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Attenuation of morphine dependence and withdrawal by glycine$_B$ site antagonists in rats

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Abstract

Numerous data indicate that noncompetitive and competitive *N*-methyl-D-aspartate (NMDA) receptor antagonists inhibit the development of physical dependence on opioids when these substances are administered together, and NMDA receptor antagonists are used at lower range of doses. Higher doses of these antagonists can enhance some opioid-induced effects. The present study extends these findings to the effects of NMDA/glycine (glycine$_B$) site antagonists. Wistar rats were rendered dependent on morphine by implantation of morphine pellets. Both of the glycine$_B$ site antagonists used, 7-chloro-4-hydroxy-3-(3-phenoxy)-phenyl-2(*H*)-quinolone (L-701,324; 2.5 and 5.0 mg/kg) and 5,7-dichlorokynurenic acid (5,7-DCKA; 25, 50, and 100 mg/kg), suppressed the expression of morphine withdrawal syndrome estimated as wet dog shakes. Furthermore, L-701,324 (2.5 and 5 mg/kg), given twice a day during the development of morphine dependence, attenuated the development of morphine dependence, and the results were comparable to those obtained after administration of noncompetitive NMDA receptor antagonist — MK801 (0.1 mg/kg). Our data suggest that glycine$_B$ site antagonists may attenuate wet dog shakes (withdrawal) and the development of dependence, both being induced by chronic morphine administration in rats.

Keywords: Morphine dependence and withdrawal; MK801; Glycine$_B$ site antagonists; L-701,324; 5,7-DCKA

1. Introduction

So far, no effective drugs have been developed for the therapy of the opioid dependence. Commonly applied methadone replacement is not effective, and often causes recidivism after discontinuation of the therapy (Ball and Ross, 1991). Because of these disadvantages, there is a strong motivation for the search for novel methods for treatment of the opioid abuse. A promising target seems to involve compounds inhibiting glutamatergic transmission. Glutamatergic transmission has been shown to play a significant role in opioid dependence and withdrawal. Microdialysis studies have shown a dramatic increase of the release of glutamate within the pontine locus coeruleus during withdrawal from morphine (Aghajanian et al., 1994; Zhang and Feng, 1995) or butorphanol (Hoshi et al., 1991). Moreover, direct intracerebroventricular (icv) or locus coeruleus injection of glutamate, dose-dependently induced withdrawal signs in the opioid-dependent animals (Tokuyama et al., 1996, 1998).

As it has been shown, the noncompetitive *N*-methyl-D-aspartate (NMDA) receptor antagonists such as ketamine, dextromethorphan (Koyuncuoğlu et al., 1990), MK801 (Cappendijk et al., 1993; Koyuncuoğlu et al., 1992; Tanganelli et al., 1991; Tokuyama et al., 1996), and LY274614, a competitive NMDA receptor antagonist (Rasmussen et al., 1991), have been reported to attenuate signs of the naloxone-precipitated withdrawal syndrome in the morphine-dependent animals, when administered immediately before naloxone. It has also been shown that MK801 inhibited signs of the naloxone-precipitated opioid withdrawal after coadministration with morphine during the development of physical dependence (Fundytus and Coderre, 1994; Trujillo and Akil, 1991). Unfortunately, most of these compounds, particularly NMDA-channel blockers, such as phencyclidine (PCP) and MK801, display psychotomimetic and pathomorphological side-effects that preclude their use in human (Fix et al., 1994; Willetts et al., 1990). Potentially more attractive drug candidates are glycine$_B$ site antagonists of the NMDA receptors (Danysz et al., 1995; Konieczny et

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al., 1999), which in contrast to the other NMDA receptor antagonists, fail to produce neurodegenerative changes in the cingulate/retrosplenial cortex (Berger et al., 1994; Chen et al., 1993), psychotomimetic-like effects (Bristow et al., 1996a), or impairment of learning at anticonvulsive doses (Chiamulera et al., 1990).

The aim of the present experiment was to determine whether glycine$_B$ receptor antagonists, 5,7-dichlorokynurenic acid (5,7-DCKA) (Corbett and Dunn, 1993; Palfreyman and Baron, 1990) and potent, orally active 7-chloro-4-hydroxy-3-(3-phenoxy)-phenyl-2(*H*)-quinolone (L-701,324) (Kulagowski et al., 1994), affected the expression of the naloxone-precipitated morphine withdrawal. The influence of L-701,324 on the development of morphine dependence was also examined, and this effect was compared to the results received after administration of the noncompetitive NMDA receptor antagonist — MK801. Among various naloxone-precipitated morphine withdrawal signs (Bläsing et al., 1973), the wet dog shakes were chosen as a representative and quantitative withdrawal symptom in rats because they occurred more often than the other withdrawal signs (e.g. jumpings).

2. Method

2.1. Animals

The experiments were approved by the Institutional Review Committee for the Use of Animal Subjects, and the experimental procedures are in compliance with the European Communities Council Directive of 24 November 1986 (86/609/EEC). Male Wistar rats (180–230 g) were housed in plastic cages in groups of four per cage, and had unlimited access to water and lab chow (Bacutil, Motycz, Poland). The rats were kept under constant temperature (25°C) and on a controlled light–dark cycle (lights on between 7 AM and 7 PM). The animals were adapted to the laboratory conditions for at least 1 week and then handled once daily for 5 days before the start of the experiments.

2.2. Drugs

The glycine$_B$ receptor antagonist, 7-chloro-4-hydroxy-3-(3-phenoxy)-phenyl-2(*H*)-quinolone sodium salt (L-701,324; Merck Sharp and Dohme, Rahway, USA), was given as a suspension prepared using 0.5% solution of methyl cellulose. Other glycine$_B$ receptor antagonist, 5,7-DCKA (RBI, Natick, USA), was prepared as a suspension consisting of a few drops of Tween 80 and distilled water. The noncompetitive NMDA receptor antagonist, dizocilpine (MK801, RBI), and opioid receptors antagonist, naloxone hydrochloride (Sigma, USA), were also dissolved in physiological saline.

Naloxone, MK801, and 5,7-DCKA were administered intraperitoneally (ip); L-701,324 was given per os (po); and morphine pellets were implanted subcutaneously (sc).

2.3. Procedure

Morphine dependence was induced by subcutaneous implantation of morphine pellets. Morphine pellets were prepared according to (Way et al., 1969) and consisted of 75 mg morphine base, 75 mg microcrystalline cellulose, 1.5 mg magnesium stearate, and 0.75 mg silica gel. Constituents were carefully mixed and highly compressed.

The pellets were implanted in rats (8–10 per group) under light ether anesthesia (one pellet per rat). The skin was cut crosswise, 1 cm backwards the line connecting both back side of ear laps. The pellets were inserted under the skin and pushed on 1 cm towards the head. After 72 h, the morphine withdrawal syndrome were precipitated by naloxone (5 mg/kg) and scored by observation of rats separately in glass cylinder, and the number of wet dog shakes during a 45-min session was counted.

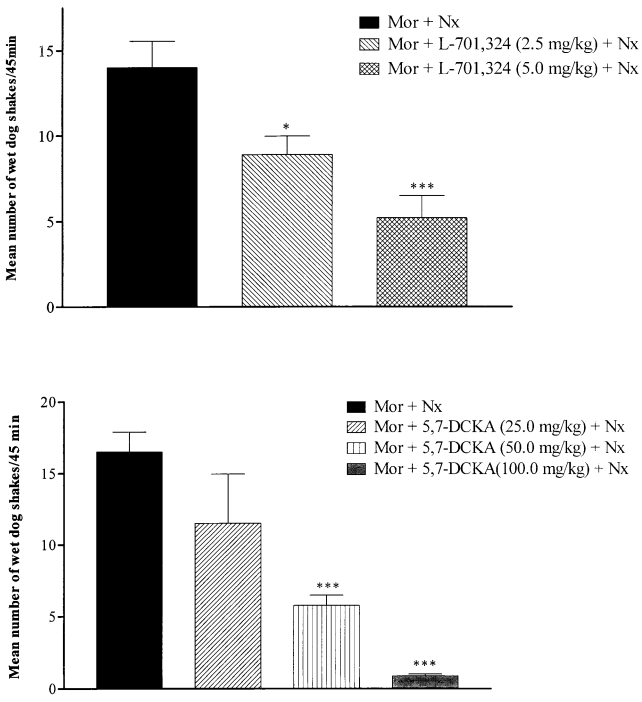

Fig. 1. Acute effect of increasing doses of the glycine$_B$ site antagonists, L-701,324 (upper panel; 2.5 and 5.0 mg/kg, po) and 5,7-DCKA (bottom panel; 25, 50, and 100 mg/kg, ip) on expression of morphine (Mor) dependence (withdrawal syndrome) precipitated by naloxone injection (Nx) — wet dog shakes in Wistar rats. Shown are the means ± S.E.M. of 8–10 rats in each group. $*P<.05$ and $***P<.001$ as compared to control (morphinized) animals only given the drug vehicle, instead of substances, during the expression of morphine dependence.

Two types of experiments using morphine-dependent animals were performed: (1) the influence of the acute administration of the glycine$_B$ receptor antagonists on the expression of morphine withdrawal syndrome, and (2) the influence of the chronic administration of glycine$_B$ receptor antagonist (L-701,324) and a noncompetitive NMDA receptor antagonist (MK801) on the development of morphine dependence.

The acute administration (Experiment 1) of L-701,324 (2.5 and 5 mg/kg) was performed 30 min, and 5,7-DCKA (25, 50, and 100 mg/kg), 15 min prior to naloxone injection. During chronic administration of the drugs (Experiment 2), L-701,324 (2.5 and 5 mg/kg) was injected twice a day for the first 2 days (every 12 h), followed by a single injection on the third day (9 AM). MK801 (0.1 mg/kg) was administered two times daily for 3 days (every 12 h).

2.4. Data analysis

The morphine withdrawal syndrome, wet dog shakes, was analysed using one-way analysis of variance (ANOVA) followed by a post hoc Bonferroni's *t* test for multiple comparisons.

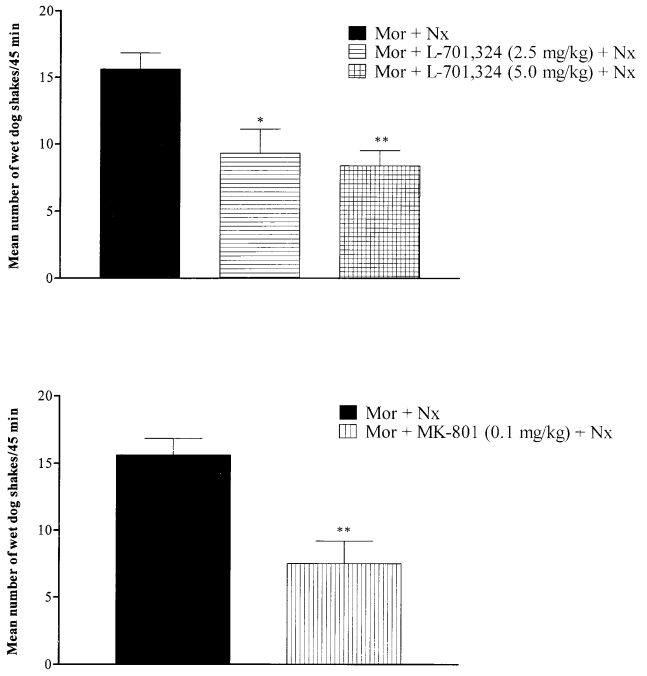

Fig. 2. Chronic effect of glycine$_B$ site antagonist, L-701,324 (upper panel; 2.5 and 5.0 mg/kg, po) and noncompetitive NMDA receptor antagonist, MK801 (bottom panel; 0.1 mg/kg, ip) on the acquisition of morphine dependence. The morphine (Mor) withdrawal syndrome, wet dog shakes, was estimated after naloxone (Nx) injection. Shown are the means ± S.E.M. of 8–10 rats in each treatment group. * $P<.05$ and * * $P<.01$ as compared to control (morphinized) animals only given the drug vehicle, instead of substances, during the development of morphine dependence.

3. Results

3.1. Experiment 1

Data depicted in Fig. 1 demonstrate the acute effects of increasing doses of the glycine$_B$ receptor antagonists, L-701,324 (upper panel) and 5,7-DCKA (bottom panel), on the wet dog shake frequency. As can be seen in Fig. 1, L-701,324 at doses 2.5 ($P<.05$) and 5.0 mg/kg ($P<.001$) and 5,7-DCKA at doses 50.0 ($P<.001$) and 100 mg/kg ($P<.001$) significantly suppressed the expression of morphine withdrawal syndrome in a dose-dependent manner.

3.2. Experiment 2

Data shown in Fig. 2 present the effects of a chronic administration of the glycine$_B$ site antagonist, L-701,324 (upper panel), and a noncompetitive NMDA receptor antagonist, MK801 (bottom panel) on the development of morphine dependence. L-701,324 at doses 2.5 ($P<.05$) and 5.0 mg/kg ($P<.01$), and MK801 at dose 0.1 mg/kg ($P<.01$), significantly suppressed the development of morphine dependence, which was expressed as a wet dog shake behavior. However, the dose of MK801 (0.1 mg/kg) increased mortality in morphinized rats during the first few hours after implantation of the pellet, wherein two animals died.

4. Discussion

The present data indicate that either glycine$_B$ receptor antagonist, L-701,324 or 5,7-DCKA, suppresses expression of the naloxone-precipitated morphine withdrawal in rats in a dose-dependent manner. Furthermore, L-701,324 also suppresses the development of the morphine dependence, and these results are comparable with the data received after administration of MK801 — the noncompetitive NMDA receptor antagonist (Figs. 1 and 2).

Withdrawal from morphine, following its chronic administration in rats, elicits abstinence syndromes indicating dependence expressed by the wet dog shake: jumping, flying, diarrhea, ptosis, teeth chattering, and abdominal stretching (Bläsing et al., 1973). Dependence can be rapidly and fully exposed by treatment with the μ-opioid receptor antagonist naloxone, which abruptly removes morphine from its receptors. In the present study, wet dog shakes were only estimated as a naloxone-precipitated morphine withdrawal syndrome. Dominant symptoms of abstinence, such as jumpings and teeth chattering, were hardly observable, or absent (flying). None of the used NMDA receptor antagonists enhanced morphine withdrawal syndrome in our experiments.

Morphine withdrawal signs precipitated by naloxone are inhibited by the NMDA receptor antagonists such as

MK801, memantine, and dextromethorphan (and many others), although specificity of these effects can be questioned, since the myorelaxant/ataxic activity of NMDA receptor antagonists could simply obscure the behavioral expression (Manning et al., 1996; Popik and Danysz, 1997). These findings were also extended on the glycine$_B$ receptor antagonists such as felbamate (Kosten et al., 1995), MRZ 2/570, or L-701,324 (Popik et al., 1998) or partial agonist such as R-(+)-HA-966 (Bristow et al., 1997), which inhibited morphine effects related to drug abuse. The expression of morphine dependence was reduced or blocked by these substances, but the effects of MRZ 2/570 or L-701,324 were not dose dependent (Popik et al., 1998) in mice. Although glycine$_B$ receptor antagonists are devoid of PCP-like effects, most characteristic of MK801, and related to the noncompetitive NMDA antagonists (hyperlocomotion, head weaving, and body rolling) in animals, majority of them have a very poor access to the central nervous system (CNS) and induce ataxia, myorelaxation, and sedation in animals (for review, see Danysz and Parsons, 1998).

L-701,324 is a potent, orally active antagonist acting at the glycine modulatory site of the NMDA receptor complex, with high per os bioavailability and a long plasma half-life (Bristow et al., 1996a; Bristow et al., 1996b). Apart from its anticonvulsant (Bristow et al., 1996b), anxiolytic (Kotlińska and Liljequist, 1998), and atypical neuroleptic profile (Bristow et al., 1996a), L-701,324 induces ataxia and myorelaxation (Bristow et al., 1996b; Konieczny et al., 1999) but not at the doses used in present experiments (below 5 mg/kg, po) (Kotlińska and Liljequist, 1998). Therefore, we can anticipate that inhibition of the development of morphine dependence and the expression of withdrawal syndrome by L-701,324, presented in this study, are not the result of its side-effects, but rather occur due to the interaction between NMDA receptors and opioid neurotransmission. The attenuation of the number of wet dog shakes by another glycine$_B$ site antagonist, 5,7-DCKA, may support the importance of NMDA structures in expression of morphine withdrawal.

Many recent data indicate that MK801, a noncompetitive NMDA receptor antagonist, prevented the development of morphine dependence (for review, see Trujillo and Akil, 1995). However, this substance causes a very strong side-effect (in our studies, few animals died during concomitant administration of MK801 and morphine). MK801 also induces an increase of the locomotor activity in animals, and this effect is probably associated with the increase of dopamine (DA) release in mesocorticolimbic structures (Rao et al., 1990). These structures are commonly known to be involved in rewarding effects of the drugs of abuse (Nutt, 1996; Wise, 1989), and NMDA receptors presented in these structures are probably also implicated in the morphine reward and dependence (Popik and Kolasiewicz, 1999). In contrast to MK801, L-701,324 fails to alter significantly the DA function in any brain areas (Bristow et al., 1996a) or stimulate locomotor activity in rodents (Johnson and Ascher, 1987), and even suppresses the effects induced by a stimulation of DA function (Hutson and Barton, 1997). However, in our studies, L-701,324, similar to MK801, inhibited the development of morphine dependence in rats.

In conclusion, our experiments indicate that glycine$_B$ site antagonists attenuate the development of morphine dependence and the expression of withdrawal syndrome in rats, and may suggest that NMDA structures play some role in these phenomena.

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