Stimulatory effects of opioids

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Opioid receptors

Opioid receptors exist as three principle types, known as μ, δ and κ and have been recently reclassified by an International Union of Pharmacology subcommittee as OP1(δ), OP2(κ) and OP3(μ). All three receptors have been cloned, and have pharmacological characteristics consistent with those of endogenous receptors. Multiple isoforms of each subtype have been proposed on pharmacological grounds but this remains controversial, as no subtypes have been identified using molecular biology techniques, although the possibility of post-translational modification of a common gene product remains.

Opioid receptors belong to the family of guanine-nucleotide-binding protein (G-protein) coupled receptors, consisting of seven transmembrane-spanning domains, an extracellular N-terminus, and an intracellular C-terminus. Such cell surface receptors have no direct contact with their final effector molecules. In this process receptors relay information via G proteins to activate effector proteins (fig. 1).

G proteins are heterotrimeric membrane-associated proteins consisting of α, β and γ subunits. They are able to activate effector enzymes by a relatively straightforward process, the “G protein cycle” (fig. 2). Historically, G proteins are classified using two types of bacterial toxin, pertussis (PTX) and cholera (CTX). Pertussis toxin adenosine di-phosphate ribosylates inhibitory G proteins (eg. G) and prevents interaction with the effector molecule. Cholera toxin prevents the GTP (Guanine–triphosphate)–adenosine diphosphate (ADP) ribosylation inhibitory G proteins (eg. G) and activates stimulatory G proteins (G). The released calcium may then be involved in a wide range of events; for example, it may bind to calcium-binding proteins such as calmodulin, or cause further release of calcium. DAG may then be cleaved to produce arachidonic acid or cause the activation of protein kinase C (PKC). This enzyme also phosphorylates cellular proteins, such as ion channels in neurons.

Opioids and neurotransmitter release

Opioids produce their analgesic effects via an inhibition of excitatory neurotransmission that involves substance P and glutamate. This is achieved by coordinated changes at a cellular level, namely closure of voltage-sensitive calcium channels, activation of potassium currents, resulting in slowing of the action potential, and a reduction in cAMP, which may inhibit the inwardly rectifying cation current I$_h$. Opioids also phosphorylate cellular proteins, such as ion channels in neurons.

Keywords: metabolism, second messengers; opioids; opioids, tolerance; receptors, opioid
cause release of a variety of neurotransmitters from DRG neurones and stimulate noradrenaline release from SK-N-SH cells. Indeed, opioids have been shown to increase the duration of the action potential and reduce $K^{+}$ current in DRG neurones and to increase aeuronal firing rate.

It is thought that opioid stimulation of a variety of second-messenger systems, including adenylyl cyclase activity, phosphoinositide hydrolysis and elevations in intracellular calcium, may underlie the opioid stimulation of neuronal activity, and we shall discuss this in the remainder of the present review. The physiological role of these stimulatory actions is unclear, and the subject of much debate.

**Opioid stimulation of adenylyl cyclase**

A stimulatory effect of opioids on adenylyl cyclase activity was first shown more than two decades ago, after chronic opioid treatment. In the same year a stimulatory effect of morphine on adenylyl cyclase was shown in rat corpus striatum. Later it was demonstrated that opioids positively regulated adenylyl cyclase activity, in chick heart cells, rat heart and DRG neurones. Some studies have found that the stimulation of adenylyl cyclase was $G_{i/o}$ linked, whereas in other studies it was found to occur in a PTX-sensitive $(G_{i/o})$ manner involving $G_{i/o}$ subunit stimulation of type II adenylyl cyclase. It has been shown that chronic opioid treatment of the $\mu$ receptor induces type V adenylyl cyclase super-activation in COS-7 cells co-transfected with the $\mu$ opioid receptor and type V adenylyl cyclase, which is time dependent, reversible and may involve $\beta$ subunits. In contrast to this, acute opioid inhibition of adenylyl cyclase in the same cell line occurs via $\alpha_{i/o}$ subunits. The same group found that opioid action of adenylyl cyclase is isozyme specific. Acute activation of the $\mu$ receptor stimulates type II, IV and VII adenylyl cyclase while inhibiting the activity of types I, V, VI and VIII. Superactivation of adenylyl cyclase stimulated types I, VI and VIII. In SK-N-SH neuroblastoma cells, activation of the $\mu$ opioid receptor stimulates the formation of cAMP in a process that is calcium/calmodulin (Ca$^{2+}$/CaM) dependent and is secondary to the opening of voltage-sensitive calcium channels (VOCCs). The stimulatory effect is seen
only at nM concentrations of μ agonist, whereas at higher concentrations (0.1 μM) an inhibitory effect is seen. This bimodal effect of stimulation at low concentrations of agonist and inhibition at higher doses, was also noted in myenteric plexus. In rat olfactory bulb and rat brain slices, Xenopus oocytes transfected with the μ receptor and human kidney cells transfected with the δ receptor, activation of adenyl cyclase activity also occurs in F11 neuroblastoma sensory neuron hybrid cells, rat brain slices, Xenopus oocytes transfected with the κ receptor and human kidney cells transfected with the δ receptor.

Activation of adenyl cyclase will lead to accumulation of cAMP, which is responsible for the activation of cAMP-dependent protein kinase A. PKA activity will produce an increased level of protein phosphorylation, potentially leading to altered cellular responses. An example would be phosphorylation of synaptic proteins in nerve terminals or phosphorylation of ion channels. In addition, opioid activation of adenyl cyclase leading to production of cAMP may activate the inwardly rectifying cation current (I)_2, the consequence of which is increased neuronal firing.

Opioid stimulation of phosphoinositide hydrolysis

Activation of both recombinant and endogenous opioid receptors stimulates phosphoinositide turnover, leading to the production of Ins(1,4,5)P_3, in a variety of cell lines. In SH-SY5Y cells, activation of PLC was shown to occur secondary to opening of L-type calcium channels. As in all cases tested the breakdown was PTX sensitive, the role of PLC activation does not seem to be dependent upon calcium influx. These differences in PLC signalling between NG108-15 cells but the activation of PLC does not seem to be dependent upon calcium influx. These differences in PLC signalling between NG108-15 cells...
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Both recombinant \( \mu \) and \( \delta \) opioid receptors have also been shown to stimulate PLC when expressed in Chinese hamster ovary (CHO) cells, as have transiently transfected \( \delta \) receptors in human kidney cells, the \( \delta \) receptor expressed in Xenopus oocytes and in COS-7 cells transiently expressing the \( \mu \) receptor. Other reports of opioid stimulation of phosphoinositide hydrolysis include rat-brain aggregates and spinal cord-DRG neurones. Opioid stimulation of phosphoinositide hydrolysis has been proposed as a possible mechanism for the opioid-induced release of adenosine in dorsal root ganglion neurons. Examples of opioid-receptor stimulation of Ins(1,4,5)P₃ are shown in Figure 5.

Opioid stimulation of Ins(1,4,5)P₃ formation also causes concomitant activation of PKC in SH-SY5Y cells and NG108-15 cells, presumably via DAG formation. It has also been shown that this occurs with the \( \delta \) receptor in Xenopus oocytes and rat spinal trigmenial neurons. The activation of PKC may lead to the phenomenon of tolerance, that is the reduced ability of opioids to produce pharmacological effects, particularly analgesia, after repeated or prolonged exposure to them. Various studies have provided evidence indicating that the stimulatory effects of opioids may be involved in the development of tolerance to such drugs. The development of tolerance was prevented by blocking the stimulatory effects of opioids with etorphine or dynorphin fragments in dorsal root ganglia. Chronic inhibition of intracellular calcium release or PKC activation significantly reduced the development of morphine tolerance in rats. G-protein-coupled receptors contain PKC phosphorylation sites on the third intracellular loop and on the C terminus. Indeed it is known that the C terminus of the \( \delta \)-opioid receptor is required for agonist-mediated phosphorylation.
of these sites may cause receptor–G protein uncoupling, resulting in reduced efficacy of an opioid. Various studies have shown that potential phosphorylation sites on the C terminus are important in desensitization and down-regulation of opioid receptors, which may be involved in the development of tolerance. Acute application of opioids to δ receptors lacking C-terminal amino acids (that is, loss of PKC phosphorylation sites) converts a sustained Ins(1,4,5)P3 response to a monophasic response when such receptors are expressed in CHO cells. Therefore the C-terminus of the δ receptor appears to be important in acute PLC coupling. In contrast to this, another study found that the C-terminus of the δ-opioid receptor may not be important in agonist-mediated activation. PKC activation was shown to trigger the onset of stimulatory effects to opioids on electrically induced cAMP formation, as seen in tolerant tissue. Other studies have illustrated that increased PKC activity contributes to the development of tolerance through various mechanisms. Opioid-induced increases in PKC activity have been shown to modulate K+ channels; μ-opioid-induced activation of PKC in SH-SY5Y cells may inhibit Ca2+-activated K+ channels, which would prevent hyperpolarization, while in Xenopus oocytes transfected with the μ receptor, PKC activity causes desensitization of inwardly rectifying K+ channels, although this is controversial. In addition to the possible role of PKC in the development of opioid tolerance (for a review, see Smart and Lambert 1996), other mechanisms cannot be excluded. There is evidence that bimodal actions of opioids may be of importance in this phenomenon, as discussed later.

**Opioid induced increases in intracellular calcium**

Calcium ions are one of the most ubiquitous signal transduction elements, and regulate a range of diverse cellular functions from neurotransmission to cell proliferation. An intracellular calcium concentration of ~100 nM compared with an extracellular concentration of ~2.5 mM provides a concentration gradient to make calcium ions an ideal second messenger. The cell is able to use both intra- and extracellular calcium as a signalling molecule. Extracellular calcium can enter the cell through numerous types of calcium channels, such as voltage-operated calcium channels that allow calcium influx after depolarization. Intracellular calcium is released from stores within the cell after agonist stimulation, as described earlier.

Opioid-induced increases in intracellular calcium may come from intracellular or extracellular sources or both. In NG108-15 cells there is a close temporal and dose–response relationship between Ins(1,4,5)P3 formation and increases in [Ca2+]i suggesting that the induced increase in Ins(1,4,5)P3 formation is sufficient to cause a mobilization of intracellular calcium, and in the same cell line inhibition of PLC blocks the increase in [Ca2+]i. Smart and colleagues found that in SH-SY5Y cells μ opioids allow calcium influx to stimulate PLC activity, resulting in Ins(1,4,5)P3 formation, and μ opioid receptor activation in the same cells causes a modest increase in [Ca2+]i, which may be attributable to calcium entry. The EC50 values obtained in the studies above for increases in [Ca2+]i were much greater than for Ins(1,4,5)P3 formation. This prompted the hypothesis that that localized increases in [Ca2+]i, in the vicinity of the plasma membrane may activate PLC, but these would be too small to be detected measuring [Ca2+]i, in whole-cell suspensions. However, another study found that synergism with other agents was necessary to produce an opioid-mediated response in SH-SY5Y cells. Elevation of intracellular calcium by δ and μ receptors occurred only when there was ongoing muscarinic receptor activation, although the nature of the link between muscarinic occupancy and opioid mobilization of [Ca2+]i, was not clear. “Priming” of PLC, this time with bradykinin, was also necessary in a study carried out by Okajima and colleagues in NG108-15 cells. It remains unclear why there is a discrepancy between the results of the studies using SH-SY5Y cells; it may be that differing cell culture techniques cause some cell lines to lack certain types of VOCCs. Factors increasing the stimulatory effects of opioids on intracellular calcium in NG108-15 and SK-N-SH cells have been studied by Sarne and Gafni, who concluded that synchronization of the cell cultures, differentiation of the cells and synergism with other stimulatory agents increased the probability and the amplitude of the response to the opioid agonist. Of these, synchronization had the most pronounced effect, possibly by producing a more homogenous cell population, and might explain the differences between the SH-SY5Y cell studies described above. Endogenous δ-opioid receptors in undifferentiated NG108-15 cells can cause release of intracellular calcium from thapsigargin-sensitive stores in a PTX-sensitive manner. When expressed in CHO cells, the recombinant δ receptor couples to PLC and causes an increase in [Ca2+]i, as does the μ receptor. In Xenopus oocytes the κ receptor causes mobilization of intracellular calcium, as it does when expressed in CHO cells (Harrison and Lambert, unpublished observation). There is evidence to suggest that there may be interaction between the Ins(1,4,5)P3-Ca2+ and the adenylyl cyclase–cAMP signalling systems in rat heart, as blockade of increases in [Ca2+]i, induced by κ opioid receptors also results in suppression of cAMP accumulation.

As well as opioid mobilization of intracellular calcium from Ins(1,4,5)P3-sensitive stores, there has been a report to suggest that the δ opioid receptor can regulate the activity of ryanodine receptors on intracellular calcium stores in SK-N-BE cells. Like insulin, the insulin (1,4,5)P3 receptors, ryanodine receptors are calcium-release channels located on intracellular calcium stores. Cyclic adenosine diphosphate (cADP) ribose, synthesized from ATP, may be the endogenous trigger for calcium release. In this report the mobilization of [Ca2+]i occurred only at μM concentration of agonist, was independent of extracellular calcium and did not follow a biphasic or monophasic pattern typical of increases in [Ca2+]i, seen in other studies. The response was neither CTX- nor PTX-sensitive. It was suggested that there could be direct coupling between the δ receptor and the ryanodine receptor on the endoplasmic reticulum, or the δ receptor could stimulate the generation of cADP ribose.

Mobilization of calcium from ryanodine-sensitive stores in rat myocytes has also been observed.
The physiological consequences of opioid-induced mobilization of intracellular calcium remain unclear. In one study it was shown that the rise in [Ca\(^{2+}\)], was insufficient to cause a release of noradrenaline.\(^{46}\) It may be that release of intracellular calcium activates a calcium-sensitive isoform of PKC or augments increases in intracellular calcium from other sources.

Opioids can cause increases in intracellular calcium by stimulating calcium influx into the cell. Morphine was shown to stimulate the Ca\(^{2+}\) component of the action potential\(^{27}\) and to activate calcium channels.\(^{24}\) Moreover, opioids were shown to increase the Ca\(^{2+}\) spike of the action potential in dorsal root ganglion neurons.\(^{57}\) The increase in intracellular calcium is dependent upon extracellular calcium in several cases. For example, \(\kappa\) opioids,\(^{28,84,85}\) \(\delta\) opioids\(^{82,86}\) and \(\mu\) opioids\(^{82,83,92}\) have been reported to cause calcium influx in a variety of cell types and uptake of \(\text{Ca}^{2+}\) has been reported in neuronal cell lines.\(^{30}\)

Sarne and colleagues\(^{23}\) showed that concentrations of \(\mu\) opioids that cause calcium influx also released norepinephrine in SK-N-SH neuroblastoma cells. The same group also showed that dopamine release was potentiated by the modulation of N-type VOCCs by \(\mu\) and \(\delta\) opioids.\(^{44}\) This effect did not involve PTX-sensitive G proteins and was reversed to an inhibitory effect upon blockade of the channels with omega-conotoxin. Other studies have provided evidence that L-type calcium channels may be involved in the opioid-induced increase in [Ca\(^{2+}\)].\(^{29,83,86}\) Opioid-induced increases in intracellular calcium resulting from calcium entry may therefore be sufficient to produce neurotransmitter release, but clearly further work is needed to clarify this.

A comparison of \(\mu\), \(\delta\) and \(\kappa\) opioid-receptor coupling to adenyl cyclase and Ins(1,4,5)P\(_3\)-[Ca\(^{2+}\)], is shown in table 1. Typical increases in Ins(1,4,5)P\(_3\) and [Ca\(^{2+}\)], are shown in fig. 5.

**Table 1**

<table>
<thead>
<tr>
<th>Recombinant receptors</th>
<th>Endogenous receptors</th>
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<tr>
<td>CHO(_\mu)</td>
<td>CHO(_\delta)</td>
</tr>
<tr>
<td>Fentanyl</td>
<td>DPDPE</td>
</tr>
<tr>
<td>IC(_{50}) CAMP</td>
<td>38 nM</td>
</tr>
<tr>
<td>EG(_{50})Ins(1,4,5) P (_3)</td>
<td>11 nM</td>
</tr>
<tr>
<td>Increased Ca(^{2+})</td>
<td>✓</td>
</tr>
<tr>
<td>CHOx</td>
<td>SH-SY5Y ((\mu))</td>
</tr>
<tr>
<td>Spiradoline</td>
<td>Fentanyl</td>
</tr>
<tr>
<td>IC(_{50})</td>
<td>2 nM</td>
</tr>
<tr>
<td>Cyclic AMP</td>
<td>ND</td>
</tr>
<tr>
<td>(3nM)</td>
<td>✓</td>
</tr>
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Refs: 38, 39, 48, 78, 81, 82, 93

**Table 2**

<table>
<thead>
<tr>
<th>Conditions required to bring about bimodality of opioid action</th>
<th>Selected references</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low vs high concentrations of opioid</td>
<td>Fields and Sarne 1997(^{31})</td>
</tr>
<tr>
<td>Acute vs chronic opioid administration</td>
<td>Crain and Shen 1990(^{31})</td>
</tr>
<tr>
<td>Change in experimental conditions</td>
<td>Crain and Shen 1992(^{22})</td>
</tr>
<tr>
<td>Change in experimental conditions</td>
<td>Avidor-Reiss and others 1996(^{7})</td>
</tr>
<tr>
<td>Change in experimental conditions</td>
<td>Same and Gafni 1996(^{7})</td>
</tr>
<tr>
<td>Change in experimental conditions</td>
<td>Olian and Onali 1994(^{67})</td>
</tr>
</tbody>
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**Bimodal actions of opioids and their possible role in tolerance**

In some cases (but not all) both inhibitory and stimulatory effects of opioids have been observed. In most, this could be for one of three reasons (table 2): low vs high concentrations of opioid agonist; acute vs chronic administration of agonist; and a change in experimental conditions.\(^{11}\)

**LOW VS HIGH CONCENTRATIONS OF OPIOID AGONIST**

In DRG neurones, for example, neuronal stimulation\(^{21}\) was enhanced by mM concentrations of opioid but inhibited by \(\mu\)M concentrations. Stimulation of adenyl cyclase activity in SK-N-SH cells occurred only at mM concentrations of \(\mu\) agonist, and was reversed to inhibition by application of \(\mu\)M doses.\(^{31}\)

**ACUTE VS CHRONIC ADMINISTRATION OF ANTAGONIST**

Crain and Shen\(^{23}\) showed that chronic treatment with opioids caused sensory neurones to be supersensitive to the excitatory effects of opioids. In COS-7 cells transfected with the \(\mu\) receptor and type V adenyl cyclase, prolonged treatment with agonist caused stimulation of adenyl cyclase whereas acute administration decreased adenyl cyclase activity.\(^3\)

**Nociceptin/orphanin FQ**

In addition to the classical opioid receptors (\(\mu\), \(\delta\) and \(\kappa\)), cloning studies have revealed an atypical opioid receptor with 50% homology to \(\mu\), \(\delta\) and \(\kappa\) opioid receptors, termed ORL(opioid-receptor-like)-1.\(^{82,98}\) This receptor does not bind conventional opioid ligands but is found in the CNS in areas involved in pain perception, although there is debate about the overall role of its ligand in the production of analgesia or hyperalgesia.\(^{36}\) An endogenous ligand, nociceptin or orphanin FQ, has been isolated.\(^{60,67}\) Most reports to date indicate that nociceptin has inhibitory actions at a cellular level, although in SH-SY5Y cells, nociceptin increases intracellular calcium concentrations in the presence of carbachol.\(^{10}\) In addition, in guinea pig myenteric plexus low (nM) concentrations of nociceptin enhance enkephalin release in the presence of opioid receptor blockade.\(^{74}\)
A CHANGE IN EXPERIMENTAL CONDITIONS

In NG108-15 and SK-N-SH cells, the probability and amplitude of an increase in [Ca\(^{2+}\)], caused by opioids was increased by synchronization of the cultures, differentiation of the cells or synergism with other stimulatory agents. In rat olfactory bulb, \(\mu\) and \(\delta\) opioids stimulate basal cAMP but inhibit stimulated cAMP formation. There is evidence to suggest that the bimodal actions of opioids may be involved in tolerance and dependence on opioids. Wang and Gintzler showed that the opioid stimulation of cAMP in opioid-naive tissue differed from that seen in tolerant tissue. In naïve tissue, inhibition was \(G_{\text{i}}\) linked, but if this pathway was inactivated by PTX treatment then facilitation occurred in a CTX-sensitive manner. However in tolerant tissue, previously inhibitory doses (micromolar) of opioid lead to facilitation of cAMP formation in a CTX-sensitive manner. By treating opioid-naive tissue with protein phosphatase inhibitors or PKC activators, opioid inhibition of cAMP formation can be reversed to facilitation, as seen in tolerant tissue. In DRG neurones treated long term with opioids, low concentrations of opioids produce excitatory effects (lengthening of action potential duration) which is \(G_{\text{i}}\) linked and produce a counter-activation of inhibitory effects. It was proposed that the balance between excitatory and inhibitory effects is dependent upon the levels of the glycolipid, GM1 ganglioside, which is found on neurons. 

Conclusion

In summary, it can be seen that opioids exert a variety of stimulatory effects on signal transduction. These include stimulation of cyclic AMP formation, phosphoinoside hydrolysis, and elevation of intracellular calcium, by mobilization from intracellular stores and by stimulating influx. At a cellular level these changes may underlie an opioid stimulation of neuronal activity. In addition, the stimulatory effects of opioids may play a part in the development of tolerance to opioid drugs. Studies aimed at unraveling these intricate signalling events may contribute better to our understanding of opioid action.

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References

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