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COMMENTARY

Has the sun set on κ_3-opioid receptors?

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μ-Opioid receptor agonists are a mainstay of clinical analgesia, despite the significant unwanted effects and dependence liability associated with drugs like morphine. The quest for opioids that produce analgesia with fewer undesirable effects has lead to the putative identification of multiple opioid receptor subtypes, despite the identification of only four opioid-related receptor genes. One such putative receptor subtype is the κ_3 receptor, activation of which supposedly produces analgesia in animals. In the present issue of this Journal, Olianas and co-workers have demonstrated that the prototypic κ_3 agonist naloxone benzoylhydrazone is actually a partial agonist at the cloned μ, δ, and κ opioid receptors and an antagonist at opioid-like NOP receptors. Together with a recent study that showed that high-affinity naloxone benzoylhydrazone binding is abolished in triple $\mu/\delta/\kappa$ receptor knockout mice, the present study provides strong evidence that *in vivo* effects attributed to κ_3 receptor activation probably just reflect the combined actions of a particularly nonselective opioid drug. Indeed, molecular identification of any of the proposed subtypes of μ, δ, and κ opioid receptors has proven elusive, suggesting that it is perhaps time to retire the notion of opioid receptor subtypes until definitive evidence for their existence is provided.
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Morphine is one of the oldest and most useful drugs in Western medicine. Attempts to produce nonaddictive and nonsedating analgesics free of side effects such as nausea, constipation and respiratory depression lead to the synthesis of an enormous number of opioid drugs with diverse *in vivo* and *in vitro* effects. It seemed inconceivable that all these actions could be mediated by a single receptor, and besides, the notion that the unwanted and therapeutic effects of morphine might be mediated by different receptors that could eventually be distinguished pharmacologically was almost irresistible. Thus, shortly after the pharmacological delineation of μ, κ and δ receptors (Martin *et al*., 1976, Lord *et al*., 1977), a plethora of pharmacological subtypes began to be reported. In particular, heterogeneity of κ-opioid receptors was proposed (Clark *et al*., 1989; Traynor, 1989). These diverse κ receptors could be distinguished on the basis of the differential reversal of agonist effects by different antagonists, the lack of complete cross tolerance between supposed κ agonists and, of course, the apparent presence of multiple binding sites for opioid radioligands in brain tissue (Clark *et al*., 1989). When reviewing the topic of multiple κ-opioid receptors in 1989, Traynor concluded that confirmation of multiple κ subtypes would have to wait for the development of more selective ligands and better physiological assays and, of course, for the cloning of the κ-opioid receptor(s).

The κ_3 receptor subtype was originally proposed as one of four κ-subtypes defined by radioligand binding studies with a variety of ligands in a variety of tissues (Clark *et al*., 1989). Naloxone benzoylhydrazone (NalBzoH) was the prototypic κ_3 agonist, and while it was recognized that NalBzoH also bound to μ-, κ_1- and to a lesser extent δ-opioid receptors (Price *et al*., 1989), the analgesia produced by NalBzoH was attributed to κ_3 receptor activation (Paul *et al*., 1990). Although a 'κ_3-related gene' was cloned from SH-SY5Y neuroblastoma cells, this turned out to be the human homolog of the gene for the nociceptin receptor (Pan *et al*., 1995), and no other candidates for a gene corresponding to the κ_3- (or indeed κ_2-) receptors have been identified.

It is now more than 10 years since the cloning of the classical opioid receptors, including a single κ-receptor. While better ligands and a greater understanding of the role of κ receptors in physiology have emerged, it is molecular biology that has apparently laid to rest the issue of κ-opioid receptor subtypes. In a study reported in the current issue of this Journal, Olianas and co-workers have used recombinant opioid receptors to assess the efficacy of NalBzoH at μ, δ, κ and NOP receptors, while in a recent study by Cox *et al*. (2005), the radioligand binding profile of [^{3}H]-NalBzoH was determined in mice with a genetic deletion of all three classical opioid receptor types.

This study in opioid receptor knockout mice had two important findings. Firstly, it established beyond doubt that NalBzoH binds to the NOP receptor but only at relatively high concentrations, as a complete lack of binding of [^{3}H]NalBzoH at 4 nM was observed in the triple μ, δ and κ opioid knockout mice and clear NOP labelling was only evident at concentrations more than 10-fold higher (Cox *et al*., 2005). The estimates of the relative contribution of opioid receptor to opioid related receptor (NOP) binding from this study was 2 : 1. Secondly, complete regional mapping of the binding of [^{3}H]NalBzoH clearly showed a distinct labelling of opioid receptors when compared to [^{3}H]naloxone labelling, pointing to much more δ-binding for [^{3}H]NalBzoH than has previously been suggested (Price *et al*., 1989). The most recent paper by Olianas *et al*.

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(2006) also clearly supports a major component of interaction with δ-opioid receptors.

Although collectively the radioligand binding data confirms that NalBzoH is likely to bind to all opioid receptors, some functional studies have suggested that it may have agonist activity at μ, κ and δ receptors (e.g. Onali & Olianas, 2004), while others have reported that NalBzoH effectively blocks μ and δ receptor effects *in vivo* and *in vitro* (Paul *et al*., 1990; Bertzetei-Gurke *et al*., 1995). Olianas and co-workers have now directly addressed these apparently conflicting data by examining the ability of NalBzoH to activate or inhibit recombinant human μ, δ- and κ-opioid receptors and the NOP receptor expressed in chinese hamster ovary (CHO) cells. NalBzoH was a partial agonist at all three classical opioid receptors, with an efficacy considerably less than prototypic agonists such as DAMGO and morphine (μ), U50,488 (κ) and DPDPE (δ). NalBzoH was a pure antagonist at NOP receptors, although other studies have reported weak agonist activity. The finding that NalBzoH is a partial agonist can explain why it blocks μ- or κ- agonist analgesia *in vivo* (Paul *et al*., 1990) yet can mimic opioid agonists actions in native tissue *in vitro* (Onali & Olianas, 2004). The realization that analgesia produced by higher doses of NalBzoH could be explained through actions at the NOP receptor (Noda *et al*., 1998), an inference now confirmed in mice lacking the classical opioid receptors (Cox *et al*., 2005), means that the complex pharmacological profile of NalBzoH results from its interaction with all opioid receptors and its capacity to act as an apparent agonist or antagonist depending on the receptor reserve in the tissue.

So where does this leave κ_3 receptors? The prototypic κ_3 agonist NalBzoH is in fact a ligand for all known opioid receptors, and can act as a functional agonist or antagonist at most or all of them. No unique κ_3 protein has been identified, and there are no known splice variants of the cloned κ_1 receptor. Deleting all three classical opioid receptors abolishes high-affinity NalBzoH radioligand binding, and deleting the NOP receptor blocks the antinociceptive effects of the drug. No selective κ_3 agents have been reported. While it is possible that receptors with a novel pharmacology could be generated through heterodimerization of κ receptors, this is the only hypothesis left to test. However, it seems likely that the 'κ_3 receptor' represents the combined actions of a nonselective drug at the four different opioid receptors – a conclusion that sits comfortably with suggestions made many years ago (Traynor, 1989).

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