Morphine Tolerance in Mice Changes Response of Heroin from μ to δ Opioid Receptors (44520)

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Abstract. **Heroin produced antinociception in the tail flick test through μ receptors in the brain of ICR and CD-1 mice, a response inhibited by 3-O-methylnaltrexone. Tolerance to morphine was produced by subcutaneous morphine pellet implantation. By the third day, the heroin response was produced through δ opioid receptors. The response was inhibited by simultaneous intracerebroventricular (i.c.v.) administration of naltrindole, a δ opioid receptor antagonist. More specifically, δ_1 rather than δ_2 receptors were involved because 7-benzylidenenaltrexone, a δ_1 receptor antagonist, inhibited but naltriben, a δ_2 antagonist, did not. Also, antinociception produced by i.c.v. heroin was inhibited by intrathecal administration of bicuculline and picrotoxin consistent with the concept that δ_1 receptors in the brain mediated the antinociceptive response through descending neuronal pathways to the spinal cord to activate $GABA_A$ and $GABA_B$ receptors rather than spinal α_2-adrenergic and serotonergic receptors activated originally by the μ agonist action in naive mice. The μ response of 6-monoacetylmorphine, a metabolite of heroin, was changed by morphine pellet implantation to a δ_2 response (inhibited by naltriben but not 7-benzylidenenaltrexone). The agonist action of morphine in these morphine-tolerant mice remained μ. Thus, the opioid receptor selectivity of heroin and 6-monoacetylmorphine in the brain is changed by production of tolerance to morphine. Such a change explains how morphine tolerant mice are not cross-tolerant to heroin.** [P.S.E.B.M. 2000, Vol 224:93–101]

Substantial evidence exists to support the concept that the major pharmacological actions of heroin are due to the formation of active metabolites, 6-monoacetylmorphine(6MAM), and morphine (1–3). Differences in pharmacokinetics such as the more rapid penetration of the blood brain barrier by heroin serve to explain the greater potency of systemically administered heroin compared with morphine (1, 4, 5). Competitive binding studies with ^{3}H-naltrexone in rat brain membrane preparations suggest that 6MAM and morphine act on μ opioid receptors (6). In the face of such evidence, we have found some fundamental pharmacological differences between the antinociceptive actions of heroin and morphine in certain mice. Heroin-induced antinociception involves μ receptors in inbred DBA/2J, CBA/J, and C3H/HeJ mice, but δ receptors in inbred C57BL/6J mice (7). Also, intracerebroventricular (i.c.v.) heroin and 6MAM responses were mediated by μ receptors in outbred ICR and CD-1 mice but by δ receptors in Swiss Webster mice (8–10). These strain differences indicate that genetic factors contribute to the opioid receptor selectivity of heroin and 6MAM even though the response to morphine is mediated by μ receptors in all of these mice. A puzzling finding is that Swiss Cox mice on Day 3 after morphine pellet implantation are highly tolerant to subcutaneous morphine but not cross-tolerant to subcutaneous (s.c.) heroin (determined at 20 min in the tail flick test) (11). If heroin action were due to morphine, cross

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tolerance should have occurred. One explanation for this finding might be similar to that recently given for Swiss Webster mice (which are δ responders to heroin). Tolerance to the μ response of morphine in these mice does not confer cross-tolerance to the δ agonist action of heroin (Rady JJ, Fujimoto JM, unpublished data).

In the present study, another aspect of the opioid receptor action of heroin and 6MAM was demonstrated. Heroin and 6MAM antinociceptive tail flick responses in ICR and CD-1 mice, which initially are through brain μ opioid receptors, were shown to become δ receptor mediated after morphine pellet implantation even though the morphine response stayed μ. Participation of δ opioid receptors was determined by the inhibitory effect of i.c.v.-administered δ opioid receptor antagonists. To reinforce the concept of δ agonist function, GABA receptor antagonists were given intrathecally (i.t.) because δ agonist action in the brain inhibits the tail flick response through activation of descending pathways mediated by spinal GABA receptors (12–14).

Materials and Methods

Animals and Measurement of Antinociception. Male ICR and CD-1 mice weighing 25–30 g were obtained from Sasco, Inc. (Omaha, NE) and Charles River Laboratories (Wilmington, MA), respectively. The original studies were performed in ICR mice, but when Charles River took Sasco Inc. over, the ICR mice were replaced with the CD-1 mice of Charles River. Even though these mice are designated differently, they have a common origin (both are derived from the Institute of Cancer Research mice). The mice used for each experiment are given in the text, figure legends, and table. Each animal was used in only one experimental group. In the radiant heat tail flick test for antinociception (15), two tail flick test trials were conducted prior to the administration of drugs and the average (2–4 sec) was employed as the predrug latency. An automatic cut-off time set at 10 sec prevented trauma to the tail and was used as the maximal antinociceptive response. The percentage maximum possible effect (% MPE) was calculated from the tail flick latencies by the formula (16):

$$\%\ \text{MPE} = \frac{(\text{Postdrug}) - (\text{Predrug}) \times 100}{(10 - \text{Predrug})}$$

Drug Administration. The basic protocol consisted of measuring antinociception 10 min after i.c.v. administration of heroin and other opioid agonists by the tail flick response. Opioid receptor antagonists were given i.c.v. in the same solution as the agonists. This 10-min time favored heroin and 6MAM remaining in their native form rather than metabolites. Initially, 3-O-methylnaltrexone (MeNTX) was used to assess the μ response to heroin and 6MAM in naive mice and confirm the results reported in the literature (17–19). A morphine pellet containing 75 mg morphine base or placebo pellet was implanted s.c. as used previously (11). Four μl of drug solution were administered i.c.v. to the mouse (20) under light halothane anesthesia. Dose-response curves for the agonists (7–10 mice at each dose) were obtained in the absence and presence of a fixed dose of the δ receptor antagonists (naltrindole, 7-benzylidenenaltrexone, and naltriben) and were used to derive ED_{50} values as in previous studies (7, 21). In one experiment, the ED_{50} values were obtained for heroin at 15 min after s.c. administration with naltrindole given i.c.v. 10 min before the tail flick test. To determine the descending neuronal pathways through which the opioids given i.c.v. inhibited the tail flick response (7, 12–14), antagonists for spinal $GABA_A$ (bicuculline and picrotoxin), $GABA_B$ (2-hydroxysaclofen), α_2-adrenergic (yohimbine), and serotonergic (methysergide) receptors were administered intrathecally in 5 μl volume (22) 5 min before the tail flick test (i.e., 5 min after the i.c.v. administration of the agonist). Stimulation of brain μ receptors activates spinal noradrenergic and serotonergic receptors; δ_1 and δ_2 receptor responses activate $GABA_A$ receptors, but only δ_1 receptors activate $GABA_B$ receptors (12–14). Appropriate vehicle solutions were administered as required. Drugs were dissolved in a 0.9% saline solution with 6MAM free base and bicuculline requiring a few drops of 0.1 *M* hydrochloric acid and slight heating to dissolve; slight heating was also needed to dissolve 2-hydroxysaclofen. The opioid peptides, [D-Pen^2-D-Pen^5]enkephalin (DPDPE) and [D-Ser^2, Leu^5]enkephalin-Thr (DSLET), were dissolved in a 0.9% saline solution containing 0.01% Triton X-100. All studies were done in compliance with the Institutional Animal Care and Use Committee (Animal Studies Subcommittee).

Statistical Analysis. ED_{50} values were compared by deriving the potency ratio for parallel curves with a computerized version of the method of Litchfield and Wilcoxon (23) as described by Dewey *et al.* (16) and used previously (7). Analysis of single-dose experiments that involved a comparison between two groups was by Student's *t* test, and those involving more than two groups were evaluated by analysis of variance followed by Dunnett's test (24) for comparison of several groups to a given control group. $P \leq 0.05$ indicated significant differences between mean values.

Source of Drugs. The drugs and commercial sources were as follows: morphine sulfate·$5H_2O$ (Mallinckrodt Chemical Works, St. Louis, MO); heroin hydrochloride and 6MAM (free base) (National Institute on Drug Abuse, Rockville, MD); DSLET (Peninsula Laboratories, Inc., Belmont, CA); naltrindole hydrochloride (Research Biochemical Inc., Natick, MA); and DPDPE, (+)-bicuculline and yohimbine hydrochloride (Sigma Chemical Co., St. Louis, MO). 7-Benzylidenenaltrexone (BNTX) and naltriben, δ_1 and δ_2 receptor antagonists, respectively, were synthesized as described previously (25, 26). 3-O-methylnaltrexone was synthesized according to Brown *et al.* (17). The doses of the

drugs, stated with each experiment, were for the forms stated above.

Results

The Initial Opioid Receptor Response to i.c.v. Heroin and 6MAM. The results in Figure 1A indicated that MeNTX given i.c.v. over a wide dose range inhibited the antinociceptive response to i.c.v. heroin in CD-1 mice. The duration of action for a 0.1-μg dose of MeNTX was at least 30 min, but the effect was not evident at 1 hr (Fig. 1C). As expected (17–19), MeNTX at 10 and 20 ng inhibited the i.c.v. 6MAM response (Fig. 1B) in CD-1 mice, but even at 10 μg, MeNTX had no effect on the i.c.v. morphine response (Rady JJ *et al.*, unpublished data) in CD-1 mice. The ability of MeNTX to inhibit the antinociceptive action of heroin and 6MAM but not morphine suggests the possibility that a somewhat different μ receptor might exist from that on which morphine acts (17–19).

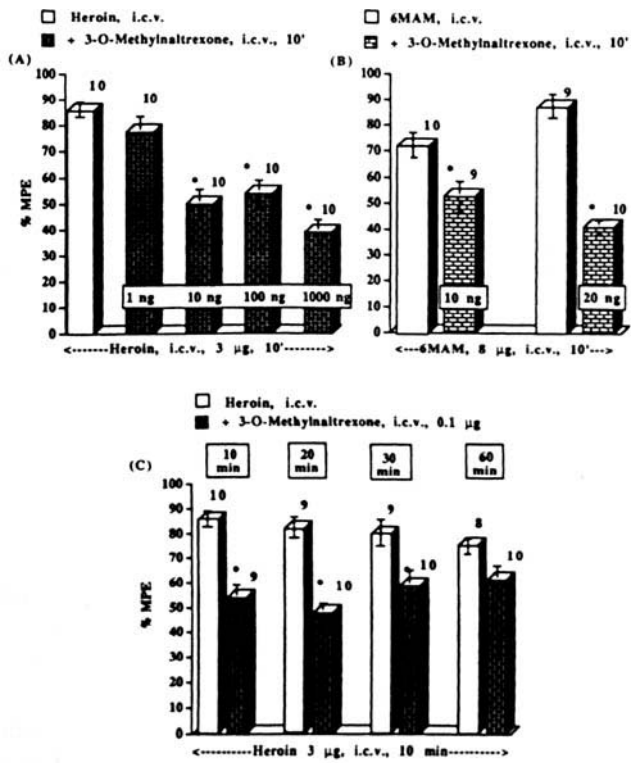

Figure 1. The inhibitory action of i.c.v. 3-O-methylnaltrexone (MeNTX) on i.c.v. heroin-induced antinociception in CD-1 mice. (A) Heroin, 3 μg, and MeNTX at various doses were given together, i.c.v., and the tail flick response evaluated 10 min later. The number of mice in each group is given at the top of each bar along with the standard error of the mean indicated by the vertical line. The asterisk, *, indicated a significant difference ($P \leq 0.05$) between the mean and the control group mean by Dunnett's test. (B) MeNTX (10- and 20-ng doses) given i.c.v. along with 6MAM (8 μg) 10 min before the tail flick test. The asterisk, *, indicates a significant difference ($P \leq 0.05$) from the matched control group using Student's *t* test. (C) Heroin was given i.c.v. as above to all groups, and 0.1 μg (100 ng) of MeNTX was given i.c.v. at the various times indicated before the tail flick test. The asterisk, *, indicated a significant difference between the paired groups ($P \leq 0.05$) by Student's *t* test; each mouse was used for only one group.

Opioid Receptor Response to i.c.v. Heroin and 6MAM in Morphine Pellet-Implanted Mice. Normally, the response to i.c.v. heroin and 6MAM given 10 min before the tail flick test is μ receptor mediated in ICR and CD-1 mice (8–10). On Day 1 after implantation of the 75 mg pellet of morphine in ICR mice, there was no difference in response to i.c.v. heroin as compared with the heroin with naltrindole treatment, indicating that no δ agonist action was evident (Fig. 2A). On Day 2, the heroin response was significantly inhibited by the co-administration of naltrindole. Similar inhibition of the heroin response was produced by naltrindole on the third and seventh day of the morphine pellet implantation. The response to i.c.v. heroin (without naltrindole administration) remained the same between the first and seventh days, that is, no evidence of cross-tolerance to heroin was seen in the morphine pellet–implanted animals.

For the experiment depicted in Figure 2B, the morphine pellet was removed on the third day, and the groups were evaluated as before at various times thereafter. The dose of i.c.v. heroin was increased 3-fold over that in the previous panel because the removal of the morphine pellet produced withdrawal tolerance to i.c.v. heroin (discussed later). The inhibitory effect of naltrindole on heroin antinociception continued to occur for the second and fourth hours after morphine pellet removal. By the sixth hour after pellet removal, the i.c.v. naltrindole produced no inhibition, suggesting that the heroin response had reverted back to the original μ receptor–mediated response. In Figure 2C, naltrindole had no effect on the heroin response in placebo pellet–implanted mice.

Similar experiments were performed for 6MAM. The response to 6MAM is μ receptor mediated in normal ICR and CD-1 mice (9, 10). The results of morphine pellet implantation on the first three days were similar to those for heroin. The response to i.c.v. 6MAM was not inhibited by i.c.v. naltrindole on Day 1 but was inhibited on Days 2 and 3 (Fig. 3A). However, in contrast to the heroin response, on Day 7, there was no significant effect of the naltrindole. The response to 6MAM alone remained the same on Days 1–7; no development of cross-tolerance was seen. Figure 3B gives the results for when the morphine pellet was removed on the third day. As for heroin, the dose of 6MAM needed to be increased, and inhibition by naltrindole was present at 2 hr after pellet removal. In contrast to the heroin response, the 6MAM response was no longer inhibited by naltrindole at 4 hr after pellet removal. The major effects of interest were that the morphine pellet implantation caused the manifestation of δ opioid receptor responses to heroin and 6MAM as indicated by the inhibitory effect of naltrindole. These effects were investigated further. In Figure 3C, naltrindole had no effect on 6MAM-induced analgesia in 3-day placebo pellet–implanted ICR mice. Because of the lack of effect of naltrindole against heroin and 6MAM, no further placebo pellet–implanted mice were included in the subse-

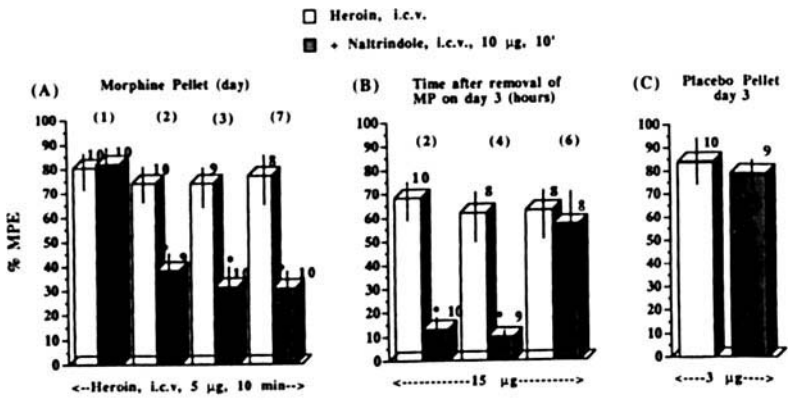

Figure 2. Onset and offset of heroin δ agonist response in ICR mice. (A) Morphine pellet–implanted mice: Onset of change from μ to δ opioid receptor-mediated response indicated by inhibition of i.c.v. heroin-induced antinociception by co-administration of naltrindole 10 min before the tail flick test. All mice were implanted s.c. with a 75-mg morphine pellet; at the day designated in parentheses (), paired groups were given either i.c.v. heroin or i.c.v. heroin together with naltrindole. (B) Offset of heroin δ response indicated by loss of effectiveness of naltrindole. All mice were implanted with a morphine pellet for 3 days. The pellet was removed and at the hour designated in parentheses (), the paired groups were given i.c.v. heroin or i.c.v. heroin together with naltrindole as above. Note that the heroin dose was increased to 15 µg. (C) Placebo pellet–implanted mice tested on Day 3 by administration of i.c.v. heroin or i.c.v. heroin together with naltrindole. The * asterisk indicates a significant difference ($P \leq 0.05$, Student's t test) between the paired groups; each mouse was used for only one group.

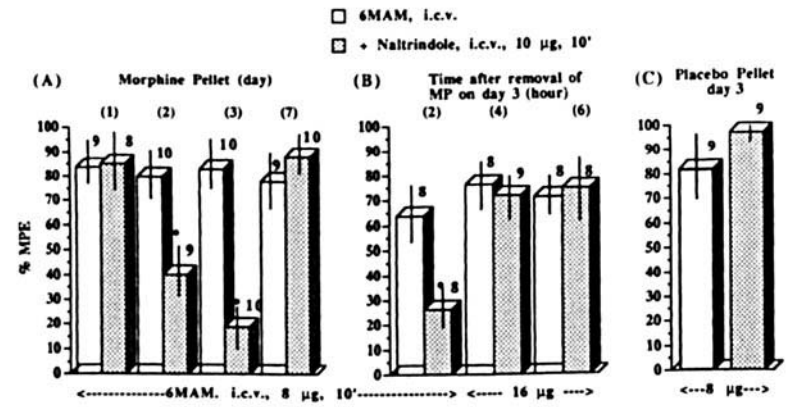

Figure 3. Onset and offset of 6MAM δ agonist response in ICR mice. (A) Morphine pellet–implanted mice: Onset of change from μ to δ receptor response for i.c.v. 6MAM evaluated as in previous figure. (B) Offset of 6MAM δ response after morphine pellet removal evaluated as in previous figure. Note increase in 6MAM dose to 16 µg. (C) Placebo pellet–implanted mice tested on Day 3 by administration of i.c.v. 6MAM or i.c.v. 6MAM together with naltrindole. Designations are as in previous figure, and again each mouse was used for only one group.

quent experiments that determined the subtype of δ receptor involved in heroin and 6MAM responses.

Possible Subtypes of δ Receptor Acted on by Heroin and 6MAM in Morphine-Pelleted Mice. Naltrindole inhibits both δ_1 and δ_2 subtypes (defined pharmacologically) of δ opioid agonist actions. To differentiate between the possible subtypes involved in heroin and 6MAM actions, experiments using the δ_1 and δ_2 opioid receptor antagonists, BNTX and naltriben, respectively, were performed. Studies with DPDPE and DSLET, δ_1 and δ_2 agonists, respectively, were performed to establish that the sensitivity toward the δ agents did not change. The data in Table I show that the ED_{50} value for i.c.v. DPDPE was increased with co-administration of BNTX, and the ED_{50} value for i.c.v. DSLET was increased with co-administration with naltriben in placebo pelleted CD-1 mice. The sets of ED_{50} values for DPDPE and DSLET in the three day morphine pelleted mice showed that the values remained unchanged compared with the placebo pellet–implanted groups. That is, the morphine pelleting did not produce cross-tolerance to the δ agonist actions of DPDPE and DSLET. Furthermore, the shifts in the ED_{50} values produced by BNTX and naltriben, respectively, in the morphine and placebo pellet-implanted groups were of the same magnitude. Thus, the morphine pellet implantation did not change the sensitivity of the mice to the antagonists or agonists.

BNTX and naltriben were used to determine the subtype of δ receptors involved in the action of heroin and 6MAM in the morphine pellet–implanted ICR mice. The ED_{50} value for i.c.v. heroin was increased by BNTX but not naltriben, indicating that heroin was acting on δ_1 receptors. The action of i.c.v. 6MAM was inhibited by naltriben but not BNTX; therefore, 6MAM acted on δ_2 receptors.

The last set of data in Table I is for an experiment where the heroin was given s.c. (instead of i.c.v.) in CD-1 mice. The ED_{50} values for s.c. heroin in placebo pelleted and 3-day morphine pellet–implanted groups were the same. Thus, there was no cross-tolerance to s.c. heroin in the morphine-tolerant mice. The ED_{50} value for heroin in the presence of i.c.v. treatment with naltriben was unchanged; heroin was not acting through δ_2 receptors. The i.c.v. administration of BNTX caused a significant rightward shift in the ED_{50} value according to the potency ratio calculation so that a major effect of s.c. heroin appeared to be mediated through brain δ_1 receptors. However, overlap of the confidence intervals for the ED_{50} values indicates caution in interpretation. Perhaps, in this particular experi-

Table I. ED_{50} Values (95% Confidence Intervals) for Opioids with the Tail Flick Test Determined on Day 3 in Subcutaneous Morphine (75 mg) Pellet (MP) and Placebo Pellet (PP) Implanted Mice

	Opioid/ antagonist, i.c.v.	ED_{50} (95% CI)	Mouse strain
PP	DPDPE	5.2 (3.4–7.9)[a] μg	CD-1
PP	DPDPE + BNTX, 3 pmol	20.9 (11.8–37.0)[b]	
MP	DPDPE	4.9 (2.7–0.9)[a]	
MP	DPDPE + BNTX, 3 pmol	15.4 (7.8–30.3)[b]	
PP	DSLET	0.12 (0.06–0.25)[a] μg	CD-1
PP	DSLET + Naltriben, 35 pmol	0.38 (0.24–0.60)[b]	
MP	DSLET	0.16 (0.10–0.25)[a]	
MP	DSLET + Naltriben, 35 pmol	0.60 (0.33–1.12)[b]	
MP	Heroin	1.9 (0.9–3.9)[a] μg	ICR
MP	Heroin + BNTX, 1 pmol	6.2 (2.9–13.4)[b]	
MP	Heroin + Naltriben, 25 pmol	1.6 (0.9–2.9)[a]	
MP	6MAM	3.9 (2.3–6.5)[a] μg	ICR
MP	6MAM + BNTX, 1 pmol	3.6 (2.4–5.5)[a]	
MP	6MAM + Naltriben, 25 pmol	8.3 (5.9–11.6)[b]	
Heroin s.c./antagonist i.c.v.			
PP	Heroin + saline	0.26 (0.14–0.49)[a] mg/kg	CD-1
MP	Heroin + saline	0.29 (0.11–0.73)[a]	
MP	Heroin + BNTX, 1 pmol	1.09 (0.59–1.79)[b]	
MP	Heroin + Naltriben, 25 pmol	0.31 (0.15–0.63)[a]	

Note. The ED_{50} values for i.c.v. opioids were determined at 10 minutes in the absence and presence of a fixed dose of opioid receptor antagonist (given in the same solution with the opioid). In one experiment, the ED_{50} was determined for subcutaneous heroin at 15 min, and the antagonist or saline was given i.c.v. at 10 min before the tail flick test.

[a] ED_{50} value was significantly different from that marked with [b] $P \leq 0.05$ for the potency ratio (17) for each comparison.

ment, there may be sufficient contribution from sites other than the brain to weaken the effect of i.c.v. BNTX.

The Descending Systems Involved in the δ Agonist Actions of Heroin and 6MAM. The descending systems involved in modulating the antinociceptive responses were evaluated in 3-day morphine pellet–implanted ICR mice. The results in Figure 4 indicated that the i.c.v. heroin-induced analgesia was inhibited by i.t. administration of picrotoxin and bicuculline (which inhibit $GABA_A$ receptors). The response to i.c.v. heroin was also inhibited by the i.t. administration of 2-hydroxysaclofen, an inhibitor of $GABA_B$ receptors. These results were consistent with heroin-activating δ_1 receptors (12–14). The i.c.v. heroin-induced response was not inhibited by i.t. yohimbine or methysergide.

In 3-day morphine pellet–implanted ICR mice, the antinociceptive action of i.c.v. 6MAM was inhibited by i.t. picrotoxin and bicuculline but not 2-hydroxysaclofen (Fig. 5); this is consistent with 6MAM acting on supraspinal δ_2 receptors to activate spinal $GABA_A$ but not $GABA_B$ receptors (12–14). Like the results for heroin, i.t. administration of yohimbine and methysergide did not inhibit i.c.v. 6MAM-induced analgesia, indicating that the descending adrenergic and serotonergic systems were not involved. The i.c.v. morphine-induced response in 3-day morphine pellet–implanted ICR mice was inhibited by both i.t. yohimbine and methysergide (Fig. 5), suggesting activation of spinal α_2-adrenergic and serotonergic receptors. This finding is consistent with the μ agonist action for morphine that has been shown previously (27, 28). When heroin and 6MAM act on brain μ receptors, spinal serotonergic and α_2-adrenergic receptors are activated (7–9).

Discussion

The μ receptor action of heroin in ICR and CD-1 mice appears to be different from that for morphine (17–19). In the present study, i.c.v. MeNTX inhibited the response to heroin and 6MAM but not morphine. Naloxonazine, which is a μ_1 receptor antagonist (29, 30), inhibited the analgesic action of morphine but not that of heroin and 6MAM (10).

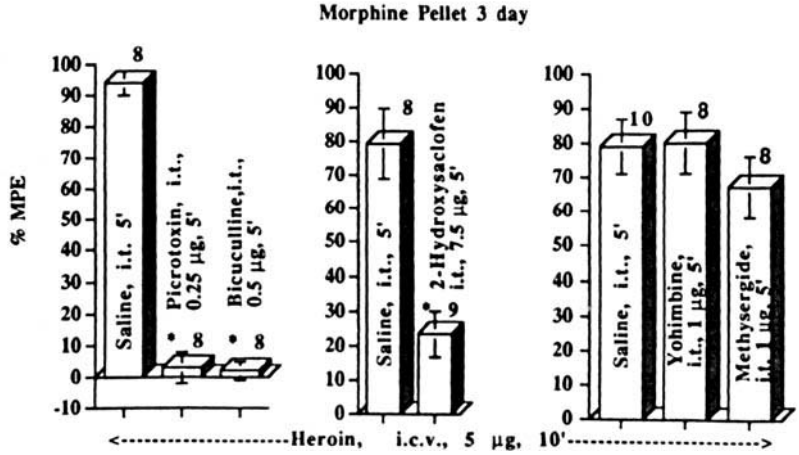

Figure 4. Descending systems in ICR mice: Examination of the descending neuronal system(s) involved in i.c.v. heroin-induced antinociception on Day 3 in morphine pelleted mice. Inhibition of the antinociceptive action of i.c.v. heroin by i.t. bicuculline, picrotoxin, and 2-hydroxysaclofen indicated involvement of spinal $GABA_A$ and $GABA_B$ receptors after activation of δ receptors in the brain. Absence of inhibition by i.t. yohimbine and methysergide indicated that spinal α_2-adrenergic and serotonergic receptors, respectively, were not involved as would be expected for brain μ receptor activation in control mice (8). The * asterisk indicates a significant difference ($P \leq 0.05$) from the control group by Dunnett's test (left panel) or Student's *t* test (middle panel); each mouse was used for only one group.

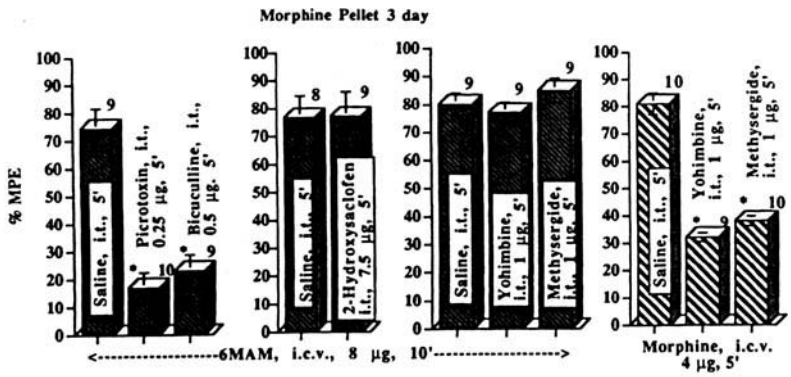

Figure 5. Descending systems in ICR mice. Examination of the descending neuronal system(s) involved in i.c.v. 6MAM-induced antinociception on Day 3 in morphine-pelleted mice. Spinal $GABA_A$ but not $GABA_B$ receptors were activated by i.c.v. administration of 6MAM. The response to i.c.v. morphine was inhibited by i.t. yohimbine and methysergide indicating that brain µ receptors were activated. The * asterisk indicates a significant difference ($P \leq 0.05$) from the control group by Dunnett's test; each mouse was used for only one group.

Yet, like the µ response to i.c.v. morphine, the heroin and 6MAM µ receptor action was transmitted through descending adrenergic and serotonergic systems to the spinal cord to inhibit the tail flick response (7–9).

Morphine pellet implantation caused i.c.v. heroin and 6MAM to act through δ opioid receptors. First, the δ receptor antagonist, naltrindole, coadministered i.c.v. with heroin and 6MAM, inhibited the responses. Also, the heroin response was inhibited by the i.c.v. co-administration of BNTX but not naltriben, indicating that a δ_1 but not δ_2 subtype of receptor was involved. The 6MAM response was inhibited by i.c.v. naltriben but not BNTX, indicating a δ_2 receptor subtype involvement. These interpretations are based on significant differences found with the potency ratio analysis of the ED_{50} values. A caveat is that in a few of these cases the 95% confidence intervals overlapped so that it would be desirable to obtain more robust differences. These results are concordant with the previous finding that in Swiss Webster and C57BL/6J mice where heroin and 6MAM act as δ agonists, they show the same respective δ_1 and δ_2 selectivity (7, 21). Second, the i.c.v. heroin- and 6MAM-induced antinociceptive actions in the 3-day morphine pellet–implanted mice were inhibited by i.t. administration of picrotoxin and bicuculline, indicating the participation of spinal $GABA_A$ receptors (12–14). The i.t. administration of 2-hydroxysaclofen, a $GABA_B$ receptor antagonist, inhibited the δ_1 agonist action of heroin but not the δ_2 agonist action of 6MAM; δ_1 action involves spinal $GABA_A$ and $GABA_B$ receptors whereas δ_2 action only involves $GABA_A$ receptors. These results are again concordant with expectations (12–14). It is possible that the morphine pellet implantation has a slightly different effect on the expression of the two δ receptor subtypes. With the morphine pellet in place, δ agonist action was present on Day 7 for heroin but not for 6MAM. Also, the times of offset of the δ response after morphine pellet removal on Day 3 were different. Heroin δ agonist action was present at 2 and 4 hr and disappeared at 6 hr after pellet removal whereas the δ agonist action for 6MAM, though present at 2 hr, was gone at 4 hr. However, it should be noted that evidence for the existence of different subtypes of δ receptors comes from pharmacological studies, but the subtypes have not been cloned (31).

Several accompanying findings need discussion. A previous study demonstrates that morphine pellet implantation in ICR mice results in 7-fold tolerance to the analgesic action of morphine (32), and the present results suggested that the sensitivity to the δ_1 and δ_2 agonists DPDPE and DSLET, respectively, was not different between placebo and morphine pellet–implanted mice. Thus, development of tolerance to the µ agonist action of morphine did not produce cross-tolerance to the δ agonists. The present study also demonstrated that the sensitivity to BNTX and naltriben was not altered by the morphine pelleting. In light of these results, the ability of the δ agonist actions of heroin and 6MAM to be detected in morphine pellet–implanted mice was not due to enhanced efficacy of the δ receptor antagonists. If one assumes that the morphine pellet induced tolerance to the µ agonist action of heroin and 6MAM, this tolerance might have uncovered the presence of the latent δ agonist action of heroin and 6MAM. Because the ED_{50} values for i.c.v. heroin and 6MAM were not changed by the morphine pellet, the loss of µ activity through tolerance would have been replaced by an equivalent amount of δ agonist action. The morphine pellet implantation did not produce cross-tolerance to the δ agonist action of DPDPE and DSLET; therefore, we presume cross-tolerance was not produced to the δ agonist actions of heroin and 6MAM. An analogous situation may exist in diabetic animals that have decreased sensitivity to the µ agonist action of morphine (33, 34) but retain sensitivity to the δ agonist peptides (35). Thus, streptozotocin-induced diabetic mice and rats (both of which were originally µ responders to heroin) have δ agonist responses to heroin (10, 36). An additional factor that might contribute to expression of δ agonist action is sensitization to δ agonist action. Induction of diabetes increases sensitivity to the δ_1 agonist action of DPDPE but not the δ_2 agonist (35). But, differential sensitization is not obtained in diabetic mice to the δ_1 heroin over δ_2 6MAM action (10). An increase in the low-affinity binding of δ agonist may occur in morphine tolerance (37–40). High- and low-affinity binding for DSLET is found in the cortex of C57BL/6 mice, but the high-affinity binding is not found in DBA/2 mice (41). This difference might have something to do with 6MAM having a δ_2 agonist action in C57BL/6 mice but µ action in DBA/2 mice. However, it would not explain

why heroin should have δ_1 and not μ action in C57BL/6 because heroin acts on δ_1, not δ_2, receptors. It is not known whether the receptor binding of heroin and 6MAM to δ receptors is increased or not. The strain differences between heroin δ (Swiss Webster, C57BL/6 J) and μ (ICR/CD-1, CBA, DBA/2, C3H) responding mice indicate genetic control of the expression of receptor selectivity for heroin. Whether morphine pellet implantation and streptozotocin-induced diabetes alter genetic control of heroin receptor selectivity might be worth investigating.

Quantification of the amounts of μ and δ agonist actions simultaneously present for heroin and 6MAM were not performed in the present study. It should not be assumed that the μ agonist actions of morphine, heroin, and 6MAM develop the same degree of tolerance since the μ agonist actions of heroin and 6MAM are presumably different from morphine. Also, if δ and μ agonist actions were present together, there might be a synergistic interaction according to the concept of μ and δ receptor coupling (31). To address these problems, more sophisticated approaches than those used here are necessary. Also, it is known that morphine has latent δ agonist activity (42), and it would be of interest to find out what happens to this component in morphine pellet–implanted mice.

In the present study, 15 min after s.c. heroin administration in morphine-pelleted mice, the antinociceptive action was partially (significant difference by potency ratio calculation but overlap in ED_{50} 95% confidence interval) antagonized by i.c.v. administration of BNTX. This finding is interpreted to mean that even at 15 min, substantial heroin δ_1 action is present. Even if 6MAM and morphine were present, their actions would not be inhibited by BNTX. Presence of a major effect of heroin at this time point is hard to fathom because after this s.c. administration, heroin would be expected to have been converted in large part to 6MAM and morphine (1–3). Also, the finding that morphine pellet–implanted mice were not cross-tolerant to the δ_1 and δ_2 agonist actions of DPDPE and DSLET was interpreted to mean that cross-tolerance to the δ agonist actions of heroin and 6MAM did not occur. Even though further experiments were not performed to test these views, some earlier findings support them. In Swiss Webster mice (where both 6MAM and heroin are δ agonists), acute pretreatment with a combination of i.c.v. plus s.c. morphine produces tolerance to the μ agonist action of morphine but not to the δ agonist action of heroin and 6MAM (Rady JJ, Fujimoto JM, unpublished data). Also, the combinations of i.c.v. plus s.c. heroin or i.c.v. plus s.c. 6MAM produce tolerance to themselves and other δ agonists but not to morphine μ action (Rady JJ, Fujimoto JM, unpublished data). Morphine pellet implantation produces tolerance to s.c. morphine but not s.c. heroin (tested at 20 min). As a generalization, we would expect that in mice where morphine produces tolerance, there would be little cross-tolerance to heroin if heroin is acting as a δ agonist. The finding mentioned earlier that Swiss Cox mice tested on Day 3 of morphine pelleting are 28-fold tolerant to s.c. morphine but not tolerant to s.c. heroin (11) would now be explained by the δ receptor action of heroin. There are other examples of asymmetric tolerance to opiates that might be explained by the present concept (43, 44).

The present discussion is based on mice in which the morphine pellet was left in place when the challenging agents were tested for tolerance and cross-tolerance. With the pellet in place, by the third day, the concentrations of morphine in the brain had reached a steady plateau (45). In spite of this persistence of morphine, the tail flick latency was back to control values at this time (another indication of development of tolerance to morphine without having to administer challenging doses of morphine) so that homeostasis had been reestablished for the tail flick response. Thus, lack of cross-tolerance to heroin occurred at this point in a relatively homeostatically stable animal. After morphine pellet removal, cross-tolerance to heroin would be expected, a so-called withdrawal cross-tolerance (43). When the morphine pellet is removed, the morphine concentration in the brain rapidly declines (45), and the animal is far from homeostatically stable (43, 45). This situation explains the need to increase the dose of i.c.v. heroin and 6MAM after the morphine pellet was removed to test the offset of δ agonist action of heroin and 6MAM.

A major assumption we made in using the opioid receptor antagonists in the morphine pellet–implanted mice was that their relative selectivities remain as in the morphine naive animal. Support for the relative selectivity of naltrindole, BNTX, and naltriben is not only based on our own experience (8, 9, 21) but on reports from the literature (25, 26, 46). This view was supported not only by the similarity in results in placebo as compared with morphine pellet–implanted mice in response to BNTX and naltriben as discussed above but also by the supportive results given by determinations of the descending systems involved. However, these approaches do not eliminate the complications. For instance, naloxone increases in potency to precipitate acute withdrawal effects as tolerance to morphine develops (43), and even the pA2 value for naloxone antagonism of morphine analgesia may increase (47, 48). It is not known whether the δ opioid receptor antagonists potency to precipitate acute withdrawal is increased in the present situation. Thus, the present report should be looked upon as preliminary findings that need to be studied further.

In summary, development of tolerance to morphine converts the μ agonist actions of heroin and 6MAM to δ agonist actions. These findings are used to explain the lack of cross-tolerance to heroin in morphine-tolerant mice. Receptor selectivity of the agents should be evaluated when tolerance and cross-tolerance studies are performed.

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