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Antistereotypic effects of dopamine D-1 and D-2 antagonists after intrastriatal injection in rats. Pharmacological and regional specificity

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Summary. Apomorphine antagonistic effects of a range of dopamine (DA) antagonists were studied after intracerebral and after peripheral injection. Inhibitory activity was found selectively within the ventral striatum with a D-1 antagonist (SCH 23390), D-2 antagonists (benzamides, butyrophenones) and mixed D-1/D-2 antagonists (thioxanthenes, phenothiazines), whereas α-adrenoceptor antagonists, muscarinic- and serotonin S_2-antagonists were ineffective. Great differences in absolute potencies and in peripheral versus intrastriatal potency ratios were observed. High peripheral versus central selectivity ratios and high intrastriatal potencies were found with the hydrophilic compounds (−)-sulpiride, veralipride and domperidone which do not readily cross the blood-brain barrier. High intrastriatal potency was also observed for the benzamide, YM 09151-2, haloperidol and spiroperidol although these compounds had lower peripheral versus intrastriatal selectivity ratios. Neuroleptic potency after intracerebral administration did not depend solely on DA receptor affinity but additionally on physicochemical properties. On the basis of the peripheral vs. intrastriatal potency ratios, it is concluded that only few of the neuroleptics tested in this study are suited for topographical studies of DA receptor function using intracerebral injection but that (−)-sulpiride is one example combining high potency, high central selectivity, high DA D-2 receptor specificity, stereoselectivity and long duration of action. The site-selectivity of apomorphine-antagonistic effects was further studied using (−)-sulpiride as a model compound. Inhibitory activity against oral stereotypy was preferentially found after injection into the ventral striatum, whereas the low-component patterns of apomorphine stereotypy (sniffing, rearing, motility) were blocked equally well in the ventral striatum and nucleus accumbens. In contrast, a facilitation of oral stereotypy was induced by (−)-sulpiride in the dorsal striatum. No effect on apomorphine-stereotypy was found after injection into frontal cortex, supragenual cortex, septum, amygdala, substantia nigra or thalamus. These results support data obtained in lesion studies indicating the ventral striatum as the important site mediating inhibition of oral stereotypy after DA receptor blockade. However, the differentiation between striatum and accumbens in mediation of stereotypy and hyperactivity was not as absolute as has been suggested by lesion studies

Key words: Dopamine D-1 receptors – Dopamine D-2 receptors – Apomorphine – Neuroleptics – Antistereotypic effect – Striatum – Nucleus accumbens – Rats

1. Introduction

The striatum has for several years been regarded as the main target of the antistereotypic effects of neuroleptic compounds in animals (review by Fog 1972) although also other dopamine (DA)-rich regions and in particular nucleus accumbens have been implicated (review by Costall and Naylor 1980).

One reservation, however, in accepting this hypothesis is that in studies with intracerebral application, most neuroleptic compounds are used in relatively high doses in order to inhibit DA-mimetic-induced effects (Pijnenburg et al. 1975; Costall et al. 1983). The doses required are often close to those reported to be effective after peripheral injection (see e.g. Niemegeers and Janssen 1979) thus questioning the selectivity of the local effect.

Two factors may be responsible for the weak activity of neuroleptics after intrastriatal injection: 1) Most classical neuroleptics are highly lipophilic leading to a rapid diffusion away from the injection site (e.g. Fleminger et al. 1983) and 2) Topographical localization of the antistereotypic effect *within* a limited region of the striatum thus making the exact injection site important. Several studies have indicated that the striatum is organized as a functionally heterogeneous structure (Costall et al. 1980; Cools and Van Rossum 1980; Joyce 1983) with the ventral portion as a candidate for mediation of oral stereotypies (Iversen and Koob 1977; Scheel-Krüger 1983).

Only very little work has been reported where larger numbers of DA antagonists have been studied after intrastriatal injection, but recently Costall et al. (1983) compared 8 different neuroleptics for ability to induce circling behaviour. No regional selectivity within striatum was mentioned but it was reported that the hydrophilic benzamide neuroleptic, sulpiride, was very potent, thus making it suitable for investigation of regional selectivity. In the present study the antagonistic effect of 14 different, intrastriatally administered, DA antagonists against the stereotyped behaviour induced by apomorphine has been studied. Furthermore, (−)-sulpiride has been used as a model compound for investigation of the regional distribution of the antistereotypic effect within the striatum and some extra-striatal regions.

2. Materials and methods

2.1 Animals and surgery. Male Wistar (Mol:Wist) SPF rats weighing 250–275 g at the time of surgery were used. They were housed conventionally in groups of 4–5 in Macrolon

type III cages in animal rooms with automatic control of temperature (21 ± 1°C), relative humidity (55 ± 5%), air exchanges (16 times per h) and day/night cycle (6 a.m. – 6 p.m.). They had free access to a commercial pelleted diet and tap water. The rats were implanted with bilateral stainless steel guide cannulae (23 G) [under pentobarbital (50 – 55 mg/kg i.p.) anaesthesia] at different coordinates (see section 2.4). The tips of the guide cannulae terminated 3 mm above the actual injection site.

The rats were allowed a 7 days recovery period before the experiment. Injections were made (into conscious rats) using 30 G needles connected to Hamilton syringes placed in an infusion pump (Bio-Invent, Stockholm) allowing simultaneous bilateral injections of 0.5 µl volume at a rate of 1 µl/min.The needles were kept in position for further 20 s before slow withdrawal. The rats were usually tested twice with at least 1 week between each experiment.

2.2 Behavioural methods. The day before the experiment the rats were transferred to individual wire mesh observation cages for adaptation. Bilateral intracerebral injections were made and the rats received immediately an injection of apomorphine (1.6 µmol/kg = 0.5 mg/kg s.c.). Apomorphine was again injected to the same rats 90 min and in some experiments also at 4 h after the intrastriatal injection. In comparable experiments apomorphine was injected simultaneously with, 90 min and 4 h after peripheral (s.c.) injection of test compounds in rats with guide cannulae.

Behavioural observations were made every 5 min for 45 – 50 min and each pattern (licking, biting, rearing, sniffing, head movements, motility) of the apomorphine response was scored as absent, mild or intense. For simplification the data were reduced to two responses, oral stereotypy (licking/biting) and "low-component" stereotypy (hyperactivity patterns). The results were expressed as per cent of the maximum possible scores for each group of at least 5 rats. For determination of dose-response relations each rat was assigned a score 0, 1 or 2 for oral and low-component stereotypy, respectively. Score 2 required the presence of continuous stereotypy at least during 2 consecutive observation times, whereas score 1 was given to rats showing periodical stereotypy also during at least 2 consecutive observation times. The total score for each dose group was expressed as per cent of that of a parallel control group. Control groups were either rats receiving 0.5 µl saline intrastriatally or unoperated rats of matched weights, receiving apomorphine only. Intrastriatal saline injection slightly reduced (8%, $n = 60$) the apomorphine-induced oral stereotypy when apomorphine was given immediately after the intrastriatal injection), without affecting low-component stereotypy scores. When unoperated rats were used as controls a correction was made for this difference in oral stereotypy, ED_{50} values were calculated by log-probit analysis. Differences between individual groups were calculated by Kruskal-Wallis one-way analysis of variance (Siegel 1956).

2.3 Histology. After the experiments the rats were killed, the brains were removed and fixed in formalin for histological verification of the localization of the injection site. They were examined using cryostat sections (100 µm). In regional studies all brains were sectioned, whereas in the structure-activity studies using fixed coordinates every 3rd – 4th brain was examined. The following coordinates (König and Klippel 1963) are referred to in the text: Ventral striatum (A 7.0 – 8.3 L 2.0 – 3.0 V −0.2 −1.0), dorsal striatum (A 7.4 – 8.6 L 2.0 – 3.0 V +0.5 −1.5), nucleus accumbens (A 8.6 – 9.8 L 0.8 – 1.2 V −0.5 −1.3), frontal cortex (A 9.8 – 10.3 L 0.3 – 0.8 V +1.8 −2.5), supragenual cortex (A 7.0 – 8.5 L 2.2 – 2.8 V +3.0 −3.3), septum (A 7.8 – 8.4 L 0.5 V +1.0), amygdala (A 3.8 – 4.4 L 3.2 – 3.5 V −2.5 −3.0), substantia nigra (A 1.3 – 1.8 L 2.0 V −2.8). If injections were localized outside the defined regions they were omited from data analysis. Accuracy rate was at least 90%.

2.4 Drugs. The following compounds were dissolved in saline: Apomorphine, HCl (Ph. N., containing 0.02% ascorbic acid), cis(Z)-flupentixol, 2 HCl (Lundbeck), trans(E)-flupentixol, 2 HCl (Lundbeck), cis(Z)-clopenthixol, 2 HCl (Lundbeck), chlorprothixene, HCl (Lundbeck), teflutixol, 2 HCl (Lundbeck), tefludazine, 2 HCl (Lundbeck), fluphenazine, 2 HCl (Squibb), SCH 23390 (Schering, USA), lidocaine, HCl (Mecobenzon) and cinanserin, HCl (Squibb). Prazosin, HCl (Pfizer) and clebopride, maleate (Almirall) were dissolved in distilled water. Domperidone, haloperidol, spiroperidol and ketanserin (all Janssen) were dissolved in an equivalent amount of 0.1 M tartaric acid and diluted with 0.9% w/v NaCl solution. (±)-Sulpiride, (−)-sulpiride, (+)-sulpiride (Delagrange) and YM 09151-2 (Yamanouchi) were dissolved in an equivalent amount of dilute acetic acid. Clozapine (Wander) and veralipride (Delagrange) were dissolved using 0.2 N hydrochloric acid. Phentolamine methansulphonate was prepared from commercially available ampoules. All solutions for intracerebral injections were neutralized as far as possible using 0.1 N NaOH. Peripheral injections were made in volumes of 5 ml/kg.

3. Results

3.1 Effect of (−)-sulpiride injected into striatum and adjacent areas

Following injection into the ventral striatum, (−)-sulpiride (0.006 – 0.37 nmoles = 2 – 125 ng, bilaterally) dose-dependently inhibited both the oral and low component (hyperactivity patterns) stereotypy induced by apomorphine (1.6 µmol/kg = 0.5 mg/kg s.c.). The time-curve for selected doses is shown in Fig. 1 for the oral stereotypy. Although the inhibitory effect of (−)-sulpiride was present immediately after its injection the inhibitory potency was slightly increased when apomorphine was administered once more 1.5 h or 4 h after (−)-sulpiride (see also the decrease in ED_{50} value in Table 3). Although the apomorphine response in the control group was slightly increased and had a more rapid onset, (−)-sulpiride completely blocked the oral stereotypy (Fig. 1) as well as hyperactivity (not shown). In Fig. 2 the dose-response curves for both parameters obtained during the first apomorphine session are shown. It is seen that the oral stereotypy was more sensitive to (−)-sulpiride than the low-component patterns.

Dorsal intrastriatal injections (1.5 – 2.5 mm above the ventral injections) of (−)-sulpiride (0.09 nmoles) did not block the apomorphine-induced stereotypy (Table 1). Rather an increased oral stereotypy response was observed. This increase appeared primarily as a change from stereotyped licking to continuous biting activity and will be described in detail elsewhere (Scheel-Krüger and Arnt 1985).

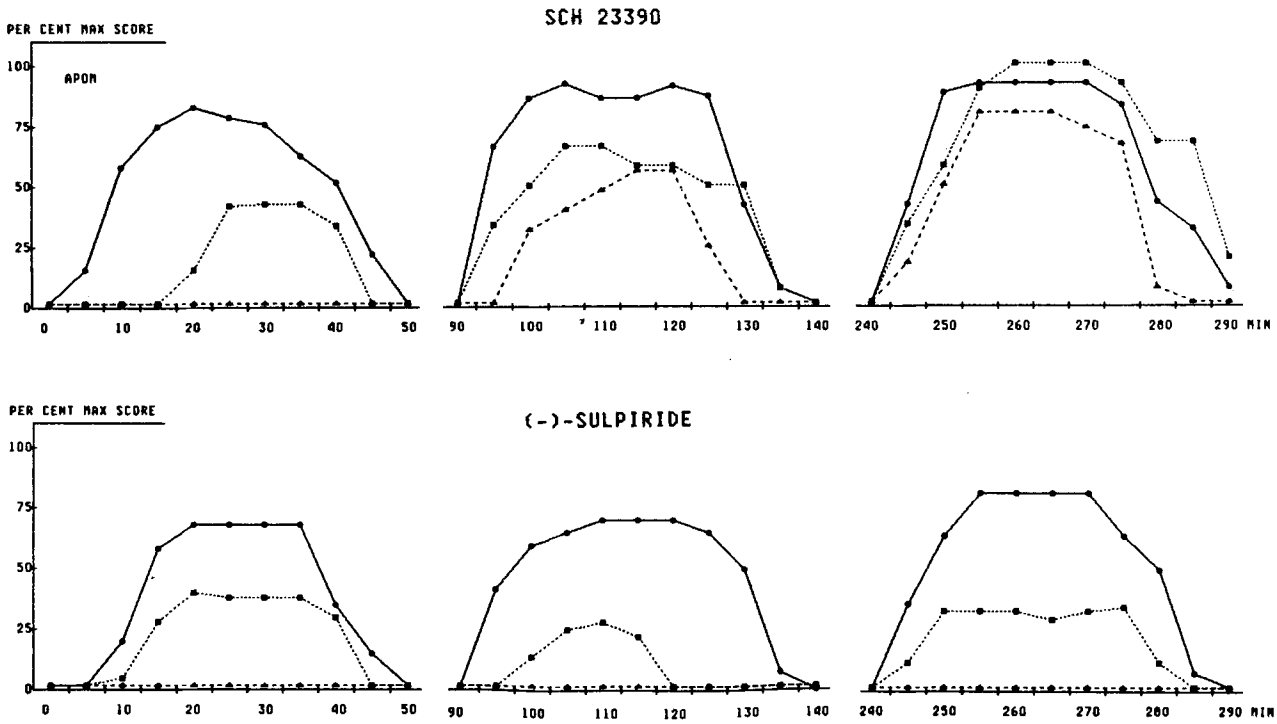

Fig. 1. Effect of SCH 23390 (*upper part*) and (−)-sulpiride (*lower part*) injected into ventral striatum on apomorphine-induced licking and biting. SCH 23390 0.7 nmol/striatum (■----■), 2.9 nmol (▲----▲), (−)-sulpiride 0.02 nmol/striatum (■ ----■), 0.09 nmol (▲-----▲) or saline 0.5 µl (●——●) was injected at time 0. Apomorphine (1.6 µmol/kg) was injected immediately after the intrastriatal injection. Apomorphine was again injected to the same rats 90 min and 240 min later (middle and right part of the figure). The values are given as per cent of the maximum possible oral stereotypy score for each group consisting of 6−12 rats

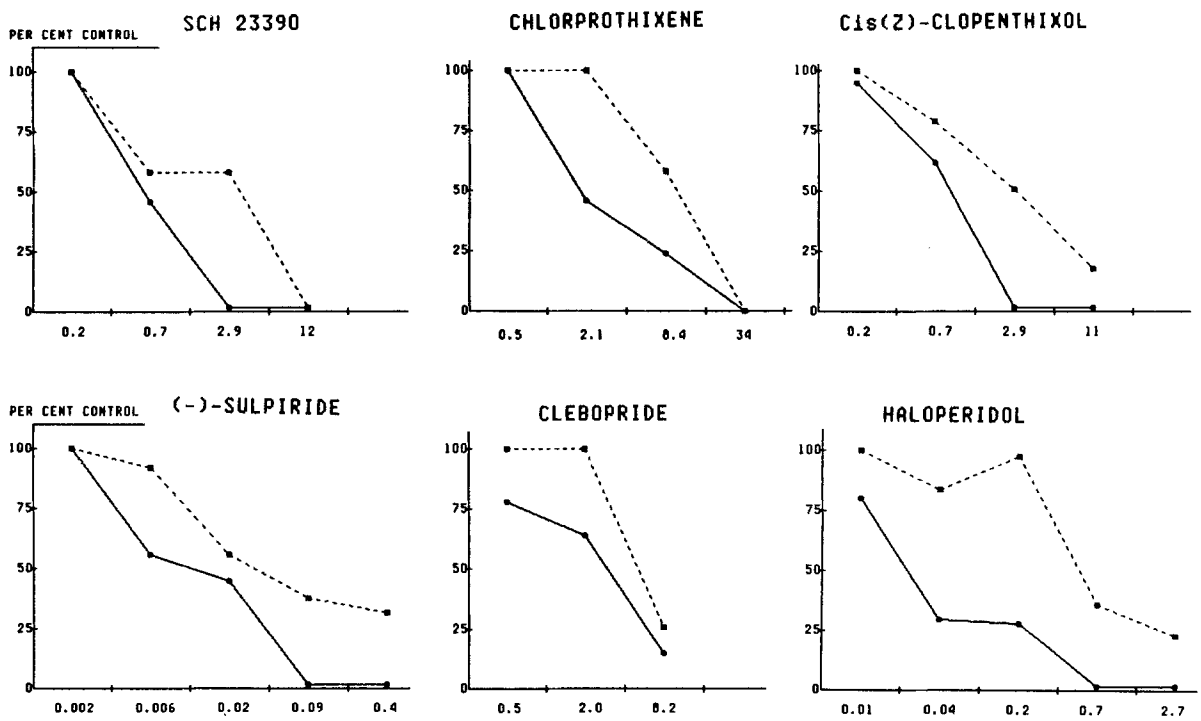

Fig. 2. Inhibition of apomorphine-induced stereotypy after bilateral injection of dopamine antagonists into ventral striatum of rats. The antagonists were injected immediately before apomorphine (1.6 µmol/kg s.c.), except for cis(Z)-clopenthixol which was injected 1.5 h before apomorphine. Oral stereotypy (■——■) and low component stereotypy (motility, sniffing and rearing, ■----■) were scored as described in methods section. The abscissa indicates the dose (nmol/0.5 µl) injected into each striatum. The values indicate per cent of the score of a parallel control group. Each group consisted of 5−12 rats

Table 1. Effect of (−)-sulpiride on apomorphine-induced stereotypy after injection into various brain regions. (−)-sulpiride (0.09 or 0.37 nmol, bilaterally) was injected immediately before apomorphine (1.6 μmol/kg s.c.) and behaviour was scored as described in Materials and methods. The number of rats in each group are shown in parentheses

Injection site	Per cent inhibition			
	Oral stereotypy (−)-sulpiride		Low component stereotypy (−)-sulpiride	
	0.09 nmol	0.37 nmol	0.09 nmol	0.37 nmol
Ventral striatum	100* (6)	n.t.	62*	n.t.
Dorsal striatum	0** (7)	n.t.	0	n.t.
Nucleus accumbens	12 (8)	47* (4)	81*	75*
Frontal cortex	25 (5)	14 (5)	0	0
Supragenual cortex	0 (8)	n.t.	0	n.t.

* $P < 0.01$ significant inhibition compared with parallel control groups
** $P < 0.01$ increased oral stereotypy (see section 3.1)
n.t. not tested
Injection of similar doses of (−)-sulpiride into septum, thalamus, substantia nigra and amygdala did not significantly change apomorphine-induced stereotypy

Table 2. Latency of the antagonism, by ventral intrastriatal injection of (−)-sulpiride, of ongoing stereotypy induced by apomorphine. Apomorphine (8.2 μmol/kg s.c.) was injected to groups of 6 rats. Fifteen minutes later the rats received bilateral injection of 0.9% w/v NaCl solution 0.5 μl or (−)-sulpiride (0.4 or 1.5 nmol/0.5 μl) into the ventral striatum. Stereotypy was rated every 5 min. The results indicate the mean ±SEM latency (in minutes) to disappearance of oral and low-component stereotypy, respectively

	Minutes (mean ± SEM)		
	Saline 0.5 μl	(−)-Sulpiride 0.37 nmol	(−)-Sulpiride 1.5 nmol
Oral stereotypy	77 ± 6	53 ± 2*	51 ± 5*
Low-component stereotypy	90 ± 5	60 ± 2*	63 ± 2*

* $P < 0.01$ with respect to the group treated with saline solution

Injection of (−)-sulpiride into the nucleus accumbens was less effective than ventral intrastriatal injections in reducing oral stereotypy. The licking/biting was reduced by 47% only when a large dose was administered (Table 1). However, the hyperactivity components of the apomorphine stereotypy were effectively reduced by (−)-sulpiride injected into the nucleus accumbens with a sensitivity similar to or greater than that found after ventral intrastriatal injection (Table 1).

In contrast, (−)-sulpiride had no effect on the apomorphine-induced stereotypy when injected into the frontal cortex, the supragenual cortex above the striatum, thalamus, amygdala or substantia nigra. Similarly, no blocking effect was found after intraseptal injection, although the hyperactivity patterns appeared to be of slightly lower intensity (Table 1).

In order to define further the most sensitive site for the antistereotypic activity the effect of (−)-sulpiride, injected at a time when the maximal apomorphine stereotypy was occurring, was investigated. The latency of apomorphine inhibition was used as parameter. Surprisingly, it was found that (−)-sulpiride was much weaker when administered in this way. Even very high doses (0.37 and 1.5 nmol, bilaterally) only blocked the effect of apomorphine after very long latencies of about 50 min (Table 2) although the injections were localized to the same very sensitive area as those reported in Figs. 1 and 2. Since the dose of apomorphine in these experiments (8.2 μmol/kg s.c.) was 5 times higher than that used in the other experiments (in order to obtain a prolonged duration of action) it was checked whether this difference could explain the loss of (−)-sulpiride potency. After simultaneous injection or 4 h pretreatment with (−)-sulpiride (0.37 and 1.5 nmol/striatum) and apomorphine (8.2 μmol/kg s.c.) a dose-dependent and immediate block of stereotypy was found after both pretreatment schedules (50 and 100% block of all stereotypic patterns, respectively, data not shown).

Essentially similar results were obtained using d-amphetamine (54 μmol/kg s.c.) instead of apomorphine. Ongoing stereotypy was relatively insensitive to (−)-sulpiride, whereas simultaneous injections revealed high (−)-sulpiride potency (data not shown).

3.2 Apomorphine-antagonistic effect of drugs injected into ventral striatum

A range of DA antagonists, of different classes, was studied using injection into the site which was most sensitive to (−)-sulpiride, i.e. the ventral part of the striatum. Apomorphine (1.6 μmol/kg s.c.) was injected immediately, 90 min and 4 h (not shown) after the intrastriatal injection.

Pilot studies revealed that the injection of 0.9% w/v NaCl solution (0.5 μl) induced a slight decrease of the maximal oral stereotypy score (8%, $n = 60$) compared with parallel injections of apomorphine in unoperated rats. This was only seen in the first apomorphine observation period immediately after the intrastriatal injection. Injection of distilled water (0.5 μl, $n = 6$) or 0.1 M tartaric acid (0.5 μl, $n = 5$) did not change the apomorphine-induced stereotypy. Injection of the local anaesthetic, lidocaine (1.7−28 nmol, bilaterally) induced a slight (10−30%) decrease of the oral stereotypy (which was not dose-dependent) and was found only during the first apomorphine session immediately after intrastriatal injection (data not shown).

The neuroleptics differed greatly in their inhibitory potency against apomorphine-induced stereotypy. Figure 2 shows dose-response curves for some selected compounds, and Table 3 gives ED_{50} values for all the drugs studied. The most potent compounds were the benzamides, sulpiride, veralipride and YM 09151-2, which in low doses antagonized all patterns of the apomorphine-induced stereotypy, although the oral part was the most sensitive. However, it should be noted that benzamide, clebopride, was much less potent.

The butyrophenones haloperidol and spiroperidol were also potent blockers of oral activity, whereas blockade of low component stereotypies required somewhat higher doses.

Other classes of neuroleptics also blocked the apomorphine-induced stereotypy, and included the thioxanthenes, cis(Z)-flupentixol, cis(Z)-clopenthixol, chlorprothixene and

Table 3. Effect of dopamine antagonists on apomorphine-induced stereotypy after injection into the ventral striatum. Bilateral injections were made immediately before and 1.5 h before apomorphine (1.6 μmol/kg s.c.) and behaviour was scored as described in Materials and methods. At least 3 doses each tested on at least 5 rats were used with each drug

	ED_{50} (nmoles/striatum)			
	Oral stereotypy		Low component stereotypy	
	Simultaneous treatment	1.5 h pretreatment	Simultaneous treatment	1.5 h pretreatment
Thioxanthenes/phenothiazine/phenylindans				
Cis(Z)-flupentixol	2.5	1.0	>8	8
Trans(E)-flupentixol	>16	>16	>16	>16
Cis(Z)-clopenthixol	2.1–17[a]	2.5	15	9.1
Chlorprothixene	0.77	2.1	6.5	15
Teflutixol	9.9	4.2	16	15
Fluphenazine	5.7	3.9	12	7.8
Tefludazine	1.1	1.9	6.6	5.4
Butyrophenones/benzamides				
Haloperidol	0.037	0.056	0.72	2.2
Spiroperidol	0.043	0.051	5.1	1.8
Domperidone	0.54	0.40	2.1	3.5
(−)-Sulpiride	0.013	0.0062	0.062	0.019
(±)-Sulpiride	0.032	0.020	0.28	0.053
(+)-Sulpiride	1.8	0.47	11	2.4
Veralipride	0.010	0.015	0.026	0.026
Clebopride	2.2	>8.2	5.5	>8.2
YM 09151-2	0.054	0.041	0.080	0.64
Others				
SCH 23390	0.64	1.1	1.3	3.8
Clozapine	>12	>12	>12	>12

[a] Irregular dose-response

teflutixol, the phenothiazine, fluphenazine and the phenylindane, tefludazine. Most of these drugs were less potent immediately after intracerebral injection but showed higher antagonistic activity 1.5 h after injection (Table 3).

Furthermore, the dopamine (DA)D-1 antagonist, SCH 23390 (Fig. 1 and Table 3) effectively blocked all signs of apomorphine-induced stereotypy but the duration of action was shorter than seen with most other compounds.

Finally, clozapine was tested and was found to be ineffective. Stereoselectivity was found in two cases: The neuroleptically inactive trans(E)-isomer of flupentixol was ineffective in a dose 16 times above the ED_{50} value found with the active cis(Z)-isomer. For sulpiride, the (−)-isomer was about 100 times more potent than the (+)-isomer and the racemate was 2–3 times weaker than the (−)-enantiomer (Table 3).

Other neurotransmitter antagonists were also studied under similar conditions (data not shown): Prazosin (2.4 nmoles/striatum), phentolamine (11 nmoles), cinanserin (11 nmoles), ketanserin (10 nmoles) and methylatropine (2.6 nmoles) did not significantly change apomorphine-induced stereotypy. However, the cholinergic agonist, carbachol, significantly antagonized apomorphine-induced licking/biting by 12 and 67% after doses of 5.5 and 22 nmoles, respectively (n = 6 per group).

3.3 Effects of peripherally injected test drugs

Comparable studies using subcutaneous injections of antagonists were made using similar experimental conditions. In Table 4 the peak ED_{50} values for inhibition of apomorphine-induced stereotypy are presented. Most neuroleptics were, so expected, potent blockers. Exceptions were domperidone, (±)-sulpiride and veralipride which were weak or inactive even after high doses. The oral stereotypy was more sensitive to blockade, but the differences between oral and low-component ED_{50} values were only small, in most cases, the ratios of the ED_{50}'s being, between one or two. Prazosin was the only compound which inhibited low-component stereotypy without affecting oral stereotypy.

The ratios of potency after subcutaneous injection and intrastriatal administration are also shown in Table 4. This ratio was obtained after multiplication of intrastriatal ED_{50} values by a factor of 6 in order to correct for bilateral injections and for body weights, since mean body weight of the rats was 330 g. As shown in Table 4 large differences in this ratio were obtained. The lowest ratios were found with clebopride, fluphenazine and teflutixol (ratio 2.8–3.8), intermediate values with cis(Z)-clopenthixol, cis(Z)-flupentixol, YM09151-2, SCH23390, tefludazine, haloperidol and spiroperidol and very high ratios (>29,000) with domperidone, (±)-sulpiride and veralipride.

4. Discussion

The present results indicate that a wide range of DA antagonists (but not clozapine) are able to block all signs of apomorphine-induced stereotypy when they are injected bilaterally into the ventral part of striatum of rats. However,

Table 4. Effect of peripherally (s.c.) injected test compounds on apomorphine-induced stereotypy. Test conditions were similar to those described in Table 2, but only the lowest ED50 value (peak effect) is shown. At least 3 dose groups each consisting of 4–8 rats were studied for each compound. The ratio between subcutaneous (s.c.) and intrastriatal (i.s.) potency was calculated after converting intrastriatal ED50 values (Table 3) from nmol/striatum to nmol/kg

		ED50 (μmol/kg s.c)		ED_{50} (s.c.)/-ED_{50} (i.s.)
		Oral stereotypy	Low component stereotypy	Oral stereotypy
b	Cis(Z)-flupentixol	0.11	0.11	18
b	Cis(Z)-clopenthixol	0.11	0.14	7.3
b	Chlorprothixene	0.27	>1.8	58
b	Teflutixol	0.095	0.12	3.8
b	Fluphenazine	0.037	0.043	3.2
a,b	Haloperidol	0.029	0.11	131
b	Spiroperiol	0.018	0.030	70
a,b	Domperidone	>94	>94	> 29,000
b	(±)-Sulpiride	56	76	46,000
a,b	Veralipride	>104	>104	>1,700,000
a	Clebopride	0.037	0.088	2.8
b	YM 09151-2	0.0064	0.0064	26
a	SCH 23390	0.038	0.041	10
b	Tefludazine	0.071	0.098	11
a	Prazosin	>6.0	3.1	–

a Antagonist injected immediately before apomorphine
b Antagonist injected 1.5 h before apomorphine
a,b Similar ED50 value was obtained at both dose regimens

the oral stereotypy (primarily licking after the dose of apomorphine employed) was inhibited by doses of neuroleptics lower than those necessary for inhibition of "low-component"-stereotypies (motility, rearing and sniffing). The apomorphine antagonism could not be differentiated on basis of the relative DA D-1/D-2 receptor selectivity (Kebabian and Calne 1979) of the DA antagonists, since a selective D-1 antagonist, SCH 23390 (Iorio et al. 1983; Hyttel 1983; Arnt and Hyttel 1984), selective D-2 antagonists, e.g. the benzamides and butyrophenones (Fleminger et al. 1983; Hyttel 1980, 1983; Terai et al. 1983) and mixed D-1/D-2 antagonists, e.g. thioxanthenes (Hyttel 1983) all blocked the effect of apomorphine after intrastriatal as well as after peripheral injection. The similar profile of a DA D-1 and D-2 antagonist is also found in experiments when antagonists were injected systemically (Iorio et. al 1983; Christensen et al. 1984), and thus suggests that both receptor types are localized at the same efferent systems.

In addition to the DA-antagonistic effect, most neuroleptics, with the exception of benzamides (Terai et al. 1983) block other neurotransmitter receptors, in particular α_1-adrenoceptors and 5-HT_2 receptors (Peroutka and Snyder 1980; Hyttel 1983). Therefore, the effect of reference agents blocking these types of receptors was studied separately. However, apomorphine-induced stereotypy was not affected by intrastriatal injection of the α_1-adrenoceptor antagonist prazosin and the mixed α_1-/α_2-antagonist, phentolamine (Delini-Stula et al. 1979; Massingham et al. 1981). The 5-HT_2 receptor antagonists, ketanserin and cinanserin (Leysen et al. 1981) also did not block the effect of apomorphine. Thus, the DA-antagonistic effect of intrastriatally administered neuroleptics can be regarded as responsible for the antistereotypic effect. After peripheral injection, prazosin showed partial apomorphine antagonism, since the low-component stereotypy was antagonized without any inhibitory effect on oral activity. This profile was not seen with any of the neuroleptics.

In spite of the common effect of the DA antagonists, great differences in potency were detected after intrastriatal injection and these were without any correlation to their affinity to DA receptors labelled by ^{3}H-spiroperidol or ^{3}H-piflutixol in vitro (Fleminger et al. 1983; Hyttel 1983). Among the benzamides (−)-sulpiride and YM 09151-2 were almost equipotent despite a 500-fold difference in DA D-2 receptor affinity (Fleminger et al. 1983; Terai et al. 1983). Clebopride, in contrast, was much less potent after intrastriatal injection despite high in vitro affinity (Hyttel 1980; Fleminger et al. 1983). Similarly fluphenazine was much weaker than e.g. haloperidol despite similar DA D-2 receptor affinity in vitro (Hyttel 1983) and equipotency as apomorphine antagonists after peripheral administration. These discrepancies may primarily depend on differences in the lipophily of the compounds. Highly lipophilic compounds probably diffuse more rapidly away from the intracerebral injection site and may also be removed through the blood stream due to ready passage of the blood-brain barrier thus leading to a dilution of the concentration at active site. Conversely, highly hydrophilic compounds which less readily penetrate the blood-brain barrier are more likely to remain at the injection site. If the ratio between the potency after peripheral and that after intrastriatal injection is considered, large differences between compounds were noted. Highly hydrophilic compounds, e.g. (−)-sulpiride (Honda et al. 1977; Fleminger et al. 1983), veralipride (Perrault et al. 1981) and domperidone (Costall et al. 1979) showed very low potency or no effect after peripheral injection, whereas they were among the most potent after intrastriatal injection. Selectivity ratios above 29,000 were found with these drugs. In contrast, highly lipophilic compounds, e.g. clebopride (Fleminger et al. 1983) showed much lower ratios ranging from 2.8 to about 100. From these results it is seen that most neuroleptics are unsuited for use in intracerebral drug injection studies with (−)-sulpiride as one of the exceptions. In addition, stereospecificity can be checked with the (+)-enantiomer of sulpiride which in the present study was found to be about 100 times weaker than (−)-sulpiride. This is in agreement with in vitro binding studies (for references see Hyttel et al. 1984).

Our experiments with (−)-sulpiride injected into different striatal and extrastriatal sites confirmed the highly localized effect. Only in a narrow band in the ventral striatum (−)-sulpiride showed the marked apomorphine-antagonistic activity. Intrastriatal injections 1–2 mm more dorsally had no inhibitory effect. Conversely, an increase in oral stereotypy was observed after bilateral (−)-sulpiride injections (0.02–0.4 nmol/striatum) or SCH 23390 (5.8 nmol/striatum) into the anterior part of dorsal striatum (Scheel-Krüger and Arnt 1985). Although this again indicates a similar profile of a DA D-1 and D-2 antagonist, these results confirm that striatum is functionally heterogenous (see Introduction), but this distinction has not previously been described after intrastriatal neuroleptic administration. This may be due to the fact that in most studies the center of the striatum or the dorsal part was intended as target for the injection and that high doses of antagonists

and maximally effective doses of DA stimulants have been used (e.g. Fog 1972; Pijnenburg et al. 1975). These high doses are close to those effective after peripheral injection and probably diffuse rapidly throughout the whole striatum. Furthermore it should be noted that high (−)-sulpiride potency depended on injection of (−)-sulpiride *before* or simultaneously with apomorphine. This marked difference was surprising and may explain the need of very high intrastriatal dose of haloperidol necessary in the study of Pijnenburg et al. (1975), since haloperidol was injected *after* apomorphine or amphetamine. No explanation can be given but it may be speculated that agonists and antagonists, respectively, stabilize the conformation of DA receptors in two different states. This is parallel to the marked affinity differences in binding affinities of DA agonists when using ^{3}H-agonists and -antagonists, respectively, as ligands (review by Seeman 1981).

Although the striatum for long time has been considered as the main target for stereotypic effects and the mesolimbic system for locomotor activity, some overlaps have also been described as shown by lesion and intracerebral drug injection studies (Kelly et al. 1975; Pijnenburg et al. 1975; Costall et al. 1980). In this study the effect of a low and a high dose of (−)-sulpiride was studied after injection into the nucleus accumbens. It was found that the "low-component" stereotypy induced by apomorphine was equally well antagonized following injection into the accumbens as it was when (−)-sulpiride was injected into the ventral striatum, whereas the oral stereotypy was preferentially inhibited by the injection into the ventral striatum. Only a very high dose of (−)-sulpiride caused partial antagonism of the apomorphine-induced oral stereotypy when injected into the nucleus accumbens. Thus the results suggest that oral stereotypy seems to be preferentially mediated through the striatum, whereas the hyperactivity components are regulated by both structures. This is in agreement with a topographical analysis of DA agonist actions in guinea pig striatum indicating that sites mediating locomotor activity also are localized in the striatum (Costall et al. 1980).

Other DA containing structure have also been implicated in the regulation or mediation of stereotypic effects, e.g. frontal cortex (Carter and Pycock 1980) and substantia nigra (e.g. Scheel-Krüger 1983). However, injection of (−)-sulpiride into different regions of the brain including the above-mentioned sites and also the amygdala, septum and thalamus did not induce any modification of apomorphine-induced stereotypy. In this connection it is interesting to note that injections of neuroleptics into the amygdala in doses comparable to those used in the present study blocked the acquisition of a conditioned avoidance response, a characteristic feature of neuroleptic action (Petty et al. 1984).

In conclusion, the present study has shown that the choice of a neuroleptic for topographical drug action studies not only depends on DA receptor affinity and specificity, but also on the physico-chemical properties of the compound, since many lipophilic compounds were only marginally more effective after intrastriatal compared with peripheral administration. Furthermore, the dose-schedule is of importance. Ideally, site specific effects after intracerebral drug injections should be studied as early as possible after the injection of low doses of a hydrophilic and potent compound. (−)-Sulpiride seems to fulfill these criteria and with this compound highly specific site-localization of inhibition of the apomorphine-induced stereotyped behaviour was found. The specific involvement of dopamine in oral stereotypy was confirmed using various reference compounds from other drug classes. Further studies using this approach should lead to a better understanding of the regional differentiation of various behaviours mediated by DA receptors.

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