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BRIEF COMMUNICATION

Effects of the Dopamine D-1 Antagonist SCH 23390 on Water Intake, Water-Rewarded Operant Responding and Apomorphine-Induced Decrease of Water Intake in Rats

TOMAS LJUNGBERG[1,2]

Department of Pharmacology, Karolinska Institute
P.O. Box 60 400, S-104 01, Stockholm, Sweden

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LJUNGBERG, T. *Effects of the dopamine D-1 antagonist SCH 23390 on water intake, water-rewarded operant responding and apomorphine-induced decrease of water intake in rats.* PHARMACOL BIOCHEM BEHAV **33**(3) 709–712, 1989.—The specific dopamine (DA) D-1 receptor antagonist SCH 23390 was found to attenuate operant lever-pressing with water as reward in a dose-dependent manner and more potently than drinking itself. This effect occurred in the same fashion as previously reported for DA D-2 antagonists. In contrast to the DA D-2 antagonist haloperidol, the attenuated operant lever-pressing induced by the DA D-1 antagonist SCH 23390 was not counteracted by the anticholinergic drug scopolamine. The decreased water intake in thirsty animals caused by a low dose of apomorphine was not antagonised by SCH 23390. This has previously been found with DA D-2 antagonists, such as haloperidol and sulpiride. The results show that in spite of some similarities in the behavioural effects of DA D-1 and D-2 antagonists, a closer pharmacological analysis is able to reveal pronounced differences.

Neuroleptics SCH 23390 Apomorphine Scopolamine Water intake Operant behavior Rat

ON the basis of pharmacological data, brain dopamine (DA) receptors have been divided into two distinct classes; D-1 and D-2 receptors (14,27). The classical effects of neuroleptics in behavioral pharmacological models, like induction of catalepsy, inhibition of DA agonist-induced hypermotility or stereotyped behaviors and the attenuation of operant responding, were initially thought to be related to the blockade of the D-2 receptor (5,24).

The benzazepine derivative SCH 23390 is the first selective DA D-1 antagonist (11–13). Surprisingly, SCH 23390 was found to exert potent effects in the models presumed to reflect classical neuroleptic, i.e., DA D-2 antagonistic, activity. For example, SCH 23390 causes a selective inhibition of conditioned avoidance responding (CAR), it antagonises DA agonist-induced locomotion, stereotyped behaviors and rotation in unilateral 6-OH-DA-lesioned animals and it produces catalepsy (5,13).

In recent papers we have described the development of a new behavioral-pharmacological paradigm where we can study in parallel the effects of neuroleptics on water-rewarded operant lever-pressing and on the corresponding consummatory act, i.e., unconditioned water intake. We have found that the operant lever-pressing response is more potently attenuated by DA D-2 antagonists than water intake (15) in the same fashion as the conditioned avoidance response is more potently antagonised than the escape reaction (1). We have also found that the attenuation of the lever-pressing response produced by the DA D-2 antagonist haloperidol could be counteracted by pretreatment with the anticholinergic drug scopolamine. However, the haloperidol-induced attenuation of the water intake could not be counteracted by scopolamine (16).

After administration of a low dose (i.e., 0.05–0.1 mg/kg SC) of the mixed D-1/D-2 receptor agonist apomorphine (APO) to water-deprived animals, subsequent water intake is decreased. We have also found that this effect of apomorphine can be completely antagonised by the DA D-2 antagonist sulpiride, indicating the

[1]Present address: Institut de Physiologie, Université de Fribourg, Rue du Musée 5, CH-1700 Fribourg, Switzerland.
[2]Requests for reprints should be addressed to Tomas Ljungberg, Department of Pharmacology, Karolinska Institute, P.O. Box 60 400, S-104 01, Stockholm, Sweden.

importance of D-2 receptors in this effect of APO (17).

In order to characterise further the dopaminergic control of instrumental and consummatory behaviors and the functional role of DA D-1 receptors we investigated in the present study the DA D-1 antagonist SCH 23390 in the various tests described above. Thus, we tested A) whether SCH 23390 could attenuate the operant lever-pressing and the unconditioned water intake in rats made thirsty by water deprivation, B) whether scopolamine could counteract the attenuation found under heading A, and C) whether SCH 23390 could counteract the effects of a low dose of APO.

METHOD

Animals

The experiments were performed on 107 male Sprague-Dawley rats (ALAB, Sollentuna) which arrived at the animal colony at least 1 week prior to the start of the experiments. During the experiments, the animals were housed under conditions of controlled temperature and humidity on a 12-hr light/12-hr dark schedule (light on 7 a.m.–7 p.m.) with lab chow ad lib. The weights of the animals were between 220–350 grams and each animal was only used in one of the five separate experiments described below.

Effects of SCH 23390 on Water Intake (n = 12) and Lever-Pressing (n = 10)

Apparatus. Water intake and the ability to press the lever were tested in slightly modified Skinner boxes (length = 32 cm, width = 20 cm, height = 20 cm). All boxes were placed inside sound-protecting boxes equipped with one-way observation windows. Electric fans ventilated the boxes and provided a constant background noise.

In the boxes where water intake was tested, the levers and the dipper mechanisms were removed and water nipples were mounted in place of the dipper cups. The animal thus only needed to lick the nipple to obtain water, not to perform or learn any operant response. The total amount of water consumed was registered.

A specially developed lever was used. It resembled a "mill-wheel" and its four wings were 4 cm long and 3 cm wide and made out of 5 mm black plastic. One quarter of a turn, which was signalled to the animal as a distinct click and as a sudden and transient drop in resistance, was defined as one lever press. The weight necessary to turn the lever was set to 20 g [see (18)]. A dipper of standard type delivered 0.05 ml every time it was activated (in this experiment after each lever press). The accumulated number of lever presses obtained during a session was registered.

Experimental procedure. The experiments were performed according to methodology developed earlier and described in greater detail elsewhere (15,16). In short, the animals were kept individually in ordinary laboratory cages. Except for receiving water in the 30-min long daily experimental session, the animals also had access to water in the home cages for 15 minutes, 45 minutes after the end of the test session. During weekends the animals had free access to water.

The animals were their own controls. When they had reached a stable baseline response they were injected with the drug vehicle alone for 1 to 2 days and then on the following day tested on SCH 23390. SCH 23390 was administered 30 minutes before the start of the experiments. No animal was tested with drug on more than three occasions, nor with the same dose more than once and at least one week elapsed between each drug test. As control performance (shown as 100% in Fig. 1) the mean total number of lever presses during a session was 369 ± 27 (n = 8) and the mean total amount of water consumed was 13.8 ± 0.9 ml (n = 8).

Calculation of the result. The baseline response for an animal was calculated by taking the median value (amount of water consumed or number of lever presses) on each day of control injections. To obtain a measure of the effect of drug we first calculated the change in response after drug injection for each animal, expressed as a percentage of its own control value. The group mean ± S.E.M. was then calculated and used as a measure of the effect of the drug treatment. The level of significance was tested with the Student's *t*-test for paired samples. As the Student's *t*-test was used for multiple comparisons and as a mixed dependent-independent experimental design was used the Bonferroni method was applied to adjust the *p*-level (30). By this method the *p*-value of the Student's *t*-test is adjusted according to the formula $p^* = p/m$, where the p^* is the adjusted *p*-value, *p* is the *p*-level set by the researcher (in this case 0.05, two-tailed), and m is the number of comparisons.

Reversal With Scopolamine of the Effects of SCH 23390 on Lever-Pressing (n = 28) and Water Intake (n = 8)

Apparatus, experimental procedure and calculation of the result. Doses of SCH 23390 were selected from the experiments described above and the decrease in performance was aimed at being in the same range as in our previous study where the behavioral effects of haloperidol were reversed with scopolamine (16). Scopolamine was administered 60 minutes and SCH 23390 30 minutes before the start of the experiment. The mean and the S.E.M. were calculated and the level of significance was tested with the Student's *t*-test (for paired samples when tested against the own control values and for unpaired samples when tested against the SCH 23390 controls). The *p*-value was adjusted with the Bonferroni method as described above. Apparatus, experimental procedure and calculation of the results were otherwise as described above.

Effects of SCH 23390 on Decreased Water Intake Caused by 0.05 mg/kg of Apomorphine (n = 54)

Apparatus and experimental procedure. The same modified Skinner boxes as described above were used. The animals were kept two in each home cage. Except for getting water in the 10-min long experimental session, the animals also had access to water in the home cages for 15 min, 1 hr after the end of the test session. The animals were tested on three consecutive days and were their own controls. The total amount of water consumed during the session on the second day was used as a control value and the amount of water consumed on the experimental day (i.e., day 3) was for each animal compared against its control value (total amount of water consumed on day 3 − day 2 was thus calculated for each animal and used in the statistical evaluations). SCH 23390 was administered 40 minutes and apomorphine 10 minutes before the start of the experiment. The animals were tested alone and used only once [see (17)].

Calculation of the result. The mean and the S.E.M. were calculated and the level of significance was tested with one-way ANOVA followed by Dunnett's test. The only APO-treated group was selected as the "control" and the significance level was set at $p < 0.05$.

Drug Treatments

SCH 23390 (Schering Plough Co., USA) and scopolamine-hydrochloride (Sigma) were dissolved in saline. Apomorphine-HCl was dissolved in saline. The doses of SCH 23390 refer to the above mentioned form while the doses of scopolamine and apo-

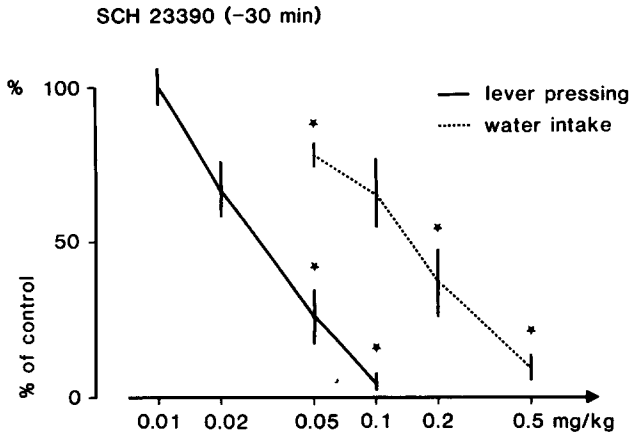

FIG. 1. Effects of SCH 23390 on lever-pressing and water intake. The results are shown as group means ± S.E.M. (*$p<0.05$). Five to seven animals were tested at each dose. SCH 23390 was administered 30 min before the start of the experiments.

morphine refer to the base. All drugs were injected subcutaneously in the flank in a volume of 1 ml/kg.

RESULTS

Effects of SCH 23390 on Water Intake and Lever-Pressing

SCH 23390 attenuated in a dose-dependent manner both the lever-pressing and the water intake. The lever-pressing was more potently attenuated than the water intake (Fig. 1).

Reversal of the Effects of SCH 23390 With Scopolamine

A wide range of scopolamine doses (0.03–1.0 mg/kg), taken from our previous study (16), was tested. It was not possible to counteract the attenuation in performance caused by SCH 23390 by pretreatment with scopolamine. The results are shown in Table 1.

Effects of SCH 23390 on the Decreased Water Intake Caused by 0.05 mg/kg of Apomorphine

The one-way analysis of variance was significant, $F(8,45) = 12.51$, $p<0.0001$. APO 0.05 mg/kg caused a significant reduction in water intake (controls = 1.14 ± 0.58; n = 14; and APO 0.05 mg/kg = −3.49 ± 0.45; n = 9). It was not possible to counteract the APO-induced decrease in water intake with SCH 23390 (2 μg/kg = −2.6 ± 0.76; 5 μg/kg = −1.68 ± 0.73; 10 μg/kg = −3.46 ± 0.44; 20 μg/kg = −3.72 ± 0.82; 50 μg/kg = −2.84 ± 1.17— all nonsignificant; n = 4–5 at each dose). At higher doses of SCH 23390, the water intake was even further reduced (0.1 mg/kg = −6.0 ± 1.0; 0.2 mg/kg = −7.18 ± 0.94; $p<0.05$), indicating an effect of SCH 23390 of its own (cf. Fig. 1).

DISCUSSION

In agreement with previous publications, SCH 23390 was found to attenuate in a dose-dependent manner both operant lever-pressing and unconditioned water intake (2, 10, 21–23). However, it was further found that the operant lever-pressing was attenuated more potently than the water intake (Fig. 1). These findings are in agreement with our previous results concerning DA D-2 antagonists in the same experimental paradigm (15) and with previous results concerning the effects of both DA D-1 and D-2 antagonists on the CAR (1, 5, 13).

TABLE 1

EFFECTS OF SCOPOLAMINE ON SCH 23390-INDUCED BEHAVIORAL ATTENUATION

Experiment	Treatment	Dose(s) in mg/kg	Performance in % of Control	N
Lever-pressing	SCH 23390	0.05	26.1 ± 9.1	(6)
	+Scopolamine	0.03	9.0 ± 4.1	(6)
	+Scopolamine	0.1	6.2 ± 1.6	(6)
	+Scopolamine	0.3	2.4 ± 1.1	(6)
	+Scopolamine	1.0	2.6 ± 0.6	(6)
Lever-pressing	SCH 23390	0.1	4.6 ± 3.1	(6)
	+Scopolamine	0.03	3.5 ± 1.8	(4)
	+Scopolamine	0.1	2.0 ± 1.3	(5)
	+Scopolamine	0.3	<1	(5)
	+Scopolamine	1.0	9.9 ± 7.9	(4)
Water intake	SCH 23390	0.5	9.2 ± 4.5	(6)
	+Scopolamine	0.03	<1	(4)
	+Scopolamine	0.1	1.1 ± 0.9	(5)
	+Scopolamine	0.3	<1	(5)
	+Scopolamine	1.0	<1	(4)

SCH 23390 caused a significant reduction in performance (control performance = 100%). It was not possible to counteract this effect of SCH 23390 with scopolamine. Numbers of tested animals are given in parentheses and the data are shown as mean ± S.E.M. In no case were the results obtained after scopolamine + SCH 23390 significantly different from the respective only SCH 23390-treated control group and in all cases were the performance of the scopolamine + SCH 23390-treated animals significantly lower than the control performance.

It has previously been shown in rats that DA D-2 antagonist-induced attenuation of operant responding as well as the DA D-2 antagonism of DA agonist-induced stereotyped behaviors and induced catalepsy can be counteracted by anticholinergic drugs [for references, see (16)]. In contrast, haloperidol-attenuated water intake in thirsty rats has not been found to be counteracted by scopolamine (16).

In the present study, a large range of scopolamine doses, selected from our previous investigation (16), was tested against the attenuating effects of SCH 23390 on the operant lever-pressing and water intake. In contrast to our results obtained previously with haloperidol, it was not possible to counteract the effects of SCH 23390 on operant lever-pressing with scopolamine [see Table 1 and (16)].

Acetylcholine (Ach) is used as a transmitter in a small population of interneurons in the striatum. It has previously been shown that the release of Ach from these interneurons is inhibited by dopamine via a D-2, but not a D-1, receptor [see (27)]. The obtained results in the present study can tentatively be explained by the idea that scopolamine can reinstate a behavioral function attenuated by a D-2 antagonist by counteracting the increased release of Ach caused by the D-2 antagonist.

However, different results concerning the actions of scopolamine on behavioural effects of SCH 23390 in rats have previously been reported. It has, for example, both been reported that scopolamine can antagonise catalepsy induced by SCH 23390 (19)

and that it cannot (4). It has further been reported that scopolamine can counteract the attenuating effect of SCH 23390 on DA agonist-induced stereotypies (3) as well as it cannot (4). It has further been shown in primates that both the D-1 antagonist SCH 23390 and the D-2 antagonist haloperidol induce acute dystonias which can be counteracted by anticholinergic drugs [(5, 8, 9) and Gerlach, personal communication]. It is, therefore, at the present time difficult to outline the direct functional importance of the proposed D-2-Ach link in the striatum and its relation to extrapyramidal side-effects induced by neuroleptics in man.

Low doses of the mixed D-1/D-2 agonist APO induce a specific behavioural syndrome characterised by, for example, yawning, decreased motor activity and decreased water intake in rats made thirsty by water deprivation [see (7,17)]. We found it not possible to counteract the decrease in water intake caused by a low dose of apomorphine by a pretreatment with the D-1 antagonist SCH 23390. This is in line with previous studies showing that motor activity and food intake decreased by a low dose of APO cannot be counteracted by SCH 23390 (6, 26, 28, 29). However, our results are in contrast to the findings that APO-induced yawning can be, at least partially, blocked by SCH 23390 (20,25). This might indicate that the behavioural syndrome induced by a low dose of APO is not a unitary phenomenon and that different behavioral components in the syndrome might have different pharmacological characteristics.

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