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Co-treatment with MK-801 potentiates naloxone-precipitated morphine withdrawal in the isolated spinal cord of the neonatal rat

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Abstract

The effects of acute and chronic administration of (MK-801; [(+)-5-methyl-10,11-dihydro-5*H*-dibenzo[*a*,*d*]cyclo-hepten-5,10-imine hydrogen maleate) were assessed on morphine dependence in the isolated spinal cord of the neonatal rat and on behavioral measures in intact adult rats. Neonatal rats were treated chronically (3 or 4 days) with injections of either morphine, morphine + MK-801, or saline. Naloxone (10 μM) which increased baseline ventral root spontaneous firing, induced more activity in spinal cords from morphine-treated neonates than in saline controls. In spinal cords from neonates receiving MK-801 with morphine, naloxone-induced spontaneous firing was significantly greater than in saline-treated and morphine alone-treated neonates. Acute MK-801 attenuated naloxone-induced firing in the morphine-treated group. Chronic co-treatment with MK-801 increased locomotor signs of withdrawal and decreased mastication in intact adult rats which had been treated chronically with morphine. MK-801-induced enhancement of morphine withdrawal is consistent with upregulation of NMDA receptors.

Keywords: MK-801; Morphine withdrawal; Spinal cord; Naloxone; Morphine dependence

1. Introduction

Chronic administration of opioids leads to the development of opioid dependence. Recent studies utilizing the *N*-methyl-D-aspartate (NMDA)-type glutamate receptor antagonist (MK-801; [(+)-5-methyl-10,11-dihydro-5*H*-dibenzo[*a*,*d*]cyclo-hepten-5,10-imine hydrogen maleate) have proposed that NMDA receptors play an important role in the development and expression of opioid physical dependence (Lutfy et al., 1993; Trujillo and Akil, 1991b). These findings suggest NMDA-type glutamate receptor-mediated neurotransmission is important in producing physical dependence following chronic opioid administration. However, MK-801 produces movement disorders (Tricklebank et al., 1989; Trujillo and Akil, 1991a,b) that could interfere with the assessment of motor signs of physical dependence. In the present study, we examined the role of spinal glutamatergic neurotransmission in opioid dependence using an in vitro preparation independent from the confounding effects of motor impairment. The neonatal rat spinal cord preparation (Otsuka and Konishi, 1974) allows electrophysiological evaluation of synaptic manifestations of opioid withdrawal. For example, the spinal cord is an important locus subserving opioid withdrawal (Delander and Takemori, 1983; Yaksh et al., 1977). Also, electrophysiological experiments have demonstrated that the acutely dependent isolated neonatal rat spinal cord is capable of generating withdrawal symptoms (Bell and Jaffe, 1986). In the present study, we sought to validate this preparation as an in vitro model following chronic administration of morphine to neonates and to examine the effects of MK-801 on the development of opioid physical dependence at the spinal level.

2. Materials and methods

2.1. Animals

Newborn Sprague-Dawley rats were housed in cages with the maternal lactating mother rat (Charles River

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Breeding Laboratories) until dissections were to be performed.

For behavioral studies, male, Fischer-344 rats (Charles River Breeding Laboratories), shipped at 86 days of age, were housed in a temperature controlled vivarium for 1–3 months with access to food and water ad libitum before being used in experiments.

2.2. Drugs

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

Morphine (75 mg free base) or placebo pellets (University of North Carolina School of Pharmacy, Drug Products Program, Chapel Hill, NC) were used for behavioral studies.

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

In behavioral studies, morphine pellets were implanted subcutaneously while rats were anesthetized with halothane (5% < 3 min). One pellet was implanted on day 1; two pellets were implanted 3 days later. MK-801 (0.1 mg/kg or 0.3 mg/kg) was injected once daily (s.c.).

2.4. Spinal cord preparation

On day 4 or 5 after the neonatal rat was weighed it was anesthetized with ether and the spinal column from T10 to the tail was removed and placed in artificial cerebrospinal fluid (ACSF). The ACSF consisted 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. After the spinal cord was exposed and the dura 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 into a 1 ml controlled temperature bath and was superfused with ACSF at a rate of 5 ml/min. The bath temperature was maintained at 27°C.

2.5. Electrophysiology preparation

Glass capillary suction electrodes were used to 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.

2.6. Electrophysiological measurements of opioid withdrawal

In electrophysiological studies of withdrawal, spinal cords from neonates treated with chronic saline, morphine or morphine + MK-801 were used. As part of a companion study of tolerance each preparation received morphine for 1 h in concentrations of 10 nM, 100 nM, 1 μM and 10 μM sequentially. After 1 h of superfusion with the highest concentration of morphine it was replaced in the superfusate with naloxone (10 μM). Spontaneous activity was recorded from the ventral root for 10 min subsequent to the introduction of naloxone into the bath. The spontaneous activity was digitized and the integral of the activity above baseline was measured. The area of the integral of spontaneous activity obtained was defined as the measure of physical dependence.

2.7. Measurement of withdrawal in adult intact rats

In intact adult rats, on day 5 of morphine administration, withdrawal was precipitated with naloxone (0.5 mg/kg, s.c.). Each rat was weighed and then allowed to acclimate for 15 min to a Plexiglas observation chamber (46 cm × 46 cm × 26 cm) with lines dividing the bottom into quadrants. After naloxone injection, rats were returned to the chamber, and withdrawal signs were scored on individual rats for 15 min by an investigator who was blinded to the treatments. The number of occurrences of each of the following withdrawal signs was recorded: quadrant crossing, rearing, jumping, abnormal posturing, wet dog shakes, teeth chattering, ejaculation/penile licking and excessive grooming. An abnormal posture was defined as one in which the rat lay on its abdomen or side with its spine in an unusually straight position. Exploratory activity was quantified as the sum of quadrant crossings and rearing. Presence of diarrhea and loss of body weight were recorded.

2.8. Data analysis

One-way analysis of variance (ANOVA) followed by post-hoc comparisons using the Mann-Whitney 'U'-test were used to test significance of drug effects among

different treatment groups. Effects were considered significant if $P < 0.05$.

3. Results

3.1. Effect of chronic MK-801 on development of physical dependence

We used spinal cords from a companion tolerance study for examination of electrophysiological manifestations of morphine withdrawal. These spinal cords received four concentrations of morphine incrementing by an order of magnitude from 10 nM to 10 μM at 1 h intervals. Following the generation of morphine dose-response curves in these preparations naloxone (10 μM) produced increases in spontaneous firing recorded from the ventral root. The integral (10 min) of the increased spontaneous firing immediately after introduction of naloxone into the superfusate was considered proportional to the degree of physical dependence (Fig. 1). In spinal cords from saline-treated neonates naloxone increased baseline firing rates after acute morphine demonstrating acute withdrawal. Chronic treatment of neonates with morphine significantly (Mann-Whitney 'U'-test $P < 0.05$) amplified naloxone-induced increases in baseline firing (Fig. 1). Chronic treatment of neonates with morphine + MK-801 caused a significantly greater increase in naloxone-induced spontaneous firing than that produced by chronic treatment with morphine alone (Mann-Whitney 'U'-test $P < 0.01$). In spinal cords from neonates that received chronic morphine, addition of MK-801 (300 nM) to the spinal cord superfusate significantly (Mann-Whitney 'U'-test $P < 0.01$) suppressed electrophysiological manifestation of physical dependence as naloxone-induced firing recorded from the ventral root was almost completely blocked (Fig. 1).

3.2. Effect of chronic MK-801 on withdrawal signs in adult rats

Because chronic MK-801 co-treatment with morphine augmented electrophysiologic signs of withdrawal in the isolated neonatal rat spinal cord, we wanted to investigate whether this was manifested in adult rat withdrawal behavior. Therefore, the effect of co-administration of MK-801 with chronic morphine (pellets) on naloxone-precipitated withdrawal was examined in intact adult rats. In this part of the study, we examined the effects of naloxone-precipitated withdrawal on a wide range of motor and autonomic behaviors (see Methods). We looked specifically at the comparison between rats that received a regimen of morphine pellets and rats that received morphine pellets and daily injections of MK-801 (0.3 mg/kg and 0.1

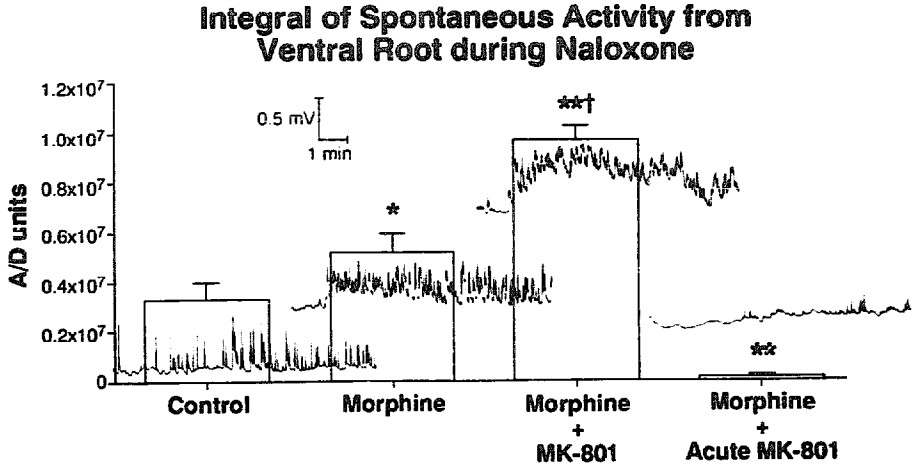

Fig. 1. Potentials were recorded extracellularly from the L5 ventral root of an isolated spinal cord preparation from a 5 to 6 day old rat. Following generation of morphine concentration curves the isolated spinal cord was superfused with artificial cerebral spinal fluid containing naloxone (10 μM) for 10 min. The control spinal cord was from a neonate which received saline injections for 4 days. Chronic morphine spinal cord was from a neonate that 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. Spontaneous firing for 10 min was digitized, and the integral was expressed in analog to digital (A/D) units. The insets represent digitized examples of the spontaneous firing for the three treatment conditions and the bars represent the average integral of activity (A/D units) recorded for the 10 min. period. The recording period began immediately upon entry of the naloxone into the recording chamber. Chronic treatment with morphine (*, Mann-Whitney 'U'-test $P < 0.05$) and morphine + MK-801 significantly (**, Mann-Whitney 'U'-test $P < 0.01$) increased the amount of naloxone-induced spontaneous firing recorded from the ventral root when compared to control. Chronic morphine + MK-801 treatment caused greater naloxone-induced spontaneous firing than chronic treatment with morphine alone (dagger indicates $P < 0.05$ when compared to chronic morphine alone, Mann-Whitney 'U'-test). Chronic treatment with morphine followed by acute addition of MK-801 (300 nM) to the superfusate significantly (**, Mann-Whitney 'U'-test $P < 0.01$) attenuated the spontaneous firing generated by naloxone in spinal cords from neonates treated chronically with morphine when compared to the chronic morphine group.

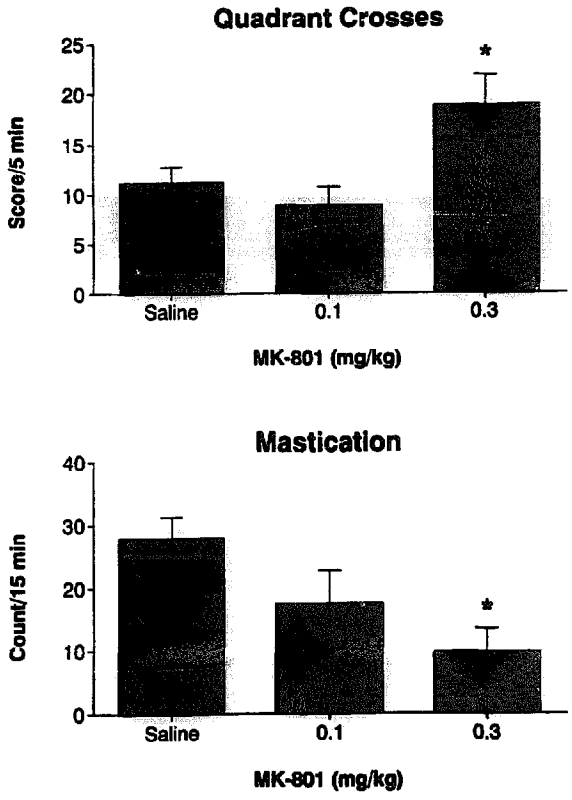

Fig. 2. Upper panel: effect of MK-801 (0.01 or 0.3 mg/kg i.p.) on total movement of the rat in a plexiglas chamber (Quadrant Crosses) observed for 5 min following 0.5 mg/kg naloxone. Each bar represents the mean ± S.E.M. for six rats. Rats were made morphine-dependent over a 5 day period (one 75 mg pellet for 3 days, two pellets for 2 days); MK-801 was administered once per day (i.p.). No MK-801 was administered on the day of naloxone challenge. * $P < 0.05$ versus control (0 mg/kg MK-801) Dunnett's test (one tailed). Lower panel: effect of MK-801 (0.1 or 0.3 mg/kg i.p.) on mastication observed for 15 min following 0.5 mg/kg naloxone. * $P < 0.05$ versus control (0 mg/kg MK-801) Dunnett's test (one tailed). Conditions were the same as the upper panel.

mg/kg). We found no significant difference between the three treatment groups (saline, 0.1 mg/kg and 0.3 mg/kg MK-801) on most signs of withdrawal. In the first 5 min of observation, motor activity increased significantly ($P < 0.05$, $n = 6$), as measured by quadrant crossing, in the group that received the high dose of MK-801 (Fig. 2, upper panel). Conversely, the control group demonstrated a greater increase in mastication over the 15 min time period (Fig. 2, lower panel). There was no significant difference between the groups in jumping behavior although a marked ataxia was observed in the group receiving the highest dose of MK-801 (data not shown).

4. Discussion

The present study produced no evidence that administration of chronic MK-801 along with chronic morphine attenuates the development of physical dependence manifested either in electrophysiological signs from the isolated spinal cord of the neonatal rat or in adult rat behavioral signs. This finding contrasts with many recent results that demonstrate an attenuation of physical dependence development by co-treatment with MK-801 (Lutfy et al., 1993; Trujillo and Akil, 1991a). The reason for this difference is not clear, but it could relate to the effects of glutamate receptor antagonists on motor behavior. MK-801 has effects on motor behavior (Tricklebank et al., 1989) which can interfere with the interpretation of expression of physical dependence. For instance, Trujillo and Akil (1991b) used jumping behavior as an index of physical dependence and reported that a decrease in jumping behavior, following chronic co-treatment with morphine + MK-801, indicated attenuated physical dependence. In the present study we found in adult rats that jumping per se was affected in rats that had received chronic MK-801. The rats seemed to initiate jumps during naloxone-precipitated withdrawal, but were too ataxic to complete them. Therefore in the study of Trujillo and Akil (1991b) the ataxia produced by chronic MK-801 administration may have masked their measure of physical dependence.

In the present study using the isolated spinal cord, chronic co-treatment with morphine + MK-801 caused an increased naloxone-induced spontaneous firing of motoneurons when compared to chronic treatment with morphine alone. Thus, co-treatment with MK-801 enhanced the effects of naloxone-precipitated withdrawal. Conversely, addition of MK-801 to the superfusate of isolated spinal cords from neonates that received chronic morphine injections attenuated naloxone-induced spontaneous firing. Regarding motor activity, the present findings are consistent with the studies of Koyuncuoglu et al. (1992) done in intact adult rats in which chronic MK-801 administered in conjunction with morphine pellets intensified naloxone-induced motor signs of withdrawal, whereas acute MK-801 administered just prior to naloxone reduced withdrawal signs. In the present study, unlike Koyuncuoglu et al. (1992) we did not observe increases in ptosis. Opiates have been demonstrated to block the release of glutamate in the spinal cord (Malmberg and Yaksh, 1995), and MK-801 blocks postsynaptic NMDA-type glutamate receptors (Wong et al., 1986). These two factors could lead to an upregulation and supersensitivity (McDonald et al., 1990) of glutamate receptors which could lead to a more intense opioid withdrawal.

Previous studies have shown that behavioral signs of opioid withdrawal can be attenuated by acute MK-801 administration (Higgens et al., 1992; Koyuncuoglu et al., 1992) and the competitive NMDA receptor antagonist LY 274614 (Rasmussen et al., 1991a). The present study demonstrates that electrophysiological manifestations of physical dependence can be blocked in the

neonatal cord by acutely administered MK-801. This may be due to a blockade of opioid withdrawal-induced increases in spinal glutamatergic activity. Increases in glutamatergic transmission can contribute to expression of physical dependence (Aghajanian et al., 1994; Oleskevich et al., 1993; Rasmussen and Aghajanian, 1989; Rasmussen et al., 1991b). NMDA receptor levels peak transiently on days 6-10 in neonatal mice (Gonzalez et al., 1993). If this is true in neonatal rats it may explain the intensity of the effect which we observed. Therefore, if excessive glutamatergic neurotransmission plays a role in withdrawal then administration of a glutamate receptor antagonist such as MK-801 could attenuate signs of withdrawal.

We conclude that the isolated spinal cord from neonatal rats chronically treated with opioids is a useful preparation for examining the role of neurotransmitters in the development and expression of physical dependence. We further conclude that co-treatment with MK-801 potentiates morphine withdrawal and this is probably due to upregulation of glutamate receptors. Furthermore postsynaptic blockade of excessive glutamatergic neurotransmission by MK-801 can mask the expression of morphine physical dependence.

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