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MK-801 blocks the expression but not the development of tolerance to morphine in the isolated spinal cord of the neonatal rat

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Abstract

This study investigated the role of (MK-801; [(+)-5-methyl-10,11-dihydro-5*H*-dibenzo[*a*,*d*]-cyclo-hepten-5,10-imine hydrogen maleate) in the development and expression of tolerance to morphine in the isolated spinal cord of the neonatal rat. Neonatal rats were treated chronically (3 or 4 days) with either morphine, morphine + MK-801, MK-801 alone or saline. Morphine, in a concentration-dependent manner, depressed a delayed ventral root potential produced by supramaximal electrical stimulation of an ipsilateral dorsal root. Chronic treatment of neonates with morphine alone, morphine with MK-801 and MK-801 alone produced tolerance to morphine depression of the ventral root potential. Acute MK-801 (300 nM) did not depress the ventral root potential but enhanced the depressant effects of acute morphine on the ventral root potential in saline-treated controls. Acute MK-801 (300 nM) appeared to reverse tolerance in all of the drug-treated groups. We conclude that MK-801 can mask the expression of morphine tolerance by enhancing the acute depressant effects of morphine.

Keywords: MK-801; Spinal cord; Morphine tolerance; NMDA receptor antagonist; Ventral root potential

1. Introduction

The spinal cord is an important site of opioid analgesia (Yaksh and Rudy, 1976) and the chronic administration of opioids systemically or intrathecally results in the development of tolerance to opioids at the spinal cord level (Yaksh et al., 1977). The study of tolerance in a specific region of the central nervous system can be accomplished by chronic opioid treatment of an animal followed by isolation of a portion of the central nervous system. One such region that has been utilized for in vitro electrophysiological study is the locus coeruleus (Andrade et al., 1983). The isolated spinal cord preparation from the neonatal rat (Otsuka and Konishi, 1974) could be similarly utilized as an in vitro model of spinal opioid tolerance. In the present study, we sought to validate this preparation as such an in vitro model. We subsequently used the isolated spinal cord of the neonatal rat to examine the role of the non-competitive glutamate receptor antagonist (MK-801; [(+)-5-methyl-10,11-dihydro-5*H*-dibenzo[*a*,*d*]-cyclo-hepten-5,10-imine hydrogen maleate) in the development of opioid tolerance at the spinal level. Previous studies suggest the *N*-methyl-D-aspartate (NMDA)-type glutamate receptor antagonist MK-801 attenuates the development of opiate tolerance (Elliot et al., 1994; Gustein and Trujillo, 1993; Kest et al., 1993; Lutfy et al., 1993; Marek et al., 1991a; Trujillo and Akil, 1991a, 1994). These findings indicate that NMDA-type glutamate receptor-mediated neurotransmission is important in producing tolerance. The spinal cord has been shown to be an important site of MK-801-mediated blockade of morphine tolerance since intrathecal administration of MK-801 (Kest et al., 1993) and administration of MK-801 to spinal rats (Gustein and Trujillo, 1993) inhibit the development of morphine tolerance.

The isolated spinal cord of the neonatal rat should be a useful model for studies of interactions between MK-801 and morphine in the development of mor-

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phine tolerance. Spinal cord nociceptive reflexes are mediated by glutamate released from primary afferent fibers (Jeftinija et al., 1991; Kangrga and Randić, 1991). Furthermore, prolonged ventral root potentials which are electrophysiological manifestations of spinal nociception can be evoked by high threshold electrical stimulation of nociceptive primary afferents in the isolated spinal cord of the neonatal rat (Thompson et al., 1994). These prolonged ventral root potentials are partially blocked by NMDA-type glutamate receptor antagonists (Brugger et al., 1990; Gibbs and Kendig, 1992; Jeftinija et al., 1991; Nagy et al., 1993; Thompson et al., 1992, 1993; Woodley and Kendig, 1991) an observation implying that NMDA receptors are critical in glutamatergic nociceptive transmission.

Opioids block the release of glutamate from spinal cord nociceptive afferents (Kangrga and Randić, 1991; Malmberg and Yaksh, 1995). In the isolated spinal cord of the neonatal rat, opioids attenuate electrically evoked high threshold prolonged ventral root potentials (Yanagisawa et al., 1985). Therefore the isolated spinal cord of the neonatal rat is a synaptic model rich in NMDA and opioid receptors interacting at the level of nociceptive reflexes. Thus, the primary objectives of the present study were (1) to establish a model of tolerance to morphine in the isolated spinal cord of the neonatal rat, and (2) to examine the interaction of MK-801 with the development of tolerance to opioids in the isolated spinal cord.

2. Materials and methods

2.1. Animals

Newborn male and female Sprague-Dawley rats were housed in cages with the maternal lactating mother rat (Charles River Breeding Laboratories) until dissections were to be performed.

2.2. Drugs

Morphine sulfate was obtained from the Division of Intramural Research pharmacy. MK-801 (also known as dizocilpine) was purchased from Research Biochemicals International. Naloxone was purchased from Sigma Chemicals. For injections, drugs were dissolved in physiological saline.

2.3. Administration of drugs

Starting on day 1, neonatal rats were weighed and received subcutaneous injections with the assigned treatment twice daily. The morphine dose for day 1 was 20 mg/kg. Morphine (20 mg/kg) + MK-801 (0.3 mg/kg) was also given on day 1 to a second group. The MK-801 dose was held constant at 0.3 mg/kg throughout the treatment period. Saline was injected as a vehicle control. On days 2 and 3, the morphine dose in the morphine alone group and the morphine + MK-801 group was incrementally increased by 20 mg/kg per day until days 4–5 when morphine doses were held constant at 60 mg/kg.

2.4. Spinal cord preparation

On day 4 or 5 after the neonatal rat was weighed it was anesthetized with carbon dioxide and the spinal column from T10 to the tail was removed and placed in artificial cerebrospinal fluid (ACSF) consisting of (mM) NaCl 138.6, KCl 3.35, $NaHCO_3$ 21.0, Na_2HPO_4 0.58, $CaCl_2$ 2.5, $MgCl_2$ 1.25, glucose 12.0, and was bubbled constantly with 95% oxygen-5% CO_2. The spinal cord was exposed and the dura was carefully removed from the dorsal surface of the cord. The attached dorsal and ventral roots were cut distal to their entry into the cord. The spinal cord was then placed in a 1 ml controlled temperature bath and was superfused with ACSF 27°C at a rate of 5 ml/min.

2.5. Electrophysiology preparation

Glass capillary suction electrodes were used to stimulate the dorsal root and record from the ventral root. The tip of the recording electrode was placed near the ventral root (L4 or L5), and the root was sucked carefully into the electrode until the tip of the electrode pressed lightly against the spinal cord. The recording electrode was connected through an AgCl pellet to a DC preamplifier. The dorsal root was connected to an electrode in the same manner. To activate high threshold reflexes the dorsal root was stimulated intermittently with a square wave pulse of 50 V/1 ms duration. This stimulus produced fast mono- and polysynaptic responses that were overwhelmed by the stimulus artifact, followed by a long lasting slow ventral root potential of 0.5–1.0 mV and lasting 10–30 s. The slow depolarization corresponds to a nociceptive reflex produced by tail pinch in the same preparation (Yanagisawa et al., 1985).

2.6. Measurement of tolerance in the isolated spinal cord

After baseline ventral root potential responses were established, morphine concentration-response curves were constructed by superfusing increasing concentrations of morphine by orders of magnitude from 10 nM up to 10 μM. Concentrations of morphine above 10 μM exhibited excitatory effects (not shown). These concentration-response curves can be considered cumulative. Ventral root potentials were generated once every 30 min. Voltage data were digitized (10 Hz) using

the Basic-Fastlab data acquisition program run on a 386 PC and analyzed off-line. Drug responses were expressed as percentage inhibition of the response with respect to baseline, of the electrically induced depolarization of the ventral root.

2.7. Data analysis

Baseline ventral root potential measurements were established before morphine was added to the spinal superfusate. Morphine-induced depression of the ventral root potential was expressed as percentage depression of the baseline ventral root potential. The area under the concentration-response curves produced by adding acute morphine to the spinal superfusate was calculated using the trapezoidal rule (Tallarida and Murray, 1987). The area values for each concentration-response curve were analyzed by one-way analysis of variance (ANOVA) comparing treatment groups. This was followed by post-hoc comparisons using the Newman-Keuls multiple comparisons test to assess significance of drug effects on the areas of the individual concentration-response curves among different treatment groups. Effects were considered significant if $P < 0.05$.

3. Results

3.1. Effect of chronic treatment with morphine or morphine + MK-801 on development of tolerance

Fig. 1 shows typical examples of ventral root potentials recorded from the L5 ventral root following single shock stimulation of the ipsilateral L5 dorsal root. Stimulation of the dorsal root at low intensity (5 V, 200 μs) activates Aβ/ group I/II range fibers and produces a short latency ventral root potential lasting about 2 s (Thompson et al., 1992). We used single shock stimulation of the dorsal root at high stimulus intensities (50 V, 1 ms) to activate nociceptive C-fibers (Thompson et al., 1992) and evoke a prolonged ventral root potential. Fig. 1 demonstrates that in isolated spinal cords from saline-treated controls acute morphine did not depress the Aβ fiber-mediated short latency ventral root potential (first 2 s) but selectively depressed the C-fiber-mediated prolonged portion of the ventral root potential. Acute morphine did not markedly depress the prolonged portion of the ventral root potential (Fig. 1, middle panel) in chronic morphine-treated spinal cords indicating the development of tolerance. Chronic treatment of neonates with mor-

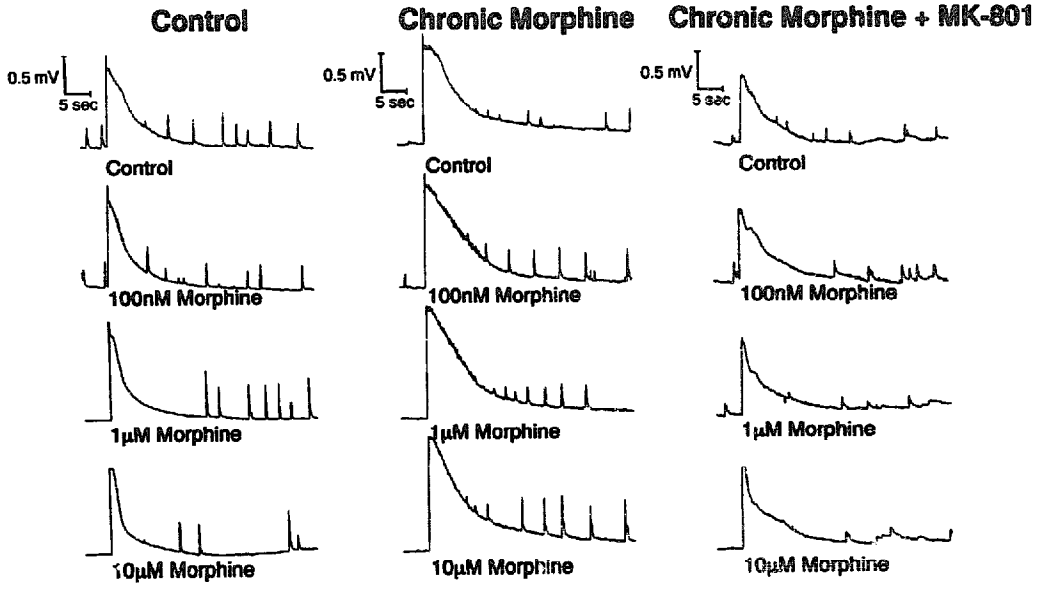

Fig. 1. Effects of four concentrations of morphine on electrically induced ventral root potentials in spinal cords from three groups of neonatal rats. Potentials were recorded extracellularly from the L5 ventral root of an isolated spinal cord preparation from a 5 to 6 day old rat. The L5 dorsal root was stimulated with a single shock (50 V 1 ms) every 30 min. The control spinal cord was from a neonate that received saline injections for 4 days. Chronic morphine spinal cord was from a neonate which received morphine injections twice daily of 20 mg/kg on day 1, 40 mg/kg on day 2 and 60 mg/kg on the last 2 days. Chronic morphine + MK-801 was from a neonate that received the same dosage of morphine as above as well as MK-801 (0.3 mg/kg) with each injection. Traces demonstrate the depressant effects of various concentrations of morphine after different chronic treatments of the neonates. Treatment with chronic morphine or chronic morphine + MK-801 produced significant tolerance to the depressant effects of morphine.

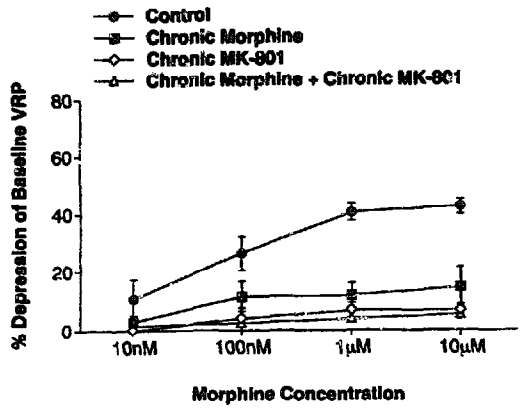

Fig. 2. Effects of morphine on electrically induced ventral root potentials. An L5 dorsal root from the isolated spinal cord of a 5 to 6 day old rat was stimulated once every 30 min. Concentration-response curves show the effects of morphine on the ventral root potential to single shocks. Percentage inhibition of the area of the response was plotted against the concentration of morphine. Each point and vertical bar show mean and S.E.M. ($n = 6$). Experimental procedures were the same as in Fig. 1. The area under the concentration-response curves produced by adding acute morphine to the spinal superfusate was calculated using area under the curve: trapezoidal and Simpsons rules (Tallarida and Murray, 1987). The area values for each concentration-response curve were analyzed by one-way analysis of variance (ANOVA) comparing treatment groups. This was followed by post-hoc comparisons using the Newman-Keuls multiple comparisons test to assess significance of drug effects on the areas of the individual concentration-response curves among different treatment groups. Prior treatment of the neonates with morphine alone, morphine + MK-801 and MK-801 alone caused a statistically significant attenuation of morphine-induced depression of the ventral root potential when compared to saline-treated controls.

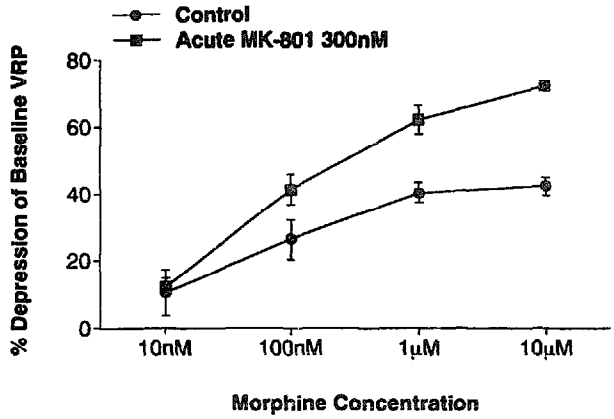

Fig. 3. Effects of acute morphine on electrically induced ventral root potentials. An L5 dorsal root from the isolated spinal cord of a 5 to 6 day old rat was stimulated once every 30 min. Concentration-response curve showing the effects of morphine on the ventral root potential to single shocks. Percentage inhibition of the area of the response was plotted against the concentration of morphine. Each point and vertical bar show the mean and S.E.M. ($n = 6$). These experiments were performed in 5 to 6 day old rats that had received saline injections for 4 days. Data analysis on area of concentration-response curves same as in Fig. 2. Addition of MK-801 (300 nM) to the superfusate significantly (Newman-Keuls $P < 0.01$) enhanced morphine's depressant effects on the ventral root potential.

phine + MK-801 (0.3 mg/kg) also produced tolerance to acute morphine-induced depression of the prolonged ventral root potential (Fig. 1, right panel).

In Fig. 2 the overall data for morphine concentration-response curves are shown as percent depression of mean baseline values of the integral of the ventral root potentials. Surprisingly chronic treatment of neonates with MK-801 alone as well as morphine and morphine + MK-801 caused a significant attenuation of the depressant effects of acute morphine (Fig. 2). Statistical analysis of the area under the dose-response curves for the various drug treatments revealed that they were significantly different from the chronic saline group (Newman-Keuls $P < 0.01$), but not from each other.

3.2. Effect of acute MK-801 after chronic saline treatment

Although NMDA receptor antagonists are known to reduce the ventral root potential, we examined acute synergistic MK-801 effects on morphine-induced depression of the ventral root potential. In order to do this we choose a physiologically relevant concentration of MK-801 (300 nM) (Scheller et al., 1989) which by itself had no depressant effect on the ventral root potential, whereas MK-801 (3 μM) depressed the slow ventral root potential by 42.0 ± 5.6% ($n = 4$). In spinal cords from neonates chronically treated with saline, acute MK-801 (300 nM) significantly (Newman-Keuls

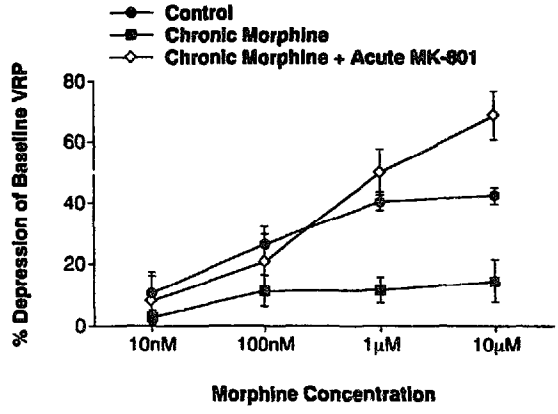

Fig. 4. Effects of morphine on electrically induced ventral root potentials. An L5 dorsal root from the isolated spinal cord of a 5 to 6 day old rat was stimulated once every 30 min. Concentration-response curve showing the effects of morphine on the ventral root potential to single shocks. Percentage inhibition of the area of the response was plotted against the concentration of morphine. Each point and vertical bar show the mean and S.E.M. ($n = 6$). Experimental procedures were the same as in Figs. 1 and 2. Data analysis of area under the concentration curve was same as in Fig. 2. Prior treatment of the neonates with morphine caused a statistically significant ($P < 0.01$) attenuation of morphine-induced depression of the ventral root potential. Addition of MK-801 to the superfusate significantly ($P < 0.01$) amplified the depressant effects of morphine in spinal cords from neonates that had been chronically treated with morphine.

$P < 0.01$, area under the concentration-response curve) enhanced the depressant effects of morphine on the electrically evoked ventral root potential (Fig. 3). The depressant effects were increased by 15%, 20% and 30% at the 100 nM, 1 μM and 10 μM concentrations of morphine, respectively.

3.3. Effect of acute MK-801 after chronic morphine treatment

As demonstrated above, in saline-treated rats, acute MK-801 enhanced morphine-induced depressant effects on the electrically evoked ventral root potential. Consequently, we examined the effect of acute MK-801 on morphine-induced depression of the ventral root potential in spinal cords from neonates receiving chronic drug administration. In chronic morphine-treated isolated spinal cords, tolerance was shown by a flattening of the concentration-response curve to the depressant effect of morphine on the ventral root potential (Fig. 2). In spinal cords from chronic morphine-treated neonates, addition of MK-801 to the spinal superfusate apparently reversed tolerance to the depressant effects of acute morphine (Fig. 4). The area under the chronic morphine with acute MK-801 concentration-response curve is significantly different (Newman-Keuls $P < 0.01$) from the chronic morphine curve. The chronic saline and the chronic morphine with acute MK-801 curves are not significantly different.

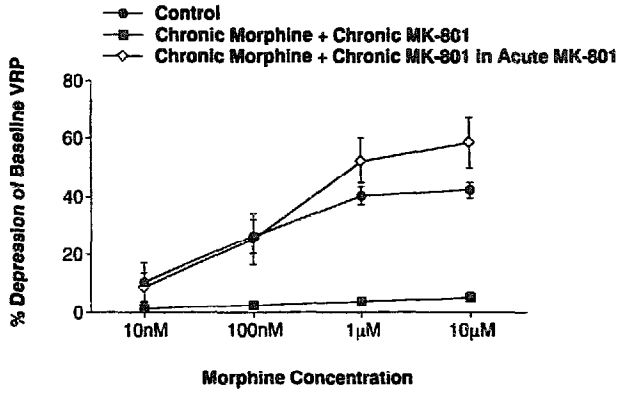

Fig. 5. Effects of morphine on electrically induced ventral root potentials. An L5 dorsal root from the isolated spinal cord of a 5 to 6 day old rat was stimulated once every 30 min. The concentration-response curve shows the effects of morphine on the ventral root potential to single shocks. Percentage inhibition of the area of the response was plotted against the concentration of morphine. Each point and vertical bar show mean and S.E.M. ($n = 6$). Experimental procedures were the same as in Figs. 1 and 2. Data analysis of area under the concentration-response curves was the same as in Fig. 2. Co-treatment with morphine + MK-801 caused a statistically significant (Newman-Keuls $P < 0.01$) attenuation of morphine-induced depression of the ventral root potential. Addition of MK-801 (300 nM) to the superfusate produced a significant reversal of the tolerance to morphine's depressant effects that is produced by chronic co-treatment with morphine + MK-801 (Newman-Keuls $P < 0.01$).

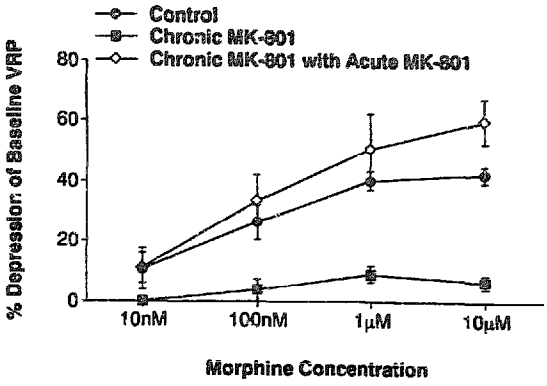

Fig. 6. Effects of morphine on electrically induced ventral root potentials. An L5 dorsal root from the isolated spinal cord was stimulated once every 30 min. Concentration-response curve showing the effects of morphine on the ventral root potential to single shocks. Percentage inhibition of the area of the response was plotted against the concentration of morphine. Each point and vertical bars show mean and S.E.M. ($n = 6$). Experimental procedures were the same as in Figs. 1 and 2. Data analysis of area under the concentration-response curves was the same as in Fig. 2. Chronic treatment of neonates with MK-801 alone caused significant tolerance to the depressant effects of morphine. Acute MK-801 (300 nM) significantly reversed the tolerance to the depressant effects of acute morphine (Newman-Keuls $P < 0.01$).

3.4. Effect of acute MK-801 after chronic morphine + MK-801 treatment

Co-chronic treatment of neonates with morphine + MK-801 produced a marked tolerance to the depressant effect of acute morphine on the ventral root potential. In this experiment, MK-801 (300 nM) was added to the superfusate of spinal cords from neonates chronically treated with both morphine and MK-801. Addition of acute MK-801 to the superfusate reversed tolerance produced by chronic morphine + MK-801 (Fig. 5). The area under the chronic morphine + MK-801 with acute MK-801 concentration-response curve was significantly different from the chronic morphine + MK-801 curve (Newman-Keuls $P < 0.01$). The chronic saline and the chronic morphine + MK-801 with acute MK-801 curves were not significantly different.

3.5. Effect of acute MK-301 after chronic MK-801 treatment

In spinal cords from neonates treated chronically with MK-801 alone acute morphine did not depress the ventral root potential. In experiments where acute MK-801 (300 nM) was added to the superfusate mor-

phine significantly depressed the ventral root potential in cords from neonates receiving chronic MK-801 alone (Fig. 6).

4. Discussion

In the present study, we established an electrophysiological model of opiate tolerance in the isolated spinal cord of the neonatal rat. In the isolated spinal cord from morphine-treated neonatal rats tolerance was defined as a decreased depressant effect of acute morphine on an electrically evoked delayed ventral root potential. Tolerance to morphine-induced decreases in the firing rate of spontaneously active isolated neurons has been demonstrated in the locus coeruleus slice from morphine-treated rats (Andrade et al., 1983; Christie et al., 1987, Nestler, 1992). As an isolated electrophysiological model of opiate tolerance, the spinal cord preparation from morphine-treated neonatal rats seems comparable to the locus coeruleus slice preparation obtained from morphine-treated adult rats. The locus coeruleus (Andrade et al., 1983; Christie et al., 1987) and the spinal cord (DeLander and Takemori, 1983; Yaksh et al., 1977) are principal central nervous system sites of opioid tolerance. The locus coeruleus represents a single cell model of tolerance in which postsynaptic mechanisms of tolerance can be studied (Andrade et al., 1983; Christie et al., 1987). The ventral root potential from the isolated spinal cord represents activity of pre- and postsynaptic elements in an integrated synaptic circuitry amenable to in vitro study (Thompson et al., 1994). Therefore the isolated spinal cord preparation could be a useful adjunct to the locus coeruleus slice wherein pre- and postsynaptic mechanisms of the development and expression of morphine tolerance can be examined.

Chronic treatment of neonatal rats with morphine, morphine + MK-801, and MK-801 alone caused tolerance to morphine-induced depression of the electrically evoked delayed ventral root potential. The results of the present study contrast to recent findings demonstrating that co-chronic treatment with MK-801 and morphine attenuated the development of tolerance to morphine analgesia (Elliot et al., 1994; Gustein and Trujillo, 1993; Kest et al., 1993; Lutfy et al., 1993; Marek et al., 1991a; Trujillo and Akil, 1991a, 1994). Therefore, the results of the present study do not support a hypothesis that chronic MK-801 administered with chronic morphine attenuates the development of tolerance. It should be noted, however that these discrepant findings could be due to differences in experimental procedures or the use of neonatal spinal cords in which NMDA receptor levels and distribution are different from adult rats (Gonzalez et al., 1993).

The major finding of the present study was that addition of MK-801 to the solution superfusing the isolated cord reversed tolerance produced by chronic morphine, morphine + MK-801 and MK-801 alone. This finding is consistent with the results of studies of Tiseo and Inturrisi (1993) demonstrating reversal of morphine tolerance by a competitive glutamate receptor antagonist. However, chronic pretreatment with the competitive glutamate receptor antagonist did not prevent subsequent development of morphine tolerance (Tiseo et al., 1994). The apparent reversal of tolerance may be related to synergistic actions of morphine and MK-801. In the present study, we utilized a low concentration of MK-801 (300 nM) that did not depress the ventral root potential (data not shown) but acted synergistically with morphine to increase the depression of the ventral root potential produced by a given concentration of morphine. Glutamate receptor antagonists have previously been shown to synergistically increase opioid analgesia. For example, kynurenic acid, a non-specific glutamate receptor antagonist, and MK-801 caused a robust augmentation of morphine analgesia as demonstrated by an increased latency to paw lick in the hot plate test (Ben-Eliyahu et al., 1992; Marek et al., 1991b). Regarding non-analgesic morphine effects, MK-801 has also been reported to increase morphine toxicity (Trujillo and Akil, 1991b), catalepsy (Ben-Eliyahu et al., 1992; Trujillo and Akil, 1991b) and it amplified morphine effects on the EEG (Haberny and Young, 1994).

Tolerance is manifest when after repeated administration, a given concentration of a drug produces a decreased response (Jaffe, 1970). In the present study, MK-801 acted synergistically with morphine to increase the depression of the ventral root potential produced by a given concentration of morphine. Therefore, in the present experiments, acute MK-801 probably attenuated the expression of tolerance because it potentiated acute morphine effects. Thus, it is possible that in some previous studies (Elliot et al., 1994; Gustein and Trujillo, 1993; Kest et al., 1993; Lutfy et al., 1993; Marek et al., 1991a; Trujillo and Akil, 1991a, 1994) the development of tolerance may not have been attenuated but instead its expression masked by the presence of residual MK-801. Although the plasma half life of MK-801 is relatively short (Scheller et al., 1989), its hydrophobicity may cause prolonged sequestration in central nervous system lipids. In the present study, for example, addition of MK-801 (300 nM) to the isolated spinal cord perfusate consistently attenuated NMDA-induced depolarizations for up to 8 h (data not shown).

We found that chronic treatment of neonates with MK-801 alone produced tolerance to acute morphine administration. This was an unexpected finding because previous studies utilizing tail flick or hot plate have demonstrated no effect of chronic MK-801 on morphine-induced antinociception (Elliot et al., 1994;

Gustein and Trujillo, 1993; Kest et al., 1993; Lutfy et al., 1993; Marek et al., 1991a; Trujillo and Akil, 1991a, 1994). However, the tail flick and hot plate test may be measures of only epicritic pain whereas, the prolonged ventral root potential may represent an NMDA receptor-mediated protopathic pain. Chronic MK-801 upregulates NMDA receptors (McDonald et al., 1990). Opiates block the release of glutamate in the spinal cord (Malmberg and Yaksh, 1995). Therefore an apparent tolerance may develop because morphine blockade of glutamate-mediated transmission may be canceled by upregulated NMDA receptors in neonates treated with chronic MK-801.

In conclusion, the isolated spinal cord is a useful preparation for examining the role of neurotransmitters as well as pre- and postsynaptic mechanisms involved in the development and expression of tolerance. We further conclude that facilitation of morphine analgesia by MK-801 can mask the expression of morphine tolerance leading to the misunderstanding of its role in tolerance development.

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