Opioid Receptor Selectivity of Heroin Given Intracerebroventricularly Differs in Six Strains of Inbred Mice

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ABSTRACT

Heroin administered i.c.v. acts on supraspinal μ opioid receptors in ICR mice but on δ receptors in Swiss Webster mice. The purpose of this study was to determine the degree to which genotype plays a role in the opioid receptor selectivity of heroin across a range of fully inbred strains of mice. Six inbred strains were given heroin i.c.v. 10 min before the tail-flick test. Differences in the descending neurotransmitter systems involved in supraspinal opioid-induced analgesia were evaluated as the first step. Antagonism by bicuculline given intrathecally indicated the involvement of supraspinal δ receptors in activating spinal γ-aminobutyric acid (GABA) receptors; antagonism by intrathecal methysergide indicated either μ or κ receptor involvement. Antagonism by intrathecal yohimbine implicated μ and eliminated κ receptor involvement. Intracerebroventricular opioid antagonists (β-funaltrexamine, 7-benzylidenenaltrexone, naltrexiben, or nor-binaltorphimine) provided further differentiation. Based on these initial results, receptor selectivity was determined by more extensive ED50 experiments with i.c.v. administration of heroin with opioid antagonists, β-funaltrexamine (for μ), naltindole (for δ), and nor-binaltorphimine (for κ). The combined results indicated that heroin analgesia was predominantly mediated in C57BL/6J by δ, in DBA/2J and CBA/J by μ, and in BALB/cByJ and AKR/J by κ receptors. The response in C3H/HeJ appeared to involve μ receptors. The results indicate that the opioid receptor selectivity of heroin is genotype-dependent. Because these genotypes are fully inbred, the genetically determined molecular and neurochemical substrate mediating the different opioid receptor selectivities of heroin can be studied further.

An unexpected difference in opioid receptor selectivity occurs in the antinociceptive (tail-flick test) action of heroin between two strains of commonly used laboratory mice. Heroin given i.c.v. activates μ opioid receptors in ICR (and CD-1) mice but δ opioid receptors in Swiss Webster mice (Rady et al., 1991). This difference in receptor selectivity occurs even though both sets of mice show no difference to the μ-agonist action of morphine or the δ agonist action of (D-Pen2,5)enkephalin (DPDPE). A potential pitfall associated with using randomly outbred mice such as the Swiss Webster and ICR is that the trait and genotype may vary across vendors or the strain may become commercially unavailable, and ICR is that the trait and genotype may vary across laboratories and time. The advantage of inbred strains is that much is known about genetic composition and origin of the strains (origins of inbred). In addition, in many cases much is known about the behavioral and neurochemical profile of the genotype (Belknap and O'Toole, 1991). Furthermore, the ability to determine genetic loci (and possibly the transcribed proteins) that account for genotype-dependent variations in response to a drug makes genetic control both a short- and long-term advantage (Crabbe et al., 1994). Because previous research had suggested that genotype significantly influences the pharmacological effects of heroin, one purpose of the current study was to determine the degree to which genotype governs opioid-receptor selectivity for heroin in inbred mice.

A related purpose of this series of studies was to identify genotypes in which heroin acts through different opioid receptors. Identification of genotypes with distinct pharmacological reactions to heroin has several advantages. First, it

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ABBREVIATIONS: DPDPE, (D-Pen2,5)enkephalin; DAMGO, Tyr-d-Ala2-Gly-N-MePhe4-Gly-ol2; U50,488H, trans-3,4-dichloro-N-methyl-N-[2-(1-pyrrolidinyl)-cyclohexyl]-benzene acetamide methanesulfonate; β-FNA, β-funaltrexamine; BNTX, 7-benzylidenenaltrexone; N-BNI, nor-binaltorphimine; GABA, γ-aminobutyric acid; % MPE, percent maximum possible effect; i.c.v., intracerebroventricularly; IT, intrathecally.
clearly establishes the degree to which genotype determines the pharmacological actions of heroin. Second, it characterizes the pharmacology of heroin in a broad population. Third, it provides a system in which the biochemical and molecular mechanisms underlying agonist receptor selectivity can be further analyzed. Finally, it establishes a database to explore the pharmacological basis of vulnerability to drug use and abuse.

The most direct way to evaluate the type of opioid receptor involved is to use selective opioid receptor antagonists and perform major ED50 studies for heroin. Initial single-dose studies with opioid antagonists would provide information for the design of the subsequent ED50 experiments. However, such initial results would not provide information beyond those derived from the major studies. On the other hand, intial studies on descending pathways of heroin-induced antinociception would provide complementary results useful beyond those from the ED50 studies. The tail-flick test depends on a spinal reflex (it remains intact after transection of the cervical spinal cord [Wang et al., 1994]), which can be inhibited by activating descending antinociceptive pathways to the spinal cord. The loci of action of opioids in the brain, the opioid receptors there, and the descending systems with neurotransmitters in the spinal cord have been reviewed by Yaksh and Malmberg (1994). Differential activation of spinopetal antinociceptive pathways occurs after i.c.v. administration of opioid receptor agonists (Fig. 1). In mice, activation of \(\kappa\) receptors in the brain leads to antinociception modulated by spinal serotonin receptors (Ho and Takemori, 1989). Spinal serotonin and the \(\alpha_2\)-action of norepinephrine are involved in the analgesic action of \(\mu\) agonists given i.c.v. (Hyl- den and Wilcox, 1980; Arts et al., 1991). Activation of \(\delta\) receptors in the brain involves GABA receptors in the spinal cord (Holmes and Fujimoto, 1994; Rady and Fujimoto, 1995, 1996). Supraspinal opioid-induced antinociception can be selectively inhibited by IT administration of antagonists to the neurotransmitter mediating each descending system: methysergide for serotonin, yohimbine for \(\alpha_2\) noradrenergic and bicuculline for GABA\(_A\) receptors. In Swiss Webster mice, where i.c.v. heroin acts on \(\delta\) receptors, IT bicuculline shifts the dose-response curve for heroin to the right in parallel fashion (Rady and Fujimoto, 1995), while IT yohimbine and methysergide produce parallel rightward shifts for i.c.v. morphine (Suh et al., 1989). The initial results were used in designing more extensive experiments, wherein heroin dose-response relationships were generated after i.c.v. administration of opioid receptor antagonists: \(\beta\)-funtrelxamine (\(\beta\)-FNA) for \(\mu\), naltrindole for \(\delta\), and N-BNI for \(\kappa\) receptors.

Materials and Methods

Animals. Adult male AKR/J, BALB/cByJ, C57BL/6J, C3H/HeJ, CB6J, and DBA/2J mice (Jackson Laboratories, Bar Harbor, ME), 60 to 120 days old and weighing approximately 23 to 30 g at the start of the experiment, were used. Animals were housed in groups of three to five in a temperature-controlled room (21°C) with a 12-h light/dark cycle (lights on at 7:00 AM). Animals had free access to Purina Laboratory Chow and tap water at all times. The studies were conducted in accordance with the Guide for Care and Use of Laboratory Animals provided by the National Institutes of Health and adopted by National Institute on Drug Abuse and were in compliance with the Institutional Animal Care and Use Committee (Animal Studies Subcommittee).

Antinociceptive Response. The radiant heat tail-flick test (D’Amour and Smith, 1941) was used to measure antinociception. The lamp intensity was set to provide a predrug response time of 2 to 4 s, and a cut-off time of 10 s was used as the maximal response. The postdrug tail-flick latency was converted to percent maximum possible effect (% MPE) as calculated by Dewey et al. (1970); % MPE = (postdrug time – predrug time) \times 100/predrug time.

Intracerebroventricular and IT Drug Administration. The basic protocol involved administration of heroin or other opioid agonists i.c.v. to inhibit the tail-flick response. The approach to peak action of i.c.v. agonists was assumed to be 10 min after administration based on previous studies in outbred mice (Rady et al., 1991, 1994a, b; Holmes and Fujimoto, 1994; Rady and Fujimoto, 1995, 1996). This 10-min time favored heroin as the primary antinociceptive agent rather than its metabolites, 6-monoacetylmorphine and morphine (Rady et al., 1991; Rady and Fujimoto, 1995, 1996). Intracerebroventricular administration was by the method of Haley and McCormick (1957) under light halothane anesthesia. In later experiments opioid antagonists were administered i.c.v. along with heroin. IT administration of antagonists (bicuculline, methysergide, yohimbine) was by the method of Hyliden and Wilcox (1980).

Single-Dose Studies Involving Descending Systems. The most direct way to determine the opioid receptors involved in heroin analgesia would be to use selective opioid receptor antagonists i.c.v. and determine the shifts they produce in the dose-response curves for heroin. However, \(\beta\)-FNA (a nonequilibrium \(\mu\) antagonist), N-BNI (a \(\kappa\) receptor antagonist), and naltrindole (a \(\delta\) receptor antagonist) have long duration of action (Ward et al., 1982; Takemori et al., 1988; Horan et al., 1992) making reuse of the mice difficult. Reuse was desirable because some inbred strains were not readily available. The use of the nonopioid antagonists for the neurotransmitters of the descending systems (see Introduction), which had short duration of action (Suh et al., 1989; Arts et al., 1991; Holmes and Fujimoto, 1994) allowed the mice to be used more than once. The mice were used a total of 3 times with 5 to 7 days between uses. The treatments were randomized and no order effects were found. Previously Mickley et al. (1990) reported results from C57BL/6J mice reused 3 days after i.c.v. administration of \(\mu\) and \(\delta\) receptor agonists.

The sequence for the single-dose experiments is depicted in Fig. 1. In the first step, heroin was given i.c.v. at 10 min and followed by bicuculline or methysergide given IT at 5 min before the tail-flick test. The inhibitory action of bicuculline in the spinal cord differentiates the antinociceptive action of i.c.v. \(\delta\) receptor agonists from...
other opioid receptor agonists; IT bicuculline does not affect μ and κ agonist actions (Holmes and Fujimoto, 1994; Rady and Fujimoto, 1995).

Second-step studies were carried out to further delineate receptor selectivity as necessary (Fig. 1). Inhibition by bicuculline in the first step indicated δ receptor involvement; in the second step, inhibition by i.c.v. BNTX (7-benzylidenenaltrexone) or naltrilene delineated between δ₁ and δ₂ receptor subtypes respectively (Rady et al., 1994b).

When inhibition by IT methysergide in the first step suggested μ or κ receptor involvement, differentiation was obtained by i.c.v. N-BNI, a κ receptor antagonist, or IT yohimbine, an alph₃ adrenergic antagonist. A response to yohimbine was followed up by a test with β-FNA to indicate μ receptors. Mice treated with β-FNA and N-BNI were not retested. To demonstrate the functional existence of the descending systems the ability of the neurotransmitter antagonists to inhibit the effects of other agonists given i.c.v. [DPDPE, DAMGO (Tyr-D-Pen²⁵,N-Me-Phe₄-Gly-ol³), U50,488H (trans-3,4-dichloro-N-methyl-N-(1-pyrrolidinyl)-cyclohexyl]-benzene acetamide methanesulfonate), and morphine] was investigated for comparative purposes.

Dose-Response Relationship Experiments for i.c.v. Heroin with Opioid Receptor Antagonists (ED₅₀ Determinations). Additional pharmacological determination of receptor selectivity for i.c.v. heroin was based on the use of opioid receptor antagonists given i.c.v. These mice were experimentally naive and were not retested. The results from the single-dose experiments with the descending systems indicated that all three of the known opioid receptor types (μ, δ, and κ) had to be considered. The opioid antagonists used were β-FNA for μ, naltrilene for δ, and N-BNI for κ receptors. Doses were based on our previous publications (Rady et al., 1991, 1994a, b). Naltrilene and N-BNI were administered i.c.v. at 10 min in the same solution with heroin; β-FNA was given i.c.v. as a 24-h pretreatment.

Statistical Analysis. Student’s t test (comparison between two groups) and analysis of variance (ANOVA) followed by Dunnett’s test (comparison of each group to a control group) were used for determining significant differences between group means as indicated by a P < 0.05 (Steel and Torrie, 1960). The ED₅₀ values with the 95% confidence intervals were determined and compared for significant differences (P < 0.05) according to the method of Litchfield and Wilcoxon (1949) as described for the computerized version by Dewey et al. (1970) and used previously (Roerig and Fujimoto, 1989).

Source of Drugs and Drug Solutions. DAMGO (Peninsula, Belmont, CA); heroin hydrochloride (National Institute on Drug Abuse, Rockville, MD); morphine sulfate (Mallinckrodt Chemical Works, St. Louis, MO); (-)-bicuculline, DPDPE and yohimbine hydrochloride (Sigma Chemical Co., St. Louis, MO); U50,488H (Up John, Kalamazoo, MI); methysergide maleate (Sandoz, Berne, Switzerland), β-FNA and N-BNI (RBI, Natick, MA); naltrilene and BNTX were obtained from Takemori and Portoghese (University of Minnesota, Minneapolis, MN). The doses of the drugs were for the forms stated above. Drugs were dissolved in a 0.9% sodium chloride solution or in a 0.01% Triton X-100 in 0.9% sodium chloride solution (DPDPE, DAMGO). Slight heating was used to dissolve bicuculline. Doses and times of administration of the drugs were taken from our previous publications and are given with each experiment under Results.

Results

The results are grouped according to the receptor selectivity found for heroin. In group A, heroin acted on δ receptors, in B on μ receptors, and in C on κ receptors.

Single-Dose Studies Involving Descending Systems. Group A (δ Receptor Response). The results in Fig. 2 for the C57BL/6J mice indicate that a 3-μg dose (used as the standard dose in all strains except AKR/J) of heroin given i.c.v. 10 min before the tail-flick test produced about an 80% MPE. This antinociception was inhibited by IT bicuculline (0.5 μg), but not by methysergide (1 μg), given 5 min before the tail-flick test. These results indicated that the response involved supraspinal δ and not μ or κ receptors. In the test for the subtype of δ receptor (see Fig. 3), the δ₁ receptor antagonist BNTX, 1 pmol (0.465 ng) given with the heroin, inhibited the response while naltrilene, 25 pmol (12.8 ng), a δ₂ receptor antagonist, did not. These doses of BNTX and naltrilene were shown previously in Swiss Webster mice to be effective in inhibiting the δ₁ and δ₂ response, respectively (Rady et al., 1994). Thus, the i.c.v. heroin receptor response involved δ₁ receptors. The methysergide treatment was effective in inhibiting the antinociceptive action of i.c.v. morphine (Table 1). Therefore, the serotonergic pathway was present but was not involved in heroin action.

Group B (μ Receptor Response). CBA/J, DBA/2J, and C3H/HeJ. Bicuculline did not affect heroin action in CBA/J, DBA/2J, and C3H/HeJ, indicating that δ receptors were not involved (Fig. 2). Inhibition by methysergide indicated that in CBA/J and DBA/2J either μ or κ receptors were involved. The effectiveness of IT yohimbine (Fig. 3) indicated μ receptor participation in the CBA/J response. The lack of response of DBA/2J to N-BNI eliminated the alternative of the κ receptor and classified DBA/2J as μ responders. The lack of response to IT yohimbine (Fig. 3) was considered to be a reflection of the different amounts of participation between serotonergic and noradrenergic systems in the μ response. In this regard, the response in C3H/HeJ was insensitive to methysergide (Fig. 2) but sensitive to yohimbine inhibition (Fig. 3), making this strain μ responders. The positive yohimbine response eliminated κ receptors. Thus, μ receptors were involved in the action of heroin in CBA/J, DBA/2J, and C3H/HeJ mice.

Responses to other standard agonists were evaluated in these three strains for limited comparative purposes. Antinociception from i.c.v. DPDPE, a δ agonist, was inhibited by IT bicuculline in all three strains (Table 1). These results meant that δ receptors and the descending GABA systems were present even though they were not activated by heroin. That the stimulation of μ receptors can lead to different amounts of activation of serotonergic and noradrenergic descending pathways was seen with morphine. Yohimbine inhibited the analgesic action of morphine in DBA/2J and heroin in C3H/HeJ, consistent with the presence of descending noradrenergic involvement in μ receptor action. However, note that in C3H/HeJ, neither the action of morphine nor DAMGO (a peptide with greater selectivity for μ receptors than morphine) was inhibited by IT methysergide (Table 1). Evidently, spinal serotonin mediation of supraspinal μ receptor activation appeared not to occur in this mouse. Administration of i.c.v. U50,488H, 30 and 60 μg, at 15 min produced % MPE (± S.E.M.) of 22.9 (7.8) and 17.7 (7.0), respectively. This indicated that μ receptor mediated responsiveness was poor, suggesting that the descending serotonergic pathway might not be elicited in these mice.

Group C (κ Receptor Response): BALB/cByJ, AKR/J. The action of i.c.v. heroin was not inhibited by IT bicuculline but was inhibited by IT methysergide in both BALB/cByJ and AKR/J (Fig. 2). The antinociceptive action of heroin was also not inhibited by administration of yohimbine IT (Fig. 3).
Because, \( \mu \) receptor action is not always associated with the descending noradrenergic activation, lack of yohimbine effect did not necessarily rule out \( \mu \) receptor action in these strains. A 24-h pretreatment with 25 ng of i.c.v. \( \beta \)-FNA in BALB/cByJ was not effective (Fig. 3); thus, \( \mu \) receptors were not involved. This negative finding alerted us to the need to consider \( \kappa \) receptors, even though there was no hint previously that heroin could have \( \kappa \) agonist activity. Strikingly, i.c.v. administration of N-BNI was effective in both BALB/cByJ and AKR/J strains (Fig. 3). Therefore, heroin action was due to activation of \( \kappa \) receptors, which are associated with the descending serotonergic system. Note that in AKR/J, the dose of heroin was 6 \( \mu \)g i.c.v., compared with 3 \( \mu \)g in all the previous experiments. These mice were less sensitive to i.c.v. heroin. IT bicuculline inhibited the antinociceptive action of i.c.v. DAMGO (Table 1) in BALB/cByJ and AKR/J, indicating that \( \delta \) receptors and the descending GABA systems were present but not acted on by heroin.

**Intracerebroventricular Heroin ED\(_{50}\) Determinations and Effect of Opioid Antagonists.** The ED\(_{50}\) values and 95% confidence intervals derived for i.c.v. heroin alone or in the presence of the opioid antagonists for the six strains are given in Table 2. The dose-response curves are given in Fig. 4. These experiments give more directly focused results than those derived from the initial single-dose experiments.

**Group A.** Treatment with naltrindole shifted the dose-response curve of heroin in C57BL/6J mice to the right (Table 2 and Fig. 4). Twenty-four hour pretreatment with i.c.v. \( \beta \)-FNA or administration of N-BNI along with heroin did not

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**TABLE 1**

Antinociceptive response (in the tail-flick test) to standard agonists in the presence of vehicle or antagonist in different strains of inbred mice

<table>
<thead>
<tr>
<th>Strain</th>
<th>Agonist, route, dose</th>
<th>% MPE (S.E.M.)</th>
<th>Antagonist, route, dose</th>
<th>% MPE (S.E.M.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C57BL/6J</td>
<td>Morphine, i.c.v., 12 ( \mu )g</td>
<td>84.2 (5.0)</td>
<td>Methysergide, IT, 1 ( \mu )g</td>
<td>40.2 (10.8)(^a)</td>
</tr>
<tr>
<td>DBA/2J</td>
<td>DPDPE, i.c.v., 10 ( \mu )g</td>
<td>85.8 (7.1)</td>
<td>Bicuculline, IT, 1 ( \mu )g</td>
<td>15.5 (5.6)(^a)</td>
</tr>
<tr>
<td>CBA/J</td>
<td>Morphine, i.c.v., 4 ( \mu )g</td>
<td>89.8 (10.2)</td>
<td>Yohimbine, IT, 1 ( \mu )g</td>
<td>39.4 (8.9)(^a)</td>
</tr>
<tr>
<td>C3H/HeJ</td>
<td>DPDPE, i.c.v., 10 ( \mu )g</td>
<td>77.7 (8.7)</td>
<td>Bicuculline, IT, 0.5 ( \mu )g</td>
<td>37.5 (11.3)(^a)</td>
</tr>
<tr>
<td>BALB/cByJ</td>
<td>Morphine, i.c.v., 4 ( \mu )g</td>
<td>83.4 (9.4)</td>
<td>Bicuculline, IT, 0.5 ( \mu )g</td>
<td>56.9 (8.3)(^a)</td>
</tr>
<tr>
<td>AKR/J</td>
<td>DAMGO, i.c.v., 25 ( \mu )g</td>
<td>80.8 (8.0)</td>
<td>Methysergide, IT, 1 ( \mu )g</td>
<td>66.1 (16.1)</td>
</tr>
<tr>
<td></td>
<td>DPDPE, i.c.v., 10 ( \mu )g</td>
<td>72.2 (9.4)</td>
<td>Bicuculline, IT, 0.5 ( \mu )g</td>
<td>94.8 (5.2)</td>
</tr>
<tr>
<td></td>
<td>DPDPE, i.c.v., 10 ( \mu )g</td>
<td>73.3 (6.5)</td>
<td>Bicuculline, IT, 0.5 ( \mu )g</td>
<td>31.7 (8.1)(^a)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Methysergide, IT, 1 ( \mu )g</td>
<td>14.8 (4.2)(^a)</td>
</tr>
</tbody>
</table>

Eight mice were used in each group. i.c.v. and IT injection were made 10 and 5 min, respectively, before the tail-flick test.

\(^a\) Significantly different by Student’s \( t \)-test, \( P \leq .05 \), between the vehicle-treated control given the agonist versus group given agonist + antagonist.

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**Fig. 2.** The ability of IT bicuculline and methysergide (step 1) to inhibit i.c.v. heroin in six strains of inbred mice determined with the tail-flick test. Heroin, 3 \( \mu \)g (6 \( \mu \)g in AKR/J) was given i.c.v. to all groups 10 min before the tail-flick test. The other treatments given IT at 5 min before the tail-flick test are indicated vertically for each group. Heroin antinociception was attenuated by IT bicuculline only in C57BL/6J mice to implicate \( \delta \) receptors. IT methysergide inhibited heroin antinociception in CBA/J, DBA/2J BALB/cByJ, and AKR/J mice, suggesting either \( \mu \) or \( \kappa \) receptor mediation. Additional analyses were performed in the second step, next figure. The bars represent the mean % MPE, with the S.E.M. indicated by the vertical line at the top of the bar with the number of mice in each group given along side. * Indicates that the mean was significantly different from the control group mean using Dunnett’s test (more than one group compared with control); \( P \leq .05 \).
affect the ED$_{50}$ value. Thus, the response to heroin in C57BL/6J mice involved $\delta$ but not $\mu$ or $\kappa$ opioid receptors.

**Group B.** The ED$_{50}$ values and dose-response curves for heroin in DBA/2J and CBA/J mice indicated that 24-h $\beta$-FNA treatment inhibited the analgesic response of i.c.v. heroin (Table 2 and Fig. 4). N-BNI and naltrindole treatments had no effect. These two strains gave $\mu$ receptor responses to heroin. The results for the C3H/HeJ mice did not give a clear differentiation. Even though the data analysis in Table 2 indicated that $\beta$-FNA had a small but significant inhibitory
effect on heroin antinociception, it was not significantly different from the heroin response in the presence of N-BNI. Thus, these results gave weak support to the conclusion that C3H/HeJ mice were μ receptor responders to heroin.

**Group C.** Both the BALB/cByJ and AKR/J strains showed a robust inhibition of the heroin response by N-BNI (Table 2 and Fig. 4). Naltrindole and β-FNA treatments had no effect on the heroin ED₅₀ values. Thus, both strains were designated as δ receptor responders to heroin.

### Discussion

Previous findings in outbred mice suggest that genotype significantly influences the pharmacology of heroin-induced antinociception (Rady et al., 1991, 1994b). The current studies confirmed these findings and characterized the degree to which genotype governs opioid-receptor selectivity in standardized inbred mice. Because the inbred strains used in these experiments were studied under identical conditions (environments), differences observed in receptor selectivity and descending control mechanisms were due to genetic differences across individual strains. The initial single-dose experiments to determine descending pathways require cautious interpretation, particularly when an antagonist did not affect the heroin response. Larger doses of heroin might activate more than one system. When the MPE is near 100%, an antagonist selective for the low-dose effect of heroin might be ineffective because of the presence of the second action of heroin. Such concern is diminished in part if the results from the descending systems and the ED₅₀ determinations lead to the same conclusion. The results of both studies are discussed together.

#### δ Agonist Action: C57BL/6J.

In these mice, IT biculteline but not methysergide inhibited the response to heroin. This was consistent with i.c.v. heroin-activating supraspinal δ receptors to activate spinal GABA_A receptors. The robust shift in the heroin ED₅₀ value produced by i.c.v. naltrindole and lack of effect of N-BNI or β-FNA treatment provided direct evidence for a predominant δ receptor response. Additional differentiation with i.c.v. BNTX suggested that i.c.v. heroin acted on supraspinal δ₁ receptors. These findings are similar to those in Swiss Webster mice (Rady and Fujimoto, 1995; Rady et al., 1991).

#### μ Agonist Action: CBA/J, DBA/2J, C3H/HeJ.

The antinociceptive responses to i.c.v. heroin in these three inbreds were ascribed predominantly to supraspinal μ receptor involvement. The descending system could be serotonergic (DBA/2J), noradrenergic (C3H/HeJ), or both (CBA/J). Different μ agonists show heterogeneity for activation of serotonergic and noradrenergic systems (Arts et al., 1991). Even though IT yohimbine inhibited i.c.v. heroin-induced analgesia in C3H/HeJ mice, the μ receptor assignment was weakened because the shift of the heroin ED₅₀ value produced by β-FNA was very small. Increasing the dose of β-FNA did not improve this result (data not given). The ED₅₀ results for both of the other two strains agreed with the findings of the single-dose studies.

A caveat not covered in these experiments is that heroin acts on a different μ receptor than does morphine and DAMGO. The analgesic action of morphine can be differentiated from heroin in mice with deletion of different exons in the μ receptor (Schuller et al., 1997). Also, in normal mice, 3-methoxynaltrexone inhibits heroin-induced analgesia without affecting other μ agonists (Brown et al., 1997). The use of 3-methoxynaltrexone in C3H/HeJ may be more revealing than β-FNA.

**κ Agonist Action: AKR/J, BALB/cByJ.** Heroin antinociception was inhibited by IT methysergide in both strains. Intracerebroventricular N-BNI shifted the dose-response curve of heroin well to the right, while i.c.v. β-FNA and naltrindole had no effect. Because the tail-flick test is generally not sensitive to κ agonists (Taber, 1974), obtaining analgesia for i.c.v. heroin as κ receptor responses was unusual. An ED₅₀ value of 2 μg (about 5 nmol) suggests that heroin is about 4 times more potent than U50,488H as a κ agonist. Because κ agonists have dysphoric effects, it would be of interest to determine if the κ agonist analgesic action of heroin were not limited by dysphoric actions.

These groupings for heroin receptor selectivity are consistent with the ancestry of the strains (Fox and Witham, 1997). The μ responders (DBA/2J, CBA/J, and C3H/HeJ) are closely related (Little’s mice), while the δ responder (C57 BL/6J) arose separately from Lathrop’s albino, and the κ responders (BALB/cByJ and AKR/J) originated from Bagg’s albino and Forth’s A&R stocks, respectively.

Even though only single opioid receptor action was evaluated here, heroin could be acting simultaneously on more than one opioid receptor (as on multiple descending pathways). One way to uncover multiple receptor actions is to inhibit the major action and then test for the minor action as done for morphine (Takekori and Portoghese, 1987). A related consideration was the apparent “all or nothing” nature of the opioid receptor selectivity of heroin. This consideration becomes a major factor when different strains are cross-bred to evaluate the mode of inheritance of heroin receptor selectivity. If there is phenotypic expression of two receptor responses to heroin in the same mouse, quantification of the

### Table 2

<table>
<thead>
<tr>
<th>Strain</th>
<th>i.c.v. heroin</th>
<th>+β-FNA*</th>
<th>+Naltrindole*</th>
<th>+N-BNI*</th>
</tr>
</thead>
<tbody>
<tr>
<td>C57BL/6J</td>
<td>1.2 (0.6–2.2)</td>
<td>1.4 (0.8–2.5)</td>
<td>5.5 (3.1–10)</td>
<td>1.9 (1.1–3.3)</td>
</tr>
<tr>
<td>CBA/J</td>
<td>0.5 (0.2–1.2)</td>
<td>1.5 (1.1–2.1)</td>
<td>0.4 (0.2–0.9)</td>
<td>0.7 (0.4–1.0)</td>
</tr>
<tr>
<td>DBA/2J</td>
<td>0.7 (0.3–1.5)</td>
<td>2.6 (1.3–5.1)</td>
<td>0.4 (0.2–1.0)</td>
<td>0.8 (0.3–2.3)</td>
</tr>
<tr>
<td>C3H/HeJ</td>
<td>0.8 (0.4–1.3)</td>
<td>1.5 (1.2–2.0)</td>
<td>0.8 (0.5–1.5)</td>
<td>1.3 (1.0–1.7)</td>
</tr>
<tr>
<td>BALB/cByJ</td>
<td>1.9 (1.1–3.2)</td>
<td>1.9 (1.1–3.3)</td>
<td>1.9 (1.1–3.2)</td>
<td>9.5 (4.3–21)</td>
</tr>
<tr>
<td>AKR/J</td>
<td>2.1 (1.0–5.4)</td>
<td>2.6 (1.5–4.7)</td>
<td>2.8 (1.6–4.8)</td>
<td>7.7 (5.9–10.1)</td>
</tr>
</tbody>
</table>

* Indicates significant difference from heroin alone (P ≤ .05) according to comparison of ED₅₀ values, as in Methods.
receptor responses will be required. In the present results, examination of the control ED_{50} values for i.c.v. heroin suggests a rank order (although not significant) where the μ responders were the most sensitive, the δ responder was intermediate, and the κ responders were the least sensitive. This ordering might affect the effectiveness of each of the antagonists used.

Differential agonist selectivity may be a function of a) qualitative or quantitative differences in the opiate receptors, b) regional differences in the brain B_{max}, c) genetic differences in the intrinsic activity of the receptors in stimulating second messenger systems, d) genotype-dependent structural differences in the receptors, or e) some combination of these factors. The limited testing of standard agonists in the present study, as well as in previous work (Elmer et al., 1995a,b; 1996; 1998; Elmer et al., unpublished observations), and a review of the literature suggest that the six strains used do not lack any of the specific opioid receptors and that the opioid receptor selectivity of heroin response is not correlated with the strength of response to other standard agonists. Quantified differences in receptor parameters reported for standard opioids are considered now for these strains. Most of the work involves the hot-plate test (exceptions are noted) and are generally systemic rather than i.c.v. administration.

High affinity δ receptor binding is greater in the cortex of C57BL/6J than DBA/2J mice (Yukhananov et al., 1994). High and low affinity binding of [3H]DSLET (l-Ser^2, Leu^5)-enkephalin-Thr, a δ agonist) is present in C57BL/6J mice, but is absent in DBA/2J mice. Having high affinity binding for δ receptors in combination with low affinity binding for μ receptors in C57BL/6J mice may be sufficient for heroin to have δ, rather than μ, agonist action. However, high affinity binding of [3H]DSLET likely involves δ_{2} rather than the δ_{1} receptor subtype, while in the present study, the antinociceptive action of heroin was through δ_{1} receptors. Thus, the literature does not suggest a reason for the differential action of heroin on δ receptors in C57BL/6J mice.

Quantitatively, the argument for μ receptor selectivity for heroin would be based on high affinity or on capacity for μ receptors over other receptors. In a review, Frischknecht et al. (1988), present a comparison of the effects of morphine between C57BL/6J and DBA/2J mice. DBA/2J mice are more sensitive to the antinociceptive effect of systemic and i.c.v. morphine than are C57BL/6J mice (Castellano and Olivero, 1975; Gwynn and Domino, 1984; Belknap et al., 1989; Sennova et al., 1995; Elmer et al., 1998). In the abdominal constriction test there is no difference (Brase et al., 1977). Morphine, presumably acting on μ receptors, is more potent in BALB/c and CBA mice than in C57BL/6J mice (Castellano and Olivero, 1975). However, C3H/HeJ mice are less sensitive to the antinociceptive effect of systemic and i.c.v. morphine than are C57BL/6J mice (Castellano and Olivero, 1975; Gwynn and Domino, 1984; Belknap et al., 1989; Sennova et al., 1995; Elmer et al., 1998). In the abdominal constriction test there is no difference (Brase et al., 1977). Morphine, presumably acting on μ receptors, is more potent in BALB/c and CBA mice than in C57BL/6J mice (Castellano and Olivero, 1975). However, C3H/HeJ mice are less sensitive to the antinociceptive effect of systemic and i.c.v. morphine than are C57BL/6J mice (Castellano and Olivero, 1975; Gwynn and Domino, 1984; Belknap et al., 1989; Sennova et al., 1995; Elmer et al., 1998). In the abdominal constriction test, κ agonists (U50,488H, trifluadom, and bremazocine) are more potent in BALB/c than in C57BL/6J mice (Brase et al., 1977), while C57BL/6J and DBA/2J have similar sensitivity to ethylketocyclazocine, another κ receptor agonist, in the hot-plate test (Gwynn and Domino, 1984). AKR mice have low sensitivity to morphine (Elmer et al., 1998), which might contribute to increased sensitivity to the κ agonist action of heroin. However, a robust κ receptor response to heroin occurs in BALB/cBy mice, which are sensitive to morphine (Elmer et al., 1998). The current literature cannot explain the κ receptor selectivity of heroin in BALB/cByJ and AKR/J.

Characterization of the molecular and pharmacological mechanisms that account for differential agonist selectivity across these genotypes remains to be done. These studies clearly establish the large degree to which genotype is a determinant of the pharmacological actions of heroin, and they provide a system to further analyze the biochemical and molecular mechanisms underlying agonist receptor selectivity.

References


Rady JJ, Aksu F and Fujimoto JM (1994a) The heroin metabolite, 6-monoacetylmorphine, activates delta opioid receptors to produce antinociception in Swiss Webster mice. J Pharmacol Exp Ther 266:1222–1231.


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