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SCH 23390 MAY ALTER DOPAMINE-MEDIATED MOTOR BEHAVIOUR VIA STRIATAL D-1 RECEPTORS

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Abstract—SCH 23390 potently displaced the specific binding of ^{3}H-piflutixol to D-1 sites in striatal membranes but haloperidol was only weakly effective. SCH 23390 weakly displaced specific ^{3}H-spiperone binding to D-2 sites, but haloperidol was potent. SCH 23390 was more effective than haloperidol in inhibiting dopamine stimulated striatal adenylate cyclase activity. These results confirm the D-1 selectivity of SCH 23390. However, SCH 23390 inhibited apomorphine-induced stereotypy and climbing behaviour in rats with equal potency to haloperidol. Haloperidol dose-dependently increased striatal HVA and DOPAC concentrations without altering dopamine content. Low doses of SCH 23390 elevated striatal DOPAC concentrations but higher doses were without effect; striatal dopamine and HVA overall was unaffected by administration of SCH 23390. Haloperidol did not affect basal ^{3}H-acetylcholine release from striatal slices but reversed the apomorphine-induced inhibition of ^{3}H-acetylcholine release. SCH 23390 did not affect basal ^{3}H-acetylcholine release nor did it reverse the apomorphine-induced inhibition of ^{3}H-acetylcholine release.

The ability of SCH 23390 to inhibit motor behaviour in the rat may be due to its action on D-1 receptors since the drug does not cause typical changes in parameters of striatal D-2 receptor function.

Multiple types of dopamine receptors may exist in the striatum [1]. Receptor populations are divided into those linked to the enzyme adenylate cyclase (D-1 sites) and those which are not directly linked to this enzyme (D-2 sites) [2]. Various subpopulations of adenylate cyclase independent receptors have been proposed but there is dispute over their existence [3, 4]. The functional effects of dopamine receptor action in the brain until now have been attributed to drug action at D-2 receptor sites. Thus, good correlations exist between the ability of neuroleptic drugs to inhibit apomorphine-induced stereotyped behaviour and their ability to displace ^{3}H-spiperone from its specific binding site on D-2 receptors [5], and between the antipsychotic activity of neuroleptic drugs and their ability to displace ligands from D-2 sites [6]. No obvious function for D-1 sites in brain is known but, recently, evidence for an interaction between D-1 and D-2 sites has accumulated. Stoof and Kebabian [7] showed the ability of the D-2 selective antagonist sulpiride to potentiate the ability of dopamine or dopamine agonist drugs to stimulate striatal cyclic AMP formation, presumably via D-1 sites. Such data suggest that some D-2 receptor sites within the striatum may be inhibitory on D-1 function.

The major problem in determining a role for D-1 receptors has been the lack of selective drugs for this site. Most neuroleptic compounds show a preference for D-2 sites and even the thioxanthenes, which act potently on D-1 receptors, show equal affinity for D-2 sites [8]. However, recently a benzazepine compound, SCH 23390 (R-(+)-8-chloro-2,3,4,5-tetrahydro-3-methyl-5-phenyl-1 H-3-benzazepine-7-ol) was described which appears highly selective for D-1 receptor sites in striatum [9]. Thus, SCH 23390 potently displaced ^{3}H-piflutixol from its specific binding site to D-1 receptors and inhibited dopamine stimulation of adenylate cyclase activity, but only weakly displaced either ^{3}H-haloperidol or ^{3}H-spiperone from their specific binding sites on rat striatal preparations [10, 11]. SCH 23390 also acts on 5HT-2 and α-2 receptor sites, but at concentrations 20 and 500 times greater respectively than at D-1 sites, as judged by ligand binding assays. Despite the apparent selectivity for D-1 receptors, SCH 23390 blocked apomorphine-induced stereotyped behaviour in rats, an accepted D-2 behavioural response [9]. This might suggest that D-1 receptors are of functional significance in the rat. However, SCH 23390 is not without effect on D-2 sites, so it is possible that this behavioural antagonism was due to this action of the drug.

In the present experiments we set out to confirm the D-1 receptor selectivity of SCH 23390 and to examine the drug in three accepted models of D-2 receptor action namely, inhibition of apomorphine-induced motor behaviours, elevation of brain dopamine turnover, and alteration of ^{3}H-acetylcholine release. We find that SCH 23390 is selective for D-1 sites in binding and adenylate cyclase studies, but that the behavioural antagonism of apomorphine stereotypy and climbing it produces is not paralleled by changes in other D-2 parameters.

MATERIALS AND METHODS

Specific ^{3}H-spiperone and ^{3}H-piflutixol binding to striatal membranes. Ligand binding studies with ^{3}H-spiperone and ^{3}H-piflutixol were carried out using washed membrane preparations prepared from pooled striatal tissue from female Wistar rats (150 ± 10 g; Bantin & Kingman Ltd., Hull, U.K.).

Final membrane preparations were suspended in 500 vol. of incubation buffer (Tris–HCl 50 mM containing 120 mM NaCl, 2.5 mM $CaCl_2$, 1 mM $MgCl_2$, 5 mM KCl, 0.1% ascorbic acid, pH 7.4 at 4°).

Specific binding of ^{3}H-spiperone (21 Ci/mmole; Amersham International, Amersham, U.K.) was determined using a ligand concentration of 0.052 nM (added in 50 μl 0.1% ascorbic acid). Specific binding was defined by incorporation of 3×10^{-5} M sulpiride (Delagrange). Specific binding of ^{3}H-piflutixol (11.7 Ci/mmole; Lundbeck, Copenhagen, Denmark) was determined at a ligand concentration of 0.25 nM (added in 50 μl 0.1% ascorbic acid). Specific binding was defined by incorporation of 10^{-6} M *cis*-flupenthixol in the presence of 3×10^{-5} M sulpiride to prevent the binding of the ligand to D-2 receptors, and so define the component of binding to D-1 receptors. The effects of haloperidol (Janssen Pharmaceutica, Beerse, Belgium) or SCH 23390 (Schering, Bloomfield, N.J.) were determined by incorporation of the drug in a range of concentrations between 10^{-10} and 10^{-5} M.

Samples were incubated at 37° for up to 15 min (in the case of ^{3}H-spiperone) or 25 min (in the case of ^{3}H-piflutixol). Bound ligand was separated from that free in solution by vacuum filtration over Whatman GF/C filters.

Adenylate cyclase assay. Dopamine (100 μM)-stimulated adenylate cyclase activity was determined according to the method of Miller *et al.* [12] using the saturation assay of Brown *et al.* [13]. Using striatal tissue from male Wistar rats (250 ± 20 g; Bantin & Kingman Ltd.). Basal and dopamine (100 μM)-stimulated adenylate cyclase were determined in triplicate in striatal tissue homogenates from 3 animals on 3 separate occasions. SCH 23390 or halperidol were incorporated into incubates in concentrations between 10^{-9} and 10^{-4} M. Regression analysis of inhibition plots was used to obtain the IC_{50} value for each drug.

Apomorphine induced stereotypy and climbing behaviour. Female Wistar rats (180–220 g; Bantin & Kingman Ltd.) were pre-selected for their ability to exhibit climbing behaviour in response to the administration of apomorphine hydrochloride (0.5 mg/g s.c.) as described elsewhere [14].

Apomorphine-induced stereotypy and apomorphine-induced climbing behaviour were determined simultaneously. Animals were placed in wire mesh climbing cages and allowed to acclimatise for a 1-hr period. Animals then received either SCH 23390 (0.0375–1.25 mg/kg i.p.) 30 min prior to the administration of apomorphine hydrochloride (0.15 mg/kg s.c.) or haloperidol (0.00625–0.2 mg/kg i.p.) 1 hr prior to apomorphine administration. These times were chosen to agree with those used previously to demonstrate pharmacological effects of SCH 23390 compared to haloperidol [9]. Other animals received apomorphine hydrochloride (0.15 mg/kg s.c.) alone. The climbing and stereotyped response to the administration of apomorphine was assessed simultaneously in a period 10–20 min following apomorphine administration at the time of peak drug effect. Climbing was assessed on a scale of 0–2 as follows: 0 = all paws on floor; 1 = two paws on cage wall; 2 = all paws on cage wall. Stereotypy was scored on the following scale: 0 = normal behaviour; 1 = periodic sniffing and locomotion; 2 = continuous sniffing and discontinuous locomotion; 3 = periodic licking or biting; 4 = continuous licking or biting. Scores were given for each animal at 2 min intervals during the period 10–20 min following apomorphine administration. A final score for stereotypy and climbing behaviour for each animal was obtained from the mean of the scores attributed during the 10 min observation period.

Measurement of striatal dopamine HVA and DOPAC concentrations. Striatal dopamine, HVA and DOPAC concentrations were measured using a modification of the fluorimetric technique of Westerink and Korf [15]. Animals were killed by cervical dislocation and decapitation and the paired corpora striata removed, weighed and homogenised in 0.4 M perchloric acid. Samples were applied to Sephadex G-10 columns, and dopamine was measured according to the fluorimetric method of Laverty and Sharman [16] and HVA and DOPAC by the technique of Murphy and colleagues [17]. Determinations were made in six control animals and in animals receiving either SCH 23390 (0.05–0.4 mg/kg i.p. 30 min previously) or haloperidol (0.05–0.4 mg/kg i.p. 1 hr previously) again six animals being used at each dose level.

^{3}H-acetylcholine release from striatal slices. The technique utilised for ^{3}H-acetylcholine release from rat striatal slices was that of Kerwin and Pycock [18]. Striatal tissue was removed from female Wistar rats (151–175 g; Charles River, Margate, Kent, U.K.). Striatal slices (0.2 × 0.2 mm) were pre-labelled for 15 min in Krebs bicarbonate buffer (pH 7.4) containing 0.13 μM ^{3}H-choline (78 Ci/mmole; Amersham International). Tissue was then placed in a chamber and superfused at 0.8–1.0 ml/min with Krebs bicarbonate buffer. Following a 30-min washout period to remove free radioactivity, fractions of perfusate were collected every 2 min. Six min after the start of the collection potassium chloride (25 mM) was incorporated as a depolarising stimulus over a 4-min period, and fractions collected for a further 12 min. For the superfusion part of experiments hemicholinium (10 μM) was included. Apomorphine hydrochloride (100 μM) was dissolved in Krebs bicarbonate buffer and introduced into the perfusion chamber for a 6-min period commencing 4 min after the start of collection. SCH 23390 or haloperidol (5 μM) were added to the superfusion medium 15 min prior to the start of the collection.

Radioactivity in each fraction (250 μl aliquots) and in the tissue at the end of the experiment was measured by addition to ES299 scintillant (4 ml; Packard, Caversham, Bucks, U.K.) and subsequent liquid scintillation spectrometry using a Packard 460C liquid scintillation spectrometer.

Total tissue radioactivity during each collection period was determined by serially subtracting fractional radioactivity from total radioactivity. The amount of evoked release was calculated from values for individual collection periods and was taken as the total amount of radioactivity released during a 6-min period from the beginning of K^+ stimulation, after the total amount of radioactivity released during the 6-min period immediately prior to K^+ stimu-

Table 1. The effect of SCH 23390 and halperidol on specific ^{3}H-spiperone and ^{3}H-piflutixol binding to striatal membranes and dopamine (100 μM) stimulated adenylate cyclase activity in striatal homogenates

	K_i (nM)		IC_{50} (nM)
	^{3}H-spiperone	^{3}H-piflutixol	adenylate cyclase
Haloperidol	1.3 ± 0.2	570 ± 43	189 ± 44
SCH 23390	300 ± 60	1.3 ± 0.3	15 ± 4

The results are the mean (± 1 S.E.M.) of at least three individual determinations using separate tissue pools. Specific binding of ^{3}H-spiperone (0.052 nM) was defined by incorporation of 3×10^{-5} M sulpiride. Specific binding of ^{3}H-piflutixol (0.25 nM) was defined by the incorporation of 10^{-6} M *cis*-flupenthixol in the presence of 3×10^{-5} M sulpiride. Basal adenylate cyclase activity was 36.5 ± 6.7 pmole/2 mg tissue/2.5 min which was elevated to 77.7 ± 1.6 pmole/2 mg tissue/2.5 min by incorporation of 100 μM dopamine.

lation had been subtracted; this was expressed as the percentage of the total recovered radioactivity.

Statistical analysis. Biochemical data were analysed using a two-tailed Student's *t*-test. Climbing and stereotypy data were examined using the Mann-Whitney U test.

RESULTS

Displacement of specific ^{3}H-spiperone or ^{3}H-piflutixol binding

The incorporation of haloperidol (10^{-10}–10^{-5} M) into striatal membrane preparations potently displaced the specific binding of ^{3}H-spiperone (0.052 nM) but was only weakly effective in displacing ^{3}H-piflutixol (0.25 nM) binding to striatal membranes (Table 1). In contrast, incorporation of SCH 23390 was only weakly effective in displacing ^{3}H-spiperone binding but potently displaced the specific binding of ^{3}H-piflutixol to striatal membranes (Table 1). In all cases competitive displacement of ^{3}H-spiperone or ^{3}H-piflutixol occurred.

Dopamine stimulated cyclic AMP formation

Incorporation of haloperidol (10^{-9}–10^{-5} M) into striatal homogenates was only weakly effective in inhibiting dopamine (100 μM) stimulation of cyclic AMP formation but SCH 23390 potently inhibited cyclic AMP formation (Table 1).

Apomorphine-induced stereotypy and climbing behaviour

Apomorphine (0.15 mg/kg s.c. 10–20 min previously)-induced stereotypy and climbing behaviour were both potently inhibited by SCH 23390 (0.0313–1.2 mg/kg 10 min prior to apomorphine) and by haloperidol (0.0063–0.2 mg/kg i.p. 30 min prior to apomorphine) (Fig. 1). Haloperidol (ID_{50}: stereotypy 0.042 mg/kg; climbing 0.036 mg/kg) was approximately twice as potent as SCH 23390 (ID_{50}: stereotypy 0.10 mg/kg; climbing 0.10 mg/kg) in inhibiting these motor behaviours.

Striatal dopamine, HVA and DOPAC concentrations

Administration of haloperidol (0.05–0.4 mg/kg i.p. 1 hr previously) caused a dose-related increase in striatal HVA and DOPAC concentrations with no change in content of dopamine (Table 2). SCH 23390 (0.05–0.4 mg/kg 30 min previously) did not alter striatal dopamine content and there was no dose-related

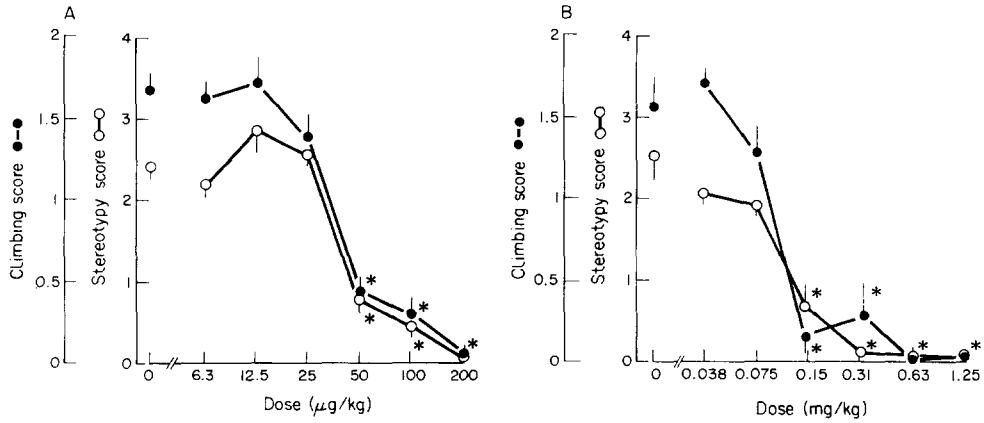

Fig. 1. The effect of prior treatment with (A) haloperidol (0.0063–0.2 mg/kg i.p. 1 hr previously) or (B) SCH 23390 (0.0313–1.2 mg/kg i.p. 30 min previously) on apomorphine (0.15 mg/kg s.c.)-induced climbing behaviour (—●—) or stereotyped behaviour (—○—) in rats. Values are the mean (± 1 S.E.M.) of overall scores obtained for six individual rats at each dosage level. * $P < 0.05$ compared to apomorphine alone

Table 2. The effect of administration of haloperidol (0.05–0.4 mg/kg i.p. 1 hr previously) of SCH 23390 (0.05–0.4 mg/kg i.p. 30 min previously) on striatal concentrations of dopamine, homovanillic acid (HVA) and 3,4-dihydroxyphenylacetic acid (DOPAC) compared to tissue from vehicle treated control animals

Drug treatment	Dose (mg/kg)	Striatal content Dopamine (μg/g)	HVA (ng/g)	DOPAC (ng/g)
Control		11.0 ± 1.4	892 ± 67	1163 ± 106
SCH 23390	0.05	9.6 ± 1.6	973 ± 109	1562 ± 109*
	0.1	8.2 ± 1.3	695 ± 61	1661 ± 205*
	0.2	11.1 ± 0.9	553 ± 50*	1400 ± 193
	0.4	9.1 ± 2.6	938 ± 72	1038 ± 84
Haloperidol	0.05	11.8 ± 1.1	1402 ± 191*	2006 ± 210*
	0.1	8.8 ± 0.7	1362 ± 251*	2046 ± 421*
	0.2	11.4 ± 1.2	1567 ± 328*	2568 ± 699*
	0.4	11.0 ± 0.6	2165 ± 419*	3246 ± 643*

Values are the mean (± 1 S.E.M.) of results obtained for six animals at each dosage level.
* $P < 0.05$ compared to values for control animals.

change in striatal HVA or DOPAC concentrations; DOPAC concentrations were elevated at low doses of SCH 23390 but values returned to control with increasing dose.

^{3}H-*acetylcholine release*

Potassium chloride (25 mM) enhanced the evoked release of ^{3}H-acetylcholine and this effect was partially inhibited by the incorporation of apomorphine hydrochloride (100 μM) (Table 3). Haloperidol (5 μM) reversed the apomorphine-induced inhibition of ^{3}H-acetylcholine release but SCH 23390 (5 or 15 μM) was without effect. Neither drug had an effect on basal ^{3}H-acetylcholine release (% radioactivity released in fraction collected 17–19 mm following addition of antagonist: no addition 1.7 ± 0.1, $N = 26$; 5 μM haloperidol 1.9 ± 0.2, N = 8, $P > 0.05$; 5 μM SCH 23390 1.8 ± 0.2, N = 8, $P > 0.05$; 15 μM SCH 23390 1.7 ± 0.3, N = 8, $P > 0.05$).

DISCUSSION

The behavioural effects of neuroleptic drugs have been attributed to their actions on the D-2 dopamine receptor population in brain; the function of cerebral D-1 receptors remains unknown. The importance of

Table 3. The effect of SCH 23390 (5 and 15 μM) and haloperidol (5 μM) on apomorphine (100 μM) induced inhibition of potassium chloride (25 mM) evoked ^{3}H-acetylcholine release from striatal slices

	(%) evoked release in 6 min
Haloperidol	
25 mM KCl	11.7 ± 1.2 (6)
25 mM KCl + 100 μM apomorphine	5.4 ± 0.8* (6)
25 mM KCl + 100 μM apomorphine + 5 μM haloperidol	8.1 ± 0.7*† (8)
SCH 23390	
25 mM KCl	11.7 ± 2.1 (6)
25 mM KCl + 100 μM apomorphine	6.0 ± 0.8* (8)
25 mM KCl + 100 μM apomorphine + 5 μM SCH 23390	4.8 ± 0.6* (8)
25 mM KCl + 100 μM apomorphine + 15 μM SCH 23390	5.5 ± 0.9* (8)

Values represent the amount of radioactivity released subtracted from baseline levels, during a 6-min period following potassium stimulation. Apomorphine (100 μM) was included in the perfusing solution for a period of 2 min before and then during potassium addition. SCH 23390 (5 or 15 μM) or haloperidol (5 μM) were included from 15 min prior to collection of perfusate.
Values are the mean (± 1 S.E.M.) of 6–8 separate determinations.
* $P < 0.05$ compared to KCl alone.
† $P < 0.05$ compared to KCl and apomorphine.

D-2 receptors, however, has been based upon the actions of those neuroleptic drugs currently available, their common mechanism of action being on D-2 sites rather than D-1. Thus, substituted benzamide drugs and butyrophenone neuroleptics are selective for D-2 receptors; phenothiazine drugs act predominantly on D-2 receptors, but also affect D-1 sites, and thioxanthenes have equal affinity for both D-1 and D-2 receptors [8, 19]. Until now, a selective D-1 receptor antagonist has not been available.

SCH 23390, however, appears to be the first selective D-1 receptor antagonist. The selective effect on D-1 sites is demonstrated by its potent ability to displace specific ^{3}H-piflutixol binding (defined using *cis*-flupenthixol in the presence of sulpiride) and to inhibit dopamine-stimulated adenylate cyclase activity compared to its relatively weak inhibition of ^{3}H-spiperone binding. This contrasts with the actions of haloperidol which is potently active in displacing ^{3}H-spiperone binding from D-2 sites but only weakly active in displacing ^{3}H-piflutixol from D-1 receptors. In these experiments SCH 23390 was approximately 240 times more active on D-1 sites than D-2 sites. This contrasts with the greater selectivity previously reported [10, 11], which may relate to differences in the ligand concentration and tissue dilution employed and the method of defining specific binding.

Despite its selectivity for D-1 receptors, SCH 23390 potently inhibited both apomorphine-induced climbing and stereotyped behaviour [9]. Indeed, its potency in blocking these behaviours was equivalent to that of haloperidol. However, SCH 23390 is not specific for D-1 receptors but merely exhibits selectivity. The possibility exists, that the effects of this compound are due to its lesser but not insignificant D-2 actions.

A number of dopamine related parameters are presently associated with an effect of drugs on D-2 receptors. All classical neuroleptic drugs cause a compensatory increase in dopamine turnover as a result of blocking post-synaptic dopamine receptors, presumably the D-2 population. Haloperidol increased the striatal concentrations of HVA and DOPAC in a dose-related manner but, in contrast, SCH 23390 increased striatal DOPAC concentrations only at low doses of the compound. This effect might appear to parallel the actions of haloperidol but if this represented a D-2 action of SCH 23390 it might be expected to be apparent at higher doses where selectivity for D-1 and D-2 would be less evident.

Dopamine is inhibitory on acetylcholine release in the striatum and this effect is thought to be mediated via D-2 receptor sites [20]. As expected, we found that haloperidol in the concentration used could partially reverse the apomorphine-induced inhibition of ^{3}H-acetylcholine release from striatal slices, but SCH 23390 was without effect.

So, although SCH 23390 does block apomorphine-induced motor behaviours in rats, these effects are not associated with classical D-2 receptor actions when assessed using increased dopamine turnover and alterations of ^{3}H-acetylcholine release. This suggests that the behavioural effects of SCH 23390 are due to its action on D-1 receptors on which the drug is selectively effective. This conclusion is enhanced by the finding that SCH 23390 does not inhibit apomorphine-induced vomiting in dogs nor does it elevate circulating prolactin levels in rodents [9], both of which are thought to be mediated via classical D-2 receptor effects.

In conclusion, SCH 23390 has selective effects on D-1 receptors in rat striatum and this action, rather than an effect on D-2 sites, would appear responsible for the ability of this drug to inhibit motor behaviours in rodents. If this finding can be confirmed then the ability of neuroleptic drugs to alter motor behaviours can no longer be attributed entirely to their effects on D-2 sites. Clearly, the concept of the functional differentiation between D-1 and D-2 sites will have to be revised in the light of the findings with this drug. Recently, Stoof and Kebabian [7] have shown that a complex interaction exists in striatum between D-2 and D-1 receptors. So it may be that the relationship between D-1 and D-2 sites is more intimate than was initially conceived. It is even conceivable that some motor events previously attributed to D-2 receptor action might be mediated by a DA→D-2→D-1→action chain of events. SCH 23390 may provide one of the first clues as to the role of D-1 receptor sites in striatum and may have relevance to out understanding of how alterations in dopamine receptor function can be involved in the aetiology of illness such as schizophrenia and Parkinson's disease.

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