INHIBITORY EFFECT OF
THE NMDA RECEPTOR ANTAGONIST,
DIZOCILPINE (MK-801), ON THE
DEVELOPMENT OF MORPHINE DEPENDENCE

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ABSTRACT — We investigated the effect of a noncompetitive N-methyl-D-aspartate (NMDA) receptor antagonist, (+)-5-methyl-10,11-dihydro-5H-dibenzo[a,d]cyclohepten-
5,10-iminehydrogen maleate (dizocilpine, MK-801), on hippocampal norepinephrine release in morphine-treated rats in order to clarify the relationship between NMDA receptors and the development of morphine dependence. Naloxone hydrochloride injected subcutaneously (s.c.) into morphine-dependent rats, induced an immediate increase in hippocampal norepinephrine release, which was associated with a typical morphine withdrawal syndrome. The increased norepinephrine levels persisted for at least 2 hr, even after the disappearance of the behavioral withdrawal syndrome. This striking effect of naloxone on hippocampal norepinephrine release was dependent on the duration of the intracerebroventricular (i.c.v.) morphine infusion. Pretreatment with dizocilpine (s.c.) before naloxone challenge reduced the rate of the rise in hippocampal norepinephrine release induced by naloxone in morphine-treated rats. Concurrent infusion (i.c.v.) of dizocilpine and morphine decreased the level of hippocampal norepinephrine release after a naloxone challenge. Both pretreatment with dizocilpine (s.c.) before naloxone injection and infusion (i.c.v.) of dizocilpine suppressed rearing and teeth-chattering signs, but not wet-dog shakes in morphine-treated rats. These results suggest that dizocilpine attenuates the development of morphine dependence through NMDA receptors, and thus that interaction between opioid receptors and NMDA receptors may be involved in the development of morphine dependence.

KEY WORDS: Morphine dependence, Naloxone-precipitated withdrawal, Nor
epinephrine, Dizocilpine (MK-801), Hippocampus.
INTRODUCTION

There is an evidence that central noradrenergic neurons are involved in opiate-modified function, that is, opiate dependence and tolerance (Redmond and Krystal, 1984). In particular, precipitation of withdrawal in morphine-dependent rats by the administration of opioid antagonists such as naloxone causes an elevation in the firing rate of noradrenergic neurons in the locus coeruleus (LC) and an increase in norepinephrine turnover in the rat forebrain, as well as causing a behavioral syndrome which has been associated with these effects (Aghajanian, 1978; Rasmussen et al., 1990). Furthermore, evidence has been presented that this striking effect of naloxone on norepinephrine release is markedly attenuated by pretreatment with an alpha₂-adrenergic agonist, clonidine (Taylor et al., 1988; Done et al., 1992), and that (+)-5-methyl-10,11-dihydro-5H-dibenzo[a,d]cyclohepten-5,10-imine hydrogen maleate (dizocilpine, MK-801) inhibits tolerance to the analgesic effect of morphine and morphine dependence as assessed by naloxone-precipitated withdrawal in morphine-treated rats (Trujillo and Akil, 1991). Since it is possible that dizocilpine is acting at a site distal from LC cell bodies, we examined the effects of dizocilpine on norepinephrine release in the hippocampus, which receives its norepinephrine input solely from the LC (Foote et al., 1983).

The aim of the present study was to elucidate the role of N-methyl-D-aspartate (NMDA) receptors in the development of morphine dependence by measuring the extracellular norepinephrine in the hippocampus of freely moving rats.

MATERIALS AND METHODS

Animals: Seven- to eight-week-old male Wistar rats (210–250 g) were purchased from Sankyo Laboratory Service Corp., Inc. (Shizuoka, Japan). The animals were housed in a room with an ambient temperature of 21 ± 2°C on a 12 hr light: 12 hr dark schedule and were allowed free access to food and water.

Chemicals: Morphine hydrochloride was purchased from Sankyo Co. Ltd. (Tokyo, Japan), naloxone hydrochloride was from Sigma Chemical Co. Ltd. (St Louis, MO, USA), dizocilpine (MK-801) was from Research Biochemicals International (Natick, MA, USA) and sodium 1-octane sulfonate was from Nacarai Tesque Inc. (Kyoto, Japan). Other reagents were purchased from Wako Pure Chemical Industries Ltd. (Tokyo, Japan). All reagents were of analytical grade.

Surgical Procedures: Rats were anesthetized with pentobarbital sodium (50 mg/kg, i.p.). An indwelling stainless steel guide cannula (26 gauge, 10 mm long) was stereotaxically implanted into the lateral cerebral ventricle (AP: −0.5 mm from the bregma, L: ±1.3 mm from the midline, and DV: −4.5 mm below the skull surface; Paxinos and Watson, 1986) for the infusion of morphine via an osmotic minipump, which allowed direct, continuous administration to the brain.

In addition, a guide cannula for use as a microdialysis probe was implanted into the ventral hippocampus (AP: −5.8 mm from the bregma, L: ±4.8 mm from the midline, DV: −4.5 mm below the skull surface) and cemented in place. After surgery, the rats were allowed at least 4 days to recover before the morphine infusion began.

Administration Schedule and Induction of Morphine Dependence: Each animal was infused (i.c.v.) with saline or 50 nmol/μl morphine hydrochloride at a rate of 1 μl/h for 24 hr or 72 hr via an osmotic minipump (Alzet 2001; Alza Corp., Palo Alto, CA, USA) which had been implanted subcutaneously between the scapulae under ether anesthesia. A 4-cm piece of tygon tubing (0.38 mm inner diameter) was used to attach the minipump to an ‘L’-shaped piece of stainless steel injector tubing (32 gauge, 30 mm long), which was then placed into an i.c.v. guide cannula. Both normal saline and the morphine solution were passed through a 0.25-μm filter before being introduced into the pumps, and the delivery apparatus was assembled under sterile conditions. Minipumps were primed overnight at room temperature using normal saline so that an optimal flow rate (1 μl/h) was obtained. Rats receiving the morphine regimen displayed characteristic morphine withdrawal signs, including rearing, teeth-chattering, wet-dog shakes, salivation, diarrhea and ptosis, when naloxone hydrochloride
(3 mg/kg, s.c.) was administered 1 hr after the termination of saline or morphine infusion. Of these signs rearing, teeth-chattering and wet-dog shakes were evaluated in this study.

**Microdialysis Procedure and Norepinephrine Analysis:** Infusion of morphine was terminated after 24 hr or 72 hr by cutting the tubing from the osmotic minipump. A 3-mm concentric dialysis probe was inserted into the hippocampus 2 hr before the termination of morphine or saline infusion and then was continuously perfused (2 μl/min) with Ringer's solution. Successive 40-μl samples were collected at 20-min intervals in tubes containing 10 μl 0.1 M acetic acid, and were analyzed immediately for norepinephrine using high-performance liquid chromatography (HPLC) with an electrochemical detection (ECD) system. The HPLC-ECD system consisted of a pump (Eicom 300) connected to a reverse-phase column (CA-5 ODS, 2.1 mm × 150 mm) and an ECD unit (ECD-300; Eicom Co. Ltd., Kyoto, Japan). A graphite working electrode was maintained at 450 mV with a Ag/AgCl reference electrode. The mobile phase, consisting of 0.1 M disodium hydrogenphosphate, including 0.15 mM disodium EDTA and 1.85 mM sodium 1-octane sulfonate, was adjusted to pH 6.0 with 0.1 M sodium dihydrogenphosphate, and methanol was added to 5% (v/v). The column was eluted at a flow rate of 0.2 ml/min and regulated at 25°C in a column oven unit.

**Statistics:** Statistical calculations were performed on the raw data. Norepinephrine levels were evaluated by analysis of variance (ANOVA) followed by the Newman-Keuls post-hoc test, and the numbers of withdrawal signs were compared by unpaired t-test.

**RESULTS**

**Naloxone-precipitated Morphine Withdrawal Syndrome:** Administration of naloxone precipitated typical morphine withdrawal signs which were measured during the 10 min-period immediately following naloxone injection. As shown in Fig. 1, the number of rearing, teeth-chattering and wet-dog shakes after naloxone injection was significantly higher in morphine-treated rats than in saline-treated rats. Dizocilpine attenuated the rearing and teeth-chattering signs, but not wet-dog shakes, when it was infused (i.c.v.) concurrently with morphine, and

![Fig. 1. Effects of infusion (i.c.v.) or injection (s.c.) of dizocilpine on naloxone-precipitated morphine withdrawal signs.](image)

Naloxone hydrochloride was injected (3 mg/kg, s.c.) into rats that had received a 72 hr i.c.v. infusion of either saline, morphine (50 nmol/μl/h), morphine (50 nmol/μl/h) together with dizocilpine (1 or 10 nmol/μl/h) or morphine (50 nmol/μl/h) and a dizocilpine injection (0.1 mg/kg, s.c.) 20 min before naloxone injection. Each point represents the mean ± S.E.M. of the number of typical withdrawal signs per rat, observed in the 10 min period immediately after naloxone injection. These were 3–10 rats in each group.

\*p<0.05, \**p<0.01 vs. the value for the morphine-treated group. □, saline; ■, morphine (50 nmol/μl/h); □, morphine (50 nmol/μl/h) plus dizocilpine (1 nmol/μl/h); ★, morphine (50 nmol/μl/h) plus dizocilpine (10 nmol/μl/h); ★, morphine (50 nmol/μl/h) plus dizocilpine injection (0.1 mg/kg, s.c.).
when it was injected (s.c.) 20 min before the naloxone injection.

**Development of Morphine Dependence in Terms of Norepinephrine Levels in the Hippocampus** : Injection of naloxone hydrochloride (3 mg/kg, s.c.) induced an immediate and long-lasting (>120 min) increase in hippocampal nor- epinephrine output in morphine-treated rats (Figs. 2–4). This naloxone-induced elevation of hippocampal norepinephrine release depended on the duration of the morphine infusion: the increase in 72 hr morphine-infused rats was greater than that in 24 hr morphine-infused rats (Fig. 2). In contrast, hippocampal norepinephrine output in saline-treated rats was not affected by naloxone administration.

The mean basal output of norepinephrine in rats infused (i.c.v.) with morphine for 24 hr was 1.46±0.032 pg/40 μl/20 min sample, which was significantly less than that in rats infused with saline (1.85±0.023 pg/40 μl/20 min sample). Morphine infusion for 72 hr caused the basal output of norepinephrine to return to the level seen in the saline-treated rats (Fig. 2).

**Effect of Dizocilpine on Norepinephrine Release Augmented by Naloxone** : Different doses of dizocilpine were infused together with morphine via the osmotic minipump for 72 hr into the lateral cerebral ventricle. A low dizocilpine dose (1 nmol/μl/h) reduced the naloxone-induced rise in norepinephrine release and a high dizocilpine dose (10 nmol/μl/h) blocked the naloxone-induced increase in norepinephrine release (Fig. 3). Although this inhibitory effect of dizocilpine was also observed in rats which were infused with dizocilpine (1 nmol/μl/h) together for 24 hr, there was a slight, but sustained rise in norepinephrine release after the naloxone injection which gradually decreased (Fig. 3). On the other hand, when morphine-treated rats were

![Fig. 2](image1.png)

**Fig. 2.** Effect of naloxone on norepinephrine release in the hippocampus of morphine-dependent rats. Morphine hydrochloride (50 nmol/μl/h) or saline (1 μl/h) was continuously infused (i.c.v.) via an osmotic minipump. Naloxone hydrochloride was injected (3 mg/kg, s.c.) into rats that had received a 24 hr or 72 hr morphine i.c.v. infusion. Each point represents the mean±S.E.M. obtained from 3–5 rats. ANOVA showed a significant effect of naloxone on both the 24 hr and the 72 hr morphine-treated groups (F=3.05; d.f.=9, 29; p<0.05 and F=27.82; d.f.=9, 40; p<0.01 respectively). Post-hoc analysis revealed that norepinephrine levels at each time point between 20 and 120 min post-naloxone were significantly different compared to the level of each basal output before naloxone challenge and that at the 20 min point results for the 24 hr and 72 hr morphine-treated groups were significantly different (p<0.05). ○, saline (72 hr); □, morphine (24 hr); ●, morphine (72 hr).

![Fig. 3](image2.png)

**Fig. 3.** Effect of infusion (i.c.v.) of dizocilpine together with morphine on naloxone-induced norepinephrine release in the hippocampus of morphine-dependent rats.

Dizocilpine was infused together with morphine for 72 hr or 24 hr through an osmotic minipump. Each point represents the mean±S.E.M. obtained from 3–10 rats. ANOVA revealed a significant effect of naloxone on both the 72 hr morphine- and morphine plus dizocilpine (1 nmol/μl/h)-treated groups (F=34.79; d.f.=9, 90; p<0.01 and F=3.03; d.f.=9, 20; p<0.05, F=32.71; d.f.=9, 20; p<0.01 and F=3.03; d.f.=9, 20; p<0.05 respectively). ○, saline (1 μl/h) plus dizocilpine (1 nmol/μl/h); ●, morphine (50 nmol/μl/h); □, morphine (50 nmol/μl/h) plus dizocilpine (1 nmol/μl/h); ■, morphine (50 nmol/μl/h) plus dizocilpine (10 nmol/μl/h); ○—●, morphine (50 nmol/μl/h) plus dizocilpine (1 nmol/μl/h); ———, for 72 hr; ···, for 24 hr.
injected with dizocilpine (0.1 mg/kg, s.c.) 20 min before naloxone challenge, dizocilpine caused a slight reduction in the rate of increase of norepinephrine release after the naloxone injection, but the level of norepinephrine release quickly rose to the level seen in rats not treated with dizocilpine (Fig. 4).

Neither dizocilpine injection before naloxone injection nor continuous infusion (i.c.v.) of dizocilpine affected the behavioral signs (data not shown) or the hippocampal norepinephrine release of saline-treated rats (Figs. 3 and 4). The inhibitory effect of acute morphine injection (5 mg/kg, s.c.) on the hippocampal norepinephrine release was not affected by dizocilpine injection (0.1 mg/kg, s.c.) in naive rats (data not shown).

DISCUSSION

In this study, we measured endogenous norepinephrine release from the hippocampus of freely moving rats using brain microdialysis coupled with HPLC-ECD, while observing their behavior. We found that dizocilpine (MK-801), a noncompetitive NMDA-receptor antagonist, decreased the naloxone-precipitated withdrawal signs and reduced the naloxone-induced rise in hippocampal norepinephrine release after naloxone injection in morphine-treated rats.

In rats continuously receiving morphine (i.c.v.) via osmotic mini pumps for 72 hr, the injection of naloxone (s.c.) induced an immediate and long-lasting increase in extracellular norepinephrine in the hippocampus (Figs. 2–4), which was associated with characteristic morphine withdrawal signs (Fig. 1). The increase in the hippocampal norepinephrine output after naloxone challenge paralleled the onset of the behavioral signs of withdrawal. The behavioral signs were most frequent during the first 10 min after naloxone injection and disappeared within 60 min. Therefore, we counted the signs during the initial 10 min after the injection, particularly rearing, wet-dog shakes and teeth-chattering.

The effect of naloxone on norepinephrine release was dependent on the duration of the morphine infusion (Fig. 2). The number of morphine withdrawal signs after naloxone injection was also dependent on the duration of morphine infusion (data not shown). Both the norepinephrine output of the hippocampus and the number of morphine withdrawal signs observed in rats that had received morphine for 72 hr were greater than in rats treated with morphine for 24 hr. Although morphine infusion for 24 hr reduced the hippocampal basal output of norepinephrine, morphine infusion for 72 hr caused the basal level of hippocampal norepinephrine release to return to the level seen in saline-treated rats (Fig. 2). These findings suggest that morphine dependence and tolerance develop gradually and depend on the duration of the morphine infusion. The marked and long-lasting increase in hippocampal norepinephrine output after naloxone injection might have been due to the effects of chronic morphine treatment.
on subcellular components of noradrenergic neurons such as adenylate cyclase, cAMP-dependent protein kinase (Nestler and Tallman, 1988; Rasmussen et al., 1990) and protein kinase C (Narita et al., 1994).

There have been several reports showing that the LC is involved in the development of morphine withdrawal signs (Rasmussen et al., 1990; Maldonado et al., 1992; Maldonado and Koob, 1993), although it is unclear whether hippocampal norepinephrine mediates the behavioral withdrawal signs. It has been reported that stimulation of the LC produces several behavioral and physiological signs (Rasmussen et al., 1990) and that signs such as jumping, rearing and locomotor activity are particularly frequent after methylsaloxonium injections into the LC in morphine-dependent rats (Maldonado et al., 1992). The hippocampus is one of the regions receiving input from the LC, and represents the largest cluster of noradrenergic neurons in the brain (Foote et al., 1983). The LC possesses a high density of opioid receptors, particularly of the mu and kappa types (Tempel and Zukin, 1987). A direct relationship between the neuronal activity of LC neurons and neurotransmitter release in morphine-dependent rats is supported by the immediate rise in hippocampal norepinephrine output following naloxone administration, and also by the simultaneous occurrence of opiate withdrawal signs. An immediate rise in the norepinephrine output of the hippocampus after naloxone challenge persisted even after disappearance of the behavioral withdrawal syndrome. This discrepancy between norepinephrine output and behaviors might be due to the neuronal activation at certain areas including the LC, but not the development of withdrawal signs that were induced by the hippocampal norepinephrine release after naloxone challenge.

Recently, it has been suggested that hyperactivity of LC neurons after naltrexone challenge in morphine-dependent rats is due to the input of excitatory amino acids from the nucleus para-gigantocellularis (Akaoka and Aston-Jones, 1991; Rasmussen, 1991). In order to determine whether the rise in hippocampal norepinephrine release following naloxone injection was mediated by glutamate, we examined the effect of dizocilpine. Although dizocilpine, when injected (s.c.) before naloxone challenge to morphine-dependent rats, attenuated the initial rate of the naloxone-induced rise in norepinephrine release, it failed to suppress the level and duration of the lasting rise in norepinephrine release (Fig. 4). This finding indicates that glutamate acts solely as a trigger, to induce the release of norepinephrine. Pretreatment with dizocilpine also attenuated the development of rearing and teeth-chattering, but unexpectedly did not affect wet-dog shakes (Fig. 1). However, our data coincide with a report that pretreatment with dizocilpine does not decrease the number of wet-dog shakes among other morphine withdrawal signs (Rasmussen et al. 1991).

On the other hand, dizocilpine, when infused together with morphine for 72 hr, decreased hippocampal norepinephrine release dose-dependently, in association with a reduction in withdrawal signs, after a naloxone challenge (Fig. 3), and the level of hippocampal norepinephrine release did not increase to the level seen in rats not treated with dizocilpine. The naloxone-induced rise in norepinephrine release, which was partially attenuated by a low dose of dizocilpine, was long-lasting. These results confirm and extend those of a previous study (Trueillo and Akil, 1991) on the inhibition of tolerance to the analgesic effect of morphine and inhibition of the development of physical dependence by dizocilpine, although it is unclear whether dizocilpine specifically attenuates the expression of physical dependence produced by continuous administration of morphine.

It is well known that NMDA receptors are involved in neuronal development, long-term potentiation, kindling, learning and memory (Collingridge and Lester, 1989). The inhibition of norepinephrine release by continuous dizocilpine infusion indicates that NMDA receptors are involved in the behavioral changes and presumably the neuronal adaptations caused by chronic morphine exposure.

In summary, naloxone induced an immediate and long-lasting increase in the hippocampal norepinephrine release in morphine-treated rats, which was associated with withdrawal signs. This rise in norepinephrine release was reduced by concurrent i.c.v. infusion of dizocilpine and morphine. These results suggest that dizocilpine
inhibits not only the development of naloxone-precipitated withdrawal signs, but also the expression of morphine dependence through NMDA receptors.

REFERENCES


