125}I]-3,14-dihydroxy-17-cyclopropylmethyl-4,5α-epoxymorphinan ([^{125}I]OXY).[4,4] So were defined the κ_{2a1}- and κ_{2a2}- sites, having relatively high and low affinities respectively for nor-BNI and enadoline (CI-977), and κ_{2b1}- and κ_{2b2}-sites with high and low affinities for DAMGO and α-neo-endorphin.

Definitive functional pharmacological evidence supporting the existence of this confusing number of putative subtypes of the κ-receptor is lacking, because of the absence of subtype-specific antagonists. It has been reported however, that pretreatment with the isothiocyanate analogue of U-50,488 called (-)-UPHIT, was able to produce a long-lasting block of the antinociceptive effect of U-69,593 in the mouse without affecting the action of bremazocine, while treatment with the non-selective antagonist WIN 44,441 (quadazocine) blocked selectively the antinociception with bremazocine.[4,5,4,6] These findings provide obvious support for the κ_1-κ_2 subdivision; the pharmacological corollary is that (-)-UPHIT and WIN 44,441 are antagonists with selectivity for the κ_1-and κ_2-subtypes respectively, at least in the mouse.

Correlating genes with μ-, δ- and κ-receptor subtypes

Although there is as yet little evidence for different genes encoding the different subtypes of μ-, δ- and κ-receptor these subtypes may result from different post-translational modifications of the gene product (glycosylation, palmytoylation, phosphorylation, etc), from receptor dimerization to form homomeric[4,7] and heteromeric complexes,[4,8,4,9,5,0] or from interaction of the gene product with associated proteins such as RAMPs.[5,1]

The Orphan Receptor

Extending the screening of genomic and cDNA libraries, perhaps in an effort to identify putative subtypes of the classical opioid receptors, resulted in the identification of a novel receptor that bore as high a degree of homology towards the classical opioid receptor types, as they shared among each other. The receptor was identified in three species: rat, mouse and man, with the degree of homology among the species variants more than 90%. Although the putative receptor has had as many names as the number of groups who reported its identification, [5,2] there is some consensus for the use

of the original designation for the human form, "ORL_1". Workers in the field are, however, divided in their preferred terminology for the endogenous peptide agonist for ORL_1 with both "nociceptin"[53] or "orphanin FQ"[54] being used with roughly equal frequency.

Although the ORL_1 receptor was accepted as a member of the "family" of opioid receptors on the basis of its structural homology towards the classical types, there is no corresponding pharmacological homology. Even non-selective ligands that exhibit uniformly high affinity towards μ-, κ- and δ-receptors, have very low affinity for the ORL_1 receptor, and for this reason as much as for the initial absence of an endogenous ligand, the receptor was called an "orphan opioid receptor". Close comparison of the deduced amino-acid sequences of the four receptors highlights structural differences that may explain the pharmacological anomaly. Thus there are sites near the top of each of the trans-membrane regions, that are conserved in the μ-, κ- and δ-receptors, but are altered in ORL_1. Work with site-directed mutants of ORL_1 (rat) has shown that it is possible to confer appreciable affinity on the non-selective benzomorphan bremazocine by changing Ala^{213} in TM5 to the conserved Lys of μ, κ and δ, or by changing the $Val\text{-}Gln\text{-}Val^{277278}$ sequence of TM6 to the conserved Ile-His-Ile motif.[55]

A splice variant of the ORL_1 receptor from rat has been reported ("XOR")[56] with a long form (XOR1L) containing an additional 28 amino acids in the third extracellular loop. In the homologous receptor from mouse (also sometimes referred to as "KOR-3") five splice variants have been reported to date.[57]

ORL_1-Receptor subtypes

Selective high affinity ligands with which to attempt pharmacological definitions of the ORL_1 receptor are few in number (Table 2). Besides the natural heptadecapeptide agonist nociceptin/orphanin FQ and some closely related peptides, the only other ligands offering high affinity and selectivity belong to a class of peptides obtained by a positional scanning approach to combinatorial libraries of hexapeptides.[58] Being basic peptides highly susceptible to degradation, all of those agents are chancy tools in the hands of the unwary. So the paucity of safe and sure pharmacological tools may partly explain some of the confusion in the literature regarding the effect of nociceptin in tests of response latency to noxious stimulation; antinociception, pro-nociception/hyperalgesia, allodynia, or no overt effect, have all been reported.

Although the results of some studies have been interpreted as pointing to the existence of subtypes of ORL_1, this conclusion is so far premature in most cases. The most reliable pharmacological definition of receptors is based on differences in antagonist affinity, and in this context the absence of useful antagonists for ORL_1 is particularly galling to pharmacologists. Although the synthetic analogue of the N-terminal tridecapeptide of nociceptin, $[Phe^1\psi(CH_2\text{-}NH)Gly^2]$nociceptin(1-13)$NH_2$ was first reported to be a selective antagonist,[59] increased use of this peptide points to it having agonist actions. There are no grounds for saying that this peptide is an antagonist at ORL_1 receptors in the periphery, but an agonist in the brain (not least because agonist actions in the periphery, and antagonist actions in the brain have been reported) and that these differences in *efficacy* point to differences in the receptors. Although differences in the *affinity* for $[Phe^1\psi(CH_2\text{-}NH)Gly^2]$nociceptin(1-13)$NH_2$ may be found between central and peripheral sites,[60] and there may indeed be different "subtypes" of ORL_1 in the brain and periphery, the safest conclusion for the moment is just that $[Phe^1\psi(CH_2\text{-}NH)Gly^2]$nociceptin(1-13)$NH_2$ is a partial agonist, and that the observed differences in efficacy are consistent with differences in receptor reserve.

Very recently a peptide related to the combinatorial hexapeptide library hit acetyl-Arg-Tyr-Tyr-Arg-Trp-Lys-NH_2 (Ac-RYYRWK-NH_2; Table 2), but with isoleucine substituting for tryptophan, was reported to block the effects of nociceptin/orphanin FQ in rat cortex (stimulation of $GTP\gamma^{35}S$ binding) or heart (positive chronotropic effect in isolated myocytes). Although this peptide, like all of its structural homologues, was originally reported to be a potent agonist, but with somewhat less than full efficacy,[58] it will be important to see if the antagonist activity of Ac-Arg-Tyr-Tyr-Arg-Ile-Lys-NH_2 (Ac-RYYRIK-NH_2) at the ORL_1 receptor[61] is confirmed.

Table 2. Selective opioid ligands

Receptor type	μ-Receptor	δ-Receptor	κ-Receptor	ORL_1
Selective agonists	**endomorphin-1** **endomorphin-2** **DAMGO**	[D-Ala^2]-deltorphin I **[D-Ala^2]-deltorphin II** DPDPE **SNC 80**	enadoline **U-50488** U-69593	**nociceptin / OFQ** Ac-RYYRWK-NH_2*
Selective antagonists	CTAP	**naltrindole** TIPP-ψ ICI 174864	**nor-binaltorphimine**	None as yet**
Radioligands	[^{3}H]-DAMGO	**[^{3}H]-naltrindole** [^{3}H]-pCl-DPDPE **[^{3}H]-SNC 121**	[^{3}H]-enadoline [^{3}H]-U69593	[^{3}H]-nociceptin

(bold text denotes compounds available from Tocris)

*Related combinatorial library hits are also selective agonists.[58] **Ac-RYYRIK-NH_2 has been proposed to be an ORL_1 antagonist[61] whereas the putative antagonist $[Phe^1\psi(CH_2\text{-}NH)Gly^2]$nociceptin(1-13)$NH_2$[59] appears to be a partial agonist.

Less Well-Characterised Opioid Receptors

In addition to the μ-, δ-, κ- and ORL_1-receptors, several other types of opioid receptor have been postulated. Since the contractions of the isolated vas deferens of the rat are much more sensitive to inhibition by β-endorphin than by other opioid peptides, it was suggested that this tissue contains a novel type of opioid receptor, the ε-receptor, that is specific for β-endorphin.[62] The rabbit ileum has been proposed to possess ι-receptors, for which the enkephalins have high affinity but which are distinct from δ-receptors.[63] A very labile λ-binding site with high affinity for 4,5 epoxymorphinans has been found in freshly-prepared rat membrane fragments[64] and there is evidence that opioids inhibit growth in S20Y murine blastoma cells by an action at yet another receptor type called the ζ-receptor.[65] The ε-, λ-, ι-and ζ-receptors are poorly characterised and wider acceptance of their existence awaits further experimental evidence, in particular isolation of their cDNAs.

Although originally classified as such, the σ-receptor appears not to be an opioid receptor but rather the target for another class of abused drugs, phencyclidine (PCP) and its analogues.[66] Phencyclidine is an effective blocker of the ion channel associated with the N-methyl-D-aspartate (NMDA) receptor where it binds to the same site as MK 801.[67]

Endogenous Ligands

In mammals the endogenous opioid peptides are mainly derived from four precursors: pro-opiomelanocortin, pro-enkephalin, pro-dynorphin and pro-nociceptin/orphanin FQ.[68,69,70,53] Nociceptin/orphanin FQ is processed from pro-nociceptin/orphanin FQ and is the endogenous ligand for the ORL_1-receptor; it has little affinity for the μ-, δ- and κ-receptors.[53,54] The amino acid sequence of nociceptin/orphanin FQ has homology with other opioid peptides especially the prodynorphin fragment dynorphin A (Table 3), suggesting a close evolutionary relationship between the precursors. Nociceptin/orphaninFQ, however, has a C-terminal phenylalanine (F) whereas peptides derived from the other precursors all have the pentapeptide sequence TyrGlyGlyPheMet/Leu (YGGFM/L) at their N-termini. These peptides vary in their affinity for μ, δ- and κ-receptors, and have negligible affinity for ORL_1-receptors, but none binds exclusively to one opioid receptor type.[71,53] β-endorphin is equiactive at μ-and δ-receptors with much lower affinity for κ-receptors; the post-translational product, N-acetyl-β-endorphin, has very low affinity for any of the opioid receptors.[72,27,5] [Met]- and [Leu]enkephalin have high affinities for δ-receptors, ten-fold lower affinities for μ-receptors and negligible affinity for κ-receptors.[71] Other products of processing of pro-enkephalin, which are N-terminal extensions of [Met]enkephalin, have a decreased preference for the δ-receptor with some products, e.g. metorphamide displaying highest affinity for the μ-receptor.[71] The opioid fragments of pro-dynorphin, particularly dynorphin A and dynorphin B, have high affinity for κ-receptors but also have significant affinity for μ- and δ-receptors.[71]

Endomorphin-1 and endomorphin-2 are putative products of an as yet unidentified precursor, that have been proposed to be the endogenous ligands for the μ-receptor where they are highly selective.[74] The endomorphins are amidated tetrapeptides and are structurally unrelated to the other endogenous opioid peptides (Table 3) . Although the study of the cellular localisation of these peptides is at an early stage, endomorphin-2 is found in discrete regions of rat brain, some of which are known to contain high concentrations of μ-receptors.[75] Endomorphin–2 is also present in primary sensory neurones and the dorsal horn of the spinal cord where it could function to modulate nociceptive input.[76]

In comparison to the mainly non-selective mammalian opioid peptides (the exceptions being the endomorphins), amphibian skin contains two families of D-amino acid-containing peptides that are selective for μ- or δ-receptors. Dermorphin is a μ-selective heptapeptide Tyr-D-Ala-Phe-Gly-Tyr-Pro-Ser-NH_2

Table 3. Mammalian endogenous opioid ligands

Precursor	Endogenous peptide	Amino acid sequence
Pro-opiomelanocortin	β-Endorphin	YGGFMTSEKSQTPLVTL-FKNAIIKNAYKKGE
Pro-enkephalin	[Met]enkephalin	YGGFM
	[Leu]enkephalin	YGGFL
		YGGFMRF
		YGGFMRGL
	Metorphamide	YGGFMRRV-NH_2
Pro-dynorphin	Dynorphin A	YGGFLRRIRPKLKWDNQ
	Dynorphin A(1-8)	YGGFLRRI
	Dynorphin B	YGGFLRRQFKVVT
	α-neoendorphin	YGGFLRKYPK
	β-neoendorphin	YGGFLRKYP
Pro-nociceptin / OFQ	**Nociceptin**	FGGFTGARKSARKLANQ
Pro-endomorphin[1]	**Endomorphin-1**	YPWF-NH_2
	Endomorphin-2	YPFF-NH_2

(bold text denotes compounds available from Tocris)

[1]Presumed to exist, awaiting discovery

without significant affinity at δ- and κ-receptors.[77] In contrast, the deltorphins - deltorphin (dermenkephalin; Tyr-D-Met-Phe-His-Leu-Met-Asp-NH_2), [D-Ala^2]-deltorphin I and [D-Ala^2]-deltorphin II (Tyr-D-Ala-Phe-Xaa-Val-Val-Gly-NH_2, where Xaa is Asp or Glu respectively) - are highly selective for δ-opioid receptors.[78]

Effector Mechanisms

The opioid receptor family, in common with the somatostatin receptor family, is somewhat unusual in that all of the cloned opioid receptor types belong to the G/G_o-coupled superfamily of receptors. Opioid receptors do not couple directly with G_s or G_q and none of the cloned receptors forms a ligand-gated ion channel. It was originally thought that μ- and δ-receptors coupled through G/G_o proteins to activate an inwardly rectifying potassium conductance and to inhibit voltage-operated calcium conductances whereas κ-receptors only inhibit voltage-operated calcium conductances. However it is now known that the κ-receptor is, in some cell types, also coupled to activation of an inwardly rectifying potassium conductance.[79] It seems highly likely, therefore, that all of the opioid receptors will share common effector mechanisms. Indeed, many papers have recently appeared demonstrating that the ORL_1-receptor couples to the same effector systems as the other more extensively studied opioid receptors. It should be borne in mind that, given the heterogeneity of α_i, α_o, β and γ subunits which may combine to form a trimeric G protein, there may well be some subtle differences in the downstream effector mechanisms to which opioid receptors are coupled if one type of opioid receptor is unable to interact with a certain form of G/G_o heterotrimer. However, different responses evoked in different cell types in response to activation of different opioid receptors or even in response to activation of the same receptor are likely to reflect changes in the expression of G proteins and effector systems between cell types rather than any inherent differences in the properties of the receptors themselves.

Opioid receptor activation produces a wide array of cellular responses (Table 4). Although the pertussis toxin sensitivity has not been assessed in all instances it is highly likely that in each the first step is activation of G_i or G_o . The functional significance of many of these opioid receptor-mediated effects is still unclear, but two recent observations on changes in neurotransmitter release following acute and chronic exposure to opioids are worthy of special mention because they provide potential solutions to long-asked questions.

The periaqueductal grey region (PAG) is a major anatomical locus for opioid activation of descending inhibitory pathways to the spinal cord and is thus an important site for μ-receptor-induced analgesia. Opioids do not excite descending fibres directly but disinhibit them by inhibiting spontaneous GABA release from local GABAergic interneurones.[80] This inhibition of transmitter release results from activation of a dendrotoxin-sensitive, voltage-sensitive potassium conductance. The mechanism by which the voltage-sensitive potassium conductance is activated appears to be through activation of phospholipase A_2 (PLA_2) with subsequent metabolism of arachidonic acid along the 12′-lipoxygenase pathway because the inhibition of GABA release can be inhibited by quinacrine and 4-bromo-phenacylbromide, inhibitors of PLA_2, and by baicalein, an inhibitor of 12′-lipoxygenase. This proposed mechanism of opioid action also explains the synergy between opioids and non-steroidal analgesic drugs (NSAIDs) in producing analgesia because in the presence of a NSAID, with the cyclo-oxygenase enzymes inhibited, more of the arachidonic acid produced by opioid activation of PLA_2 can be diverted down the 12′-lipoxygenase pathway.

Table 4. Opioid receptor-evoked cellular responses

Direct G-protein βγ or α subunit-mediated effects
- activation of an inwardly rectifying potassium channel
- inhibition of voltage operated calcium channels (N, P, Q and R type)
- inhibition of adenylyl cyclase

Responses of unknown intermediate mechanism
- activation of PLA_2
- activation of PLCβ (possibly direct G protein βγ subunit activation)
- activation of MAPKinase
- activation of large conductance calcium-activated potassium channels
- activation of L type voltage operated calcium channels
- inhibition of T type voltage operated calcium channels
- direct inhibition of transmitter exocytosis

Responses which are a consequence of opioid-evoked changes in other effector pathways
- activation of voltage-sensitive potassium channels (activation of PLA_2)
- inhibition of M channels (activation of PLA_2)
- inhibition of the hyperpolarisation-activated cation channel (Ih) (reduction in cAMP levels following inhibition of adenylyl cyclase)
- elevation of intracellular free calcium levels (activation of PLCβ, activation of L type voltage operated calcium conductance)
- potentiation of NMDA currents (activation of protein kinase C)
- inhibition of transmitter release (inhibition of adenylyl cyclase, activation of potassium channels and inhibition of voltage operated calcium channels)
- decreases in neuronal excitability (activation of potassium channels)
- increases in neuronal firing rate (inhibition of inhibitory transmitter release - disinhibition)
- changes in gene expression (long-term changes in adenylyl cyclase activity, elevation of intracellular calcium levels, activation of cAMP response element binding protein (CREB))

The cellular locus of opiate withdrawal has long been the Holy Grail of opioid biologists. Over 20 years ago, it was shown that following chronic exposure of NG108-15 neuroblastoma x glioma hybrid cells to opiates, withdrawal resulted in a rebound increase in adenylyl cyclase;[81] the functional significance of this observation for opiate withdrawal in brain neurones has remained obscure. Recently, Williams and colleagues[82] have observed an increase in the release of the inhibitory neurotransmitter GABA, in the nucleus accumbens during opiate withdrawal. This effect could be mimicked by the adenylyl cyclase activator, forskolin, and inhibited by protein kinase A inhibitors. Therefore, as proposed over 25 years ago by the late Harry Collier, rebound adenylyl cyclase activity in withdrawal may be the fundamental step in eliciting the withdrawal behaviour.[83]

Development and Clinical Applications of Opioid Ligands

Among the receptors for the many neuropeptides that exist in the nervous system, the opioid receptors are unique in that there existed before the discovery of the natural agonists, an abundance of non-peptide ligands with which the pharmacology of the receptors was already defined. In current terms relating to the drug-discovery process, we would consider the 4,5-epoxy-methylmorphinan opioid alkaloids morphine, codeine and thebaine as "natural-product hits" on which were based chemical programmes to design analogues with improved pharmacology (Figure 1). The effects of morphine to reduce sensitivity to pain or to inhibit intestinal motility and secretion, have continued to be exploited clinically, however the presence of other undesirable effects (e.g. depression of respiration, tolerance/dependence, effects on mood) provided the stimulus to seek analogues that were selective in producing analgesia. Thus a semi-synthetic di-acetylated analogue of morphine was introduced in the 19th century in the mistaken belief that this compound (heroin) had those desired properties. More radical changes to the morphinan nucleus were subsequently explored in various synthetic programmes, in many early cases resulting in the development of low efficacy partial agonists.

With the benefit of hindsight, it is possible to conceive an evolution of those opioid analogues, with a progressive simplification of chemical structure from the epoxymorphinans (nalorphine, nalbuphine) through the morphinans such as levorphanol, and the benzomorphans such as pentazocine, to the phenyl-piperidines including pethidine and the 4-anilino-piperidines as exemplified by fentanyl (Figure 1). The ultimate simplification of the morphine structure was in the methadone class, with methadone itself and d-propoxyphene (Darvon). Although thebaine is virtually inactive, the compound itself was an important chemical precursor in the synthesis of 14-hydroxy derivatives of morphine, most particularly the antagonists naloxone and naltrexone. Also derived from thebaine were the oripavine derivatives, and here the trend of chemical "simplification" was reversed with the introduction of an additional six-membered ring that appeared to enhance biological potency. For example, etorphine is about one thousand times more potent than morphine as an analgesic, but its use is limited to veterinary medicine as a sedative for large animals.

For the most part, such compounds have highest affinity for the μ-receptor, and to a greater or lesser extent produce the full panoply of effects, good and bad, obtained with morphine. Depending on the level of affinity and efficacy, such compounds have been used acutely or chronically, to provide analgesia in cases of mild, through moderate to severe pain, alone or with adjuncts. The piperidines related to fentanyl include the most potent non-peptide μ-agonists known, and are generally used peri-operatively, often for the induction and maintenance of anaesthesia. The use of many of the benzomorphans (as had been found with the first of the "duallists" nalorphine) has been associated with dysphoric and psychotomimetic effects in man, a property originally thought to be attributable to affinity at the non-opioid σ-site.

The attractiveness of the prospect for development of selective κ-agonists as analgesics was based on the preclinical pharmacology in animals of the 6,7-benzomorphans such as ketazocine and its derivatives (Figure 1). Although those agents are not selective in terms of *affinity*, their utility as pharmacological tools is based on their functional selectivity for the κ-receptor, where their *efficacy* is high. Such agents produced a powerful antinociceptive effect, but did not substitute for morphine in dependent animals. A full biochemical and pharmacological characterisation of the κ-receptor was not possible until the discovery of highly selective agonists in the aryl-acetamides that appear unrelated structurally to any of the morphine derivatives. The first compound of this class was U-50,488, but its importance was also as a chemical lead for the attempted design of related compounds of greater selectivity and potency. At least two such compounds have entered clinical trials as centrally acting analgesics, Spiradoline (U-62,066) and enadoline (CI-977). Although CNS-mediated, mechanism-related side effects of sedation and dysphoria may limit the potential for development of such compounds, the prospects for analogues with limited brain penetration to produce a peripherally mediated analgesic effect in inflammatory conditions is under exploration, with at least one compound (asimadoline, EMD-61753) in clinical trials for osteoarthritis. The observation of neuroprotective properties of κ-agonists in pre-clinical models of cerebral ischaemia has lead to consideration of the possible clinical development of selective κ-agonists for stroke or traumatic head injury. In this context the sedative properties of κ-agonists, and even perhaps their characteristic diuretic action, may be advantageous.

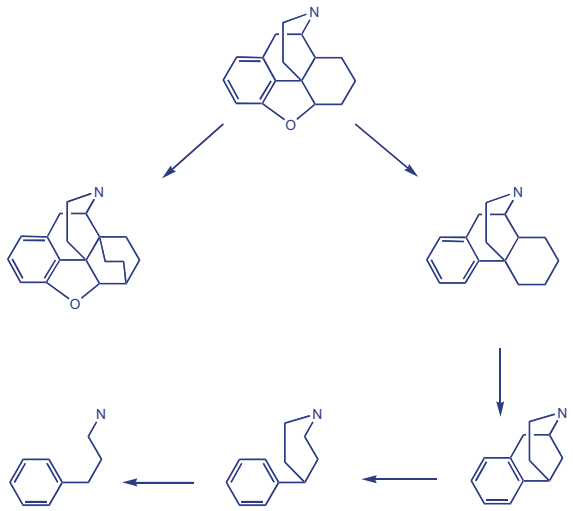
The discovery of the enkephalins and of the δ-receptor, led to the idea that the peptides themselves might be taken as "leads" for the synthesis of a new class of opioid agonist that lacked the addictive properties of morphine. Although such synthetic activities produced many useful experimental tools, no direct benefit in the form of a drug appeared, in spite of the attempted development of several enkephalin analogues. It did become clear from the work of a number of laboratories that activation of the δ-receptor is associated with antinociception in animals, and the development of a selective non-peptide agonist is under consideration by a number of commercial drug houses. In some cases the synthetic strategy is based directly on structural considerations of the first non-peptide with significant selectivity, the 6,7-indole analogue of naltrexone, naltrindole (Figure 1).[84] Applying the "message-address" concept that produced the antagonist naltrindole to a novel series of octahydroisoquinoline derivatives has been successful in producing non-peptide δ-selective agonists TAN-67[85] or SB 213698.[86] Similar considerations do not serve to explain the existence of another series of novel piperazine derivatives δ-agonists, BW 373U86[87] or SNC 80.[88] Preclinical studies suggest that δ-agonists may have a superior profile as analgesics, but this will only

be established when such an agent is successfully introduced into clinical investigation; other possible applications of selective ligands for this receptor may emerge from clinical experience.

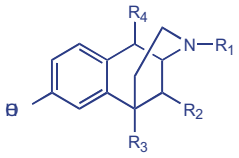
The prospects for clinical utilities of agonists or antagonists for the ORL_1 receptor can only be the subject of speculation. Elucidation of the role of the nociceptin/ORL-receptor system in pain control (and in other areas, for the peptide and its receptor have a dense and wide investment in the nervous system) must await the initial results of the drug-discovery process. Only with the availability of non-peptide selective agonists, and perhaps more particularly antagonists, will it be possible to undertake the definitive pre-clinical studies that will serve for the identification of possible clinical targets. There is some agreement that activation of the ORL_1 receptor in the brain leads to a motor impairment, so it may be that the development of ORL_1 agonists would be difficult.

Figure 1. Structures of non-peptide agonists and antagonists

a) Analgesic morphine derivatives

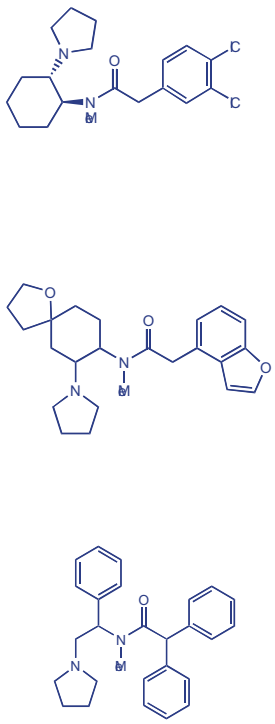


b) Benzomorphans

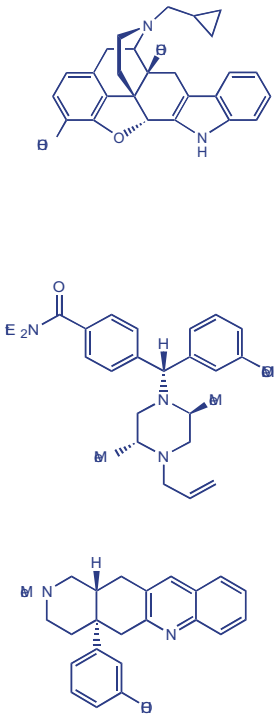


	R_1	R_2	R_3	R_4
PENTAZOCINE	$H_2CHC=C(Me)_2$	Me	Me	H_2
SKF 10,047	$H_2CHC=CH$	Me	Me	H_2
KETAZOCINE	H_2C	Me	Me	O
BREMAZOCINE	H_2C	$(Me)_2$	Me	H_2

c) Selective κ-opioid agonists



d) Selective δ-receptor non-peptide ligands



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Opioid Receptor Ligands Available from Tocris

μ Receptor

agonists

1171	DAMGO	Selective μ agonist
1055	Endomorphin-1	Potent and selective μ agonist
1056	Endomorphin-2	Potent and selective μ agonist

antagonists

0898	Clocinnamox	Irreversible μ antagonist
0516	Etonitazenyl isothiocyanate	Irreversible affinity label (μ selective)
0926	β-Funaltrexamine	Irreversible μ-selective antagonist
0591	Naloxonazine	Selective μ_1 antagonist

δ Receptor

agonists

1180	[D-Ala^2]-Deltorphin II	Selective δ agonist peptide
1170	DSLET	Selective δ agonist peptide
0764	SNC 80	Highly selective non-peptide δ agonist
1008	SNC 121	Potent analogue of (0764)
R1008	[^{3}H]-SNC 121ÊÊ☢	Radiolabeled form of (1008)

antagonists

0827	ICI-154,129	δ selective peptide antagonist
0820	ICI-174,864	δ selective peptide antagonist
0740	Naltrindole	δ selective non-peptide antagonist
R740	[^{3}H]-NaltrindoleÊÊ☢	Radiolabeled form of (0740)
0899	BNTX	Standard δ_1 selective antagonist
0754	N-Benzylnaltrindole	δ_1 selective non-peptide antagonist
0892	Naltriben	Standard δ_2 selective antagonist

κ Receptor

agonists

0699	BRL-52537	Potent and selective κ agonist
0778	ICI-199,441	Potent κ agonist
0822	ICI-204,448	κ agonist, acts peripherally
0783	N-Methyl-N-[(1S)-1-phenyl-2-(1-pyrrolidinyl)-ethyl]phenylacetamide	Selective κ agonist
0700	(±)-1-(4-Trifluoromethylphenyl)acetyl-2-(1-pyrrolidinyl)methylpiperidine	Very potent and selective κ agonist
0495	(±)-U-50488	Standard selective κ agonist
0471	(+)-U-50488	Less active enantiomer of (0495)
0496	(-)-U-50488	More active enantiomer of (0495)
0498	U-54494A	κ agonist

antagonists

0347	nor-Binaltorphimine	Standard κ selective antagonist
0794	DIPPA	Selective irreversible κ antagonist

Orphan Opioid Receptor Ligands

0910	Nociceptin	Endogenous ORL_1 agonist
1092	[Phe^{1}ψ(CH_2-NH)Gly^2]Nociceptin(1-13)NH_2	Selective nociceptin partial agonist
1118	Nocistatin (bovine)	Opposes action of nociceptin
1198	Nocistatin (human)	Human putative counterpart of nocistatin
1119	NocII	Orphan neuropeptide

Other / Miscellaneous Opioid Compounds

0840	Loperamide	Opioid ligand, Ca^{2+} channel blocker
0599	Naloxone	Broad spectrum opioid antagonist
0677	Naltrexone	Broad spectrum opioid antagonist

Tocris Cookson Ltd.
Northpoint Fourth Way
Avonmouth BS11 8TA UK
Tel: + 44 (0)117 982 6551
Fax: + 44 (0)117 982 6552
customerservice@tocris.co.uk

www.tocris.com
technicalsupport@tocris.co.uk

Tocris Cookson Inc.
16144 Westwoods Business Park
Ellisville MO 63021 USA
Tel: (800) 421-3701
Fax: (800) 483-1993
customerservice@tocrisusa.com