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Stimulating dopamine D_1 receptors increases the locomotor activity of developing rats

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Abstract

To determine the role of dopamine D_1 receptors in the locomotor activity of developing rats, male offspring were habituated to an animal activity monitor and were then injected with the dopamine D_1 receptor antagonist, SCH 23390 (*R*(+)-7-chloro-8-hydroxy-3-methyl-1-phenyl-2,3,4,5-tetrahydro-(1*H*)-3-benzazepine), or vehicle and returned to the activity monitors. 30 min later, they were injected with the dopamine D_1 receptor agonist, SKF 38393 (*R*(+)-1-phenyl-2,3,4,5-tetrahydro-(1*H*)-3-benzazepine-7,8-diol), or vehicle and were again placed in the activity boxes where their locomotor activity was monitored individually for 1 h. The litter was used as the unit for statistical analyses. There was a significant increase in the locomotor activity of 10- and 21-day-old offspring injected with SKF 38393. This effect was antagonized by pretreatment with SCH 23390. These data provide the strongest evidence to date that stimulation of dopamine D_1 receptors increases the locomotor activity of habituated developing rats

Keywords: Dopamine D_1 receptor; Development; Locomotor activity; SKF 38393; SCH 23390; (Rat)

1. Introduction

When studying the effects of perinatal exposure to a κ-opioid receptor agonist on the locomotor activity of developing rats, we observed a significant 5-fold increase in the locomotor activity of 10-day-old control offspring injected with the dopamine D_1 receptor agonist, SKF 38393, 10 mg/kg, compared to control offspring injected with vehicle (Shieh and Walters, 1994). Previous results on the effects of this dose of SKF 38393 on the locomotor activity of developing rats have been inconclusive. For example, in one study, Moody and Spear (1992a) reported a significant increase in the activity of 3- and 21-day-old rats but not 10-day-old rats injected with a 10-mg/kg dose of SKF 38393 whereas in another study this dose of SKF 38393 had no significant effect on forward locomotion at 21 days of age (Moody and Spear, 1992b). McDevitt and Setler (1981) reported a significant increase in the locomotor activity of 5–8-day-old rats but not 30-day-old rats injected with SKF 38393, 10 mg/kg. Lastly, SKF 38393, 5–15 mg/kg, had no significant effect on the locomotor activity of 11- or 17-day-old rats (McDougall et al., 1990).

The role of dopamine D_1 receptors in the locomotor activity of adult animals is also unclear. For example, SKF 38393 and other dopamine D_1 receptor agonists (Murray and Waddington, 1989) have been reported to significantly increase the locomotor activity of habituated (Mazurski and Beninger, 1991; Molloy and Waddington, 1985, 1987) and non-habituated (Bruhwyler et al., 1991; Chagraoui et al., 1990) animals. However, in other studies, SKF 38393 had no significant effect on the locomotor activity of either habituated or non-habituated animals (Gandolfi et al., 1988; Starr and Starr, 1987; Arnt, 1985).

Because of these ambiguous and sometimes conflicting results and because of our previous findings, we attempted to clarify the role of dopamine D_1 receptors in the locomotor activity of developing rats.

2. Materials and methods

2.1. Animals

Most previous studies on the effects of dopamine D_1 receptor agonists on the locomotor activity of developing

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rats assigned only 1 pup per litter to each test condition for a total of 7–8 observations per treatment. However, whether due to the experimental design or some other factor, the results of these studies have been inconsistent and in some cases not reproducible. Thus, to try and clarify the role of dopamine D_1 receptors in locomotor activity, a different approach was taken by using the litter as the unit for analyses. With this method, an n of 1 represented the response of 4–9 pups instead of the response of just 1 pup. This will also make it easier to compare the present results with the results of a previous study in which the litter was used as the unit for analyses (Shieh and Walters, 1994).

Sprague-Dawley male (225–249 g) and female (150–174 g) (Harlan Sprague-Dawley, Indianapolis, IN, USA) rats were housed at 25 ± 2°C with lights on from 05:00 to 17:00 h. Food and water were available ad libitum. Each night, 3–4 females and 1 male were housed together for mating. At birth, the offspring were sexed by anal-genital distance and culled to 9 pups per litter. Each litter contained 4–9 male offspring.

2.2. Procedure

At 10 or 21 days of age, male offspring were adapted singly for 1 h to a Digiscan animal activity monitor (Omnitech Electronics, Columbus, OH, USA) containing 16 photobeams in each horizontal direction spaced 2.5 cm apart. The offspring were then injected subcutaneously with the dopamine D_1 receptor antagonist, SCH 23390, 0.1 or 0.5 mg/kg, or 0.9% saline vehicle, 1 ml/kg, and returned to the activity monitors. 30 min later, they were injected subcutaneously with the dopamine D_1 receptor agonist, SKF 38393, 10 or 30 mg/kg, or vehicle and were again placed in the activity boxes where their locomotor activity was monitored for 1 h. Locomotor activity was defined as the total distance traveled in cm. To prevent the offspring from losing body heat, heating pads were placed beneath the cages to gently warm the cage floor. This did not measureably alter the ambient temperature of 25 ± 2°C inside the cages. All animals were humanely sacrificed by CO_2 asphyxiation at the end of the experiments.

2.3. Statistics

The unit for statistical analyses was the litter. Therefore, at each time interval, the individual values for 4–9 male offspring in a given litter were averaged for an n of 1. Thus, an n of 5 represents the responses of 20–45 individual animals. The data were analyzed by an analysis of variance and a least significant difference (LSD) test. Statistical significance implies a $P < 0.01$.

2.4. Drugs

Hydrochloride salts of SKF 38393 (*R*(+)-1-phenyl-2,3,4,5-tetrahydro-(1*H*)-3-benzazepine-7,8-diol) and SCH 23390 (*R*(+)-7-chloro-8-hydroxy-3-methyl-1-phenyl-2,3,4,5-tetrahydro-(1*H*)-3-benzazepine) were obtained from Research Biochemicals (Natick, MA, USA). All drug doses were calculated as the free base and the drugs were dissolved in 0.9% saline vehicle.

3. Results

There was a significant increase in the locomotor activity of 10-day-old offspring injected with SKF 38393, 10 or 30 mg/kg (Fig. 1). Significant increases in activity were

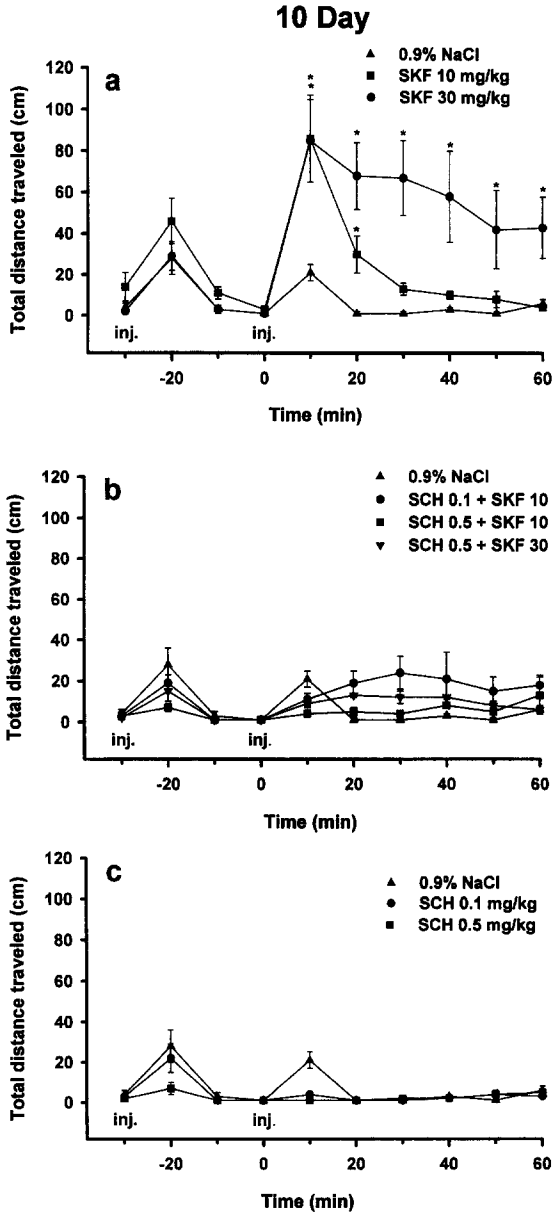

Fig. 1. Time-course of the effects of (a) the dopamine D_1 agonist SKF 38393, 10 and 30 mg/kg, (b) the dopamine D_1 antagonist SCH 23390, 0.1 and 0.5 mg/kg, followed by SKF 38393, 10 or 30 mg/kg, and (c) SCH 23390, 0.1 and 0.5 mg/kg, given alone on the locomotor activity of 10-day-old rats. The litter was used as the unit for statistical analyses. * $P < 0.01$.

observed 10 and 20 min after injecting the lower dose of SKF 38393 whereas the higher dose significantly increased activity at every time-point sampled over the 1-h test period. The maximal effect of both doses of SKF 38393 was similar, each increasing locomotor activity by 4-fold 10 min after injection (Fig. 1a). Pretreatment with SCH 23390 antagonized in a dose-related fashion the locomotor response of 10-day-old offspring to both doses of SKF 38393 (Fig. 1b). Given alone, SCH 23390 had no significant effect on the locomotor activity of 10-day-old offspring (Fig. 1c).

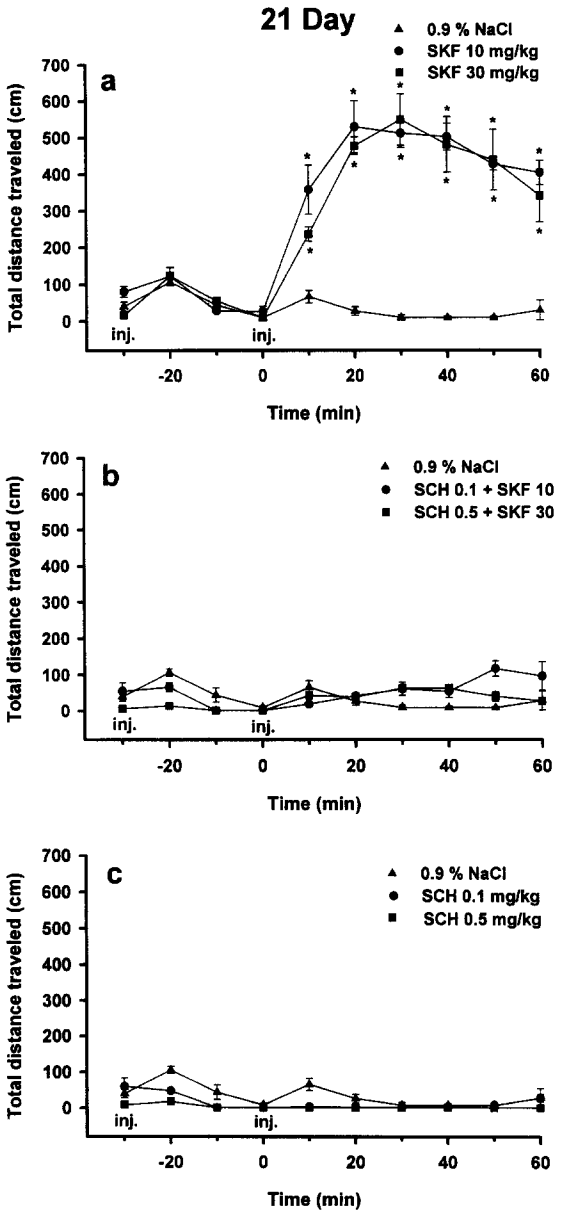

Fig. 2. Time-course of the effects of (a) the dopamine D_1 agonist SKF 38393, 10 and 30 mg/kg, (b) the dopamine D_1 antagonist SCH 23390, 0.1 and 0.5 mg/kg, followed by SKF 38393, 10 or 30 mg/kg, and (c) SCH 23390, 0.1 and 0.5 mg/kg, given alone on the locomotor activity of 21-day-old rats. The litter was used as the unit for statistical analyses. $^*P < 0.01$.

The 10- and 30-mg/kg doses of SKF 38393 both significantly increased the locomotor activity of 21-day-old offspring (Fig. 2). However, unlike at 10 days of age, the time-courses of the reponses to the lower and higher doses were similar throughout the test period (Fig. 2a). The maximal total distance traveled was similar for both doses of SKF 38393 and the peak response to either dose occurred 20–30 min after injection. As before, pretreatment with SCH 23390 antagonized the effect of SKF 38393 (Fig. 2b). Given alone, SCH 23390 had no significant effect on locomotor activity at 21 days of age (Fig. 2c).

4. Discussion

In 1981, McDevitt and Setler reported a significant increase in the motor activity of 5–8-day-old rats injected with SKF 38393, 10 mg/kg (McDevitt and Setler, 1981). In that study, motor activity was determined by observation and the scores obtained 15 and 30 min after injection were added together to obtain a total score. The present results generally agree with those of McDevitt and Setler (1981) in that in this study the locomotor activity of 10-day-old rats was significantly increased 10 and 20 min after injecting SKF 38393, 10 mg/kg. They are also consistent with results obtained 1 h after injecting 10-day-old rats with a 10-mg/kg dose of SKF 38393 (Shieh and Walters, 1994). Unfortunately, in the study by McDevitt and Setler (1981), data from rats 5–8 days of age were combined making it impossible to determine the contribution of each of these ages to their results. Regardless, the above results differ from those of other studies in which SKF 38393, 10 or 15 mg/kg, had no significant effect on the locomotor activity of 10- or 11-day-old rats (McDougall et al., 1990; Moody and Spear, 1992a,b). There was, however, a significant increase in activity at these ages after injecting 30 mg/kg of SKF 38393 (present results; McDougall et al., 1990).

In this and another study (Shieh and Walters, 1994), SKF 38393, 10 mg/kg, significantly increased the locomotor activity of 21-day-old rats at each time-point measured over a 60-min test period. Moody and Spear (1992a) also reported a significant increase in activity at this age and dose of SKF 38393. However, in another study, this dose had no significant effect on forward locomotion at this age (Moody and Spear, 1992b). Similarly, SKF 38393, 15 mg/kg, had no significant affect on locomotion at 17 days of age whereas at 30 mg/kg, significant increases in activity were observed for 17- (McDougall et al., 1990) and 21-day-old rats (this study). Interestingly, a 40-mg/kg dose of SKF 38393 had no significant effect on the locomotor activity of 30-day-old rats (McDevitt and Setler, 1981).

There are several possible explanations for the differences in the results of these studies, one of which is the method by which locomotor activity was measured. In

both of our studies, photobeam sensors were used to detect locomotor activity which was defined as the total distance traveled in cm. Moody and Spear (1992a,b) used a time-sampling procedure in which animals were observed for 5 s every 20 s over a 5- or 10-min period whereas McDougall et al. (1990) used the number of line crossings as the dependent variable. The method of McDevitt and Setler (1981) is described above. Another important factor that might contribute to differences in results between studies is whether or not the animals were habituated to the test environment prior to experimentation. In our studies, the animals were habituated to the activity monitors for 1 h before testing thus lowering the 'background noise'. This might explain why we consistently observe an increase in locomotion in response to SKF 38393 whereas some investigators do not (present results; Shieh and Walters, 1994). Although SKF 38393 has been reported to stimulate locomotion in non-habituated animals, the locomotor activity of habituated animals often responds to dopamine D_1 receptor agonists whereas that of non-habituated animals often does not (Chandler et al., 1990; Molloy and Waddington, 1985, 1987; Tirelli and Terry, 1993).

Perhaps the greatest difficulty in comparing results between studies is relating the effects of different drug doses on different aged animals. In some studies, insufficient information is provided for the reader to determine the actual drug dose received by the animal. If meaningful comparisons are to be made, it is important to know if the dose is expressed as the salt or free base. In addition, the dose of 'active drug' will also depend on whether the racemate or (*R* +)-enantiomer is used, as in this and our previous study. Studies in adult animals indicate that the locomotor response to the R(+)-enantiomer is more robust than that of the racemate (Molloy et al., 1986; Murray and Waddington, 1989). Unfortunately, the other developmental studies cited above did not indicate whether the racemate or R(+)-enantiomer was used or if the salt or free base was used to calculate the doses. Differences in dose due to such factors might also partly explain why some investigators did not see a significant effect of SKF 38393 at certain ages or time-points.

In summary, with the litter as the unit for statistical analyses, the dopamine D_1 receptor agonist SKF 38393 significantly increased the locomotor activity of habituated 10- and 21-day-old rats. This effect was antagonized by pretreatment with the dopamine D_1 receptor antagonist, SCH 23390. These data provide the strongest evidence to date that stimulating dopamine D_1 receptors increases the locomotor activity of developing rats. The results are also consistent with results that indicate dopamine D_1 receptors in rat brain attain near-adult levels between 14 and 21 days of age. (Rao et al., 1991; Schambra et al., 1994). It is possible, however, that the effect of SKF 38393 on locomotor activity is dependent upon co-stimulation of dopamine D_1 and D_2 receptors because significant locomotor activation in response to SKF 38393 is not observed with habituated 10- or 21-day-old offspring depleted of dopamine (Moody and Spear, 1992b). This is consistent with results that indicate SKF 38393 significantly increases dopamine release in the 10- and 21-day-old rat brain (Walters and Howard, 1990). Lastly, the overwhelming majority of studies on the role of dopamine D_1 receptors in locomotor activity have used SKF 38393 as the agonist. Therefore, the present results and others must be confirmed when other selective dopamine D_1 receptor agonists become routinely available.

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