Opioid receptors

J McDonald PhD
DG Lambert PhD FRCA

Revalidation
FOR ANAESTHETISTS
RCA Revalidation matrix
Matrix reference 1A02, 2E01, 3E00

Key points

The opioid system comprises four types of receptor: μ- (MOP), δ- (DOP), κ-opioid and nociceptin (NOP).

Opioid receptors all have selective endogenous peptides.

Analgesia elicited by clinically applied opioids act predominantly via the MOP receptor.

Tolerance to MOP receptor analgesics maybe attenuated by both NOP and DOP receptor antagonism.

Mixed-opioids represent a new focus in opioid receptor pharmacology.

Opium and its derivatives have been used for centuries; findings of fossilized opium poppy seeds dating as far back as 30 000 yr suggest the use of opium by Neanderthal man. In 1799, Friedrich Serturner discovered the major active ingredient of opium, which he named morphine and opioid pharmacology was born. Morphine and other opioid drugs act on an endogenous opioidergic system which is not only involved in setting pain (nociceptive) threshold and controlling nociceptive processing but also participates in modulation of gastrointestinal (GI) function, endocrine and autonomic function, and a possible role in cognition.

Evidence for the existence of multiple opioid receptor types was reported based on the different anatomical location and the pharmacological profiles of compounds that were eventually used to name them, that is, *m*orphine (*m*u), *k*etocyclazocine (*k*appa), and vas *d*eferens (*d*elta). In subsequent years, the molecular cloning, functional expression, and characterization of these receptors has been reported and it is understood that each receptor is the product of a single gene; OPRM1 (μ), OPRK1 (κ), and OPRD1 (δ). The nociceptin orphanin FQ peptide receptor was first reported as an opioid receptor-related clone, formerly named LC132 or ORL-1, and with no known endogenous ligand, the receptor was dubbed 'orphan'.[1 2] Its orphan status remained until the formal identification of an endogenous ligand, nociceptin/orphanin FQ (N/OFQ), isolated from brain extracts, utilizing a process known as reverse pharmacology (the searching of a drug to activate a molecular cloned receptor), simultaneously by Meunier and colleagues[3] and Reinscheid and colleagues.[2]

The International Union of Basic and Clinical Pharmacology (IUPHAR) nomenclature regards MOP (μ), KOP (κ), and DOP (δ) as 'classical' opioid receptors, a distinction based on the sensitivity to the opioid antagonist naloxone. Nociceptin receptor (NOP) is used for the N/OFQ peptide receptor which is currently classified as a non-opioid member of the opioid receptor family, based on similarities in structure and localization to the classical opioid receptors but insensitivity to naloxone. Putative subtypes of the classical opioid receptors have been suggested, $\mu_{1,\ 2,\ \text{and}\ 3}$ for MOP, $\delta_{1\ \text{and}\ 2}$ for DOP, and $\kappa_{1a,\ 1b,\ 2a,\ 2b,\ \text{and}\ 3}$ for KOP, based on a variety of evidence such as the differential modulation by drugs on functional responses *in vivo* and *in vitro*, along with incomplete tolerance of functional responses seen when using different drugs targeting the same receptor. However, the legitimacy of these pharmacologically defined subtypes is deficient when placed alongside the molecular research which reveals how individual opioid receptors are encoded by a single respective gene, and that the knock-out of a single receptor gene leads to the loss of all functionality associated with the receptor. Pharmacologically defined subtypes of receptor are the consequence of a number of processes including alternative splicing of opioid receptor genes.

To understand the process of alternative splicing, and how it may result in different subtypes of receptor, it is necessary to understand how opioid receptor genes are encoded in genomic DNA which consists of introns and exons. Introns represent nucleotide sequences encoded by the gene that are removed in the formation of RNAs, while exons represent nucleotide sequence encoded by the gene that remains in the final RNA. In the case of opioid receptors, gene splicing removes the intron sequences leaving a messenger RNA that is translated into the final protein structure of the receptor. Alternative splicing concerns how, in this process, after the removal of the introns, the exons can be reattached in different ways resulting in different RNAs and therefore leading to different opioid receptor proteins, that is, opioid receptor subtypes.

Cellular mechanisms of action

All four receptors are G-protein-coupled receptors sharing a similar seven transmembrane topology (Fig. 1). G-protein-coupled receptors have no direct link with effector proteins, instead the message is relayed via a G-protein. All four subtypes of receptors preferentially couple to inhibitory G-proteins and the activation of opioid

J McDonald PhD
Department of Cardiovascular Sciences
Division of Anaesthesia, Critical Care and Pain Management
RK-CSB, Leicester Royal Infirmary
Leicester LE2 7LX
UK

DG Lambert PhD FRCA
Department of Cardiovascular Sciences
Division of Anaesthesia, Critical Care and Pain Management
RK-CSB, Leicester Royal Infirmary
Leicester LE2 7LX
UK
Tel: +44 116 258 5291
Fax: +44 116 285 4487
E-mail: dgl3@le.ac.uk
(for correspondence)

doi:10.1093/bjaceaccp/mku041
Continuing Education in Anaesthesia, Critical Care & Pain | 2014

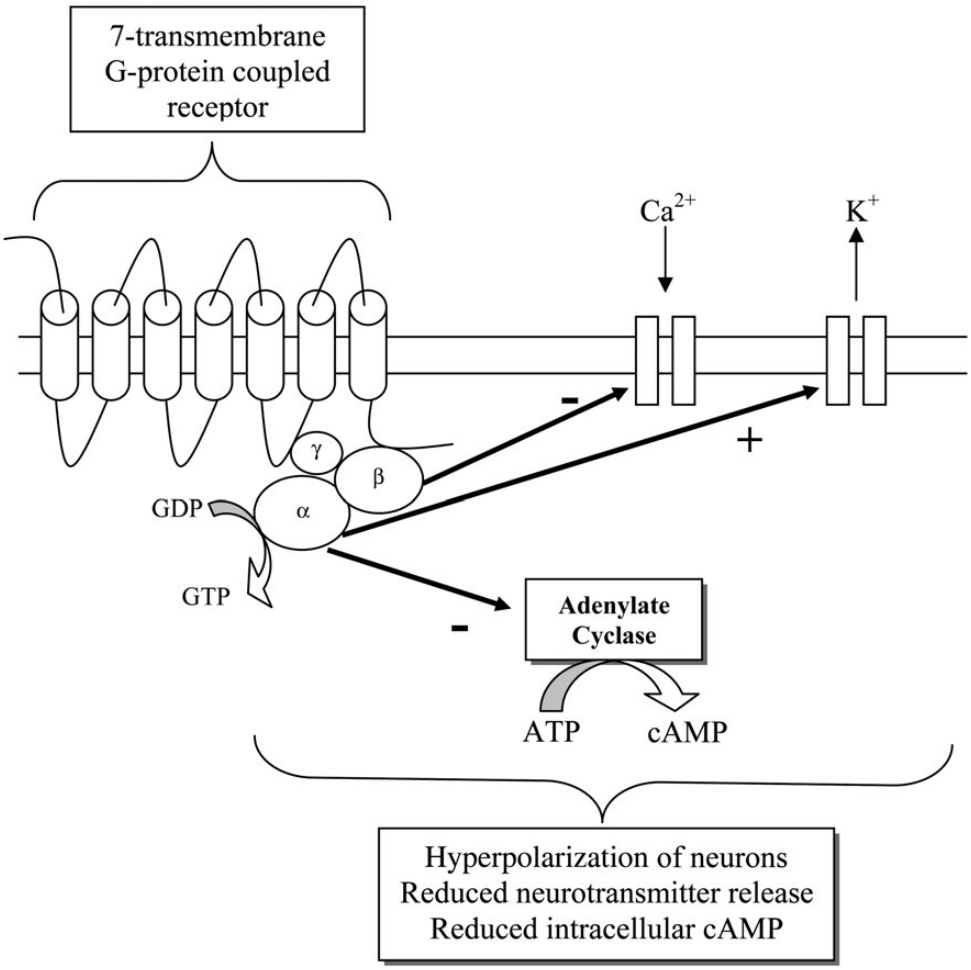

Fig 1 The seven transmembrane structure of opioid G-protein-coupled receptors. Activation by opioid receptor ligands leads to initiation of intracellular transduction pathways that include stimulation of potassium efflux, inhibition of VSCCs, and inhibition of adenylyl cyclase.

receptors, for example, MOP with morphine leads to a variety of cellular processes; (i) closing of voltage-sensitive calcium channels (VSCCs), (ii) stimulation of potassium efflux leading to hyperpolarization, and (iii) reduced cyclic adenosine monophosphate (cAMP) production via inhibition of adenylyl cyclase. Overall, this results in reduced neuronal cell excitability leading to a reduction in transmission of nerve impulses and inhibition of neurotransmitter release (Fig. 1).

Endogenous and exogenous ligands

Endogenous opioid peptides are cleaved from pro-hormone precursors. The endogenous DOP receptor peptides met-enkephalin and leu-enkephalin (cleaved from proenkephalin and prodynorphin) give rise to the KOP receptor agonists dynorphin A and B, while N/OFQ is derived from the polypeptide precursor pre-pro−N/OFQ. Proopiomelancortin encodes the peptide β-endorphin which has agonist activity at the three classical opioid receptors. It is assumed that the endogenous MOP receptor peptides endomorphin 1 and 2 are cleaved from a larger precursor protein(s); however, to date, this protein remains elusive (Tables 1 and 2).

The majority of opioid drugs used clinically elicit their action through activation of the MOP receptor, and are mainly used to produce analgesia, being effective against high-intensity mechanical, thermal, and chemical stimuli. The prototypical MOP agonist is the alkaloid morphine, purified from opium. Semi-synthetic compounds, such as diamorphine and codeine, are the result of chemical modifications to the natural opiate morphine. Codeine represents a partial agonist, with reduced efficacy compared with a full agonist like morphine acting at the MOP receptor, and for this reason is used for the treatment of less severe pain. Fully synthetic MOP agonists, with structures unrelated to morphine, have also been identified and are used clinically including the piperidine series such as meperidine, fentanyl, and methadone along with the benzomorphan series which include pentazocine (Table 1).

Selective antagonists are available for the classical opioid receptors, naltrindole for DOP, norbinaltorphimine for KOP, and D-Phe-Cys-Tyr-D-Trp-Orn-Thr-Pen-Thr-NH_2 (CTOP) for MOP. As

Table 1 Opioid receptor nomenclature including information on various agonists and antagonists selective for different receptor subtypes. *Spiradoline (U-62,066E) and *Enadoline (CI-977) are KOP selective ligands that have undergone clinical trials but are not currently in use[4 5]

	Current receptor nomenclature			
	DOP	KOP	MOP	NOP
Previous nomenclature	OP_1, δ	OP_2, κ	OP_3, μ	OP_4, LC132, ORL_1
G-protein coupling	$G_{i/o}$	$G_{i/o}$	$G_{i/o}$	$G_{i/o}$
Endogenous ligand	Leu-enkephalin, Met-enkephalin	Dynorphin A	β-Endorphins, endomorphin 1 and 2	Nociceptin/OrphaninFQ (N/OFQ)
Synthetic agonists	DPDPE DSTBULET SNC-80	U69593	DAMGO	Ro64-6198
Selective antagonists	Naltrindole	Nor-BNI	CTOP	J-113397 (synthetic) UFP-101 (peptide)
Clinical drugs	None	*Spiradoline, *Enadoline	Morphine, fentanyl, diamorphine, methadone, codeine	None
Mixed action	Pentazocine (partial agonist) Buprenorphine (inactive)	Pentazocine (partial agonist) Buprenorphine (antagonist)	Pentazocine (antagonist) Buprenorphine (partial agonist)	Pentazocine (inactive) Buprenorphine (partial agonist)
Naloxone sensitivity	Antagonist	Antagonist	Antagonist	Inactive

Table 2 The key clinical effects mediated by opioid receptor subtypes and selectivity of endogenous opioid peptides. N/OFQ, nociceptin orphanin FQ; ×, no affinity/effect; √, low affinity/effect; √√, intermediate affinity/effect; √√√, high affinity/effect (modified from Rang and colleagues)[6]

Clinical effect	Receptor subtype			
	MOP	KOP	DOP	NOP
Supraspinal: analgesia	√√√	×	×	×
Spinal: analgesia	√√	√	√√	√√
Respiratory depression	√√√	×	√	×
Euphoria	√√√	×	×	×
Endogenous ligand				
β-Endorphins	√√√	√√√	√√√	×
Dynorphin A	√√	√√√	√	√
Leu-enkephalin	√	×	√√√	×
Met-enkephalin	√√	×	√√√	×
Dynorphin A/B	√√	√√√	√	√
Endomorphin 1/2	√√√	×	×	×
N/OFQ	×	×	×	√√√

previously described, the actions of all three receptors can be inhibited by the non-selective antagonist naloxone, used traditionally in defining opioid receptors. While the actions of N/OFQ at NOP are not inhibited by naloxone, NOP selective antagonist drugs have been developed, including a synthetic ligand J-113397 and UFP-101, a peptide developed through modification to the endogenous N/OFQ peptide (Table 1).

Opioid receptor types

μ-Opioid receptor

The MOP receptor was the last of the classical opioid receptors to be cloned and is located throughout the central nervous system (CNS) in areas involved in sensory and motor function, including regions concerned with the integration and perception of these senses, for example, the cerebral cortex and amygdala (part of the limbic system).

The highest density of MOP receptors are found in the caudate putamen (of the basal ganglia). MOP receptors are located presynaptically on primary afferent neurones within the dorsal horn of the spinal cord where they inhibit glutamate release and hence transmission of nociceptive stimuli from C- and Aδ-fibres.

The periaqueductal grey (PAG) is an area of the midbrain involved in the central control of nociceptive transmission. Efferent outflow from the PAG descends to the spinal cord where it acts to inhibit nociceptive transmission in afferent fibres; this pathway is known as the descending inhibitory control pathway. The efferent outflow from the PAG is constrained under resting conditions by the actions of γ-amino butyric acid (GABA). GABA is the main inhibitory transmitter in the brain, preventing neurotransmission through hyperpolarizing cell membranes which inhibit action potential firing, here GABA acts to reduce antinociceptive outflow from the PAG (Fig. 2). High densities of MOP receptor are found in the PAG and the analgesia of some opioid drugs is proposed to come about by block of the resting inhibitory GABA activity into this region of the brain. The inhibitory effect of MOP receptor firing blocks the inhibitory actions of GABA, removing its tonic block and stimulating antinociceptive outflow to the spinal cord.

Major side-effects that come about from the use of MOP agonists include respiratory depression through a reduction in the sensitivity of chemoreceptors (CNS/PNS) to hypercapnia. MOP agonists also inhibit GI tract secretions and peristalsis often causing constipation, and have predominantly inhibitory effects on the cardiovascular system, thermoregulation, hormone secretion, and immune function.

Studies using MOP receptor knockout mice have defined the role MOP plays tonically and when stimulated by exogenously applied ligands. MOP receptor knockout mice show increased sensitivity to thermal pain, implicating the receptor in this mode of nociception. However, no change in threshold from pain elicited via mechanical stimuli was seen. None of the predicted effects or side-effects of morphine were seen in mice lacking the MOP receptor. MOP receptor knockout mice showed no change in respiratory function demonstrating no tonic role in this system. Analgesia and reward were absent and investigation of acute morphine showed no respiratory action. This genetic approach indicates that both the wanted and unwanted effects of morphine are due to action at the MOP receptor.

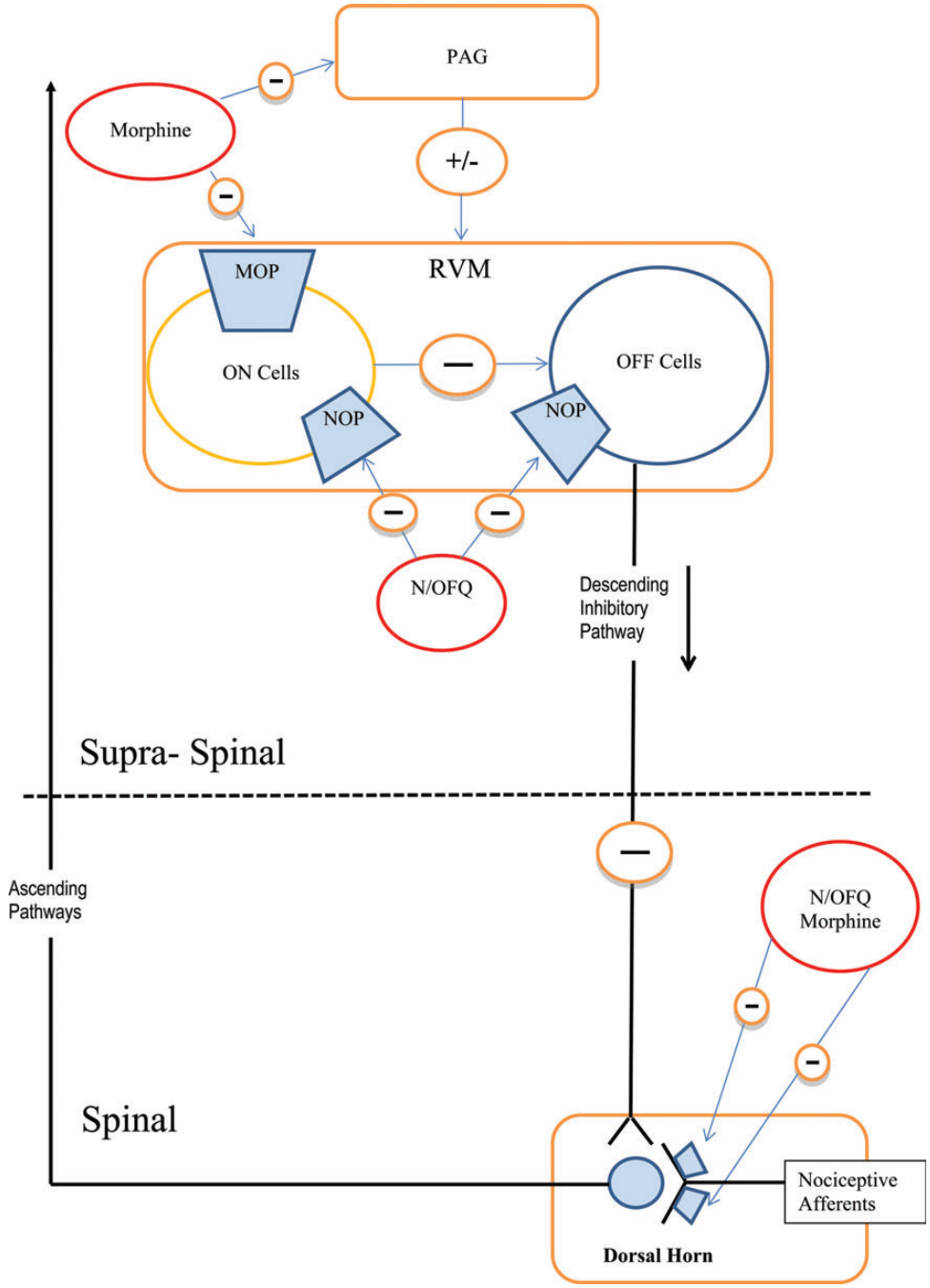

Fig 2 In the rostral ventromedial medulla (RVM), ON cells tonically inhibit the firing of OFF cells, through the release of the inhibitory neurotransmitter GABA. OFF cell firing induces analgesia via activation of descending inhibitory control pathways to the spinal cord. MOP agonists (e.g. morphine) cause analgesia supraspinally by inhibiting ON cells, that is, removal of the GABA-mediated inhibition resulting in the firing of OFF cells and activation of descending inhibitory control and analgesia. NOP receptors situated at ON and OFF cells give rise to N/OFQ-mediated anti-opioid action through a direct inhibition of the OFF cells, thus preventing MOP receptor lead activation of the descending inhibitory control pathway.

While the main analgesic effects of opioids are elicited by central activation of opioid receptors, a number of the common side-effects including reduced GI motility, urinary retention, and pruritus are regulated by activation of peripherally located opioid receptors. The use of peripherally acting opioid receptor antagonists may reduce a number of these side-effects. Indeed, methylnaltrexone, a peripherally acting opioid antagonist, in clinical trials was effective at treating opioid-induced constipation, while alvimopan, another peripherally acting MOP antagonist, was shown to reduce the duration of postoperative ileus and postoperative nausea and vomiting.

MOP opioids are used for analgesia in both acute pain and chronic pain; however, opioids produce tolerance where dose escalation is required to maintain the same degree of analgesia received. Accompanying escalating doses of MOP agonist is the increased prevalence of MOP-mediated side-effects. Tolerance that develops to MOP agonists is influenced by the DOP receptor. In animals in which the DOP receptor is blocked, either with the use of DOP selective antagonists or through genetic knock-out, there is a reduction in MOP tolerance. Analgesic responses to a fixed daily morphine dose were lost after 5 days in wild-type mice; however, in DOP-receptor knockout mice, no such tolerance could be determined after 8 days administration.[7] In humans, this strategy could be achieved through the administration of two drugs, a MOP agonist and DOP antagonist; however, there are currently no DOP antagonists licensed for clinical use in man, and with this in mind, the challenge has been to discover non-selective drugs that can act as both an agonist at the MOP receptor while antagonizing the DOP. Those drugs of interest include UFP-505 a non-selective bi-functional compound which interacts with both the DOP and MOP receptor through distinct binding interactions, and MDAN-21 a bivalent ligand in which the pharmacologically active region (pharmacophore) of oxymorphone (MOP agonist) is attached to a naltrindole (DOP antagonist) pharmacophore by way of a 21-atom linker.[8 9] Bivalent ligands with MOP agonism and DOP antagonism not only have a reduced tolerance profile but also have improved antinociception, along with decreased physical dependence when compared with morphine. Hence, the development of 'mixed-opioids' represents a potential strategy to design novel classes of analgesics.

While the focused development and use of mixed opioids represents a new therapeutic strategy, there are already a number of opioid drugs in current use which have mixed sites of action. For example, pentazocine behaves as a partial agonist at DOP and KOP receptors resulting in analgesia, while at MOP receptors, it is an antagonist, the consequence of which is reduced risk of respiratory depression. Other mixed opioids in clinical use include buprenorphine, a drug with partial agonist activity at MOP and NOP receptors. Buprenorphine has a bell-shaped response curve for its analgesic activity *in vivo* such that at low and intermediate doses, an analgesic response results, at higher doses, the analgesic response is decreased back to baseline. The complex pharmacology associated with buprenorphine may be explained by its agonist activity at MOP, resulting in analgesia at low and intermediate doses, and NOP, resulting in an anti-opioid/anti-analgesic action at higher doses (see later).

δ-Opioid receptor

The DOP receptor was the first to be cloned and shows restricted distribution relative to the other opioid receptors. Highly selective agonists that are peptide (DSTBULET), cyclic peptide (DPDPE), and synthetic/non-peptidic (SNC-80) in structure are available for the DOP receptor, along with high-affinity antagonists, for example, the non-peptide naltrindole. All these ligands are experimental only.

Highest densities of the receptor are found in the olfactory bulb, cerebral cortex, nucleus accumbens, and the caudate putamen. DOP receptors are located presynaptically on primary afferents where they inhibit the release of neurotransmitter. Through both spinal and supraspinal sites, the receptor is involved in the antinociceptive/analgesic actions of some opioids. DOP receptor agonists have also been shown to reduce GI tract motility and cause respiratory depression, limiting clinical interest.

Interactions between MOP and DOP receptors have been suggested based on the pharmacology of DOP agonists *in vivo* and *in vitro*, and the effects of DPDPE have been tested in mice lacking the MOP receptor which showed, dependent on the response measured, that DPDPE displayed various actions from ineffective to fully and partially effective. In mice lacking the MOP receptor, respiratory depression of DOP agonists was absent which further suggests cross-talk between the MOP and DOP receptors or that DOP agonists have activity at the MOP receptors *in vivo*, the latter is unlikely since even the most selective DOP compound deltorphin showed attenuated responses in MOP receptor knock-out animals. Interestingly, co-administration of a MOP agonist such as morphine with a sub-antinociceptive dose of leu-enkephalin, a DOP agonist, has a synergistic analgesic action. Such an approach could be useful in limiting the dose of morphine required for analgesia, therefore attenuating the development of tolerance. To re-emphasize, there are currently no clinically available DOP ligands.

κ-Opioid receptor

The KOP receptor was the second of the opioid receptor family to be cloned. The prototypical agonist of the κ-receptor is the non-peptide benzomorphan, ketocyclazocine the actions of which have been shown to be distinct from those elicited by stimulation of the MOP receptor (e.g. sedation without marked effects on heart rate). Two synthetic KOP receptor agonists, spiradoline (U-62,066E) and enadoline (CI-977), have undergone clinical trials for their analgesic actions. While spiradoline produced promising analgesia in animals, clinical data show that spiradoline produces adverse effects such as diuresis, sedation, and dysphoria at doses lower than those needed for analgesic effects. Enadoline produced similar side-effects, including sedation, confusion, dizziness along with increased urinary output, and feelings of depersonalization. The side-effects elicited by these and other KOP receptor agonists have as yet limited their effective clinical usage. However, it has been shown recently that KOP agonists, such as enadoline, may have neuroprotective actions via their ability to inhibit post-ischaemic glutamate release. The advantage of KOP receptor agonists over other opioid ligands is that they do not cause respiratory depression, although their effective use may be limited by dysphoria; this is not seen in all subjects.

Nociceptin receptor

At the cellular level, N/OFQ produces similar actions to those of the classical opioids resulting in reduced neuronal excitability and

inhibition of transmitter release. Initial studies concentrated on the role of N/OFQ and NOP in pain. However, exogenous administration of N/OFQ has been shown to have effects on locomotion, stress and anxiety, feeding, learning and memory, reward/addiction, and urogenital activity.[10]

N/OFQ under laboratory conditions has a pronociceptive, anti-analgesic effect when applied supraspinally, while spinally, N/OFQ causes analgesia at high doses and hyperalgesia at low doses. Nociceptin earned its name from the conclusions of the original paper, in which intracerebroventricular (i.c.v.) injection of N/OFQ caused hyperalgesia. This finding is now refuted, i.c.v. N/OFQ does not cause hyperalgesia, the pain threshold of mice administered with i.c.v. N/OFQ is the same as vehicle-treated animals. N/OFQ has anti-opioid actions, such that when administered supraspinally, it reverses the action of exogenous applied opioid drugs (Fig. 2). N/OFQ also reverses opioid-mediated stress-induced analgesia. It is now known that stress-induced release of endogenous opioids takes place in the animal paradigms utilizing i.c.v. administration, and administering N/OFQ via this route would reverse this action and accounts for the original observations of a hyperalgesia (animals were compared with vehicle-treated groups in which the stress-induced release of endogenous opioids was active).

The N/OFQ/NOP system is believed to play a role in the development of tolerance to morphine analgesia. NOP receptor 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. This action has also been confirmed through the actions of potent selective NOP antagonists, which also attenuate morphine tolerance. Combined use of NOP receptor antagonists in conjunction with MOP agonists is an exciting possibility.

The field of opioid pharmacology currently has a new focus, a move away from the highly selective drugs of old towards bivalent, bi-functional, mixed-opioids. This represents an important shift and one that will hopefully lead to new clinically effective analgesics with reduced side-effect profiles.

Declaration of interest

None declared.

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