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PHARMACOLOGICAL EVIDENCE FOR THE SUBCLASSIFICATION OF CENTRAL DOPAMINE RECEPTORS IN THE RAT

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1 The relative potencies of dopamine receptor agonists in causing stereotypy in rats when injected into the olfactory tubercles, and contralateral rotation when injected unilaterally into the caudate nucleus of rats with lesions of the nigro-striatal dopamine pathway, were determined. The actions of some agonists in eliciting these responses following peripheral injection, and the relative potencies of dopamine receptor antagonists in inhibiting them were also determined.

2 Dopamine, apomorphine and 2-amino-5,6 and 2-amino-6,7-dihydroxy-1,2,3,4-tetrahydronaphthalene (A-5, 6 DTN, A-6, 7 DTN) and N,N dipropyl A-5, 6DTN induced both responses. In contrast, 2,3,4,5-tetrahydro-7,8-dihydroxy-1-phenyl-1H-3-benzazepine HCl (SK & F 38393) whether injected intracerebrally or peripherally, induced contralateral rotation but not stereotypy.

3 Contralateral rotation and stereotypy induced by apomorphine or N,N dipropyl A-5,6 DTN were inhibited by haloperidol, pimozide and fluphenazine but these drugs failed to inhibit rotation induced by SK & F 38393. Clozapine inhibited rotation induced by SK & F 38393, apomorphine or N,N dipropyl A-5,6 DTN but failed to inhibit stereotypy. Loxapine was more potent in inhibiting stereotypy than rotation, whereas clothiapine inhibited rotation and stereotypy at similar doses irrespective of the agonist used to elicit the response.

4 Contralateral rotation induced by SK & F 38393 was not inhibited by yohimbine, prazosin, atropine, methysergide, mepyramine or propranolol.

5 The results provide evidence that contralateral rotation induced by dopamine receptor agonists is mediated by two different classes of dopamine receptors and that these receptors differ from those mediating the stereotypy response.

6 The receptors mediating these responses appear classifiable in terms of their sensitivity to the agonist actions of SK & F 38393 or apomorphine respectively. SK & F 38393-sensitive receptors are susceptible to blockade by clozapine but are not blocked by haloperidol, pimozide or fluphenazine. Apomorphine-sensitive receptors are susceptible to blockade by haloperidol, pimozide and fluphenazine but appear divisible into two sub-classes depending on whether or not they are blocked by clozapine and on their sensitivity to blockade by loxapine.

Introduction

A number of dopamine receptor agonists and antagonists have selective actions which suggest that they may differentiate between central dopamine receptors. For example, the neuroleptic drug, clozapine, differs from other dopamine receptor antagonists in that it fails to inhibit apomorphine-induced stereotypy or to block autoreceptors on dopaminergic nigro-striatal nerve terminals (Stille, Lauener & Eichenberger, 1971; Roth, 1980). The new dopamine receptor agonist, SK & F 38393, has been shown to cause contralateral rotation in rats prepared with unilateral nigro-striatal lesions but, unlike other dopamine receptor agonists it does not induce stereotypy in this species or vomiting in dogs (Setler, Sarau, Zirkle & Saunders, 1978). In the present study we have examined the possibility that these and other dopamine receptor agonists and antagonists could provide useful information for a functionally related subclassification of central dopamine receptors. The agonists were injected directly into dopamine-rich areas of the brain which have been associated with defined dopaminergically mediated responses. Relative potencies were determined in causing stereotypy in rats when injected bilaterally into the olfactory tubercles (see Costall, Naylor & Neumeyer, 1975; Krieger, 1980) and contralateral rotation when injected unilaterally into the caudate nucleus of rats with unilateral lesions of the nigro-striatal dopamine pathway (see Ungerstedt, 1971). The actions of some dopamine agonists known to penetrate the blood brain barrier were determined following peripheral injection. The relative potencies of dopamine receptor antagonists in inhibiting the agonist-induced responses were also determined.

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Methods

Animals

Male hooded rats (AH/H strain) were used for rotation experiments. Male albino rats (AH/A strain) gave better stereotypy responses than hooded rats and were used for all stereotypy experiments. Rats used in rotation experiments weighed 140–160 g at the time of lesion placement; all rats weighed 230–290 g at the time of experimental use. The rats were maintained on 41B cube diet and water *ad libitum*. Housing and laboratory temperatures were maintained at 20–21°C.

Intracerebral injections

Intracerebral injections in conscious rats were administered via stereotaxically implanted stainless steel guide cannulae similar to those described by Costall & Naylor (1974). The cannulae were kept patent by stainless steel stylets which extended 0.5 mm below the tips. For injections the stylets were replaced with stainless steel injection cannulae which extended either 1.5 mm (caudate nucleus) or 2.5 mm (olfactory tubercles) below the guide cannulae tips. Drugs were injected over a 30 s period in a dose volume of 1–2 μl. Nialamide, (100 mg/kg i.p.) was routinely injected 2 h before intracerebral injections of agonists but was omitted when agonists were injected peripherally. Injection sites were checked by injecting 1 μl Evans blue dye intracerebrally and examining frozen brain sections microscopically. All cannulae placements were located in the areas specified in the text.

Rotation experiments

Nigro-striatal lesions in rats used for rotation experiments were induced under pentobarbitone anaesthesia by infusing 6-hydroxydopamine into the substantia nigra on one side as described by Ungerstedt & Arbuthnott (1970). Only rats that subsequently rotated contralaterally following apomorphine (0.5 mg/kg i.p.) or ipsilaterally following (+)-amphetamine (2.5 mg/kg i.p.) were used. The rotation response was measured in rotometers similar to those described by Ungerstedt & Arbuthnott (1970).

Agonists were injected intraperitoneally or were injected directly into the caudate nucleus via a single guide cannula implanted on the same side as the nigro-striatal lesion. Intra-caudate injection site coordinates were: frontal 7.5 mm; hor. 1.0 mm; sag 2.5 mm (König & Klippel, 1963). Each agonist was investigated in a separate group of 6–12 rats. The various doses of agonists or a control injection of saline (0.9% w/v NaCl solution) were administered to each rat in the group during several weekly sessions, no rat being injected more frequently than once in 7 days. Measurement of rotation started immediately after injection and was continued for up to 7 h. Log dose-response curves were plotted at the time of peak rotation for each agonist.

In antagonist studies each antagonist was investigated in a separate group of 6–12 rats. Over the course of several weekly experimental sessions each dose of antagonist and a control injection of vehicle were administered intraperitoneally to each rat in the group. Each dose of antagonist or vehicle was followed by a fixed, intraperitoneal dose of agonist. Measurement of rotation started immediately after agonist injection and was continued for 1 h. Antagonist dose-test intervals, timed from the injection of the antagonist to the injection of the agonist are given in the text. Percentage inhibition of the agonist response by each dose of the antagonist was calculated with reference to the control response following treatment with vehicle plus agonist.

Stereotypy experiments

Experimentally naive rats were used. For stereotypy assessment each rat was placed in a 2 litre glass beaker containing a 1 cm layer of sawdust. Stereotyped movements were scored as follows, using a 'blind' procedure in which the observer did not know the nature of the drug treatments:

Repetitive licking movements Repetitive sniffing of sawdust Repetitive side to side head movements	Discontinuous: Continuous:	Score = 1 Score = 2
Repetitive chewing movements Active chewing of sawdust	Discontinuous: Continuous:	Score = 3 Score = 4

Agonists were injected intravenously or were injected bilaterally into the olfactory tubercles via bilateral guide cannulae implanted 7 days previously. Coordinates for the olfactory tubercle injections were: A 8.6, H −2.4, L ± 2.5 (De Groot, 1959). Dose-groups of 6 rats were used. Control rats received an equivalent volume of saline. Stereotypy was assessed in each rat at 5 min intervals starting immediately after injection and continuing until the response terminated. The 5 min scores were summed for each rat over each 30 min of the observation period and means for consecutive 30 min periods calculated in each group. Log dose-response curves at peak effect were plotted by using the highest 30 min mean score obtained for each dose of agonist.

In each antagonist study, each dose of antagonist was administered intraperitoneally to a dose-group

of 6 rats and a control group of 6 rats injected intraperitoneally with an equivalent volume of antagonist vehicle. All rats then received a fixed intravenous dose of agonist. Antagonist dose-test intervals, timed from the injection of the antagonist to the injection of the agonist are given in the text. The stereotypy response was assessed in each rat at 5 min intervals for 1 h starting immediately after agonist injection. The scores over this period were summed for each rat and mean values obtained for each group. The percentage inhibition of the agonist response by each dose of antagonist was calculated with reference to the response in the control group.

Statistics

Comparisons between responses following drug and control treatments in rotation experiments were made using the non-parametric Wilcoxon matched-pairs signed-ranks test for related data (Siegel, 1956). Comparisons between responses in drug and control groups in stereotypy experiments were made using the non-parametric Mann-Whitney U test (Siegel, 1956). In all agonist and some antagonist experiments, for ease of comparison, means ± s.e. are presented as the index of response. ED_{50} values for antagonists were the doses producing a 50% reduction in the response to the agonist when compared with vehicle plus agonist controls.

Drugs and solutions

The drugs used were apomorphine HCl (Macfarlan Smith); 2,3,4,5-tetrahydro-7,8-dihydroxy-l-phenyl-1H-3-benzazepine HCl (SK & F 38393, Smith, Kline & French); dopamine HCl (Koch-Light); 2-amino-5,6 and 2-amino-6,7-dihydroxy-1,2,3,4-tetrahydronaphthalene (A-5,6 DTN and A-6,7 DTN), N,N di-*n*-propyl A-5,6 DTN (synthesized at Glaxo Group Research); haloperidol base, pimozide base (Janssen); fluphenazine HCl (Allen & Hanbury); clozapine base, clothiapine base, and methy-sergide bimaleate (Sandoz); loxapine succinate (Lederle); yohimbine HCl (Sigma); prazosin HCl (Pfizer); atropine SO_4 monohydrate (Koch-Light) and 6-hydroxydopamine HBr (Aldrich Chemicals).

For intracerebral injection apomorphine, SK & F 38393, and doses of the other agonists above 10 μg were dissolved in nitrogen-bubbled distilled water; lower doses of dopamine and the aminotetralins were dissolved in nitrogen-bubbled sterile saline solution. Solutions were freshly prepared.

For parenteral injection apomorphine, SK & F 38393, N,N dipropyl A-5,6 DTN, atropine, fluphenazine and loxapine were dissolved in saline; haloperidol, pimozide, clozapine clothiapine, prazosin and methysergide were suspended in 5.0% gum acacia solution in distilled water; yohimbine was dissolved in distilled water. Nialamide was dissolved in a minimal volume of 2 N HCl, neutralized to pH 4–5 with saturated $NaHCO_3$ solution and made up to volume with distilled water. The dose volume for parenteral injections was 2 ml per kg body weight. All doses of drugs mentioned in the text refer to the free acid or base.

Results

Agonist studies

The effects of intracerebrally injected dopamine receptor agonists in causing contralateral rotation and stereotypy are shown in Figure 1. Like dopamine, the 2-aminotetralins, A-5,6 DTN and A-6,7 DTN were effective in both tests and showed no selectivity of action in that their potencies relative to dopamine were of a similar order in both tests, N,N dipropyl A-5,6 DTN was also effective in both tests but its potency, relative to dopamine, was considerably greater in causing sterotypy than contralateral rotation. In this respect this agonist showed some selectivity in its actions. Clear selectivity of action was apparent with SK & F 38393; this agonist caused rotation but failed to cause stereotypy. Following intracerebral injection apomorphine had only a week action in both tests.

Dopamine, A-5,6 DTN and A-6,7 DTN do not penetrate the blood brain barrier and are inactive in these tests following peripheral injection. The effects of N,N dipropyl A-5,6 DTN, SK & F 38393 and apomorphine in causing contralateral rotation and stereotypy following peripheral injection were determined and are shown in Figure 1. Apomorphine was active in both tests but was clearly much more effective in eliciting contralateral rotation and stereotypy when injected peripherally than centrally. N,N dipropyl A-5,6 DTN was also effective in both tests following peripheral injection but its potency, relative to apomorphine, was considerably greater in causing stereotypy than contralateral rotation. This agonist therefore showed similar selectivity in its actions whether injected centrally or peripherally. SK & F 38393 caused contralateral rotation but not stereotypy. These results with SK & F 38393 were the same as those obtained following direct injection into the brain.

Antagonist studies

In these studies the possibility that selected neuroleptic drugs known to be dopamine antagonists might differentially inhibit responses elicited by SK & F

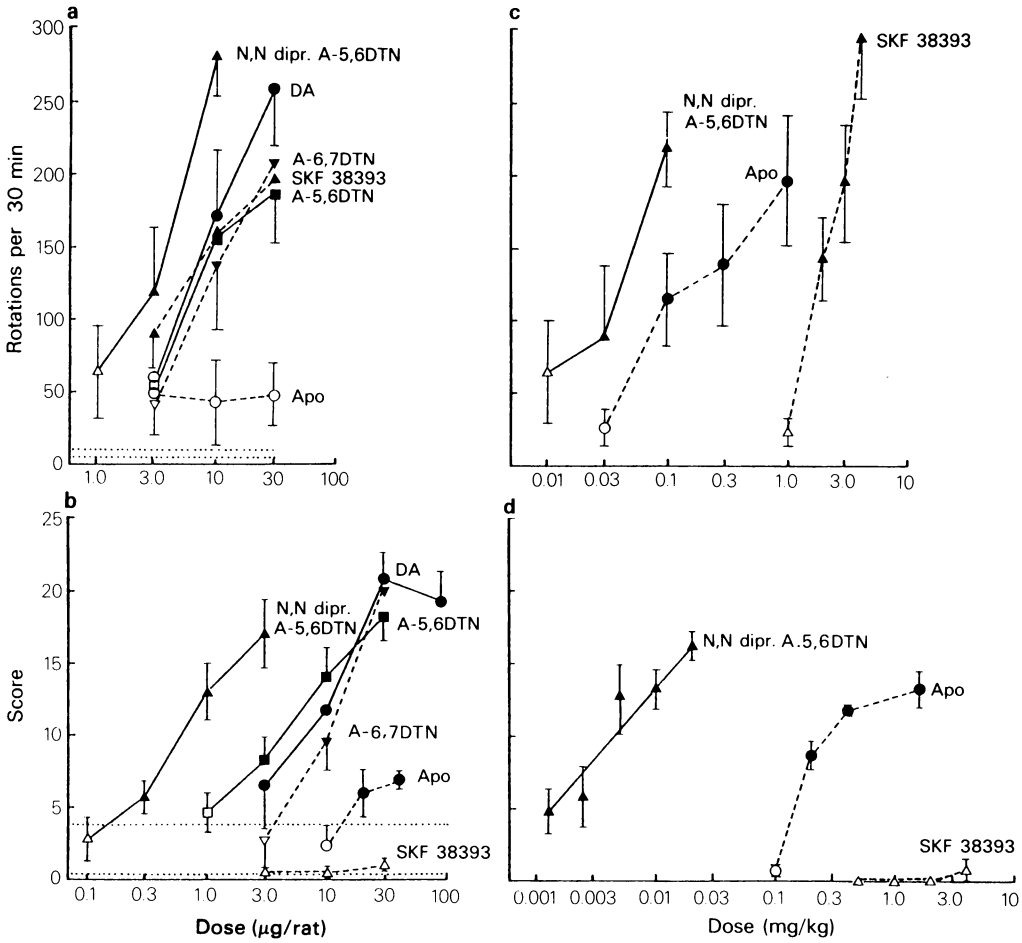

Figure 1 Effects of dopamine receptor agonists in causing (a) contralateral rotation following injection into the caudate nucleus on the same side as a unilateral lesion of the nigro-striatal pathway; (b) stereotypy following bilateral injection into the olfactory tubercles; (c) contralateral rotation following intraperitoneal injection in rats with nigro-striatal lesions; (d) stereotypy following intravenous injection. Values plotted are mean responses at the time of peak effect with vertical bars indicating s.e.means. In (a) $n = 8-12$, (b) $n = 6$, (c) $n = 8$, (d) $n = 6$. Filled symbols indicate significant differences from control responses in rats injected with normal saline solution ($P < 0.05$, 2-tailed). Dotted horizontal lines indicate ranges of control means. Control means in (c) and (d) were zero. Apo = apomorphine DA = dopamine.

38393 compared with responses elicited by apomorphine or N,N dipropyl A-5,6 DTN was examined. The results obtained are shown in Tables 1 and 2. The antagonist profiles of haloperidol, pimozide and fluphenazine resembled each other in that these antagonists inhibited contralateral rotation and stereotypy induced by apomorphine or N,N dipropyl A-5,6 DTN. Over the same dose ranges however, these drugs failed to inhibit contralateral rotation induced by SK & F 38393.

The antagonist profile obtained with clozapine was quite different. This drug inhibited contralateral rotation induced by SK & F 38393, apomorphine or N,N dipropyl A-5,6 DTN but did not inhibit stereotypy induced by apomorphine or N,N dipropyl A-5,6 DTN. The selective inhibition of the rotation response by clozapine was not due to motor incapacitation or sedation. When rats with nigro-striatal lesions were carefully observed following drug treatment, using a procedure similar to that described by Irwin (1968), clozapine caused no visible sedation in doses of 5–20 mg/kg (i.p.) and only very marginal sedation at 40 mg/kg (i.p.) Pimozide at doses of 4–8 mg/kg (i.p.) caused considerably more overt sedation than clozapine (mainly reduced alertness, reduced spontaneous activity and muscular hypotonia)

Table 1 Effects of dopamine receptor antagonists on contralateral rotation induced by SK & F 38393, (3 mg/kg i.p.), apomorphine (0.5 mg/kg i.p.) or N,N-dipropyl A-5,6 DTN (0.1 mg/kg i.p.)

Antagonist (dose-test interval)	*Dose* (mg/kg i.p.)	*vs SK & F 38393* % *Inhib.*	*vs SK & F 38393* ED_{50} (mg/kg)	*vs Apomorphine* % *Inhib.*	*vs Apomorphine* ED_{50} (mg/kg)	*vs N,N dipr. A-5,6 DTN* % *Inhib.*	*vs N,N dipr. A-5,6 DTN* ED_{50} (mg/kg)
Haloperidol	0.05	—		22.4		24.8	
(1 h)	0.1	0.0		55.5*		51.8*	
	0.2	6.9	Inactive	51.4*	0.09	47.3*	0.2
	0.4	10.4		61.0*		62.2*	
	0.8	0.0		51.0*		78.6*	
Pimozide	0.5	—		0.0		29.0	
(4 h)	1.0	—		0.0		39.0*	
	2.0	0.5	Inactive	28.2*	3.6	65.0*	1.8
	4.0	0.0		53.9*		64.8*	
	8.0	0.5		39.0*		77.0*	
Fluphenazine	0.0125	—		22.4		24.6	
(4 h)	0.025	13.5		31.7*		48.8*	
	0.05	0.0		74.9*		59.6*	
	0.1	0.0	Inactive	80.4*	0.04	83.6*	0.03
	0.2	5.8		—		—	
	0.4	20.7		—		—	
	0.8	15.4		—		—	
Clozapine	1.25	—		—		17.0	
(1 h)	2.5	38.1		—		45.0*	
	5.0	45.6	6.2	35.5	12.0	48.2*	5.3
	10.0	57.0*		47.9*		63.0*	
	20.0	67.2*		51.1*		69.9*	
	40.0	78.6*		81.2*		—	
Clothiapine	0.5	23.8		—		10.4	
(1 h)	1.0	42.1*	1.4	37.4	1.65	53.9*	1.0
	2.0	56.3*		49.0*		77.3*	
	4.0	—		76.9*		—	
Loxapine	0.25	—		39.9*		14.2	
(1 h)	0.5	39.9*		54.6*		33.7*	
	1.0	54.6*	0.8	63.3*	0.5	66.9*	0.7
	2.0	75.2*		77.3*		—	

Antagonist dose-test intervals were timed from the injection of the antagonist to the injection of the agonist. Contralateral rotation was recorded for 1 h starting immediately after SK & F 38393, apomorphine or N,N dipropyl A-5,6 DTN injection. Percentage inhibition was calculated with reference to control responses in rats injected with antagonist vehicle plus agonist. Dose-groups of 6–8 rats were used. *Significantly different from vehicle-agonist response ($P < 0.05$, 1-tailed).

and yet at these doses failed to inhibit contralateral rotation induced by SK & F 38393.

The antagonist profiles of clothiapine and loxapine, two neuroleptic drugs structurally related to clozapine, differed from that obtained with clozapine. Clothiapine was non-selective in its blocking actions and inhibited contralateral rotation and stereotypy at similar doses, irrespective of the agonists used to elicit the responses. Clothiapine caused little or no overt sedation at 2 mg/kg (i.p.) but at 4 mg/kg (i.p.) caused similar sedation to that induced by pimozide, 4 mg/kg (i.p.). Like clozapine, loxapine inhibited contralateral rotation induced by all three agonists but, unlike clozapine, was clearly more potent in inhibiting stereotypy than rotation.

In order to investigate whether rotation induced by SK & F 38393 was mediated by receptors other than dopamine receptors, the effects of yohimbine, prazosin, atropine, methysergide, mepyramine and propranolol on SK & F 38393-induced rotation were determined. The results are shown in Figure 2. The effects of clozapine, which is known to possess antihistamine, anti-5-hydroxytryptamine, cholinoceptor blocking and α-adrenoceptor blocking properties (Stille *et al.*, 1971), are included for comparative purposes.

Table 2 Effects of dopamine receptor antagonists on stereotypy induced by apomorphine (1 mg/kg i.v.), or N,N dipropyl A-5,6 DTN (0.02 mg/kg i.v.)

Antagonist (dose-test interval)	*Dose* (mg/kg o.p.)	*vs Apomorphine* % *Inhib.*	*vs Apomorphine* ED_{50} (mg/kg)	*vs N,N-dipr. A-5,6 DTN* % *Inhib.*	*vs N,N-dipr. A-5,6 DTN* ED_{50} (mg/kg)
Haloperidol (1 h)	0.05	18.0*	0.13	0.0	0.16
	0.1	34.2*		21.4	
	0.2	63.2*		45.4*	
	0.4	100.0*		100.0*	
Pimozide (4 h)	0.25	22.9*	1.10	34.4*	0.41
	0.5	31.3*		53.2*	
	1.0	41.7*		83.7*	
	2.0	64.6*		98.0*	
Fluphenazine (4 h)	0.025	22.0	0.05	0.0	0.03
	0.05	43.9*		40.0*	
	0.1	82.9*		78.0*	
	0.2	100.0*		91.0*	
Clozapine (1 h)	10.0	—	Inactive	0.0	Inactive
	20.0	22.6		0.0	
	40.0	0.0		0.0	
	80.0	5.6		6.0	
Clothiapine (1 h)	0.25	—	0.81	36.6*	0.52
	0.5	22.6		38.1*	
	1.0	62.0*		72.7	
	2.0	100.0*		90.2*	
Loxapine (1 h)	0.015	—	0.07	19.9	0.03
	0.03	6.0		52.9*	
	0.063	57.0*		83.0*	
	0.125	74.0*		94.8*	
	0.25	88.0*			

Antagonist dose-test intervals were timed from the injection of the antagonist to the injection of the agonist. Stereotypy was measured for 1 h starting immediately after apomorphine or N,N dipropyl A-5, 6 DTN injection. Percentage inhibition was calculated with reference to control responses in rats injected with antagonist vehicle plus agonist. Dose-groups of 6 rats were used. *Significantly different from vehicle-agonist control response ($P<0.05$, 1-tailed).

Yohimbine and prazosin caused a transient inhibition of the response, the inhibition was confined to the first 30 min after the injection of SK & F 38393. No inhibition occurred after this period. This transient inhibition was not related to the time course of action of these drugs because, in further experiments in which the same doses of yohimbine or prazosin were injected 10 min instead of 1 h before SK & F 38393, identical results were obtained. In all these experiments, if recording of the rotation response was continued up to 4 h after SK & F 38393 administration, it could be seen that yohimbine and prazosin caused a slight shift to the right in the time course of the SK & F 38393 response (Gower & Marriott, unpublished observations). These drugs therefore appeared to delay rather than to inhibit the response. None of the other drugs tested inhibited the response to SK & F 38393.

Discussion

In the present study we have confirmed previous findings (Setler *et al.*, 1978) that the dopamine receptor agonist SK & F 38393, when injected peripherally, induces contralateral rotation in rats with unilateral lesions of the nigro-striatal dopamine pathway but does not cause stereotypy. We have further shown that this agonist, like other dopamine receptor agonists, causes contralateral rotation in rats when injected directly into the caudate nucleus on the same side as a unilateral nigro-striatal lesion but, unlike other dopamine receptor agonists, does not cause stereotypy in rats when injected bilaterally into the olfactory tubercle. This selective action of SK & F 38393 strongly suggests that the receptors mediating the rotation response to this agonist are not the same as those that mediate stereotypy. Some selectivity,

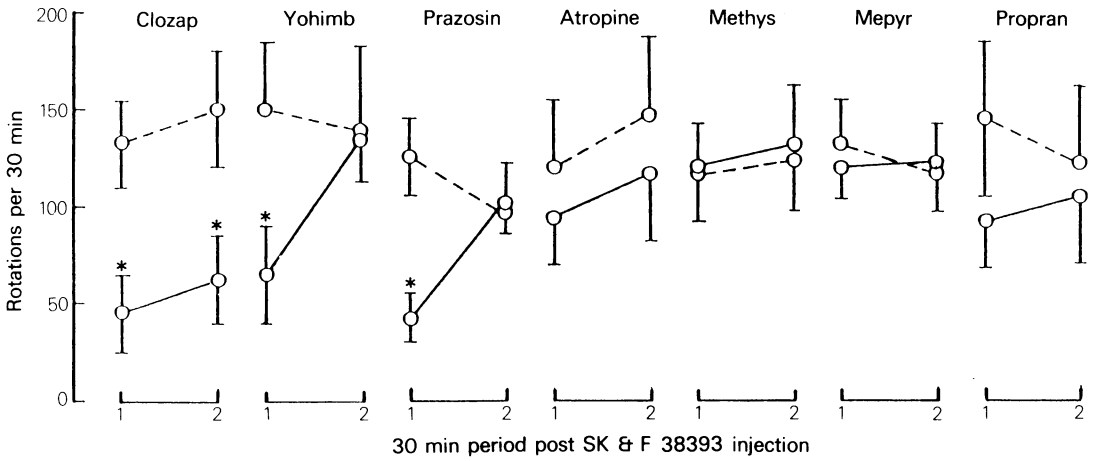

Figure 2 Effects of clozapine (Clozap) (5 mg/kg), yohimbine (Yohimb) (2 mg/kg), prazosin (2 mg/kg), atropine (20 mg/kg), methysergide (Methys) (10 mg/kg), mepyramine (Mepyr) (10 mg/kg) and propranolol (Propran) (20 mg/kg) on contralateral rotation induced by SK & F 38393 in rats with nirgro-striatal lesions. Drug or vehicle were injected intraperitoneally 1 h before the injection of SK & F 38393, 3 mg/kg i.p. Mean rotations recorded during each of two consecutive 30 min periods starting immediately after SK & F 38393 injection are shown. Dashed line = vehicle plus SK & F 38393; complete line = drug plus SK & F 38393. Groups of 6–8 rats were used. Vertical bars indicate s.e.mean. *Significantly different from vehicle plus SK & F 38393 control response ($P<0.05$, 2-tailed).

although in the opposite direction, was also apparent in the actions of N,N dipropyl A-5,6 DTN. Whether injected intracerebrally or peripherally, the relative potency of this agonist tended to be greater in causing stereotypy than contralateral rotation, a finding which again suggested a difference in the receptors mediating these responses.

Following intracerebral injection, dopamine and the 2-aminotetralins A-5,6 DTN and A-6,7 DTN were of similar potency to each other in both tests. These agonists, unlike SK & F 38393 or N,N dipropyl A-5,6 DTN did not therefore appear to discriminate between the receptors mediating the two responses.

Apomorphine had a much weaker action in eliciting rotation or stereotypy following intracerebral injection than it had following peripheral injection. It is unlikely that the greater activity of apomorphine following peripheral injection was due to the generation of an active metabolite, since there is good evidence that this does not occur in the rat (Baldessarini, Arana, Kula, Campbell & Harding, 1981). The weaker action of apomorphine intracerebrally suggests that the drug may have a more restricted access to appropriate receptors when injected by this route. As in other reports, (Cannon, Lee, Goldman, Costall & Naylor, 1977; Makanjuola, Dow & Ashcroft, 1980) we found that dopamine receptor agonists required a considerable time, 1–2 h, to reach peak effect following intracerebral injection, suggesting a slow rate of diffusion to active sites. In this context, apomorphine may diffuse less readily than other agonists following localised injection into brain tissue. There are also indications that apomorphine may have a lower intrinsic activity than other dopamine receptor agonists (Miller, Kelly & Neumeyer, 1976; Costall & Naylor, 1976); this could lead to a lower maximum response where there is limited access to receptors. Whatever the explanation for the weaker action of apomorphine intracerebrally, this drug, whether injected peripherally or intracerebrally, showed no selectivity in eliciting rotation or stereotypy and did not therefore appear to discriminate between the receptors mediating these responses.

In order to investigate further the nature of the receptors mediating the contralateral rotation and stereotypy responses induced by peripherally injected dopamine receptor agonists, the effects of several neuroleptic dopamine antagonists on the responses induced by SK & F 38393, apomorphine and N,N dipropyl A-5,6 DTN, injected peripherally, were next examined. In these experiments it was found that haloperidol, pimozide and fluphenazine resembled each other in that they inhibited rotation and stereotypy induced by N,N dipropyl A-5,6 DTN or apomorphine but, at similar or slightly higher doses, failed to inhibit SK & F 38393-induced rotation. These results again demonstrated that the receptors mediating SK & F 38393-induced rotation were different from those mediating stereotypy and,

in addition, showed that they were also different from the receptors that mediated rotation induced by apomorphine or N,N-dipropyl A-5,6 DTN.

A different profile of antagonism was obtained with clozapine. This drug inhibited contralateral rotation irrespective of the agonist used to elicit the response but it did not inhibit stereotypy. Failure of clozapine to inhibit stereotypy confirmed earlier findings with this drug (Stille *et al.*, 1971). There was a possibility that the selective blockade of the rotation response was due to the known sedative and muscle relaxant effects of the drug (Stille *et al.*, 1971) affecting the motor component of this response. However, this possibility was discounted because, despite careful observation, there were no visible signs of such effects following doses of clozapine that were effective in inhibiting rotation. In other experiments (Gower & Marriott, unpublished observations), we found that the sedative benzodiazepine drug nitrazepam, like clozapine selectively inhibited the contralateral rotation response at doses which did not inhibit stereotypy. However, in contrast to clozapine, nitrazepam only inhibited the rotation response at doses that caused very obvious behavioural sedation and muscular hypotonia. It therefore seemed reasonable to conclude that the differential blocking actions of clozapine were due to receptor differences. The implications were that the receptors mediating apomorphine or N,N dipropyl A-5,6 DTN rotation, as well as those mediating SK & F 38393 rotation, differed from the receptors mediating stereotypy. The effects of two drugs structurally related to clozapine, loxapine and clothiapine, were also examined in these tests. Loxapine, was clearly more potent in inhibiting stereotypy than it was in inhibiting contralateral rotation. This profile of antagonism; although unexpected, strengthened the evidence that the receptors mediating stereotypy differed from both types of receptors mediating contralateral rotation. In contrast to both clozapine and loxapine, clothiapine inhibited all responses at similar dose-levels and did not therefore appear to discriminate between any of the receptors mediating these responses.

Finally, it has been suggested that the receptors mediating SK & F 38393-induced rotation in rats with nigro-striatal lesions may not be dopamine receptors (Seeman, 1980). It was therefore of interest to determine the effects of antagonists other than dopamine antagonists on contralateral rotation induced by SK & F 38393. The lack of inhibition by relatively high doses of atropine, methysergide, mepyramine or propranolol excluded the possibility that cholinoceptors, 5-HT receptors, histamine receptors or β-adrenoceptors were involved in this response. Whilst yohimbine and prazosin caused a transient delay in the onset of the response they did not inhibit it. Thus contralateral rotation induced by SK & F 38393 was clearly not mediated by α-adrenoceptors although there did appear to be some α-adrenoceptor involvement in the early phase of the response. This is not peculiar to rotation induced by SK & F 38393. In other experiments (Gower & Marriott, unpublished observations) we have found that yohimbine and prazosin also cause a transient delay in contralateral rotation induced by apomorphine.

The present results provide evidence for the subclassification of central dopamine receptors in the rat. Two receptor classes appear to be involved in contralateral rotation induced by dopamine receptor agonists. These are definable in terms of sensitivity to the agonist actions of SK & F 38393 or apomorphine respectively. The SK & F 38393-sensitive receptors are susceptible to blockade by clozapine but are not blocked by haloperidol. The apomorphine-sensitive receptors are susceptible to blockade by haloperidol and by clozapine. The receptors that mediate stereotypy resemble these latter receptors in their sensitivity to apomorphine and haloperidol but they are not blocked by clozapine. Apomorphine-sensitive receptors therefore appear divisible into two sub-classes depending on whether or not they are blocked by clozapine. In terms of this classification, N,N dipropyl A-5,6 DTN stimulates apomorphine-sensitive receptors and appears to have some selectivity for the sub-class not blocked by clozapine.

Pimozide and fluphenazine show the same selectivity in their antagonist actions as haloperidol. Loxapine has greater antagonist potency at apomorphine-sensitive receptors that are not blocked by clozapine than it has at the other receptors.

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