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Opposite Effects of SK&F 38393, a Dopamine D-1 Agonist, and SCH 23390, a Dopamine D-1 Antagonist, in Tests of Salt Preference/Aversion in the Rehydrating Rat

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GILBERT, D. B. AND S. J. COOPER. *Opposite effects of SK&F 38393, a dopamine D-1 agonist, and SCH 23390, a dopamine D-1 antagonist, in tests of salt preference/aversion in the rehydrating rat.* PHARMACOL BIOCHEM BEHAV **32**(4) 945–948, 1989.—Male rats were adapted to a 22-hr water-deprivation schedule, and to a 15-min choice test, in which water was available in one drinking tube, and water, 0.064%, 0.16%, 0.4%, 1.0%, or 2.5% NaCl solution, respectively, was available in a second. A typical saline preference-aversion function was obtained. The selective dopamine D-1 agonist, SK&F 38393 (3.0 mg/kg, IP), significantly depressed choice of hypertonic saline solutions (1.0% and 2.5% NaCl solutions), without affecting preference for hypotonic saline solutions. In contrast, the selective dopamine D-1 antagonist, SCH 23390 (0.1 mg/kg, SC), significantly increased the preference measure in the case of hypertonic solutions. These data indicate a role for D-1 receptors in dopaminergic mediation of the descending limb of the saline preference-aversion function.

Dopamine Salt SCH 23390 SK&F 38393 Thirst Rats

MOGENSON and Wu (15) provided the first evidence implicating central dopamine mechanisms in the control of saline preference in the rat. Extending their work, we reported recently that several dopamine antagonists significantly affected saline preference in male and female rats (8). Pimozide and the 'atypical' neuroleptic, clozapine, produced dose-dependent reductions in fluid intake, but also increased salt preference, particularly in the case of a hypertonic NaCl solution. The substituted benzamide, sulpiride (a selective dopamine D-2 receptor antagonist), did not reduce fluid intake, but did increase saline preference in some circumstances. The results were interesting in that there was no occasion when the dopamine antagonists reduced saline preference.

A distinction has been drawn between the two dopamine receptors, D-1 and D-2 (14). The introduction of selectively-active D-1 and D-2 agonists and antagonists has allowed behavioural consequences of drug actions at the receptor subtypes to be investigated (20). Since there was no existing evidence for a possible role for the D-1 receptor in relation to saline preference, the present experiments were designed to investigate the effects of a selective D-1 agonist, SK&F 38393 (18,19), and a selective D-1 antagonist, SCH 23390 (2, 10–12) in a salt preference test.

A two-choice preference procedure was used (3), and a range of salt concentrations, from 0.064% to 2.5% NaCl, allowed assessment of drug effects over the saline preference-aversion function to be made.

METHOD

Animals

Adult male hooded rats (General strain, bred in our laboratory) were used. They were housed in pairs in stainless steel cages, and were given free access to food pellets (modified Diet 41B, Heygate and Sons, U.K.). They were maintained under a 12-hr light:12-hr dark schedule (lights on at 7 a.m.) and the room temperature was kept constant at 21°C. Before testing, the animals were thoroughly adapted to a 22-hr water-deprivation schedule, and to handling and injection procedures over a period of two weeks. The animals weighed 250–400 g at testing.

Apparatus

The rats were individually tested in eight wooden boxes, the

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dimensions of which were 25 × 25 × 22.5 cm. Each box had two holes (11 cm apart, 6 cm above the floor, and equidistant from the sides), at the front of the box through which the stainless steel spouts of the drinking tubes were presented. The saline solutions and water were available in inverted clear plastic 50 ml graduated cylinders, which were clipped in position to the outside of each drinking box. Each box had an opaque cover, to minimize disturbance to the animal during the drinking test. Sawdust covered the floor, and food was never presented to the animals in these boxes.

Drugs

(±)SK&F 38393 (2,3,4,5-tetrahydro-7,8-dihydroxy-1-1H-3-benzazepine), supplied courtesy of Smith Kline & French Laboratories, Philadelphia, was suspended ultrasonically in isotonic saline, and was administered IP 5 min before the saline preference-aversion tests.

SCH 23390 [R-(+)-8-chloro-2,3,4,5-tetrahydro-3-methyl-5-phenyl-IH-3-benzazepine 7-ol] hemimaleate, supplied courtesy of Schering Plough Corp., Bloomfield, NJ, was dissolved in 0.9% saline. SCH 23390, at doses of 0.03, 0.1, 0.3 and 1.0 mg/kg, was injected by subcutaneous route, 30–35 min before the saline preference-aversion tests.

Procedure

The animals were allocated at random to six equal groups. Each group was then assigned to one of the following tests conditions: water in both tubes, or 0.064%, 0.16%, 0.4%, 1.0% and 2.5% (w/v) NaCl solution versus water, respectively. The concentrations of the saline solutions were established on the basis of pilot experiments to give a range of values covering ascending and descending portions of the saline preference-aversion function.

Within each group, each animal was placed in the drinking box for 15 min following the period of water deprivation. The fluid levels in both drinking tubes were measured to the nearest 0.5 ml before and after the 15-min test, and the intakes were calculated. Spillage was negligible and therefore was not taken into account. Following the test period, animals were returned to their home cages and were allowed access to tap water for the next 1.75 hr with their food. All animals had been well adapted to the choice procedure before the drug tests began. The position of water and saline tubes were randomly assigned at each trial to avoid the development of position habits.

Each animal received each dose of SCH 23390 in a randomized order, and at least two days separated consecutive tests. Tests of baseline intake were carried out on days between drug testing. All tests were carried out in the morning. After a one-week interval, testing was resumed using the same procedure, and each animal received each dose of SK&F 38393 in a randomized order.

Data Analysis

The intake (ml) data were analysed using a 3-way ANOVA of mixed design, with saline concentration as a between subject factor (six levels), drug dose as a repeated measure factor (five levels), and fluid condition as repeated measure (two levels). Dunnett's *t*-test was used to assess the significance of differences of individual dose means from control values. The preference measure was defined as the intake of the saline solution as a percentage of the total fluid consumption within a drinking trial. Values above 50% were taken to indicate preference for saline, and values below, an aversion to the saline.

TABLE 1

EFFECTS OF THE DOPAMINE D1 ANTAGONIST SCH 23390 ON TOTAL FLUID INTAKE (ml) IN A SALINE PREFERENCE TEST

	SCH 23390 (mg/kg)					
	0	0.03	0.1	0.3	1.0	ED_{50} (mg/kg)
Water	10.9 ±0.5	8.9 ±0.8	3.2‡ ±1.2	1.8‡ ±0.5	1.2‡ ±0.5	0.06
Saline						
0.064%	11.8 ±0.9	8.8* ±1.7	3.3‡ ±1.4	1.6‡ ±0.8	0.4‡ ±0.1	0.06
0.16%	13.2 ±0.7	10.9 ±0.9	6.2‡ ±1.4	3.1‡ ±1.2	2.1‡ ±1.3	0.09
0.4%	14.7 ±0.6	12.9 ±1.5	5.8‡ ±1.4	3.4‡ ±1.9	0.7‡ ±0.2	0.08
1.0%	17.8 ±0.7	14.8* ±0.8	9.1‡ ±1.3	3.9‡ ±1.6	1.9‡ ±0.7	0.1
2.5%	12.9 ±0.5	11.4 ±1.8	4.9‡ ±1.3	1.3‡ ±0.7	0.6‡ ±0.3	0.07

Results represent means ± S.E.M. (N = 8 per group)
Dunnett's *t*-test: *$p<0.05$; †$p<0.01$; ‡$p<0.005$.
The ED_{50} dose was calculated as the dose required to reduce intake by 50% of the control value.

RESULTS

Total Fluid Intake: SCH 23390

Analysis of the total fluid intake results revealed a significant effect of saline concentration, $F(5,42) = 6.13$, $p<0.001$. As Table 1 shows, intake increased as a function of saline concentration up to 1%, and dropped again at 2.5%. SCH 23390 (0.03–1.0 mg/kg) produced a dose-dependent suppression of drinking, $F(4,168) = 158.2$, $p<0.0001$, and did so independently of saline concentration, $F(2,168)<1.0$, not significant. At a dose of 0.03 mg/kg, SCH 23390 significantly reduced total fluid intake in the 0.064% and 1.0% NaCl conditions. However, at 0.1 mg/kg, intake was reduced to a major extent in all conditions (Table 1). Drinking was virtually abolished at 0.3 and 1.0 mg/kg.

Saline Preference: SCH 23390

As expected, the level of saline preference was a function of the concentration of the saline available in the two-choice test. Values (mean ± S.E.M.) after vehicle injections were as follows: 57 ± 1.9% (0.065% NaCl); 64 ± 7.5% (0.16% NaCl); 69 ± 2.9% (0.4% NaCl); 52 ± 7.6% (1.0% NaCl); 22 ± 3.3% (2.5% NaCl). Thus, the peak preference was for the 0.4% NaCl solution, while animals displayed an aversion to the 2.5% NaCl.

At lower doses, SCH 23390 (0.03 and 0.1 mg/kg) had no significant effect on the preferences for 0.064%, 0.16% or 0.4% NaCl solutions. However, at 0.1 mg/kg, SCH 23390 significantly *increased* the preference measure for the two strongest salt solutions, 1.0% and 2.5% NaCl, respectively (Fig. 1). It is especially noteworthy that the aversion to the 2.5% salt solution was completely blocked by SCH 23390. Since 0.3 and 1.0 mg/kg SCH 23390 suppressed drinking to a considerable extent, the preference data were not considered at these doses.

Total Fluid Intake: SK&F 38393

SK&F 38393 had a significant effect on fluid intake, $F(3,126) =$

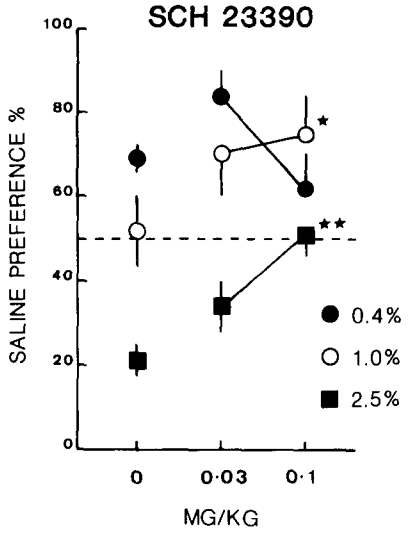

FIG. 1. Saline preference (%) measures in a two-choice test following administration of the selective dopamine D-1 receptor antagonist SCH 23390 (0.03 and 0.1 mg/kg). SCH 23390 did not significantly affect the preference for dilute NaCl solutions (0.064% and 0.16%) (data not shown). The results indicate mean ± S.E.M. (N = 8 per group). Levels of significance: *$p<0.05$; **$p<0.01$ (Dunnett's t-test).

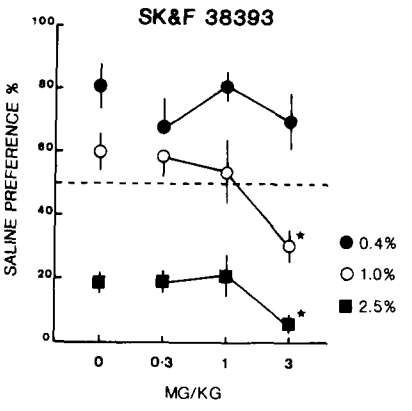

FIG. 2. Saline preference (%) measures in a two-choice test after administration of the selective dopamine D-1 receptor agonist, SK&F 38393 (0.3, 1.0 and 3.0 mg/kg). SK&F 38393 did not significantly affect the preference for dilute NaCl solutions (0.64% and 0.16%) (data not shown). The results indicate mean ± S.E.M. (N = 8 per group). Level of significance: *$p<0.05$ (Dunnett's t-test).

5.0, $p<0.003$. As Table 2 shows, SK&F 38393 produced slight but significant reductions in total intake, when 0.4%, 1.0% and 2.5% NaCl solutions were available in the preference test. The reductions were not markedly dose-related. On the other hand, it had no effect on water consumption, or on total intake when either 0.064% or 0.16% NaCl solutions were available in the preference test. Hence, there appeared to be no general antidipsogenic action of SK&F 38393 over the dose range 0.3–3.0 mg/kg.

TABLE 2

EFFECTS OF THE DOPAMINE D1 AGONIST (±) SK&F 38393 ON TOTAL FLUID INTAKE (ml) IN A SALINE PREFERENCE TEST

	SK&F 38393 (mg/kg)			
	0	0.3	1.0	3.0
Water	10.5 ±1.0	9.2 ±0.9	11.0 ±0.8	10.6 ±0.8
Saline				
0.064%	11.3 ±1.1	10.7 ±1.1	10.9 ±0.6	12.2 ±0.5
0.16%	10.7 ±0.8	9.1 ±0.4	9.9 ±0.8	9.8 ±0.5
0.4%	14.8 ±0.8	12.3† ±1.1	12.5† ±0.7	12.4† ±0.8
1.0%	16.3 ±1.2	15.0 ±1.3	14.2* ±1.2	12.5‡ ±1.5
2.5%	12.5 ±1.7	11.8 ±0.5	10.3* ±0.5	10.3* ±1.0

Results represent means ± S.E.M. (N = 8 per group).
Dunnett's t-test: *$p<0.05$; †$p<0.01$; ‡$p<0.005$.

Saline Preference: SK&F 38393

SK&F 38393 had no effect on saline preference for any of the hypotonic saline concentrations (0.064%–0.4% inclusive). Nevertheless, as Fig. 2 indicates, SK&F 38393 did have an effect on saline preference in the case of the 1.0% and the 2.5% NaCl solutions, respectively. At 3.0 mg/kg, SK&F 38393 significantly enhanced the relative aversion to the hypertonic saline.

DISCUSSION

The present results indicate an involvement of dopamine D-1 receptors in the control of hypertonic saline intake in a two-bottle test. The selective D-1 agonist, SK&F 38393, increased aversion to hypertonic saline solutions, whereas the selective D-1 antagonist, SCH 23390, had the opposite effect to increase the relative preference for hypertonic saline solutions. The major implication of these data is that dopamine activity at D-1 receptors underlies, at least in part, the relative aversion expressed for hypertonic saline solutions in rehydrating rats. Neither compound significantly affected the positive preference for hypotonic saline, and therefore it appears that D-1 receptors are not involved in the preference for dilute salt solutions. Different neuropharmacological mechanisms are likely to be responsible for the ascending and descending limbs of the saline preference-aversion function.

SK&F 38393 (0.3–3.0 mg/kg) had no marked antidipsogenic effect, and at these doses we observed no signs of general behavioural impairment over this range of doses. There have been two recent reports of the discriminative stimulus properties of SK&F 38393, but higher training doses of 8.0 mg/kg and 10 mg/kg were used (4,13). At higher doses still, SK&F 38393 has been reported to produce excessive grooming in rats (16). Thus, the increased aversion to hypertonic saline produced by SK&F 38393 reflects a sensitive and behaviorally-specific index of D-1 receptor activation.

The selective D-1 antagonist SCH 23390 reduced fluid intake dose-dependently, confirming results we obtained previously using a single-bottle test of saline and water acceptance (6,7). This

depression of drinking may be due to performance impairment (9,17), although we have recently observed that SCH 23390 selectively reduced drinking, without affecting feeding responses, in free-feeding and drinking rats in home cages (Rusk, Clifton and Cooper, submitted for publication). Hence, there may be a component of SCH 23390's actions which is specifically antidipsogenic. Several earlier studies showed that dopamine antagonists (pimozide, haloperidol, chlorpromazine) reduced fluid ingestion in rats (5). It is interesting that the present data suggest that there may be a D-1 receptor basis for at least part of the antidipsogenic effects of classical neuroleptics. There has also been a recent suggestion that the antipsychotic efficacy of neuroleptics may be due to blockade of brain D-1 receptors (1).

In summary, the present results are consistent with a dopamine D-1 receptor-mediated aversion to hypertonic salt solutions, as determined in two-choice preference tests. The selective D-1 agonist SK&F 38393 increased the aversion to hypertonic saline; conversely, the selective D-1 antagonist SCH 23390 decreased the aversion. In contrast, preferences for hypotonic saline solutions remained unaffected either by SK&F 38393 or by SCH 23390. Additionally, SCH 23390 had a marked antidipsogenic effect, which occurred across all test conditions, irrespective of salt concentration.

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