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Opioids and their receptors: Are we there yet?

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Abstract

Opioids have an important place in pharmacology. While their clinical use as analgesics is fundamental in medicine, their use is constrained by their side-effects and abuse potential. Pharmacologists have sought analgesics lacking side-effects and the abuse liability of the current agents. The identification of the opioid receptors in 1973 marked the beginning of our understanding of the molecular mechanisms of these agents. The isolation of the opioid peptides quickly followed, along with the classification of three families of opioid receptors. Clinicians have long been aware of subtle differences among the mu opioids that were not easily reconciled with a single receptor and selective antagonists implied two subdivisions of mu receptors. However, the cloning of the mu opioid receptor MOR-1 has led to the realization of the extensive complexity of the mu opioid receptor gene and its vast array of splice variants. Many of these splice variants are truncated and do not conform to the structure of traditional G-protein coupled receptors. Yet, evidence now shows that they are quite important and may prove valuable targets in the development of potent analgesics lacking the undesirable properties of current opioids.

Keywords

opioid receptor; mu receptor; morphine; truncated; G-protein coupled receptor; splice variant; MOR-1

1.0 Opioid Receptors

Opiates have a special place in pharmacology due to their importance in the management of pain and the societal impact of their abuse. They have been at the leading edge of our understanding of the neuropeptides and their receptors for decades. Since the initial isolation of morphine in 1805, we have seen the generation of a wide range of analogs that have established strong structure-activity relationships, including some of the earliest synthetic pharmacological antagonists, starting with an N-allylnorcodeine in 1917 and evolving to the

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highly selective agents used today (Pasternak and Pan, 2013). Indeed, these antagonists have been crucial in defining the pharmacology of these drugs.

The synthesis of thousands of analogs and their strict structure-activity relationships led to the proposal of specific recognition sites, or receptors (Beckett and Casy, 1965; Portoghese, 1965; Portoghese, 1966) many years before their demonstration biochemically in 1973 (Pert et al., 1973; Simon et al., 1973; Terenius, 1973), based upon the concept of stereoselectivity described by Goldstein (Goldstein et al., 1971). Subsequent studies of opioid binding sites were the first to demonstrate the sodium effect and its ability to discriminate between the binding of agonists and antagonists, in addition to a variety of other treatments with similar effects (Pasternak et al., 1975b; Pasternak et al., 1975c; Pasternak and Snyder, 1975b; Wilson et al., 1975). These observations have since been extended to an array of other G-protein coupled receptors. Forty years later, crystal structures actually identified the sodium ion binding site within these receptors (Liu et al., 2012).

Since those initial descriptions, the field of opioid receptors has become increasingly complex. There is a long history of receptors in pharmacology, going back over a century, but the opiates were unique in that their receptor was identified without a known endogenous ligand. Looking back, it is remarkable how quickly the endogenous peptides were uncovered following those initial reports. Although several groups identified opioid-like materials in brain (Hughes, 1975; Pasternak et al., 1975a; Terenius and Wahlstrom, 1975), Kosterlitz was the first to identify the materials as pentapeptides (Hughes et al., 1975), followed soon afterwards by his identification of receptors selective for these enkephalins, which he termed delta (Lord et al., 1976). The enkephalins were soon followed by the isolation of dynorphin A (Goldstein et al., 1979) and β-endorphin (Li et al., 1976). Remarkably, these three classes of endogenous opioids share identical amino acid sequences at the first four positions, followed by either methionine ([Met^5]enkephalin and β-endorphin) or leucine ([Leu^5]enkephalin and the dynorphins). Dynorphin A has its own receptor (Chavkin and Goldstein, 1981), termed kappa$_1$, which corresponds to the kappa receptor proposed by Martin (Martin et al., 1976), while β-endorphin has high affinity for both mu and delta receptors. Each of the three opioid peptides is generated by processing a different precursor confirming that they were separate families of peptides (Berezniuk and Fricker, 2011). Within the enkephalin and dynorphin precursors, there are a number of opioid-like peptides, raising the question as to whether or not these additional opioid-like peptides have their own distinct receptors. This would be quite intriguing since it might offer the opportunity of multiple new targets and drugs with unique pharmacologies.

2.0 Multiple Mu Receptors

There is a rich clinical history of opioids (Eddy, 1973). The opioid field is unique from most others in pharmacology due to the extensive clinical experience with a wide range of drugs, most of them mu agonists, prior to the identification of the receptors. Thus, with opioids, the clinical experience predated the studies of mechanism. This clinical experience provided a depth of understanding of the actions of these agents that was not possible with animal models due to the subtle differences among various drugs. For example, clinicians have long known that the opioids do not work equally well in every patient (Foley, 1985; Foley, 1996;

Payne and Pasternak, 1992). Some patients respond better to one drug while another patient may be better managed with a different one. Side-effects seen with a specific drug also can vary from patient to patient independently of its analgesic activity. Finally, studies at the Addiction Research Center in Lexington Kentucky found that patients with a history of opioid abuse were able to distinguish one opioid from another (Eddy, 1973). Together, these observations raised the question of how to reconcile them with the existence of a single receptor, particularly since almost all the drugs were mu opioids.

The first experimental studies suggesting multiple mu opioid receptors came from detailed binding studies that revealed a second morphine binding site of even higher affinity with a distinctive selectivity profile (Lutz et al., 1985; Munson et al., 1984; Pasternak and Snyder, 1975a; Wolozin and Pasternak, 1981). The synthesis of antagonists capable of selectively blocking this second site provided pharmacological tools to distinguish the actions of the proposed subtypes of mu receptors. In brief, these antagonists, naloxonazine and naloxazone, dissociated the supraspinal and spinal analgesic actions of morphine (Ling et al., 1986; Ling and Pasternak, 1983; Paul et al., 1989), as well as separating analgesia from respiratory depression (Ling et al., 1983; Ling et al., 1985), most aspects of physical dependence (Ling et al., 1984), inhibition of gastrointestinal transit (Heyman et al., 1988; Paul and Pasternak, 1988) and even the release of prolactin and growth hormone (Spiegel et al., 1982). These were among the first examples that opioid analageisa could be dissociated from troublesome side-effects.

3.0 Molecular biology of mu opioid receptors

The concept of multiple opioid receptors was first proposed by Martin (Martin, 1967) based upon interactions between morphine and nalorphine (Houde and Wallenstein, 1956; Lasagna and Beecher, 1954). In this proposal, he suggested the existence of morphine, or “M”, receptors and nalorphine, or “N” receptors. He subsequently proposed that the “M” receptors be named mu and the “N” termed kappa, based upon the pharmacology of ketocyclazocine (Martin et al., 1976). The third member of the opioid receptor family was proposed by Kosterlitz based upon the enkephalins and termed delta (Lord et al., 1977), followed by the closely related peptide orphanin FQ/nociceptin (Meunier et al., 1995; Reinscheid et al., 1995) and its receptor, ORL_1 (also known as KOR-3) (Bunzow et al., 1994; Chen et al., 1994; Fukuda et al., 1994; Keith, Jr. et al., 1994; Mollereau et al., 1994; Pan et al., 1994). Much effort has been focused upon targeting these additional receptor classes to develop analgesics lacking the problems of the traditional opiates used clinically – which are almost all mu. These efforts have not yet produced clinically useful agents. $Kappa_1$ agents were found to have psychotomimetic actions while early delta compounds were complicated by seizure activity. However, recent results suggest that drugs selective for mu opioid receptor subtypes may prove valuable in the development of superior analgesics. Targeting the ORL_1 receptor may yield interesting compounds, but they have not yet been examined clinically.

MOR-1 was first cloned in 1993 (Chen et al., 1993; Eppler et al., 1993; Thompson et al., 1993; Wang et al., 1993), soon after the delta receptor (Evans et al., 1992; Kieffer et al., 1992). The receptor was comprised of four exons (Fig. 1). The first exon encodes the N-

terminus and the first transmembrane domain while the second and third exons each encoded an additional three transmembrane domains, yielding the seven transmembrane structure of traditional G-protein coupled receptors. The delta (DOR-1) and kappa$_1$ (KOR-1) receptors have analogous structures with their three exons. However, MOR-1 differs from the others in that it also contains a fourth exon responsible for coding only 12 amino acids at the tip of the intracellular C-terminus. Over the years, a large array of 31 splice variants have been isolated from mice (Doyle et al., 2006; Doyle et al., 2007; Pan et al., 2009a; Pan et al., 1999; Pan et al., 2000; Pan et al., 2005b; Pan, 2005; Pan, 2000; Pan et al., 2001), sixteen from rats (Pasternak et al., 2004; Xu et al., 2011; Zimprich et al., 1994) and nineteen from humans (Bare et al., 1994; Cadet et al., 2003; Choi et al., 2006; Pan et al., 2003; Pan, 2005; Shabalina et al., 2009; Xu et al., 2009), with similar gene structures and splicing patterns in each species.

There are three major classes of MOR-1 splice variants in mice, rats and humans. The first are the full length variants, in which 3'splicing leads to the replacement of the 12 amino acids encoded by exon 4 with alternative sets of exons which generate distinct amino acid sequences in the tip of the C-terminus (Fig. 1). The second set are associated with exon 11 and its promoter, located approximately 30 kbases upstream of exon 1. These variants, which lack exon 1 and thus the first transmembrane (TM) domain, are truncated and contain only the last 6 TM domains. The third set involve exon skipping with the loss of exon 2 or of exons 2 and 3 to generate a single TM protein encoded by exon 1. All three classes are functionally relevant.

4.0 Functional assessment of MOR-1 and its splice variants

There are many indications that these variants are functionally important. Their regional distributions at both the mRNA and protein levels are quite distinct from one another. Immunohistochemical studies using C-terminus epitopes clearly illustrated these differences (Abbadie et al., 2000a; Abbadie et al., 2000b; Abbadie et al., 2000c; Abbadie et al., 2004), as well as at the ultrastructural level (Abbadie et al., 2001). Whereas mMOR-1 was localized both presynaptically and postsynaptically, the splice variant mMOR-1C was almost exclusively presynaptic. Unlike many mu opioids, morphine does not internalize MOR-1 (Keith et al., 1996; Keith et al., 1998). However, morphine effectively internalizes MOR-1C in vivo, showing a clear difference in their trafficking (Abbadie and Pasternak, 2001). The full length variants also vary in their sensitivity towards activation by a series of opiates, with drugs showing varying efficacies and potencies among the variants as determined by stimulation of ^{35}S-GTPγS binding (Bolan et al., 2004; Pan et al., 2005a; Pan et al., 2009a; Pan et al., 1999; Pan et al., 2000; Pan et al., 2003; Pan et al., 2001). However, a series of knockout models have provided the best insights into their actions.

Several groups have generated knockouts of the *OPRM1* gene, targeting exon 1(Schuller et al., 1999; Sora et al., 1997), exon 2 or exons 2/3 (Loh et al., 1998; Matthes et al., 1996), or exon 11 (Pan et al., 2009b). All the knockout models targeting exons 1, 2 or 3 eliminated morphine actions. However, one knockout animal targeting exon 1 retained heroin and morphine-6β-glucuronide (M6G) analgesia, implying that their analgesic mechanisms were distinct from those of morphine (Schuller et al., 1999). This knockout model still expressed

a second set of MOR-1 variants associated with exon 11 that did not contain exon 1, suggesting that these variants may play a role in heroin and M6G analgesia. If so, disruption of exon 11 and its associated variants should selectively diminish heroin and M6G analgesia. When this hypothesis was tested in an exon 11 knockout mouse (Pan et al., 2009b), morphine analgesia was fully retained despite the loss of the exon 11-associated splice variants. Yet, the analgesic activity of heroin and M6G were significantly reduced, as predicted.

The importance of the truncated 6TM exon 11-associated variants became clearer with the development of a novel series of ligands (Majumdar et al., 2011; Majumdar et al., 2012). The significance of the truncated 6TM variants initially was uncertain. Lacking the full 7 transmembrane domains associated with traditional G-protein coupled receptors, initial studies examining the variants expressed in cell lines failed to demonstrate binding. However, the pharmacology of these variants was clarified by a recently developed compound, 3-iodobenzoyl-6β-naltrexamide (IBNtxA). Using an ^{125}I-radiolabeled version of the compound, we demonstrated a very high affinity binding site in brain with a pharmacological profile unlike any of the traditional opioid receptors. Furthermore, the binding was still present in an exon 1 knockout mouse lacking all the full length MOR-1 splice variants as well as in a “triple knockout mouse” in which the delta and kappa$_1$ receptors were also eliminated (Majumdar et al., 2011; Majumdar et al., 2012). However, disruption of exon 11 with the loss of the exon 11-associated variants eliminated the binding (Fig. 2). Although it was structurally related to the antagonist naltrexone, IBNtxA was a potent analgesic and it retained full analgesic activity in the triple knockout mice (Fig. 3a,b). Like the binding site, IBNtxA analgesia was completely lost in the exon 11 knockout mouse, clearly showing that the 6TM exon 11-associated variants played a critical role in both the binding site and its functions. Pharmacologically, IBNtxA had other distinctions. While IBNtxA and a number of related analogs were potent analgesics, they lacked respiratory depressant activity (Fig. 3c), had minimal effect on gastrointestinal transit and showed no evidence of physical dependence with chronic dosing. They showed no cross tolerance to morphine analgesia and, perhaps most intriguing, showed no reinforcing or aversive activity in a conditioned place preference paradigm (Fig. 3d) (Majumdar et al., 2011; Majumdar et al., 2012). These studies clearly established the relevance of these truncated receptors, but many questions remain. While the composition of the target of IBNtxA clearly contains exon 11-associated variants, it appears that in brain it may be a heterodimer between the 6TM variants and other G-protein coupled receptors. Since the binding site and the analgesic actions of IBNtxA remained in the triple knockout mice remained, it seems unlikely that these partners involve any of the traditional opioid receptors, raising interesting questions on the potential partner(s). ORL$_1$ is among the possibilities. While IBNtxA does not label ORL$_1$ receptors with high affinity, we already know that a heterodimer between ORL$_1$ and MOR-1 yield displays differing binding profiles than either receptor alone. Finally, it is important to consider that there may be more than one partner for the exon 11 variants and to remember that there are multiple 6TM exon 11 variants in all the species.

5.0 Conclusions

Much has happened since the initial biochemical description of opioid receptors forty years ago. Research has uncovered a complexity far exceeding early estimates. Pharmacological approaches using receptor binding and selective antagonists initially suggested mu_1 and mu_2 receptors, but molecular biological approaches have now identified over 30 splice variants of the mu receptor in mice, 16 in rats and 19 in humans. Much work will be needed to understand the full significance of all these variants.

The most intriguing aspect of the field, however, remains our continued progress towards the “Holy Grail” – opioid analgesics with potent analgesic activity that lack side-effects and abuse potential. With the identification of delta and $kappa_1$ receptors, efforts focused upon agents targeting these sites. Although these led to many candidates, their clinical potential has been impaired by psychotomimetic or epileptogenic activity. Ironically, it appears that the most promising agents target mu opioid receptor splice variants, but not necessarily the traditional ones initially observed in 1973. Biased agonism might prove useful in drug development (Raehal et al., 2011), but the truncated variants may prove better targets to yield highly potent analgesics lacking most of the typical side-effects of opioids as well as physical dependence and reward and aversive behavior, at least in a conditioned place preference assay. This is a major step forward, both in drug development and in our understanding of G-protein coupled receptors in general where a vast array of truncated forms of these receptors have been uncovered over the years.

Are we there yet? Maybe we are close….

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Highlights

- Morphine and most clinical opiates act through mu opioid receptors
- The mu opioid receptor MOR-1 was cloned twenty years ago
- The mu opioid receptor undergoes extensive splicing to generate three major classes of variants
- MOR-1 splice variants may prove useful in the development of analgesics lacking the undesirable actions of current opioid drugs.

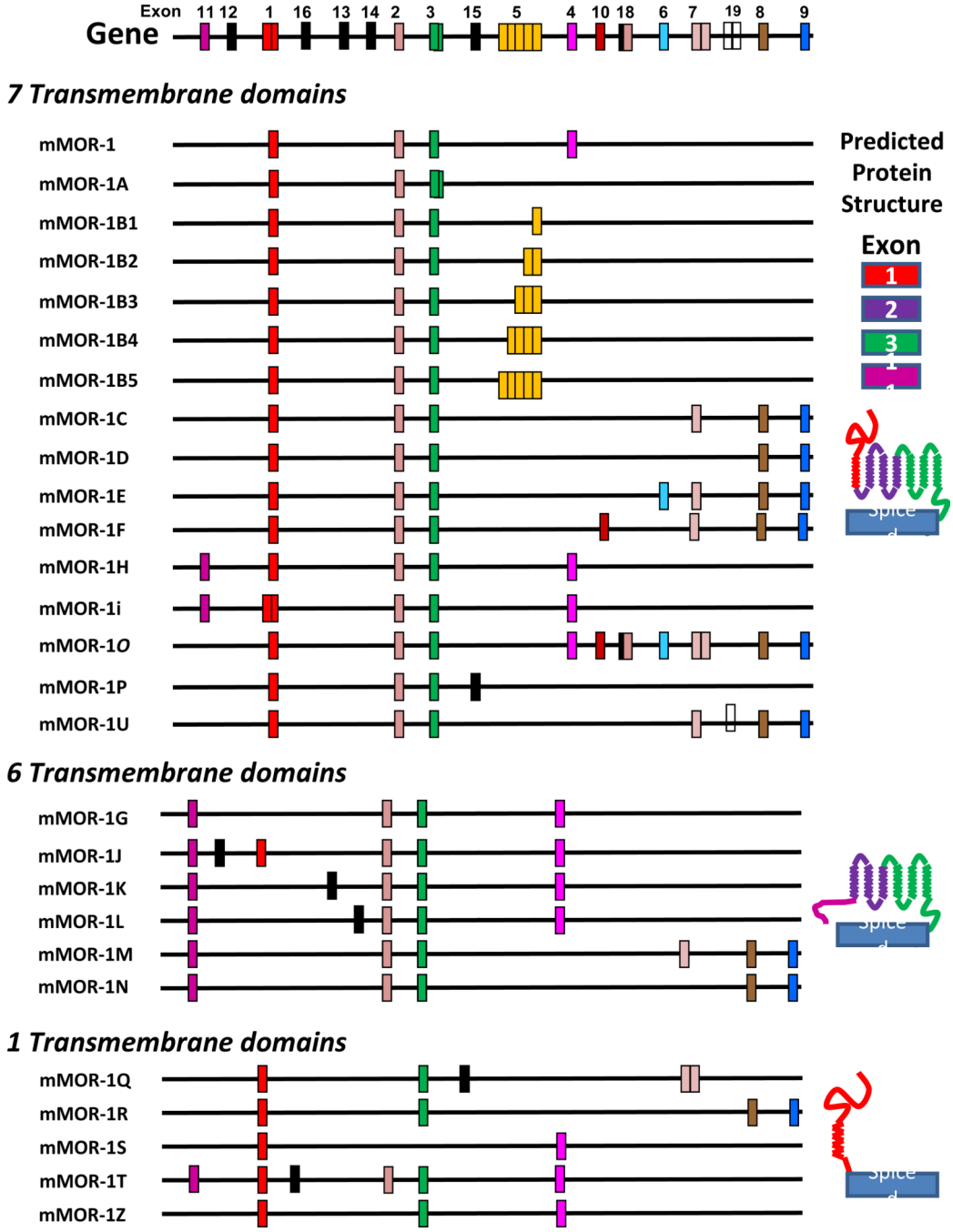

Figure 1. Schematic of the mouse *Oprm1* gene and the MOR-1 splice variants

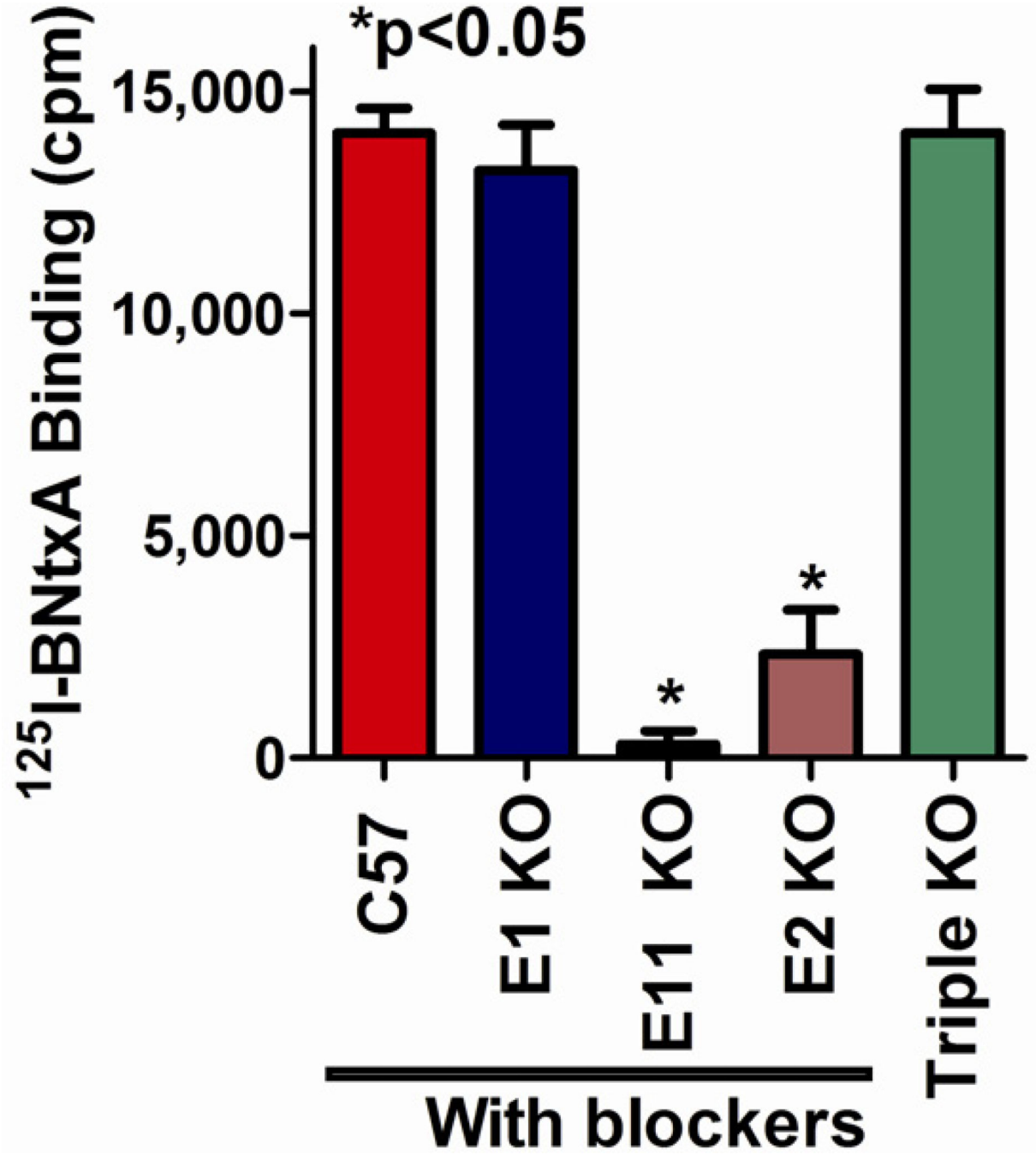

Figure 2. ^{125}I-BNtxA binding in knockout mice

Mice with the indicated exon disruption were tested for ^{125}I-BNtxA binding. Wildtype and mice with disruptions of exons containing within MOR-1 were assayed in the presence of blockers to eliminate binding to the traditional opioid receptors (mu: CTAP 1 μM; delta: DPDPE 1 μM; kappa$_1$: U50,488H 1 μM). Blockers were not used in the triple knockout mice since they had no traditional opioid binding due to the knockouts. From the literature (Majumdar et al., 2011).

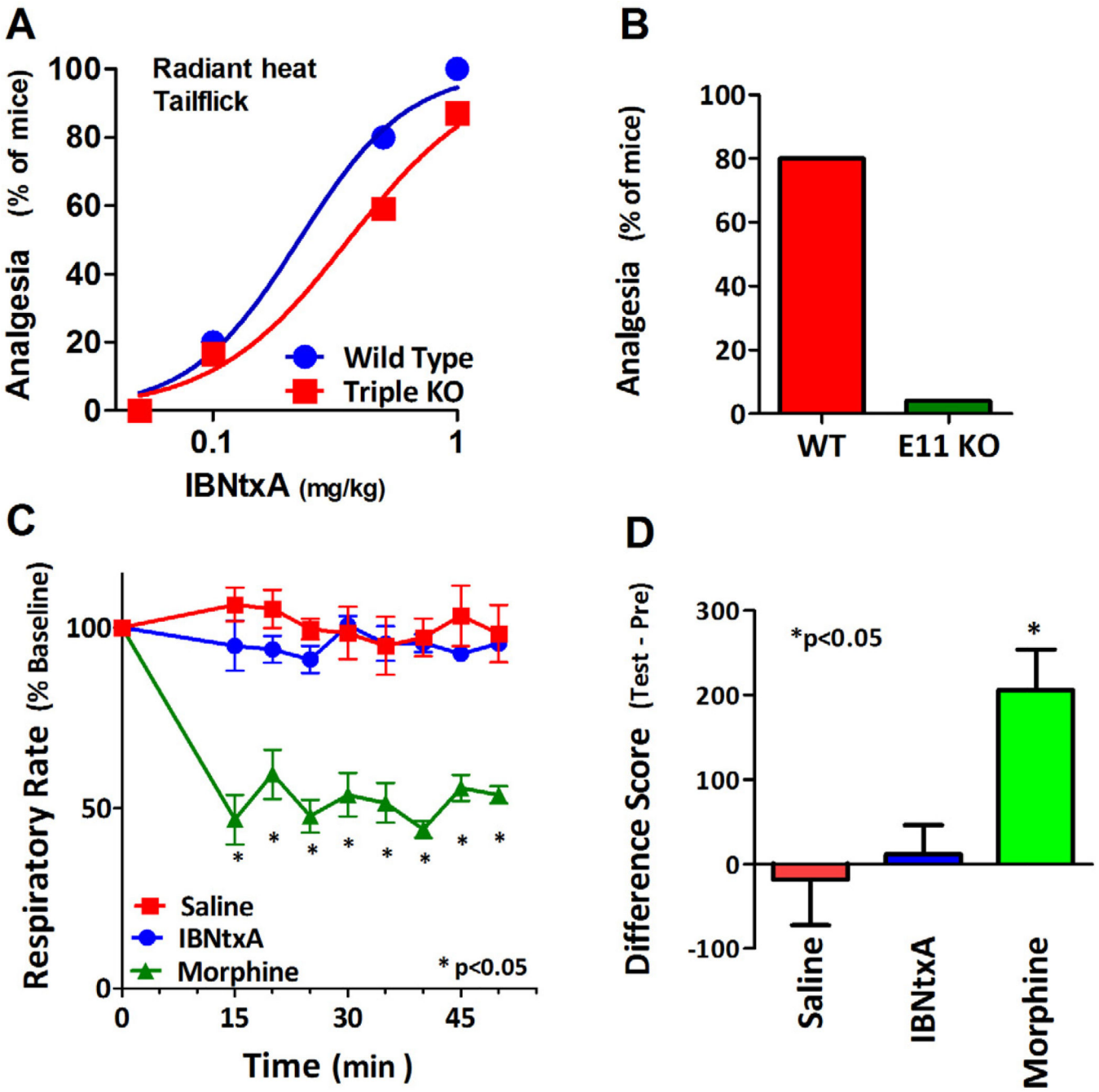

Figure 3. Pharmacological characterization of IBNtxA actions in vivo

a) IBNtxA analgesia was assessed in wildtype mice and triple knockout mice at the indicated dose. The ED_{50} values for the two groups were not significantly different. b) IBNtxA (0.5 mg/kg, s.c.) was given and analgesia assessed using the radiant heat tailflick assay in wildtype and exon 11 knockout mice. No observable analgesia could be detected in the knockout animals. c) Respiratory rate was determined in groups of mice given saline or high equianalgesic doses of morphine (20 mg/kg, s.c.)or IBNtxA (2.5 mg/kg, s.c.). d) Conditioned place preference was carried out and the activity of morphine (10 mg/kg, s.c.)

and IBNtxA (1 mg/kg, s.c.) compared to saline. Results are from the literature (Majumdar et al., 2011).