Methadone and Heroin Antinociception: Predominant δ-Opioid-Receptor Responses in Methadone-Tolerant Mice

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ABSTRACT—Antinociceptive tail flick responses to heroin and 6-monoacetylmorphine mediated in the brain by μ-opioid receptor are switched by morphine pellet implantation to δ1- and δ2-opioid-receptor responses. Present results showed that the μ-receptor response (inhibited by β-funaltrexamine) to methadone was changed by morphine pellet implantation to δ1 (inhibited by 7-benzylidenenaltrexone)- and δ2 (inhibited by naltriben)-opioid-receptor responses. Methadone pellet implantation likewise changed mediation from μ- to δ-opioid receptors for heroin and methadone but not for morphine (β-funaltrexamine continued to inhibit). Methadone μ action in the brain was linked through a descending system to activate spinal serotonin receptors (inhibited by methysergide), but this link was gone in the methadone-pellet-implanted group. In the latter group, the new δ1- and δ2-receptor responses were mediated by spinal GABA_A (inhibited by bicuculline) and GABA_B receptors (inhibited by 2-hydroxy-saclofen) receptors. These shifts in neuronal systems meant that μ receptors on a given neuron were not changed into δ receptors. Preliminary results showed that δ-agonist action for methadone was prevented from appearing by MK801, a NMDA-receptor antagonist, and did not occur in 129S6/SvEv mice which lack NMDA responsiveness. Could methadone maintenance treatment in humans uncover δ-agonist actions?

Keywords: Methadone, Heroin, Antinociception, δ-Opioid receptor, Chronic methadone

Heroin and its metabolites, 6-monacetylmorphine (6MAM) and morphine act on μ-opioid receptors in the brain to produce antinociception in CD-1 and ICR mice (1, 2). These μ-receptor actions are inhibited by β-funaltrexamine (β-FNA). Further differentiation between μ-opioid actions is possible because the μ actions of heroin and 6MAM but not morphine are inhibited by 3-O-methyl-naltrexone, OMeNTX (3 – 5), while morphine but not heroin and 6MAM action is inhibited by naloxonazine (3, 6). The explanation for these differences is given as alternative splicing of the μ receptor (7 – 9). Morphine pellet (MP) implantation, which produces tolerance to morphine, changes the receptor selectivity in the brain for heroin and 6MAM to δ-opioid-receptor-mediated responses, but the response to morphine remains μ-receptor-mediated (5). In this situation, heroin activates δ1 (inhibited by 7-benzylidenenaltrexone, BNTX) and 6MAM activates δ2 (inhibited by naltriben) receptors in the brain. In the tail flick test in mice, the μ responses from the brain for heroin, 6MAM and morphine are mediated by spinal serotonergic and adrenergic receptors and δ responses by spinal GABA_A and GABA_B receptors (1, 2, 5, 10, 11). Thus, when the changes in opioid receptor selectivity from μ to δ for heroin and 6MAM are produced by MP implantation, the descending pathway shifts from spinal serotonergic to GABAergic receptors (5). These effects of MP implantation further differentiate heroin and 6MAM analgesic actions from that of morphine.

Methadone maintenance is used to treat heroin abuse in human subjects (12). A question brought up by our morphine tolerance work above was would chronic treatment of mice with methadone (in analogy to methadone maintenance) cause heroin and 6MAM to become δ agonists? Another question was, would chronic methadone treatment of mice cause methadone to become a δ agonist? In the present study, chronic methadone treatment was simulated by s.c. implantation of a methadone pellet (MdP) and
compared in part to MP implantation. The antinociceptive response to an intracerebroventricular, i.c.v., challenging dose of heroin, 6MAM or methadone was determined and the opioid receptor selectivity of the response was evaluated by use of μ (β-FNA, OMeNTX)- and δ (naltrindole, BNTX, naltriben)-opioid-receptor antagonists given i.c.v. Selectivity for corresponding descending neuronal systems were evaluated by intrathecal (i.t.) administration of antagonists: methysergide for serotonergic, yohimbine for adrenergic, bicuculline for GABA_A and 2-hydroxysaclofen for GABA_B receptors (1, 2, 5, 10). An insight into a mechanism involved in expressing δ receptor action was obtained by considering the role of NMDA-receptor activation because it is known that NMDA receptors are involved in the mechanism of development of tolerance to morphine (13).

MATERIALS AND METHODS

Animals: tail flick test

Male CD-1 mice (Charles River, Wilmington, MA, USA) weighing 25 to 30 g were used to demonstrate the major effect (change in opioid receptor selectivity imposed by MP or MdP implantation). In a few experiments, 25 – 30 g male Swiss Webster mice (Hilltop Laboratories, Scottdale, PA, USA) and inbred 9-week-old male 129/SvEv mice (Tacoinc, Germantown, NY, USA) were used to take advantage of certain features of opioid actions. Each animal was used for only one experiment. In the radiant heat tail flick test for antinociception, two tests were conducted before the administration of drugs and the average (2 – 4 s) was employed as the predrug latency. An automatic cut-off time set at 10 s prevented trauma to the tail and was used as the maximal antinociceptive response. The percent maximum possible effect (% MPE) was calculated from the tail flick latencies by the formula:

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

All studies were performed in compliance with the Institutional Animal Care and use Committee (Animal Studies Subcommittee).

Protocols: routes of drug administration

The usual protocol consisted of measuring antinociception 15 min after i.c.v. administration of methadone. A 4-μl aliquot of drug solution was administered i.c.v. to the mouse (14) under light halothane anesthesia. The opioid receptor response to methadone was evaluated by giving antagonists to μ (β-FNA, OMeNTX) and δ (naltrindole, BNTX, naltriben) receptors. Selective activation of opioid receptors in the brain by i.c.v. administration of opiates is associated with activation of different descending spinal pathways to inhibit the tail flick response. Thus, activation of brain μ-opioid receptors is mediated spinally by serotonergic and α2-adrenergic receptors, which in the present case was examined by i.t. administration of methysergide and yohimbine, respectively (15, 16). Activation of δ receptors is mediated by spinal GABA_A and GABA_B receptors which were tested by selective inhibitory action produced by i.t. bicuculline and 2-hydroxysaclofen, respectively (5, 6, 11). Antinociception produced by δ-receptor agonists in the brain is associated with both spinal GABA_A and GABA_B receptors, while that by δ2 agonists involves only GABA_A receptors (17, 18). A change in opioid receptor selectivity from μ to δ1 and δ2 receptors in the brain demonstrated by cross breeding studies (19), MP implantation (5) or streptozotocin-induced diabetes (6) is linked to corresponding shifts in the descending spinal system. To determine whether a shift in the descending system might occur, the i.c.v. administration of an opiate was followed by the i.t. administration of methysergide, yohimbine, bicuculline or 2-hydroxysaclofen 5 min before the tail flick test. The i.t. administration of 5 μl of drug solution was by the method of Hylden and Wilcox (20).

In an initial experiment, the effect of s.c. MP (75 mg base) implantation on methadone analgesia was evaluated. In subsequent experiments, the effects of s.c. MdP (5 mg base) implantation were studied. Implantation of the 10-mg MdP resulted in deaths so the pellet was cut in half to provide a 5-mg dose. The tail flick latencies with MdP as compared to the placebo-pellet (PP) implanted group (generally 7 – 10). Three or more dose levels were used to construct each of the dose response curves.

Protocols: routes of drug administration

A 0.9% (w/v) sodium chloride solution (hereafter designated as saline) was used to dissolve all of the drugs; slight heating was needed to dissolve 2-hydroxysaclofen and bicuculline (the latter with a few drops of 0.1 M hydrochloric acid). Saline rather than acidified saline was used as the i.t. control injection for the bicuculline experiment since heating was needed to dissolve 2-hydroxysaclofen and bicuculline (the latter with a few drops of 0.1 M hydrochloric acid). Saline rather than acidified saline was used as the i.t. control injection for the bicuculline experiment as in earlier experiments (11, 17). The opioid peptide DPDPE, used in one experiment, was dissolved in a 0.9% sodium chloride solution containing 0.01% Triton X-100.

In the figures given in the Results, pretreatments (s.c. PP,
MP, MdP and drug) are stated at the top of the figure. The antinociceptive agent given as an acute challenge is designated at the bottom and the antagonists used to determine receptor activity for the challenging agent are stated vertically in the middle. The bar represents the mean with the vertical line at the top of the bar representing the S.E.M. and the number at its side, the mice/group.

Data analyses

Heroin s.c. ED_{50} values and 95% confidence intervals were derived from the quantal response values transformed to probits and plotted against the log dose; comparison for parallelism of the curves and ED_{50} potency ratio were made according to Litchfield and Wilcoxon (21). Analysis of single dose experiments, which involved comparison of means 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 for comparison of several groups to a given control group (22). A P≤0.05 indicated significant differences between mean values.

Source of drugs

MdP, MP and PP were made by Lange et al. (23) by the method of Gibson and Tingstadt (24). The drugs and commercial sources were as follows: morphine sulfate · 5H_{2}O (Mallinckrodt Chemical Works, St. Louis, MO, USA); methadone hydrochloride (S.B. Penick and Company, Newark, NJ, USA); heroin hydrochloride and 6MAM (free base) (National Institute on Drug Abuse, Rockville, MD, USA); naltrindole hydrochloride, β-FNA (Research Biochemical Inc., Natick, MA, USA); DPDPE, (+)-bicuculline, yohimbine hydrochloride, 2-hydroxysaclofen and MK801 (Sigma Chemical Co., St. Louis, MO, USA); and methysergide maleate (Sandoz Pharmaceutical Co., Berne, Switzerland). The BNTX and naltriben methanesulfonate were synthesized as described and used previously (25 – 27). The OMeNTX was synthesized according to Brown et al. (4). The doses of the drugs for the forms described above are stated along with the routes and time of administration (given as time before the tail flick test) with each experiment.

RESULTS

Antinociceptive response to i.c.v. methadone in PP- and MP-implanted CD-1 mice

The purpose of the first experiment was to determine the opioid receptor involved in antinociception produced by i.c.v. methadone and see if MP implantation changed the opioid receptor selectivity of methadone as it does for heroin and 6MAM (5). The antinociceptive action of i.c.v. methadone in CD-1 mice implanted with a PP for 3 days (72 h) was inhibited by 24-h pretreatment with i.c.v. β-FNA (Fig. 1A) as for morphine (5) and indicated that methadone antinociception was mediated by δ-receptors in the brain. Naltrindole given i.c.v. at a dose effective in inhibiting δ-opioid receptors (5) was without effect. In MP-implanted mice on day 3 (72 h), methadone antinociception was no longer inhibited by β-FNA and now i.c.v. naltrindole was effective (Fig. 1B). These data indicated that the opioid receptor selectivity for methadone had changed from μ to δ receptors. Furthermore, the δ-receptor action was inhibited by i.c.v. BNTX and naltriben, which are δ_{1} and δ_{2}-opioid-receptor antagonists, respectively (Fig. 1B). Thus, MP implantation that changes the δ_{1}- and δ_{2}-opioid-receptor antagonists, respectively (5), conferred both δ_{1}- and δ_{2}-receptor activity on methadone.

Even though in Fig. 1, significant tolerance (though small) occurred to i.c.v. methadone (left panel of B compared to A), the corresponding group in the right panel.

**Fig. 1.** Determination of opioid receptors for i.c.v. methadone antinociception in placebo pellet (PP)- and morphine pellet (MP)-implanted CD-1 mice. A: In PP-implanted mice, β-FNA given i.c.v. 24 h before the tail flick test inhibited i.c.v. methadone action, but naltrindole given along with the methadone had no effect. B: In MP-implanted CD-1 mice, i.c.v. naltrindole but not β-FNA inhibited methadone action. Also, BNTX and naltriben both inhibited methadone action. * indicates a significant difference from the methadone-control group using ANOVA followed by Dunnett’s test, P≤0.05. Values are the means ± S.E.M. Numbers of mice tested are given at the top of each column.
showed no significant tolerance. This aspect was not a major point of this study, but is noted further in the Discussion.

**MdP implantation in CD-1 mice**

The next series of experiments tested whether MdP implantation produced changes in opioid receptor selectivity similar to those produced earlier by MP implantation. In MdP-implanted CD-1 mice on day 3, the antinociceptive action of i.c.v. heroin was inhibited by i.c.v. BNTX but not naltriben (Fig. 2A). The action of 6MAM was inhibited by naltriben but not BNTX (Fig. 2B). These results were similar to those obtained with MP implantation in that the antinociception from i.c.v. heroin and 6MAM was obtained, respectively, through $\delta_1$ and $\delta_2$ receptors in the brain (5). In addition, the results support a view that 10 min after administration, little of the heroin was transformed to 6MAM and little of the 6MAM was transformed to morphine in that each of the three compounds gave responses that could be attributed to the compound initially administered and not due to metabolites. In the same opiate naive mice, the antinociceptive actions of heroin and 6MAM are mediated by $\mu$-receptor actions that are inhibited by both $\beta$-FNA and OMeNTX (4 – 6). Note that the $\mu$ action of heroin and 6MAM still appeared to be present in the MdP implanted mice as indicated by the inhibition produced by i.c.v. OMeNTX (Fig. 2: A and B). The MdP implantation did not change the $\mu$-receptor activity involved in i.c.v. morphine-induced antinociception in that $\beta$-FNA inhibited while naltrindole and OMeNTX did not (Fig. 2C). These findings for morphine parallel those in opiate naive mice (5). Previously heroin antinociception was shown not to act through $\delta$ receptors (not affected by naltrindole) in PP-implanted groups (5), so PP-implanted groups were not included in the present experiment.

Taking these results as a model for what might happen to heroin action in methadone-maintained human subjects, the next experiment was performed to see if in MdP-implanted mice, the action of systemic (rather than i.c.v.) heroin could be shown to involve $\delta_1$ receptors. Dose-response curves were obtained for heroin 15 min after s.c. administration (Fig. 3). First, tolerance occurred to heroin in the MdP compared to the PP groups because the dose response curve was shifted significantly ($P<0.05$) to the right about fivefold (ED$_{50}$ value for the PP group was 0.26 (0.14 – 0.49, 95% confidence interval) mg/kg; for the MdP group, 1.26 (0.82 – 1.93)). The administration of BNTX s.c. produced a further significant shift to the right ($P<0.05$, with MdP + BNTX, ED$_{50}$ = 3.07 (1.83 – 5.17) mg/kg) compared to the MdP group. Thus, at 15 min after s.c. administration, heroin was acting as itself (because 6MAM and morphine antinociception are not inhibited by BNTX, Fig. 2 and (5)).

Because MP implantation caused methadone to manifest a $\delta$-receptor selectivity (Fig. 1) and MdP like MP implantation caused heroin and 6MAM to become $\delta$ agonists,
the next experiment evaluated whether MdP like MP implantation would cause methadone to express \( \delta \) agonist action. In PP-implanted mice, the antinociceptive action of i.c.v. methadone was inhibited by i.c.v. OMeNTX but not naltrindole, indicative of \( \mu \)-agonist action (Fig. 4A). At days 1, 2 and 3 of MdP implantation, naltrindole (which acts on both \( \delta_1 \) and \( \delta_2 \) receptors) inhibited i.c.v. methadone antinociception (Fig. 4B), suggesting participation of \( \delta \) receptors. OMeNTX continued to inhibit methadone antinociception in the MdP groups (Fig. 4B); this result indicated that the \( \mu \)-receptor activity was still present, a point to be brought up in the Discussion. The results shown in Fig. 5, A and B, indicated that both \( \delta_1 \) and \( \delta_2 \) receptors were involved in the \( \delta \)-receptor response following MdP implantation because both BNTX and naltriben inhibited the antinociception in the MdP-implanted groups. These effects produced by MdP were similar to those for MP implantation shown in Fig. 1. Results similar to those for i.c.v. methadone were obtained for s.c. methadone antinociception (Fig. 5: C and D). However, in contrast to i.c.v. methadone challenge where the responses were similar (no manifestation of tolerance) between PP- and MdP-implanted groups, note that in Fig. 5D, the dose of s.c. methadone of 30 mg/kg gave a response no different than that of the 5 mg/kg dose used in the PP-implanted mice (Fig. 5C), indicating that tolerance of about sixfold was manifested to s.c. methadone challenge. These findings of differences in manifestation of tolerance between i.c.v. and s.c challenges occurs for morphine as explained in the Discussion. Also, in Fig. 5D, the inhibition produced by OMeNTX against s.c. methadone in the MdP group was consistent with the results in Fig. 4B.

**Descending spinal system involved in i.c.v. methadone antinociception**

As mentioned in the introduction, antinociceptive actions produced by \( \mu \)-opioid-receptor stimulation in the brain are mediated through spinal serotonergic and \( \alpha \)-adrenergic receptors, while \( \delta \)-receptor actions involve spinal GABA\(_{\Lambda}\).
and GABA\(_{\beta}\) receptors. Thus, in the present study, changes in opioid receptor selectivity for methadone produced by MdP implantation were evaluated for corresponding shifts in the descending systems. The \(\mu\)-receptor response to i.c.v. methadone in PP-implanted CD-1 mice was inhibited by i.t. methysergide (Fig. 6A), indicating that a descending serotonergic pathway was involved in the inhibition of the tail flick response produced by the \(\mu\) action of i.c.v. methadone. The \(\mu\)-receptor-mediated response to i.c.v. methadone did not involve activation of spinal noradrenergic receptors because i.t. yohimbine had no inhibitory effect. Even though the antinociceptive tail flick response to i.c.v. morphine is inhibited by both i.t. methysergide and yohimbine, it is known that differing amounts of activation of the descending serotonergic and adrenergic systems occurs for different \(\mu\) agonists. For instance, i.c.v. DAMGO \((\alpha\text{-Ala}^2\text{-N-methylPhe}^4\text{-Gly-ol}^5)\)-induced antinociception is mediated by the serotonergic more than the noradrenergic system (15). On day 3 of MdP implantation, this inhibition by methysergide was no longer present. In PP-implanted groups, the \(\delta_1\) and \(\delta_2\)-receptor involvement. A: Antinociception produced by i.c.v. methadone was not affected by i.c.v. BNTX or naltriben in PP-implanted CD-1 mice. B: Both BNTX and naltriben inhibited methadone-induced antinociception in the MdP-implanted mice. C: In control mice, the antinociception produced by s.c. methadone was not affected by BNTX or naltriben and was inhibited by OMeNTX. D: In MdP-implanted (day 3) mice, methadone antinociception was inhibited by s.c. administration of either naltriben or BNTX. The inhibitory action of OMeNTX against methadone antinociception was still present. The 5 and 30 mg/kg dose of methadone produced similar antinociceptive responses in the control and MdP groups, respectively. * and \# indicate a significant difference, \(P<0.05\), by Dunnett’s test and Student’s \(t\)-test, respectively. Values are the means ± S.E.M. Numbers of mice tested are given at the top of each column.

Fig. 5. Further evaluation for \(\delta_1\) and \(\delta_2\)-receptor involvement. A: Antinociception produced by i.c.v. methadone was not affected by i.c.v. BNTX or naltriben in PP-implanted CD-1 mice. B: Both BNTX and naltriben inhibited methadone-induced antinociception in the MdP-implanted mice. C: In control mice, the antinociception produced by s.c. methadone was not affected by BNTX or naltriben and was inhibited by OMeNTX. D: In MdP-implanted (day 3) mice, methadone antinociception was inhibited by s.c. administration of either naltriben or BNTX. The inhibitory action of OMeNTX against methadone antinociception was still present. The 5 and 30 mg/kg dose of methadone produced similar antinociceptive responses in the control and MdP groups, respectively. * and \# indicate a significant difference, \(P<0.05\), by Dunnett’s test and Student’s \(t\)-test, respectively. Values are the means ± S.E.M. Numbers of mice tested are given at the top of each column.
Thus, these results were consistent with i.c.v. methadone induced antinociception involving brain $\delta$ receptors being mediated by spinal serotonergic receptors, while MdP implantation that changed the brain opioid receptor selectivity to $\delta_1$ and $\delta_2$ was correspondingly associated now with spinal GABA$_A$ and GABA$_B$ receptors.

In MdP-implanted groups, i.c.v. morphine antinociception was inhibited by i.t. yohimbine and methysergide, but not bicuculline and 2-hydroxysaclofen (Fig. 6C). Thus, these results were similar to those for opiate naive control mice (5). MdP-like MP implantation did not cause the $\mu$-agonist action of morphine to change in opioid receptor selectivity or descending pain control system activation.

**NMDA receptor involvement in the change of methadone to $\delta$-receptor action**

Evidence suggests that NMDA activation is involved in the effect of chronic morphine treatment to change the antinociceptive response of heroin from $\mu$ to $\delta$ receptors (5). Thus, studies were performed to see whether activation of NMDA receptors might be involved in the methadone-induced $\delta$-agonist action of methadone. Methadone at an antinociceptive dose of 5 mg/kg was given s.c. at 24 and 16 h before the tail flick test. The antinociceptive action of i.c.v. methadone challenge was not affected by naltrindole in the saline-treated group, but the two dose treatment with s.c. methadone was sufficient to cause methadone to become a $\delta$ agonist as indicated by the inhibitory action of naltrindole (Fig. 7A). In the next experiment (Fig. 7B), a s.c. treatment with MK801 was given 30 min before the 24- and 16-h methadone pretreatment. Now the two dose methadone treatment was ineffective in producing the $\delta$ response as seen by lack of effect of naltrindole. The two MK801 treatments by themselves had no effect on the acute antinociceptive action of i.c.v. methadone (the saline-treated groups in Fig. 7B). The effect of MK801 to inhibit the expression of $\delta$-agonist action indicated that NMDA receptors may be involved in producing this expression. However, in these and the remainder of the experiments, numbers of mice used in each group were small and must be considered as preliminary results.

Treatment of CD-1 mice with s.c. NMDA, 1 mg/kg, once a day for 5 days, which produces tolerance to morphine (28), produced a significant $\delta$-receptor response for i.c.v. methadone; naltrindole inhibited the antinociceptive response (Fig. 8A). However, the effect was not robust. In another protocol, a 24.5-h single dose pretreatment with NMDA did not produce a naltrindole-sensitive response to methadone (Fig. 8B). A 24-h single dose (5 mg/kg) pretreatment with s.c. methadone was also ineffective (Fig. 8C). When these two ineffective pretreatments were combined, the antinociceptive response to i.c.v. methadone response was inhibited by i.c.v. naltrindole (Fig. 8C).
use of one-half the doses of methadone and NMDA in combination produced the same change (Fig. 8D).

Lack of ability of MdP to produce δ-receptor responses to methadone in 129S6/SvEv mice

Kolesnikov et al. (28) have shown that morphine tolerance does not develop in 129S6/SvEv mice because these
mice lack NMDA receptor responsiveness. Even though i.c.v. methadone produced antinociception in these mice, MdP implantation was ineffective in producing a \( \delta \)-response; naltrindole did not inhibit methadone-induced antinociception (Fig. 9A). The lack of change in the MdP-implanted group implicated the need for an intact NMDA-receptor response. The lack of conversion to a \( \delta \)-response for methadone cannot be attributed to a lack of \( \delta \)-receptors in the brain because there was a robust antinociceptive response to i.c.v. DPDPE (Fig. 9B).

**Antinociceptive response to methadone in Swiss Webster mice based on a strain difference**

Swiss Webster mice served as a model to see if methadone had the potential to act as a \( \delta \)-receptor agonist without the need for MdP implantation. Heroin- and 6MAM-induced antinociception in Swiss Webster mice are \( \delta_1 \) and \( \delta_2 \)-receptor-mediated, respectively (27). The antinociceptive response to i.c.v. methadone in Swiss Webster mice was inhibited by naltrindole but not by OMeNTX (Fig. 10A). These data indicated that methadone was acting as a \( \delta \)- and not as a \( \mu \)-receptor agonist in Swiss Webster mice. The \( \delta \)-receptor response involved both \( \delta_1 \)- and \( \delta_2 \)-receptor subtypes because both BNTX and naltriben inhibited the responses. Similar results were obtained for systemic administration of methadone, BNTX and naltriben (Fig. 10B), suggesting that the results from i.c.v. experiments reflected what happens systemically.

*Fig. 9.* Lack of change in receptor response to i.c.v. methadone in MdP-implanted 129S6/SvEv mice. A: The antinoiciceptive response to i.c.v. methadone was not inhibited by naltrindole in either the PP or MdP-implanted 129S6/SvEv mice. B: i.c.v. DPDPE in control 129S6/SvEv mice produced an antinociception that was inhibited by i.c.v. naltrindole. * indicates a significant difference by Student’s *t*-test, *P*≤0.05. Values are the means ± S.E.M. Numbers of mice tested are given at the top of each column.

*Fig. 10.* Opioid receptors involved in i.c.v. methadone-induced inhibition of the tail flick response in Swiss Webster mice. A: Antinociception from i.c.v. methadone was not inhibited by OMeNTX but was inhibited by naltrindole and by both BNTX and naltriben. B: Antinociception from s.c. methadone was inhibited by s.c. naltriben and BNTX, but not OMeNTX. * indicates a significant difference by Dunnett’s *t*-test, *P*≤0.05. Values are the means ± S.E.M. Numbers of mice tested are given at the top of each column.
DISCUSSION

The antinociceptive response to i.c.v. methadone in PP-implanted CD-1 mice was inhibited by i.c.v. pretreatment with β-FNA. Also, OMeNTX, i.c.v. at a dose that inhibits heroin and 6MAM antinociception (5) inhibited i.c.v. methadone antinociception. These findings indicated that methadone action was mediated by μ receptors as are heroin and 6MAM antinociception (1–6). These μ responses to heroin and 6MAM are similar to those of morphine-6β-glucuronide and different from those for morphine (3, 4, 7, 8). The antinociceptive action of i.c.v. morphine is not inhibited by i.c.v. OMeNTX at as high a dose as 10 μg (5). Morphine-6β-glucuronide produces antinociception through an alternatively spliced isoform of the MOR-1 receptor (7–9). The possibility that methadone, heroin and 6MAM may act through an isoform of the μ receptor might be associated with the ability of these opiates in the present study to act through δ receptors following MP and MDP implantation.

In MDP-implanted mice, i.c.v. heroin-induced antinociception was selectively inhibited by BNTX and not naltriben, indicating the appearance of δ-receptor-subtype response. This δ1 selectivity is similar to that produced by MP implantation (5). Also, the δ2-receptor selectivity manifested for i.c.v. 6MAM by MDP implantation is similar to that after MP implantation. For methadone antinociception, MDP and MP implantation elicited δ1- and δ2-receptor responses together. Thus, MP and MDP implantation produced the same expression of δ1 (heroin)- and δ2 (6MAM)-receptor subtypes separately or together (methadone, present study), depending on the agonist tested. Correspondingly, δ1- and δ2-receptor activities were obtained for methadone in Swiss Webster mice without the need of opiate pellet implantation (Fig. 10). This was similar to the δ1 and δ2 activity obtained in Swiss Webster mice for heroin and 6MAM, respectively (1, 2).

As given in the rationale in the Methods stimulation of μ receptors in the brain by i.c.v. opioids activates descending systems mediated by spinal serotonergic and α2-adrenergic receptors that are inhibited by methysergide and yohimbine, respectively. Antinociception produced through δ1 receptors in the brain is mediated by spinal GABAδ and GABAβ receptors (inhibited by i.t. bicuculline and 2-hydroxyasaclofen, respectively), while that produced by δ2 receptors is mediated by spinal GABAδ receptors only (inhibited by bicuculline). Methadone stimulated both δ1 and δ2 receptors in the brain of the MDP groups (Fig. 5A) and activated both spinal GABA receptors (Fig. 6B). Thus, the change produced by MDP implantation shifted the antinociceptive response for i.c.v. methadone from the μ-receptor-linked activation of spinal serotonergic receptors to δ-receptor-linked spinal systems mediated by GABAδ and GABAβ receptors. These coordinated changes were not due to the absence of the μ-linked serotonergic system because the μ response produced by i.c.v. morphine still activated spinal serotonin receptors (Fig. 7C). Thus, the major finding that MP and MDP implantation uncovered the δ agonist actions for methadone, heroin and 6MAM was robust.

The finding that the inhibitory action of i.c.v. OMeNTX remained for heroin, 6MAM (Fig. 2) and methadone (Fig. 4) in the MDP-implanted mice requires further clarification. This finding suggested that the μ-agonist actions for these three opiates persisted after MDP implantation. Because OMeNTX did not inhibit the antinociceptive action of i.c.v. methadone in Swiss Webster mice (where methadone acted primarily on δ receptors), a non-selective inhibitory action of OMeNTX through δ receptors on methadone, heroin, and 6MAM responses in the MDP implanted mice was an unlikely alternative. Note, however, that the OMeNTX-sensitive μ response to methadone was not inhibited by β-FNA (Fig. 1), indicating that mediation of its antinociceptive response through μ receptors had been eliminated. The latter indication was consistent with the result that the antinociceptive action of i.c.v. methadone was no longer inhibited by i.t. methysergide in the MDP-implanted group (Fig. 7A). There is no explanation for why OMeNTX continued to be effective in the MDP-implanted groups.

An explanation was needed for how MDP and MP implantation uncovered δ-receptor activities. The present evidence suggested that NMDA receptors participated in the mechanism for the change of methadone antinociception to a δ-receptor response. MK801 administration inhibited the ability of a two-dose paradigm of methadone pretreatment (24 and 16 h) to produce the δ response. Furthermore, the administration of a single dose of NMDA in combination with a single dose of methadone was effective in producing δ agonism for i.c.v. methadone. Lastly, MDP implantation in 129S6/SvEv mice was not effective in producing the change. A lack of NMDA responsiveness in these mice exists (28). Thus, these indications of NMDA-receptor involvement by which MDP implantation produced the δ response to methadone aligned themselves with the concept that the development of tolerance to morphine requires NMDA-receptor participation (13). However, Crain and Shen (29) find that 129S6/SvEv are deficient in GM δ ganglioside. Their evidence is that GM δ ganglioside is involved in the excitatory action of morphine, which in turn is responsible for the development of tolerance to morphine. Thus, our present results on implicating NMDA receptors should be considered as being preliminary and require further investigation.

Factors other than NMDA receptors may contribute to the expression of δ-receptor action for methadone, heroin
and 6MAM. First, genetic contributions exist. Even though methadone produced μ-receptor-mediated antinociception in CD-1 mice, Swiss Webster mice gave δ- and δ-receptor responses to i.c.v. methadone as primary responses (Fig. 10). Similarly, Swiss Webster mice give primary δ-receptor responses without μ-receptor ones for heroin and 6MAM (1, 2, 27). Inbred C57BL/6J mice give a primary δ-receptor response to heroin, while the inbred DBA/2J, C3H/HeJ and CBA/J mouse strains give μ-receptor-mediated rather than δ-receptor-mediated responses to heroin (10). When Swiss Webster mice are cross bred with ICR mice (which give a μ response to i.c.v. heroin), the F1 male and female offspring give μ- and δ-receptor responses to i.c.v. heroin, respectively (19). This sex specific influence is produced through autosomal rather than sex-chromosome linked inheritance. Second, another means of controlling receptor selectivity is to induce diabetes by streptozotocin treatment that changes the usual μ response for i.c.v. heroin in CD-1 and ICR mice (6) and Sprague Dawley rats (30) to a δ-receptor-mediated antinociceptive responses. A factor common to these various treatments and the strain differences is not evident.

In situations where the μ- and δ-agonist responses occur for heroin, 6MAM or methadone (whether it be difference between mouse strains, cross breeding, streptozotocin-induced diabetes or MP and MdP implantation), the matching of the activation of brain opioid receptor to the appropriate descending spinal antinociceptive system as discussed above carries implications regarding mechanisms involved in the receptor selectivity change. These associations indicate that when the μ-receptor responses to methadone, heroin or 6MAM were changed from μ- to δ-receptor responses, the responses physically shift from serotonergic or α2-adrenergic neurons associated with μ-agonist action to GABAergic neurons associated with δ-agonist actions. These findings make it unlikely that μ receptors change into δ receptors in the same neuron. Furthermore, the μ-receptor action for morphine through its associated descending systems after MdP, MP or streptozotocin treatment still persists. Also, DPDPE δ1- and DSLET δ2-receptor responses and their associated descending GABA systems are present in control CD-1 mice so it is puzzling why MdP and MP implantation should cause the μ actions of certain agonists to change to δ actions while the receptor selectivity for morphine remains unchanged. These changes in receptor selectivities are due to shifts from one to another neuronal system and not due to μ receptors changing into δ receptors in given neurons. However, this statement does not preclude the possibility of a more subtle change in opioid receptors occurring within different sets of neurons.

This latter possibility involves the interaction between μ and δ receptors to form dimers or oligomers. The synergistic enhancement of μ-receptor-induced antinociception and binding by δ agonists has been the subject of investigation for over 20 years (31–34), and recent studies provide biochemical evidence for the existence of μ-δ dimers in cells expressing μ and δ receptors simultaneously (35, 36). Given the critical involvement of δ receptors in μ-mediated tolerance (35) and the finding that the selectivity of an antagonist or agonist may depend upon the organization of opioid receptors as monomers or heterodimers (37, 38), the transition from μ to δ selectivity may reflect changes in the distribution of μ-δ dimers relative to monomers. In this connection, it is conceivable that the observed “δ” agonism induced by chronic methadone and morphine treatment, may actually be mediated via the μ receptor component of the dimer. This would be analogous to norbinaltorphimine-induced antagonism of a δ agonist bound to the δ component of a κ-δ heterodimer (39). These changes in heterodimeric distributions need not involve major changes in the monomers involved in μ and δ agonist actions consistent with the evidence cited in the paragraph above. Probes with well-established selectivity for heterodimeric opioid receptors would be required for the rigorous investigation of these possibilities.

Progressing onto considerations reflecting secondary findings, an insight relevant to tolerance was present. First, sixfold tolerance to s.c. methadone was manifested on day 3 in MdP-implanted mice compared to control CD-1 mice. A fivefold tolerance to s.c. heroin was seen with MdP. The development of tolerance would be an expected finding that is attributable classically to the development of tolerance to the μ-agonist action of methadone and heroin. Note by contrast that i.c.v. methadone and heroin responses did not show tolerance. A similar phenomenon occurs for morphine where MP implantation produces a large degree of tolerance to s.c. but not to i.c.v. or i.t. morphine challenge (40– 42). These findings with morphine are the basis for the proposal that morphine tolerance is in part due to reduction in the synergistic interaction that occurs between morphine-sensitive sites in the brain and spinal cord sites after systemic morphine administration as contrasted to lack of reduction of sensitivity of the individual sites (i.c.v. and i.t.). On the average about 50% of the tolerance to morphine can be attributed to the decrease in synergistic interaction (41).

Another notable feature is that morphine-tolerant mice are not cross-tolerant to methadone (43) and morphine-tolerant rats show low cross-tolerance to methadone (44). Swiss Cox mice made highly tolerant to morphine are not cross-tolerant to heroin (45). In MP implanted CD-1 mice, tolerance develops to the μ action of heroin, but the appearance of δ-agonist action induced by MP implantation counter balances the tolerance so that the net result is no manifestation of overall tolerance to heroin (5). Such a
counter-balancing effect might be occurring also in the present situation. The dose-response curve for s.c. heroin was shifted to the right 4.3-fold, a shift attributed to development of tolerance to the \(\mu\)-agonist action of heroin. However, a substantial \(\delta\)-receptor response to heroin was occurring because BNTX produced a further rightward shift in the dose response curve for s.c. heroin. It is likely that tolerance to \(\mu\)-agonist action does not confer cross-tolerance to \(\delta\)-agonist action of heroin (5). Thus, the development of tolerance to the \(\mu\) action of s.c. heroin in MdP-implanted mice was counterbalanced at least in part by the new appearance of the \(\delta\)-agonist action for heroin. Having brought up these considerations, it is possible that the reason for methadone, heroin and 6MAM becoming \(\delta\) agonists is that tolerance developed to their \(\mu\)-receptor actions but not to their \(\delta\)-receptor actions. This argument would not explain why in Swiss Webster mice and streptozotocin-treated CD-1 mice, \(\delta\)-agonist action would predominate for these selected agonists but not for morphine. Another implicit realization was that s.c. heroin was acting as heroin and not as 6MAM or morphine because BNTX effectively shifted the s.c. heroin dose-response curve to the right in the MdP-implanted CD-1 mice. BNTX does not inhibit 6MAM or morphine antinociception in control or MP-implanted mice (5, 27). Here, heroin at 15 min after s.c. administration acted as itself in MdP-implanted mice. These results are not in accord with the studies that indicate that the major pharmacological actions of heroin in mice can be attributed to morphine, which is formed as a metabolite, and perhaps to the intermediate metabolite, 6MAM (46 – 49). The present results suggest that a reexamination of the pharmacological action of heroin and methadone in methadone-maintained human subjects would be of interest. Unpublished evidence suggests that the presence of \(\delta\)-agonist action of methadone in chronic methadone-treated rats (G. Gross, Medical College of Wisconsin, personal communication) and possibly human addicts on methadone maintenance treatment (R. Maslansky, Bellevue Hospital, New York, and A. Penn, Louisiana State University; personal communication) is cardioprotective. The \(\delta\)-receptor action of certain other opioids is cardioprotective against experimentally induced myocardial infarction (50, 51).

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REFERENCES


2. 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)


11. Holmes BB and Fujimoto JM: [\(\text{D-NmethylPhe}^2,\text{D-Pen}^5\text{enkephalin} \] a delta opioid agonist given intracerebroventricularly in the mouse produces antinociception through mediation of spinal GABA receptors. Pharmacol Biochem Behav 49, 675 – 682 (1994)


17. Rady JJ and Fujimoto JM: Spinal GABA receptors mediate brain delta opioid analgesia in Swiss Webster mice. Pharmacol...
19 Rady JJ and Fujimoto JM: Analgesic response in offspring of crosses between heroin δ (Swiss Webster) and μ (ICR) responding mice. Pharmacogenetics 7, 429 – 433 (1997)