Pharmacogenetic Evidence for the Involvement of 5-Hydroxytryptamine (Serotonin)-1B Receptors in the Mediation of Morphine Antinociceptive Sensitivity

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ABSTRACT

Morphine antinociception has been shown to be influenced significantly by genetic factors, now beginning to be identified in mice. A recent quantitative trait locus analysis revealed a significant statistical association between morphine antinociceptive magnitude and a region of mouse chromosome 9. This region contains the Htr1b gene, which encodes the 5-hydroxytryptamine (serotonin)-1B (5-HT1B) receptor subtype. To investigate the possibility that Htr1b represents the quantitative trait locus, C57BL/6 and DBA/2 inbred strains, the progenitors of the original quantitative trait locus mapping populations, were administered a novel 5-HT1B receptor antagonist (GR127935) concomitant with morphine. These mice are known to differ in morphine antinociceptive sensitivity on thermal pain assays (DBA/2 high; C57BL/6 low). GR127935 caused a dose-dependent antagonism (both reversal and prevention) of morphine antinociception in DBA/2 mice but had no effect in C57BL/6 mice. However, a 5-hydroxytryptamine-1A subtype (5-HT1A) receptor agonist, 8-hydroxydipropylaminotetralin, reversed morphine antinociception equally in the two strains. DBA/2 mice also exhibited significantly greater antinociception than did C57BL/6 mice from the administration of a 5-HT1B agonist, CGS12066. These data collectively support a role for 5-HT1B receptors in the mediation of morphine antinociception and support the contention that polymorphisms in the Htr1b gene may underlie individual differences in morphine sensitivity.

The antinociceptive effects of morphine differ widely among individuals (Lasagna and Beecher, 1954; Levine et al., 1981). Evidence in animal models suggests that much of this variability is genetic in origin. For instance, large differences in morphine antinociceptive magnitude have been observed in genotypically distinct mouse populations. Inbred strains such as C57BL/6 and DBA/2 mice consistently display low and high antinociception, respectively, when injected with morphine (see Belknap and O'Toole, 1991; Mogil et al., 1996a for reviews). Similarly, the BXD/Ty series of recombinant inbred (RI) strains created from C57BL/6 and DBA/2 progenitors differ widely in their morphine antinociceptive sensitivity on the hot-plate test, with the heritability estimated at $h^2 = 0.44$ (Belknap et al., 1995). Although these findings indicate that morphine antinociception has genetic determinants, the genes influencing this trait are just beginning to be identified.

Quantitative trait locus (QTL) analysis is a technique aimed at mapping genes affecting quantitative or continuously distributed traits to broad chromosomal regions. QTL analyses search for significant statistical associations, or linkage, between quantitative trait variation and allelic variation at mapped DNA marker loci. If significant associations are found, the implication is that the QTL is located in the same chromosomal region as the marker and that one or more genes in that region are affecting the trait of interest (Belknap et al., 1995). A recent QTL analysis of BXD/Ty RI strains and (C57BL/6 × DBA/2)/F$_2$ hybrids conducted in our laboratory yielded two chromosomal regions significantly associated with morphine antinociception on the hot-plate test of thermal nociception. The first is the Mpmv5/D10Mit51 region [0–20 centiMorgans (cM)], which accounts for the highest percentage of the genetic variance (28–33%; Belknap et al., 1995). This region also contains the Oprm gene (7 cM) that encodes the µ-opioid receptor, making it an excellent candidate gene for the mapped QTL. The results of pharmacological and transgenic studies have provided ample support for the crucial role of µ-opioid receptors in the mediation of antinociceptive effects.

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ABBREVIATIONS: RI, recombinant inbred; QTL, quantitative trait locus; 5-HT1B, 5-hydroxytryptamine (serotonin)-1B subtype; 5-HT1A, 5-hydroxytryptamine 1A subtype; 8-OH-DPAT, 8-hydroxydipropylaminotetralin; %MPE, percentage of the maximum possible effect; AUC, area under the curve; AD$_{50}$, half-maximal antinociceptive dose.
morpheine’s multiple biological actions (Pasternak, 1993; Matthes et al., 1996; Sora et al., 1997).

However, other genetic factors contribute importantly to morphine sensitivity. This fact is evidenced by the lack of a significant correlation (r = 0.33) between morphine inhibition of thermal (hot-plate test) and chemical (acetic acid abdominal constriction) nociception in inbred mice (see also Mogil et al., 1996b; Elmer et al., 1998). If µ-opioid receptor function were the only relevant factor in morphine antinociception, strains sensitive to morphine on one assay would necessarily be sensitive on another assay. Yet this is not the case. Our attention has turned, therefore, to the chromosomal region accounting for the next highest percentage of genetic variation (18%) in morphine antinociception, on chromosome 9 (40–60 cM). A gene in this region, Htr1b (46 cM), encodes the 5-hydroxytryptamine (serotonin)-1B (5-HT1B) receptor subtype, which has been implicated in mediating effects of serotonin on opioid nociceptive processing (Crisp et al., 1991a). Given these data, Htr1b is an attractive candidate gene for the morphine antinociceptive QTL on chromosome 9.

The recent development of a highly specific 5-HT1B antagonist, GR127935 (Clitherow et al., 1994), has allowed the evaluation of Htr1b as a candidate gene for morphine antinociception. Because most of the existing ligands for the 5-HT1B receptor bind with varying affinities to the 5-hydroxytryptamine 1A subtype (5-HT1A receptor; see Hoyer et al., 1994 for review), also implicated in pain modulation mechanisms, this antagonist could distinguish the roles of each receptor subtype. If C57BL/6 and DBA/2 strains exhibit low and high morphine antinociception, respectively, caused by actions at 5-HT1B receptors and not 5-HT1A receptors, a 5-HT1A agonist, 8-hydroxydipropylaminotetralin (8-OH-DPAT), to produce differential effects of 5-HT1B receptor antagonist on 5-HT1A receptor function were the only relevant factor in morphine antinociception between strains. The ability of GR127935 to both reverse and prevent the development of morphine antinociception was thus evaluated. To further confirm that any attenuation of morphine antinociception was caused by actions at 5-HT1B receptors and not 5-HT1A receptors, a 5-HT1A agonist, 8-hydroxydipropylaminotetralin (8-OH-DPAT), was administered to examine its effects on morphine antinociception in the two strains. Finally, we investigated whether an agonist with considerable selectivity for 5-HT1B receptors, CGS12066 (Neale et al., 1987), would produce differential antinociception by itself in these two strains.

5-HT1B receptors have been implicated in both supraspinal and spinal mechanisms of antinociception (Crisp et al., 1991b; Alhaider and Wilcox, 1993). Thus, although the chromosome 9 QTL was identified by the hot-plate test of nociception, which involves a supraspinally organized behavioral response to thermal stimulation, the present studies were conducted with the reflexive tail-withdrawal assay.

### Materials and Methods

**Subjects.** C57BL/6 and DBA/2 mice were bred in a Veterans Affairs Medical Center colony room or in the vivarium of author J.S.M. at the University of Illinois. Both sexes were used in all experiments. No significant interactions of sex with drug were observed in any case; therefore, data from both sexes were combined for all reported analyses. Animal rooms were maintained on a 12-h light/dark cycle with lights on at 6:00 AM. Mice were housed in polyurethane boxes in same-sex groups of two to five, with ad libitum access to laboratory rodent diet (PMI Feeds, Inc. or Purina chow) and water. In all experiments, mice were tested in genotype- and dose-matched pairs.

**Drugs.** Morphine sulfate was acquired from the Research Technology Branch, National Institute of Drug Abuse (Bethesda, MD). GR127935 was a generous gift from GlaxoWellcome (Hertfordshire, UK). CGS12066 was obtained from Research Biochemicals, Inc. (Natick, MA). 8-OH-DPAT was purchased from Research Biochemicals, Inc. and Torciss Cookson Inc. (Ballwin, MO). All drugs were dissolved in physiological saline, and delivered in a volume of 10 μl/kg.

**Tail-Withdrawal Test.** A modified version of the assay described by Janssen et al. (1983) was used. Mice were placed in a restrainer made of cloth and cardboard for testing. Baseline sensitivity was assessed by placing the distal half of the tail into a 49 ± 0.2°C water bath and recording the latency to vigorous, escape-directed tail withdrawal. Two such measurements were made and averaged. An i.p. injection of drug followed within 15 s, and retesting occurred as described below. A cut-off latency of 15 s was used in all cases.

**Effects of 5-HT1B Receptor Antagonism on Prevention of Morphine Antinociception in C57BL/6 and DBA/2 Mice.** Morphine dose-response curves on the tail-withdrawal test were constructed with these strains in the presence and absence of GR127935. After assessment of baseline latencies, mice received either saline or GR127935 (10 mg/kg, s.c.), followed by one of the following doses of morphine (0, 1, 5, or 10 mg/kg i.p.) 15 min later. Tail-withdrawal latencies were measured 20, 40, and 60 min after injection of the morphine.

**Effects of 5-HT1B and 5-HT1A Receptor Ligands on Reversal of Morphine Antinociception in C57BL/6 and DBA/2 Mice.** In a separate experiment, the ability of GR127935 and 8-OH-DPAT to reverse existing morphine antinociception was tested. After baseline testing, mice of the C57BL/6 and DBA/2 strains were administered separate doses of morphine (10 and 5 mg/kg i.p., respectively) that were found in the previous experiment to produce equipotent antinociception in these two strains. All mice were retested at 20 min after injection to confirm the presence of morphine antinociception in each subject. Immediately thereafter, mice were administered GR127935 (10 mg/kg s.c.), 8-OH-DPAT (1 mg/kg s.c.), or saline, and tested again for noxious sensitivity 15 min later.

**Effects of 5-HT1B and 5-HT1A Receptor Agonists in C57BL/6 and DBA/2 Strains.** The ability of a 5-HT1b-selective agonist, CGS12066, and a 5-HT1A-selective agonist, 8-OH-DPAT, to produce antinociception in these strains was evaluated. The tail-withdrawal assay was used because this test is not confounded by motor dysfunction caused by high doses of CGS12066 and 8-OH-DPAT. Doses ranged from 25 to 75 mg/kg (i.p.) for CGS12066 and 5 to 15 mg/kg (i.p.) for 8-OH-DPAT, and each subject received only one dose. Mice were retested at 20, 50, 120, and 240 min after injection.

**Statistical Analyses.** Percent maximum possible effect (%MPE) scores were calculated as [(postinjection latency − baseline latency) / (cutoff latency − baseline latency)] × 100. This transformation takes into account and compensates for the strain difference in baseline latencies. For experiments with multiple postinjection latency determinations, areas under the time × latency curve (AUCs; min × s) were calculated according to the trapezoidal rule. %MPEs were then calculated by comparing the obtained AUC to the maximal AUC that would be obtained from a subject displaying cut-off tail-withdrawal latencies (>15 s) at all postinjection time points. Half-maximal antinociceptive dose (AD50) estimates and corresponding 95% confidence intervals were calculated by using linear regression of %MPE scores at each dose (SYSTAT 7.0; SPSS Inc., Evanston, IL). For the experiments investigating the reversal of morphine, raw latency scores were used because different drugs were administered at the second time point, precluding calculations of AUC. ANOVA was used to determine whether differences existed between doses and strains, followed by t tests where appropriate. All statistical tests were two-tailed and used a criterion for significance of p < .05.
Dunnett’s post hoc tests were used to determine whether GR127935 and 8-OH-DPAT, separately, reversed morphine antinociception, compared with saline controls.

**Results**

**Effects of 5-HT1B Antagonism on Prevention of Morphine Antinociception in C57BL/6 and DBA/2 Mice.** GR127935 had no significant effect by itself on baseline tail-withdrawal latencies in either strain (data not shown). A three-way ANOVA performed on morphine %MPE data revealed significant main effects of strain ($F_{1,51} = 7.54, p < .01$), morphine dose ($F_{2,51} = 36.68, p < .001$), and GR127935 dose ($F_{1,51} = 5.45, p < .05$); the three-way interaction approached significance ($F_{2,51} = 3.05, p = 0.056$). As shown in Fig. 1, saline-treated DBA/2 mice exhibited significantly increased morphine + saline antinociception relative to C57BL/6 mice at all doses (1 mg/kg: $t = 4.34, df = 7, p < .005$; 5 mg/kg: $t = 2.42, df = 8, p < .05$; 10 mg/kg: $t = 4.73, df = 7, p < .005$). Whereas the morphine + GR127935 dose-response curve in C57BL/6 mice was essentially unchanged from that of morphine + saline, concurrent administration of morphine + GR127935 to DBA/2 mice resulted in a robust and significant decrease in antinociceptive magnitude at the 5 mg/kg ($t = 2.96, df = 10, p < .05$) and 10 mg/kg ($t = 4.52, df = 10, p < .001$) doses. In this strain, the AD$_{50}$ of morphine was shifted to the right by a factor of 4.0 by GR127935 (see Table 1). Intriguingly, in the presence of GR127935, the AD$_{50}$ displayed by both strains were equivalent.

**Effects of 5-HT1B and 5-HT1A Agents on Reversal of Morphine Antinociception in C57BL/6 and DBA/2 Mice.** Fig. 2 illustrates the reversal of morphine antinociception by GR127935 in DBA/2 but not C57BL/6 mice. Morphine antinociception was reversed in both strains, however, by 8-OH-DPAT. A two-way (strain × condition) ANOVA performed on raw latency data revealed an expected significant main effect of strain on baseline latencies ($F_{1,29} = 37.14, p < .001$). Both strains displayed equivalent tail-withdrawal latencies at 20 min postmorphine (from 2-fold different morphine doses, however). A significant main effect of condition ($F_{2,29} = 5.33, p < .05$) and significant interaction of strain × condition ($F_{2,29} = 5.83, p < .01$) were revealed at 35 min after morphine injection. One-way Dunnett’s tests show that morphine antinociception continued to develop in C57BL/6 mice despite GR127935 administration ($t = 2.59, df = 15, N.S.$), but this manipulation reversed morphine antinociception in DBA/2 mice ($t = -4.43, df = 14, p < .05$) at 35 min after morphine injection, compared with saline controls. In contrast, an injection of 8-OH-DPAT reversed morphine antinociception in both C57BL/6 ($t = -3.48, df = 15, p < .05$) and the DBA/2 mice ($t = -4.51, df = 14, p < .05$), compared with saline controls.

**Effects of 5-HT1B and 5-HT1A Agonists in C57BL/6 and DBA/2 Mice.** As with morphine, DBA/2 mice exhibited an increased antinociceptive response from the 5-HT$_{1B}$ agonist, CGS12066, relative to C57BL/6 mice (see Fig. 3). A significant main effect of dose ($F_{3,40} = 12.37, p < .001$) and strain ($F_{1,40} = 5.48, p < .05$) were revealed by ANOVA. The AD$_{50}$ values were compared (DBA/2: 64.5 mg/kg; C57BL/6: 133.1 mg/kg), and a significant difference between strains was found ($t = 16.42, df = 4, p < .001$).

The 5-HT$_{1A}$ agonist did not produce analgesia in either the C57BL/6 or the DBA2 strain (data not shown). ANOVA revealed an expected significant difference of baseline latencies ($F_{1,29} = 69.35, p < .001$), but no significant effect of dose.

**TABLE 1**

<table>
<thead>
<tr>
<th>Strain</th>
<th>Drug</th>
<th>AD$_{50}$</th>
<th>Potency Ratio</th>
</tr>
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<tbody>
<tr>
<td></td>
<td></td>
<td>mg/kg</td>
<td></td>
</tr>
<tr>
<td>C57BL/6</td>
<td>Saline</td>
<td>7.3 ± 2.4</td>
<td></td>
</tr>
<tr>
<td></td>
<td>GR127935</td>
<td>5.5 ± 2.6</td>
<td>0.8</td>
</tr>
<tr>
<td>DBA/2</td>
<td>Saline</td>
<td>2.0 ± 3.6*</td>
<td></td>
</tr>
<tr>
<td></td>
<td>GR127935</td>
<td>7.8 ± 3.4</td>
<td>3.9**</td>
</tr>
</tbody>
</table>

*p < 0.05; **p < 0.001; ***p < 0.001; N.S. = not significant.

a Calculated as GR127935 AD$_{50}$/Saline AD$_{50}$.

b See text for calculation.
Serotonin and Antinociception. Several studies have examined the role of serotonin in nociceptive and antinociceptive processing, both at the spinal and supraspinal level (for reviews see Hamon et al., 1990; Richardson, 1990). When injected intrathecally, serotonin by itself produces antinociception (Yaksh and Wilson, 1979; Solomon and Gebhart, 1988; Bardin et al., 1997). 5-HT1A and 5-HT1B receptors are the two most likely serotonin receptor subtypes involved (Fasmer et al., 1986; Eide et al., 1990; Schlicker, 1992; Xu et al., 1994). 5-HT1B receptor agonists have been demonstrated to cause antinociception at the spinal level (Alhaider and Wilcox, 1993; Ali et al., 1994; but see Solomon and Gebhart, 1988). A variety of actions have been documented concerning 5-HT1A receptor agonists at the spinal level, including antinociception (Eide et al., 1990; Xu et al., 1994), hyperalgesia (Crisp et al., 1991a; Alhaider and Wilcox, 1993; Ali et al., 1994), and no effect on nociceptive responding (Fasmer et al., 1986; Solomon and Gebhart, 1988). The 5-HT1A receptor agonist 8-OH-DPAT at moderate doses (5 or 10 mg/kg) did not produce antinociception in the C57BL/6 or DBA/2 mice in our hands (data not shown). Higher doses (15 or 20 mg/kg) caused tremors, which precluded using the tail-withdrawal test. Millan (1994) contends the antinociceptive action seen with 8-OH-DPAT, a 5-HT1A receptor agonist commonly used in these studies, is actually caused by an adrenergic component, because this compound also binds with moderate affinity to the α-adrenoreceptor.

Fig. 3. CGS12066 dose-response curves for DBA/2 and C57BL/6 mice on the tail-withdrawal assay. Symbols represent mean %MPE scores (calculated from AUC) ± S.E. A significant main effect of dose (p < .001) and strain (p < .05) was revealed.

Discussion

The present data provide multiple lines of pharmacogenetic evidence that 5-HT1B receptors can modulate morphine antinociception. The 5-HT1B receptor antagonist, GR127935, significantly attenuated morphine antinociception in DBA/2 inbred mice but not C57BL/6 mice. Likewise, the 5-HT1B receptor agonist, CGS12066, had significantly greater antinociceptive activity in DBA/2 mice than in C57BL/6 mice. The 5-HT1A receptor agonist, 8-OH-DPAT, in contrast, did not differentially reverse morphine antinociception in the two inbred strains. These data suggest, therefore, that the divergent morphine antinociception exhibited by these strains is at least partially mediated by differences in 5-HT1B receptor function, presumably the result of differences in the Htr1b gene sequence or expression.
whereas 5-HT$_{1A}$ agonists and partial agonists have been shown to attenuate morphine antinociception (Berge et al., 1985; Millan and Colpaert, 1990, 1991). Results in the present study, in which 8-OH-DPAT had no effect by itself but attenuated morphine antinociception in both the C57BL/6 and DBA/2 mice, seem to support these studies. A mixed 5-HT$_{1A/1B}$ antagonist, pindolol, has been shown to attenuate morphine antinociception as well (Crisp et al., 1991a). However, the ligands used in some of these studies were not particularly selective, making it difficult to discern the specific role of the two receptor subtypes. To our knowledge, the present findings that the 5-HT$_{1B}$ receptor antagonist, GR127935, blocks and reverses morphine antinociception, are the first to suggest that selective antagonism of this receptor subtype inhibits the antinociceptive properties of morphine. Now that more selective agents are available, more work can and should be done in this area.

Is the Htr1b Gene the Morphine Antinociception QTL on Chromosome 9? QTL analysis of morphine antinociception on the hot-plate test in BXD/Ty RI strains and an F$_2$ population derived from their progenitor strains (DBA/2 and C57BL/6) revealed a QTL in the middle of chromosome 9 (30–50 cM) (Belknap and Crabbe, 1992). This QTL accounts for only a small amount of genetic variance (18%) in the hot-plate assay; the present results suggest that the genetic variance accounted for by this QTL (and, presumably, by the Htr1b gene) for morphine antinociception might be greater in the spinally mediated tail-withdrawal assay.

QTL analysis can distinguish a chromosomal area of about 10 to 20 cM, but not an individual gene, as related to a trait. The Htr1b gene is one possibility of many in the QTL region on chromosome 9. Our results strongly suggest that Htr1b is, in fact, the gene affecting morphine antinociception and represents this QTL. However, more studies are needed to confirm this. For instance, congenic strains, in which a genomic region from one strain is introgressed onto the background of the other, could resolve this issue. In fact, congenic strains with sections of the C57BL/6 chromosome 9 genome placed on a DBA/2 background are presently under development in the Belknap laboratory. If congenic strains with different or overlapping sections of the C57BL/6 genome are compared, the area containing the QTL affecting morphine antinociception may be narrowed. This could lead to confirmation or refutation of the contention that Htr1b is the gene affecting morphine antinociceptive magnitude. In any case, the present studies provide compelling evidence that 5-HT$_{1B}$ receptors can play a role in the modulation of morphine antinociception.

Clinical Applications. The rodent 5-HT$_{1B}$ receptor is the species homologue of the human 5-HT$_{1D}$ receptor, with approximately 95% homology (Adham et al., 1991; Maroteaux et al., 1992). Currently, selective 5-HT$_{1B/1D}$ receptor agonists such as sumitranipt and zolmitraprant, are widely used for the treatment of migraine headaches (Matthew, 1997). Sumatriptan has been shown to induce antinociception in mice on the hot-plate and abdominal constriction assays (Ghelardini et al., 1996). Both that study and the experiments described here suggest that selective 5-HT$_{1B/1D}$ receptor agonists could be useful for other types of pain, either alone or as adjuncts to standard opioid therapies.

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