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Inhibition of THIP on Morphine-Induced Hyperactivity, Reverse Tolerance and Postsynaptic Dopamine Receptor Supersensitivity

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This study was performed to investigate the effect of tetrahydroisoxazolopyridine (THIP), a $GABA_A$ agonist, on the morphine-induced hyperactivity, reverse tolerance and postsynaptic dopamine receptor supersensitivity in mice. A single administration of morphine induced hyperactivity in mice. However, the morphine-induced hyperactivity was inhibited dose-dependently by the administration of THIP (0.2, 0.4 and 0.8 mg/kg, i.p.). In contrast, daily administration of morphine resulted in a reverse tolerance to the hyperactivity caused by morphine (10 mg/kg, s.c.). THIP inhibited the development of reverse tolerance in the mice that had received the repeated same morphine (10 mg/kg, s.c.) doses. The postsynaptic dopamine receptor supersensitivity, which was evidenced by the enhanced ambulatory activity after the administration of apomorphine (2 mg/kg, s.c.), also developed in the reverse tolerant mice. THIP also inhibited the development of the postsynaptic dopamine receptor supersensitivity induced by the chronic morphine administration. These results suggest that the hyperactivity, reverse tolerance and postsynaptic dopamine receptor supersensitivity induced by morphine can be inhibited activating the $GABA_A$ receptors.

Key words: THIP, Morphine, Hyperactivity, Reverse tolerance, Postsynaptic dopamine receptor supersensitivity, $GABA_A$ receptor

INTRODUCTION

Opioid μ-receptor agonists increase the extracellular dopamine levels in the striatum and nucleus accumbens (Di Chiara and Imperato, 1988; Spanagel et al., 1990). In addition, morphine indirectly stimulates the dopaminergic system through an agonistic action on the opioid system, in particular, the μ-receptors (Rethy *et al.*, 1971). Furthermore, morphine increases dopamine (DA) synthesis from the presynaptic terminals in the striatum and increases dopaminergic activity (Babbini *et al.*, 1972; Kuschinsky and Hornykiewicz, 1974). Dopamine turnover is also increased after morphine administration. Therefore, a single dose of morphine results in hyperactivity (Babbini *et al.*, 1972). Repeated morphine doses also result in a progressive increase in locomotor activity (Babbini *et al.*, 1972; Kilbey *et al.*, 1977; Kuribara *et al.*, 1989). This phenomenon is referred to as sensitization or reverse tolerance. It is thought that this sensitization represents an animal model of drug-induced psychosis (Allen and Young, 1978; Robinson and Becker *et al.*, 1986). Chronic morphine treatment has been also reported the deveopment of postsynaptic dopamine receptor supersensitivity in the central nervous system (CNS) (Kim *et al.*, 1999; Kim *et al.*, 1995). This increase in sensitivity is defined as a hypersensitivity in the direct-acting dopamine agonists, e.g., apomorphine and as an increase in the affinity of the dopamine receptors (John and Takemori, 1986; Bhargava 1980; Eidelberg and Erspamer, 1975; Ritzmann *et al.*, 1972). In addition, the development of reverse tolerance to the hyperactivity of morphine has been suggested to be closely related to the development of postsynaptic dopamine receptor supersensitivity (Kim *et al.*, 1999).

Several studies have demonstrated that the increased GABAergic activity attenuates the psychostimulant drug-induced increases in extracellular dopamine and the behaviors associated with these biochemical changes

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(Suzuki *et al.*, 1995; Finlay *et al.*, 1992; Dewey *et al.*, 1992; Gong *et al.*, 1998). Baclofen, a $GABA_B$ receptor agonist as well as diazepam, a benzodiazepine receptor agonist, both inhibit the increased locomotor activity caused by morphine (Woo and Kim, 2001).

It was previously reported that muscimol inhibited morphine-induced hyperactivity, reverse tolerance and postsynaptic dopamine receptor supersensitivity (Yoon *et al.*, 2002). Therefore, this study was conducted to determine whether another $GABA_A$ receptor agonist, THIP, directly inhibits via the activation of $GABA_A$ receptors.

MATERIALS AND METHODS

Animals and drugs

ICR male mice (Samyuk Laboratory Animal Inc., Osan, Korea) weighing 25-30 g in groups of 10 were used. Ten mice per cage were housed in a controlled room environment (temp. 22 ± 2°C, humidity 40 ± 10%). They were kept on a 12 hours light/dark cycle and were given a solid diet and tap water *ad libitum* for 1 week prior to the experiment. This investigation carried out in accordance with the Declaration of Helsinki for human subjects and with the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

The following drugs were used; morphine hydrochloride (Samsung Pharm Co. Korea), THIP (Tocris, USA), apomorphine (Sigma, USA). Morphine and THIP were dissolved in physiological saline. Apomorphine was dissolved in saline containing 0.1% ascorbic acid immediately prior to the experiment. Both morphine and apomorphine were administered subcutaneously (s.c.) and THIP was administered intraperitoneally (i.p.).

Measurement of morphine-induced hyperactivity

Morphine-induced hyperactivity using a 10 mg/kg morphine dose was investigated according to the method reported previously (Kim *et al.*, 1999; Kim *et al.*, 1995). The ambulatory activity of the mice was measured using a tiling-type ambulometer (AMB-10; O'hara Co., Ltd., Tokyo, Japan). Each mouse was placed in an activity cage (20 cm in diameter and 18 cm in height) and 10 mg/kg of morphine was administered after an adaptation period of 10 min. The mice were pretreated with THIP (i.p.) 30 min before the morphine injection (10 mg/kg). The ambulatory activity was measured every 10 min for 1 h after the morphine injection.

Measurement of the development of reverse tolerance to the hyperactivity

Reverse tolerance to the ambulatory activity caused by morphine (10 mg/kg) had developed significantly within a period of 6 days. Thus, morphine (10 mg/kg) was administered to the mice once a day for 6 days to induced reverse tolerance according to method reported previously (Kim *et al.*, 1999; Kim *et al.*, 1995). THIP was administered 30 min before the morphine injection once a day for 6 days. To test the degree of the development of the reverse tolerance to morphine, the mice were injected only with morphine on day 7, 24 h after the final morphine injections to avoid any residual effects of the test drugs themselves. The morphine-induced reverse tolerance was measured for 1 h using a tiling-type ambulometer. The mice were first allowed to pre-ambulate for 10 min in the activity cages followed by a 1 h test period immediately after the morphine injection.

Measurement of the development of postsynaptic dopamine receptor supersensitivity

Additional groups of mice with the same chronic morphine and THIP treatment, were used to determine the effects of these treatments on the development of postsynaptic dopamine receptor supersensitivity.

To determine the development of postsynaptic dopamine receptor supersensitivity in the reverse tolerant mice, morphine (10 mg/kg, s.c.) was administered once a day for 6 days, The degree of the development of morphine-induced postsynaptic dopamine receptor supersensitivity was shown by the enhanced ambulatory activity induced by apomorphine on day 7, 24 h after the final injection of morphine. The mice were first allowed to preambulate for 10 min followed by a 20 min test period immediately after the 2 mg/kg apomorphine (s.c.) injection, a dose which produced a significant increase in ambulatory activity.

Statistics

The data were expressed as means ± S.E.M. The significance of the drug effects was assessed by an analysis of variance (ANOVA). In the case of a significant variation, the individual values were compared by a Dunnett's *t*-test

RESULTS

Inhibitory effects of THIP on morphine-induced hyperactivity

The morphine-treated group showed approximately 1500% in ambulatory activity (2156 counts, $P<0.01$), when compared to the saline group (142 counts). Meanwhile, 0.2, 0.4 and 0.8 mg/kg THIP administered 30 min prior to the morphine injection inhibited the morphine-induced hyperactivity by approximately 25% ($P<0.01$), 49% ($P<0.01$) and 88% ($P<0.01$), respectively, compared to the

morphine group (Fig. 1).

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

The morphine-induced ambulation-accelerating activity was progressively enhanced by 150% (3107 counts, $P<0.01$) by repeated morphine administration once a day for 6 days when compared to the saline group (2077 counts. This suggests that the development of reverse tolerance to the hyperactivity caused by morphine (Fig. 2).

Repeated administration of THIP alone (0.8 mg/kg) once a day for 6 days had little effect on the morphine-induced ambulatory activity. This suggests that the chronic administration of THIP did not itself produce an altered state of responsiveness to morphine. Meanwhile, THIP 0.2, 0.4 and 0.8 mg/kg administered 30 min before the morphine injection inhibited the development of morphine-induced reverse tolerance by approximately 19% (2502 counts, $P<0.05$), 28% (2255 counts, $P<0.01$) and 39% (1915 counts, $P<0.01$), respectively, when compared with the morphine group.

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

The mice that 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 218 counts ($P<0.01$), when compared to the saline group (104 counts). This suggests the development of postsynaptic dopamine receptor supersensitivity in the morphine-induced reverse tolerant mice (Fig. 3). However, the group treated with THIP (0.8 mg/kg) alone exhibited no significant apomorphine-induced ambulatory activity when compared to the saline group. These results suggest that chronic THIP administration by itself has no significant influence on the development of dopamine receptor supersensitivity in this paradigm. However, 0.2, 0.4 and 0.8 mg/kg of THIP administered 30 min prior to the morphine injection reduced the ambulatory activity of apomorphine by approximately 25% (164 counts, $P<0.05$), 31% (150 counts, $P<0.05$) and 42% (127 counts, $P<0.01$), respectively, when compared to the morphine control group. This suggests that THIP should inhibit the development of postsynaptic dopamine receptor supersensitivity in the morphine-induced reverse tolerant mice.

DISCUSSION

These results showed that pretreatment with THIP inhibited the hyperactivity induced by morphine as well as the development of reverse tolerance to this hyperactivity (Fig. 1 and 2). In addition, the development of postsynaptic dopamine receptor supersensitivity was also inhibited in the morphine-induced reverse tolerant mice (Fig. 3). These results suggest that THIP can inhibit the hyperactivity, reverse tolerance and postsynaptic dopamine receptor supersensitivity induced by morphine through the $GABA_A$ receptors.

These experiments also showed that morphine increased the ambulatory activities of mice and those effects were progressively enhanced by the repeated administration of

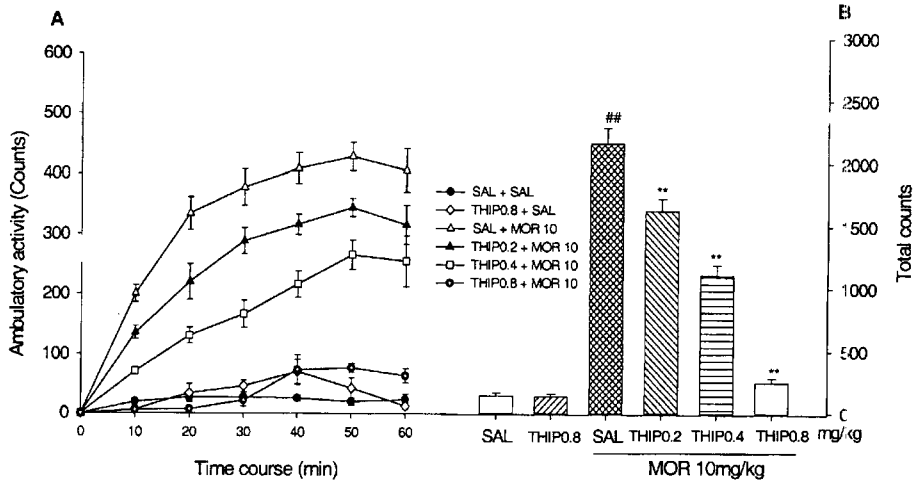

Fig. 1. Inhibition of morphine-induced hyperactivity by THIP. THIP (0.2, 0.4 and 0.8 mg/kg, i.p.) was administered 30 minutes prior to the morphine injection (10 mg/kg, s.c.). The ambulatory activities at each 10 min for 1 hr (A) after the administration of morphine are expressed as the total counts (B). ##$P<0.01$, compared with the saline (SAL+SAL) group. **$P<0.01$ compared the morphine (SAL+MOR) group. (Dunnetts *t*-test). SAL, saline; MOR, morphine; THIP, tetrahydroisoxazolopyridine.

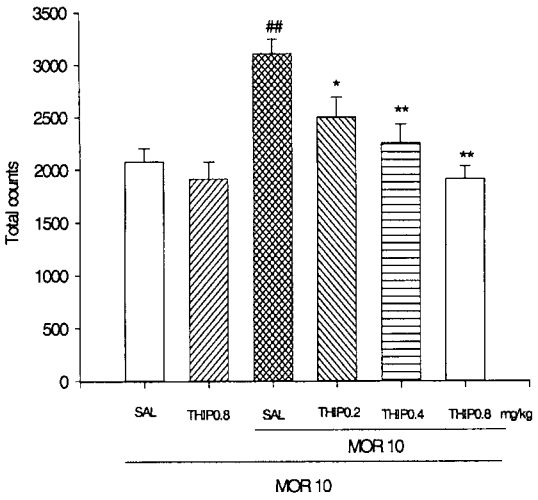

Fig. 2. Inhibition of morphine-induced reverse tolerance by THIP. Mice in a group of 10 were rendered reverse tolerant to morphine by a s.c. morphine injection (10 mg/kg). THIP was administered daily 30 min prior to the morphine injection. On day 7, 24 h after the final injection of morphine, the ambulatory activity of morphine 10 mg/kg without THIP injection was measured for 1 h. ##P<0.01, compared with the saline group. *P<0.05, **P<0.01 compared the morphine group.

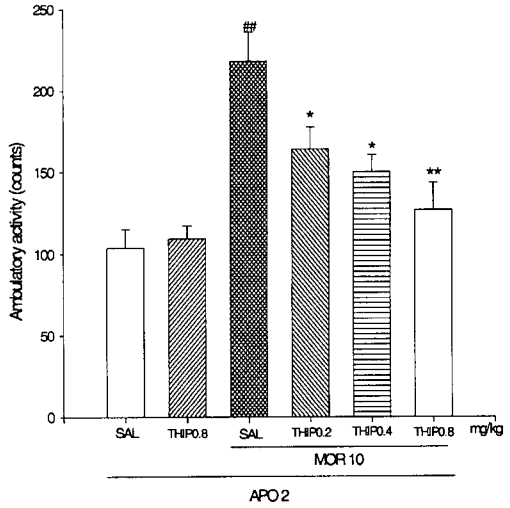

Fig. 3. Inhibitory Effects of THIP on the development of dopamine receptor supersensitivity in the morphine-induced reverse tolerant mice. The development of dopamine receptor supersensitivity was evidenced by enhanced ambulatory activity to apomorphine 24 h after the final morphine injection. Mice in a group of 10 were injected with apomorphine (2 mg/kg, s.c.) and first allowed to ambulate for 10 minutes and then tested for 20 minutes. ##P<0.01, compared with the saline group. *P<0.05, **P<0.01, compared with the morphine group.

morphine indicating the development of reverse tolerance. These results concur with those reported previously (Kuribara and Tadokoro, 1989; Kim *et al.*, 1995). The phenomenon of reverse tolerance is a model for invesgating the psychotoxicity of dependence-liable drugs (Allen and Young, 1978; Robinson and Becker, 1986).

It has been demonstrated that both the $GABA_A$ agonist, pregnanolone, and the $GABA_B$ agonist, baclofen, significantly decrease the level of extracellular dopamine in the rat striatum in vivo, while the $GABA_A$ antagonist, bicuculline, and the $GABA_B$ antagonist, phaclofen, increased the level of extracellular dopamine (Smolders *et al.*, 1995). Both baclofen and diazepam, also attenuate morphine-induced locomotor activity (hyperactivity and reverse tolerance) (Woo and Kim, 2001). Diazepam inhibits the morphine-induced position preference in dopamine turnover in the limbic forebrain (Suzuki *et al.*, 1995). Benzodiazepines, which are positive allosteric modulators of GABA activity at $GABA_A$ receptors, significantly attenuate both the dopaminergic and behavioral response to a cocaine challenge (Allen and Young, 1978; Giorgetti *et al.*, 1998; Merrinne *et al.*, 1999). Baclofen, a $GABA_B$ receptor agonist, also inhibits the reinforcing effects of morphine and cocaine (Tsuji *et al.*, 1996; Roberts *et al.*, 1996; Xi and Stein, 1999). These studies suggest that activating the $GABA_A$, $GABA_B$ and benzodiazepine receptors would inhibit the capacity of either morphine or cocaine to activate dopamine neurotransmission.

In addition, the phenomenon of the development of postsynaptic dopamine receptor supersensitivity has been suggested to be a possible mechanism for the behavioral sensitization (reverse tolerance) to psychomotor stimulatory drugs and opioids (Kim *et al.*, 1999). These results are consistent with those reports because the development of postsynaptic dopamine receptor supersensitivity was observed in the morphine reverse tolerant mice. The development of reverse tolerance and postsynaptic dopamine receptor supersensitivity was inhibited at the same time in the mice treated with THIP. The $GABA_A$ receptor directly gates a Cl^- ionophore and has modulatory binding sites for benzodiazepines, barbiturates, neurosteoids and ethanol. In contrast, baclofen couples to the Ca^{2+} and K^+ channels *via* G proteins and secondary messenger systems. Although the mechanisms of $GABA_A$ and $GABA_B$ drugs differ, the GABAergic drugs inhibited morphine-induced hyperactivity, reverse tolerance and dopamine receptor supersensitivity in mice in this experiment (Woo and Kim, 2001). It is strongly suggested that GABAergic drugs commonly interact with morphine and modulate morphine action.

Morphine binds to the μ-opioid receptors that exist on presynaptic dopaminergic structures (Ritzmann *et al.*, 1976). Therefore, the inhibitory effects of THIP on the development of reverse tolerance and postsynaptic dopamine receptor supersensitivity, might be associated with the modulation of chronic morphine action at the presynaptic dopamine receptors. This is because morphine action acts indirectly on the dopamine receptors (Puri and

Lal, 1973; Bhargava, 1980). It has also been reported that morphine-induced reverse tolerance appears to involve the activation of the dopaminergic system in the brain (Wood and Altar, 1988). In accordance with this report, the postsynaptic DA receptor supersensitivity to apomorphine, a DA receptor agonist, was developed after the repeated administration of morphine (Ritzmann *et al.*, 1976; Bhargava, 1980). This shows that the inhibitory effects of a $GABA_A$ agonist on both the morphine-induced reverse tolerance and the postsynaptic DA receptor supersensitivity may be closely related to the recovery of a dysfunction in the dopaminergic system produced by morphine in the central nervous system.

In conclusion, THIP inhibited the hyperactivity, reverse tolerance and postsynaptic dopamine receptor supersensitivity induced by morphine. These results suggest that the hyperactivity, reverse tolerance and postsynaptic dopamine receptor supersensitivity induced by morphine may be modulated by THIP by activating the $GABA_A$ receptors.

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