Lack of Morphine and Enkephalin Tolerance in 129/SvEv Mice: Evidence for a NMDA Receptor Defect

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ABSTRACT

In contrast to the rapid development of tolerance to morphine in CD-1 mice, tolerance is not seen in 129/SvEv mice implanted with morphine pellets or given daily morphine injections for 5 days. Similarly, the progressive and complete loss of analgesia in CD-1 mice seen with repeated dosing of the delta ligand [D-Pen²,D-Pen⁵]enkephalin is not observed in 129/SvEv mice. In contrast, tolerance develops normally to both the kappa drug U50,488H and the kappa agent naloxone benzoylhydrazone. N-methyl-D-aspartate (NMDA) given alone attenuates morphine analgesia in CD-1 mice and accelerates the development of tolerance in CD-1 mice when given daily with morphine. In contrast, NMDA has no significant effect in the 129/SvEv mice in either paradigm. Activation of NMDA receptors can lead to increased NO production. Sodium nitroprusside and L-arginine increase nitric oxide levels and decrease morphine analgesia in both the control CD-1 and 129/SvEv mice. Thus, the defect in the NMDA/nitric oxide cascade responsible for the loss of morphine tolerance in the 129/SvEv mice rests at the level of the NMDA receptor itself or in the steps up to the activation of nitric oxide synthase.

Morphine tolerance can be readily demonstrated in mice using a variety of experimental paradigms. Although repeated administration of morphine almost certainly leads to biochemical changes at the level of the receptor and possibly its transduction systems, some of the most interesting aspects of morphine tolerance have come from studies implicating both the NMDA and nitric oxide systems (Bhargava, 1994; Pasternak et al., 1995). The development of morphine tolerance is effectively prevented by noncompetitive or competitive NMDA antagonists (Ben-Elizayhu et al., 1992; Bhargava and Matwyshyn, 1993; Dunbar and Yaksh, 1996a; Elliott et al., 1994, 1995; Gutstein and Trujillo, 1993; Kolesnikov et al., 1993a; Manning et al., 1996; Tiseo and Inturrisi, 1993; Trujillo and Akil, 1991, 1994), or even agents acting at the glycine site (Kolesnikov et al., 1994; Lutfy et al., 1995). NMDA activation leads to increases in NO production. Inhibition of NOS, the enzyme that generates NO, also blocks the appearance of morphine tolerance (Babey et al., 1994; Bhargava and Zhao, 1996; Dunbar and Yaksh, 1996b; Kolesnikov et al., 1992, 1993b, 1997; London et al., 1994; Vaupel et al., 1995). These insights into modulatory circuits mediating morphine tolerance have opened new potential therapeutic targets.

Variations among strains of mice have provided valuable models with which to explore opioid function. The demonstration that different strains vary markedly in their sensitivity to opioid analgesics has played a large role in the identification and characterization of the various opioid receptor subtypes. For example, morphine is not an effective analgesic when given either systemically or supraspinally in CBXBK mice (Baron et al., 1975; Elmer et al., 1995; Pick et al., 1993; Raffa and Schupsky, 1993; Reith et al., 1981; Vaught et al., 1988). This insensitivity to morphine analgesia contrasts sharply with the normal potency of heroin, its active metabolite 6-acetylmorphine and morphine-6β-glucuronide in this mouse strain (Rossi et al., 1996), indicating the existence of distinct receptors for the two classes of drugs. The development of knockout mice lacking various proteins, including opioid receptors, provides important new tools in the exploration of opioid pharmacology. Because many of these genetically altered animals are derived from 129/SvEv mice, it is important to determine the pharmacology of opioids in this strain. We now describe the differences in the development of morphine tolerance between CD-1 and 129/SvEv mice.

Materials and Methods

Morphine sulfate, U50,488H and DPDPE were gifts from the Research Technology Branch of the National Institute on Drug

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ABBREVIATIONS: NalBzoH, naloxone benzoylhydrazone; DPDPE, [D-Pen²,D-Pen⁵]enkephalin; NMDA, N-methyl-D-aspartate; NOS, nitric oxide synthase; NO, nitric oxide.
Mice. Prior studies have shown many similarities in the
not in the 129/SvEv, mice. Compounds were administered intracerebroventricularly with the animals under light halothane anesthesia. Daily injections also were used in which the animals received a single injection each day. Animals receiving multiple injections always received morphine 15 min after the other agent.

All groups contained a minimum of 10 mice; most comprised at least 20. Single doses were compared using the Fisher Exact Test. Dose-response curves were generated from at least three doses of drug. Each dose was tested at least 10 mice, which were examined only once. Data were analyzed to generate ED$_{50}$ values and 95% confidence limits using the BLISS-20 program, which maximizes the log-likelihood function to fit a parallel set of gaussian normal sigmoid curves to the dose response of quantal data (Umans and Inturrisi, 1981).

**Results**

**Morphine tolerance in CD-1 and 129/SvEv mice.** First, we examined the development of morphine tolerance in both the CD-1 and 129/SvEv strains of mice. Pelleting mice with morphine produced analgesia in all the mice, followed within a few days by the rapid development of tolerance in CD-1 mice (fig. 1a). In contrast, the analgesic response in the 129/SvEv mice did not diminish over the 6 days examined. The analgesic response was 100% for 4 days; the limited decline after that was not significant. We observed a similar effect with daily subcutaneous injections of equianalgesic morphine doses (fig. 1b). As reported previously (Elliot et al., 1994; Kolesnikov et al., 1993a, 1993b, 1994), tolerance developed quite rapidly in the CD-1 mice, with the analgesic response declining from 60% to 0% within 5 days. In contrast, 129/SvEv mice receiving equianalgesic dose of morphine daily did not develop tolerance. The analgesic response remained constant at 60% over the full 5 days. Finally, we examined the effects of daily supraspinal injections of morphine (fig. 1c). Again, the morphine analgesia seen on the first day was virtually lost by the fifth day in the CD-1 but not in the 129/SvEv mice.

**Delta and kappa tolerance in CD-1 and 129/SvEv mice.** Prior studies have shown many similarities in the pharmacological sensitivity of tolerance to both delta and mu analgesia (Babey et al., 1994; Kolesnikov et al., 1992, 1993a, 1993b, 1994; Pasternak et al., 1995). With daily intrathecal injections, the analgesic activity of the delta ligand DPDPE rapidly declined from 60% to zero by 5 days in the CD-1 mice (fig. 2). In contrast, the response in the 129/SvEv mice remained relatively constant over the same period (P < .01).

Earlier work suggested that kappa tolerance was not me-
full dose-response studies (table 1). The analgesic ED50 value for 129/SvEv mice treated in the same manner retained their in an effort to induce a lowered morphine response. Treating the development of morphine tolerance, we administered NMDA cascade responsible for the loss of morphine and DPDPE.

We next assessed the location along the NMDA/NO gesia.

Kolesnikov et al., 1992, 1993a, 1993b, 1994). We therefore examined toler-
ance to the kappa1 agent U50,488H and the kappa3 drug NaBzOH in the 129/SvEv mice (fig. 3). Prior studies have repeatedly demonstrated that repeated daily dosing with either U50,488H or NaBzOH leads to a loss of analgesia within 5 days in CD-1 mice (Babey et al., 1994; Elliott et al., 1994; Kolesnikov et al., 1992, 1993b, 1994). We observed a similar loss of kappa analgesic sensitivity in the 129/SvEv mice. Using this same daily injection paradigm, the analgesic activity of U50,488H and NaBzOH in 129/SvEv mice seen on the first day, 70% and 60%, respectively, was completely lost by the fifth day. Thus, tolerance to both kappa1 and kappa3 drugs developed in the 129/SvEv mice at a rate similar to that previously reported in CD-1 mice (Babey et al., 1994; Kolesnikov et al., 1992, 1993a, 1993b).

**NMDA receptors and NO actions on morphine analgesia.** We next assessed the location along the NMDA/NO cascade responsible for the loss of morphine and DPDPE tolerance. Because blockade of NMDA receptors prevents the development of morphine tolerance, we administered NMDA in an effort to induce a lowered morphine response. Treating mice with NMDA alone for 5 days significantly lowered the response to a single morphine dose in CD-1 mice, whereas 129/SvEv mice treated in the same manner retained their analgesic sensitivity toward morphine, as demonstrated by full dose-response studies (table 1). The analgesic ED50 value was determined on the indicated day 15 min after injection. After 5 days, none of the CD-1 mice were analgesic (P < .01), whereas the 129/SvEv mice showed no significant change in their analgesic sensitivity.

**Fig. 2.** Effects of daily DPDPE dosing in CD-1 and 129/SvEv mice. Groups of mice (n ≥ 10) received DPDPE (0.5 µg i.t.) daily, and analgesia was determined on the indicated day 15 min after injection. After 5 days, none of the CD-1 mice were analgesic (P < .01), whereas the 129/SvEv mice showed no significant change in their analgesic sensitivity.

**Fig. 3.** Effects of daily kappa drugs in 129/SvEv mice. Groups of 129/SvEv mice (n ≥ 10) received daily injections of either the kappa1 drug U50,488H (5 mg/kg s.c.) or the kappa3 agent NaBzOH (50 mg/kg s.c.). Analgesia was determined on the indicated day 30 min after injection. None of the mice were analgesic after 4 days on NaBzOH or 5 days on U50,488H (P < .01).

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<th>Group</th>
<th>CD-1</th>
<th>129/SvEv</th>
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<tr>
<td>Naive</td>
<td>3.1 (1.6,4.4)</td>
<td>2.0 (1.4,2.8)</td>
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<tr>
<td>NMDA</td>
<td>12.4 (6.1,14.8)</td>
<td>4.0 (2.2,5.6)</td>
</tr>
<tr>
<td>Nitroprusside</td>
<td>8.4 (4.7,14.2)</td>
<td>2.7 (2.2,6.8)</td>
</tr>
<tr>
<td>t-Arginine</td>
<td>9.9 (7.5,12.5)</td>
<td>3.2 (5.2,1.1)</td>
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**TABLE 1**

| Effect of NMDA, t-arginine and nitroprusside on morphine analgesia in CD-1 and 129/SvEv mice
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for morphine in the CD-1 mice was shifted 4-fold, whereas the response in the 129/SvEv mice was not significantly affected.

We then examined the effects of NMDA given along with morphine. Concurrent daily administration of NMDA along with morphine accelerated the development of morphine tolerance in CD-1 mice (fig. 4a). After 3 days of treatment, analgesic activity in the CD-1 mice receiving both drugs was lost, a result that was significantly different from the response in the morphine-alone group (P < .05). NMDA coadministered with morphine had no significant effect on the analgesic activity of morphine over time in the 129/SvEv mice, even when the treatment period was extended to 10 days (fig. 4b). This inability of NMDA itself to overcome the lack of tolerance development in these mice suggested that the defect was at the level of the NMDA receptor or further downstream in the pathway.

To explore the potential role of the NO system, we used both t-arginine and sodium nitroprusside. Both agents, which increase NO levels, significantly lowered the analgesic sensitivity of mice to morphine (table 1). Sodium nitroprusside works directly as a NO donor while t-arginine requires its enzymatic conversion to NO by NOS. Activity by nitroprusside implies that the downstream pathway activated by NOS is intact. The ability of t-arginine to modulate morphine actions ensures the presence of enzymatically active NOS. Sodium nitroprusside significantly shifted the dose-response curve for morphine 2.7-fold in CD-1 mice and 2.5-fold shift in the 129/SvEv mice. t-Arginine, the substrate for NOS, is converted enzymatically to NO. Treating mice with t-arginine for 3 days significantly lowered morphine analgesia in both the CD-1 and 129/SvEv strains to a similar degree (table 1).

We then examined the effect of concurrent administration of morphine and t-arginine in 129/SvEv mice (fig. 5). Given daily for 5 days, morphine maintained a constant analgesic
Coadministering L-arginine with morphine rapidly induced a significant decrease in sensitivity to morphine (Reisine and Pasternak, 1993b). After 5 days, we added L-arginine to the regimen. Morphine injections did not significantly change over 10 days, consistent with the previous result in which daily injections of L-arginine (50 mg/kg s.c.) for an additional 5 days. After 5 days on L-arginine, analgesia was significantly lower than controls (P < .05). B, Groups of 129/SvEv mice (n = 10) received daily injections of morphine (2.5 mg/kg s.c.) alone (Control) or with daily injections of NMDA (1 mg/kg s.c.). Base-line latencies in the 129/SvEv (2.8 ± 0.2 sec) were not influenced by NMDA (2.9 ± 0.1 sec).

**Fig. 4.** Effect of NMDA on morphine tolerance in CD-1 and 129/SvEv mice. A, Groups of CD-1 mice (n = 10) received daily injections of morphine (4 mg/kg s.c.) alone (Control) or with daily injections of NMDA (1 mg/kg s.c.). After 3 days, the response in the group receiving NMDA was significantly lower than controls (P < .05). B, Groups of 129/SvEv mice (n = 10) received daily injections of morphine (2.5 mg/kg s.c.) alone (Control) or with daily injections of NMDA (1 mg/kg s.c.). Base-line latencies in the 129/SvEv (2.8 ± 0.2 sec) were not influenced by NMDA (2.9 ± 0.1 sec).

**Fig. 5.** Effect of L-arginine on the development of morphine tolerance in 129/SvEv mice. A group of 129/SvEv mice (n = 10) received morphine (2.5 mg/kg s.c.) alone for 5 days and then continued to receive the same morphine dose in conjunction with a daily dose of L-arginine (50 mg/kg i.p.) for an additional 5 days. After 5 days on L-arginine, analgesia was significantly decreased compared with the response before the addition of L-arginine (P < .01).

Response, consistent with the previous result in which daily morphine injections did not significantly change over 10 days (fig. 4b). After 5 days, we added L-arginine to the regimen. Coadministering L-arginine with morphine rapidly induced a dramatic decline in analgesic responsiveness, eliminating the analgesic response within an additional 5 days. The rate of decline of analgesic sensitivity was similar to that seen with morphine alone in CD-1 mice (fig. 1b).

**Discussion**

Opioid tolerance is a complex series of biochemical events leading to a reduction in sensitivity (Reisine and Pasternak, 1996). Although it is likely that many factors play a role, numerous studies have documented the importance of NMDA receptors (Ben-Eliyahu et al., 1992; Bhargava and Matwyslyn, 1993; Dunbar and Yaksh, 1996a; Elliott et al., 1994, 1995; Gutstein and Trujillo, 1993; Kolesnikov et al., 1994, 1993a; Lutfy et al., 1995; Manning et al., 1996; Tiseo and Inturrisi, 1993; Trujillo and Akil, 1991, 1994) and the NOS cascade (Babey et al., 1994; Bhargava and Zhao, 1996; Dunbar and Yaksh, 1996b; Kolesnikov et al., 1992, 1993b, 1997; London et al., 1994; Vaupel et al., 1995) in morphine and enkephalin tolerance. The generation of various knock-out animals has greatly facilitated studies that explore the molecular biology of behavior. However, the use of these animals requires a sound understanding of the pharmacology of the mouse strains used to generate the mutants, including the 129/SvEv mouse strain. Initially considered simple control studies, we quickly observed very unusual results in the 129/SvEv mice.

In contrast to the CD-1 strain, 129/SvEv mice did not develop tolerance to morphine regardless of whether it was administered by pellet or daily injections given subcutaneously or intracerebroventricularly. This does not appear to simply reflect a slower rate of tolerance development. The analgesic activity of morphine in the 129/SvEv mice remained similar to naïve levels even after 10 days of treatment, twice the time needed to completely lose morphine analgesia in the CD-1 mice. Prior work has suggested that delta tolerance is similar to that of morphine, as indicated by the similar sensitivity toward NMDA antagonists and NOS inhibitors (Babey et al., 1994; Elliott et al., 1994; Kolesnikov et al., 1993a, 1993b, 1994; Pasternak et al., 1995). As in these earlier studies, the activity of the delta ligand DPDPE mimicked that of morphine. DPDPE maintained its analgesic potency in the 129/SvEv mice over 5 days despite the complete loss of an equianalgesic DPDPE dose in CD-1 mice in the same period of time. Yet, tolerance develops to both kappa and delta receptors leads to the activation of nNOS, which in turn leads to the development of tolerance. We examined the role of NMDA receptors and NOS. Although NMDA effectively lowered morphine analgesia in CD-1 mice, it was inactive in the 129/SvEv mice. After 5 days, NMDA treatment shifted the morphine dose-response curve in CD-1 mice 4-fold without significantly changing the response in the 129/SvEv mice. This observation implied that the difficulty arose from the NMDA receptor itself or from a downstream step in the pathway.

The NO pathway does not appear to be impaired. The NO donor nitroprusside effectively lowered the analgesic potency of morphine as effectively in both the 129/SvEv and the CD-1...
mice, implying that the targets for NO needed to diminish the activity of morphine and the downstream pathways are intact. The ability of L-arginine to shift the morphine dose-response to a similar degree in both strains suggests that the enzymatic activity of NOS also is not impaired.

Together, these results imply that the defect in the 129/SvEv mice may lie at the NMDA receptor itself. Alternatively, it might involve steps in the pathway between the NMDA receptor and the initiation of the NO cascade. Additional studies are now needed to explore these possibilities. Clearly, the 129/SvEv mice provide a unique model to further explore the mechanisms mediating opioid tolerance and illustrate the strength of genetic models in the exploration of opioid function.

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References


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