Neuroscience Letters 457 (2009) 115–119

Contents lists available at ScienceDirect

Neuroscience Letters

journal homepage: www.elsevier.com/locate/neulet

The contribution of MOR-1 exons 1–4 to morphine and heroin analgesia and dependence

Gad Klein[a], Grace C. Rossi[b], Amanda R. Waxman[a], Carolne Arout[a], Aaron Juni[a], Charles E. Inturrisi[c], Benjamin Kest[a,d,*]

[a] Neuropsychology Doctoral Subprogram, Queens College, City University of New York, Flushing, NY 11367, United States
[b] Department of Psychology, C.W. Post College, Long Island University, New York, United States
[c] Department of Pharmacology, Weill Medical College, Cornell University, New York, NY 10021, United States
[d] Department of Psychology and Center for Developmental Neuroscience, The College of Staten Island, City University of New York, Staten Island, NY 10314, United States

ARTICLE INFO

Article history:
Received 8 February 2009
Received in revised form 22 March 2009
Accepted 6 April 2009

Keywords:
Morphine
Heroin
Dependence
MOR-1
Exons
Antisense oligodeoxynucleotides

ABSTRACT

Although morphine and heroin analgesia is mediated by μ-opioid receptors encoded by the MOR-1 gene, distinct isoforms are involved. Both opioids also induce dependence by acting at μ-opioid receptors, but which variants are utilized is not known. Here, we assayed morphine and heroin analgesia and dependence in mice treated with antisense oligodeoxynucleotides (AO) targeting MOR-1 exons 1–4. Whereas AOs targeting exons 1 and 4 blocked morphine analgesia, those targeting exons 2 and 3 blocked heroin analgesia. Neither morphine nor heroin analgesia was compromised 5 days after the last AO injection. In morphine and heroin dependent mice, only exon 1 AO significantly reduced jumping incidence during naloxone (50 mg/kg) precipitated withdrawal. Neither analgesia nor withdrawal jumping was attenuated in controls pretreated with saline or a mismatch oligodeoxynucleotide control sequence. While these data confirm previous reports that morphine and heroin analgesia are not mediated by a single μ-opioid receptor, both opiates nonetheless apparently induce dependence via a μ-opioid receptor isoform containing exon 1. For heroin, the possibility that analgesia and dependence are mediated by distinct μ-opioid receptor isoforms offers the prospect of developing potent opiate analgesics possessing reduced dependence liability.

Although morphine is widely used to treat moderate to severe pain, repeated morphine exposure can cause unwanted side effects including physical dependence. In the CNS, morphine activity is primarily mediated by the μ-opioid receptor, encoded by MOR-1 [16], and the binding of morphine to the μ-opioid receptor is a critical step in the development of physical dependence. For example, physical symptoms accompanying naloxone-precipitated withdrawal (NPW) from morphine are blocked when animals are pretreated supraspinally with the μ-selective antagonist β-funaltrexamine [2].

Like morphine, heroin is an opiate with high abuse liability where even an acute exposure can cause dependence [11,12]. Since heroin is rapidly converted to 6-monoacetylmorphine and then morphine in vivo [6], and preferentially binds and activates μ-opioid receptors [24,28], it has long been thought to have a pharmacological profile identical to that of morphine. Recent findings challenge this assumption. For example, although morphine analgesia is markedly reduced in the μ-opioid receptor deficient CXBK mouse [18], heroin analgesia is not [20]. Data from various subsequent studies, including an antisense oligodeoxynucleotide (AO) strategy to "knock-down" distinct exonic regions of the MOR-1 clone, suggest that alternative splice variants of the μ-opioid receptor may account for these differences [16]. Specifically, whereas MOR-1 exons 1 and 4 mediate systemic and supraspinal morphine analgesia in rats and mice, exon 2 is primarily involved in heroin analgesia. Since the AO targeting of exon 4 but not exon 1 attenuated the ability of morphine to inhibit gastrointestinal transit and spinal analgesia [19], it appears that identical splice variants do not mediate all of the functional effects of even a single opiate.

Common substrates are also thought to underlie morphine and heroin dependence. For example, blockade of δ_2 opioid, NMDA, and AMPA receptors attenuate NPW in morphine and heroin dependent mice [8,11,14,26,27]. Furthermore, there is a very strong genetic correlation in NPW jumping between morphine and heroin dependent inbred mouse strains [11,12], indicating their mediation by common genetic substrates. However, as with analgesia, recent studies indicate distinct mechanism for morphine and heroin dependence. For example, blocking the δ_1 opioid receptor attenuates NPW jumping in mice after acute and chronic heroin, but not morphine,

* Corresponding author at: Department of Psychology, 2800 Victory Blvd., Staten Island, NY 10314, United States. Tel.: +1 718 982 4070; fax: +1 718 982 4114.
E-mail address: kest@mail.csi.cuny.edu (B. Kest).

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doi:10.1016/j.neulet.2009.04.012

Table 1
Characterization of antisense and mismatch oligodeoxynucleotides.

Target	Oligodeoxynucleotide sequence (5′–3′)	Position	Bases[b]
Exon 1	CGC CCC AGC CTC TTC CTC T	5′-Untranslated region	195–213
Exon 2	TTG GTG GCA GTC TTC ATT TTG G	Intracelullar loop 1	572–593
Exon 3	CCA CCA GCA CCA TCC GGG	Extracellular loop 2	1118–1135
Exon 4	GGGCAATGGAGCAGTTTCTG	Intracellular COOH terminus	1402–1422
Mismatch	CGC CCC GAC CTC TTC CCT T[a]	5′-Untranslated region	195–213

[a] Underlined bases indicate a mismatch with exon 1 antisense oligodeoxynucleotide sequence.
[b] Based upon the gene structure of the mouse MOR-1 (GenBank accession no. U2691).

treatment [11]. Also, whereas morphine is capable of restoring long-term potentiation in heroin dependent rats, heroin was unable to restore long-term potentiation in morphine dependent rats, which indicate a differential modulation of hippocampal functions in morphine and heroin dependent rats [3]. Furthermore, the upregulation of protein kinase A in rat hippocampus during withdrawal from morphine can be suppressed by re-exposure to morphine but not heroin [3]. Whether morphine and heroin dependence is also mediated by different splice variants of the μ-opioid receptor is unknown.

Here, we aim to delineate the exonic regions of MOR-1 that contribute to the development of morphine and heroin dependence using AOs targeting MOR-1 exons 1–4. We also assayed separate groups of AO treated mice for morphine or heroin analgesia. These additional groups allowed us to confirm that AOs effectively downregulated opiate function, to assess the recovery of function, and to directly compare the exonic profile of morphine and heroin analgesia and dependence in mice.

Adult male CD-1 mice (Charles River Labs, Kingston, NY) were maintained in a climate-controlled room with free access to food and tap water. An $n \geq 8$ was used for all conditions and each mouse was used only once. The experimental protocol was approved by the College of Staten Island Institutional Animal Care and Use Committee.

Heroin hydrochloride, morphine sulfate (both gift of NIDA), naloxone hydrochloride (Sigma–Aldrich, St. Louis, MO), and all oligodeoxynucleotides (Midland Certified Reagent Co., Midland, TX) were delivered in a 0.9% saline vehicle. The AO sequences used here to target MOR-1 exons 1, 2, 3, and 4 (Table 1) are identical to those previously demonstrated to reduce μ-opioid receptor expression [1,22] and are the most effective in reducing morphine and heroin analgesia [19,21]. Along with a control "mismatch" oligodeoxynucleotide (MO) sequence (Table 1), they were purified according to methods previously described [19,21].

Osmotic mini-pumps (Model 2001, Alza, Mountain View, CA) provided continuous heroin and morphine infusion (12 mg/kg cumulative daily dose). Pumps were implanted subcutaneously (s.c.) in anesthetized mice through a small dorsal midline incision closed with surgical staples and remained in place for the remainder of the study. Intracerebroventricular (i.c.v.) injections were made into the lateral ventricles using the method of Haley and McCormick [5] and as previously adapted [8]. Specifically, a small midline incision was made in the scalp of mice under oxygen/isoflourane inhalant anesthesia, and lambda located. Injections (5 μl volume) were made directly through the skull at a point 2 mm rostral and lateral to lambda at a depth of 3 mm using a 10-μl Hamilton micro-syringe fitted with a 27-gauge needle. A stainless steel wound clip was used to close the incision after each injection. All s.c. injections were made using a 10 ml/kg injection volume.

Nociception was assayed using the tail-withdrawal test where each mouse is gently restrained and the distal half of the tail is immersed in water maintained at $49.0 \pm 0.2\,^{\circ}C$ by an immersion heater/circulator. Latency to reflexive withdrawal of the tail is recorded twice to the nearest 0.1 s, with each determination separated by 30 s, and averaged. A cutoff latency of 15 s was employed to prevent the possibility of tissue damage. To test for analgesia, nociception was assayed before (baseline, BL) and 30 min after an s.c. injection of morphine or heroin (4 mg/kg).

Withdrawal was precipitated by the s.c. injection of naloxone (50 mg/kg). This naloxone dose has been shown to elicit maximal withdrawal responding in morphine [9] and heroin [11] dependent mice. Immediately after naloxone injection, subjects were placed into individual Plexiglas observation cylinders (25 cm × 11 cm), and the number of jumps – defined as the simultaneous removal of all four paws from the horizontal surface – was tallied for each subject over the next 15 min. The jumping response is a commonly used and reliable index of opiate withdrawal magnitude and, alone among withdrawal measures, is morphine and heroin dose-dependent [9,11]. Thus, although symptoms such as diarrhea, ptosis, wet-dog shakes, and lacrimation were sporadically observed in the present study, they were excluded from analysis. Withdrawal was always precipitated 4–6 h into the light cycle.

The following experimental protocols were used. On pretreatment Days 1, 3, 5, and 7, all mice were injected once i.c.v. with 5 μg of AO or MO, or an equal volume of saline. On Days 6 and 12, groups of mice from each of these pretreatment conditions were tested for analgesia. Other groups of mice from each pretreatment condition were implanted on Day 6 with pumps infusing morphine or heroin. On Day 12, these mice were subjected to NPW with pumps intact.

Morphine and heroin data were always analyzed separately. Analgesia data is represented by single values indicating the increase in latencies from BL after opiate injection. These data were analyzed using two-way (pretreatment × injection interval) ANOVA. Withdrawal jumping counts were subject to a one-way ANOVA. Both tests were followed post hoc using Fisher's LSD test (protected t-tests). For all comparisons, $p < 0.05$ was considered significant.

In mice pretreated with saline, morphine injection on Day 6 significantly increased withdrawal latencies relative to BL values, indicating analgesia (Fig. 1A). Analgesia was also evident in mice pretreated with MO or AOs targeting exons 2 and 3, but not 1 and 4. In contrast, morphine injection increased latencies relative to BL values obtained prior to morphine injection in all pretreatment groups on Day 12, 5 days after their final pretreatment injection on Day 7 (Fig. 1A). Withdrawal latencies were also significantly increased relative to BL values in heroin injected mice on Day 6 in all groups except those pretreated with an AO targeting exons 2 and 3 (Fig. 1B). As with morphine injection, all pretreatment groups displayed significant heroin analgesia on Day 12. There was no significant difference in mean BL values between saline, AO, or MO groups on either Day 6 or 12 in either the morphine or heroin studies (data not shown).

Control mice pretreated with saline and subjected to saline infusion (saline–saline group) displayed minimal jumping responses (mean: 2.7 ± 1.3) during naloxone-precipitated withdrawal on Day 12 (data not shown). Relative to this saline–saline control group, jumping frequencies were significantly increased in mice pretreated with saline or a mismatch oligodeoxynucleotide sequence followed by morphine infusion (Fig. 2A) or heroin (Fig. 2B). For both opiates, significant withdrawal jumping was also tallied for all