Opioid mechanisms and opioid drugs

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Abstract

The opioid system comprises four receptor subtypes: μ (MOP), κ (KOP), δ (DOP), now called the 'classical' opioid receptors, and the 'non classical' nociceptin/orphanin FQ peptide (N/OFQ) receptor (NOP). Selective endogenous peptides, cleaved from larger precursor proteins, have been identified for all subtypes. Both classical and non-classical opioid receptors couple to inhibitory, pertussis toxin-sensitive G-proteins. Opioid receptors activate the same major intracellular pathways, which include: closing of voltage-sensitive calcium channels; opening of potassium channels and subsequent cellular hyperpolarization; and inhibition of cyclic adenosine monophosphate (cAMP) production through inhibition of the enzyme adenylate cyclase. All current, clinically used opioids work through activation of the MOP receptor. In an experimental setting, co-administration of MOP and DOP agonists has been shown to have a synergistic analgesic action. Administration of DOP-receptor antagonists has also been shown to reduce tolerance, physical dependence and other side effects of MOP-receptor agonists, without detriment to their analgesic action. In animal models NOP agonists are analgesic when administered spinally and have a pronociceptive/anti-analgesic (or anti-opioid) effect supraspinally. NOP knockout mice show a partial loss of tolerance to morphine and there is an up-regulation of N/OFQ production in chronic morphine tolerant mice. Analgesic tolerance that develops from repeated exposure to morphine is markedly attenuated in NOP knockout mice. The development of ligands with mixed action at MOP, DOP and NOP receptors offer new opportunities for opioid pharmacology.

Keywords G-protein-coupled receptor; MOP/DOP opioid receptor actions; NOP-receptor antagonist

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Opium and its derivatives have been used for centuries for medicinal and recreational purposes. Opiates refer to the non-peptide synthetic morphine-like drugs whilst the term opioid is more generic, encompassing all substances that produce morphine-like actions. Opioids can be loosely divided into four groups:
- naturally occurring endogenously produced opioid peptides (e.g. dynorphin and met-enkephalin)
- opium alkaloids such as morphine purified from the poppy *Papaver somniferum*
- semi-synthetic opioids (modifications to the natural morphine structure) such as diacetylmorphine (heroin)
- synthetic derivatives with structure unrelated to morphine, which include the phenylpiperidine series (e.g. pethidine and fentanyl), methadone series (e.g. methadone and dextropropoxyphene), benzomorphan series (e.g. pentazocine), and semi-synthetic thebaine derivatives (e.g. etorphine and buprenorphine).

Snyder and colleagues in 1973 published data showing specific binding of opioids, providing the first evidence of distinct receptors for these drugs. Multiple opioid receptor types were evident from initial studies, which showed: differences in opioid potency; selective antagonism; and stereospecificity of opiate actions. Opioid-receptor subtypes were defined from multiple studies characterizing drug action at distinct anatomical locations and through pharmacological profiles of opioids. 'Classical' opioid receptor definition is based in part on a sensitivity to naloxone and subtypes μ (MOP), κ (KOP) and δ (DOP) exist. Current International Union of Basic and Clinical Pharmacology (IUPHAR) nomenclature is included in parenthesis and will be used for the remainder of this article. Low-stringency hybridization screening using opioid receptor probes led to the discovery of a fourth 'opioid-like receptor' initially named LC132 (rat), MOR-3 (mouse) and ORL1 (human). Following the deorphanizing of the receptor and the identification of an endogenous ligand, nociceptin/orphanin FQ (N/OFQ), the receptor was classified as a 'non-opioid' branch of the opioid receptor family, owing to a lack of sensitivity to naloxone whilst sharing significant sequence homology with the classical opioid receptors. The current nomenclature for this fourth opioid receptor subtype is nociceptin/orphanin FQ (N/OFQ) peptide receptor (NOP).

All receptors are G-protein coupled and share the same general structure: seven linked transmembrane-spanning domains, an extracellular N-terminus and intracellular C-terminal tail. Based on alignment of their amino acid sequences, all four subtypes have an overall homology of about 60%; however, this increases to more than 80% in the second, third and seventh transmembrane domains.

Isoforms of all opioid receptors have been suggested based largely on pharmacological grounds, which sees the differential effect of a drug *in vivo* when compared to *in vitro* responses or incomplete tolerance profiles seen when alternating drugs acting at the same receptor. There is no evidence for multiple genes encoding opioid-receptor subtypes; opioids are encoded by single genes which when removed, as in knock-out animals, results in all those responses associated with the respective receptor becoming absent. The putative isoforms of receptors reported are accounted for in part by the alternative splicing of a single gene resulting in alternative receptor protein structures and therefore pharmacology, for the MOP receptor 15 splice variants have been reported with some distinct regional distribution within the CNS. The exact nature of the pharmacologically described subtypes remains the subject of debate.

Endogenous opioid peptides

Hughes and Kosterlitz isolated the first endogenous opiates: two peptides (met-enkephalin and leu-enkephalin) that competed with morphine-like drugs for binding to receptors in the brain. Subsequent studies identified further endogenous opioids to include dynorphin A, dynorphin B, β-endorphin, endomorphin- 1 and -2, and N/OFQ (Table 1).[1] Met- and leu-enkephalin showed preferential binding to DOP receptors, whilst dynorphin A and B favoured binding to KOP receptors, N/OFQ for NOP and endomorphin- 1 and -2 for MOP. β-endorphin has activity at all three classical subtypes but shows some preference for MOP receptors. Opioid peptides are cleaved from larger precursors: β-endorphin from preproopiomelancortin; met- and leu-enkephalin from preproenkephalin; N/OFQ from prepronociceptin; and dynorphin A and B from preprodynorphin. The precursor(s) for endomorphin 1 and 2 are yet to be identified. The precursors contain other bioactive fragments within their sequences, and in the case of preproenkephalin, multiple copies of their respective peptides (Table 1).[2]

Intracellular effectors

The four subtypes of opioid receptor couple to inhibitory, pertussis toxin sensitive G-proteins (e.g. $G_{i/o}$). Both recombinant and endogenously expressed opioid receptors activate the same major intracellular pathways, which include: closing of (predominantly) N and P/Q-type voltage-sensitive calcium channels; opening of potassium channels and subsequent cellular hyperpolarization; and inhibition of cyclic adenosine monophosphate (cAMP) production through the inhibition of the enzyme adenylyl cyclase.[2,3]

G-protein-coupled receptors (GPCRs) couple to heterotrimeric G-proteins, which are formed from three distinct subunits: α, β, and γ. There are numerous gene products encoding for the different subunits. GPCR activation, for example MOP with morphine, induces a conformational change in the receptor, which allows coupling to respective G-protein subtype(s), this will be a G-protein possessing a $G_{i/o}$ α-subunit. In their resting form G-proteins reside in a heterotrimeric complex (αβγ), with guanine diphosphate (GDP) in association with the α-subunit. G-protein association with its cognate receptor, upon ligand binding, leads to dissociation of GDP from the α subunit and binding of guanosine triphosphate (GTP) followed by the dissociation of α-GTP from the βγ complex. Both α-GTP and βγ are able to affect different intracellular pathways through activation or inhibition of enzymes and ion channels. G-protein GTPase activity, which converts GTP into GDP, leads to cessation of signalling and reforming of the α-GDP subunit with βγ (Figure 1).

N/P-type voltage-sensitive calcium channels (VSCCs) are located at synaptic terminals and play a major role in transmitter release and therefore synaptic transmission. Some G-protein-coupled receptors, including opioid receptors, negatively regulate VSCC, such that their activation inhibits calcium influx, preventing neurotransmitter release. Channel inhibition results from a positive shift in the voltage dependence of the channel coupled to a slowing of activation; the inhibition can be relieved by a strong depolarization. Classical opioid receptors have been shown to inhibit N-, P/Q-, L- and T-type calcium channels. However because of their location at presynaptic terminals, N- and P/Q-type channel modulation is thought to be of most importance. N-type and P/Q-types calcium currents are most sensitive to inhibition by N/OFQ. VSCC inhibition by opioids has been demonstrated in a variety of preparations, including the locus coeruleus, periaqueductal grey neurons and trigeminal ganglion neurons. Overall the effect of opioids on VSCCs leads to reduced transmitter release.[3]

G-protein inwardly rectifying potassium (GIRK) channels are activated by opioid receptors. GIRK channel opening is through an interaction with Gβγ subunits released from $G_{i/o}$ G-proteins and leads to membrane hyperpolarization through an efflux of potassium ions. The net effect is reduced neuronal excitability and, through opioid receptor location on nociceptive afferents, a concomitant reduction in nociceptive transmission. N/OFQ-mediated activation of GIRK channels has also been demonstrated at many central sites, including spinal cord, locus coeruleus, periaqueductal grey and hypothalamus.[4,5]

Characteristics of the more common opioid peptides.

Precursor	Ligand	Peptide sequence
Unknown	Endomorphin-1	YPWF-NH$_2$
Unknown	Endomorphin-2	YPFF-NH$_2$
Pro-opiomelanocortin	β-endorphin	**YGGF**MTSE**KS**QTPLVTL FKNAIIKNAYKKGE
Pro-enkephalin	Leu-enkephalin	**YGGF**L
		YGGFMRF
		YGGFMRGL
	Met-enkephalin	**YGGF**M
	Metorphamide	**YGGF**MRRV-NH$_2$
Pro-dynorphin	Dynorphin A	**YGGF**LRRIRPKL**KWD**NQ
	Dynorphin B	**YGGF**LRRQFKVVT
	α-neoendorphin	**YGGF**LRKYPK
	β-neoendorphin	**YGGF**LRKYP
Pre-pro-nociceptin	N/OFQ	F**GGF**TGAR**KS**AR**KL**ANQ
	Nocistatin	TEPGLEEVGEIEQKQLQ

Standard single amino acid code is used. Note that endorphins, enkephalins and dynorphins share a common Tyr-Gly-Gly-Phe (YGGF) motif which enables binding to classical opioid receptors (modified from[1])
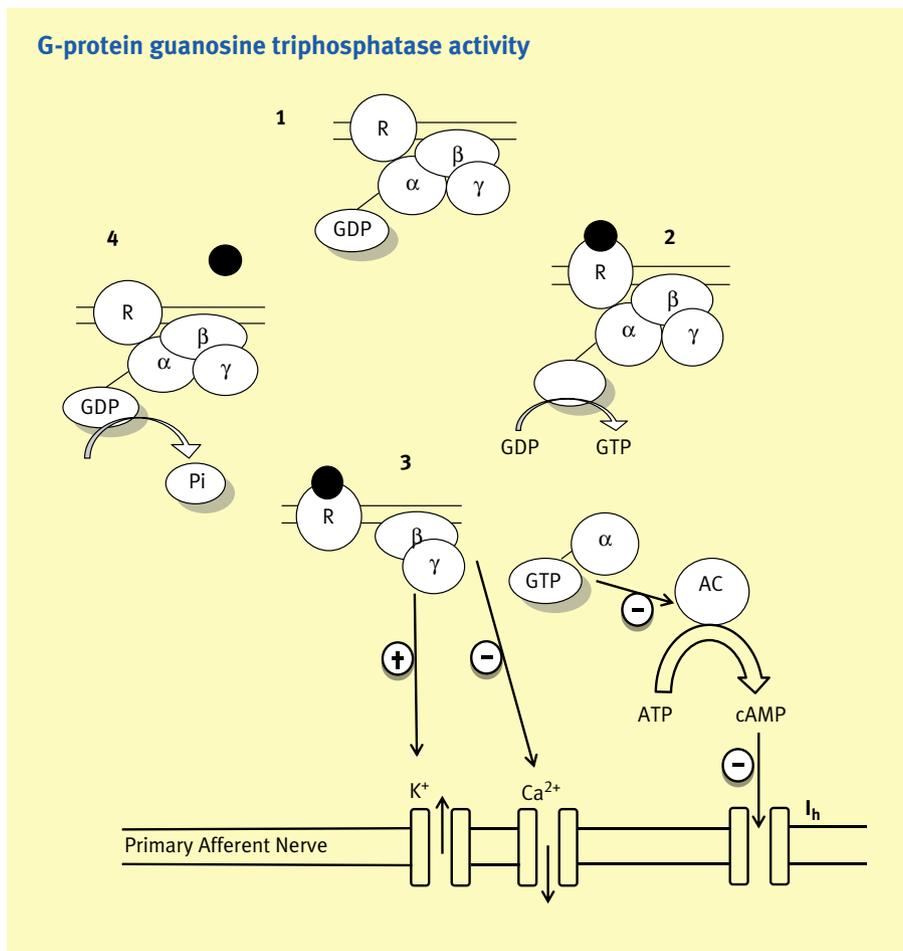
Table 1

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Figure 1 The receptor (R) and heterotrimeric G-protein ($\alpha\beta\gamma$) are depicted in (1). When ligand (black circle) binds (2) GDP is exchanged for GTP and the system is activated such that α and $\beta\gamma$ dimer dissociate (3) to interact with effectors, Ca^{2+} channels ($-$ close), K^+ channels ($+$ open) and adenylate cyclase ($-$ inhibit) which indirectly modulates I_h ($-$ close). The system is turned off when the intrinsic GTPase activity of the alpha-subunit converts GTP back to GDP (4).

Regulation of cAMP also plays a role in how opioid receptors modulate the firing of nociceptive afferents. The hyperpolarization-activated current, I_h, is an important modulator of action potential firing in excitable cells and represents an inward current activated by hyperpolarization of the resting membrane potential. It is believed that part of the action of pro-inflammatory mediators is through a capacity to increase the frequency of action potentials generated from a given inward current, and modulation of I_h is involved in this. I_h is regulated by cAMP and increases in cAMP enhance I_h currents, reducing refractory times, intensifying the firing of nociceptive afferents. Opioid receptors do not act directly on the I_h channel, but modulate it indirectly through a reduction in cAMP. In this way the opioid receptor mediated inhibition of I_h, and reduction of excitability in primary in afferent nerves, would only be relevant in the presence of agents which lead to an increase in cAMP formation.[6]

Types of opioid receptor

MOP receptors: the clinically used opioids work through activation of the MOP receptor. Respiratory depression accompanies analgesic doses of morphine and related compounds. This action is mediated by MOP receptors located within the respiratory centres of the medulla, decreasing the sensitivity of chemoreceptors to carbon dioxide. Whilst this action is one of the major side effects of opioid drugs, tolerance to its action does build. Nausea and vomiting is prevalent in patients given morphine, and is caused by stimulation of the chemoreceptor-trigger zone of the medulla. Again, tolerance to this action does develop. Opioids cause constipation through reduction of gastrointestinal-tract motility. Not only is this effect unpleasant, it can also affect the absorption of other drugs. This action is mediated via inhibition of nerves in the myenteric plexus that cause visceral smooth muscle contraction of the gut.

As many of the unwanted side effects of opioid drugs are caused by activation of peripherally located opioid receptors, there has been interest in the use of peripherally acting opioid-receptor antagonists, unable to pass the blood–brain barrier, for treating these side effects. Methylnaltrexone, a peripherally acting opioid-receptor antagonist was evaluated regarding the incidence of postoperative nausea and vomiting. It was concluded that this agent did not prevent or significantly reduce the incidence/severity of postoperative nausea and vomiting. However, methylnaltrexone given subcutaneously in patients with opioid-induced constipation (no bowel movements for 48 hours despite the use of laxatives and stool softeners) caused

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laxation within 4 hours. Alvimopan has been shown to have gastrointestinal-selective opioid-receptor antagonist activity. It improved postoperative recovery of bowel function and shortened the duration of hospital stay without compromising opioid-mediated analgesia.[7,8]

DOP receptors: were the first opioid receptors to be cloned from NG-108 (a mouse neuroblastoma hybridoma cell line) cells in 1992. Their distribution throughout the CNS is more restricted relative to the other opioid receptors. The highest DOP receptor densities can be found in the olfactory bulb, neocortex, caudate putamen and nucleus accumbens, and to a lesser degree the thalamus, hypothalamus and brainstem. DOP receptors are located presynaptically on primary afferents where they inhibit the release of neurotransmitters. Through both spinal and supraspinal sites the receptor is involved in the antinociceptive/analgesic actions of some opioids.

DOP receptors are located in bulbospinal neurons and functionally identified respiratory neurons of the ventral respiratory group. The receptor is located presynaptically and therefore modulates transmitter release from these neurons. DOP agonists have a role in central respiratory pattern generation but have a lesser role in respiratory rhythm generation. However, it has been demonstrated that DOP-respiratory depression is absent in MOP-deficient species. Also of note is a reduction in DOP-receptor mediated analgesia in MOP knockout species. Therefore, both the analgesic properties and respiratory effects of DOP receptor activation may, in part, be dependent on the presence of functionally active MOP receptors (i.e. MOP/DOP-receptor cooperativity or synergy takes place). Since DOP-mediated intracellular signalling was unaffected in mice deficient of the MOP receptor, it is likely that the MOP/DOP interaction occurs through distinct locations on neuronal networks.

Currently, there are no clinical drugs in use that work via activation of the DOP receptor. One major area of interest regarding DOP-receptor activation is an antidepressant-like effect. Indeed, DOP-deficient mice display a depressant-like phenotype and anxiogenic-like responses. Enkephalinase inhibitors have also been shown to produce antidepressant-like actions in animal paradigms, as have DOP receptor agonists.

Current interest lies in the development of drugs with agonist activity at both DOP and MOP, and drugs that have agonist activity for MOP and antagonist actions at DOP. The rationale for this approach comes from studies in which co-administration of MOP and DOP agonists revealed a synergistic analgesic action such that sub-antinociceptive doses of leu-enkephalin potentiate the analgesia elicited by morphine. The administration of DOP receptor antagonists reduces tolerance, physical dependence and other side effects of MOP-receptor agonists without detriment to their analgesic action. Development of tolerance and dependence to morphine following chronic dosing was blocked by antisense oligonucleotides directed against the DOP receptor. In addition, wild-type mice were shown to lose their analgesic response to daily morphine dosing after 5 days, whilst DOP-receptor knockout mice failed to develop tolerance after 8 days' administration.[9]

Bivalent ligands are single drug molecules with two pharmacophoric regions (motifs responsible for a drugs action). Combining the pharmacophores of oxymorphone and naltrindole in one drug, by way of a 21-atom linker, lead to MDAN-21, a bivalent ligand which when compared to morphine had improved antinociception and a reduction in the development of tolerance.[10] Bi-functional drugs, like bivalent drugs, also interact with multiple receptor targets; however they are chemically different in having only one pharmacophoric region capable of achieving multiple receptor interactions. Bi-functionals able to produce both MOP agonism and DOP antagonism have also been reported and include UFP-505 which utilizes a DMT-tic pharmacophore to achieve its mixed opioid pharmacology.[10]

KOP receptors: have been implicated in a number of functions, including nociception, diuresis and feeding. Activation of the KOP receptor has been shown to produce actions distinct from those elicited by stimulation of the MOP receptor, importantly sedation without marked effects on heart rate. Further, KOP receptors do not cause respiratory depression and therefore have been of great interest due to their potentially safer side-effect profile. Two synthetic KOP receptor agonists, spiradoline (U-62,066E) and enadoline (CI-977) have undergone clinical trials for their analgesic actions.[11,12] Spiradoline produced analgesia in animal models; however, clinical data showed that spiradoline produced adverse effects such as diuresis, sedation and dysphoria at doses lower than that needed for its analgesic effects. Enadoline had a similar side-effect profile of sedation, confusion, dizziness along with increased urinary output and feelings of depersonalization. The side effects elicited by these and other KOP receptor agonists limit their effective clinical usage.

Whilst the use of KOP ligands for the treatment of pain is currently limited there appear to be a number of potential alternative uses, including the treatment of alcohol dependence. Moreover, a role for dynorphin and the KOP receptor in epileptogenesis and epilepsy has also been recently demonstrated.

NOP receptors: the observation that NOP regulates similar transduction mechanisms to those of classical opioids, the high sequence homology of the endogenous KOP receptor peptide dynorphin A to N/OFQ and abundant overlap of NOP receptor distribution with classical opioid receptors suggests that NOP and N/OFQ are related to the opioid family.

N/OFQ has been shown to have both pre- and postsynaptic sites of action. The superficial dorsal horn expresses high levels of NOP-receptor and N/OFQ peptide mRNA. Studies mapping the binding of N/OFQ and measuring mRNA for N/OFQ indicate similar distribution. N/OFQ and its receptor are widely expressed throughout both the central and the peripheral nervous system and show broad overlap with classical endogenous opioids and receptors.[13] Application of N/OFQ has been shown to cause hyperalgesia, allodynia and analgesia.[14] However, the route of administration and nociceptive paradigm under investigation determine the observed response. Nevertheless, most studies conclude that intrathecally administered N/OFQ causes analgesia.

There has been much controversy over the supraspinal effects of N/OFQ. Original studies reported that intracerebroventricular administration caused hyperalgesia compared with vehicle treated groups.[5] It has since been shown that there is no difference between the pain threshold of intracerebroventricular N/OFQ-treated and vehicle-treated animals. Therefore, it is

assumed that N/OFQ does not cause hyperalgesia but reverses the opioid-mediated stress-induced analgesia caused by the experimental procedure.

The anti-opioid role of N/OFQ has subsequently been validated, and N/OFQ is known to counteract analgesia elicited through the endogenous opioid system and analgesia from exogenously applied morphine. The overall effect of a systemic dose of N/OFQ would be dependent on both its antinociceptive action at spinal sites, and its supraspinal pro-nociceptive/anti-opioid action. The relative contribution at both these sites, coupled with the degree of resting supraspinal endogenous-opioid tone, would influence the overall outcome of systemic N/OFQ.

Mixed opioids with activity at NOP are also of pharmacological interest. Indeed buprenorphine, which has agonist activity at both MOP and NOP, is one such drug in clinical use. 'In both animals and humans a hallmark of the antinociceptive action of buprenorphine is the production of a ceiling effect or a bell shaped curve'.[15,16] The ceiling effect, which is seen in man, may be caused simply through buprenorphine partial agonist activity. However subsequent activation of NOP receptor mediated anti-opioid activity may set the ceiling response of buprenorphine and is certainly responsible for the falling phase of buprenorphine's bell shaped curve. It may be also suggested that the supraspinal actions of NOP not only compromise the MOP mediated actions of buprenorphine but also override the possible spinal NOP mediated antinociception of buprenorphine.

The chronic use of MOP-receptor analgesics, such as morphine, results in tolerance and a reduction in analgesia from a fixed dose. The anti-analgesic action of the NOP−N/OFQ system may play a key role in development of this type of tolerance. Indeed, NOP knockout mice show a partial loss of tolerance to morphine, and there is up-regulation of N/OFQ production in chronic morphine-tolerant mice.[17] Analgesic tolerance that develops from repeated exposure to morphine is markedly attenuated in NOP knockout mice. Acute morphine analgesia is unaffected in NOP knockout species. This action has also been confirmed using potent selective NOP antagonists, which additionally attenuate morphine tolerance.[18] These findings suggest the NOP−N/OFQ system may contribute to the neuroplasticity that accompanies tolerance from chronic morphine exposure.[17] NOP blockade may prove useful in reducing tolerance to opioids and/or reducing the dose required to provide analgesia.[17] With this is mind the merits of a drug with that behaves as an NOP antagonist and MOP agonist are clear, indeed one particular experimental molecule SR-14148 has already been presented in the literature.[19] ◆

REFERENCES

1 Johnson E, Lambert DG. Molecular pharmacology of the opioid/nociceptin system. *Curr Anaesth Crit Care* 2002; **13:** 305−12.

2 Zollner C, Stein C. Opioids. *Handb Exp Pharmacol* 2007; **177:** 31−63.

3 McDonald J, Lambert DG. Opioid receptors. *Contin Educ Anaesth Crit Care Pain* 2005; **5:** 22−5.

4 New DC, Wong YH. The ORL1 receptor: molecular pharmacology and signalling mechanisms. *Neurosignals* 2002; **11:** 197−212.

5 Mogil JS, Pasternak GW. The molecular and behavioral pharmacology of the orphanin FQ/nociceptin peptide and receptor family. *Pharmacol Rev* 2001; **53:** 381−415.

6 Christie MJ, Vaughan CW, Ingram SL. Opioids, NSAIDs and 5-lipoxygenase inhibitors act synergistically in brain via arachidonic acid metabolism. *Inflamm Res* 1999; **48:** 1−4.

7 Stein C, Schafer M, Machelska H. Attacking pain at its source: new perspectives on opioids. *Nat Med* 2003; **9:** 1003−8.

8 Sinatra RS. Peripherally acting mu-opioid-receptor antagonists and the connection between postoperative ileus and pain management: the anesthesiologist's view and beyond. *J Perianesth Nurs* 2006; **21**(2A suppl): S16−23.

9 Ananthan S. Opioid ligands with mixed mu/delta opioid receptor interactions: an emerging approach to novel analgesics. *AAPS J* 2006; **8:** E118−25.

10 Dietis N, Guerrini R, Calo G, Salvadori S, Rowbotham DJ, Lambert DG. Simultaneous targeting of multiple opioid receptors: a strategy to improve side-effect profile. *Br J Anaesth* 2009; **103:** 38−49.

11 Wadenberg ML. A review of the properties of spiradoline: a potent and selective kappa-opioid receptor agonist. *CNS Drug Rev* 2003; **9:** 187−98.

12 Walsh SL, Strain EC, Abreu ME, Bigelow GE, Wadenberg ML. Enadoline, a selective kappa opioid agonist: comparison with butorphanol and hydromorphone in humans. A review of the properties of spiradoline: a potent and selective kappa-opioid receptor agonist. *Psychopharmacology (Berl)* 2001; **157:** 151−62.

13 Mollereau C, Mouledous L. Tissue distribution of the opioid receptor-like (ORL1) receptor. *Peptides* 2000; **21:** 907−17.

14 Mogil JS, Nessim LA, Wilson SG. Strain-dependent effects of supraspinal orphanin FQ/nociceptin on thermal nociceptive sensitivity in mice. *Neurosci Lett* 1999; **261:** 147−50.

15 Cowan A. Update of the general pharmacology of buprenorphine. In: Lewis J, Cowan A, eds. Buprenorphine: combating drug abuse with a unique opioid. New York: Wiley, 1995; 31−47.

16 Rothman R, Qi N, Xu H. Buprenorphine: a review of the binding literature. In: Lewis J, Cowan A, eds. Buprenorphine: combating drug abuse with a unique opioid. New York: Wiley, 1995; 19−30.

17 Kest B, Hopkins E, Palmese CA, Chen ZP, Mogil JS, Pintar JE. Morphine tolerance and dependence in nociceptin/orphanin FQ transgenic knock-out mice. *Neuroscience* 2001; **104:** 217−22.

18 Ueda H, Inoue M, Takeshima H, Iwasawa Y. Enhanced spinal nociceptin receptor expression develops morphine tolerance and dependence. *J Neurosci* 2000; **20:** 7640−7.

19 Spagnolo B, Calo G, Polgar WE, et al. Activities of mixed NOP and mu-opioid receptor ligands. *Br J Pharmacol* 2008; **153:** 609−19.

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