Effects of Rolipram, a Selective Inhibitor of Phosphodiesterase 4, on Hyperlocomotion Induced by Several Abused Drugs in Mice

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ABSTRACT—The effects of rolipram, a selective inhibitor of phosphodiesterase 4, on the hyperlocomotion induced by several abused drugs (methamphetamine, morphine and phencyclidine) and a dopamine D₂-receptor agonist (SKF81297; (±)-6-chloro-7,8-dihydroxy-1-phenyl-2,3,4,5-tetrahydro-1H-3-benzazepin hydrobromide) in mice were investigated. Methamphetamine (0.5 – 2.0 mg/kg), morphine (5.0 – 20 mg/kg), phencyclidine (1.25 – 5.0 mg/kg) and SKF81297 (2.5 – 10 mg/kg) each induced dose-dependent hyperlocomotion. A low dose (1.0 mg/kg) or moderate dose (3.2 mg/kg) of rolipram suppressed methamphetamine (2.0 mg/kg)- and morphine (20 mg/kg)-induced hyperlocomotion, but not phencyclidine (5.0 mg/kg)-induced hyperlocomotion. These results suggest that cAMP in the brain is involved in methamphetamine- and morphine-induced hyperlocomotion, while the underlying mechanism(s) of phencyclidine-induced hyperlocomotion may be different from those of methamphetamine- and morphine-induced hyperlocomotion. It is well known that methamphetamine- and morphine-induced hyperlocomotion are mediated by the dopaminergic system and that interaction between postsynaptic D₁ and D₂ receptors may play an important role in the expression of various dopamine-mediated behaviors. In the present study, SKF81297 (10 mg/kg)-induced hyperlocomotion was significantly but not completely suppressed by the highest dose of rolipram (10 mg/kg). Therefore it is unlikely that postsynaptic D₂-receptor-mediated functions are involved in the suppressive effects of rolipram on methamphetamine- and morphine-induced hyperlocomotion. These results suggest that rolipram may inhibit methamphetamine- and morphine-induced hyperlocomotion via increase cAMP levels at D₂-receptors.

Keywords: Rolipram, Methamphetamine, Phencyclidine, Morphine, SKF81297

It is well known that psychostimulants, such as amphetamine, methamphetamine and cocaine, as well as other abused drugs, such as morphine and heroin, induce hyperlocomotion in rodents, which is believed to be mediated by the dopaminergic, and especially the mesolimbic dopaminergic system (1 – 4). Furthermore, dopamine neurotransmission in the nucleus accumbens, which is the terminal region of the mesolimbic dopaminergic system, is thought to mediate the rewarding effects of abused drugs (5, 6). On the other hand, there are two superfamilies of dopamine receptors, designated D₁-like and D₂-like receptors (7). D₁-receptor agonists and D₂-like receptor agonists act synergistically in the expression of hyperlocomotion, stereotypies and rewarding effects, suggesting that the interaction between D₁ and D₂-receptors may play an important role in the expression of various dopamine-mediated behaviors (8 – 10).

Phencyclidine produces many amphetamine-like stimulant effects, particularly in rodents, including increased locomotor activity, stereotyped behavior and enhancement of the effects of amphetamine-like drugs. It is widely believed that the locomotor effects, at least, are mediated by dopamine release in the nucleus accumbens (11). However, recent studies have demonstrated that the mechanisms of phencyclidine-induced hyperlocomotion can not be explained merely by the enhancement of dopaminergic transmission (2, 4).

Rolipram is a selective inhibitor of cyclic adenosine 3',5'-monophosphate (cAMP)-specific phosphodiesterase 4 (PDE4) and enhances cAMP-mediated functions by inhibiting cAMP metabolism (12). Recently, Iyo et al. (13, 14) demonstrated that rolipram significantly attenuated various methamphetamine-induced behaviors; e.g., hyperlocomotion, rearing, repetitive head movements and sensitization to locomotor activity in rats, suggesting that cAMP is involved in methamphetamine-induced behavioral...
changes. However, the involvement of cAMP in the acute behavioral effects of abused drugs in animals is not yet clear (13–17). Therefore, to investigate the involvement of cAMP in the behaviors induced by various abused drugs, we examined the effects of rolipram on methamphetamine-, morphine- and phencyclidine-induced hyperlocomotion in mice. Since D₁-receptor agonists also increase locomotor activity (8, 10), the effect of rolipram on SKF81297-induced hyperlocomotion in mice was also examined.

Morphine has been shown to inhibit adenylyl cyclase activity and the production of cAMP (18). Thus we would expect that rolipram would attenuate morphine-induced dopamine release. Therefore, we also measured the effect of rolipram on the morphine-induced increase in dopamine turnover in the limbic forebrain.

MATERIALS AND METHODS

Animals
Male ddY mice (Nihon SLC, Hamamatsu) weighing 25–35 g were used for the following experiments. The animals were housed at a room temperature of 20–25°C and under a 12-h light-dark cycle (lights on at 7:00 AM). Food and water were available ad libitum. All of the following procedures were conducted in accordance with the guiding principles for the care and use of laboratory animals of The Japanese Pharmacological Society and with the guidelines for animal care in our laboratories, as approved by the Meiji Seika Pharmaceutical Research Center Committee on animal care and use.

Locomotor activity
The locomotor activity of the animal was measured by an infra-red sensor (NS-AS01; Neuroscience, Inc., Tokyo) placed over an open-top box (23 × 33 × 12.5 cm). The data of locomotor activity were collected and analyzed with a personal computer (PC9800; NEC, Tokyo). Animals were placed in the box on sawdust 1-cm-deep for the 75-min habituation period and then injected with saline, methamphetamine (0.5–2.0 mg/kg, s.c.), phencyclidine (1.25–5.0 mg/kg, s.c.), morphine (5.0–20 mg/kg, s.c.), SKF81297 (2.5–10 mg/kg, s.c.) or rolipram (1.0–10 mg/kg, i.p.) in a volume of 10 ml/kg. In combination tests, animals were pretreated with vehicle or rolipram (1.0–10 mg/kg, i.p.) 15 min prior to the administration of methamphetamine (2.0 mg/kg, s.c.), phencyclidine (5.0 mg/kg, s.c.) or SKF81297 (10 mg/kg, s.c.). Rolipram was administered just before the injection of morphine (20 mg/kg, s.c.). Locomotor activity was measured for 2 h, except in the case of morphine, with which locomotor activity was measured for 3 h, because our preliminary data demonstrated that hyperlocomotion-induced by each of the drugs was continued through 2 or 3 h.

Neurochemical analysis
The concentrations of dopamine, 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) were determined. Animals were sacrificed by the dislocation of cervical vertebrae 60 min after the injection of saline (s.c.), vehicle (i.p.), morphine (20 mg/kg, s.c.) or rolipram (3.2 mg/kg, i.p.), and then immersed in a dry ice/ethanol solution for 20 s to minimize postmortem changes in dopamine-related substances. The brain was quickly removed and the limbic forebrain, including the nucleus accumbens and olfactory tubercle, was dissected on an ice-cold glass plate as follows. Briefly, the brain was turned to expose the dorsal surface and a vertical cut was made through the anterior commissure. The resulting frontal part was turned to expose the ventral surface. A vertical cut was passed through the rhinal fissure and a small part including the accessory olfactory bulb and olfactory nucleus was removed. The resulting block of tissue was turned to expose the section, and the area bordered by the caudate putamen and the nucleus accumbens was cut vertically. The block of tissue that included the nucleus accumbens and olfactory tubercle was considered to be the main portion of the limbic forebrain. The tissues were stored at −80°C until analysis.

The frozen tissues were homogenized in 50 vol (w/v) of 0.1 M perchloric acid containing 3 mM EDTA-2Na and 50 ng/ml of deoxypinephrine and 5-hydroxy-Na-methyltryptamine as internal standards. To remove the proteins completely, the homogenate was placed in cold water for 30 min and then centrifuged at 20,000×g for 15 min at 5°C. The supernatants obtained were added with sufficient 1 M sodium acetate to adjust the pH to 3–4. A 10-μl aliquot of each sample was applied to a high-performance liquid chromatography (HPLC) system with an electrochemical detection (ECD) unit. The HPLC system consisted of a delivery pump (L-6020; Hitachi, Tokyo), a sample injector (Gilion 231; Gilion, Middleton, WI, USA) and an analytical column (Cosmosil SC18, 4.6×250 mm; Nacalai Tesque, Kyoto). The electrochemical detector (Coulchem II; ESA, Bedford, MA, USA) was equipped with a 5020 guard cell and 5011 analytical cell and used at the following voltage settings: guard cell electrode, +450 mV; analytical cell electrodes, +50 mV followed by +400 mV. The mobile phase consisted of 40 mM phosphate/citrate Na buffer, pH 3.0, containing 0.1 mM EDTA-2Na, 5.5 mM 1-heptanesulfonate Na, 8.7% methanol and 4.3% acetonitrile, and was delivered at a flow rate of 0.8 ml/min. The column temperature was maintained at 20°C. Control of the HPLC system and an analysis were performed by a Macintosh IIfx computer using MacIntegrator II (Rainin, Woburn, MA, USA).
**Drugs**

The drugs used in the present study were rolipram (Schering AG, Berlin, Germany), methamphetamine hydrochloride (Daunippon Pharmaceutical Co., Osaka), morphine hydrochloride (Takeda Pharmaceutical Co., Osaka), phencyclidine (synthesized in our laboratory) and (±)-6-chloro-7,8-dihydroxy-1-phenyl-2,3,4,5-tetrahydro-1H-3-benzazepin hydrobromide (SKF81297; Research Biochemicals, Inc., Natick, MA, USA). All drugs except rolipram were dissolved in saline, and rolipram was suspended in a 0.5% carboxymethylcellulose-Na solution.

**Statistical analyses**

Data are expressed as the mean with S.E.M. A one-way analysis of variance (ANOVA) followed by a post hoc Dunnett's t-test were used for the statistical analysis. Dopamine turnover was calculated as dopamine turnover = (DOPAC (ng/g tissue) + HVA (ng/g tissue)) / dopamine (ng/g tissue).

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**RESULTS**

In the present study, rolipram (1.0 - 10 mg/kg) alone slightly suppressed spontaneous locomotor activity in mice, but this effect was not significant (total counts: 551 ± 109.6, maximally: 382.3 ± 79.3). Methamphetamine (1.0 and 2.0 mg/kg), phencyclidine (2.5 and 5.0 mg/kg), morphine (20 mg/kg) and SKF81297 (2.5, 5.0 and 10 mg/kg) each significantly increased locomotor activity (Fig. 1: A-D). Furthermore, hyperlocomotion-induced by drugs used in the present study continued through about 2 h (methamphetamine, phencyclidine and SKF81297) and 3 h (morphine).

In the combination study, methamphetamine (2.0 mg/kg) and morphine (20 mg/kg)-induced hyperlocomotion were significantly suppressed by treatment with 1.0 - 10 mg/kg and 3.2 - 10 mg/kg of rolipram, respectively. In these cases, 10 mg/kg of rolipram produced almost complete inhibition. On the other hand, SKF81297 (10 mg/kg)-induced hyperlocomotion was only partially, but still significantly, suppressed by the highest dose of rolipram (10 mg/kg).

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**Fig. 1.** Effects of methamphetamine (A), morphine (B), phencyclidine (C) and SKF81297 (D) on the spontaneous locomotor activity in mice. Each column represents the mean total counts with S.E.M. of 10 animals. **P<0.01, vs saline control.**
Fig. 2. Effects of rolipram on the hyperlocomotion induced by methamphetamine (A), morphine (B), phencyclidine (C) and SKF81297 (D) in mice. Each column represents the mean total counts with S.E.M. of 6 animals. **P<0.01, vs drug control.

mg/kg), while phencyclidine-induced hyperlocomotion was only slightly, but not significantly suppressed, by the highest dose of rolipram (Fig. 2: A–D).

The effect of rolipram on the morphine-induced increase in dopamine turnover in the mouse limbic forebrain is shown in Fig. 3. Morphine (20 mg/kg) increased dopamine turnover in the mouse limbic forebrain (1.11 ± 0.09 in the morphine group vs 0.81 ± 0.05 in the control group, n = 7–9). On the other hand, rolipram (3.2 mg/kg) did not affect either basal dopamine turnover or the increased dopamine turnover induced by morphine.

DISCUSSION

Consistent with previous results in rats (13), rolipram potently suppressed methamphetamine-induced hyperlocomotion in mice. Furthermore, morphine-induced hyperlocomotion was also completely suppressed by rolipram. These results suggest that cAMP plays an inhibitory role in methamphetamine- and morphine-induced hyperlocomo-
tion. Methamphetamine and morphine increased dopamine release in the nucleus accumbens, which is the terminal region of the mesolimbic dopaminergic system. These dopamine-releasing effects may play an important role in their hyperlocomotion, rewarding and other behavioral effects (5, 6, 19). A previous microdialysis study demonstrated that rolipram has no effect on the dopamine release induced by methamphetamine (14). Furthermore, the morphine-induced increase in dopamine turnover in the limbic forebrain, probably due to an increase in dopamine release (20, 21), was not affected by 3.2 mg/kg of rolipram, which completely suppressed morphine-induced hyperlocomotion. Therefore, the effects of rolipram on methamphetamine- and morphine-induced hyperlocomotion might not be due to the modulation of dopaminergic neurons.

D1-receptors are coupled positively to adenylate cyclase, and activation of these receptors may increase intracellular cAMP, while D2-receptors are negatively coupled to this enzyme (22, 23). Previous studies have indicated that D1-receptor agonists and D2-like receptor agonists act synergistically in the expression of hyperlocomotion, stereotypy and rewarding effects postsynaptically (8–10). On the other hand, various lines of evidence show that the behavioral effects of both methamphetamine and morphine are antagonized by dopamine D1- and D2-receptor antagonists (24–28). Apomorphine (2.0 mg/kg)-induced climbing behavior, which is mediated by both D1- and D2-receptors, is suppressed by 3.2 mg/kg of rolipram (T. Mori et al., unpublished data). However, the hyperlocomotion induced by the selective dopamine D1-receptor agonist SKF81297 was only partially attenuated by 10 mg/kg rolipram (which induces potent sedation). Therefore, postsynaptic D2-receptor-mediated functions might not be involved in the suppressive effects of rolipram on methamphetamine- and morphine-induced hyperlocomotion. Considering that rolipram does not modify the dopamine release induced by methamphetamine or morphine (ref. 14 and present study), rolipram may postsynaptically inhibit the effects of methamphetamine and morphine, especially, increase cAMP levels at D2-receptors that are negatively coupled to adenyl cyclase.

The firing of dopaminergic neurons in the ventral tegmental area is negatively regulated by GABAergic interneurons (29). Morphine and other μ-opioid receptor agonists indirectly relieve this negative regulation by inhibiting the firing of these GABA neurons in the ventral tegmental area (28, 29). This mechanism has been proposed to mediate the dopamine release and locomotor enhancement induced by morphine. On the other hand, morphine has been shown to inhibit adenylate cyclase activity and the production of cAMP, and this may be involved in the dopamine release induced by morphine (18). Therefore, we expected that the dopamine release induced by morphine would be suppressed by rolipram, and that this effect may play a role in the suppression of morphine-induced hyperlocomotion by rolipram. In the present study, the morphine-induced increase in dopamine turnover in the limbic forebrain was not affected by 3.2 mg/kg rolipram, which completely suppressed morphine-induced hyperlocomotion. In accordance with our present results, the antinociceptive effects of morphine were not affected by rolipram (T. Nabeshima, personal communication). However, it is not clear why rolipram suppresses some of morphine’s effects but not others. Therefore, further examination is necessary to clarify the mechanism involved in the interaction of morphine and rolipram.

Although antagonism of the actions of psychostimulants, such as amphetamine or methamphetamine, is widely used to detect antipsychotic activity, phencyclidine is considered to be a more faithful tool for establishing a model of schizophrenia (30). We found that phencyclidine-induced hyperlocomotion is less sensitive to rolipram than methamphetamine-induced hyperlocomotion. This is an unexpected result, since several dopamine receptor antagonists can suppress both phencyclidine- and methamphetamine-induced hyperlocomotion in animals (30, 31). This suggests that the underlying mechanism of phencyclidine-induced hyperlocomotion is different from that of methamphetamine-induced hyperlocomotion. The mechanism of action of phencyclidine is complex; in addition to blocking NMDA receptors, phencyclidine can also enhance both dopaminergic and serotonergic neurotransmission (32). Recent reports have suggested that phencyclidine-induced hyperlocomotion and other behavioral changes are dependent on the 5-HT2A-receptor (2, 4, 33, 34), which is coupled to phospholipase C, but not to adenylate cyclase (35). This may explain why phencyclidine-induced hyperlocomotion is less sensitive to rolipram, unlike those induced by methamphetamine and morphine.

In summary, the results of the present study suggest that methamphetamine- and morphine-induced hyperlocomotion are mediated by cAMP in the brain, while the underlying mechanism(s) of phencyclidine-induced hyperlocomotion may be different from those of methamphetamine- and morphine-induced hyperlocomotion.

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