Comparison of \(^{3}H\)Glyburide Binding with Opiate Analgesia, Tolerance, and Dependence in ICR and Swiss-Webster Mice

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ABSTRACT

Our laboratory demonstrated that morphine exhibits a modulatory control over the glyburide-binding site (sulfonylurea receptor) of the ATP-gated \(K^+\) channel. This study evaluated the effect of chronic morphine administration on the sulfonylurea receptor during tolerance and physical dependence. ICR and Swiss-Webster mice were rendered tolerant to morphine by pellet implantation and were withdrawn by pellet removal. Alterations in the \(B_{\text{max}}\) and \(K_D\) were evaluated in mouse spinal cord using the radiolabeled ATP-gated \(K^+\) channel blocker glyburide. The \(B_{\text{max}}\) for Swiss-Webster mice shifted from 13 to 451 mg/kg and thus they were more tolerant to morphine than ICR mice (\(ED_{50}\) shift from 12 to 120 mg/kg). Swiss-Webster mice were also dependent to morphine only when the morphine pellet was in place, unlike ICR mice, which were dependent for 48 h after morphine pellet removal. Glyburide binding increased during chronic morphine treatment in Swiss-Webster mice by over 2-fold (from 294 to 635 fmol/mg of protein). This was not observed in ICR mice. In Swiss-Webster mice, chronic morphine treatment also significantly increased the \(K_D\) by 3-fold (from 0.38 to 1.1 nM), whereas there was no change in affinity for ICR mice. Both strains of mice remained tolerant for 2 days after spontaneous withdrawal from morphine. However, the only increases in the \(B_{\text{max}}\) and \(K_D\) of glyburide were observed in Swiss-Webster mice that were highly tolerant to morphine. These results indicate that a high degree of tolerance is needed to alter ATP-gated potassium channels.

Opioids produce analgesia and have been used for thousands of years to treat acute pain. However, opioids such as morphine are limited in their function due to the development of tolerance and physical dependence. The goal of this article is to evaluate the alterations that occur at the K\(_{\text{ATP}}\) channel during morphine tolerance and physical dependence to ultimately determine whether openers of K\(_{\text{ATP}}\) channels could be useful analgesic agents in patients who have undergone morphine therapy. Ocan˜a et al. (1995) evaluated the effects of the K\(_{\text{ATP}}\) opener cromakalim administered (i.c.v.) on the enhancement of the antinociceptive effects of various opioids, including morphine. They concluded that openers of the K\(_{\text{ATP}}\) channel enhance the analgesic effects of opiates. There is no cross-tolerance between morphine and potassium channel openers or displacement in receptor binding studies, suggesting that the interaction between opioids and K\(_{\text{ATP}}\) openers is indirect (Welch and Dunlow, 1993). These data suggest K\(_{\text{ATP}}\) openers may be useful during morphine tolerance to produce antinociception.

Morphine produces antinociception by binding to the \(\mu\)-opioid receptor and inhibiting cAMP, which decreases cAMP-dependent protein kinase activation via Gi/o proteins. Decreasing cAMP-dependent protein kinase activation decreases calcium entry via phosphorylation-sensitive voltage-gated channels and thus contributes to morphine-induced antinociception (Olson and Welch, 1991; Bernstein and Welch, 1995). It has been shown that acute morphine administration opens potassium channels (Werz and MacDonald, 1985; North et al., 1987; Aronsen, 1992; North, 1992) producing cellular hyperpolarization resulting in decreased calcium entry (Adamson et al., 1987; Olson and Welch, 1991). Conversely, chronic morphine administration leads to a decrease in the amount of potassium that leaves the cell. The cell does not become hyperpolarized, calcium is allowed to enter (Williams et al., 1982; Werz and MacDonald, 1985; North et al., 1987; Triggle, 1990; Aronsen, 1992), and neurotransmitters are again released (Lavidis, 1995). This series of cellular events contributes to morphine tolerance.

Openers of potassium channels produce antinociception that is attenuated by opiate antagonists, suggesting they release endogenous opioids. In 1993 Welch and Dunlow administered the K\(_{\text{ATP}}\) openers minoxidil, lemakalim, and dia-

ABBREVIATIONS: K\(_{\text{ATP}}\), adenosine 5'-triphosphate-gated potassium channel; i.t., intrathecal; M6G, morphine 6-\(\beta\)-D-glucuronide; %MPE, percentage of the maximum possible effect; MOR-1, \(\mu\)-opioid receptor-1.
zoxide (i.t.), showing they produced antinociception that was attenuated by the $K_{ATP}$ blocker glyburide, as well as the opiate antagonists naloxone (s.c.) and ICI 174,864. Glyburide (i.t.) has been shown to produce a withdrawal syndrome in morphine-tolerant mice (Welch and Dunlow, 1993), as well as a partial antagonism of morphine-induced antinociception (Ocaña et al., 1990; Welch and Dunlow, 1993). Raffa and Martinez (1995) showed that the supraspinal administration of glyburide also produces a rightward shift in the dose-response curves of morphine and methadone. Studies evaluating the effect of glyburide on other opioid receptors indicate that glyburide administered (i.e.v.) not only antagonizes $\mu$-agonists but also the $\delta$-agonist [D-Pen$_2$,D-Pen$_5$]-enkephalin, implying $K_{ATP}$ channel involvement in the antinociceptive effect of $\delta$- and $\mu$-opioid receptors (Wild et al., 1991).

The following experiments were undertaken to assess whether morphine tolerance and dependence alter ATP-gated potassium channels. Using two strains of mice shown to vary in the metabolism of opioids, studies were performed to determine 1) how the number of glyburide-binding sites (sulfonyleurea receptors) change during morphine tolerance and dependence; 2) how these receptor changes correlate with the time course of tolerance and dependence; and 3) what the possible mechanism underlying the difference in morphine potency between ICR and Swiss-Webster mice may be. Previous studies in our laboratory have shown that Swiss-Webster mice chronically treated with morphine show an increase in the $B_{\text{max}}$ and $K_D$ of $[^3\text{H}]$glyburide binding in the brain and spinal cord (Welch et al., 1997). The $K_D$ in the brain increased by 167% and the $B_{\text{max}}$ increased 63%. In the spinal cord, the $K_D$ increased by 193%, whereas the $B_{\text{max}}$ increased by 238%. Welch et al. (1997) evaluated the effect of glyburide during morphine tolerance, but not physical dependence.

We determined the ED$_{50}$ values in ICR and Swiss-Webster mice using morphine and methadone, two well studied $\mu$-agonists, as well as morphine-6-$\beta$-d-glucuronide (M6G), an active metabolite of morphine. Methadone was evaluated to determine whether the strain differences noted with morphine-induced antinociception could be replicated using another $\mu$-agonist of similar potency and efficacy. Comparison of the ED$_{50}$ values for M6G in both mouse strains was done to determine whether the differences between the strains were due to differences in the metabolism of morphine to M6G.

Materials and Methods

Chronic Morphine Treatment. Male Swiss-Webster and ICR mice (Harlan Laboratories, Dublin, VA) were implanted with either a 75-mg morphine pellet or placebo pellet according to the method of Way et al. (1969). The mice were administered supplemental (s.c.) injections of either morphine (20 mg/kg) or saline twice per day for 3 days. Animals treated in this manner were used for either behavioral studies or binding studies.

Treatment Regimen for Morphine-, Methadone-, and M6G-Treated Mice. Antinociception was determined using the tail-flick latency assay with a 2- to 4-s baseline to “flick” the tail and 10-s cut-off (D’Amour and Smith, 1941). Dose-response curves were obtained from naive, morphine-treated, placebo-treated, and spontaneously withdrawn mice that were previously placebo or morphine treated. The mice were administered (s.c.) morphine, methadone, or M6G were tested for antinociception in naive animals only. Tail-flick latencies were determined 20 min post administration of the drugs. %MPE was calculated as follows: (drug time – control time/10 – control time) $\times$ 100.

Binding Studies. Representative groups of placebo- and morphine-pelleted mice (not used in any experiments) were tested for tolerance by administering morphine (20 mg/kg s.c.) and tested for antinociception 20 min later. The remainder of the treated mice were considered tolerant if the representative group met the criteria for tolerance. The lack of antinociception (%MPE <10%) in the morphine-pelleted mice was the criterion for the development of tolerance. In the placebo-pelleted mice, the same dose of morphine produced $\sim$95% MPE.

For $[^3\text{H}]$glyburide binding studies, animals were sacrificed and spinal cord tissue was taken from mice that were either chronically treated, with morphine or placebo pellets intact, or spontaneously withdrawn by pellet removal. Synaptosomes were prepared from mouse spinal cord using subcellular fractionation techniques described by McGovern et al. (1973). Please refer to Welch et al. (1997) for a full description of the preparation of synaptosomes. Binding was performed according to the method of Mourre et al. (1989) with slight modifications. Synaptosomes from the spinal cord (0.6 mg/ml protein; 2-ml final volume) were incubated for 60 min (equilibrium binding was found to be reached at 40 min) at 4°C in 20 mM HEPES/NaOH buffer, pH 7.5, with the concentrations of $[^3\text{H}]$glyburide (50 Ci/nmol, 99% pure) from 1 PM to 1 $\mu$M. Incubations were stopped by rapid filtration through Whatman GF/B filters soaked for 1 h in polyethylenimine to reduce nonspecific binding. Filters were washed with 100 mM Tris-HCL, pH 7.5, at 4°C and counted. Nonspecific binding was measured using 0.1 mM glipizide.

Physical Dependence Studies. Mice were rendered tolerant by the method described above and once tolerance was ascertained (under Binding Studies), the placebo and morphine pellets were removed for 24, 48, or 72 h. The pellets remained intact in other groups of mice. To test for physical dependence, mice were given naloxone (1 mg/kg s.c.) and immediately placed on a circular platform approximately 1.5 ft in height for no more than 15 min (Take-mori and Sprague, 1978). Thus, precipitated withdrawal was ascertained in mice previously spontaneously withdrawn. “Percent jumped” was calculated as the number of mice that jumped divided by the total number of mice in the group.

Statistical Analysis. Significant differences between treatment groups were determined using the Student’s t test and chi square values. ED$_{50}$ values and parallelism were ascertained using the methods of Tallarida and Murray (1987) for graded dose-response data. All binding assays had an $n \geq 3$ and the results from the separate experiments were averaged. The $B_{\text{max}}$ and $K_D$ values were obtained from Scatchard plot analysis using the LIGAND program (Munson and Rodbard, 1980).

Drugs. $[^3\text{H}]$Glyburide was obtained from New England Nuclear (Boston, MA); cold glipizide, as well as the rest of the above-mentioned reagents, were purchased from Sigma Chemical Co. (St. Louis, MO). Morphine and morphine pellets, as well as the placebo pellets were obtained from National Institute on Drug Abuse (Bethesda, MD).

Results

Morphine Is More Potent in ICR Than Swiss-Webster Mice as Measured by the Tail-Flick Latency Test. A stringent protocol for inducing tolerance was used for this study. The mice were implanted with morphine pellets as well as injected twice per day with 20 mg/kg morphine. Both Swiss-Webster and ICR mice chronically treated with morphine for 72 h were tolerant to morphine as indicated by a rightward shift in the dose-response curve (Fig. 1, A and B). Basal morphine antinociception differed between the strains.
as noted by the difference in the ED50 of morphine in naïve Swiss-Webster and ICR mice (Table 1). Chronically treated Swiss-Webster mice had an ED50 potency ratio of 35.2 compared with placebo and ICR mice had an ED50 potency ratio of 10.2 compared with placebo-implanted mice. Swiss-Webster mice were therefore 3.5 times more tolerant to morphine than ICR mice.

**Duration of Tolerance after Spontaneous Withdrawal from Morphine Is the Same in Both ICR and Swiss-Webster Mice as Measured by the Tail-Flick Latency Test.** The duration of tolerance was also evaluated to determine whether there was a correlation between the time course of tolerance and changes in glyburide binding. Both ICR and Swiss-Webster mice remained tolerant to morphine for 48 h after abrupt withdrawal from morphine (Fig. 2, A and B). There was a time-dependent leftward shift in the dose-response curves for ICR and Swiss-Webster mice as tolerance began to wane. The ED50 values for chronic morphine-treated mice and morphine-treated mice withdrawn for 1 day were statistically higher than those of their corresponding placebo treatment groups. The level further decreased after 2 days, but remained significantly higher than placebo mice with pellets removed for 2 days. After 3 days of withdrawal, the morphine-treated group did not differ statistically from the corresponding placebo group (Fig. 2B). These data were consistent with those found in Swiss-Webster mice. Chronically treated Swiss-Webster mice had an ED50 of 451 mg/kg (401–507) that was significantly higher than that of the chronic placebo-treated mice. Although the ED50 of morphine-treated mice decreased after 1 day of spontaneous withdrawal, it was still significantly higher than placebo. The ED50 further decreased in morphine-treated mice after 2 days of spontaneous withdrawal yet remained higher than the corresponding placebo-implanted mice. The ED50 value of morphine-treated mice returned to a level no different from placebo after 3 days of withdrawal (Fig. 2A).

**Duration of Physical Dependence after Spontaneous Withdrawal from Morphine Was Longer in ICR Than Swiss-Webster Mice as Observed in the Platform-Jumping Test.** The time course of dependence was determined in both ICR and Swiss-Webster mice to determine whether there was a correlation with changes in glyburide binding (Fig. 3). Morphine- and placebo-treated mice were administered naloxone (1 mg/kg s.c.) and immediately placed on a jumping platform to determine whether they were dependent (jumped) or nondependent (did not jump) on morphine. ICR mice were dependent during chronic treatment and after 2 days of spontaneous withdrawal followed by precipitated withdrawal. During chronic treatment, 100% of the morphine-treated mice jumped, which was significantly higher than chronic placebo-treated mice. After 1 day of spontaneous withdrawal followed by precipitated withdrawal, 88% of the mice jumped, which again was significantly greater than placebo-treated mice after pellets were removed for 1 day. Withdrawing the mice for 2 days resulted in a decrease in the number of mice jumping, but the difference remained significant. After 3 days of withdrawal, the number of jumping mice returned to a level no different from placebo mice.

**Table 1**

Summary of ED50 values and 95% confidence limits in Swiss-Webster and ICR mice

<table>
<thead>
<tr>
<th></th>
<th>ICR Mice ED50, mg/kg (95% CL)</th>
<th>Swiss-Webster Mice ED50, mg/kg (95% CL)</th>
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<tbody>
<tr>
<td></td>
<td>Naïve</td>
<td>Placebo Pelleted</td>
</tr>
<tr>
<td>Methadone</td>
<td>4.4 (3–6)</td>
<td></td>
</tr>
<tr>
<td>M6G</td>
<td>24 (20–29)</td>
<td></td>
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<tr>
<td>Morphine</td>
<td>12 (9–15)</td>
<td></td>
</tr>
<tr>
<td>Chronic</td>
<td>12 (9–15)</td>
<td>120 (90–161)&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>treatment</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 day</td>
<td>14 (13–16)</td>
<td>49 (38–63)&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>2 days</td>
<td>11 (9–13)</td>
<td>29 (21–40)&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>3 days</td>
<td>11 (10–13)</td>
<td>17 (12–23)</td>
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</tbody>
</table>

CL, confidence limits.

<sup>a</sup> ED50 values significantly different from corresponding placebo value. ED50 values significantly different from: <sup>b</sup> ICR mice treated with morphine, <sup>c</sup> chronic ICR, <sup>d</sup> ICR mice withdrawn from morphine for 1 day, <sup>e</sup> ICR mice withdrawn from morphine for 2 days, and <sup>f</sup> ICR mice withdrawn from morphine for 3 days.
in 86% of the mice jumping, which remained significantly higher than the corresponding placebo-treated group. There was an abrupt cessation of dependence at 3 days of withdrawal. Swiss-Webster mice were physically dependent on morphine only during chronic treatment with 100% of the mice jumping from the platform as shown in Fig. 3A.

<table>
<thead>
<tr>
<th>TABLE 2</th>
<th>Summary of $B_{\text{max}}$ and $K_D$ values in Swiss-Webster and ICR mice</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>ICR Mice</td>
</tr>
<tr>
<td>$B_{\text{max}}$</td>
<td>nM</td>
</tr>
<tr>
<td>Naive</td>
<td>307 ± 33</td>
</tr>
<tr>
<td>Chronic placebo</td>
<td>573 ± 82</td>
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<tr>
<td>Chronic morphine</td>
<td>620 ± 90</td>
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<tr>
<td>Spontaneous Withdrawal</td>
<td></td>
</tr>
<tr>
<td>1 day placebo</td>
<td>437 ± 74</td>
</tr>
<tr>
<td>3 days placebo</td>
<td>531 ± 128</td>
</tr>
<tr>
<td>3 days morphine</td>
<td>474 ± 70</td>
</tr>
<tr>
<td>10 days placebo</td>
<td>447 ± 184</td>
</tr>
<tr>
<td>10 days morphine</td>
<td>476 ± 132</td>
</tr>
</tbody>
</table>

$^a$ $B_{\text{max}}$ and $K_D$ values significantly different from corresponding placebo values.

$^b$ $B_{\text{max}}$ and $K_D$ values significantly different from chronic placebo values.

**Chronic Morphine Treatment Increased Both the $K_D$ and $B_{\text{max}}$ of $[\text{H}]$Glyburide Binding to the Sulfonylurea Receptor in Swiss-Webster, but Not ICR Mice.** There was an increase in the $K_D$ in Swiss-Webster mice chronically treated with morphine, which remained significantly higher than the corresponding placebo-treated group. There was an abrupt cessation of dependence at 3 days of withdrawal. Swiss-Webster mice were physically dependent on morphine only during chronic treatment with 100% of the mice jumping from the platform as shown in Fig. 3A.

**Fig. 2.** Dose-response curves of s.c. morphine in mice allowed to spontaneously withdraw from chronic morphine for 1 (△), 2 (○), or 3 (●) days. △ represents mice implanted with a morphine pellet and chronically treated with morphine (20 mg/kg s.c.) two times per day for 72 h. A, results in Swiss-Webster mice. B, results in ICR mice. The antinociceptive effects (as %MPE) were measured as described under Materials and Methods, using the tail-flick latency test with $n = 5–8$ mice/dose.

**Fig. 3.** Platform-jumping test for dependence in Swiss-Webster and ICR mice. Mice were either chronically treated with morphine (■) or placebo-pelleted (□). They were allowed to spontaneously withdraw by cessation of treatment and pellet removal for the indicated days. Mice were administered a 1-mg/kg s.c. dose of naloxone and placed on the jumping platform for no more than 15 min. A, results in Swiss Webster mice. B, results in ICR mice. No column indicates that none of the mice in that group jumped. Withdrawal was measured as % jumped as described under Materials and Methods with $n = 5–8$ mice/dose. *$P < .05$, **$P < .01$, and ***$P < .001$. 

*Comparison of Glyburide Binding in Swiss-Webster and ICR Mice 2000*
treated with morphine (Fig. 4). The $K_D$ increased almost 3-fold from 0.38 ± 0.024 to 1.1 ± 0.113 nM. The $B_{\text{max}}$, which is the number of glyburide-binding sites, was twice as high in Swiss-Webster mice chronically treated with morphine versus those that were placebo-pelleted. The $B_{\text{max}}$ increased from 294 ± 31 to 635 ± 117 fmol/mg of protein (Fig. 5). No changes were seen in ICR mice during chronic treatment and neither strain showed changes in $B_{\text{max}}$ or $K_D$ during spontaneous withdrawal (Figs. 4 and 5). In Swiss-Webster mice, there is a statistically significant difference in the $K_D$ and $B_{\text{max}}$ between chronic placebo- and placebo-treated mice that were spontaneously withdrawn (Table 2). This increase was seen on days 1 and 3 and returned to chronically treated levels by day 10 (Table 2). Mice were exposed to metofane for pellet implantation and withdrawal. We believe that the increase in $B_{\text{max}}$ and $K_D$ is stress-induced due to the surgical procedure and possibly to the exposure to the anesthetic agent. There was not as much variability in the ICR strain. There was no correlation with the time course of tolerance and changes in the number of glyburide-binding sites for either strain. However, it appears that the time course of physical dependence in chronic morphine-treated (morphine pellets intact) Swiss-Webster mice correlated with alterations in both the $K_D$ and $B_{\text{max}}$ of glyburide for the sulfonylurea receptor.

**Differential Effects of Morphine versus Methadone and M6G in Swiss-Webster and ICR Mice.** Dose-response curves were determined in both strains of mice using methadone, a $\mu$-agonist, and M6G, a metabolite of morphine. The dose-effect curve for both M6G and methadone produced a full agonist effect. Methadone was more potent than morphine in producing antinociception. However, there was no difference in the antinociceptive effects of methadone between the two strains of mice (ED$_{50}$ potency ratio of 1.53) (Fig. 6). M6G produced an antinociceptive effect that was similar to that of morphine. Like methadone, there was no difference in the antinociceptive effect between the two strains of mice when M6G was administered (ED$_{50}$ potency ratio of 1.64) (Fig. 7). Although the dose-effect curves for methadone did not differ significantly from parallel between the two strains of mice, the dose-response curves for M6G did differ significantly from parallel between the two strains.

**Discussion**

Previous work in our laboratory has shown that morphine tolerance increases both the $B_{\text{max}}$ and $K_D$ of $[^3H]$glyburide in
What we observed instead was an increase in $B_{\text{max}}$ and $K_D$ only during chronic treatment and only in Swiss-Webster mice. Both strains were tolerant to morphine during chronic treatment, as well as 48 h after treatment ceased and morphine pellets were removed. It appears that changes in glyburide binding correlate better with dependence. In this study we show that Swiss-Webster mice are only physically dependent during chronic morphine treatment and that it is during this time that changes in glyburide binding occur. There tends to be more variability in the $B_{\text{max}}$ and $K_D$ in the Swiss-Webster strain of mice. Results indicate there is a statistically significant difference between chronic placebo-implanted mice and those in which the placebo pellet was removed for 1 day and 3 days. This indicates that the Swiss strain is extremely sensitive to stress, which may lead to alterations in glyburide-binding site number and affinity in placebo-implanted mice. The difference in $K_D$ and $B_{\text{max}}$ were statistically different between placebo groups but were not different from the paired morphine group. There were also differences in the ED$_{50}$ values of placebo-implanted mice. Again, this further implies that Swiss-Webster mice are sensitive to the surgery, pellet implantation, and pellet removal. The issue of sensitivity is addressed in these experiments by using paired comparisons between placebo and morphine-treated mice that were exposed to the same treatment regimen. Although the differences in the $K_D$ and $B_{\text{max}}$ between chronic morphine- and placebo-treated Swiss-Webster mice were modest, but significant, these data confirm that a high degree of morphine tolerance is necessary to alter glyburide binding.

Genetics has been well studied with regard to the varying effects of opioids on different strains of mice. In an early study, strain differences were attributed to the turnover rate of neurotransmitters (Maas, 1963). Eidelburg et al. (1975) examined four strains of mice, including ICR and Swiss-Webster. They showed that there was a genetic difference between strains with regard to locomotor activity, tolerance, and dependence. Evaluating the brain uptake of dihydromorphine ruled out blood-brain barrier penetration as the cause of the strain differences. Many have hypothesized as to why the strains differ. Muraki and Kato (1985) postulated that polygenes might be involved in the regulation of the effects of opioids on locomotor activity. They also found strain differences in morphine-induced hypothermia and respiration that could not be positively correlated to variations in $[^{3}H]$naloxone binding in seven different brain regions (Muraki and Kato, 1986). Rady et al. (1990) showed that Swiss-Webster mice metabolize heroin (typically a $\mu$-agonist) in such a way that it acts at the $\delta$-opioid receptor; however, heroin is a $\mu$-agonist in ICR mice. In addition, antisense mapping of MOR-1 indicates that morphine and M6G produce antinociceptive effects via distinct receptors that are splice variants of the MOR-1 gene (Rossi et al., 1995a,b, 1997). These studies showed that morphine analgesia is antagonized by MOR-1 antisense oligonucleotides directed against exons 1 and 4, whereas M6G analgesia is blocked by antisense oligonucleotides directed against exons 2 and 3 as well as exon 4.

We speculate there may be differences in morphine metabolism and/or $\mu$-receptor number between the two strains of mice. In fact, morphine is more potent in ICR than Swiss-Webster mice, suggesting that there may either be fewer $\mu$-opioid receptors in Swiss-Webster mice or differences in

Swiss-Webster mice (Welch et al., 1997). This study investigated the ability of morphine to alter the number and affinity of glyburide-binding sites during tolerance and physical dependence. The goals of this study were to determine whether there was a correlation between glyburide-binding site changes and morphine tolerance and dependence. In addition, a second goal of this project was to determine whether changes in glyburide binding could account for the increase in morphine tolerance seen in Swiss-Webster mice. Therapists treating chronic pain patients know from first-hand experience that patients respond to morphine in various ways. It is possible that these patients may differ in their ability to metabolize morphine, or the problem may lie at the level of the opioid receptor. Therefore, aside from our main objectives, a third goal of this study was to speculate why the two strains of mice differ with regard to morphine potency. Understanding these differences could be beneficial therapeutically for morphine-resistant patients. We have demonstrated that Swiss-Webster mice become more tolerant to morphine than ICR mice with respective potency ratios of 35.2 and 10.2. Thus, we believe that a high degree of morphine tolerance is necessary to alter ATP-gated potassium channels.

We expected to see an increase in glyburide-binding site number and a corresponding decrease in affinity during morphine tolerance in both ICR and Swiss-Webster mice, with this effect gradually returning to normal during dependence.
the metabolic pathway of morphine between the two strains. To determine whether there was a μ-receptor deficit, a dose-response curve using methadone was generated. Methadone is a μ-agonist that is slightly more potent than morphine, but metabolized differently. Methadone is biotransformed in the liver into pyrrolidine and pyrroline, which are excreted in the urine and bile (Reisine and Pasternak, 1996). The ED50 of morphine in naive Swiss-Webster mice was 23 mg/kg (19–28), which was statistically higher than that of naive ICR mice [12 mg/kg (9–15)]. However, the methadone dose-response curve did not differ between strains with the ED50 values of Swiss-Webster and ICR mice being 6.7 mg/kg (5–8) and 4.4 mg/kg (3–6), respectively. This indicates that the difference in potency between strains was morphine specific and may not indicate a general μ-receptor effect. In addition, this may also imply that the strain difference may be due to metabolism because methadone is metabolized differently than morphine. However, Yoburn et al. (1989) showed that opioid binding in the brain is correlated to the potency of opioid agonists. In this study two strains of Swiss-Webster mice that differ in their sensitivity to morphine were used. Taconic Farms mice are 2 times more sensitive to morphine than Charles River Laboratories mice. The more sensitive Taconic Farms mice had more opioid-binding sites in the brain than did the less sensitive Charles River mice. Binding studies have been performed in CXXBK (μ-receptor deficient) and Swiss-Webster mice, which indicate morphine is less potent in the μ-opioid receptor-deficient mice (Duttaroy et al., 1999). Bmax values using [3H]Nalorphine and [3H]naloxone on brain homogenates (minus the cerebellum) did not show a difference in μ-receptor number.

The major path of morphine metabolism is glucuronidation to form both active and inactive metabolites. M6G is a major metabolite of morphine. The data obtained from M6G showed that there was no difference in the ED50 of the metabolite in either strain of mice. Also, the two curves were not parallel, suggesting there might be a difference in the mechanism of action for the metabolite between the two strains. There is, however, a difference in the ED50 between naive ICR and Swiss-Webster mice treated with morphine. These data provide indirect evidence that there is a distinction in the metabolic pathway of the two strains of mice, which may explain the differences in sensitivity to morphine. Rossi et al. (1996) tried to distinguish the mechanism of action between morphine and M6G. They found that there was no cross-tolerance between the two drugs, indicating they work through distinct receptors. Alternatively, subtypes of the MOR-1 gene may predominate in Swiss-Webster versus ICR mice. It is also possible that similar to heroin, morphine may have a greater affinity for the δ-opioid receptor than for the μ-opioid receptor in mice of the Swiss strain. In conclusion, the results of this study indicate that mice must be highly morphine-tolerant to alter glyburide binding. Outside of our main objective, we speculated that the difference with regard to morphine potency between the two strains might be attributed to metabolism or the predominance of one opioid receptor subtype over the other, depending on the strain. Clinically, the use of ATP-gated potassium channel ligands as analgesic agents may be an excellent choice in morphine-tolerant patients because KATP openers produce antinoiception during morphine tolerance. Another important observation of note is the separation between tolerance and dependence in the Swiss strain. Many believe that tolerance and dependence are somewhat interconnected phenomena. This study demonstrated that although the Swiss strain was tolerant to morphine for 2 days, they were physically dependent only when a large degree of tolerance was present.

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