Pharmacology Biochemistry and Behavior, Vol. 45, pp. 843–847, 1993
Printed in the U.S.A.

0091-3057/93 $6.00 + .00

Effects of Intraaccumbens Dopamine Agonist SK&F38393 and Antagonist SCH23390 on Locomotor Activities in Rats

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Received 14 October 1992

MEYER, M. E. *Effects of intraaccumbens dopamine agonist SK&F38393 and antagonist SCH23390 on locomotor activities in rats.* PHARMACOL BIOCHEM BEHAV 45(4) 843–847, 1993.—The present study examined the effects of the dopamine D_1 and D_5 subtype receptors agonist, *R*(+)-1-phenyl-2,3,4,5-tetrahydro-(1*H*)-3-benzazepine-7,8-diol (SK&F38393), and antagonist, *R*(+)-7-chloro-8-hydroxy-3-methyl-1-phenyl-2,3,4,5-tetrahydro-1*H*-3-benzazepine (SCH23390), on locomotor activities after bilateral microinjection into the nucleus accumbens (Acb). SK&F38393 (0.1–10.0 μg) significantly potentiated and SCH23390 (0.01–1.0 μg) significantly attenuated locomotor activity as measured by horizontal distance in cm. The data were supportive of the hypothesis that dose-related locomotor activities induced by microinjections of SK&F38393 into the Acb are independently mediated by D_1 and D_5 subtype receptors.

Dopamine DA agonist Locomotor activity Nucleus accumbens DA antagonist SCH23390 SK&F38393 Rats

THE existence of subtypes of dopamine (DA) receptors is well accepted (26). During the last decade, the D_1 receptors were typically associated with stimulation of adenylate cyclase, while the D_2 receptors were either independent of adenylate cyclase or mediated its inhibition (12,24,28,30). However, two forms of the D_1 receptor have been determined, DA D_1 and D_5 (28). The availability of DA agonists and antagonists acting primarily at the DA receptors sites has stimulated research to characterize the functional effects of each receptor subtype. The behavioral, biochemical, and electrophysiological studies have shown that DA receptors are separate sites and each may have different and/or interactive functions (2,6,9,25,26,31).

During the last decade, it was suggested that all DA agonist-induced behaviors were mediated by the D_2 receptor and that the D_1 receptor had no known behavioral role (9,24,31). However, with the development of the selective D_1 antagonists, *R*(+)-7-chloro-8-hydroxy-3-methyl-1-phenyl-2,3,4,5-tetrahydro-1*H*-3-benzazepine (SCH23390) and *R*(+)-1-phenyl-2,3,4,5-tetrahydro-(1*H*)-3-benzazepine-7,8-diol (SK&F83566), systemic injections of these D_1 antagonists reproduced the behavioral effects of both the typical nonselective DA antagonists and the selective D_2 antagonists over a wide variety of behaviors (14,15,31). On the other hand, systemic injections of the D_1 agonist, SK&F38393, failed to produce locomotor stimulation or stereotypy effects of the nonselective DA agonists or selective D_2 agonists. With large dose levels, however, SK&F38393 enhances grooming (4,5,17,18,27,32).

Many of the behavioral-activating effects of the DA agonists are mediated by the nucleus accumbens (Acb). The Acb has a significant distribution of dopamine receptors (1,8). Behavioral effects of SK&F38393 following microinjections into the Acb are inconsistent. Anderson and Nielson (2), Arnt (3), Costall et al. (10), Dreher and Jackson (11), and Meyer et al. (16) reported motor enhancement. On the other hand, Breese et al. (7) and Plaznik et al. (21) found no effect.

In view of the findings that both SK&F38393 and SCH23390 bind to both of the D_1 and D_5 receptor subtypes (30) where a potentiated level of motor activity was elicited by SK&F38393 following microinjections in the Acb, and an attenuated effect of systemic injections of SCH23390, it was essential that these data be replicated and expanded. In the present study, we examined locomotor activity, as measured in horizontal distance, when SK&F38393 and SCH23390 were injected directly into the Acb. Dose- and time-dependent changes in locomotor activity were of particular interests.

METHOD

Animals

Male Long-Evans rats weighing 275–300 g were obtained from Charles River Laboratories (Wilmington, MA). Animals were individually housed, had food and water ad lib, and were maintained on a 12 L : 12 D cycle (light 0700–1900 h). Animals were tested in the light phase between 1000–1600 h. The room in which animals were maintained was kept at a constant temperature (21 ± 2°C). This study was carried out in compliance

with the rules set forth in the NIH Guide for the Care and Use of Laboratory Animals.

Surgery

Animals, while under Equithesin anesthesia, were cannulated with the use of a stereotaxic instrument; guide cannulae, 7 mm long, fabricated from 21-ga hypodermic needles were permanently fixed to the skull with microscrews and dental cement. The guide cannulae were implanted following the coordinates from Paxinos and Watson (20); for the nucleus accumbens, +1.7, +1.25, −2.5 mm with reference bregma, midline, and skull surface, respectively. The vertical depth of the injection cannula was 7.0 mm. Animals were allowed 2 weeks' recovery before behavioral testing.

Drugs and Drug Administration

SK&F38393 HCl (mol. wt. 291.8) and SCH23390 HCl (mol. wt. 324.1) were each dissolved in distilled water and made up daily to the appropriate concentrations. In 0.25 μl distilled water, the dosage was 0.1, 1.0, and 10.0 μg for SK&F38393 and 0.01, 0.1, and 1.0 μg for SCH23390. A 0.25-μl distilled water microinjection served as a vehicle control. For drug injections, two injection cannulae, 26-ga needles, were appropriately fabricated to extend to the ventral depth. They were linked to a microsyringe filled with the appropriate drug solution. The 0.25 μl solution was injected over a period of 60 s and the cannulae left in place for a further 30 s. All injections were bilateral. Immediately after the injection procedure, animals were individually placed in an activity chamber.

Histological Configuration of Injection Site

After completing behavioral testing for each animal, the animal was administered an overdose of sodium pentobarbital (Butler, Chicago, IL) and perfused intracardially with 0.9% saline followed by 10% buffered formalin. Brains were removed and placed in a 10% buffered formalin. Prior to sectioning, tissue was placed in 30% sucrose formalin until the brain dropped to the bottom. The brains were than frozen, sectioned, mounted on slides, stained with cresyl violet, and the locations of the injection cannulae tips verified by independent observers. Only those animals with bilateral placements in the target areas were used in the data analyses.

Apparatus and Measurement of Locomotor Activity

Activity chambers were used to objectively measure locomotor behavior (Digiscan-16 Animals Activity Monitoring System, Omniteck Electronics, Columbus, OH). In this study, the locomotor activity as measured by the total horizontal distance in cm was automatically recorded (22). The activity of animals were measured over a 2-h session where the response measures were blocked into 12 consecutive segments of 10 min each.

Research Design and Statistical Analyses

Each treatment group consisted of 10 animals chosen at random. Each rat was used only once. A two-factor mixed-design analysis of variance (ANOVA) was used to analyze the within measures (12 consecutive 10-min time blocks), between the treatment conditions (dose levels), and the dose × time block interaction effect. Significant interactions for the dose × time block were followed up within time blocks by Dunnett's multiple-comparison tests between the vehicle control group and the treatment groups. p values equal to or less than 0.05 were judged statistically significant.

RESULTS

Effects of SK&F38393 on Horizontal Activity

Microinjections of SK&F38393 directly into the Acb resulted in significant potentiation of horizontal activity as measured by the distance in cm that animals moved within an open field.

The significant dose × time interaction, $F(4, 41) = 5.15$, $p < 0.001$, is given in Fig. 1A. Further, the dose effect was significant, $F(4, 41) = 4.21$, $p = 0.006$, as well as the time block effect, $F(11, 450) = 12.52$, $p < 0.001$. The subsequent analyses revealed that beyond the 30-min time block the 0.1-μg dose group was significantly potentiated in comparison to the vehicle control group ($p < 0.05$ and 0.01); at 50 min, the 10.0-μg group was significantly greater than the vehicle control group ($p < 0.05$ and 0.01); and the 1.0-μg group differed from the vehicle control group at time blocks 50, 70, and 100–120 min ($p < 0.05$ and 0.01). All other comparisons were not significant ($p > 0.05$). All SK&F38393 dosage groups were significantly potentiated in comparison to the vehicle control group ($p < 0.05$ and 0.01; see Fig. 1B).

Effects of SCH23390 on Horizontal Activity

Microinjections of SCH23390 into the Acb resulted in significant attenuation of horizontal activity as measured by the distance in cm that animals moved.

The ANOVA resulted in a highly significant dose × time blocks interaction, $F(33, 396) = 6.26$, $p < 0.001$; dose effect, $F(3, 36) = 6.42$, $p < 0.001$; and time blocks effect, $F(11, 396) = 137.98$, $p < 0.001$. Subsequent analyses across the time blocks revealed significant attenuation by all dose levels of SCH23390 at time block 10 min; at time blocks 20 and 30 min, the 1.0-μg group was significantly attenuated, as was the 0.1 μg at time block 30 min ($p < 0.05$ and 0.01). All other comparisons were not significant ($p > 0.05$). All SCH23390 dosages were significantly attenuated in comparison to the vehicle control group ($p < 0.05$ and 0.01; see Fig. 2B).

DISCUSSION

The primary feature of locomotor activity elicited by SK&F38393 microinjected into the Acb was the highly significant dose × time interaction ($p < 0.001$). During the first 20 min, all groups were similar in their high rates of habituation. Beyond the 20-min interval, the vehicle control group continued to habituate, whereas the SK&F38393 groups typical showed facilitation of locomotor activities. The four dose groups (0.01, 0.1, 1.0, and 10.0 μg) begin to significantly diverge at some time point beyond 40 min. The dose of 0.1 μg SK&F38393 induced the largest response rate and was the only SK&F38393 dose level that was consistently significant from the vehicle controls across the time blocks. This finding may in part explain the reports where SK&F38393 was inactive following Acb injections, that is, the dosage level may be too high and the duration too short to have induced significant locomotor activity in intact, nonlesioned rats (7,21).

On the other hand, it has been reported that microinjections of 3.0 and 30.0 μg/0.5 μl SK&F38393 into the Acb resulted in extensive neurotoxic damage (13). If SK&F38393 functions as a neurotoxin at the 3.0-μg level, it is theoretically

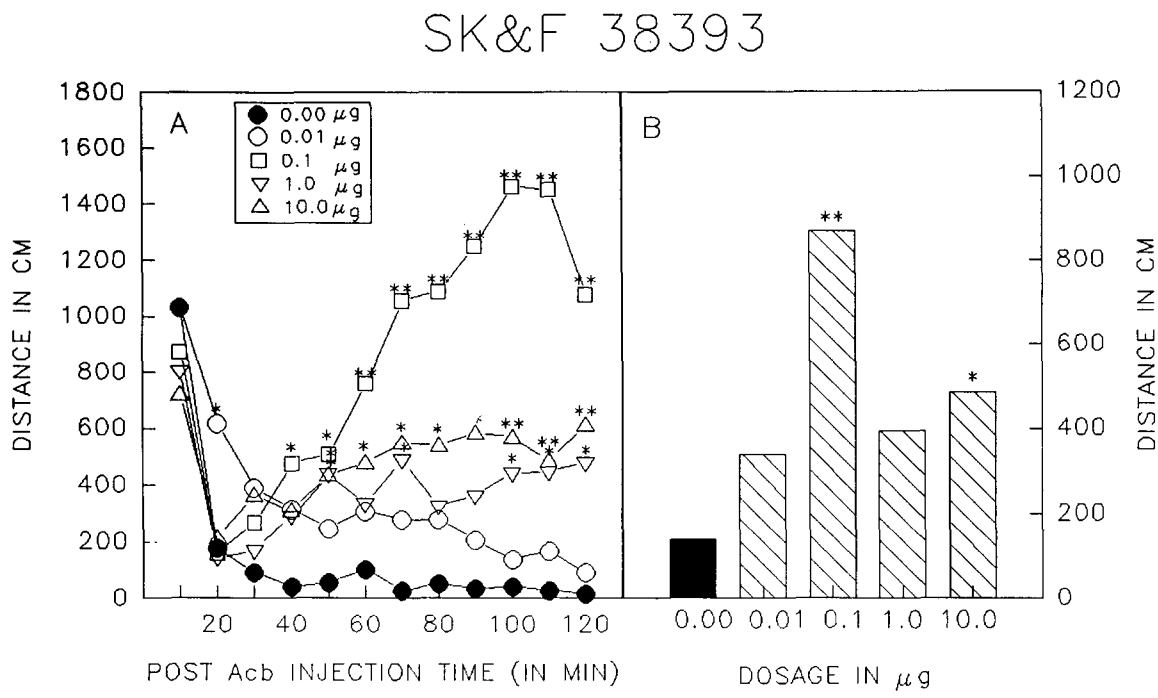

FIG. 1. (A). Effects of microinjected doses of SK&F38393 into the nucleus accumbens on horizontal locomotor activities over 120 min as measured by the distance traveled in cm. (B). Average mean effects of various dosages of SK&F38393 over the 12 10-min time blocks. The error bars have been omitted for clarity. Significant differences from the vehicle control group, $*p < 0.05$ and $**p < 0.01$.

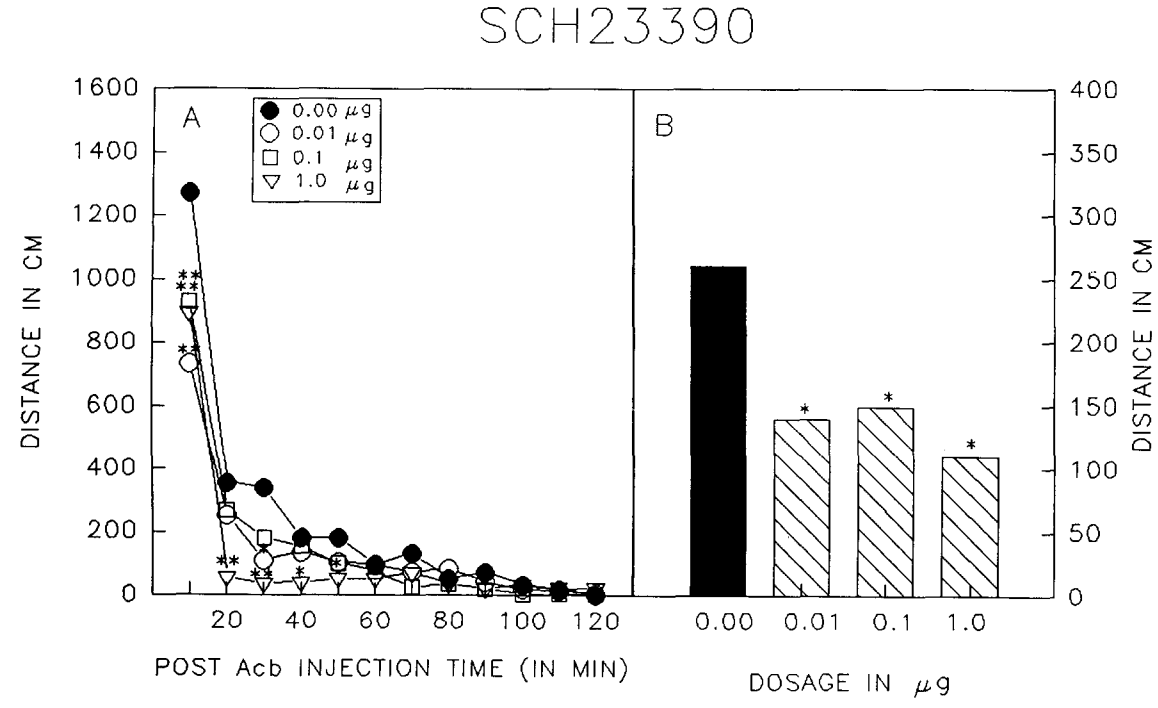

FIG. 2. (A). Effects of microinjected doses of SCH23390 into the nucleus accumbens on horizontal locomotor activity over 120 min as measured by the distance traveled in cm. (B). Average mean effects of various dosages of SCH23390 over the 12 10-min time blocks. The error bars have been omitted for clarity. Significant differences from the vehicle control group, $*p < 0.05$ and $**p < 0.01$.

possible that the D_1 and D_5 receptor subtypes may inversely respond at the higher dose levels. A posteriori inspection of the histology in the present study revealed extensive neurotoxic damage in the Acb with the 10.0 μg/0.25 μl SK&F38393 but not with any of the other dose levels or with SCH23390. The present study, however, showed behavioral effects beginning at the 20-min time block rather than following a long time course onset. This may suggest that the neurotoxic lesions seen later in the histology had a possible confounding effect on the behavior. However, the time course of the neurotoxicity of SK&F38393 has not been reported.

The attenuation of locomotor activity following microinjections of SCH23390 into the Acb replicates the systemic effects on locomotor activities (15) and was consistent with the potentiation of the durations of bar and cling catalepsy (14, 19,29).

The present data on horizontal activity are, in general, supportive of the hypothesis that dose-related locomotor activities potentiated by SK&F38393 and attenuated by SCH23390 microinjected into the Acb are independently mediated by D_1 receptor subtypes. As SK&F38393 and SCH23390 bind to both the DA D_1 and D_5 receptor subtypes, these data do not differentiate behaviorally between these two DA subtypes, nor do these data answer the theory that the main D_1 and D_2 receptors functionally interact. However, the present data support the growing evidence that the D_1 and D_5 subtypes behaviorally function independent of the D_2 receptors within the limbic system.

ACKNOWLEDGEMENTS

This research was supported by a grant to M.E.M. from the Whitehall Foundation. The author thanks Bonnie McLaurin and Thomas Potter for technical assistance and Jeanene Griffin for excellent secretarial assistance.

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