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Inhibitory Effects of Glycine on Morphine-Induced Hyperactivity, Reverse Tolerance and Postsynaptic Dopamine Receptor Supersensitivity in Mice

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The effects of glycine on morphine-induced hyperactivity, reverse tolerance and postsynaptic dopamine receptor supersensitivity in mice was examined. A single administration of morphine (10 mg/kg, s.c.) induced hyperactivity as measured in mice. The morphine-induced hyperactivity was inhibited by pretreatment with glycine (100, 200 and 400 mg/kg, i.p.). In addition, it was found repeated administration of morphine (10 mg/kg, s.c.) to mice daily for 6 days caused an increase in motor activity which could be induced by a subsequent morphine dose, an effect known as reverse tolerance or sensitization. Glycine (100, 200 and 400 mg/kg, i.p.) also inhibited morphine-induced reverse tolerance. Mice that had received 7 daily repeated administrations of morphine also developed postsynaptic dopamine receptor supersensitivity, as shown by enhanced ambulatory activity after administration of apomorphine (2 mg/kg, s.c.). Glycine inhibited the development of postsynaptic dopamine receptor supersensitivity induced by repeated administration of morphine. It is suggested that the inhibitory effects of glycine might be mediated by dopaminergic (DAergic) transmission. Accordingly, the inhibition by glycine of the morphine-induced hyperactivity, reverse tolerance and dopamine receptor supersensitivity suggests that glycine might be useful for the treatment of morphine addiction.

Key words: Morphine, Glycine, Hyperactivity, Reverse tolerance, DA receptor supersensitivity

INTRODUCTION

Morphine is a behavioral stimulant which has been shown to increase dopamine (DA) levels and DA turnover in the brain. Morphine increases extracellular DA levels in the striatum and nucleus accumbens (Babbini and Davis, 1972; Kuschinski and Hornykiewicz, 1974). The behavioral activation due to morphine, has been postulated to be mediated through the mesolimbic DA systems. Repeated doses of morphine have been shown to cause a reverse tolerance or enhanced sensitivity to the locomotor effects of a subsequent challenge dose of morphine (Kuribara and Tadokoro, 1989). This phenomenon, termed behavioral sensitization, is thought to involve enhanced dopaminergic (DAergic) transmission. Behavioral sensitization is a model for studying the psychotoxicity of dependent-liable drugs (Allen and Young, 1978; Robinson and Becker, 1986). It is also reported that chronic morphine administration causes the development of postsynaptic DA receptor supersensitivity in the central nervous systems (CNS) (Puri and Lal, 1973).

Recent studies suggest that activation of NMDA (*N*-mehtyl-D-aspartate) receptors may play an important role. For example, competitive and non-competitive NMDA receptor antagonists appear to block development of opioid tolerance (Trujillo and Akil, 1991; Bhargava and Matwyshyn, 1993; Lutfy *et al.*, 1993). It is now generally accepted that glycine is acquired for activation of NMDA receptors (Johnson and Ascher, 1987). Glycine is one of the major inhibitory neurotransmitters in the mammalian CNS, predominantly active in the spinal cord and brain stem. However, it is unclear whether glycine modulates the activated dopaminergic function after morphine administration.

Therefore, this study was investigated to research whether glycine inhibits morphine-induced hyperactivity, reverse tolerance and enhanced sensitivity at the postsynaptic DA receptors.

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MATERIALS AND METHODS

Animals and drugs

ICR male mice weighing 22-27 g, in groups of 10-15 mice were used in all experiments. They were housed, 10 mice to a cage, with water and food available *ad libitum* under an artificial 12 h light/dark cycle (light on at 07:00) with a constant environmental temperature (22± 2°C). The drugs used were morphine hydrochloride (Je-Il, Seoul, Korea), glycine (Sigma, USA) and apomorphine hydrochloride (Sigma, USA). Except for apomorphine, all drugs were dissolved in physiological saline (0.1 mL/10 g). Apomorphine was dissolved in saline containing 0.1% ascorbic acid, just prior to its use.

Measurement of hyperactivity induced by morphine

Hyperactivity of mice was measured by a tilting-type ambulometer (AMB-10, O'Hara, Tokyo, Japan). Each mouse was placed in the activity cage (20 cm in diameter, 18 cm in height) and after an adaptation period of 10 min, the drug administration protocol was implemented. Glycine (100, 200 and 400 mg/kg) was administered intraperitoneally (i.p.) 30 min prior to the subcutaneous (s.c.) administration of morphine (10 mg/kg). In the preliminary experiments, the combined effects of morphine and glycine were investigated at various time intervals. The maximal inhibitory effects of glycine on morphine-induced activity were observed when glycine was injected 30 min prior to the morphine administration. Also, preliminary experimentation indicated that the ambulatory effect of morphine produced a consistent and reliable ambulatory activity for 1 h. Therefore, the ambulatory activity was measured for 1 h after the administration of morphine.

Measurement of reverse tolerance induced by morphine

Reverse tolerance to the ambulatory activity of morphine (10 mg/kg) developed significantly within a period of 6 days. Thus, to induce reverse tolerance, morphine (10 mg/kg, s.c.) was administered once daily for 6 days according to previous methods used by these researchers (Kim *et al.*, 1999; Yoon *et al.*, 2002). Glycine was administered once daily, 30 min before the injection of morphine for 6 days. To test the degree of the development of reverse tolerance to morphine, mice were injected solely with morphine on day 7, 24 h after the previous morphine injections to avoid any residual effects of the test drugs themselves. The morphine-induced hyperactivity was measured for 1 h by using a tilting-type ambulometer. The mice were first allowed to perambulate for 10 min in the activity cages followed by a 1 h test period immediately after the injection of morphine. The development of reverse tolerance was evidenced by an enhanced reverse response to morphine and the inhibition of reverse tolerance was evidenced by a lesser ambulatory activity produced by morphine.

Measurement of the development of postsynaptic dopamine receptor supersensitivity

Additional groups of mice given the same chronic morphine and glycine treatment, for the measurement of the development of reverse tolerance as in the previous test, were used to determine the effects of these treatments on the development of postsynaptic dopamine receptor supersensitivity (Bhargava, 1980). To determine the development of postsynaptic dopamine receptor supersensitivity in the reverse-tolerant mice, morphine (10 mg/kg, s.c.) was administered once daily for 6 days. Glycine (100, 200 and 400 mg/kg) was administered once daily 30 min before the injection of morphine. The degree of the development of morphine-induced postsynaptic dopamine receptor supersensitivity was evidenced by measurement of the enhanced ambulatory activity induced by apomorphine on day 7, 24 h after the final injection of morphine according to previous methods used (Kim et al., 1999; Yoon et al., 2003). The mice were first allowed to perambulate for 10 min followed by a 20 min test period immediately after the injection of apomorphine (2 mg/kg, s.c.), a dose which significantly increased ambulatory activity.

Statistics

The data were expressed as mean± SEM. The significance of the drug effects was assessed by an analysis of variance (ANOVA). In the case of significant variation, the individual values were compared by Dunnetts *t*-test.

RESULTS

Inhibitory effects of glycine on morphine-induced hyperactivity

The morphine-treated group showed a marked increase in ambulatory activity by 573% (1,754 counts, $p<0.005$) when compared with the saline group (306 counts). Meanwhile, glycine (100, 200 and 400 mg/kg) administered 30 min prior to the morphine injection, inhibited about 9.9% (1,580 counts), 16.8% (1,460 counts) and 39.9% (1,055 counts, $p<0.05$), respectively of the morphine-induced hyperactivity, compared with the morphine group (Fig. 1).

Inhibitory effects of glycine on the development of reverse tolerance to the hyperactivity induced by morphine

Morphine-induced ambulation-accelerating activity was progressively enhanced by 148% (2,722 counts, $p<0.01$) by the repeated administration of morphine once daily for 7 days when compared with the saline group (1,845

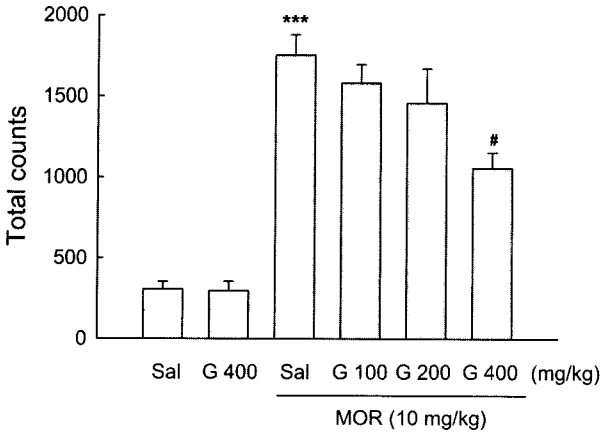

Fig. 1. Inhibitory effects of glycine on morphine-induced hyperactivity.
Glycine (G, 100, 200, 400 mg/kg i.p) was administered 30 min before morphine (MOR, 10 mg/kg) injection (s.c). The ambulatory activity was measured for 1 h after the administration of morphine. ***P<0.001, compared with that of saline group. #P<0.05 compared with that of morphine group.

counts). This suggests that the development of reverse tolerance to hyperactivity is caused by morphine. Meanwhile, glycine (100, 200 and 400 mg/kg) administered 30 min before the morphine injection inhibited the development of morphine induced reverse tolerance in about 6.4% (2,547 counts), 8.6% (2,488 counts), and 24.8% (2,048 counts, p<0.05), respectively, when compared with the morphine group (Fig. 2).

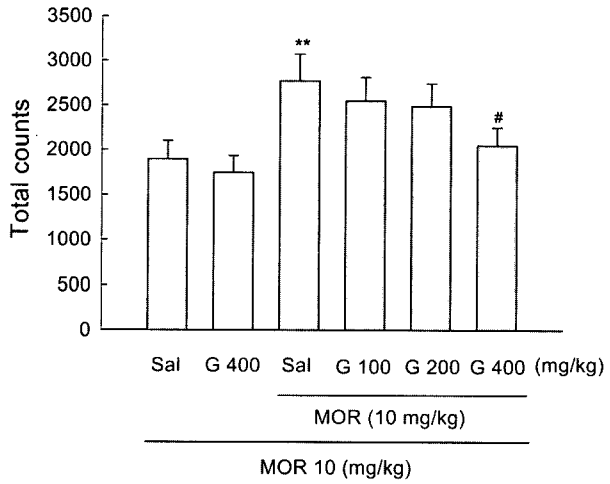

Fig. 2. Inhibitory effects of glycine on the development of morphine-induced reverse tolerance.
Glycine (G, 100, 200, 400 mg/kg, i.p.) was administered once a day 30 min before the injection of morphine (MOR, 10 mg/kg), for 6 days. To test the degree of the development of reverse tolerance of morphine, mice were injected with morphine only, on day 7. The ambulatory activity was measured for 1 h after the administration of morphine. **P<0.01, compared with that of saline group. #P<0.05, compared with that of morphine group.

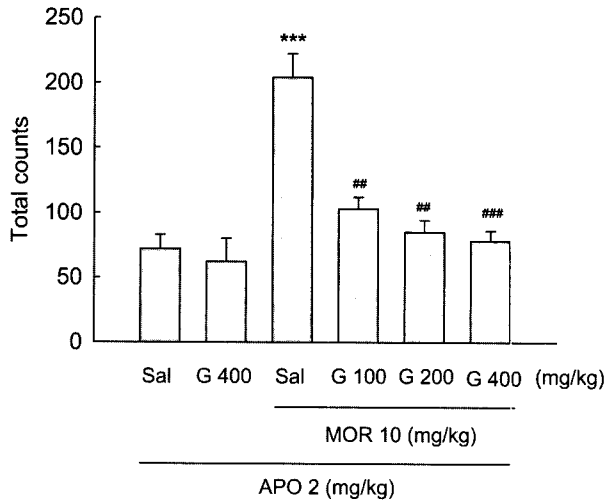

Fig. 3. Inhibitory effects of glycine on the development of dopamine receptor supersensitiviy in morphine-induced reverse tolerant mice.
Glycine (G, 100, 200, 400 mg/kg, i.p.) was administered once a day 30 min before the injection of morphine (MOR, 10 mg/kg) for 6 days. The development of dopamine receptor supersensitivity was shown by measurement of the enhanced ambulatory activity of mice to apomorphine 24 h after the final injection of morphine. Mice were injected with apomorphine (APO, 2 mg/kg, s.c.) and first allowed to ambulate for 10 min and then tested for 20 min. ***P<0.01, compared with that of saline group. ##P<0.01, ###P<0.005, compared with that of morphine group.

Inhibitory effects of glycine on the development of postsynaptic DA receptor supersensitivity in morphine-induced reverse tolerant mice

The mice which received the same chronic administration of morphine (10 mg/kg) as in the reverse-tolerance test produced an enhanced ambulatory activity to apomorphine (2 mg/kg), showing 204 counts (p<0.005), when compared with the saline group (72 counts), suggesting the development of postsynaptic DA receptor supersensitivity in morphine-induced reverse-tolerant mice. However, glycine (100, 200 and 400 mg/kg) administered 30 min before the morphine injection reduced the ambulatory activity of mice administered apomorphine in about 49.5% (103 counts, p<0.01), 58.3% (85 counts, p<0.01) and 61.8% (78 counts, p< 0.005), respectively, when compared with the morphine control group. These results suggest that glycine inhibits the development of postsynaptic DA receptor supersensitivity in morphine-induced reverse-tolerant mice (Fig. 3).

DISCUSSION

These experimental results show that pretreatment with glycine inhibited hyperactivity induced by morphine and the development of reverse tolerance to the hyperactivity of morphine (Fig. 1 and 2). It is well known that morphine increases extracellular dopamine levels in the striatum and

nucleus accumbens (Babbini and Davis, 1972; Kuschinski and Hornykiewicz, 1974). In this present experiment, it was also found that glycine inhibited postsynaptic dopaminergic supersensitivity induced by repeated morphine doses. Therefore, it is presumed that glycine inhibits the activation of dopaminergic function.

On the other hand, the strychnine-insensitive glycine site (glycine receptor) has become of special interest among the different binding sites existing within the NMDA receptors. The binding site for glycine is distinct from the glutamate binding site and has to be occupied for the channel to open. The conductance through the channel associated with the NMDA receptor is indeed strongly influenced by the presence of glycine. In cultured cerebral neurons, the frequency of the opening of this ion channel was markedly affected by the presence of glycine. This event was so relevant that in the absence of glycine, glutamate failed to activate the NMDA receptor (Johnson and Ascher, 1987). Based on these results, glycine was defined as a co-agonist of glutamate. However, there is no doubt that glycine is an inhibitory neurotransmitter in the CNS, acting on its own receptors, which functionally resembles the $GABA_A$ receptors.

It was recently reported by this team of researchers, that $GABA_A$ and $GABA_B$ receptor agonists inhibited hyperactivity, reverse tolerance and DAergic receptor supersensitivity, suggesting that the activation of DAergic receptors induced by morphine was inhibited by those drugs (Woo *et al*, 2001; Yoon *et al*., 2002). Also of note, is the phenomenon of reverse tolerance as a model for studying the psychotoxicity of dependence-liable drugs (Allen and Young, 1978; Robinson and Becker, 1986). It was reported that these effects of morphine are associated with the activation of the dopamine system (John and Takemori, 1986; Ritzmann *et al*., 1979). In association with these facts, it has been presumed that behavioral sensitization produced by repeated administration of morphine is accompanied by a change of DAergic neuronal activity.

In addition, the psychotic symptoms induced by phencyclidine (PCP) closely resembled those of schizophrenia, leading to the suggestion that PCP-induced psychoses might serve as effective clinical models for schizophrenia. In microdialysis studies, glycine significantly inhibited PCP-induced stimulation of subcortical DA release in a dose-dependent fashion. In behavioral studies, glycine reversed the effects of PCP in rodents (Daniel et al., 1999).

In conclusion, glycine inhibited hyperactivity, reverse tolerance and DA receptor supersensitivity induced by morphine. It is suggested that the inhibitory effects of glycine might be mediated by DAergic transmission. From these results, it can be presumed that glycine may be useful for the prevention and therapy of the adverse action of morphine.

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