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Tacrolimus, a specific inhibitor of calcineurin, modifies the locomotor activity of quinpirole, but not that of SKF82958, in male rats

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Abstract

In the present study, we examined the effect of tacrolimus, a specific inhibitor of calcineurin, on the locomotor activity elicited by the selective dopamine D_1 receptor agonist (±) 6-chloro-7,8-dyhydroxy-3allyl-1-phenyl-2,3,4,5-tetra-hydro-1*H*-benzazepine (SKF82958) and the dopamine D_2/D_3 receptor agonist quinpirole, in male Wistar rats. Tacrolimus (0.5, 1, 2 or 5 mg/kg, i.p.) alone had no significant effect on basal locomotor activity. The dose-related increase in locomotor activity produced by the administration of SKF82958 (0.1, 1 or 5 mg/kg, i.p.) was not significantly altered by 2 mg/kg of tacrolimus. In addition, the increase in locomotor activity produced by 1 mg/kg of SKF82958 was not significantly altered by tacrolimus (0.5, 1, 2 or 5 mg/kg, i.p.). The administration of quinpirole (0.1, 0.25, 0.5, 1 or 3 mg/kg, i.p.) produced a biphasic response, with the minimum and maximal increase in locomotor activity occurring at 0.1 and 1 mg/kg, respectively. The pretreatment of 2 mg/kg of tacrolimus, compared to vehicle-treated animals, significantly lowered the dose of quinpirole that produce a maximal effect on locomotor activity from 1 to 0.5 mg/kg but did not significantly alter the minimum response. The increase in locomotor activity produced by 0.5 mg/kg of quinpirole was significantly potentiated by 0.5, 1, 2 or 5 mg/kg of tacrolimus compared to vehicle-treated animals. Our results suggest that calcineurin may play a role in the alteration of locomotor activity produced by dopamine D_2/D_3 receptors, but not dopamine D_1 receptors.

Keywords: Tacrolimus; Calcineurin; Dopamine D1 receptor; Dopamine D2 receptor; Dopamine D3 receptor; Locomotor activity

1. Introduction

In rodents, the administration of low doses of the psychostimulants amphetamine and methamphetamine have been shown to increase locomotor activity, whereas high doses elicit stereotyped behaviors (Randrup and Munkvad, 1974; Tsukamoto et al., 2001). There is considerable evidence indicating that the behavioral effects produced by amphetamine and methamphetamine is due in part to their effect on dopaminergic neurons pathways. For example, the systemic administration of amphetamine or methamphetamine increases extracellular levels of dopamine in brain areas that receive dopaminergic nerve terminals such as the striatum and nucleus accumbens (Cass et al., 1999; Kuczenski et al., 1991; Robinson and Camp, 1990). In addition, the administration of dopamine D_1 or D_2 receptor antagonists and treatments that deplete dopamine stores in the central nervous system (CNS) significantly attenuate methamphetamine- and amphetamine-induced behaviors (Molloy and Waddington, 1984; Ujike et al., 1989).

Recently, we have shown that tacrolimus (FK506, (−)-(1*R*,9*S*,12*S*,13*R*,14*S*,17*R*,18*E*,21*S*,23*S*,24*R*,25*S*,27*R*)-17-allyl-1, 14-dihydroxy-12-[(*E*)-2-[(1*R*,3*R*,4*R*)-4-hydroxy-3-methoxycyclohexil]-1-methylvinyl]-23,25-dimethoxy-13, 19,21-27-tetramethl-11,28-dioxa-4-azatricyclo[22.3.1.$0^{4,9}$]-octacos-18-ene-2, 3,10,16-tetrone hydrate), an immunosuppressant agent that is a specific inhibitor of calcineurin in the periphery and CNS, suppresses the behavioral response induced by methamphetamine in rats (Tsukamoto et al., 2001). The methamphetamine-induced behavioral changes could in part be related to the stimulation of dopamine D_1 and/or D_2/D_3 receptors by released dopamine. Thus, it is possible that the tacrolimus-induced suppression of methamphetamine's action is due to its actions on dopamine D_1

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and or D_2/D_3 receptors. Therefore, the present study was designed to examine the effect of tacrolimus on the locomotor activity produced by the selective dopamine D_1 receptor agonist (±) 6-chloro-7, 8-dyhydroxy-3allyl-1-phenyl-2, 3,4,5-tetra-hydro-1*H*-benzazepine (SKF82958) (Andersen and Jansen, 1990; O'Boyle et al., 1989) and the dopamine D_2/D_3 receptor agonist quinpirole (*trans*-(−)-4aR-4,4a,5,6,7,8,8a,9-octahydro-5-propyl-1*H*-pyrazolo[3,4−*g*]quinoline hydrochloride) (Levant et al., 1992; Tsuruta et al., 1981) in male rats.

2. Material and methods

2.1. Animals

Male Wistar rats (200–225 g; Japan SLC, Hamamatsu, Shizuoka, Japan) were used in all experiments. The animals were housed three per cage. They were maintained under standard conditions (12 h–12 h light–dark cycle; lights on from 0700 to 1900 h; room temperature, 22 ± 2 °C; humidity 55 ± 5%) with free access to food and water for at least 1 week before the beginning of the study. Experiments were performed in accordance with the principles of laboratory animal care (NIH Publication No. 85-23, revised 1985).

2.2. Drugs

(±)-SKF82958 hydrobromide (Research Biochemicals, Natick, MA, USA) or (−)-quinpirole (Eli Lilly, Indianapolis, IN, USA) was dissolved in 0.9% saline. Tacrolimus (Fujisawa Pharmaceutical, Osaka, Japan) was dissolved in Tween 60 diluted with 0.9% saline. The volume of administration for all treatments was 1 ml/kg i.p.

2.3. Locomotor activity

Locomotor activity was measured using an animal movement analysis system (SCANET SV-10; Matys, Toyama, Japan) as previously described (Asakura et al., 1992). A rectangular, transparent plastic cage (440 × 260 × 400 mm) was placed on the SCANET system with the photosensors 30 mm above the cage floor. A single animal was placed in the cage 1 h before drug treatment to allow for acclimation. Following treatment, locomotor activity was measured every 5 min for 90 min. An intersection of the photosensors (10 mm apart) in the enclosure was counted as one unit of locomotor activity.

2.4. Effects of tacrolimus on locomotor activity produced by SKF82958 and quinpirole

2.4.1. Effect of vehicle administration on the locomotor activity of SKF82958 or quinpirole

Animals ($n = 9$ per treatment group) were administered the vehicle for tacrolimus and 10 min later, received either: (1) the vehicle for SKF82958 or quinpirole, (2) 0.1, 1 or 5 mg/kg i.p. of SKF82958, or (3) 0.1, 0.25, 0.5, 1 or 3 mg/kg i.p. of quinpirole and were placed in the observation cage and locomotor activity was measured for 90 min.

2.4.2. The effect of a single dose of tacrolimus on the locomotor activity produced by SKF82958 or quinpirole

Animals ($n = 9$ per treatment group) were given 2 mg/kg of tacrolimus and 10 min later, received either: (1) 0.1. 1 or 5 mg/kg i.p. of SKF82958, (2) 0.1, 0.25, 0.5, 1 or 3 mg/kg i.p. of quinpirole and locomotor activity was assessed as described above.

2.4.3. The effect of multiple doses of tacrolimus on the locomotor activity produced by a single dose of SKF82958 or quinpirole

Animals ($n = 9$ per treatment group) were given either the vehicle for tacrolimus or 0.5, 1, 2 or 5 mg/kg i.p. of tacrolimus and 10 min later received either: (1) 1 mg/kg i.p. of SKF82958 or (2) 0.5 mg/kg i.p. of quinpirole and locomotor activity was assessed as described above.

2.5. Statistics

A one-way analysis of variance followed by Fisher's protected least significant difference test was used to compare the effects of vehicle and tacrolimus on the locomotor activity produced by SKF82958 or quinpirole and to analyse the dose-response data for SKF82958 and quinpirole alone. The a priori significance value was set at $P < 0.05$.

3. Results

3.1. Effects of vehicle on and tacrolimus on basal locomotor activity

To establish that tacrolimus alone had no significant effect on basal locomotor activity, we examined the effect of tacrolimus (0.5, 1, 2 and 5 mg/kg, i.p.) and vehicle on locomotor activity. Overall, none of the doses of tacrolimus significantly altered total locomotor activity compared to vehicle-treated animals ($F(4,40) = 1.611$, $P = 0.190$), indicating that tacrolimus alone does not alter locomotor activity.

3.2. The effect of vehicle and tacrolimus pretreatment on locomotor activity produced by SKF82958 (Fig. 1)

The effect of vehicle and tacrolimus (2 mg/kg, i.p.) on the locomotor activity produced by 0.1, 1 or 5 mg/kg i.p. of SKF82958 is shown in Fig. 1A. SKF82958 produced a dose-related increase in locomotor activity in animals pretreated with either vehicle or 2 mg/kg tacrolimus. However, statistical analysis indicated that there was no significant difference in total locomotor activity following 0.1, 1 or 5

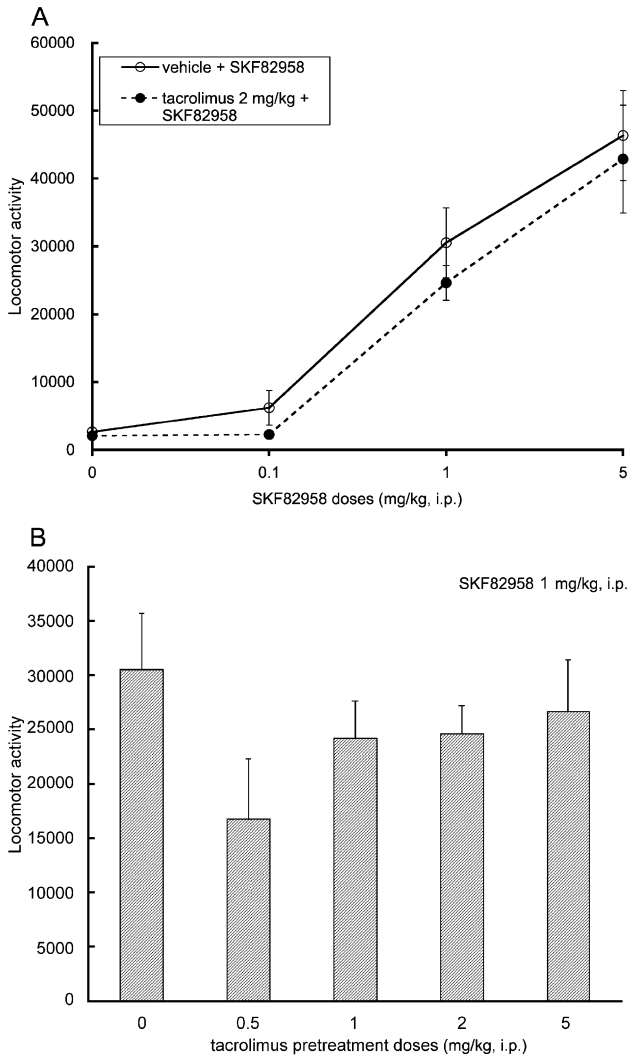

Fig. 1. Effects of pretreatment of tacrolimus on the locomotor activity produced by SKF82958 i.p. in rats. (A) Total amount of locomotor activity in 90 min after SKF82958. There was no significant differences between animals pretreated with vehicle (Tween 60) (○) and with tacrolimus (2 mg/kg) (●). All values represent the mean ± S.E.M. (B) The effect of vehicle (saline) and tacrolimus (0.5, 1, 2 or 5 mg/kg, i.p.) pretreatment on locomotor activity produced by 1 mg/kg SKF82958. All values represent the mean locomotor activity in 90 min ± S.E.M.

mg/kg i.p. SKF82958 between animals pretreated with vehicle or tacrolimus (2 mg/kg, i.p.).

The effect of tacrolimus (0.5, 1, 2 and 5 mg/kg, i.p.) on the locomotor activity induced by a fixed dose of 1 mg/kg i.p. SKF82958 is shown in Fig. 1B. There was no significant difference in locomotor activity between animals pretreated with vehicle or tacrolimus (0.5, 1, 2 or 5 mg/kg, i.p.) following 1 mg/kg i.p. SKF82958 ($F(4,40) = 1.284$, $P = 0.293$).

3.3. The effect of vehicle and tacrolimus pretreatment on locomotor activity produced by quinpirole

The effect of vehicle and tacrolimus (2 mg/kg, i.p.) on the locomotor activity produced by quinpirole (0.1, 0.25, 0.5, 1 and 3 mg/kg, i.p.) is shown in Fig. 2A. Quinpirole produced significant changes of locomotor activity compared to vehicle-treated animals (vehicle-pretreatment group, $F(5,48) = 9.377$, $P < 0.001$; tacrolimus pretreatment group, $F(5,48) = 22.686$, $P < 0.001$). Quinpirole produced a biphasic dose–response curve; i.e., decrease with 0.1 mg/kg i.p. followed by increase with larger doses. Statistical analysis indicated that there was a significant difference between vehicle and tacrolimus pre-treated animals in locomotor activity induced by 0.5 mg/kg quinpirole ($P = 0.0018$). After the administration of vehicle, the maximal effect of quinpirole was observed at 1 mg/kg, compared to 0.5 mg/kg after the administration of tacrolimus.

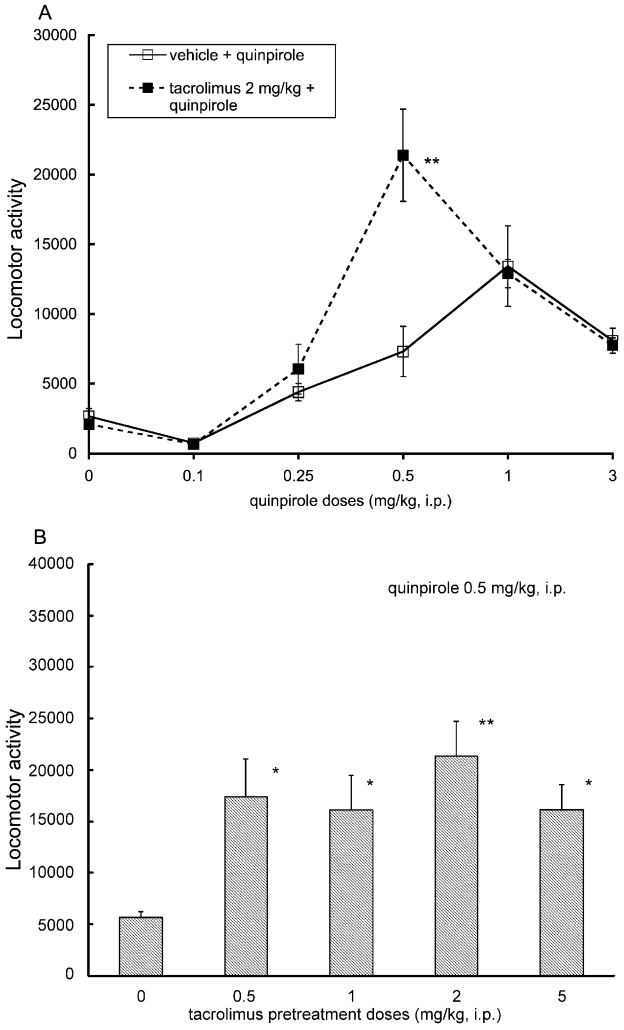

Fig. 2. Effects of pretreatment of tacrolimus on the locomotor activity produced by quinpirole i.p. in rats. (A) Total amount of locomotor activity in 90 min after quinpirole. □ and ■ represent the vehicle (Tween 60) and tacrolimus 2 mg/kg pretreatment group, respectively, followed by quinpirole. All values represent the mean ± S.E.M. $*P < 0.01$ compared to the vehicle-pretreated group. (B) The effect of vehicle (saline) and tacrolimus (0.5, 1, 2 or 5 mg/kg, i.p.) pretreatment on locomotor activity produced by quinpirole (0.5 mg/kg, i.p.). All values represent the mean locomotor activity in 90 min ± S.E.M. $*P < 0.05$ and $**P < 0.01$ compared to vehicle-pretreated animals.

Locomotor activity was significantly greater following the administration of 0.5 mg/kg i.p. quinpirole in animals pretreated with tacrolimus (0.5, 1, 2 or 5 mg/kg, i.p.) compared to vehicle-pretreated animals ($F(4,40) = 4.078$, $P = 0.0072$, Fig. 2B). The tacrolimus-induced increase in locomotor activity was not dose-dependent.

4. Discussion

In fact, we have analysed the data of locomotor activity every 5 min for 90 min. However, the detailed analysis did not reveal any different findings from the sum-score data.

As previously reported, our results indicated that the i.p. administration of the dopamine D_1 receptor agonist SKF82958 produced a dose-dependent increase in the locomotor activity of male rats following vehicle pretreatment (Chandler et al., 1990; Molloy and Waddington, 1987). The i.p. administration of the specific calcineurin inhibitor tacrolimus alone did not significantly alter the activity of animals. Pretreatment of animals with various doses of tacrolimus did not significantly alter the increase in locomotor activity produced by 1 mg/kg i.p. SKF82958 compared to vehicle-treated animals. Thus, tacrolimus did not significantly alter normal or SKF82958-induced locomotor activity. Since SKF82958 is a relatively specific agonist for dopamine D_1-like receptor (Andersen and Jansen, 1990; O'Boyle et al., 1989), and tacrolimus is a specific inhibitor for calcineurin (Hemenway and Heitman, 1999), our results suggest that calcineurin may not play a critical role in locomotor activity associated with dopamine D_1-like receptors.

The i.p. administration of quinpirole, as previously reported, produced a biphasic effect on locomotor activity over time, which was dose-related (Eilam and Szechtman, 1989; Frantz and van Hartesveldt, 1995; Gershanik et al., 1983; Horvitz et al., 2001; Van Hartesveldt, 1997). In the animals pretreated with vehicle (Tween 60), locomotor activity was lowest and highest at doses of 0.1 and 1 mg/kg, respectively, compared to vehicle-treated animals. This biphasic pattern was still observed following the pretreatment of animals with tacrolimus. However, 2 mg/kg i.p. tacrolimus significantly decreased the dose required to produce a maximal increase in locomotor activity from 1 to 0.5 mg/kg, compared to vehicle-pretreated animals. In addition, locomotor activity produced by 0.5 mg/kg i.p. quinpirole was significantly increased by 0.5, 1, 2 and 5 mg/kg of tacrolimus and there was no significant difference between the doses of tacrolimus in the magnitude of the increase. In contrast, tacrolimus did not significantly alter the effect of the 0.1 mg/kg dose. These results suggest that tacrolimus produces a leftward shift in the dose of quinpirole required to produce a maximal effect. It is known that increases in locomotor activity produced by high doses of quinpirole are due to its stimulation of postsynaptic dopamine D_2/D_3 receptors. Thus, tacrolimus may alter the postsynaptic responsiveness to quinpirole, although additional studies are required to verify this hypothesis.

Currently, the mechanism for the tacrolimus-induced potentiation of quinpirole's action remains unknown. It is unlikely that tacrolimus's potentiation of quinpirole's action is due to its stimulation of dopamine D_2/D_3 receptors as (1) it is a selective calcineurin antagonist and (2) alone, it did not significantly alter locomotor activity. Recent studies have shown that DARPP-32 (dopamine and cAMP-regulated phosphoprotein) plays an important role in mediating the effects of dopamine via D_1- and D_2-like receptors (Greengard et al., 1999; Nishi et al., 1997, 1999). Although inhibition of calcineurin increases the basal levels of phospho-DARPP-32 (Nishi et al., 1997, 1999), tacrolimus per se did not alter the pattern and intensity of locomotor activity in this study. Therefore, it is unlikely that tacrolimus enhances or modifies quinpirole-induced locomotor activity by increasing DARPP-32 phosphorylation. The activation of dopamine D_2-like receptors in striatal medium spiny neurons expressing enkephalin decreases L-type Ca^{2+} currents (Hernandez-Lopez et al., 2000) and this is blocked by inhibition of calcineurin (Hernandez-Lopez et al., 2000). It is possible that tacrolimus's potentiation of quinpirole's action is related to such a mechanism, although this remains to be proven. Finally, the possibility that the results may have been due to a drug–drug interaction cannot be ruled out.

In a recent study, we have shown that in rats, tacrolimus suppresses methamphetamine-induced locomotor activity (Tsukamoto et al., 2001). Both dopamine D_1 and D_2/D_3 receptors are reported to be involved in the development of behavioral change induced by amphetamine and methamphetamine (Kuczenski and Segal, 1999; Wang and McGinty, 1996). However, our present and previous studies suggest that the behavioral changes produced by methamphetamine via its indirect stimulation dopamine D_1 and/or D_2/D_3 receptors do not involve the calcineurin pathway.

In conclusion, tacrolimus produces a non-dose-dependent potentiation of the locomotor activity elicited by quinpirole without significantly altering SKF82958-induced locomotor activity. This suggests that tacrolimus selectively modifies the responsivity/sensitivity of postsynaptic dopamine D_2/D_3 receptors, although additional studies must be conducted to verify this hypothesis.

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