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. 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 \( \kappa \)-opioid receptor, that is different from the morphine receptor. Evidence for multiple receptors, \( \mu \), \( \kappa \) and \( \sigma \), 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). The existence of the \( \delta \)-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. 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 \( \mu \), \( \delta \) and \( \kappa \). Genes encoding for these receptors have been cloned. 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 ORL1 (opioid receptor-like). 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, \( \varepsilon \), \( \lambda \), \( \iota \), \( \varsigma \), have also been postulated. The \( \sigma \)-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 (ORL1) 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. 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. Similarly morphine did not exhibit positive reinforcing properties or an ability to induce physical dependence in the absence of the MOR-1 gene.

**μ₁ and μ₂:** The μ₁/μ₂ subdivision was proposed by Pasternak and colleagues to explain their observations, made in radioligand binding studies, that [³⁵S]-labelled-μ₁, -δ and -κ ligands displayed biphasic binding characteristics. Each radioligand appeared to bind to the same very high affinity site (μ₁) 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 μ₁-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. Subsequent work in other laboratories has failed to confirm this classification.

**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. 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-Pen², D-Pen⁵]enkephalin (DPDPE, δ-selective) or U50488 (κ-selective).

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. 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 (δ₁/δ₂ and δ₃/δ₄/δ₅) on the basis of *in vivo* and *in vitro* pharmacological experiments.

**δ₁ and δ₂:** The subdivision of the δ-receptor into δ₁ and δ₂ 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-benzylidene-naltrexone (BNTX) or [D-Ala², D-Leu⁵]enkephalyl-Cys (DALCE)¹⁸,¹⁹ whereas the antinociceptive activity of [D-Ala²]-deltorphin II (deltorphin II) and [D-Ser², Leu⁵]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, δ₁- and δ₂-receptor-induced antinociception can be differentially antagonised by blockers of different types of potassium channels.²²

Table 1. Putative ligands for δ-receptor subtypes

<table>
<thead>
<tr>
<th>Receptor subtype</th>
<th>Antagonists</th>
<th>Competitive</th>
<th>Nonequilibrium</th>
</tr>
</thead>
<tbody>
<tr>
<td>δ₁</td>
<td>DPDPE / DADLE</td>
<td>BNTX</td>
<td>DALCE</td>
</tr>
<tr>
<td>δ₂</td>
<td>Deltorphin II / DSLET</td>
<td>Naltriben</td>
<td>5'-NTII</td>
</tr>
</tbody>
</table>

N.B. DPDPE may not in fact be a selective δ₁ agonist but may also be a partial agonist at δ₂ sites.²³

The best evidence from in vitro experiments to support the δ₁ and δ₂ subdivision of δ-receptors comes from inhibition of adenyl cyclase activity in membranes from rat brain²⁴,²⁵ and from the δ-receptor-mediated elevations of intracellular Ca²⁺ in the ND8-47 cell line²⁶ 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 cells²⁸ 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 δ₁ or δ₂ subtypes. DPDPE and deltorphin II are both potent displacers of [³⁵S]-diprenorphine binding to mouse and human recombinant receptors, which is not consistent with either the δ₁ or δ₂ classifications.³¹ In contrast, [³⁵S]-diprenorphine binding to the mouse recombinant receptor is more potently displaced by naltriben than BNTX, suggesting that the cloned receptor is of the δ₂ subtype. It will be of importance to determine in the DOR-1 knockout mouse if analgesia can still be induced by either δ₁- or δ₂-receptor selective agonists.

δ cx and δ nce: The δ cx and δ nce subdivision of δ-receptors was based on the hypothesis that one type of δ-receptor (δ cx) was complexed with μ-receptors (and perhaps also κ-receptors) whereas the other type of δ-receptor (δ nce) was not associated with an opioid receptor complex.³² It was originally observed that sub-antinociceptive doses of agonists at the δ cx 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 nce-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 nce receptor i.e. δ nce-1 and δ nce-2. More recently it has been suggested that the δ nce-1 receptor is in fact synonymous with the δ₁-receptor and the δ cx-receptor synonymous with the δ₂-receptor of the previous classification.³³

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 \(\kappa\)-receptor comes almost entirely from radioligand binding assays.

The first characterisation of a \(\kappa\)-receptor binding site in brain came from work using \([^3]H\)-ethylketocyclazocine (EKO).\(^{34}\) Crucial to this success was the use of the guinea-pig brain where \(\kappa\)-sites are present in relative abundance, and of "suppression", or quenching of the binding of this non-selective ligand to \(\mu\)- and \(\delta\)-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 \(\kappa_1\)- and \(\kappa_2\)-sites distinguished by their sensitivity to DADLE.\(^{35}\) The DADLE-sensitive \(\kappa_2\) site bound \(\beta\)-endorphin with high affinity, and was later identified with the recognition site of the \(\varepsilon\)-receptor in brain.\(^{36}\) Another study using \([^3]H\)-EKC identified a \(\kappa\)-site in bovine adrenal medulla, with a pharmacology similar to that of the \(\kappa_1\)-site in guinea-pig cord\(^{37}\) but labelling with \([^3]H\)-etorphine revealed two additional sites, one resembling \(\kappa_2\) that bound \([\text{Met}]\)enkephalyl-Arg-Gly-Leu with high affinity and another termed "\(\kappa_3\)" or "MRF" that bound \([\text{Met}^5]\)enkephalyl-Arg-Phe with high affinity.

The \(\kappa_1/\kappa_2\) 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 \(\kappa\)-agonist U-50,488 and its congeners (U-69,593, PD 117302, 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 \(\mu\)- and \(\delta\)-sites) pointed to the existence of a high affinity \(\kappa_1\)-site that predominated in guinea-pig brain and was selectively sensitive to U-69,593, and a low affinity, U-69,593-insensitive \(\kappa_2\)-site that predominated in rat brain.\(^{38}\) Others resorted to the binding of \([^3]H\)-bremazocine to reveal U-69,593-insensitive \(\kappa_2\)-binding sites; in contrast to the \(\kappa_2\)-site originally defined in guinea-pig spinal cord, the \(\kappa_2\)-site in brain after suppression of \(\kappa_1\) was insensitive to DADLE.\(^{39}\)

Subdivision of the \(\kappa_1\)-site in guinea-pig brain into \(\kappa_{1a}\) and \(\kappa_{1b}\), was proposed to resolve the complex displacement of either \([^3]H\)-EKC or \([^3]H\)-U-69,593 with dynorphin B and \(\alpha\)-neo-endorphin which both preferentially bound to the proposed \(\kappa_{1b}\) sub-subtype.\(^{40}\) The same study proposed the existence of a \(\kappa_3\) subtype, insensitive to U-50,488, that was identified from the binding of \([^3]H\)-naloxone benzoylhydrazone. The pharmacology of this later "\(\kappa_3\)-site" is rather different from the \(\kappa_3\)-MRF site of bovine adrenal medulla, and has been proposed to be the receptor mediating the antinociceptive effect of nalorphine, Martin’s "N"-receptor.\(^{41}\)

Nomenclature differences appear to have arisen in the context of subtyping of the \(\kappa_1\)-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 \(\kappa_{1a}\) and \(\kappa_{1b}\). The ligand demonstrating the highest affinity, and around 30-fold preference, for the "\(\kappa_{1a}\) binding site" was \(\alpha\)-neo-endorphin.\(^{42}\) More recently putative \(\kappa_{1a}\)- and \(\kappa_{1b}\)-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 \(\kappa_1\)-sites, with that at the cloned rat KOR stably expressed in a host neuroblastoma cell line.\(^{43}\) Based on the high affinity of bremazocine and \(\alpha\)-neo-endorphin, it was deemed "consistent to term the cloned KOR a \(\kappa_{1b}\) subtype".
Rothman (1990) also reported subdivision of the \( \kappa_2 \)-binding of \([\text{H}] \)-bremazocine into 2a- and 2b-sub-subtypes.\(^{42}\) The \( \kappa_{2b} \)-site had high affinity for \( \beta \)-endorphin and DADLE, reminiscent of the original \( \kappa_2 \)-binding site of guinea-pig spinal cord. The \( \kappa_{2a} \)- and \( \kappa_{2b} \)-sites in guinea-pig brain have undergone a further subdivision (sub-sub-subtypes?) on the basis of investigations using a combination of depletion (of \( \mu \)- and \( \delta \)-sites) and suppression, against the binding of \( 6 \beta-\left[^{125}\text{I}\right]-3,14 \)-dihydroxy-17-cyclopropylmethyl-4,5\( \alpha \)-epoxymorphinan (\( \left[^{125}\text{I}\right] \))\(^{1,44}\) So were defined the \( \kappa_{2a-1} \) and \( \kappa_{2a-2} \) sites, having relatively high and low affinities respectively for nor-BNI and enadoline (CI-977), and \( \kappa_{2b-1} \) and \( \kappa_{2b-2} \) sites with high and low affinities for DAMGO and \( \alpha \)-neo-endorphin.

Definitive functional pharmacological evidence supporting the existence of this confusing number of putative subtypes of the \( \kappa \)-receptor is lacking, because of the absence of subtype-specific antagonists. It has been reported however, that pretreatment with the iso-thiocyanate analogue of U-50,488 called (\( \cdot \))-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.\(^{45,46}\) These findings provide obvious support for the \( \kappa_1 \)-\( \kappa_2 \) subdivision; the pharmacological corollary is that (\( \cdot \))-UPHIT and WIN 44,441 are antagonists with selectivity for the \( \kappa_1 \)- and \( \kappa_2 \)-subtypes respectively, at least in the mouse.

**Correlating genes with \( \mu \)-, \( \delta \)- and \( \kappa \)-receptor subtypes**

Although there is as yet little evidence for different genes encoding the different subtypes of \( \mu \)-, \( \delta \)- and \( \kappa \)-receptor these subtypes may result from different post-translational modifications of the gene product (glycosylation, palmytoylation, phosphorylation, etc), from receptor dimerization to form homomeric\(^{47}\) and heteromeric complexes,\(^{48,49,50}\) or from interaction of the gene product with associated proteins such as RAMPs.\(^{51}\)

**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,\(^{52}\) 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 \( \mu \)-, \( \kappa \)- and \( \delta \)-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 \( \mu \)-, \( \kappa \)- and \( \delta \)-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 \( \mu \), \( \kappa \) and \( \delta \), or by changing the Val-Gln-Val\(^{276-278}\) 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\(_1\))\(^{56}\) 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, pronociception/hyperalgesia, allodynia, or no overt effect, have all been reported.

### Table 2. Selective opioid ligands

<table>
<thead>
<tr>
<th>Receptor type</th>
<th>(\mu)-Receptor</th>
<th>(\delta)-Receptor</th>
<th>(\kappa)-Receptor</th>
<th>ORL(_1)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Selective agonists</td>
<td>endomorphin-1</td>
<td>[D-Ala(^4)] deltorphin I</td>
<td>enadoline</td>
<td>nociceptin / OFQ</td>
</tr>
<tr>
<td></td>
<td>endomorphin-2</td>
<td>[D-Ala(^4)] deltorphin II</td>
<td></td>
<td>Ac-RYYRK-NH(_2)*</td>
</tr>
<tr>
<td></td>
<td>DAMGO</td>
<td>DPDPE SNC 80</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Selective antagonists</td>
<td>CTAP</td>
<td>naltrindole</td>
<td>nor-binaltorphimine</td>
<td>None as yet**</td>
</tr>
<tr>
<td></td>
<td></td>
<td>TIPP-(\psi)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>ICI 174864</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Radioligands</td>
<td>(^3)H-DAMGO</td>
<td>(^3)H-naltrindole</td>
<td>(^3)H-enadoline</td>
<td>(^3)H-nociceptin</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(^3)H-pCI-DPDPE</td>
<td>(^3)H-U69593</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>(^3)H-SNC 121</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Related combinatorial library hits are also selective agonists.\(^58\)** **Ac-RYYRK-NH\(_2\) has been proposed to be an ORL\(_1\) antagonist\(^61\)** whereas the putative antagonist [Phe\(^1\)\(\psi\)(CH\(_2\)-NH)Gly\(^2\)] nociceptin(1-13)NH\(_2\)\(^59\) appears to be a partial agonist.

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\(_2\)-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\(_2\)-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\(_2\)-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-RYYRK-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,

**Less Well-Characterised Opioid Receptors**

In addition to the μ-, δ-, κ- and ORL₁-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. The rabbit ileum has been proposed to possess ε-receptors, for which the enkephalins have high affinity but which are distinct from δ-receptors. A very labile λ-binding site with high affinity for 4,5 epoxymorphinans has been found in freshly-prepared rat membrane fragments 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. The ε-, λ-, ν-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. 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.

**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. Nociceptin/orphanin FQ is processed from pro-nociceptin/orphanin FQ and is the endogenous ligand for the ORL₁-receptor; it has little affinity for the μ-, δ- and κ-receptors. 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/orphanin FQ, 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 ORL₁-receptors, but none binds exclusively to one opioid receptor type. 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. [Met]- and [Leu]enkephalin have high affinities for δ-receptors, ten-fold lower affinities for μ-receptors and negligible affinity for κ-receptors. 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. 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.

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. 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. 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.
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-NH₂ without significant affinity at δ- and κ-receptors. In contrast, the deltorphins - deltorphin (dermenkephalin; Tyr-D-Met-Phe-His-Leu-Met-Asp-NH₂), [D-Ala²]-deltorphin I and [D-Ala³]-deltorphin II (Tyr-D-Ala-Phe-Xaa-Val-Val-Gly-NH₂, where Xaa is Asp or Glu respectively) - are highly selective for δ-opioid receptors.

Table 3. Mammalian endogenous opioid ligands

<table>
<thead>
<tr>
<th>Precursor</th>
<th>Endogenous peptide</th>
<th>Amino acid sequence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pro-opiomelanocortin</td>
<td>β-Endorphin</td>
<td>YGGFMTSEKSQTPLVTL-FKNALKNAYKKGE</td>
</tr>
<tr>
<td>Pro-enkephalin</td>
<td>[Met]enkephalin</td>
<td>YGGFM</td>
</tr>
<tr>
<td></td>
<td>[Leu]enkephalin</td>
<td>YGGFGL</td>
</tr>
<tr>
<td></td>
<td>Metorphamide</td>
<td>YGGFMRF</td>
</tr>
<tr>
<td></td>
<td></td>
<td>YGGFMRRV-NH₂</td>
</tr>
<tr>
<td>Pro-dynorphin</td>
<td>Dynorphin A</td>
<td>YGGFLRRIRPKLKDWNQ</td>
</tr>
<tr>
<td></td>
<td>Dynorphin A(1-8)</td>
<td>YGGFLRRI</td>
</tr>
<tr>
<td></td>
<td>Dynorphin B</td>
<td>YGGFLRRQFKVVT</td>
</tr>
<tr>
<td></td>
<td>α-neoendorphin</td>
<td>YGGFLRKYPK</td>
</tr>
<tr>
<td></td>
<td>β-neoendorphin</td>
<td>YGGFLRYKP</td>
</tr>
<tr>
<td>Pro-nociceptin / OFQ</td>
<td>Nociceptin</td>
<td>FGGFTGARKSARKLANQ</td>
</tr>
<tr>
<td>Pro-endomorphin*</td>
<td>Endomorphin-1</td>
<td>YPW-NH₂</td>
</tr>
<tr>
<td></td>
<td>Endomorphin-2</td>
<td>YPFF-NH₂</td>
</tr>
</tbody>
</table>

*Presumed to exist, awaiting discovery

**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 Gᵢ/Gₒ-coupled superfamily of receptors. Opioid receptors do not couple directly with Gₛ or Gₐ and none of the cloned receptors forms a ligand-gated ion channel. It was originally thought that µ- and δ-receptors coupled through Gᵢ/Gₒ 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. 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 ORL₁-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 αᵢ, αₒ, 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 Gᵢ/Gₒ 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.

Table 4. Opioid receptor-evoked cellular responses

<table>
<thead>
<tr>
<th>Direct G-protein bg or a subunit-mediated effects</th>
</tr>
</thead>
<tbody>
<tr>
<td>• activation of an inwardly rectifying potassium channel</td>
</tr>
<tr>
<td>• inhibition of voltage operated calcium channels (N, P, Q and R type)</td>
</tr>
<tr>
<td>• inhibition of adenylyl cyclase</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Responses of unknown intermediate mechanism</th>
</tr>
</thead>
<tbody>
<tr>
<td>• activation of PLA$_2$</td>
</tr>
<tr>
<td>• activation of PLC b (possibly direct G protein bg subunit activation)</td>
</tr>
<tr>
<td>• activation of MAPKinase</td>
</tr>
<tr>
<td>• activation of large conductance calcium-activated potassium channels</td>
</tr>
<tr>
<td>• activation of L type voltage operated calcium channels</td>
</tr>
<tr>
<td>• inhibition of T type voltage operated calcium channels</td>
</tr>
<tr>
<td>• direct inhibition of transmitter exocytosis</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Responses which are a consequence of opioid-evoked changes in other effector pathways</th>
</tr>
</thead>
<tbody>
<tr>
<td>• activation of voltage-sensitive potassium channels (activation of PLA$_2$)</td>
</tr>
<tr>
<td>• inhibition of M channels (activation of PLA$_2$)</td>
</tr>
<tr>
<td>• inhibition of the hyperpolarisation-activated cation channel (Ih) (reduction in cAMP levels following inhibition of adenylyl cyclase)</td>
</tr>
<tr>
<td>• elevation of intracellular free calcium levels (activation of PLCb, activation of L type voltage operated calcium conductance)</td>
</tr>
<tr>
<td>• potentiation of NMDA currents (activation of protein kinase C)</td>
</tr>
<tr>
<td>• inhibition of transmitter release (inhibition of adenylyl cyclase, activation of potassium channels and inhibition of voltage operated calcium channels)</td>
</tr>
<tr>
<td>• decreases in neuronal excitability (activation of potassium channels)</td>
</tr>
<tr>
<td>• increases in neuronal firing rate (inhibition of inhibitory transmitter release - disinhibition)</td>
</tr>
<tr>
<td>• changes in gene expression (long-term changes in adenylyl cyclase activity, elevation of intracellular calcium levels, activation of cAMP response element binding protein (CREB))</td>
</tr>
</tbody>
</table>

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 $\mu$-receptor-induced analgesia. Opioids do not excite descending fibres directly but disinhibit them by inhibiting spontaneous GABA release from local GABAergic interneurones. 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'-lipooxygenase 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'-lipooxygenase. 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'-lipooxygenase pathway.

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; the functional significance of this observation for opiate withdrawal in brain neurones has remained obscure. Recently, Williams and colleagues 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.

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, naltbuphine) through the morphinans such as levorphanol, and the benzomorphans such as pentazocine, to the phenyl-piperidines including pethidine 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.

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 \( \kappa \)-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 \( \kappa \)-agonists in pre-clinical models of cerebral ischaemia has lead to consideration of the possible clinical development of selective \( \kappa \)-agonists for stroke or traumatic head injury. In this context the sedative properties of \( \kappa \)-agonists, and even perhaps their characteristic diuretic action, may be advantageous.

The discovery of the enkephalins and of the \( \delta \)-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 \( \delta \)-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. Applying the "message-address" concept that produced the antagonist naltrindole to a novel series of octahydroisoquinoline derivatives has been successful in producing non-peptide \( \delta \)-selective agonists TAN-67 or SB 213698. Similar considerations do not serve to explain the existence of another series of novel piperazine derivatives \( \delta \)-agonists, BW 373U86 or SNC 80. Preclinical studies suggest that \( \delta \)-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.

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A BRIEF HISTORY OF OPIUM

"Opium teaches only one thing, which is that aside from physical suffering, there is nothing real.”
André Malraux
MAN’S FATE

- **c.3400 B.C.**
The opium poppy is cultivated in lower Mesopotamia. The Sumerians refer to it as *Hul Gil*, the ‘joy plant.’ The Sumerians would soon pass along the plant and its euphoric effects to the Assyrians. The art of opium poppy-culling would continue from the Assyrians to the Babylonians who in turn would pass their knowledge onto the Egyptians.

- **c.1300 B.C.**
In the capital city of Thebes, Egyptians begin cultivation of *opium thebaicum*, grown in their famous poppy fields. The opium trade flourishes during the reign of Thutmose IV, Akhenaton and King Tutankhamen. The trade route included the Phoenicians and Minoans who move the profitable item across the Mediterranean Sea into Greece, Carthage, and Europe.

- **c.1100 B.C.**
On the island of Cyprus, the "Peoples of the Sea" craft surgical-quality culling knives to harvest opium, which they would cultivate, trade and smoke before the fall of Troy.

- **c. 460 B.C.**
Hippocrates, "the father of medicine", dismisses the magical attributes of opium but acknowledges its usefulness as a narcotic and styptic in treating internal diseases, diseases of women and epidemics.

- **330 B.C.**
Alexander the Great introduces opium to the people of Persia and India.
• **A.D. 400**
  *Opium thebaicum*, from the Egyptian fields at Thebes, is first introduced to China by Arab traders.

• **1020**
  Avicenna of Persia teaches that opium is "the most powerful of stupefacients."

• **A.D. 1200**
  Ancient Indian medical treatises *The Shodal Gadanigrah* and *Sharangdhar Samahita* describe the use of opium for diarrhea and sexual debility. The *Dhanvantri Nighantu* also describes the medical properties of opium.

• **1300s**
  Opium disappears for two hundred years from European historical record. Opium had become a taboo subject for those in circles of learning during the Holy Inquisition. In the eyes of the Inquisition, anything from the East was linked to the Devil.

• **1500**
  The Portuguese, while trading along the East China Sea, initiate the smoking of opium. The effects were instantaneous as they discovered but it was a practice the Chinese considered barbaric and subversive.

• **1527**
  During the height of the Reformation, opium is reintroduced into European medical literature by Paracelsus as laudanum. These black pills or "Stones of Immortality" were made of opium thebaicum, citrus juice and quintessence of gold and prescribed as painkillers.

• **1600s**
  Residents of Persia and India begin eating and drinking opium mixtures for recreational use. Portuguese merchants carrying cargoes of Indian opium through Macao direct its trade flow into China.

• **1606**
  Ships chartered by Elizabeth I are instructed to purchase the finest Indian opium and transport it back to England.

• **1620s -1670s**
  Rajput troops fighting for the Mughals introduce the habit of taking opium to Assam. Opium is given daily to Rajput soldiers. From 1637 onwards Opium becomes the main commodity of British trade with China.

• **1680**
  English apothecary, Thomas Sydenham, introduces Sydenham's Laudanum, a compound of opium, sherry wine and herbs. His pills along with others of the time become popular remedies for numerous ailments.

• **1700**
  The Dutch export shipments of Indian opium to China and the islands of Southeast Asia; the Dutch introduce the practice of smoking opium in a tobacco pipe to the Chinese.

• **1729**
  Chinese emperor, Yung Cheng, issues an edict prohibiting the smoking of opium and its domestic sale, except under license for use as medicine.

• **1750**
  The British East India Company assumes control of Bengal and Bihar, opium-growing districts of India. British shipping dominates the opium trade out of Calcutta to China.

• **1753**
  Linnaeus, the father of botany, first classifies the poppy, *Papaver somniferum* - 'sleep-inducing', in his book *Genera Plantarum*. 
• **1767**  
The British East India Company's import of opium to China reaches a staggering two thousand chests of opium per year.

• **1773**  
East India Company assumes monopoly over all the opium produced in Bengal, Bihar and Orissa. Warren Hastings introduces system of contracts. Contracts for dealing in opium were awarded through auction.

• **1793**  
The British East India Company establishes a monopoly on the opium trade. All poppy growers in India were forbidden to sell opium to competitor trading companies.

• **1796**  
The import of opium into China becomes a contraband trade. Silver was smuggled out to pay for smuggling opium in.

• **1797**  
East India Company introduced Bengal Regulation IV to enable appointment of Opium Agents for purchase of opium from cultivators and its processing at factories owned by the company at Patna and Ghazipur.

• **1799**  
China's emperor, Kia King, bans opium completely, making trade and poppy cultivation illegal.

• **1800**  
The British Levant Company purchases nearly half of all the opium coming out of Smyrna, Turkey strictly for importation to Europe and the United States.

• **1803**  
Friedrich Sertürner of Paderborn, Germany discovers the active ingredient of opium by dissolving it in acid then neutralizing it with ammonia. The result: alkaloids - *Principium somniferum* or morphine.

Physicians believe that opium had finally been perfected and tamed. Morphine is lauded as "God's own medicine" for its reliability, long-lasting effects and safety.

• **1805**  
A smuggler from Boston, Massachusetts, Charles Cabot, attempts to purchase opium from the British, then smuggle it into China under the auspices of British smugglers.

• **1812**  
American John Cushing, under the employ of his uncles' business, James and Thomas H. Perkins Company of Boston, acquires his wealth from smuggling Turkish opium to Canton.

• **1816**  
John Jacob Astor of New York City joins the opium smuggling trade. His American Fur Company purchases ten tons of Turkish opium then ships the contraband item to Canton on the Macedonian. Astor would later leave the China opium trade and sell solely to England.

• **1819**  
Writer John Keats and other English literary personalities experiment with opium intended for strict recreational use - simply for the high and taken at extended, non-addictive intervals.
• **1821**  
  Thomas De Quincey publishes his autobiographical account of opium addiction, *Confessions of an English Opium-eater*.

• **1827**  
  E. Merck & Company of Darmstadt, Germany, begins commercial manufacturing of morphine.

• **1830**  
  The British dependence on opium for medicinal and recreational use reaches an all time high as 22,000 pounds of opium is imported from Turkey and India.

  Jardine-Mathesom & Company of London inherit India and its opium from the British East India Company once the mandate to rule and dictate the trade policies of British India are no longer in effect.

• **1837**  
  Elizabeth Barrett Browning falls under the spell of morphine. This, however, does not impede her ability to write "poetical paragraphs."

• **March 18, 1839**  
  Lin Tse-Hsu, imperial Chinese commissioner in charge of suppressing the opium traffic, orders all foreign traders to surrender their opium. In response, the British send expeditionary warships to the coast of China, beginning The First Opium War.

• **1840**  
  New Englanders bring 24,000 pounds of opium into the United States. This catches the attention of U.S. Customs which promptly puts a duty fee on the import.

• **1841**  
  The Chinese are defeated by the British in the First Opium War. Along with paying a large indemnity, Hong Kong is ceded to the British.

• **1842**  
  The Treaty of Nanking between the Queen of Great Britain and the Emperor of China.

• **1843**  
  Dr. Alexander Wood of Edinburgh discovers a new technique of administering morphine, injection with a syringe. He finds the effects of morphine on his patients instantaneous and three times more potent.

• **1852**  
  The British arrive in lower Burma, importing large quantities of opium from India and selling it through a government-controlled opium monopoly.

• **1856**  
  The British and French renew their hostilities against China in the Second Opium War. In the aftermath of the struggle, China is forced to pay another indemnity. The importation of opium is legalized.

  Opium production increases along the highlands of Southeast Asia.

• **1874**  
  English researcher, C.R. Wright first synthesizes heroin, or diacetylmorphine, by boiling morphine over a stove.
In San Francisco, smoking opium in the city limits is banned and is confined to neighboring Chinatowns and their opium dens.

- **1878**
  Britain passes the Opium Act with hopes of reducing opium consumption. Under the new regulation, the selling of opium is restricted to registered Chinese opium smokers and Indian opium eaters while the Burmese are strictly prohibited from smoking opium.

- **1886**
  The British acquire Burma's northeast region, the Shan state. Production and smuggling of opium along the lower region of Burma thrives despite British efforts to maintain a strict monopoly on the opium trade.

- **1890**
  U.S. Congress, in its earliest law-enforcement legislation on narcotics, imposes a tax on opium and morphine.

  Tabloids owned by William Randolph Hearst publish stories of white women being seduced by Chinese men and their opium to invoke fear of the 'Yellow Peril', disguised as an "anti-drug" campaign.

- **1895**
  Heinrich Dreser working for The Bayer Company of Elberfeld, Germany, finds that diluting morphine with acetyl causes studies to produce a drug without the common morphine side effects. Bayer begins production of diacetylmorphine and coins the name "heroin." Heroin would not be introduced commercially for another three years.

- **Early 1900s**
  The philanthropic Saint James Society in the U.S. mounts a campaign to supply free samples of heroin through the mail to morphine addicts who are trying to give up their habits. Efforts by the British and French to control opium production in Southeast Asia are successful. Nevertheless, this Southeast region, referred to as the 'Golden Triangle', eventually becomes a major player in the profitable opium trade during the 1940s.

- **1902**
  In various medical journals, physicians discuss the side effects of using heroin as a morphine step-down cure. Several physicians would argue that their patients suffered from heroin withdrawal symptoms equal to morphine addiction.

- **1903**
  Heroin addiction rises to alarming rates.

- **1905**
  U.S. Congress bans opium.

- **1906**
  China and England finally enact a treaty restricting the Sino-Indian opium trade. Several physicians experiment with treatments for heroin addiction. Dr. Alexander Lambert and Charles B. Towns tout their popular cure as the most "advanced, effective and compassionate cure" for heroin addiction. The cure consisted of a 7 day regimen, which included a five day purge of heroin from the addict's system with doses of belladonna delirium.
U.S. Congress passes the Pure Food and Drug Act requiring contents labeling on patent medicines by pharmaceutical companies. As a result, the availability of opiates and opiate consumers significantly declines.

- **1909**
  The first federal drug prohibition passes in the U.S. outlawing the importation of opium. It was passed in preparation for the Shanghai Conference, at which the US presses for legislation aimed at suppressing the sale of opium to China.

- **February 1, 1909**
  The International Opium Commission convenes in Shanghai. Heading the U.S. delegation are Dr. Hamilton Wright and Episcopal Bishop Henry Brent. Both would try to convince the international delegation of the immoral and evil effects of opium.

- **1910**
  After 150 years of failed attempts to rid the country of opium, the Chinese are finally successful in convincing the British to dismantle the India-China opium trade.

- **Dec. 17, 1914**
  The passage of Harrison Narcotics Act which aims to curb drug (especially cocaine but also heroin) abuse and addiction. It requires doctors, pharmacists and others who prescribed narcotics to register and pay a tax.

- **1923**
  The U.S. Treasury Department's Narcotics Division (the first federal drug agency) bans all legal narcotics sales. With the prohibition of legal venues to purchase heroin, addicts are forced to buy from illegal street dealers.

- **1925**
  In the wake of the first federal ban on opium, a thriving black market opens up in New York’s Chinatown.

- **1930s**
  The majority of illegal heroin smuggled into the U.S. comes from China and is refined in Shanghai and Tietsin.

- **Early 1940s**
  During World War II, opium trade routes are blocked and the flow of opium from India and Persia is cut off. Fearful of losing their opium monopoly, the French encourage Hmong farmers to expand their opium production.

- **1945-1947**
  Burma gains its independence from Britain at the end of World War II. Opium cultivation and trade flourishes in the Shan states.

- **1948-1972**
  Corsican gangsters dominate the U.S. heroin market through their connection with Mafia drug distributors. After refining the raw Turkish opium in Marseilles laboratories, the heroin is made easily available for purchase by junkies on New York City streets.

- **1950s**
  U.S. efforts to contain the spread of Communism in Asia involves forging alliances with tribes and warlords inhabiting the areas of the Golden Triangle, (an expanse covering Laos, Thailand and Burma), thus providing accessibility and protection along the southeast border of China. In order to maintain their relationship with the warlords while continuing to fund the struggle against communism, the U.S. and France supply the drug warlords and their armies with ammunition, arms and air transport for the production and sale of
opium. The result: an explosion in the availability and illegal flow of heroin into the United States and into the hands of drug dealers and addicts.

- **1962**
  Burma outlaws opium.

- **1965-1970**
  U.S. involvement in Vietnam is blamed for the surge in illegal heroin being smuggled into the States. To aid U.S. allies, the Central Intelligence Agency (CIA) sets up a charter airline, Air America, to transport raw opium from Burma and Laos. As well, some of the opium would be transported to Marseilles by Corsican gangsters to be refined into heroin and shipped to the U.S via the French connection. The number of heroin addicts in the U.S. reaches an estimated 750,000.

- **October 1970**
  Legendary singer, Janis Joplin, is found dead at Hollywood's Landmark Hotel, a victim of an "accidental heroin overdose."

- **1972**
  Heroin exportation from Southeast Asia's Golden Triangle, controlled by Shan warlord, Khun Sa, becomes a major source for raw opium in the profitable drug trade.

  Solomon Snyder and Candace Pert discover opiate receptor in the brain.

- **July 1, 1973**
  President Nixon creates the DEA (Drug Enforcement Administration) under the Justice Dept. to consolidate virtually all federal powers of drug enforcement in a single agency.

- **Mid-1970s**

- **1975**
  Hans Kosterlitz and his colleagues isolate and purify an endogenous opioid in the brain, enkephalin.

- **1978**
  The U.S. and Mexican governments find a means to eliminate the source of raw opium - by spraying poppy fields with Agent Orange. The eradication plan is termed a success as the amount of "Mexican Mud" in the U.S. drug market declines. In response to the decrease in availability of "Mexican Mud", another source of heroin is found in the Golden Crescent area - Iran, Afghanistan and Pakistan, creating a dramatic upsurge in the production and trade of illegal heroin.

- **1982**
  Comedian John Belushi of Animal House fame, dies of a heroin-cocaine "speedball" overdose.

- **Sept. 13, 1984**
  U.S. State Department officials conclude, after more than a decade of crop substitution programs for Third World growers of marijuana, coca or opium poppies, that the tactic cannot work without eradication of the plants and criminal enforcement. Poor results are reported from eradication programs in Burma, Pakistan, Mexico and Peru.

- **1988**
  Opium production in Burma increases under the rule of the State Law and Order Restoration Council (SLORC), the Burmese junta regime.
The single largest heroin seizure is made in Bangkok. The U.S. suspects that the 2,400-pound shipment of heroin, en route to New York City, originated from the Golden Triangle region, controlled by drug warlord, Khun Sa.

- **1990**
  A U.S. Court indicts Khun Sa, leader of the Shan United Army and reputed drug warlord, on heroin trafficking charges. The U.S. Attorney General's office charges Khun Sa with importing 3,500 pounds of heroin into New York City over the course of eighteen months, as well as holding him responsible for the source of the heroin seized in Bangkok.

- **1992**
  Colombia's drug lords are said to be introducing a high-grade form of heroin into the United States.

- **1993**
  The Thai army with support from the U.S. Drug Enforcement Agency (DEA) launches its operation to destroy thousands of acres of opium poppies from the fields of the Golden Triangle region.

- **January 1994**
  Efforts to eradicate opium at its source remains unsuccessful. The Clinton Administration orders a shift in policy away from the anti-drug campaigns of previous administrations. Instead the focus includes "institution building" with the hope that by "strengthening democratic governments abroad, [it] will foster law-abiding behavior and promote legitimate economic opportunity."

- **1995**
  The Golden Triangle region of Southeast Asia is now the leader in opium production, yielding 2,500 tons annually. According to U.S. drug experts, there are new drug trafficking routes from Burma through Laos, to southern China, Cambodia and Vietnam.

- **January 1996**
  Khun Sa, one of Shan state's most powerful drug warlords, "surrenders" to SLORC. The U.S. is suspicious and fears that this agreement between the ruling junta regime and Khun Sa includes a deal allowing "the opium king" to retain control of his opium trade but in exchange end his 30-year-old revolutionary war against the government.

- **November 1996**
  International drug trafficking organizations, including China, Nigeria, Colombia and Mexico are said to be "aggressively marketing heroin in the United States and Europe."

- **1999**
  Bumper opium crop of 4,600 tons in Afghanistan. UN Drug Control Program estimates around 75% of world's heroin production is of Afghan origin.

- **2000**
  Taliban leader Mullah Omar bans poppy cultivation in Afghanistan; United Nations Drug Control Program confirms opium production eradicated.

- **July 2001**
  Portugal decriminalizes all drugs for personal consumption.

- **Autumn 2001**
  War in Afghanistan; heroin floods the Pakistan market. Taliban regime overthrown.

- **October 2002**
  U.N. Drug Control and Crime Prevention Agency announces Afghanistan has regained its position as the world's largest opium producer.
• December 2002
  UK Government health plan will make heroin available free on National Health Service "to all those with a clinical need for it". Consumers are sceptical.

• April 2003
  State sponsored heroin trafficking: Korea's attempt to penetrate the Australian heroin market hits rocky waters.

• October 2003
  US Food and Drug Administration (FDA) and Drug Enforcement Administration (DEA) launch special task force to curb in Net-based sales of narcotics from online pharmacies.

• January 2004
  Consumer groups file a lawsuit against Oxycontin maker Purdue Pharma. The company is alleged to have used fraudulent patents and deceptive trade practices to block the prescription of cheap generic medications for patients in pain.

• September 2004
  Singapore announces plans to execute a self-medicating heroin user, Chew Seow Leng. Under Singapore law, chronic heroin users with a high physiological tolerance to the drug are deemed to be "traffickers". Consumers face a mandatory death sentence if they take more than 15 grams (0.5 ounces) of heroin a day.

• September 2004
  A Tasmanian company publishes details of its genetically-engineered opium poppies. Top1 [thebaine oripavine poppy 1] mutants do not produce morphine or codeine. Tasmania is the source of some 40% of the world's legal opiates; its native crop of poppies is already being re-engineered with the mutant stain. Conversely, some investigators expect that the development of genetically-engineered plants and microorganisms to manufacture potent psychoactive compounds will become widespread later in the 21st century. Research into transgenic psychotropic botanicals and microbes is controversial; genes from mutants have a habit of spreading into the wild population by accident as well as design.

• September 2004
  The FDA grants a product license to Purdue's pain medication Palladone: high dose, extended-release hydromorphone capsules. Palladone is designed to provide "around-the-clock" pain-relief for opioid-tolerant users.

• October 2004
  Unannounced withdrawal of newly-issued DEA guidelines to pain specialists. The guidelines had pledged that physicians wouldn't be arrested for providing adequate pain-relief to their patients. DEA drug-diversion chief Patricia Good earlier stated that the new rules were meant to eliminate an "aura of fear" that stopped doctors treating pain aggressively.

• December 2004
  McLean pain-treatment specialist Dr William E. Hurwitz is sent to prison for allegedly "excessive" prescription of opioid painkillers to chronic pain patients. Testifying in court, Dr Hurwitz describes the abrupt stoppage of prescriptions as "tantamount to torture".

• May 2005
  Researchers at Ernest Gallo Clinic and Research Center in Emeryville, California, inhibit expression of the AGS3 gene in the core of nucleus accumbens. Experimentally blocking the AGS3 gene curbs the desire for heroin in addicted rodents. By contrast, activation of the reward centres of...
the nucleus accumbens is immensely pleasurable and addictive. The possible effects of overexpression and gene amplification of AGS3 remain unexplored.