Heroin Antinociception Changed From Mu to Delta Receptor in Streptozotocin-Treated Mice

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ABSTRACT—CD-1 mice were treated intravenously with streptozotocin, 200 mg/kg, and tested 2 weeks later or treated with 60 mg/kg and tested 3 days later. Both treatments changed the tail flick response of heroin and 6-monoacetylmorphine (6 MAM) given intracerebroventricularly from a μ - to δ -opioid receptor-mediated action as determined by differential effects of opioid receptor antagonists. The response to morphine remained μ . Heroin and 6 MAM responses involved δ_1 (inhibited by 7-benzylidenenaltrexone) and δ_2 (inhibited by naltriben) receptors, respectively. These δ -agonist actions did not synergize with the μ -agonist action of morphine in the diabetic mice. The expected synergism between the δ agonist, [D-Pen²-D-Pen⁵]enkephalin (DPDPE), and morphine was not obtained in diabetic mice. Thus, diabetes disrupted the purported μ/δ -coupled response. In nondiabetic CD-1 mice, heroin and 6 MAM produced a different μ -receptor response (not inhibited by naloxonazine) from that of morphine (inhibited by naloxonazine). Also, these μ actions, unlike that of morphine, did not synergize with DPDPE. The unique receptor actions and changes produced by streptozotocin suggest that extrinsic in addition to genetic factors influence the opioid receptor selectivity of heroin and 6 MAM.

Keywords: Streptozotocin, Heroin antinociception, μ - and δ -opioid, Receptor selectivity change

Heroin is rapidly biotransformed to 6-monoacetylmorphine (6MAM) and then to morphine (1–3). Because heroin is thought to act largely through these two metabolites with morphine being the major metabolite, morphine is often used to study mechanisms of tolerance and dependence even though the drug of abuse on the street is heroin. In out-bred ICR and CD-1 mice, heroin given intracerebroventricularly, i.c.v., acts on μ -opioid receptors, as does morphine, to produce antinociception (4, 5). 6MAM also acts through μ receptors in the brain of ICR and CD-1 mice (6). However, in Swiss Webster mice, heroin and 6MAM have the unique trait of acting on brain δ -opioid receptors even though morphine continues to act on μ receptors (4–6). Thus in these mice, even though the μ receptors for morphine are present, heroin and 6MAM act on δ receptors. The question of whether these unique actions occur only in Swiss Webster mice or in other situations as well arises. Even though it is logical as an initial assumption to attribute the differences in receptor selectivities for these agents to strain differences in the mice and thereby imply a genetic basis, it is also possible that there might be other factors that affect receptor selectivity.

In Swiss Webster mice, heroin and 6MAM act on δ_1 and δ_2 receptors as indicated by the inhibitory action of 7-benzylidenenaltrexone and naltriben, respectively (6–8). Kamei et al. (9) indicate that ICR mice made diabetic with streptozotocin become more sensitive to the antinociceptive action of the δ_1 -receptor peptide agonist DPDPE, [D-Pen²-D-Pen⁵]enkephalin, but not to a δ_2 -receptor peptide agonist. Furthermore, the potency of morphine as a μ -agonist decreases (10–13). Even though heroin acts on μ receptors in CD-1 mice (as in ICR mice), we felt that streptozotocin treatment might cause heroin to change from a μ - to a δ -receptor agonist. Whether the μ -agonist action of 6MAM would also change to a δ -agonist action could be examined as well.

As a further consideration, Rossi et al. (14) suggest that heroin acts on a μ receptor other than the μ_1 receptor for morphine in ICR mice. The effect of streptozotocin to reduce the potency of morphine is an effect on μ_1 -agonist action (10). If heroin acts on receptors other than the μ_1 receptors in ICR and CD-1 mice, then treating CD-1 mice with streptozotocin may not alter the receptor action of heroin. In the present study, two different protocols of streptozotocin treatment were used in CD-1 mice. The

main approach was to give the opioid agonists i.c.v. together with the various opioid receptor antagonists to determine receptor selectivity. In certain cases, non-diabetic control CD-1 mice were used for comparison.

In an additional approach, the synergistic (multiplicative, super-additive) interaction that occurs between μ and δ agonists was examined. Studies on the synergistic action have their origin in the concept of μ - δ receptor coupling first proposed by Vaught (15, 16). Further publications provide considerable insights into these interactions (8, 17–21). This μ - δ coupling implies a close association between the two receptors. Furthermore, synergism occurs between δ -opioid agonist peptides and morphine administered into several separate brain loci in rats (22). In the present study, the multiplicative interaction between the μ action of morphine and the δ action of DPDPE was taken as the basic model. In one situation, the δ -agonist actions of heroin and 6MAM (in place of DPDPE) interacting with the μ action of morphine were evaluated. In another, the μ -agonist actions of heroin and 6MAM were substituted for that of morphine and the interactions with DPDPE were evaluated.

MATERIALS AND METHODS

Animals, antinociceptive test and drug treatments

Male CD-1 mice (weighing 25–30 g) were used for all experiments. Each mouse was only used once. All studies were performed in compliance with the Institutional Animal Care and Use Committee (Animal Studies Subcommittee).

The radiant heat tail flick test (23) was used to measure antinociception. Control tail flick latencies were 2–4 sec; The tail flick apparatus had an automatic 10-sec cutoff time which prevented tissue damage. The change in tail flick latencies were converted to percent maximum possible effect (% MPE) according to the following formula (24): % MPE = (post drug latency – predrug latency)100 / (10 – predrug latency)

Opioids were given i.c.v. in 4 μ l (25).

To determine whether heroin and 6MAM act on μ receptors other than the μ_1 receptor in the brain of CD-1 mice, 10 μ g of naloxonazine was given i.c.v. along with various doses of i.c.v. morphine, heroin and 6MAM to generate dose-response curves and ED₅₀ values. Supraspinal morphine-induced antinociception is inhibited by the μ_1 -receptor antagonist, whereas the spinal μ_2 -agonist action of morphine is not inhibited by naloxonazine (26, 27). β -Funaltrexamine, β -FNA, which inhibits both μ_1 - and μ_2 -receptor actions, treated groups were included (26, 27).

Streptozotocin protocol A and B: For the main part of the study, two protocols for streptozotocin treatment

were used. In protocol A, streptozotocin, 200 mg/kg, was administered i.v. into a tail vein and the mice used 2 weeks later. This treatment was used by Kamei et al. (9, 10). Because a two-week waiting time imposed a constraint on planning of sequential experiments, a shorter pretreatment protocol was evaluated. Therefore, in protocol B, streptozotocin (commercial preparation), 60 mg/kg, was administered i.v. and the mice were used 72 hr later. Control groups were given citrate buffer or saline solution. Each gram of streptozotocin (the commercial preparation) contained 220 mg of citric acid and a similar vehicle with just the citric acid was administered as a control. Concentrations were adjusted to administer the dose on a 5 ml/kg body weight basis. In order to derive treatment parameters for protocol B, initial experiments were performed with various doses and times of administration of streptozotocin.

Determination of receptor selectivity by i.c.v. administration of opioid receptor antagonists: Opiates and opiate antagonists were administered i.c.v. to 8 to 10 mice per group. Ten minutes later (4–6), the tail flick test was performed. The mean % MPE ($\pm$ S.E.M.) was calculated. In single dose experiments, statistical comparison between two groups was performed by Student's *t*-test; for comparison of a number of groups to a control group, Dunnett's test was used. $P \leq 0.05$ was taken to indicate a significant difference (28). Dose-response relationships and ED₅₀ values were examined as stated above for the naloxonazine and β -FNA experiments (29).

Interaction between i.c.v. heroin and 6MAM (as δ -receptor agonists) with morphine (μ agonist): Once the receptors on which heroin and 6MAM acted were determined, it became of interest to determine the nature of the interaction between these two agents and morphine. To detect a super-additive (multiplicative) interaction, i.c.v. doses of each agonist which gave similar % MPE values were found. Then, the dose of each was cut in half and the half doses of the two agents were given together. Additivity between the two half doses was indicated by the % MPE not being significantly different from the % MPE of either agent given at the full dose. Multiplicative interaction was indicated by the % MPE for the combined half doses being significantly greater than the % MPE values for either of the agents at the full dose. This approach was used in earlier publications showing interactions and validated by comparison with the isobolographic method of determining interactions (4). The results were statistically evaluated by ANOVA followed by Newman-Keuls' test (28) with $P \leq 0.05$ for significant differences between groups.

Drug sources

Streptozotocin powder was obtained from Sigma

Chemical Co. (St. Louis, MO, USA) or was a commercial preparation, Zanosar (Upjohn Company, Kalamazoo, MI, USA). The antagonists (7-benzylidenenaltrexone (BNTX) and naltriben) were synthesized as previously described (30, 31). Heroin hydrochloride and 6MAM (free base) were obtained from the National Institute on Drug Abuse (Rockville, MD, USA). β -FNA, naloxonazine hydrochloride and naltrindole hydrochloride were from Research Biochemical Inc. (Natick, MA, USA). Morphine sulfate $\cdot 5\text{H}_2\text{O}$ was from Mallinckrodt Chemical Works (St. Louis, MO, USA). The opiates and antagonists were dissolved in 0.9% sodium chloride solution. For 6MAM, a few drops of 0.1 M hydrochloric acid and slight heating was needed to aid in dissolving the free base.

RESULTS

Comparison of the effect of β -FNA and naloxonazine on i.c.v. morphine, heroin and 6MAM antinociception in control CD-1 mice

Table 1 gives the results of experiments in which these μ -receptor antagonists were administered to inhibit the antinociceptive actions of i.c.v. morphine, heroin and 6MAM in CD-1 mice. Treatment of the mice 24 hr earlier with 50 ng of β -FNA, i.c.v., inhibited the response to all three agonists. The ED_{50} values were significantly different ($P < 0.05$) from the respective control ED_{50} values. Naloxonazine (a μ_1 -opioid receptor subtype antagonist) given i.c.v. along with the drugs, increased the ED_{50} value for i.c.v. morphine, but did not affect the ED_{50} value for either heroin or 6MAM. Thus, heroin and 6MAM seemed to act on a receptor other than the μ_1 receptor acted on by

morphine.

Effect of streptozotocin protocol A treatment

In protocol A, a dose of 200 mg/kg of streptozotocin was given i.v. and the mice were evaluated 2 weeks later. Even though this streptozotocin treatment seemed to decrease sensitivity to morphine somewhat, the difference was not statistically significant compared to the control group (Fig. 1). The treatment made the group more sensitive to heroin, but did not affect the sensitivity to 6MAM.

Single dose studies (data not given) showed that in the streptozotocin-treated group, antinociception produced by i.c.v. heroin decreased when 10 μg of naltrindole, a δ -receptor antagonist, was given i.c.v. along with the heroin. Thus, the heroin-induced response was δ receptor-mediated. Naltrindole did not inhibit the heroin response in control CD-1 mice; thus, δ receptors were not involved. Naltrindole also did not affect the antinociceptive action of morphine in streptozotocin-treated mice.

More extensive dose response relationship studies were performed based on the above results (Fig. 2). The dose-response curve for i.c.v. heroin in the protocol A group gave an ED_{50} value (95% confidence interval) of 0.9 (0.6–1.3) μg . When 10 μg of naltrindole was given along with the i.c.v. heroin, the curve was shifted 10-fold to the right in parallel fashion to give an ED_{50} of 9.4 (5.8–15.1) μg . The inhibition by naltrindole meant that the response to heroin in streptozotocin treated mice involved δ -opioid receptors. As an aside, the dose-response curve in the control CD-1 mice treated two weeks earlier with citrate buffer i.v. fell in between the other two curves having an

Table 1. Comparison of the effects of β -FNA and naloxonazine on ED_{50} (95% confidence interval) of morphine, heroin and 6MAM in the tail flick test in CD-1 mice

Treatment ^a	ED_{50} (μg)	95% CI (μg)	Potency ratio
Morphine, i.c.v., 10 min	0.61	0.22–1.70	
+ β -FNA, 50 ng, i.c.v., 24 hr	5.45*	2.59–11.43	8.93
+ Naloxonazine, 10 μg , i.c.v., 10 min	3.13*	1.64–5.98	5.15
Heroin, i.c.v., 10 min	1.24	0.65–2.37	
+ β -FNA, 50 ng, i.c.v., 24 hr	5.29 ^{b,*}	3.33–8.39	4.27
+ Naloxonazine, 10 μg , i.c.v., 10 min	1.23	0.83–1.81	1.01
6MAM, i.c.v., 10 min	2.10	1.03–4.28	
+ β -FNA, 50 ng, i.c.v., 24 hr	12.90*	8.47–19.64	6.14
+ Naloxonazine, 10 μg , i.c.v., 10 min	2.23	1.06–4.28	1.06

The potency ratio is the ED_{50} in presence of antagonist/ ED_{50} of agonist alone. ^aNaloxonazine was given in the same solution with the agonists. β -FNA was given 24 hr before the tail flick test. The asterisk indicates that ED_{50} value is significantly different ($P \leq 0.05$ by the procedure stated in Materials and Methods) from the corresponding value in absence of antagonist.

Fig. 1. Antinociceptive action of i.c.v. morphine, heroin and 6 MAM in vehicle (i.v. citrate buffer-treated, CD-1, open bar) and streptozotocin (i.v., 200 mg/kg, 2 weeks earlier, striped bar) protocol A-treated CD-1 mice. The bars indicate the mean % MPE along with the S.E.M. The numbers within the bar are the mice per group. The asterisk indicates a significant difference ($P \leq 0.05$ by Student's *t*-test). The main variable tested is enclosed in a rectangle.

ED_{50} of 3.4 (2.5–4.8) µg.

In a further set of experiments, the δ -receptor subtype involved in these streptozotocin treated mice was determined by use of BNTX and naltriben, δ_1 - and δ_2 -receptor antagonists, respectively. These antagonists had no effect

on the antinociceptive action of i.c.v. heroin or 6MAM in control CD-1 mice (Figs. 3 and 4). In the streptozotocin treated mice, the antinociceptive action of i.c.v. heroin was inhibited by coadministration of BNTX but not naltriben (Fig. 3). On the other hand, the antinociceptive action of 6MAM was inhibited by naltriben but not BNTX (Fig. 4). Heroin acted through δ_1 and 6MAM through δ_2 receptors.

Dose-response and time of onset of streptozotocin (protocol B)

The results in Fig. 5A indicated that three days after treatment the 28, 55 and 110 mg/kg doses of streptozotocin given i.v. produced responses to heroin that were inhibited by i.c.v. naltrindole. The 14 mg/kg dose produced no effect. Using the 55 mg/kg dose, a time course study was performed. The results in Fig. 5B indicated that at 68 and 168 hr, the heroin response was inhibited by naltrindole, while at the shorter times, 24 and 48 hr, the δ -agonist action of heroin had not yet appeared, that is, naltrindole was not inhibitory. In subsequent experiments protocol B, 60 mg/kg, 3-day treatment, was used to further assess the receptor response to heroin.

A dose response curve for i.c.v. heroin in streptozotocin protocol B treated mice gave an ED_{50} of 1.9 (1.4–2.5) µg (Fig. 6). Naltrindole, 10 µg, given along with the heroin caused the curve to shift to the right by 4.7-fold with an ED_{50} of 9.0 (6.8–12) µg. There was no difference

Fig. 2. Dose-response curve for i.c.v. heroin in groups treated with i.v. vehicle (CD-1, open square), streptozotocin (open triangle) and streptozotocin + naltrindole (open circle). $N=8-10$ per group.

Fig. 3. The effect of BNTX and naltriben on heroin antinociception in mice treated with vehicle (open bar, CD-1) and streptozotocin, protocol A (striped bar). Asterisk indicates $P \leq 0.05$ by Dunnett's test.

between the control CD-1 mice given vehicle i.v. and the protocol B-treated heroin ED_{50} values.

In the protocol B-treated groups, the response to i.c.v. heroin was not inhibited by β -FNA treatment (Fig. 7A). The response to i.c.v. morphine was inhibited by β -FNA but not naltrindole (Fig. 7B). Thus, streptozotocin treatment did not change the μ -receptor response to morphine.

δ -Receptor subtype involved in the response to heroin produced by protocol B

The δ -receptor subtype involved was defined further by the use of selective antagonists (30, 31). BNTX but not naltriben inhibited heroin antinociception. 6MAM-induced antinociception was inhibited by naltriben but not BNTX (Figs. 3, 4, 8). These results indicated that the agonist action of i.c.v. heroin involved δ_1 receptors and that of 6MAM involved δ_2 receptors.

Fig. 4. The effect of BNTX and naltriben on 6MAM antinociception in mice treated with vehicle (open bar, CD-1) and streptozotocin, protocol A (striped bar). Asterisk indicates $P \leq 0.05$ by Dunnett's test.

Determination of the type of interactions between the μ -agonist action of morphine and the μ - and δ -agonist actions of heroin and 6MAM: Selected comparisons in control Swiss Webster and control and streptozotocin-induced diabetic CD-1 mice

The 0.25- μ g dose of morphine and the 2- μ g dose of DPDPE separately produced similar % MPE values in control CD-1 mice (Fig. 9 upper left panel). One-half dose of each were combined and administered together. The response for the combined treatment was significantly greater than that for each agonist given separately.

Thus, DPDPE and morphine interacted in the expected super-additive (multiplicative) fashion in control CD-1 mice. Where i.c.v. heroin and morphine both act on μ receptors in CD-1 mice, the two interacted in an additive fashion (data not given). That is morphine and heroin given together at half the doses given separately produced a % MPE value that was not different from that of either of the drugs given alone at the full dose.

The interaction seen between morphine versus heroin and 6MAM in the Swiss Webster mice was evaluated (Fig. 9, top right two panels). Here, heroin and 6MAM that act

Fig. 5. Dose-response and time of onset of streptozotocin-induced receptor selectivity changes. A: Heroin antinociception in presence and absence of naltrindole in mice treated with various doses of streptozotocin. Asterisk indicates $P \leq 0.05$ by Student's t -test. The ability of naltrindole to inhibit antinociception indicated the presence of δ -agonist action of heroin. B: The response to heroin antinociception at various times (hours after streptozotocin treatment) at which heroin agonist action was inhibited by naltrindole. Asterisk indicates $P \leq 0.05$ by Student's t -test.

Fig. 6. Dose-response curves for heroin in mice treated with vehicle (open square, CD-1); streptozotocin, i.v., 60 mg/kg, 72 hr (protocol B, open triangle); and streptozotocin + naltrindole (open circle). $N=8-10$ per group.

on δ receptors produced a multiplicative interaction with the μ -agonist action of morphine. The μ/δ -receptor interactions were then evaluated in the streptozotocin protocol B-treated CD-1 mice. Surprisingly, the results (Fig. 9, bottom right two panels) indicated that the interaction

between the μ -agonist action of morphine and the δ -agonist action of heroin and 6MAM were additive, not multiplicative. The same failure was evident in the interaction between DPDPE and morphine in the diabetic CD-1 mice (Fig. 9, two left panels).

Streptozotocin Protocol B

Fig. 7. The effect of β -FNA and naltrindole on heroin- and morphine-induced antinociception in mice treated with streptozotocin (protocol B). A: In CD-1 mice treated with streptozotocin, protocol B mice, the response to i.c.v. heroin in control and β -FNA treatment. B: In CD-1 mice treated with streptozotocin, protocol B, the response to i.c.v. morphine in control, β -FNA and naltrindole treatments. Asterisk indicates $P \leq 0.05$ by Dunnett's test.

Because the differential inhibitory action of naloxonazine against morphine but not heroin and 6MAM action (Table 1) in control CD-1 mice indicated that heroin and 6MAM acted on a different μ receptor than morphine, the next experiment used heroin and 6MAM in place of the morphine to see if they interacted in a multiplicative fashion with DPDPE. Recall that morphine and DPDPE produced a multiplicative interaction (Fig. 9, upper left panel). The results in Fig. 10 indicated that heroin and 6MAM did not interact in this fashion with DPDPE. Instead, the interaction was additive. Thus, the μ -receptor action of heroin and 6MAM did not substitute for the μ action of morphine.

Blood glucose concentrations were determined using an ExacTech glucometer (Medisense, Inc., Waltham, MA, USA); the mean values ($\pm$ S.E., dg/ml) for treatment groups of 8–9 mice were as follows: control (citrate buffer treated two weeks earlier) = 88 ± 4 , protocol B = 106 ± 5 and protocol A = 421 ± 22 . The control and protocol B groups were not significantly different, but both groups were significantly different from the protocol A group (ANOVA followed by Newman Keuls' test, $P > 0.05$).

DISCUSSION

Supraspinal receptors involved in heroin and 6MAM antinociception in control CD-1 mice

The opioid receptor action of i.c.v. heroin and 6MAM in control CD-1 appears to involve a μ receptor other

than the μ_1 receptor responsible for i.c.v. morphine-induced antinociception. In line with the broad action of β -FNA on different μ receptors (26, 27), treatment with β -FNA increased the ED₅₀ value for morphine, heroin and 6MAM indicating that μ receptors were involved, which is consistent with our previous findings (4, 6). However, naloxonazine, the putative μ_1 -receptor antagonist (26, 27), increased the ED₅₀ of morphine but not heroin or 6MAM. Thus, heroin and 6MAM appear to have acted on a different receptor than the μ_1 receptor on which supraspinal morphine acts (26, 27). Rossi et al. (14) report that heroin acts on the same receptor as does morphine-6-glucuronide but a receptor different from the μ_1 receptor for morphine. Furthermore, the antinociceptive action of morphine can be differentiated from heroin in mice in which different μ -receptor exons are deleted (32). Also, 3-methoxynaltrexone inhibits heroin- but not morphine-induced antinociception (33). An additional piece of the present result is consistent with involvement of a different μ receptor. The results in non-diabetic CD-1 mice showed that the interaction between DPDPE and heroin and DPDPE and 6MAM were additive. On the other hand, DPDPE and morphine produced a multiplicative interaction that is consistent with reports in the literature (see review 8). That the μ -agonist actions of heroin and 6MAM cannot substitute for the μ action of morphine is suggestive of the involvement of different receptors. Taken together, the results are consistent with the concept that heroin and 6MAM act on alternative μ receptors, but does not provide definitive evidence for

Fig. 8. The effect of BNTX and naltriben on heroin (A) and 6 MAM (B) antinociception in CD-1 mice treated with streptozotocin, protocol B. Asterisk indicates $P \leq 0.05$ by Dunnett's test.

different putative subtypes of the μ receptor. The receptor actions of i.c.v. heroin and 6MAM are similar to that of morphine in so far as the descending pathways that inhibit the tail flick reflex are mediated by spinal noradrenergic and serotonergic systems (4, 6, 34).

Kamei et al. (10) conclude that the μ_1 -but not the μ_2 -receptor agonist potency of morphine is decreased by streptozotocin treatment. In the present context, neither heroin nor 6MAM are μ_1 -receptor agonists but the μ -receptor activity of these agents disappeared when the

mice were treated with streptozotocin. A decrease in μ_1 -agonist action of morphine and the μ -receptor actions of heroin and 6MAM could mean that streptozotocin treatment affects more than the μ_1 -receptor function. The effect on the alternative receptor appeared to be greater than that on the μ_1 -receptor action of morphine; the latter was not significantly reduced when the μ actions of heroin and 6MAM had been changed to δ -agonist actions.

An insight into a mechanism for the decrease in μ_1 action of morphine was given by Kamei et al. (11). A factor

Fig. 9. Interaction between combinations of μ (morphine)- and δ -receptor agonists (DPDPE, heroin and 6 MAM) in CD-1 (open bar) Swiss Webster (wave bar) and CD-1 mice treated with streptozotocin, protocol B (gray bar). Each of the δ agonists and morphine was evaluated separately to obtain a similar moderate antinociceptive response; then one half of the dose of each agonist was combined and given together. Morphine, the μ agonist, was fixed while the δ agonist was varied. Asterisk indicates a significant difference ($P \leq 0.05$) by ANOVA followed by Neuman-Keuls' test.

Fig. 10. Interaction between combinations of μ agonists, heroin and 6 MAM, and δ agonist, DPDPE, in control CD-1 mice. Here, heroin and 6 MAM acting as μ agonists were used in place of the morphine.

produced by mononuclear cells in the spleen may be involved. Sensitivity to morphine in streptozotocin-induced diabetic mice is restored by splenectomy (35) or treatment with cyclosporine (36), an immunosuppressant. Administration of mononuclear spleen cells or homogenates of spleen cells from diabetic mice to normal mice decreases sensitivity to morphine (11). The concept is analogous to that developed earlier by Raffa et al. (37, 38) to explain the decreased sensitivity of C57BL/6J-bg^J/bg^J (beige-J) mice to morphine. Decrease in μ -receptor numbers and affinity do not explain the decreased potency of morphine produced by streptozotocin treatment (12, 13).

Heroin and 6MAM given i.c.v. in streptozotocin-induced diabetic CD-1 mice act on δ -opioid receptors

The main conclusion derived from the results is that heroin and 6MAM act on δ receptors in the brain of CD-1 mice treated with streptozotocin. The selective inhibitory action of i.c.v. BNTX indicated that heroin acted on δ_1 -opioid receptors and that of i.c.v. naltriben indicated that 6MAM acted on δ_2 receptors. Streptozotocin protocol A and B treatments produced similar qualitative results. Quantitatively, naltrindole treatment produced rightward shifts of the i.c.v. heroin dose response curve of 10- and 4.7-fold with protocol A and B treatments, respectively. If the size of the shift was somehow related to the amount of functional δ receptors, it could be that protocol A had a greater effect than protocol B. However, the heroin ED₅₀ values in the presence of naltrindole were the same with protocol A (ED₅₀=9.4 μ g) as with protocol B (ED₅₀=9.0 μ g). The magnitude of the shift was determined more by the heroin ED₅₀ in the absence of naltrindole where values were 0.9 and 1.9

μ g for protocol A and B, respectively. It is not known whether this difference is produced by the larger dose (A, 200 mg/kg vs B, 60 mg/kg) of streptozotocin and/or the longer time (A, 2 weeks vs B, 3 days) of treatment.

The question as to whether the factor which decreases the sensitivity to morphine (discussed above) also increases that to DPDPE remains. When heroin acts as a δ agonist, it acts as a δ_1 -receptor agonist, in the present study as well as in previous studies (4, 5). However, when 6MAM acts as a δ agonist, it acts on δ_2 receptors here and previously (5, 6). Streptozotocin treatment alters sensitivity to δ_1 but not δ_2 agonists (9). Therefore, it would follow that the treatment might cause heroin to become a δ agonist. However, this does not explain 6MAM becoming a δ agonist. Thus, no simple explanation for the relationship that might exist between the loss in putative μ -agonist action of heroin and 6MAM and the manifestation of δ -agonist actions can be derived.

Lack of a predictable relationship seems to hold for the δ -agonist action of heroin and μ_1 action of morphine in Swiss Webster mice as well. Swiss Webster mice are not selectively more sensitive to the δ_1 -agonist actions of DPDPE or less sensitive to the μ_1 -agonist actions of morphine (4, 5, 39). Thus, the δ -receptor selective action of heroin and 6MAM does not appear to be directly dependent on the inherent sensitivity of δ receptors, and the insensitivity of μ_1 in diabetic mice is determined by factors extrinsic to the known μ - and δ -opioid receptors. Blood glucose concentrations were not directly related to the onset of δ -agonist action because the protocol B-treated group was not hyperglycemic on day 3.

Considerations regarding μ/δ -receptor interaction

The consequence of the streptozotocin treatment on the δ -agonist actions of heroin and 6MAM were also examined in terms of receptor interactions. Studies on the multiplicative interaction between morphine and δ -agonist actions have originated with the concept of μ - δ receptor coupling (15–21). A μ - δ coupling implies a close association between the two receptors. In the present study, it was shown that heroin and 6MAM acting as δ agonists in Swiss Webster mice produce a multiplicative interaction with morphine. As discussed earlier, morphine and DPDPE interacted in a multiplicative fashion in CD-1 mice. Thus, heroin and 6MAM acting as δ agonists can substitute for DPDPE in Swiss Webster mice. Because heroin and 6MAM act as δ agonists in streptozotocin (protocol B)-treated CD-1 mice, they should also interact multiplicatively with morphine as they did in Swiss Webster mice. However, they did not. Lack of a multiplicative interaction was also evident with DPDPE and morphine in the diabetic mice. Thus, in the diabetic mice, it appears that the μ - δ receptor coupling is disrupted while coupling is intact in Swiss Webster mice. The expression of heroin δ -agonist activity appears to be independent of the μ - δ receptor coupling in the diabetic mice.

Ten minutes after i.c.v. administration, both heroin and 6MAM appear to be acting as themselves. In diabetic CD-1 mice, heroin action through δ_1 receptors was evident; BNTX but not naltriben inhibited antinociception. Thus, the proximal metabolite 6MAM is not present in sufficiently high concentrations. The action of 6MAM was inhibited by naltriben but not BNTX. If morphine were present in substantial quantities, naltriben should not have been an effective inhibitor. These findings are similar to previous work in Swiss Webster mice which demonstrates that heroin and 6MAM act as themselves after i.c.v. administration (4, 6).

Summary

In control CD-1 mice, heroin and 6MAM produced antinociception through μ receptors that are different from the μ_1 receptors for morphine as indicated by the differential inhibition of morphine but not heroin and 6MAM antinociception by naloxonazine treatment. Also, the μ -agonist actions of heroin and 6MAM did not substitute for the μ_1 -agonist action of morphine in producing a multiplicative interaction with DPDPE. The major part of the present work demonstrates that treatment of CD-1 mice with streptozotocin changed the μ -receptor selectivity of heroin and 6MAM to δ_1 - and δ_2 -opioid agonist responses, respectively. The μ - δ receptor-coupled response as known for the combination of DPDPE and morphine did not occur in diabetic mice. The coupled

receptor response was also absent for δ -agonist actions of heroin and 6MAM with morphine in diabetic mice. Thus, in streptozotocin diabetic mice μ - δ receptor coupling is somehow disrupted. Also in regard to μ - δ coupling, heroin and 6MAM acting as μ agonists cannot substitute for μ -agonist action of morphine in nondiabetic CD-1 mice.

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REFERENCES

- Way EL and Adler TK: The pharmacologic implications of the fate of morphine and its surrogates. *Pharmacol Rev* **12**, 383–446 (1960)
- Way EL: Brain uptake of morphine: Pharmacological implications. *Fed Proc* **26**, 1115–1118 (1967)
- Inturrisi CE, Max MB, Foley KM, Schultz M, Shin S-U and Houde RW: The pharmacokinetics of heroin in patients with chronic pain. *N Engl J Med* **310**, 1213–1217 (1984)
- Rady JJ, Roerig SC and Fujimoto JM: Heroin acts on different opioid receptors than morphine in Swiss Webster and ICR mice to produce antinociception. *J Pharmacol Exp Ther* **256**, 448–457 (1991)
- Rady JJ, Takemori AE, Portoghesi PS and Fujimoto JM: Supraspinal delta receptor subtype activity of heroin and 6-monoacetylmorphine in Swiss Webster mice. *Life Sci* **55**, 603–609 (1994)
- Rady JJ, Aksu F and Fujimoto JM: The heroin metabolite, 6-monoacetylmorphine, activates delta opioid receptors to produce antinociception in Swiss Webster mice. *J Pharmacol Exp Ther* **266**, 1222–1231 (1994)
- Takemori AE and Portoghesi PS: Selective naltrexone-derived opioid receptor antagonists. *Annu Rev Pharmacol Toxicol* **32**, 239–269 (1992)
- Zaki PA, Bilsky EJ, Evans CJ and Porreca F: Opioid receptor types and subtypes: The δ receptors as a model. *Annu Rev Pharmacol Toxicol* **36**, 379–401 (1996)
- Kamei J, Iwamoto Y, Misawa M, Nagase H and Kasuya Y: Streptozotocin-induced diabetes selectively enhances antinociception mediated by delta 1- but not delta 2-opioid receptors. *Life Sci* **55**, PL121–PL126 (1994)
- Kamei J, Iwamoto Y, Hitosugi H, Misawa M, Nagase H and Kasuya Y: Streptozotocin-induced diabetes selectively reduces antinociception mediated by mu 1-opioid receptors, but not that mediated by mu 2-opioid receptors. *Neurosci Lett* **165**, 141–143 (1994)
- Kamei J, Kawashima N and Kasuya Y: Role of spleen or spleen products in the deficiency in morphine-induced analgesia in diabetic mice. *Brain Res* **576**, 139–142 (1992)
- Simon GS and Dewey WL: Narcotics and diabetes. I. The effects of streptozotocin-induced diabetes on the antinociceptive potency of morphine. *J Pharmacol Exp Ther* **218**, 318–323 (1991)
- Brase DA, Han Y-H and Dewey WL: Effects of glucose and

- diabetes on binding of naloxone and dihydromorphine to opiate receptors in mouse brain. *Diabetes* **36**, 1173–1177 (1987)
- 14 Rossi GC, Brown GP, Leventhal L, Yang K and Pasternak GW: Novel receptor mechanism for heroin and morphine-6 β -glucuronide analgesia. *Neurosci Lett* **216**, 1–4 (1996)
 - 15 Vaught JL and Takemori AE: Differential effects of leucine and methionine enkephalin on morphine-induced analgesia, acute tolerance and dependence. *J Pharmacol Exp Ther* **298**, 89–90 (1979)
 - 16 Vaught JL, Rothman RB and Westfall TC: Mu and delta receptors: Their role in analgesia and in the differential effects of opioid peptides in analgesia. *Life Sci* **30**, 1443–1455 (1982)
 - 17 Rothman RB, Long JB, Bykov V, Jacobson AE, Rice KC and Holaday JW: Beta-FNA binds irreversibly to the opiate receptor complex: In vitro and in vivo evidence. *J Pharmacol Exp Ther* **247**, 405–416 (1988)
 - 18 Heyman JS, Vaught JL, Mosberg HI, Haaseth RC and Porreca F: Modulation of μ -mediated antinociception by δ agonists in the mouse: Selective potentiation of morphine and normorphine by [D-Pen², D-Pen⁵]enkephalin. *Eur J Pharmacol* **165**, 1–16 (1989)
 - 19 Jiang Q, Mosberg HI and Porreca F: Modulation of the potency and efficacy of mu-mediated antinociception by delta agonists in the mouse. *J Pharmacol Exp Ther* **254**, 683–689 (1990)
 - 20 Porreca F, Takemori AE, Sultana M, Portoghesi PS, Bowen WD and Mosberg HI: Modulation of μ -mediated antinociception in the mouse involves opioid delta-2 receptors. *J Pharmacol Exp Ther* **263**, 147–152 (1992)
 - 21 Sora I, Funada M and Uhl GR: The μ -opioid receptor is necessary for [D-Pen², D-Pen⁵]enkephalin-induced analgesia. *Eur J Pharmacol* **324**, R1–R3 (1997)
 - 22 Rossi GC, Pasternak GW and Bodnar RJ: Mu and delta opioid synergy between the periaqueductal gray and the rostro-ventral medulla. *Brain Res* **665**, 85–93 (1991)
 - 23 D'Amour FE and Smith DL: A method for determining loss of pain sensation. *J Pharmacol Exp Ther* **72**, 74–79 (1941)
 - 24 Dewey WL, Harris LS, Howes JF and Nuite JA: The effect of various neurohormonal modulators on the activity of morphine and the narcotic antagonists in the tail flick and phenylquinone test. *J Pharmacol Exper Ther* **175**, 435–442 (1970)
 - 25 Haley TJ and McCormick WG: Pharmacological effects produced by intracerebral injections of drugs in the conscious mouse. *Br J Pharmacol* **12**, 12–15 (1957)
 - 26 Pick CG, Nejat RJ and Pasternak GW: Independent expression of two pharmacologically distinct supraspinal mu analgesic systems in genetically different mouse strains. *J Pharmacol Exp Ther* **265**, 166–171 (1993)
 - 27 Pick CG, Paul D and Pasternak GW: Comparison of naloxonazine and β -funaltrexamine antagonism of μ 1 and μ 2 opioid actions. *Life Sci* **48**, 2005–2011 (1991)
 - 28 Steel RGD and Torrie JH: Principles and Procedures of Statistics With Special Reference to the Biological Sciences. pp 99–111, McGraw-Hill Book Company Inc, New York (1960)
 - 29 Litchfield JT and Wilcoxon F: A simplified method for evaluating dose-effect experiments. *J Pharmacol Exp Ther* **96**, 99–113 (1949)
 - 30 Portoghesi PS, Sultana M, Nagase H and Takemori AE: A highly selective δ ₁-opioid receptor antagonist: 7-benzylidenenaltrexone. *Eur J Pharmacol* **218**, 195–196 (1992)
 - 31 Portoghesi PS, Sultana M, Nagase H and Takemori AE: Application of the message-address concept in the design of highly potent and selective non-peptide δ opioid receptor antagonists. *J Med Chem* **31**, 281–282 (1988)
 - 32 Schuller AGP, King M, Zhang J, Czick M, Unterwald E, Pasternak GW and Pintar JE: Heroin and M6G analgesia are retained in mu opioid receptor deficient mice. *Soc Neurosci Abstr* **23**, 235.6 (1997)
 - 33 Brown GP, Yang K, King MA, Rossi GC, Leventhal L, Chang A and Pasternak GW: Selective blockade of heroin but not traditional mu, delta, and kappa analgesia by 3-methoxynaltrexone. *Soc Neurosci Abstr* **23**, 235.7 (1997)
 - 34 Arts KS, Holmes BB and Fujimoto JM: Differential contribution of descending serotonergic and noradrenergic systems to central Tyr-D-Ala²-Gly-NmePhe⁴-Gly-ol⁵ (DAMGO) and morphine-induced antinociception in mice. *J Pharmacol Exp Ther* **256**, 890–896 (1991)
 - 35 Kamei J, Kawashima N and Kasuya Y: Role of spleen or spleen products in the deficiency in morphine-induced analgesia in diabetic mice. *Brain Res* **576**, 139–142 (1992)
 - 36 Kamei J, Kawashima N, Suzuki T, Misawa M and Kasuya Y: The effects of cyclosporine on morphine-induced antinociception in diabetic mice. *Neurosci Lett* **158**, 213–216 (1993)
 - 37 Raffa RB, Mathiasen JR, Kimball ES and Vaught JL: The combined immunological and antinociceptive defects of beige-J mice: The possible existence of a " μ -repressin". *Life Sci* **52**, 1–8 (1993)
 - 38 Raffa RB, Kimball ES and Mathiasen JR: The analgesic deficit of C57BL/6J-bgJ/bgJ (Beige-J; Chediak-Higashi syndrome) mice transmitted by adoptive transfer of spleen cells to normal littermates. *Life Sci* **42**, 1231–1236 (1988)
 - 39 Roerig SC and Fujimoto JM: Morphine antinociception in different strains of mice: Relationship of supraspinal multiplicative interaction to tolerance. *J Pharmacol Exp Ther* **247**, 603–608 (1988)