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Pharmacological characterization of dihydromorphine, 6-acetyldihydromorphine and dihydroheroin analgesia and their differentiation from morphine

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Abstract

The present study examined the pharmacology of dihydromorphine, 6-acetyldihydromorphine and dihydroheroin (3,6-diacetyldihydromorphine). Like morphine, dihydromorphine and its acetylated derivatives all were highly selective mu-opioids in receptor binding assays. All the compounds were potent mu-selective analgesics, as shown by their sensitivity towards the mu-selective opioid receptor antagonists naloxonazine and β-funaltrexamine. However, the actions of dihydromorphine and its analogs were readily distinguished from those of morphine, differences that were surprising in view of the very limited structural differences among them that consisted of only the reduction of the 7,8-double bond. Like heroin and morphine-6β-glucuronide, the analgesic actions of dihydromorphine and its two acetylated derivatives were antagonized by 3-*O*-methylnaltrexone at a dose that was inactive against morphine analgesia. Antisense mapping also distinguished between morphine and the dihydromorphine compounds. Antisense oligodeoxynucleotides targeting exon 2 of the cloned MOR-1 gene decreased dihydromorphine analgesia and that of its acetylated derivatives, but not morphine analgesia. Conversely, the exon 1 antisense that effectively lowered morphine analgesia was inactive against dihydromorphine and its analogs. Finally, dihydromorphine and its analogs retained their analgesic activity in a mouse model of morphine tolerance, consistent with incomplete cross-tolerance. Together, these findings imply that the mu-opioid receptor mechanisms mediating the analgesic actions of dihydromorphine and its acetylated analogs are distinct from morphine and more similar to those of heroin and morphine-6β-glucuronide.

Keywords: Heroin; Mu-opioid; Morphine; Mu-opioid receptor; MOR-1; MOP

1. Introduction

Morphine remains the prototypic mu-opioid analgesic. Yet, clinicians have long appreciated a wide range of responses from their patients for different mu-opioids (Cherny et al., 2001). Similarly, animal studies have revealed pharmacological differences between morphine and other mu-opioids, particularly morphine-6β-glucuronide (M6G), heroin and 6-acetylmorphine (Rossi et al., 1995, 1996; Pasternak and Standifer, 1995; Pasternak, 2001). Structurally, all these compounds are very similar to morphine. Heroin (3,6-diacetylmorphine) is rapidly hydrolyzed to 6-acetylmorphine by various esterases and is then more slowly metabolized to morphine (Way et al., 1960; Elliott et al., 1971). This rapid de-acetylation suggested that the analgesic activity of heroin results from 6-acetylmorphine (Inturrisi et al., 1983, 1985; Umans and Inturrisi, 1982). Differences in the pharmacological activity of heroin and morphine have been reported, and these have been largely attributed to differences in their pharmacokinetics (Way et al., 1965). However, heroin and morphine may work through different receptor mechanisms (Lange et al., 1980, 1983; Brown et al., 1997; Rossi et al., 1996; Schuller et al., 1999; Walker et al., 1999; Rady et al., 1991, 1994a). For instance, heroin and its metabolite 6-acetylmorphine show limited analgesic cross-tolerance to morphine (Rossi et al., 1996; Lange et al., 1980, 1983). In addition, morphine is inactive when given systemically in CXBK mice, while heroin and 6-acetylmorphine, like M6G,

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retain their analgesic activity (Rossi et al., 1996). Furthermore, the opioid receptor antagonist 3-*O*-methylnaltrexone selectively reverses the analgesic actions of heroin, 6-acetylmorphine and M6G at doses that are ineffective against morphine (Brown et al., 1997). Antisense mapping studies based upon the cloned mu-opioid receptor MOR-1 (MOP) also distinguish among these agonists (Rossi et al., 1995, 1996), as does a knockout mouse that has lost all analgesic sensitivity to morphine, but not to M6G, heroin and 6-acetylmorphine due to a disruption of exon 1 of the *Oprm* gene that encodes the cloned mu-opioid receptor MOR-1 (MOP) (Schuller et al., 1999). Even transduction systems seem to differ among them, as shown by their different sensitivity profiles to antisense treatments against various G-proteins (Standifer et al., 1996).

These differences between morphine and heroin are hard to explain by a single mu-opioid receptor, supporting the concept of multiple mu-opioid receptors as first proposed from traditional pharmacological approaches (Pasternak and Snyder, 1975a; Pasternak and Hahn, 1980; Wolozin and Pasternak, 1981; Hahn et al., 1982; Spiegel et al., 1982; Gintzler and Pasternak, 1983; Ling and Pasternak, 1983; Goodman and Pasternak, 1984; Ling et al., 1984, 1985) and more recently by the identification of multiple splice variants of the cloned mu-opioid receptor MOR-1 (MOP-1) (Zimprich et al., 1995; Bare et al., 1994; Pan et al., 1999, 2000, 2001).

Dihydromorphine differs structurally from morphine only by the reduction of the 7,8-double bond (Fig. 1). This reduction changes the C-ring from a boat to a chair conformation, but has few other structural effects. Binding assays have confirmed its high selectivity for mu-opioid receptors, and it has long been considered pharmacologically equivalent to morphine, although slightly more potent (Wolozin and Pasternak, 1981; Clark et al., 1988; Pasternak et al., 1975a; Pasternak and Snyder, 1975a). In the current study, we have compared the pharmacology of dihydromorphine and its acetylated derivatives to morphine.

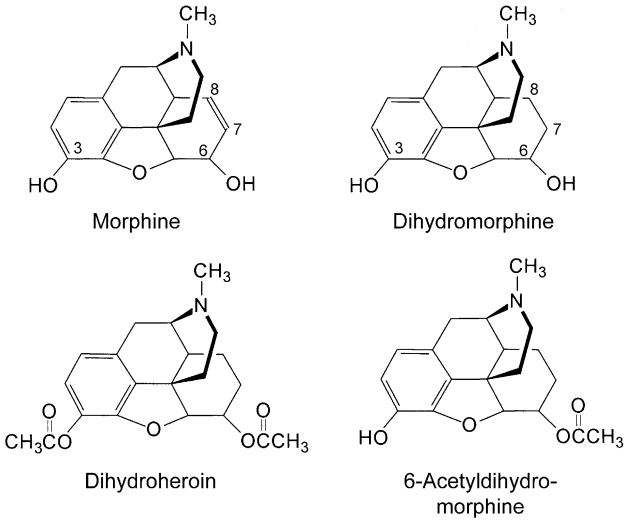

Fig. 1. Structures of dihydromorphine and its acetylated derivatives.

2. Materials and methods

All radioligands were purchased from Dupont NEN (Boston, MA), except for [^{3}H]naloxone benzoylhydrazone, which was synthesized in our lab as previously described (Price et al., 1989). Naloxonazine was synthesized as previously reported (Hahn et al., 1982). Morphine sulphate, dihydromorphine, naltrexone, heroin, 6-acetylmorphine, β-funaltrexamine and 3-*O*-methylnaltrexone were obtained from the Research Technology Branch of the National Institute of Drug Abuse (Rockville, MD). Naltrindole and nor-binaltorphimine (nor-BNI) were purchased from Sigma (St. Louis, MO, USA). 3,6-Diacetyldihydromorphine and 6-acetyldihydromorphine were synthesized and their structures were confirmed by standard chemical procedures (Hosztafi and Pasternak, in preparation). Halothane was purchased from Halocarbon Laboratory (Hackensack, NJ, USA).

All in vivo studies were carried out in accordance with the Declaration of Helsinki and with the Guide for Care and Use of Laboratory Animals, as adopted and promulgated by the National Institutes of Health. Male Crl:CD-1® (ICR) BR mice (20–25 g) were purchased from Charles River Laboratories (Raleigh, NC). Mice were housed in a 12-h light/dark cycle temperature-controlled room with food and water freely available. Intracerebroventricular (i.c.v.) and intrathecal (i.t.) injections were given under light halothane anesthesia as previously reported (Rossi et al., 1995; Haley and McCormick, 1957; Hylden and Wilcox, 1980).

2.1. Radiant heat tail-flick assay

Antinociception, referred to as ‘‘analgesia,’’ was assessed in the radiant heat tail-flick test as previously described (D’Amour and Smith, 1941; Paul and Pasternak, 1988; Paul et al., 1989). Baseline latencies typically ranged between 2 and 3 s. Analgesia was defined quantally as the doubling or greater of the baseline latency for each mouse (Paul and Pasternak, 1988; Paul et al., 1989; Rossi et al., 1996). The use of quantal analysis goes back to the original studies of D’Amour and Smith (D’Amour and Smith, 1941; Le Bars et al., 2001). Results analyzed quantally corresponded closely to those analyzed using graded responses (data not shown). Drugs were tested at peak analgesic effect after the time course for each drug was established. After systemic administration, the peak effect for all drugs was 30 min, except for 3,6-diacetyldihydromorphine and 6-acetyldihydromorphine for which the peak effect was 45 min after injection. Central injections (i.c.v. and i.t.) were assessed 15 min after injection.

2.2. Antisense mapping

Antisense oligodeoxynucleotides targeting exons 1 and 2 of the MOR-1 gene were purchased from Midland Certified Reagent Co. (Midland, TX) and purified in our lab as previously described (Rossi et al., 1995). Antisense sequences were based on mouse MOR-1 gene sequence (GenBank Accession # U26915) (Rossi et al., 1995). The antisense sequence for exon 1 was 5′-CGC CCC AGC CTC TTC CTC T-3′ and the mismatch sequence for exon 1 was 5′-CGC CCC GAC CTC TTC CCT T-3′. The MOR-1 exon 2 antisense sequence was 5′-TTG GTG GCA GTC TTC ATT TTG G-3′ and the mismatch sequence for exon 2 was 5′-TGT GTG GCA GTC TTC ATT TGT G-3′. Antisense studies were performed as previously reported (Rossi et al., 1995). Mice were injected i.c.v. (5 μg in 2 μl of 0.9% sterile saline) on days 1, 3 and 5 and analgesia was assessed on day 6.

2.3. Gastrointestinal transit

The inhibition of gastrointestinal transit was measured as previously described (Paul and Pasternak, 1988). In brief, mice that were food-deprived for 18–24 h received either saline or one of the test drugs and given a standard charcoal meal (0.5 ml) by gavage. They were sacrificed 30 min after the charcoal meal and the distance traveled by the charcoal meal was determined.

2.4. Tolerance

Groups of mice ($n \geq 20$) received either saline or morphine (5 mg/kg, s.c.) for five consecutive days. On the sixth day, mice received equianalgesic doses of either morphine (5 mg/kg, s.c.), dihydroheroin (5 mg/kg, s.c.) or 6-acetyldihydromorphine (6-AcDHM; 5.5 mg/kg, s.c.).

2.5. Receptor binding assays

Calf brain and guinea pig cerebellar membranes were prepared and binding assays were performed as previously reported (Clark et al., 1988, 1989). In brief, mu_1 binding was determined using [^{3}H][D-Ala^2,D-Leu^5]enkephalin (DADL) in calf thalamus in the presence of unlabeled [D-Pen^2,D-Pen^5]enkephalin (DPDPE) to compete delta opioid receptor binding, while mu_2 opioid binding was assessed using [^{3}H][D-Ala^2,MePhe^4,Gly(ol)^5]enkephalin (DAMGO) in the presence of unlabeled [D-Ser^2,Leu^5]enkephalin-Thr^6 (DSLET) to compete mu_1 opioid binding. Delta binding was determined in calf frontal cortex using [^{3}H]DPDPE. $Kappa_1$ binding was determined in guinea pig cerebellum with [^{3}H]U69,593 and $kappa_3$ sites were assessed with [^{3}H]naloxone benzoylhydrazone in calf striatum in the presence of EDTA. In competition studies, IC_{50} values were obtained using Graph Prism (San Diego, CA) and K_i values were determined (Cheng and Prusoff, 1973; Chou, 1974).

2.6. Reverse transcriptase-polymerase chain reactions (RT-PCR)

RT-PCR was performed as previously described (Pan et al., 1999). In brief, RNA was extracted from mouse striatum by the acid guanidium thioacyanate-phenol-chloroform extraction method as previously described (Chomczynski and Sacchi, 1987). One microgram of RNA was converted to cDNA with SuperScript II Reverse Transcriptase (GIBCO) in the presence of random primers for a total volume of 22 μl. The PCR reaction was performed in a final volume of 50 μl containing 3 μl of the cDNA and carried out in a thermal cycler (GeneAmp PCR system 9600, Perkin Elmer, Norwalk, CT) for 35 cycles. The primers spanned over exons 1 and 2 of the MOR1 gene: primer sense (5′-GAGAGGAAGAGGCTGGGGCGC-3′) and primer antisense (5′-CCGGTCAAGGCCCTGGATTTCCG-3′). Loading was verified by parallel amplification of the mouse housekeeping gene glyceraldehyde 3-phosphate dehydrogenase (G3PDH) with 5′-TGAAGGTCGGTGTGAACGGATTTGGC-3′ as sense primer and 5′-CATGTAGGCCATGAGGTCCACCAC-3′ as antisense primer (Clontech Laboratories, Palo Alto, CA, USA). The PCR products were allowed to migrate on a 1.5% agarose gel stained with ethidium bromide and visualized under UV transillumination (Bio-Rad Gel Doc, 2000, Hercules, CA, USA).

2.7. Statistical analyses

ED_{50} values and 95% confidence intervals were determined using the Litchfield–Wilcoxon method (Litchfield and Wilcoxon, 1949; Tallarida and Murray, 1987). Direct comparison of quantal results utilized the Fisher's exact test. For the gastrointestinal transit study, group differences were determined by a one-way analysis of variance followed by a Dunnett's post hoc test.

3. Results

Dihydromorphine is a potent mu-opioid that is structurally similar to morphine (Fig. 1). In binding studies, dihydromorphine showed high affinity and selectivity for mu-opioid binding sites (Table 1). It competed mu-opioid receptor binding slightly more potently than morphine and was far less active against delta and $kappa_1$ binding sites. Acetylation of the 6-position to yield 6-acetyldihydromorphine lowered its affinity for mu receptors approximately 5-fold to 10-fold while enhancing its potency for delta binding sites, although it was still over 100-fold more selective for mu-opioid binding. Dihydroheroin showed lower affinity for all the binding sites, perhaps reflecting the importance of the free 3-hydroxyl group in maintaining opioid receptor binding affinity.

All the derivatives were potent analgesics (Table 2). Systemically, heroin and its active metabolite 6-acetylmor-

Table 1
Binding affinity of morphine and dihydromorphine analogs

Binding assay	K_i values (nM)			
	Morphine	Dihydromorphine	Dihydroheroin	6-AcDHM
Mu_1	0.5 ± 0.38	0.23 ± 0.06	11.2 ± 2	1.83 ± 0.3
Mu_2	2.5 ± 0.6	1.3 ± 0.1	191 ± 15	12.3 ± 3.9
Delta	278 ± 49	>1000	2469 ± 197	273 ± 38
$Kappa_1$	49 ± 32	183 ± 30	2807 ± 519	731 ± 103
$Kappa_3$	32.8 ± 2.2	33.6 ± 13.4	536 ± 55	148 ± 14

K_i values were determined from competition studies, as described in Materials and methods. Each value represents the mean ± S.E.M. of at least three independent experiments, each of which was performed in triplicate. The values for morphine are taken from the literature (Clark et al., 1989).

phine were significantly more potent than all the other derivatives, including morphine. However, heroin was less potent than morphine supraspinally. Dihydroheroin, on the other hand, was equipotent to morphine systemically and supraspinally. Dihydromorphine and 6-acetyldihydromorphine were equipotent to morphine both systemically and supraspinally. Spinally, the relative potencies of the drugs were somewhat different, with heroin showing a significantly greater potency spinally than supraspinally ($p < 0.05$). All the other drugs tested showed a decreased potency following spinal administration, although the difference was significant ($p < 0.05$) only for dihydromorphine and dihydroheroin.

All the drugs were mu-opioid analgesics (Fig. 2). Naltrexone, a general opioid receptor antagonist, reversed the actions of all the analgesics, as did the mu-selective opioid receptor antagonists β-funaltrexamine and naloxonazine (Fig. 2). As expected, neither the $kappa_1$-selective opioid receptor antagonist norBNI nor the delta-selective opioid receptor antagonist naltrindole effectively blocked any of the agents, confirming their mu selectivity.

3-*O*-Methylnaltrexone antagonizes the analgesic actions of morphine-6β-glucuronide, heroin and its active metabolite 6-acetylmorphine at doses that are inactive against morphine (Brown et al., 1997; Walker et al., 1999). In the current studies, 3-*O*-methylnaltrexone again was inactive against morphine, but it reversed both 6-acetyldihydromorphine and dihydroheroin analgesia. Thus, all the acetylated derivatives were sensitive to 3-*O*-methylnaltrexone. However, 3-*O*-methylnaltrexone also blocked dihydromorphine analgesia, clearly distinguishing its pharmacological profile from that of morphine.

Table 2
Analgesic potency of various opioid agonists

Drug	ED_{50} (95% confidence limits)		
	Systemic (mg/kg, s.c.)	Supraspinal (μg, i.c.v.)	Spinal (μg, i.t.)
Morphine	5.2 (4.2–6.5)	0.58 (0.44–0.74)	0.98 (0.67–1.4)
Heroin	0.7 (0.68–0.8)	1.86 (1.5–2.2)	1.13 (0.87–1.4)
6-Acetylmorphine	0.8 (0.78–0.88)	0.68 (0.49–0.94)	0.91 (0.70–1.18)
Dihydromorphine	4.3 (3.6–5.1)	0.41 (0.32–0.52)	0.74 (0.58–0.94)
Dihydroheroin	3.0 (2.5–3.6)	0.57 (0.47–0.69)	1.54 (1.06–2.26)
6-Acetyldihydromorphine	3.3 (2.7–4.0)	0.55 (0.43–0.72)	0.64 (0.44–0.93)

ED_{50} values and 95% confidence intervals were determined using the Litchfield–Wilcoxon method (Litchfield and Wilcoxon, 1949; Tallarida and Murray, 1987).

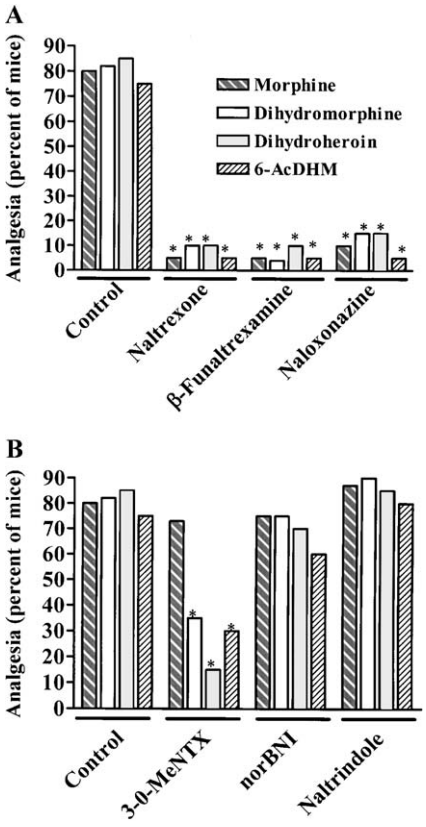

Fig. 2. Effects of selective opioid receptor antagonists on the opioid analogs. Groups of mice ($n \geq 20$) received equianalgesic doses of morphine (5 mg/kg, s.c.), dihydroheroin (5 mg/kg, s.c.) or 6-acetyldihydromorphine (6-AcDHM; 5.5 mg/kg, s.c.). immediately prior to the administration of naltrexone (1 mg/kg, s.c.), 3-*O*-methylnaltrexone (3-*O*-MeNTX, 0.25 mg/kg, s.c.), norBNI (10 mg/kg, s.c.) or naltrindole (20 mg/kg, s.c.). The mu-selective antagonists β-funaltrexamine (40 mg/kg, s.c.) and naloxonazine (35 mg/kg, s.c.) were administered 24 h before testing. Asterisks indicate decreased analgesia compared to control for the corresponding agonist ($P < 0.05$) (6-AcDHM, 6-acetyldihydromorphine).

Cross-tolerance is a powerful tool capable of distinguishing subtle differences in the mechanisms of action of opioids. We looked for cross-tolerance to morphine using a daily-dosing paradigm over 5 days that produces approximately a 2-fold shift of the morphine dose–response curve (Kolesnikov et al., 1993). In this paradigm, morphine's response decreased over 5 days from 70% to only 10% (Fig. 3). However, equianalgesic doses of 6-acetyldihydromorphine and dihydroheroin retained their analgesic activity in the morphine tolerant mice, implying the lack of complete cross-tolerance.

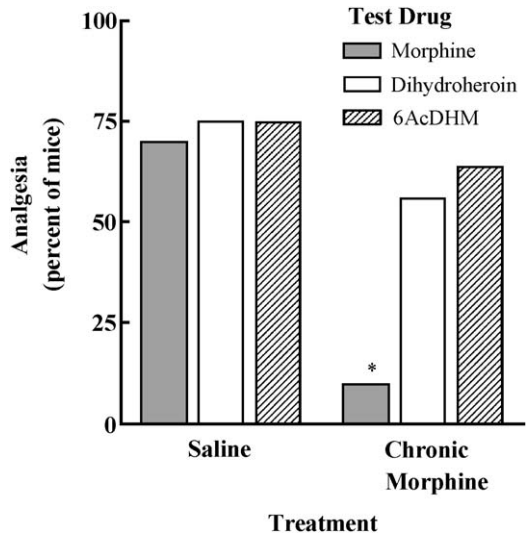

Fig. 3. Cross-tolerance of 3,6-diacetyldihydromorphine and 6-acetyldihydromorphine with morphine. Groups of mice ($n \geq 20$) received either saline or morphine (5 mg/kg, s.c.) for five consecutive days. On the sixth day, mice received an equianalgesic dose of either morphine (5 mg/kg, s.c.), dihydroheroin (5 mg/kg, s.c.) or 6-acetyldihydromorphine (6-AcDHM; 5.5 mg/kg, s.c.). The asterisk indicates a significant decrease in analgesic response compared to control mice treated with saline for 5 days and morphine on testing day ($P < 0.05$).

Antisense mapping of the various exons of MOR-1 has revealed differences among various mu-opioid drugs (Pasternak and Standifer, 1995; Rossi et al., 1995, 1996). We therefore examined the effects of antisense probes targeting exons 1 or 2 of MOR-1 on the analgesic actions of the agents. First, we confirmed the efficacy of the antisense approach using RT-PCR using an upstream exon 1 primer and a downstream exon 2 primer. Each antisense probe effectively and selectivity down-regulated mRNA expression (Fig. 4). The primers used in this study will only measure mRNA transcripts that contain both exons 1 and 2. However, the exon 2 antisense would still be expected to down-regulate only transcripts that contain exon 2 and vice versa. Splice variants lacking either exon 1 or exon 2 have been cloned (Pan et al., 1999, 2001; Du et al., 1997). The inactivity of the mismatch primers confirms the specificity of the down-regulation.

We then examined the behavioral effects of the antisense treatments. Mismatch antisense probes, in which the sequence of four of the bases were scrambled, were ineffective, ensuring the specificity of the paradigm. The exon 1 antisense probe decreased the analgesic actions of morphine (Fig. 5), as previously reported (Rossi et al., 1995, 1996, 1997), but not the analgesia induced by any of the other compounds. In contrast, the exon 2 antisense probe significantly lowered the responses of dihydromorphine and both of its acetylated derivatives, but not morphine. Thus, the selectivity profile of dihydromorphine and its acetylated derivatives in antisense mapping studies also differentiated them from morphine.

Finally, opioids have long been known to inhibit gastrointestinal transit. In the current study, morphine inhibited gastrointestinal motility, lowering transit from approximately 30 to under 10 cm (Fig. 6). The other compounds given at equianalgesic doses also lowered transit to a similar extent.

4. Discussion

Dihydromorphine is a potent mu-selective opioid analog of morphine that has long been used in receptor binding studies (Pasternak et al., 1975a,b; Pasternak and Snyder, 1975b; Pert et al., 1973; Wilson et al., 1975). Likewise, its acetylated derivatives also were mu-selective, although their actual affinities were lower than dihydromorphine. They all showed poor affinity for delta opioid receptors and their ability to compete kappa opioid binding remained at least 20-fold poorer than mu binding.

Dihydromorphine was an effective analgesic, with a potency similar to that of morphine. As previously reported (Umans and Inturrisi, 1981; Inturrisi et al., 1983), heroin and 6-acetylmorphine were far more potent than morphine after systemic administration in the current studies, but the acetylated derivatives of dihydromorphine were no more effective than morphine or dihydromorphine itself. It has been suggested that the enhanced potency of systemic heroin compared to morphine was due to its greater lipophilicity and penetration of the blood–brain barrier. However, if this were the case, we would have expected a similar enhanced activity of dihydroheroin and 6-acetyldihydromorphine compared to dihydromorphine. Since the lipophilicity of morphine and dihydromorphine is very similar (Kutter et al., 1970), the lipophilicity differences between their acetylated derivatives also should be quite minimal. Yet, dihy-

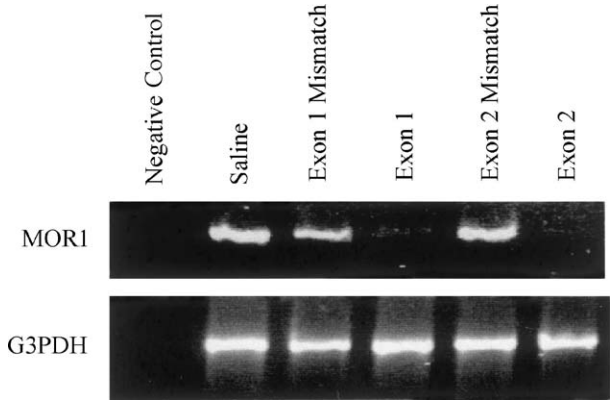

Fig. 4. Effects of antisense treatment of MOR-1 mRNA levels. Down-regulation of MOR-1 mRNA levels by antisense treatments was determined by RT-PCR, as described in Materials and methods. Antisense oligodeoxynucleotides targeting exon 1 and exon 2 of the MOR-1 gene decreased mRNA levels compared to mismatch probes or saline. As predicted, the size of the amplified cDNA fragments were 568 bp. Loading was verified by PCR of glyceraldehyde 3-phosphate dehydrogenase (G3PDH) which gave the expected 982 bp fragments with similar intensity in all lanes.

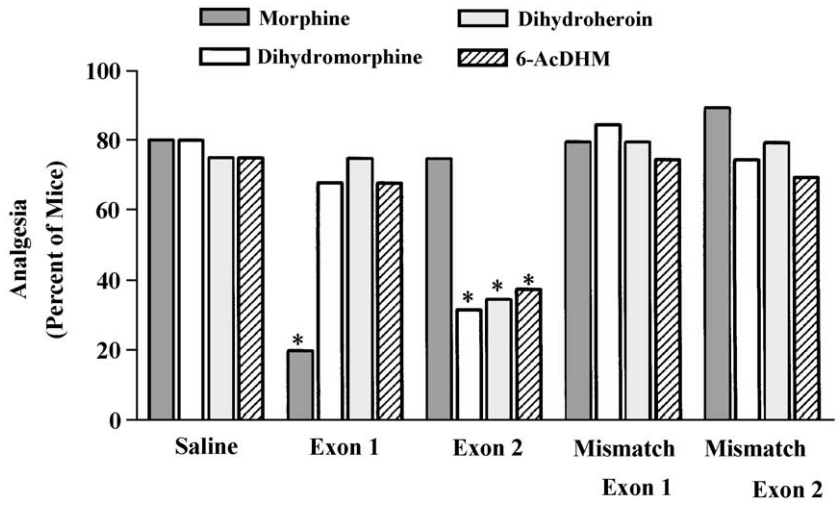

Fig. 5. Effects of antisense treatments on opioid analgesia. Groups of mice ($n \geq 20$) were treated supraspinally with saline or one of the four antisense probes on days 1, 3 and 5 and tested with either dihydromorphine (6 mg/kg, s.c.), dihydroheroin (5 mg/kg, s.c.), 6-acetyldihydromorphine (6-AcDHM; 5.5 mg/kg, s.c.) or morphine (5 mg/kg, s.c.) on day 6. Asterisks indicate a decreased analgesic effect compared to saline-treated mice that received the corresponding agonist on testing day ($P < 0.05$).

droheroin did not demonstrate the enhanced potency seen with heroin following systemic administration. Thus, it seems that lipophilicity alone cannot explain the enhanced potency of systemic heroin compared to morphine.

All the derivatives were mu-opioid-selective in the analgesic assays, as demonstrated by their sensitivity towards β-funaltrexamine and naloxonazine. However, the pharmacological profile of dihydromorphine and its acetylated derivatives differed from morphine in several aspects. Previously, we demonstrated that 3-*O*-methylnaltrexone at doses that are ineffective against morphine selectively blocks the analgesic actions of heroin and 6-acetylmorphine (Brown et al., 1997; Walker et al., 1999). The current studies revealed a similar blockade of 6-acetyldihydromorphine and dihydroheroin at an antagonist dose inactive against morphine. However, 3-*O*-methylnaltrexone also antagonized the actions of dihydromorphine. This difference between dihydromorphine and morphine was quite unexpected since they differ structurally only by the reduction of the 7,8-double bond. This suggests that subtle structural differences, such as changing the conformation of the C-ring from the "boat" conformation in morphine to the "chair" conformation in dihydromorphine, can impact on the pharmacology of the drug.

Antisense mapping studies also distinguished dihydromorphine and its derivatives from morphine. Prior work from our group reported that morphine was sensitive to antisense probes targeting exon 1, but not exon 2 (Rossi et al., 1995). In contrast, heroin analgesia was sensitive to both exon 1 and exon 2 antisense probes (Rossi et al., 1996). Dihydromorphine and dihydroheroin were different, with their analgesic actions lowered only by the exon 2 antisense probe, a sensitivity similar to that of morphine-6β-glucuronide (Rossi et al., 1995). Thus, antisense mapping also illustrated pharmacological differences between morphine and dihydromorphine, further supporting the importance of the C-ring conformation in defining the pharmacology of the drug.

In the present study, dihydroheroin and 6-acetyldihydromorphine do not show cross-tolerance to morphine. This probably reflects the limited level of morphine tolerance in the current model. It is likely that in animals showing a far greater tolerance, morphine would show partial cross-tolerance to dihydroheroin and 6-acetyldihydromorphine. Lack of cross-tolerance between heroin and morphine has been

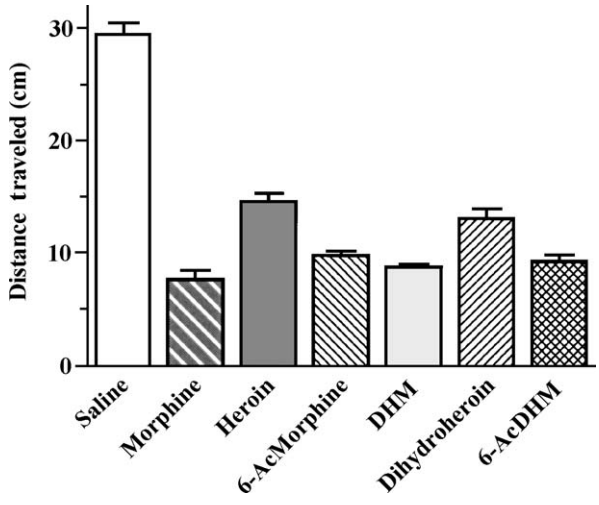

Fig. 6. Inhibition of gastrointestinal transit by various opioid agonists. Groups of mice ($n \geq 10$) received either morphine (5 mg/kg, s.c.), heroin (0.9 mg/kg, s.c.), 6-acetylmorphine (6-AcMorphine; 1 mg/kg, s.c.), dihydromorphine (DHM; 6 mg/kg, s.c.), dihydroheroin (5 mg/kg, s.c.) or 6-acetyldihydromorphine (6-AcDHM; 5.5 mg/kg, s.c.), followed by the charcoal meal and gastrointestinal transit determined. Results are the means ± S.E.M. The groups treated with opioids were significantly different from the saline control, as determined by ANOVA ($P < 0.001$).

reported previously, and it was proposed that heroin might be acting through delta receptors in morphine tolerant mice, in part due to the activity of naltrindole (Lange et al., 1980; Rady et al., 1991, 1994b, 1998; Holmes et al., 1998;). Although the change in antagonist sensitivity of heroin in morphine tolerant animals is interesting and deserves further study, it cannot account for many of the pharmacological differences between heroin and morphine seen in naive mice (Rossi et al., 1996; Schuller et al., 1999) and the differences in the current studies with dihydromorphine and its analogs. These differences imply the possibility of a distinct receptor mechanism(s) of action, although all compounds act through mu-opioid receptors. The idea that different receptors mediate the analgesic effect of mu-opioids is further supported by the observation that both drug abusers and animals easily distinguish morphine from heroin (Martin and Fraser, 1961). The differences between dihydromorphine itself and morphine suggest that even subtle structural differences induced by reduction of the 7,8-double bond can change the selectivity of the ligand. The possibility of multiple mu-opioid receptors is more interesting in view of the recent cloning of a large number of mu-opioid receptor splice variants (Zimprich et al., 1995; Bare et al., 1994; Pan et al., 1999, 2000, 2001). While none have yet been associated with the actions of specific mu ligands, their presence indicates that this possibility must be considered and explored further.

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