Arresting the development of morphine tolerance and dependence

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Agonists of the μ-opioid receptor, such as the prototypical opiate morphine, are highly effective analgesic agents for the treatment of acute pain and chronic pain associated with cancer. However, their effectiveness for treating chronic non-cancer pain is compromised by a range of side-effects, including respiratory depression, constipation, hyperalgesia, and tolerance. Opioid use can also lead to physical and psychological dependence, complicating attempts to withdraw patients from their medication. The well-known hedonic effects of opioids are popular among recreational drug users and legislation to prevent the diversion of opioids from clinical practice for illicit use restricts their availability to patients suffering from pain. The adverse consequences associated with opioid use reinforce efforts to find alternative analgesic approaches.

The μ-opioid receptors are G-protein-coupled receptors (GPCRs) distributed throughout the pain pathway from primary afferent nociceptive neurones, through the spinal cord and thalamus, into the cortex. There are numerous other potential targets within these regions for the future development of drugs that may produce analgesia without the drawbacks associated with μ-agonists, including the δ- and κ-opioid receptors. However, despite considerable effort, no new drugs have usurped the position of μ-receptor agonists as the preeminent analgesic agents for treating severe pain. Rather than throwing the μ-receptor out ‘with the bath water’, several strategies have been used in an attempt to alleviate the side-effect profile of opioid analgesics. The opioid antagonist methylnaltrexone does not cross the blood–brain barrier and inhibits the peripheral actions of morphine, alleviating constipation without blocking centrally mediated morphine analgesia. Ketamine, an antagonist of the N-methyl-D-aspartate subtype of the glutamate receptor, shows some promise reducing the development of morphine tolerance. Furthermore, a recent animal study suggests that inhibition of the 5-hydroxytryptamine type 3 receptor by ondansetron may also attenuate the development of both morphine hyperalgesia and tolerance. Ondansetron is often prescribed to suppress nausea and vomiting associated with chemotherapy. In the light of these findings, it would be interesting to determine whether ondansetron treatment influences morphine tolerance in cancer patients.

Simultaneously, targeting μ- and δ-opioid receptors may also provide a beneficial approach for achieving analgesia without tolerance. Activation of μ-receptors while antagonizing δ-receptors in animal models of pain provides analgesia with a reduction in both tolerance and gastrointestinal motility associated with the administration of a μ-receptor agonist alone.

Polypharmacy is one option for improving the side-effect profile of opioid analgesics. Alternatively, it may be possible to activate μ-receptors, or components of their downstream signalling pathways, in a way that achieves analgesia without inducing the negative consequences associated with opioid agonist-evoked activation. A detailed knowledge of the μ-receptor signalling cascade is needed to achieve this goal.

The activation of μ-receptors inhibits the activity of both adenylyl cyclase and high threshold voltage-dependent Ca2+ channels while activating K+ channels. Coupling to all three effectors occurs through G-proteins, which upon
activation dissociate into component $G_{ai/o}$ and $\beta\gamma$ subunits (Fig. 1). The inhibition of presynaptic $\text{Ca}^{2+}$ channels is particularly important for opioid-mediated reduction of excitatory neurotransmission in the pain pathway. However, additional signalling mechanisms occur in parallel with those mediated through $G$-protein activation.

Opioid receptor activation leads to the recruitment of $\beta$-arrestin 2, a scaffolding protein that interacts with signal transducers including the kinases c-Src, Akt, and MAP kinase (Fig. 1). Mounting evidence, including a study by Yang and colleagues in this issue of the *British Journal of Anaesthesia*, suggests that signalling orchestrated by $\beta$-arrestin 2 contributes to morphine’s side-effect profile.

The first arrestin protein to be identified interacts with rhodopsin, a GPCR that transduces light in the retina. This discovery was followed by the identification of three additional arrestins, two of which preferentially interact with GPCRs outside the visual system including the $\beta$-adrenergic receptors. As a result of this interaction, arrestins 2 and 3 are commonly referred to as $\beta$-arrestin 1 and 2. Additional research revealed that the $\beta$-arrestins interact with additional GPCRs, including opioid receptors. Stimulation of the $\mu$-receptor causes its phosphorylation via GPCR kinase (GRK) and the subsequent recruitment of $\beta$-arrestin 2, an event that precedes receptor endocytosis (Fig. 1). Mice lacking $\beta$-arrestin 2 ($\beta$-arr2−/− mice) exhibit a striking resistance to the development of tolerance to morphine analgesia. Furthermore, a lack of $\beta$-arrestin 2 reduces morphine-induced respiratory depression and constipation. Surprisingly, an initial report suggested that a lack of $\beta$-arrestin 2 was without effect on morphine dependence (assessed by the severity of naloxone-precipitated withdrawal symptoms), suggesting that tolerance and dependence occur through distinct molecular mechanisms. However, recent studies have forced a re-evaluation of this important finding.

A potentially confounding aspect of studying mice genetically modified to lack specific genes is the possibility for developmental compensation. It is reassuring to corroborate such studies with experiments in which genes are knocked down or deleted in mature animals. One such conditional approach is the use of small interfering RNA (siRNA) which forms a double strand with the messenger RNA encoding the protein of choice, in this case $\beta$-arrestin 2, leading to degradation of the transcript. Yang and colleagues demonstrated that the intrathecal administration of $\beta$-arrestin 2 siRNA to rats in the lumbar region of the spinal cord reduced $\beta$-arrestin 2 mRNA and protein but had no effect on $\beta$-arrestin 1 expression. As predicted by the earlier studies of $\beta$-arr2−/− mice, the reduction in $\beta$-arrestin 2 levels was associated with attenuation of the development of analgesic tolerance to morphine. Interestingly, intrathecal $\beta$-arrestin 2 siRNA administration also reduced the withdrawal symptoms associated with the administration of naloxone to rats chronically administered morphine.
suggestions that, at the level of the spinal cord, both the development of tolerance and dependence to chronic morphine analgesia involves β-arrestin 2. The inconsistency between this finding and the earlier observation of unaltered dependence in β-arre2−/− mice could be due to differences in the experimental design which include the dose of morphine and the route of administration. There could also be a role for developmental compensation caused by deletion of the β-arrestin 2 gene from mice. Yang and colleagues administered morphine systemically from a subcutaneous pellet. A recent study using subcutaneous pump infusion demonstrated that withdrawal symptoms in β-arre2−/− mice associated with naloxone administration during chronic infusion of all but the very highest concentration of morphine were substantially diminished compared with those of wild-type mice.17 Induction of dependence through a β-arrestin 2-independent mechanism only occurs with very high doses of chronic morphine.

Interestingly, a reduction in the expression of spinal β-arrestin 2 in rats caused a prolonged latency for tail withdrawal from noxious heat even in the absence of morphine treatment.10 Basal analgesia has also been observed in β-arre2−/− mice.13 In common with several other GPCRs, μ-receptors exhibit a low level of agonist-independent constitutive activity. The absence of β-arrestin 2 enhances μ-receptor constitutive activity leading to tonic inhibition of voltage-activated Ca2+ channels in primary afferent dorsal root ganglion neurones.18 19

How does the knowledge that decreasing β-arrestin 2 levels in the spinal cord produces analgesia and reduces both morphine tolerance and dependence, provide hope for improving the control of chronic pain? It seems unlikely that β-arrestin 2 siRNA will be approved in the foreseeable future to clinically reduce tolerance and subsequent withdrawal in individuals receiving morphine to treat chronic pain. However, the study by Yang and colleagues adds to the mounting body of evidence which suggests that inhibiting β-arrestin 2 signalling provides prolonged analgesia with a superior side-effect profile. There is increasing interest in the possibility that some GPCR agonists may preferentially activate either G-protein-mediated signalling or signalling through β-arrestins. Such ligands are termed biased agonists.20 Herkinorin, derived from the plant product salvinorin A, is an example of a biased μ-receptor agonist.21 Herkinorin activates μ-receptors without recruiting β-arrestin 2 and provides hope for the realization of the goal to produce analgesic agents that lack the side-effects of morphine and other opioids.

Conflict of interest
None declared.

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