Pharmacology Biochemistry & Behavior, Vol. 26, pp. 687–691. Pergamon Journals Ltd., 1987. Printed in the U.S.A. 0091-3057/87 $3.00 + .00

Effects of the Dopamine D-1 Antagonist SCH 23390 and the D-2 Antagonist Sulpiride on Saline Acceptance-Rejection in Water-Deprived Rats

DAVID B. GILBERT[1] AND STEVEN J. COOPER[2]

Department of Psychology, University of Birmingham, Birmingham, B15 2TT, U.K.

Received 6 August 1986

GILBERT, D. B. AND S. J. COOPER. *Effects of the dopamine D-1 antagonist SCH 23390 and the D-2 antagonist sulpiride on saline acceptance-rejection in water-deprived rats.* PHARMACOL BIOCHEM BEHAV **26**(4) 687–691, 1987.—Separate groups of water-deprived rats were familiarised with drinking water or one of a range of NaCl solutions (0.45–2.7%) in a 30 min test. The substituted benzamide, sulpiride, a selective dopamine D-2 receptor antagonist, significantly increased the consumption of water and hypotonic saline at 30 mg/kg. In contrast, the selective dopamine D-1 receptor antagonist, SCH 23390 (0.01–0.1 mg/kg SC) significantly reduced the intake of water and of saline at different concentrations in a dose-dependent manner. Consumption of water and 0.45% saline were most sensitive to the antidipsogenic effect of SCH 23390. These results suggest that previously-reported antidipsogenic effects of neuroleptics may depend, to at least some degree, on dopamine D-1 receptor blockade. The increase in drinking produced by sulpiride indicates that dopamine may act at D-2 receptors to inhibit the consumption of water and hypotonic saline, but not of stronger salt solutions.

Dopamine D-1 and D-2 receptors Fluid intake NaCl intake Rats SCH 23390 Sulpiride

IT is well-established that dopamine receptor antagonists (e.g., haloperidol, spiroperidol, pimozide) reduce the consumption of water [3, 14, 22, 24, 25, 27, 29]. This, together with data showing that intracerebral administration of dopamine can increase water consumption [23,27], suggests some involvement of central dopamine mechanisms in the control of drinking responses [6]. However, experiments with dopamine agonists or antagonists have not previously been extended to include a study of saline acceptance-rejection functions in rehydrating animals [7, 8, 30]. Hence, there are few data currently available concerning the possible involvement of dopaminergic mechanisms in the control of saline drinking. Furthermore, the antidipsogenic effect of dopamine receptor antagonists was described before the crucial pharmacological distinction had been drawn between D-1 and D-2 receptor subtypes [18]. Consequently, there is presently no indication of which receptor subtype mediates the antidipsogenic effects of dopamine antagonists; there is the possibility, of course, that both are involved.

The recent introduction of dopamine receptor antagonists which are selective for one or other type of dopamine receptor subtype [28] allows us to investigate D-1/D-2 receptor involvement in the antidipsogenic effects of dopamine antagonists. Exploring this question in the context of saline acceptance-rejection functions also permits us to determine effects on the consumption of NaCl solutions over a range of concentrations. In the present work, the selective D-1 receptor antagonist, SCH 23390, and the substituted benzamide, sulpiride, which is a selective D-2 receptor antagonist [4, 15–17, 28] were chosen for study. [^{3}H] SCH 23390 specific binding sites and [^{3}H] sulpiride specific binding sites have been described in the central nervous system, and have been taken to correspond to D-1 and D-2 receptors, respectively [2, 5, 10–12].

METHOD

Animals

The subjects were 96 male, black hooded rats (General strain) bred in the Psychology Department, University of Birmingham. They were housed in pairs in stainless steel cages, and were given free access to food pellets (modified

[1]Present address: Department of Anatomy and Neurobiology, College of Medicine, University of California, Irvine, CA 92717.
[2]Requests for reprints should be addressed to S. J. Cooper.

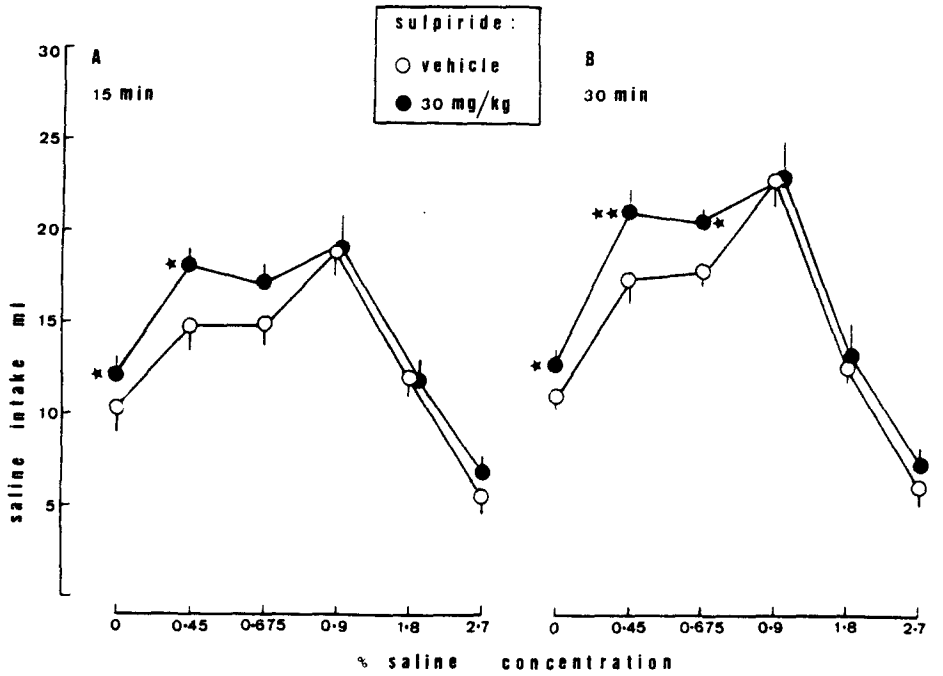

FIG. 1. Effect of sulpiride (30 mg/kg IP) on consumption of water and NaCl solutions (0.45–2.7%) after 15 min (panel A) and 30 min (panel B). Results are shown as mean±S.E.M. N=8 per group. Levels of significance in comparison with vehicle condition: $*p<0.05$; $**p<0.01$ (Dunnett's t-test).

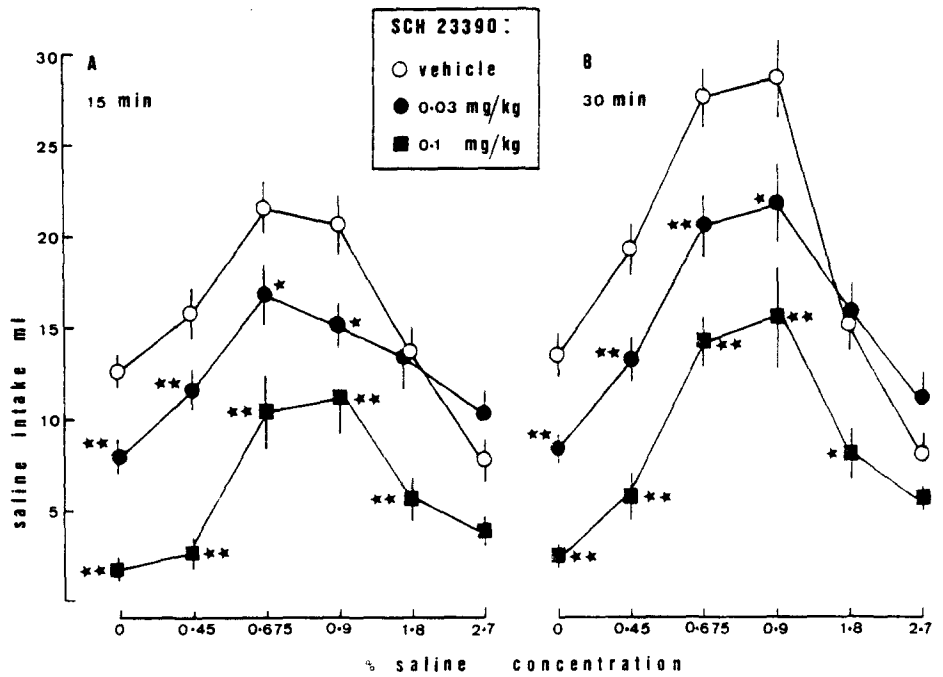

FIG. 2. Effects of SCH 23390 (0.03 and 0.1 mg/kg SC) on consumption of water and NaCl solutions (0.45–2.7%) after 15 min (panel A) and 30 min (panel B). For other details, see legend to Fig. 1.

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 21–22°C. Before testing, the rats 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 between 250–400 g at testing.

Apparatus

The rats were individually tested in eight wooden boxes, the dimensions of which were 25×25×22.5 cm. Each box had a hole (6 cm above the floor) in the front of the box, through which the spout of a drinking tube was presented. Saline solutions and water were available in inverted clear plastic 50 ml graduated cylinders, which were clipped into position on the outside of each box. Each box had an opaque cover to minimise disturbance to the animal during the drinking test.

Drugs

Sulpiride, supplied as base (Chemitechna, Surrey, U.K.) was suspended in isotonic saline, and a few drops of 0.1 N HCl were added until the suspension clarified. The sulpiride solution was buffered with a few drops of 10% KOH. Sulpiride, at doses of 3, 10 and 30 mg/kg, was injected by intraperitoneal route 1 hour before acceptance tests. SCH 23390 (R-(+)-8-chloro-2,3,4,5-tetrahydro-3-methyl-5-phenyl-1H-3-benzazepine-7-ol) maleate (supplied by Schering Plough Corp., Bloomfield, NJ) was dissolved in 0.9% saline. SCH 23390, at doses of 0.01, 0.03 and 0.1 mg/kg, was injected by subcutaneous route 35 mm before acceptance tests. All injections were given in a volume of 1 mg/kg body weight. The doses and injection-test intervals were selected on the basis of published reports.

Procedure

Before drug testing, all animals were adapted to drinking in the test boxes, for a 30 min period, until steady baseline levels of drinking were obtained. The animals were divided randomly into two groups: 48 rats to be tested with sulpiride, and 48 with SCH 23390. Each group was further sub-divided into 6 groups of 8, and each of these sub-groups was assigned to water, 0.45%, 0.675%, 0.9%, 1.8% or 2.7% saline conditions, respectively. Drinking water or one of these saline solutions, following 22 hr water-deprivation experience, each rat was familiarised with its allocated fluid condition. Each animal was injected with each dose within its drug condition, and doses were tested in random order. At least a 48 hr interval intervened between consecutive drug test days. Intake data (ml) were measured at 15 min and 30 min from the start of the test period.

Data Analysis

The acceptance data were analysed using a 2-way analysis of variance, with saline concentration as an independent factor (six levels), and drug dose as a repeated measure (four levels). Tests on individual dose treatments against control values were made using Dunnett's t-test, following one-way analysis of variance [31]. ED50 values for SCH 23390 were determined as doses calculated to reduce fluid intake to 50% of control baseline levels.

RESULTS

Sulpiride: D-2 Antagonist

The rehydrating animals showed typical saline acceptance/rejection functions at 15 and 30 min (Fig. 1). The peak intake occurred for the 0.9% NaCl solution, at both intervals. The 2.7% NaCl solution was significantly less acceptable than water ($p<0.01$).

After 15 min, sulpiride had a significant effect on fluid consumption, $F(3,126)=4.20$, $p<0.01$, and also after 30 min, $F(3,126)=3.87$, $p<0.05$. The smaller doses, 3.0 and 10.0 mg/kg, were ineffective, but at 30 mg/kg, sulpiride produced significant increases in fluid consumption. As Fig. 1 indicates, the significant effects were entirely specific to the water and hypotonic saline (0.45% and 0.675% NaCl) conditions; $F(3,36)=3.81$, $p<0.05$ (15 min intake) and, $F(3,63)=5.34$, $p<0.01$ (30 min intake). There was no effect of sulpiride on the consumption of isotonic or hypertonic saline.

SCH 23390: D-1 Antagonist

The second group of animals also showed typical saline acceptance-rejection functions (Fig. 2). After 15 min, the overall main effect of SCH 23390 was to reduce fluid consumption in rehydrating rats, $F(3,126)=60.43$, $p<0.001$ (Fig. 2). At the highest dose of 0.1 mg/kg, SCH 23390 produced a general depression of the saline acceptance-rejection function. Only the intake of the 2.7% NaCl solution was not significantly reduced. However, its effects at 0.03 mg/kg were more selective. There were significant reductions in the consumption of water, hypotonic and isotonic solutions, but there was no significant effect on the intake of either of the two hypertonic solutions. This differential effect cannot be attributed to baseline differences. SCH 23390 (0.03 mg/kg) reduced water intake from a control level of 12.7 ml down to 7.9 ml. However, it had no effect on the consumption of 1.8% saline, for which the baseline level of consumption was 13.7 ml. At the smallest dose tested, 0.01 mg/kg, SCH 23390 had no effect on intake for any fluid condition. The two conditions most sensitive to the suppressant effect of SCH 23390 were water and 0.45% saline, respectively.

There was a similar profile for the effect of SCH 23390 on saline acceptance after 30 min. There was a significant overall main effect, $F(3,126)=52.0$, $p<0.001$, and a significant drug × salt concentration interaction, $F(15,126)=2.02$, $p<0.05$. At 0.01 mg/kg, SCH 23390 had no effect on consumption, at 0.03 mg/kg it selectively reduced the intake of water, hypotonic and isotonic saline, and at 0.1 mg/kg it significantly reduced intake in all fluid conditions, except for the 2.7% NaCl solution.

DISCUSSION

The selective dopamine D-1 antagonist, SCH 23390, produced dose-dependent reductions in the ingestion of water and NaCl solutions. Blockade of D-1 receptors, therefore, was sufficient to produce a pronounced antidipsogenic effect. Nevertheless, there was some specificity in the effects of SCH 23390. Consumption of water and the weakest salt solution (0.45%) were most sensitive to its antidipsogenic effect. At the highest dose tested, however, the specificity of its effect was largely lost, and drinking was depressed generally across all but the most concentrated salt solution.

In contrast, the selective D-2 antagonist, sulpiride, lacked

antidipsogenic activity. At 30 mg/kg, it produced significant increases in fluid consumption, cf. [13]. In contrast to benzodiazepines and barbiturates, which elevate the entire acceptance-rejection function across a range of NaCl concentrations [9,26], sulpiride's hyperdipsic effect was more limited. Its effect was to raise selectively the ascending limb of the acceptance-rejection function, increasing the intake of water and hypotonic NaCl. These data suggest that dopamine may act at D-2 receptors to inhibit the consumption of water and weak salt solutions, but not of stronger salt solutions. It is interesting to note that, after IP administration, sulpiride accumulates preferentially in the hypothalamus, hippocampus, cerebellum and medulla oblongata [20]. An effect in one or more of these regions may underlie sulpiride's hyperdipsic effect.

The behavioral distinctions between SCH 23390 and sulpiride may be relevant to the distinction between D-1 and D-2 receptors. A number of neuroleptics, including chlorpromazine, haloperidol, spiroperidol and clozapine, displace [^{3}H] SCH 23390 binding [1]. Blockade of D-1 receptors may, therefore, account for at least some of the antidipsogenic effects of neuroleptics. SCH 23390 does not bind to sites labelled with [^{3}H] sulpiride [11], and therefore blockade of dopamine D-2 receptors may be necessary to induce a hyperdipsic response. Nevertheless, these distinctions have to be made tentatively, until additional compounds with D-1 and D-2 selective binding characteristics have been tested in the salt acceptance-rejection paradigm.

The opposite effects of sulpiride and SCH 23390 we report here emphasize the potential importance of the D-1/D-2 dopamine receptor distinction in accounts of the behavioural effects of neuroleptics. In further support of the view that dopamine receptor subtype is relevant to the consequences of dopamine receptor blockade, a recent study has shown that SCH 23390 suppressed responding for brain stimulation, while sulpiride (50 mg/kg) had no effect [21]. It is worth noting that SCH 23390, like haloperidol and other neuroleptics, has some cataleptogenic activity in rats [19]. This may contribute to behavioural decrements produced by the compound, but our data showed that SCH 23390 can exert a selective effect on fluid ingestion. At this stage, we cannot account for the selectivity of the drug's effect, although it is not readily explained in terms of either reduced reward [21], or motor impairment [19].

ACKNOWLEDGEMENTS

We thank the pharmaceutical companies for generously donating sulpiride and SCH 23390. Mr. D. J. Barber provided excellent technical assistance. D.B.G was supported by a grant from the University of Birmingham.

REFERENCES

1. Andersen, P. H., E. B. Nielsen, F. C. Gronvald and C. Braestrup. Some atypical neuroleptics inhibit [^{3}H] SCH 23390 binding *in vivo*. *Eur J Pharmacol* **120:** 143–144, 1986.
2. Billard, W., V. Ruperto, G. Crosby, L. C. Iorio and A. Barnett. Characterization of the binding of ^{3}H-SCH 23390, a selective D-1 receptor antagonist ligand, in rat striatum. *Life Sci* **35:** 1885–1893, 1984.
3. Block, M. L. and A. E. Fisher. Cholinergic and dopaminergic blocking agents modulate water intake elicited by deprivation, hypovolemia, hypertonicity and isoproterenol. *Pharmacol Biochem Behav* **3:** 251–262, 1975.
4. Christensen, A. V., J. Arnt, J. Hyttel, J.-J. Larsen and O. Svendsen. Pharmacological effects of a specific dopamine D-1 antagonist SCH 23390 in comparison with neuroleptics. *Life Sci* **34:** 1529–1540, 1984.
5. Cross, A. J., R. D. Marshall, J. A. Johnson and F. Owen. Preferential inhibition of ligand binding to calf striatal dopamine D1 receptors by SCH 23390. *Neuropharmacology* **22:** 1327–1329, 1983.
6. Dourish, C. T. Dopaminergic involvement in the control of drinking behaviour: a brief review. *Prog Neuropsychopharmacol Biol Psychiatry* **7:** 487–493, 1983.
7. Epstein, A. N. and E. Stellar. The control of salt preference in the adrenalectomised rat. *J Comp Physiol Psychol* **48:** 167–172, 1955.
8. Falk, J. L. Determining changes in vital functions: ingestion. In: *Methods in Psychobiology, Vol 1*, edited by R. D. Myers. New York: Academic Press, 1971, pp. 301–329.
9. Falk, J. L. and M. Tang. Chlordiazepoxide injection elevates in NaCl solution acceptance-rejection function. *Pharmacol Biochem Behav* **21:** 449–451, 1984.
10. Freedman, S. B., J. A. Poat and G. N. Woodruff. ^{3}H-Sulpiride, a ligand for neuroleptic receptors. *Neuropharmacology* **20:** 1323–1326, 1981.
11. Freedman, S. B. and G. N. Woodruff. Brain dopamine receptors. In: *Neurobiology of Dopamine Systems*, edited by W. Winlow and R. Markstein. Manchester: Manchester University press, 1986, pp. 25–39.
12. Gehlert, D. R. and J. K. Wamsley. Autoradiographic localization of [^{3}H] sulpiride binding sites in the rat brain. *Eur J Pharmacol* **98:** 311–312, 1984.
13. Gilbert, D. B. and S. J. Cooper. Naloxone antagonizes the hyperdipsic effect of sulpiride in a salt-preference test in male and female rats. *Neuropharmacology* **25:** 743–747, 1986.
14. Grupp, L. A. Time dependent action of pimozide on deprivation induced water intake: Evidence for a direct drug effect. *Pharmacol Biochem Behav* **4:** 725–728, 1976.
15. Hyttel, J. SCH 23390—the first selective dopamine D-1 antagonist. *Eur J Pharmacol* **91:** 153–154, 1983.
16. Iorio, L. C., A. Barnett, F. H. Leitz, V. P. Houser and C. A. Korduba. SCH 23390, a potential benzazepine antipsychotic with unique interactions on dopaminergic systems. *J Pharmacol Exp Ther* **226:** 462–468, 1983.
17. Jenner, P. and C. D. Marsden. The substituted benzamides, a novel class of dopamine antagonists. *Life Sci* **25:** 479–486, 1979.
18. Kebabian, J. W. and D. B. Calne. Multiple receptors for dopamine. *Nature* **277:** 93–96, 1979.
19. Morelli, M. and G. DiChiara. Catalepsy induced by SCH 23390 in rats. *Eur J Pharmacol* **117:** 179–185, 1985.
20. Mizuchi, A., N. Kitagawa and Y. Miyachi. Regional distribution of sultopride and sulpiride in rat brain measured by radioimmunoassay. *Psychopharmacology (Berlin)* **81:** 195–198, 1983.
21. Nakajima, S. and G. M. McKenzie. Reduction of the rewarding effect of brain stimulation by a blockade of dopamine D-1 receptor with SCH 23390. *Pharmacol Biochem Behav* **24:** 919–923, 1986.
22. Peres, V. L., C. G. Gentil, F. G. Graeff and M. R. Covian. Antagonism of the dipsogenic action of intraseptal angiotensin II in the rat. *Pharmacol Biochem Behav* **2:** 597–602, 1974.
23. Poat, J. A., C. Summers and G. N. Woodruff. The effects of centrally administered dopamine and 2-amino-6,7-dihydroxy-1,2,3,4-tetra-hydronapthalene (ADTN) on water and food intake in the rat. *Br J Pharmacol* **70:** 151P, 1980.

24. Rolls, E. T., B. J. Rolls, P. H. Kelly, S. G. Shaw, R. J. Wood and R. Dale. The relative attentuation of self-stimulation, eating and drinking produced by dopamine-receptor blockade. *Psychopharmacologia* **38:** 219–230, 1974.
25. Rowland, N. and D. J. Engle. Feeding and drinking interactions after acute butyrophenone administration. *Pharmacol Biochem Behav* **7:** 295–301, 1977.
26. Schmidt, H. Barbiturate effects on saline acceptance and post-ingestion variables. *Pysiol Behav* **1:** 183–189, 1966.
27. Setler, P. E. The role of catecholamines in thirst. In: *The Neuropsychology of Thirst*, edited by A. N. Epstein, H. R. Kissileff and E. Stellar. Washington, DC: Winston, 1973, pp. 279–291.
28. Stoof, J. C. and J. W. Kebabian. Two dopamine receptors: biochemistry, physiology and pharmacology. *Life Sci* **35:** 2281–2296, 1984.
29. Summers, C., G. N. Woodruff, J. A. Poat and K. A. Munday. The effect of neuroleptic drugs on drinking induced by central administration of angiotensin or carbachol. *Psychopharmacology (Berlin)* **560:** 291–294, 1979.
30. Weiner, I. H. and E. Stellar. Salt preference of the rat determined by a single-stimulus method. *J Comp Physiol Psychol* **44:** 394–401, 1951.
31. Winer, B. J. *Statistical Principles in Experimental Design*. 2nd edition. New York: McGraw-Hill, 1971.