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Prevention of methamphetamine-induced behavioral sensitization in rats by a cyclic AMP phosphodiesterase inhibitor, rolipram

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Abstract

Effects of an interaction between rolipram, a cyclic adenosine 3′,5′-monophosphate (cyclic AMP) phosphodiesterase inhibitor, and methamphetamine on the development of behavioral sensitization were observed in rats. In vivo microdialysis showed that a single dose of 4 mg/kg methamphetamine (i.p.) significantly increased striatal dopamine levels while coadministration with 4 mg/kg rolipram (i.p.) did not affect these levels. Also, methamphetamine alone did not alter striatal cyclic AMP levels but coadministration with rolipram and rolipram alone significantly increased these levels. The administration of 4 mg/kg methamphetamine (i.p.) once a day for 5 days significantly enhanced hyperlocomotion and rearing induced by a 2-mg/kg methamphetamine challenge (i.p.) after a 1-week withdrawal period, compared with controls or coadministration with 4 mg/kg rolipram (i.p.). Striatal dopamine levels, detected by in vivo microdialysis, were increased following the challenge but were comparable between the groups. These findings suggest that rolipram prevents methamphetamine-induced behavioral sensitization by increasing cyclic AMP levels while not affecting dopamine-releasing processes.

Keywords: cAMP; Behavioral sensitization; Rolipram; Methamphetamine; Microdialysis, in vivo

1. Introduction

Repeated administration of psychostimulants such as amphetamine or methamphetamine to experimental animals augments hyperlocomotion and stereotyped behavior, i.e., produces behavioral sensitization (Randrup and Munkvad, 1970; Segal and Mandell, 1974; Post and Rose, 1976; Nishikawa et al., 1983). This sensitization appears to be mediated by drug-induced alterations of both pre- and postsynaptic mechanisms in the mesolimbic and/or nigrostriatal dopamine systems (Robinson and Becker, 1986; Kalivas and Stewart, 1991; Henry and White, 1992; Wolf et al., 1994). It has been reported that repeated administration of methamphetamine or amphetamine enhances stimulant-induced dopamine efflux in the striatum and nucleus accumbens, in association with behavioral sensitization (Robinson et al., 1988; Kazahaya et al., 1989; Hamamura et al., 1991). However, a diminished amphetamine-induced dopamine response has also been reported after behavioral sensitization (Segal and Kuczenski, 1992). It has been reported that the development of sensitization is prevented by the selective dopamine D_1 receptor antagonist, SCH23390 (*R*(+)-7-chloro-8-hydroxy-1-phenyl-2,3,4,5-tetrahydro-1*H*-3-benzazepine hydrochloride) (Stewart and Vezina, 1989; Vezina and Stewart, 1989; Ujike et al., 1989; Drew and Glick, 1990). In contrast, the results of co-administration of dopamine D_2 receptor antagonists have been inconsistent. YM-09151-2 (nemonapride; *cis*-5-chloro-2-methoxy-4-(methylamino)-*N*-[2-methyl-2-(phenylmethyl)-3-pyrrolidinyl]benzamide) prevents the development of sensitization (Ujike et al., 1989), whereas sulpiride or Ro-22-2586 (*R*(−)-2,6-dimethyl-3-ethyl-4,4a,5,6,7,8,8a,9-octahydro-4a,8a-trans-1*H*-pyrrolo[2,3-g]isoquinolin-4-one) (Vezina and Stewart, 1989; Drew and Glick, 1990) does not.

It is known that dopamine D_1 receptors increase the level of cyclic adenosine 3′,5′-monophosphate (cyclic AMP) by stimulating adenylate cyclase activity, whereas dopamine D_2 receptors are either not linked to, or inhibit,

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this enzyme (Kebabian and Calne, 1979; Seeman, 1980). A relationship between amphetamine- or methamphetamine-induced behavioral sensitization and enhanced c-fos (one of the immediate-early genes) expression in rat striatum has been suggested (Norman et al., 1993; Ohno et al., 1994). Recent studies have also indicated that cyclic AMP may play an important role in c-fos induction (Berretta et al., 1992). As the synthesis of certain proteins (Karler et al., 1993) may be involved in the development of sensitization, molecular events involving cyclic AMP may trigger the synthesis of proteins which may be associated with behavioral sensitization. Furthermore, it has been reported that amphetamine treatments alter dopamine-sensitive adenylate cyclase activity (Barnett et al., 1987; Roseboom et al., 1990). Thus, intracellular cyclic AMP may play an important role, not only in the expression of the behavioral consequences of a single injection of a drug, but also in the development of behavioral sensitization.

Rolipram is a selective cyclic AMP phosphodiesterase type IV inhibitor (Lowe and Cheng, 1992), which enhances the availability of cyclic AMP in the brain by inhibiting its metabolism (Wachtel, 1982) in the absence of direct stimulation of neurotransmitter receptors (Schneider et al., 1986) or alteration of dopamine release and metabolism (Kehr et al., 1985). We have previously reported that this drug suppresses some of the behavioral effects induced by a single injection of methamphetamine in rats (Iyo et al., 1995).

In the present study, in order to understand the interaction between methamphetamine and rolipram and its effect on cyclic AMP levels, we evaluated the effects of methamphetamine and rolipram on striatal dopamine and cyclic AMP levels in rat striatum following a single administration of methamphetamine using an in vivo microdialysis technique. We then investigated the effects of rolipram on the development of behavioral sensitization to methamphetamine. We also observed striatal dopamine release in response to a methamphetamine challenge, using in vivo microdialysis in rats repeatedly given methamphetamine and rolipram.

2. Materials and methods

2.1. Animals

Male Wistar rats (weighing 180–240 g) were used. The animals were housed in groups of 3 per cage. They were maintained under standard conditions (12 h-12 h light-dark cycle: light on from 06:00 to 18:00 h, room temperature 23 ± 0.5°C, humidity 55 ± 5%) with free access to food and water for at least 1 week before being subjected to experimental manipulation.

2.2. Drugs

Rolipram (a gift from Meiji Seika Co.) was suspended in physiological sodium chloride solution containing 10% w/v Cremophor (polyethoxylated castor oil, BASF, Ludwigshafen, Germany). Methamphetamine was dissolved in physiological saline. The injection volume was 0.1 ml per 100 g body weight on each occasion. Chemicals used were purchased commercially.

2.3. Test for behavioral sensitization

Thirty rats were divided into four groups for behavioral assessment of the effects of repeated drug administration. The groups were treated intraperitoneally (i.p.) once daily with 4 mg/kg methamphetamine + vehicle (methamphetamine group; $n = 6$), 4 mg/kg methamphetamine + 4 mg/kg rolipram (methamphetamine + rolipram group; $n = 6$), saline + 4 mg/kg rolipram (rolipram group; $n = 6$), or saline + vehicle (control group; $n = 12$) for 5 consecutive days. Counts of locomotor activity and rearing were performed using the method of Iyo et al. (1995) with an animal movement analysis system (SCANET SV-10, MATYS, Toyama, Japan), 1 week after the final injection. The system consisted of 2 rectangular enclosures (440 × 300 mm). The side walls (60 mm height) of the enclosure were equipped with 144 pairs of photosensors located at intervals of 5 mm at a height of 30 mm from the bottom edge. The upper and lower enclosures were set so that their photosensors were 30 mm and 150 mm above the cage floor, respectively. Each pair of photosensors was scanned every 0.1 s to detect animal movements. An intersection of 2 paired photosensors (10 mm interval) in the lower enclosure was counted as one unit of locomotor activity. An intersection of photosensors in the higher enclosure was counted as one rearing movement. The rats were placed individually in a square, transparent plastic cage with dimensions of 287 × 287 mm internal floor area and 350 mm in height, which was set on the SCANET SV-10 40 min before drug injection. Following injection of 2 mg/kg methamphetamine, each rat was replaced in the cage and behavioral measurements were started. Half of the rats ($n = 6$) in the control group received a saline challenge and the movements were counted in the same manner as for the methamphetamine-challenged rats. Data collected for 5-min intervals over 60 min after the challenge injection of methamphetamine were used for analysis.

2.4. In vivo microdialysis study

Using the in vivo microdialysis method, we first evaluated the effect of rolipram on methamphetamine-induced changes in the levels of striatal dopamine and cyclic AMP (acute administration study), and second, the response of striatal dopamine to methamphetamine challenge in the rats repeatedly given methamphetamine with and without rolipram (repeated administration study). The results were expressed as percentage changes relative to the level before drug injection (baseline level) in each rat.

2.4.1. Surgery

The rats were anesthetized with pentobarbital (50 mg/kg i.p.) and placed in a stereotaxic frame. The skull was exposed and a hole was drilled for implantation of a guide cannula in the anterior striatum (coordinates: anterior +0.2 mm, lateral +3.0 mm from the bregma and ventral −3.5 mm from the dural surface). A dummy cannula was placed in the guide cannula until the experiment was performed. After surgery, the rats were housed individually, and the microdialysis experiment was performed 3 days later. The dummy cannula was withdrawn and a probe (3.5 mm, EICOM, Japan) was inserted into the guide cannula. The rats were then placed individually into the square, transparent plastic cage of the SCANET SV-10 apparatus.

2.4.2. Measurement of dopamine

For the acute administration study, 16 rats underwent surgery 3 days before the experiment. The rats were divided into four groups, i.e., a 4 mg/kg methamphetamine + vehicle group (methamphetamine group, $n = 4$), a 4 mg/kg methamphetamine + 4 mg/kg rolipram group (methamphetamine + rolipram group, $n = 4$), a saline + 4 mg/kg rolipram group (rolipram group, $n = 4$), and a saline + vehicle group (control group, $n = 4$) for evaluation of the effects of methamphetamine and rolipram. All injections were administered intraperitoneally (i.p.).

For the repeated administration study, rats were treated i.p. once daily with 4 mg/kg methamphetamine + vehicle (methamphetamine group, $n = 8$), 4 mg/kg methamphetamine + 4 mg/kg rolipram (methamphetamine + rolipram group, $n = 8$), or saline + vehicle (control group, $n = 6$) for 5 consecutive days. Four days after the final injection, the rats underwent surgery as above, and a methamphetamine challenge experiment using microdialysis was performed 3 days later.

Striatal perfusate assay samples were collected in both experiments as follows. Artificial cerebrospinal fluid (NaCl 147 mM, $CaCl_2$ 2.3 mM, KCl 4.0 mM) was perfused at a constant flow rate of 2.0 ml/min using a microinjection pump (EP-60, EICOM, Japan). A minimum of 2 h was allowed for adaptation following probe insertion. For assay of dopamine, the dialysate was sampled every 30 min for 60 min before and 120 min after injection of the appropriate drug (i.p.). Each sample was injected directly into a high pressure liquid chromatography (HPLC)-electrochemical detector (ECD) system (SCL-6A, Shimadzu, Japan: ECD-100, EICOM, Japan).

2.4.3. Measurement of cyclic AMP

Seventeen rats underwent the surgical procedure 3 days before the experiment. Striatal cyclic AMP levels were

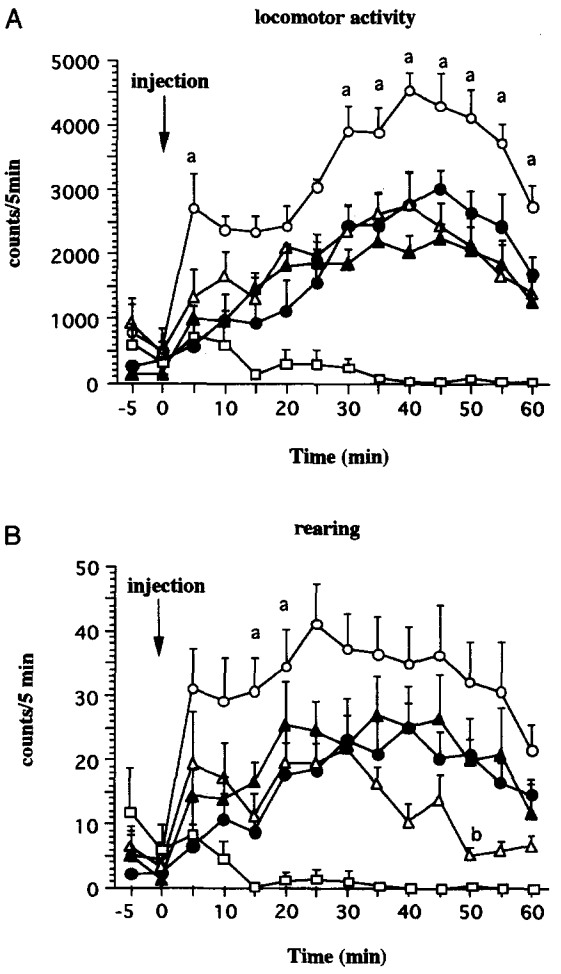

Fig. 1. (A) Locomotor activity response to 2-mg/kg methamphetamine or saline challenge. ○, ●, ▲ and △ indicate methamphetamine challenge of the rats pretreated with 4 mg/kg methamphetamine + vehicle (methamphetamine group), 4 mg/kg methamphetamine + 4 mg/kg rolipram, saline + rolipram and saline + vehicle (control), respectively. □ indicates the response to saline challenge of the rats pretreated with saline + vehicle. The locomotor activity in the groups challenged with methamphetamine increased compared with that in the saline-challenged group. ANOVA indicated that methamphetamine pretreatment significantly increased locomotor activity compared with other groups ($P < 0.001$) and that coadminstration of rolipram significantly decreased the effect of methamphetamine pretreatment ($P < 0.001$). Locomotor activity in the methamphetamine group was significantly increased compared with that in the other three groups at 5 min and at all time points from 30 to 60 min ($P < 0.05$ by Fisher PLSD). (B) Rearing response to 2-mg/kg methamphetamine or saline challenge. ○, ●, ▲ and △ indicate methamphetamine challenge of the rats pretreated with 4 mg/kg methamphetamine + vehicle (methamphetamine group), 4 mg/kg methamphetamine + 4 mg/kg rolipram, saline + rolipram and saline + vehicle (control), respectively. □ indicates the response to saline challenge of the rats pretreated with saline + vehicle. The rearing in the groups challenged with methamphetamine increased compared with that in the saline-challenged group. ANOVA indicated that methamphetamine pretreatment significantly increased rearing compared with that in other groups ($P < 0.001$) and that coadminstration of rolipram significantly decreased the effect of methamphetamine pretreatment ($P < 0.01$). Rearing in the methamphetamine group was significantly increased compared with that in the other three groups at 15 and 20 min ($P < 0.05$ by Fisher PLSD). Values are expressed as means with bars indicating S.E.M. ($n = 6$). The data were analyzed by one-way ANOVA followed by Fisher's PLSD test. [a] $P < 0.05$, methamphetamine vs. the other groups, [b] $P < 0.05$ control vs. the other groups.

measured by the method of Stone and John (1992). Artificial cerebrospinal fluid containing rolipram (10^{-3} M) to avoid cyclic AMP metabolism in the collection tube was perfused at a constant flow rate of 1.0 ml/min using the microinjection pump for measurement of cyclic AMP levels. A minimum adaptation period of 2 h was allowed following probe insertion. The dialysate was sampled every 30 min for 60 min before and 120 min after injection of the appropriate drugs (i.p.), i.e., 4 mg/kg methamphetamine + vehicle (methamphetamine group), 4 mg/kg methamphetamine + 4 mg/kg rolipram (methamphetamine + rolipram group) or saline + 4 mg/kg rolipram (rolipram group). Each dialysate sample was collected into an ice bath, frozen immediately and stored at −30°C until required. Cyclic AMP levels were measured using a radioimmunoassay kit (125I-cAMP SPA system, Amersham).

2.5. Statistical analysis

The results of the behavioral assessment or microdialysis were evaluated by one-way or two-way analysis of variance (ANOVA) followed by the Fisher's Protected Least Significant Difference (PLSD) test, respectively. The level of significance was $P < 0.05$.

3. Results

3.1. Test for behavioral sensitization

Fig. 1A shows the locomotor activity response to a 2-mg/kg methamphetamine challenge. The rats in each group challenged with methamphetamine exhibited a significant increase in locomotor activity compared with the saline-challenged rats ($P < 0.001$). Two-way ANOVA (factors: methamphetamine and rolipram) indicated significant pretreatment effects of methamphetamine, rolipram and methamphetamine + rolipram ($P < 0.005$). One-way ANOVA (factor: rolipram) indicated a significant inhibitory effect of coadministration of rolipram on locomotor activity (methamphetamine + rolipram group) compared with the methamphetamine group ($P < 0.001$). Locomotor activity in the methamphetamine group was significantly increased compared with that in the other three groups at 5 min and at all time points from 30 to 60 min ($P < 0.05$, Fisher s PLSD). One-way ANOVA of results at each time point also indicated that there was no significant difference between the methamphetamine + rolipram, rolipram and control groups ($P > 0.1$). We also compared cumulative data collected over 60 min between the groups (data not shown). The rats in the methamphetamine group exhibited significantly more locomotor activity than did those in the control group ($P < 0.01$, Fisher's PLSD). In the methamphetamine + rolipram group, the rats showed significantly less locomotor activity than did those in the methamphetamine group ($P < 0.01$, Fisher's PLSD). There was no significant difference between the control and rolipram groups.

Fig. 1B shows the rearing response to a 2-mg/kg methamphetamine challenge. The rats in each group challenged with methamphetamine exhibited a significant increase in rearing compared with the saline-challenged group ($P < 0.001$). Two-way ANOVA (factors: methamphetamine and rolipram) indicated significant pretreatment effects of methamphetamine, rolipram and methamphetamine + rolipram ($P < 0.005$). One-way ANOVA (factor: rolipram) indicated a significant inhibitory effect of coadministration of rolipram (methamphetamine + rolipram group) on rearing compared with the methamphetamine group ($P < 0.001$). Rearing in the methamphetamine group was significantly increased compared with that in the other three groups at 15 and 20 min ($P < 0.05$ by Fisher s PLSD). One-way ANOVA of results at each time point again indicated that there was no significant difference between the methamphetamine + rolipram, rolipram and control groups ($P > 0.1$), except that the counts in the control group decreased significantly compared with those in the methamphetamine + rolipram or rolipram groups at 50 min ($P < 0.05$, Fisher's PLSD). We also compared cumulative data collected over 60 min between the groups (data not shown). The rats in the methamphetamine group exhibited significantly more rear-

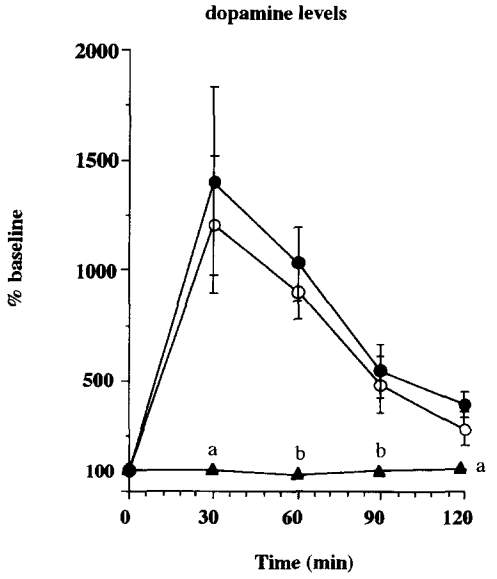

Fig. 2. Effects of methamphetamine (4 mg/kg, i.p.) and rolipram (4 mg/kg, i.p.) on extracellular dopamine levels in rat striatum measured by in vivo microdialysis. Dopamine levels were increased by methamphetamine both without and with rolipram compared with the baseline level (time 0), whereas rolipram alone produced no alteration. Dialysate samples were collected every 30 min and dopamine levels were expressed as the percentage change compared with the baseline level (time 0: level before the challenge). ○, ●, and ▲ indicate methamphetamine + vehicle administration ($n = 4$), methamphetamine + rolipram ($n = 4$) and saline + rolipram ($n = 4$), respectively. Bars indicate S.E.M. [a] $P < 0.05$ and [b] $P < 0.01$ by Fisher's PLSD test: saline + rolipram vs. methamphetamine + vehicle or methamphetamine + rolipram.

ing than did the controls ($P < 0.01$, Fisher's PLSD). In the methamphetamine + rolipram group, the rats showed significantly less rearing than did those in the methamphetamine group ($P < 0.05$, Fisher's PLSD). No significant difference was observed between the control and rolipram groups.

3.2. Striatal dopamine

Fig. 2 shows the results of the acute administration study. Striatal dopamine levels increased following drug injection in the methamphetamine group (Fig. 2). The baseline (time = 0) levels were 6166 ± 2238, 60266 ± 1400 and 30806 ± 943 area under peak (AUP: means ± S.E.M.) in the methamphetamine, methamphetamine + rolipram and rolipram groups, respectively, there being no significant difference ($P > 0.1$). There was no significant difference in response pattern between the methamphetamine and methamphetamine + rolipram groups.

The responses of extracellular dopamine to a challenge injection of methamphetamine (2 mg/kg i.p.) in the repeated administration study are shown in Fig. 3. The baseline levels were 6137 ± 802, 8002 ± 829 and 5452 ± 1119 AUP (mean + SEM) in the methamphetamine, methamphetamine + rolipram and rolipram groups, respectively, there being no significant difference ($P > 0.1$). The dopamine concentration in each group increased to about

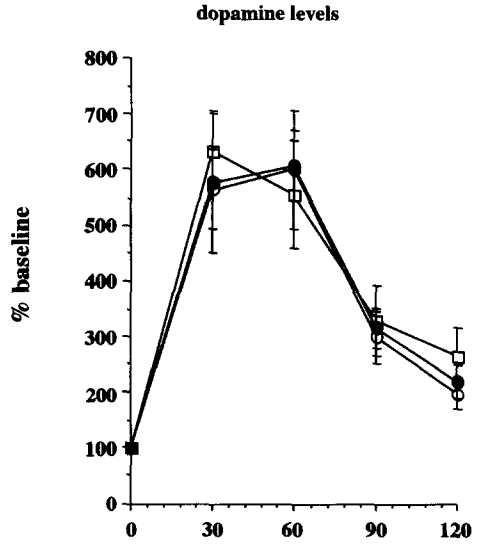

Fig. 3. Extracellular dopamine response following a 2-mg/kg methamphetamine challenge in rat striatum, detected by in vivo microdialysis, one week after the final injection of methamphetamine (4 mg/kg, i.p.) + vehicle, methamphetamine + rolipram (4 mg/kg, i.p.) or saline + vehicle (control) for 5 consecutive days. Dialysate samples were collected every 30 min and dopamine levels were expressed as the percentage change compared with the baseline level (time 0: level before the challenge). There was a significant time effect ($P < 0.05$), but no pretreatment effect. ○, ●, and □ indicate the methamphetamine group ($n = 8$), methamphetamine + rolipram group ($n = 8$) and control group ($n = 6$), respectively. Bars indicate S.E.M. The data were analyzed by two-way ANOVA.

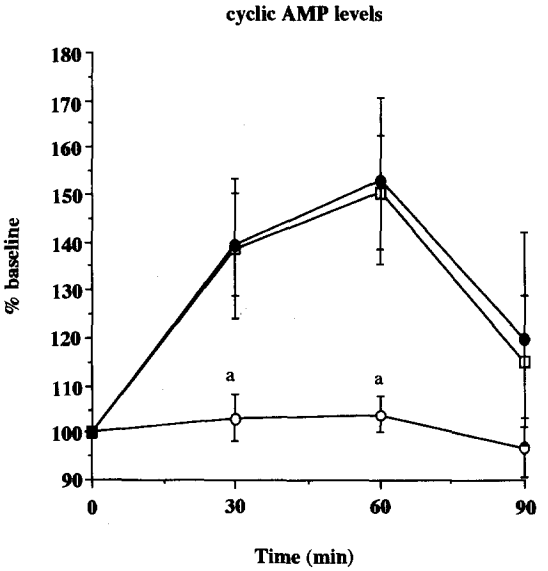

Fig. 4. Effects of methamphetamine (4 mg/kg, i.p.) and rolipram (4 mg/kg, i.p.) on extracellular cyclic AMP levels in rat striatum measured by in vivo microdialysis. Methamphetamine did not alter the levels, whereas rolipram significantly increased them either with or without methamphetamine, compared with the baseline level (time 0). Dialysate samples were collected every 30 min and cyclic AMP levels were expressed as the percentage change compared with the baseline level (time 0: level before drug administration). Open circles, closed circles and open squares indicate methamphetamine + vehicle ($n = 6$), methamphetamine + rolipram ($n = 6$) and saline + rolipram ($n = 5$) groups, respectively. Bars indicate S.E.M. The methamphetamine + rolipram (4 mg/kg, i.p.) and saline + rolipram groups show a significant time effect (one-way ANOVA). [a] $P < 0.05$ by Fisher's PLSD test: saline + rolipram vs. methamphetamine + vehicle or methamphetamine + rolipram.

600% of the baseline level in the first 30 to 60 min following the methamphetamine challenge, and then decreased rapidly. There was no significant difference in response patterns between the three groups, i.e., methamphetamine, methamphetamine + rolipram and the control groups.

3.3. Striatal cyclic AMP levels

Fig. 4 shows the alterations in cyclic AMP levels in the striatum. The baseline levels were 926 ± 61, 738 ± 116 and 994 ± 77 fmol/ml (means ± S.E.M.) in the methamphetamine, methamphetamine + rolipram and rolipram groups, respectively, there being no significant difference ($P > 0.1$). In the rolipram and methamphetamine + rolipram groups, the cyclic AMP levels were increased significantly 30 and 60 min after drug injection, by 40% and 50% ($P < 0.05$, Fisher s PLSD), respectively, whereas in the methamphetamine group there were no changes throughout the postadministration observation period. There were significant differences in the levels of cyclic AMP between the methamphetamine group and both the methamphetamine + rolipram and rolipram groups 30 and 60 min after injection of the drugs ($P < 0.05$, Fisher's PLSD).

4. Discussion

The rats which had been treated with 4 mg/kg methamphetamine once a day for 5 consecutive days exhibited a significantly greater augmentation of locomotion and rearing than the control after a challenge injection of methamphetamine (2 mg/kg, i.p.) given 1 week after the final injection. These results indicate that behavioral sensitization developed during repeated administration of methamphetamine. In contrast, the intensities of both types of challenge-induced movement shown by the rats which had received methamphetamine with 4 mg/kg rolipram were significantly lower than those shown by the rats pretreated with methamphetamine alone, and comparable to those shown by the rats pretreated with vehicle or rolipram alone. These results indicate that coadministration of rolipram prevents the development of behavioral sensitization to methamphetamine.

As facilitation of the mesolimbic and/or nigrostriatal dopamine systems by methamphetamine is crucial to the induction of behavioral sensitization (Robinson and Becker, 1986; Kalivas and Stewart, 1991; Henry and White, 1992), and it was unknown whether rolipram influences methamphetamine-induced dopamine release during repeated methamphetamine injections, it was necessary to investigate this possibility in order to understand the mechanisms underlying the inhibitory effect of rolipram on the development of behavioral sensitization. In the present study, we investigated the effects of a single dose of either 4 mg/kg methamphetamine alone, 4 mg/kg methamphetamine + 4 mg/kg rolipram or rolipram alone on striatal dopamine levels using in vivo microdialysis linked to a HPLC-ECD system, and the responses were described as percentage change relative to the baseline level. Methamphetamine significantly increased the dopamine level, whereas, following saline injection, there was no change compared with the baseline level (data not shown). Coadministration of rolipram with methamphetamine did not affect the pattern or level of methamphetamine-induced dopamine increase and its metabolites, i.e. 3,4-dihydroxyphenylacetic acid, homovanillic acid, decrease (data not shown), indicating that there was no interaction in terms of dopamine release and metabolism between methamphetamine and rolipram. Rolipram alone did not alter the dopamine level from its baseline, in agreement with the results of a previous study using rat homogenates (Kehr et al., 1985).

To explain the mechanisms underlying the present behavioral results, it was also necessary to investigate the effects of methamphetamine and rolipram on cyclic AMP in the brain after each injection during repeated administration, to determine whether methamphetamine and rolipram affect cyclic AMP levels by synergistic or opposing interactions. It has been reported that the rate of egress of cyclic AMP from cells is proportional to its intracellular concentration (Stoof and Kebabian, 1981; Lazareno et al., 1985) and that extracellular cyclic AMP in rat cortex can be detected using in vivo microdialysis and radioimmunoassay (Stone and John, 1992). Therefore, in the present study, we also investigated the response of cyclic AMP in rat striatum to single doses of methamphetamine and rolipram using these techniques. The results indicated that rolipram alone increased cyclic AMP levels by 40–50% compared with the baseline level, in accordance with results of a study indicating that rolipram increases cyclic AMP levels in rat striatal homogenates (Schneider, 1984). As dopamine D_1 receptors are linked to adenylate cyclase (Kebabian and Calne, 1979), it was originally thought that as methamphetamine increased dopamine, D_1 receptors would be stimulated and might increase cyclic AMP levels. However, like vehicle alone (data not shown), methamphetamine did not alter striatal cyclic AMP levels, while coadministration with rolipram increased the level only to the same extent as did rolipram alone. We previously reported inhibitory effects of rolipram (0.5–4 mg/kg i.p.) on behavioral changes induced by a single injection of 4 mg/kg methamphetamine (Iyo et al., 1995). Rearing was dose dependently inhibited and this inhibition was complete at 4 mg/kg rolipram, whereas locomotor hyperactivity was also dose dependently inhibited but the maximal inhibition was about 50%. Both of these types of movement have been suggested to be mediated mainly via dopamine D_2 receptors (Christensen et al., 1984; Iorio et al., 1983; Mailman et al., 1984; Ujike et al., 1989), though dopamine D_1 receptors may also be involved in locomotor hyperactivity (Dreher and Jackson, 1989; Essman et al., 1993) and rearing (Xu et al., 1994; Drago et al., 1994). In support of this theory, we have previously reported that rolipram dose dependently (0.1–1.0 mg/kg, i.p.) suppresses involuntary oro-facial movements, which may be accompanied by an increase in striatal dopamine D_2 receptors (Sasaki et al., 1995a,b). Moreover, it is known that dopamine D_2 receptors inhibit adenylate cyclase (Kebabian and Calne, 1979; Seeman, 1980), and it has been suggested that a high dopamine level may not facilitate cyclic AMP (Undie and Friedman, 1994). Therefore, we suggest that rolipram may inhibit methamphetamine-stimulated dopamine transmission, which is mainly mediated via dopamine D_2 receptors, at the stage of the second messenger, i.e., by alteration of cyclic AMP levels. However, it is known that cyclic AMP is widely distributed throughout many organs including the brain and plays many other roles besides those linked to dopamine receptors. Furthermore, we injected rolipram systemically in the present study, then investigated striatal cyclic AMP levels. Since the sensitivity of detection of cyclic AMP levels by in vivo microdialysis may be limited, the levels we measured may reflect gross cyclic AMP release from the cells surrounding the dialysis probes and not selective release from target cells, though it has been reported that cyclic AMP in only specific regions may be involved in behavioral sensitization to cocaine (Miserendino and Nestler, 1995). Therefore, it will be necessary to investigate the cyclic AMP

response in other regions or to administer the drugs to more specific area in further studies.

One possible explanation of the mechanism underlying methamphetamine-induced behavioral sensitization could be enhanced dopamine release (Robinson et al., 1988; Kazahaya et al., 1989; Hamamura et al., 1991). We investigated the dopamine response in the striatum to a 2-mg/kg methamphetamine challenge in the rats pretreated with methamphetamine, methamphetamine + rolipram or vehicle 1 week after the final pretreatment injection, using in vivo microdialysis. The methamphetamine challenge increased striatal dopamine levels in all three groups. There was no significant difference in the striatal dopamine response to the challenge between rats pretreated with methamphetamine and controls, i.e., there was no significant difference between rats with and without methamphetamine-induced behavioral sensitization in the present study. Furthermore, repeated coadministration of rolipram with 4 mg/kg methamphetamine did not alter the dopamine response pattern after the challenge in rats pretreated with methamphetamine alone. It has been suggested that the enhancement of amphetamine-stimulated dopamine release may be time-dependent, that is, it does not occur over the first 3 to 4 days of withdrawal, but may be involved in the persistence of the phenomenon after longer withdrawal periods (Segal and Kuczenski, 1992; Wolf et al., 1992). The lack of a significant difference in the striatal dopamine response to the challenge between the methamphetamine and the vehicle groups after 7 days withdrawal, despite the fact that behavioral sensitization was found, may indicate that the stages of behavioral sensitization occurred earlier in the present study, but involved alterations in postsynaptic sensitivity or other neurotransmitter systems. Therefore, as methamphetamine increased dopamine levels after a single dose, even when coadministered with rolipram, and as methamphetamine challenge did not enhance dopamine levels in rats exhibiting behavioral sensitization though the challenge test might have been performed at too early a stage and as there is still the possibility of different responses to methamphetamine or rolipram in other brain areas, we suggest that the inhibitory effects of rolipram on the development of behavioral sensitization are due to an increase in postsynaptic intracellular cyclic AMP levels.

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