Psychopharmacology (1984) 84: 512–519

Psychopharmacology
© Springer-Verlag 1984

Differential alterations in striatal dopamine receptor sensitivity induced by repeated administration of clinically equivalent doses of haloperidol, sulpiride or clozapine in rats

N. M. J. Rupniak, G. Kilpatrick, M. D. Hall, P. Jenner, and C. D. Marsden

University Department of Neurology, Institute of Psychiatry and The Rayne Institute, King's College Hospital Medical School, Denmark Hill, London SE5, UK

Abstract. Rats received therapeutically equivalent doses of either haloperidol (1.7–1.9 mg/kg/day), sulpiride (112–116 mg/kg/day) or clozapine (30–35 mg/kg/day) continuously for 4 weeks. Treatment with haloperidol, but not sulpiride or clozapine, caused inhibition of stereotyped behaviour induced by apomorphine (0.125–0.25 mg/kg SC). Following drug withdrawal for up to 7 days, haloperidol and sulpiride, but not clozapine treatment caused an exaggeration of stereotyped behaviour induced by apomorphine.

B_{max} values for striatal ^{3}H-spiperione binding were elevated in animals treated for 2 and 4 weeks with haloperidol, but not with sulpiride or clozapine. Following drug withdrawal, haloperidol, but not sulpiride or clozapine, treatment caused an increase in B_{max} for striatal ^{3}H-spiperone binding.

B_{max} values for striatal ^{3}H-NPA binding revealed no change during haloperidol or clozapine treatment. Sulpiride treatment for 1 week caused an increase in B_{max} for ^{3}H-NPA binding, which returned to control levels at 2 and 4 weeks. Following drug withdrawal, there was an increase in B_{max} for ^{3}H-NPA binding in rats treated with haloperidol and sulpiride, but not clozapine.

On continuous treatment and following withdrawal from haloperidol, sulpiride, or clozapine the ability of dopamine to stimulate striatal adenylate cyclase activity did not differ from that in control animals.

Repeated administration of sulpiride or clozapine may not induce striatal dopamine receptor supersensitivity when given in clinically relevant doses, although haloperidol does.

Key words: Clozapine – Sulpiride – Haloperidol – Dopamine receptors – Stereotypy

---

Chronic therapy with classical neuroleptic agents, including haloperidol, is associated with the production of extrapyramidal side-effects such as tardive dyskinesia. However, the use of two structurally distinct antipsychotic agents, clozapine, and sulpiride, has been claimed to cause only a low incidence of motor side effects (Simpson and Varga 1974; Matz et al. 1974; Gerlach et al. 1974; Alberts 1983).

Repeated administration of classical neuroleptics to rodents for several weeks, followed by drug withdrawal, causes cerebral dopamine receptor supersensitivity. For example, subchronic treatment with haloperidol increases the number of striatal dopamine receptors (B_{max}) identified by neuroleptic ligands such as ^{3}H-haloperidol (Burt et al. 1977), and causes an exaggerated stereotyped response to apomorphine (Tarsy and Baldessarini 1974). Induction of such changes may be responsible for the emergence of drug-induced tardive dyskinesias in man.

If these changes are related to tardive dyskinesia, it would be expected that drugs with a low propensity to induce this unwanted effect might not produce altered striatal dopamine receptor function on repeated administration to rodents. However, the literature in this field is controversial. In some studies, repeated administration of sulpiride followed by drug withdrawal caused an exaggerated stereotyped response to apomorphine and an increase in B_{max} for striatal dopamine receptors identified by ^{3}H-spiperone or ^{3}H-N,*n*-propylnorapomorphine (NPA) (Costall et al. 1978; Jenner et al. 1982; Fleminger et al. 1983). Others found that sulpiride administration did not alter B_{max} for striatal ^{3}H-spiperone binding (Trabucchi et al. 1980; Bannet et al. 1980; Fuxe et al. 1980). Similarly, repeated administration of clozapine may cause either no change in apomorphine-induced stereotypy or B_{max} for striatal ^{3}H-spiperone binding (Sayers et al. 1975; Gnegy et al. 1977; Kobayashi et al. 1978; Racagni et al. 1980; Seeger et al. 1982), or, alternatively, an increased intensity of stereotyped behaviour, and an increase in B_{max} for ^{3}H-spiperone binding (Smith and Davis 1976; Allikmets et al. 1981).

Interpretation of these findings is complicated by the use of different doses and durations of drug treatment in different studies. In addition, the relevance of these findings to man is not clear, since in many cases the doses and route of administration employed (IP rather than PO) were not based on those used therapeutically, and observations were carried out only following withdrawal of drug treatment. In this study, we have, therefore, examined the effects of repeated oral treatment with haloperidol, sulpiride, or clozapine in equivalent dose ratios based on their relative therapeutic potencies, on striatal dopamine receptor function both during treatment and following drug withdrawal. The average amounts of these drugs used in the control of schizophrenia are approximately 0.9 mg/kg/day for haloperidol, 20 mg/kg/day for sulpiride, and 6 mg/kg/day for clozapine (see Titeler and Seeman 1980; Martindale 1982).

Offprint requests to: C. D. Marsden

Materials and methods

Drug administration. Male Wistar rats (153 ± 5 g at the start of the experiment; Bantin and Kingman) were housed in groups of eight under standard conditions of laboratory lighting (12 h light/dark cycle) and temperature (21 ± 3° C). Animals received food and drinking water as required. Rats were randomly allocated to treatment with haloperidol (2 mg/kg/day), sulpiride (100 mg/kg/day) or clozapine (30 mg/kg/day) via their daily drinking water. Target drug doses were based on the daily clinical doses used in the control of schizophrenia (see Titeler and Seeman 1980; Martindale 1982), increased five times to offset the generally greater drug metabolising ability of the rat. A stock solution of 10 mg/ml haloperidol (Janssen Pharmaceutica, Belgium) was dissolved in a minimum quantity of glacial acetic acid and diluted to volume with distilled water. Sulpiride (SESIF, France) was dissolved in a minimum quantity of 2% v/v sulphuric acid and diluted with distilled water to give a stock solution of 50 mg/ml. Clozapine (Sandoz Products) was dissolved in a minimum quantity of 2 N hydrochloric acid and again diluted to volume with distilled water to give a 10 mg/ml solution. In each case, the pH of the stock solution was adjusted to between 5.5 and 7.0 using 2 N sodium hydroxide prior to further dilution with distilled drinking water. Housed alongside the drug-treated animals were age-matched control rats which received distilled drinking water alone.

Animals were assessed behaviourally and biochemically after 1, 2, and 4 weeks of drug treatment. No animal was examined behaviourally on more than one occasion, and animals examind biochemically had not been tested behaviourally within the previous week. Rats were also examined behaviourally and biochemically following withdrawal of drug treatment for between 5 and 7 days.

Apomorphine-induced stereotyped behaviour. Stereotyped behaviour induced by apomorphine hydrochloride (0.0625–2.0 mg/kg SC in the neck, 15 min previously; Sigma) was scored in six animals at each dose of apomorphine from each drug treatment group as follows: 0 = animals indistinguishable from saline-treated controls; 1= discontinuous sniffing and continuous locomotor activity; 2 = continuous sniffing, discontinuous locomotor activity; 3 = continuous sniffing with occasional licking, gnawing or biting; 4 = continuous licking, gnawing or biting; 5 = compulsive oral manipulation of faeces.

Specific striatal ^{3}H-spiperone binding. Specific ^{3}H-spiperone (15.5 Ci/mmole, Amersham International) binding was determined using a modification of the method of Leysen et al. (1978). Rats from control and drug-treated groups were killed by cervical dislocation and decapitation, and the brains rapidly removed and placed on ice. The paired corpora striata were dissected out and placed into ice-cold 50 mM Tris-HCl buffer (pH 7.6). Specific binding of ^{3}H-spiperone was determined using a range of six ligand concentrations (0.1–4.0 nM) and defined in the presence and absence of 10^{-5} M (−)-sulpiride. Specific binding was determined in triplicate at each ligand concentration on pooled striatal tissue from five animals from each group and on three separate occasions. The final incubation buffer contained 50 mM Tris-HCl (pH 7.4) with 120 mM sodium chloride. Data were examined by linear regression and Scatchard analysis to obtain estimates of the number of binding sites (B_{max}; pmoles/g wet weight tissue) and the equilibrium dissociation constant (K_D; nM).

Specific striatal ^{3}H-N,n-propylnorapomorphine (NPA) binding. Specific ^{3}H-NPA (60 Ci/mmole; New England Nuclear) binding was determined using a modification (Hall et al. 1981) of the technique of Leysen and Gommeren (1981) for ^{3}H-apomorphine binding. EDTA (1 mM) was incorporated into the homogenates and incubation buffers throughout the procedure to reduce non-specific binding. A range of six ligand concentrations (0.05–2.0 nM) was employed and specific binding was defined in the presence and absence of (±)-ADTN (10^{-6} M; 2-amino-6,7-dihydroxytetranaphthalene; Wellcome Laboratories). This concentration of ADTN is approximately 60 times the IC_{50} value for displacement of ^{3}H-NPA binding; displacement occured to the same level as found in the presence of an excess of dopamine antagonist drugs. Higher concentrations of ^{3}H-NPA were not employed because experiments in control animals had shown the presence of a lower affinity site appearing at ligand concentrations above 2.0 nM. Specific binding was determined in triplicate at each ligand concentration on pooled striatal tissue from five animals, and on three separate occasions. Data were examined by linear regression and Scatchard analysis to determine estimates of the number of binding sites (B_{max}; pmoles/g wet weight of tissue) and equilibrium dissociation constant (K_D; nM).

Assessment of free neuroleptic drug levels in brain using a reverse receptor binding technique. Free neuroleptic drug concentrations in striatal homogenates from rats treated for 1 week with haloperidol, sulpiride, or clozapine were estimated by the ability of samples of supernatant from such preparations to displace in vitro ^{3}H-spiperone binding (0.15 nM). The paired striata from three rats from each drug group were homogenised in 2 ml 50 mM Tris HCl buffer (pH 7.6) and centrifuged at 48,000 *g* for 10 min. Striatal tissue from untreated control animals was prepared in a similar manner; the supernatant was removed and serial dilutions of haloperidol (10^{-6}–10^{-12} M), sulpiride (10^{-5}–10^{-10} M) or clozapine (10^{-5}–10^{-10} M) were added to samples of supernatant from control animals. These concentrations of drug were employed for displacement of specific striatal ^{3}H-spiperone binding [defined in the presence and absence of (−)-sulpiride (10^{-5} M)]. Free concentrations of drug in the supernatant of tissue obtained from rats treated with haloperidol, sulpiride, or clozapine were determined by comparison of the displacement of ^{3}H-spiperone binding with that induced by known amounts of haloperidol, sulpiride, or clozapine added to supernatant from control tissue. Results are expressed as the concentration of drug per gram striatal tissue.

Dopamine-stimulated striatal adenylate cyclase activity. Dopamine stimulation of striatal adenylate cyclase activity was determined according to the method of Miller et al. (1974). Three rats from each drug treatment and from the control group were killed by cervical dislocation and decapitation. The brain was rapidly removed, placed on ice, and the paired corpora striata removed. Each pair of striata was separately homogenised in ice-cold buffer

(2 mM Tris maleate containing 2 mM EGTA pH 7.4) and basal and dopamine hydrochloride (1–150 μM; Sigma)-stimulated adenylate cyclase activity determined in duplicate at each dopamine concentration. The linear portions of the sigmoid concentration-response curves were subjected to linear regression analysis, and the stimulation of cyclic AMP formation over basal activity produced by 50 μM dopamine expressed as pmoles cyclic AMP formed/2.5 min/2 mg tissue.

Statistical analysis. Non-parametric stereotypy scores at each dose of apomorphine were compared between control and drug-treated groups by Kruskal-Wallis analysis of variance of ranks; in cases where resulting H scores were associated with a probability of less than 5%, groups were further analysed by paired Mann Whitney *U*-tests. Receptor binding data were subjected to linear regression analysis to determine estimates of the slope (K_D) and the intercept (B_{max}). Estimated binding parameters in control and drug-treated animals were compared by two-tailed Student's *t*-test. The stimulation of cyclic AMP formation produced by 50 μM dopamine in striatal tissue from control and drug-treated animals was compared by two-tailed Student's *t*-test.

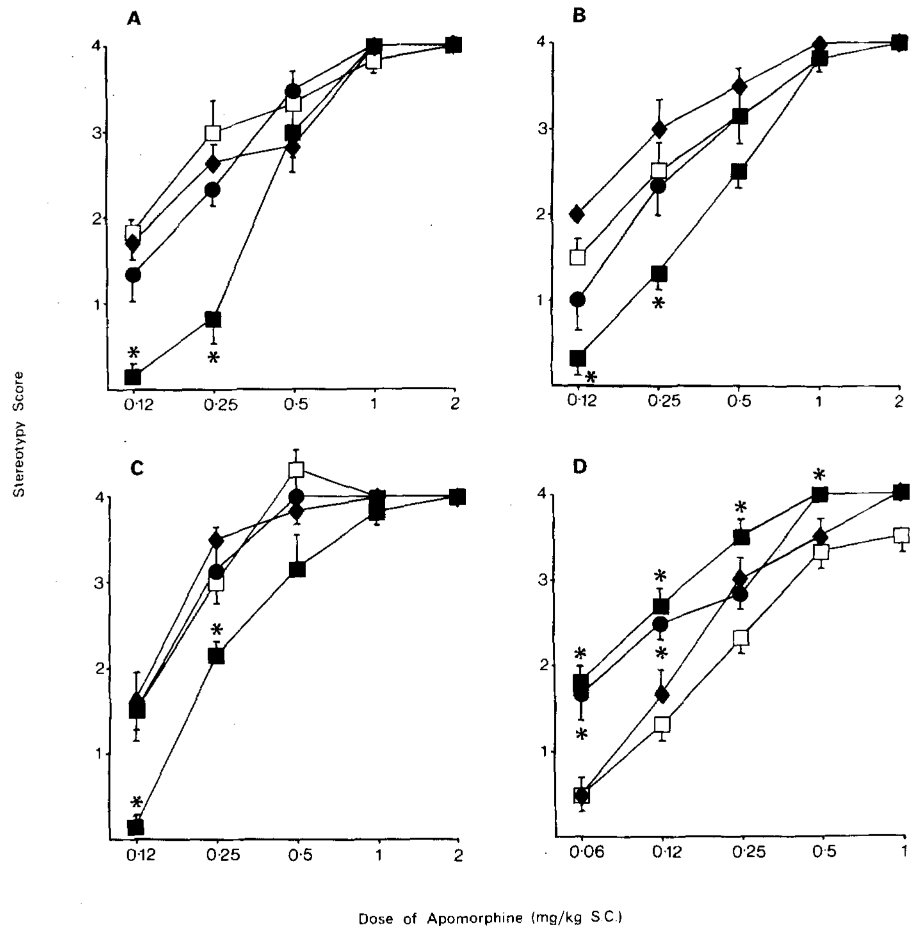

Fig. 1. Stereotyped behaviour induced by apomorphine hydrochloride (0.0625–2.0 mg/kg SC 15 min previously) in animals treated continuously in drinking water for 1 **(A)**, 2 **(B)**, or 4 **(C)** weeks and following subsequent drug withdrawal for 5 days **(D)** of haloperidol (1.7–1.9 mg/kg/day), sulpiride (112–126 mg/kg/day) or clozapine (30–35 mg/kg/day) compared to age-matched control animals. –□– Control; –■– haloperidol; –●– sulpiride; –◆– clozapine. Overall differences between the four groups were analysed using the Kruskal-Wallis analysis of variance of ranks at each time point

The following H scores and associated prolabilities at each dose of apomorphine were obtained

Dose of apomorphine (mg/kg)	Duration of treatment (weeks)			
	1	2	4	Withdrawal
0.0625				12.3, $P < 0.01$
0.125	11.2, $P < 0.01$	12.1, $P < 0.01$	10.8, $P < 0.05$	10.1, $P < 0.05$
0.25	12.4, $P < 0.01$	9.6, $P < 0.05$	9.0, $P < 0.05$	7.2, $P < 0.05$[a]
0.50	2.4, $P > 0.05$	5.1, $P > 0.05$	4.8, $P > 0.05$	5.9, $P = 0.05$[a]
1.0	1.2, $P > 0.05$	1.2, $P > 0.05$	1.2, $P > 0.05$	4.4, $P > 0.05$
2.0	0, $P > 0.05$	0, $P > 0.05$	0, $P > 0.05$	

[a] Values were obtained by comparison of control, haloperidol, and sulpiride-treated groups only. For data where H values were associated with a $P < 0.05$, groups were compared by paired Mann Whitney *U*-tests

* $P < 0.05$ compared to age-matched control animals, Mann Whitney *U*-test. Values are the mean ± 1 SEM scores obtained in six animals at each dose of apomorphine per drug-treatment group

Results

Drug intake. Neuroleptic drug solutions presented as drinking water were readily acceptable to the rats. The following average drug intakes were achieved over the 4-week period (mean ± 1 SEM): haloperidol, 1.7–1.9 mg/kg/day; sulpiride, 112–126 mg/kg/day, and clozapine 30–35 mg/kg/day. At the end of the study, animals had approximately doubled in body weight to 315 ± 8 g; there was no difference in weight between control and drug-treated animals. Throughout the study, neuroleptic-treated animals were indistinguishable from control animals in appearance and health.

Free concentrations of neuroleptic drug in striatal tissue. Specific binding of ^{3}H-spiperone to striatal tissue from control animals was displaced in a concentration-dependent manner by the supernatant from striatal homogenates taken from control rats containing either haloperidol ($10^{-6}-10^{12}$ M), sulpiride ($10^{-5}-10^{10}$ M) or clozapine ($10^{-5}-10^{-10}$ M). Supernatant from striatal tissue from rats treated for 1 week with haloperidol, sulpiride, or clozapine also displaced in vitro ^{3}H-spiperone binding. Examination of the displacement curves obtained with known amounts of haloperidol, sulpiride, or clozapine revealed the following free concentrations of neuroleptic drugs in the supernatant of striatal tissue from haloperidol, sulpiride, or clozapine-treated animals respectively: haloperidol, 1.3×10^{-10} M; sulpiride, 5.2×10^{-8} M, and clozapine, 2.4×10^{-7} M per gram striatal tissue.

Apomorphine-induced stereotyped behaviour. After 1, 2, and 4 weeks of continuous haloperidol treatment (1.7–1.9 mg/kg/day), the stereotyped response to low doses of apomorphine (0.125–0.25 mg/kg SC 15 min previously) was reduced compared to control animals. Stereotypy induced by higher doses of apomorphine (0.5–2.0 mg/kg) was not antagonised by haloperidol treatment (Fig. 1). Administration of sulpiride (112–126 mg/kg/day) or clozapine (30–35 mg/kg/day) to rats for 1, 2, or 4 weeks did not alter stereotyped behaviour induced by any dose of apomorphine (Fig. 1). When animals were withdrawn for 5 days from haloperidol treatment, the dose-response curve for apomorphine-induced stereotypy (0.0625–0.5 mg/kg) was shifted to the left. Stereotyped behaviour induced by the highest dose of apomorphine (1.0 mg/kg) was not exaggerated owing to the near maximal response obtained in control rats (Fig. 1). In rats withdrawn from sulpiride treatment for 5 days, stereotyped behaviour was enhanced with some, but not all doses of apomorphine (0.0625, 0.125, and 0.5 mg/kg). Following withdrawal from clozapine treatment, there was no change in stereotyped behaviour by comparison to control animals (Fig. 1).

Specific striatal ^{3}H-spiperone binding. In control animals, total binding of ^{3}H-spiperone to striatal membranes increased with increasing ligand concentration (0.1–4.0 nM). Specific ^{3}H-spiperone binding was saturable at high ligand concentrations (2.0–4.0 nM) which on Scatchard analysis showed a single site. Similarly, following continuous treatment for up to 4 weeks with haloperidol, sulpiride, or clozapine and following drug withdrawal, evidence of saturability, and linearity of specific binding was obtained (see Table 1).

Table 1. Specific striatal ^{3}H-spiperone (0.1–4.0 nM) binding sites (B_{max}) and equilibrium dissociation constant (K_D) after 1, 2, and 4 weeks continuous administration and subsequent withdrawal of haloperidol (1.7–1.9 mg/kg/day), sulpiride (112–126 mg/kg day) or clozapine (30–35 mg/kg/day) compared to age-matched control animals

Treatment	Duration of treatment (weeks)	B_{max} (pmoles/g wet weight of tissue)	K_D (nM)	Correlation coefficient
Control	1	28.2 ± 2.4	0.12 ± 0.001	0.97 ± 0.02
	2	23.5 ± 1.4	0.09 ± 0.003	0.99 ± 0.01
	4	22.5 ± 1.9	0.09 ± 0.005	0.98 ± 0.01
	Withdrawal	23.4 ± 1.2	0.10 ± 0.006	0.97 ± 0.01
Haloperidol	1	33.8 ± 2.4	0.17 ± 0.005*	0.98 ± 0.01
	2	35.1 ± 1.4*	0.18 ± 0.003*	0.98 ± 0.01
	4	38.7 ± 1.0*	0.17 ± 0.010*	0.98 ± 0.01
	Withdrawal	33.2 ± 1.2*	0.13 ± 0.004*	0.98 ± 0.01
Sulpiride	1	30.2 ± 1.2	0.13 ± 0.011	0.98 ± 0.01
	2	27.4 ± 1.3	0.13 ± 0.008*	0.97 ± 0.01
	4	28.3 ± 0.9	0.12 ± 0.080	0.97 ± 0.02
	Withdrawal	24.3 ± 1.4	0.10 ± 0.008	0.97 ± 0.01
Clozapine	1	31.7 ± 1.1	0.15 ± 0.007	0.98 ± 0.01
	2	26.6 ± 1.4	0.10 ± 0.007	0.97 ± 0.01
	4	26.2 ± 1.8	0.10 ± 0.003	0.98 ± 0.01
	Withdrawal	25.0 ± 1.2	0.11 ± 0.012	0.98 ± 0.01

Values are the mean (± 1 SEM) for determinations carried out on three separate tissue pools from five animals, examined in triplicate at each ligand concentration. Data were subjected to linear regression and Scatchard analysis of mean data for each ligand concentration to obtain B_{max} and K_D values
Specific binding of ^{3}H-spiperone was defined using (−)-sulpiride (10^{-5} M)
* $P < 0.05$ compared to values in age-matched control animals, Student's *t*-test

Table 2. Specific striatal ^{3}H-N,*n*-propylnorapomorphine (0.05–2.0 nM) binding sites (B_{max}) and equilibrium dissociation constant (K_D) after 1, 2, and 4 weeks continuous administration and subsequent withdrawal of haloperidol (1.7–1.9 mg/kg/day), sulpiride (112–126 mg/kg/day) or clozapine (30–35 mg/kg/day) compared to age-matched control animals

Treatment	Duration of treatment (weeks)	B_{max} (pmoles/g wet weight of tissue)	K_D (nM)	Correlation coefficient
Control	1	9.9 ± 0.6	0.34 ± 0.80	0.91 ± 0.03
	2	13.2 ± 0.6	0.32 ± 0.01	0.93 ± 0.02
	4	9.3 ± 0.4	0.24 ± 0.02	0.94 ± 0.02
	Withdrawal	15.9 ± 0.6	0.46 ± 0.02	0.97 ± 0.01
Haloperidol	1	9.9 ± 1.2	0.42 ± 0.04	0.94 ± 0.01
	2	15.7 ± 1.8	0.60 ± 0.08*	0.88 ± 0.04
	4	10.0 ± 0.1	0.39 ± 0.01*	0.91 ± 0.03
	Withdrawal	23.4 ± 1.2*	0.70 ± 0.10*	0.97 ± 0.01
Sulpiride	1	13.2 ± 0.8*	0.58 ± 0.13	0.95 ± 0.01
	2	13.0 ± 0.9	0.31 ± 0.02	0.91 ± 0.01
	4	10.3 ± 0.3	0.28 ± 0.02	0.93 ± 0.01
	Withdrawal	18.7 ± 0.8*	0.53 ± 0.03	0.97 ± 0.01
Clozapine	1	11.6 ± 0.7	0.48 ± 0.12	0.95 ± 0.01
	2	12.4 ± 0.3	0.31 ± 0.01	0.93 ± 0.03
	4	9.2 ± 0.2	0.24 ± 0.01	0.96 ± 0.01
	Withdrawal	17.2 ± 1.7	0.51 ± 0.03	0.95 ± 0.02

Values are the mean (± 1 SEM) for determinations carried out on three separate tissue pools from five animals examined in triplicate at each ligand concentration. Data were subjected to Scatchard and linear regression analysis of mean data for each ligand concentration to obtain B_{max} and K_D values
Specific binding of ^{3}H-NPA was defined using ADTN (10^{-6} M)
* $P < 0.05$ compared to value for age-matched control animals, Student's *t*-test

After 1 week of haloperidol administration, B_{max} for striatal ^{3}H-spiperone binding was not different from values obtained in striatal preparations from control animals. After 2 and 4 weeks of haloperidol treatment, B_{max} values were elevated (Table 1). In contrast, treatment with either sulpiride or clozapine did not alter B_{max} values for specific striatal ^{3}H-spiperone binding during the course of 4 weeks treatment (Table 1). Following 7 days drug withdrawal, B_{max} was elevated in striatal tissue from rats treated with haloperidol, but not sulpiride or clozapine (Table 1).

The dissociation constant (K_D) was elevated at 1, 2, and 4 week of haloperidol treatment, and after 2 weeks of sulpiride treatment, but not at other times (Table 1). K_D was unaltered during clozapine treatment. After drug withdrawal, K_D was elevated in tissue from haloperidol but not sulpiride or clozapine-treated animals (Table 1).

Specific striatal ^{3}H-NPA binding. Total and specific striatal ^{3}H-NPA binding in tissue from control and drug-treated animals increased with increasing ligand concentration (0.05–2.0 nM). Specific ^{3}H-NPA binding was saturable at high ligand concentrations (1.0–2.0 nM). Saturability and linearity of plots for ^{3}H-NPA binding was obtained after drug treatment for up to 4 weeks with haloperidol, sulpiride, or clozapine, and following drug withdrawal (see Table 2).

Scatchard analysis of these data indicated that during continuous treatment of rats with haloperidol for 1, 2, or 4 weeks, there was no change in B_{max} for ^{3}H-NPA binding (Table 2). Treatment with sulpiride for 1 week caused an increase in B_{max} which had returned to normal levels after 2 and 4 weeks. Clozapine administration for up to 4 weeks had no effect on B_{max} (Table 2). After 7 days drug withdrawal, B_{max} for ^{3}H-NPA binding was elevated in striatal tissue from rats treated with haloperidol and sulpiride, but not clozapine (Table 2).

K_D for ^{3}H-NPA binding was unchanged after 1 week of haloperidol treatment, but was elevated after 2 and 4 weeks (Table 2). K_D was not affected by treatment for up to 4 weeks with either sulpiride or clozapine (Table 2). K_D was also elevated in tissue from animals withdrawn from haloperidol, but not sulpiride or clozapine treatment (Table 2).

Dopamine-stimulated striatal adenylate cyclase activity. Dopamine (1–150 μM) caused a concentration-dependent increase in cyclic AMP formation in striatal tissue from control and drug-treated animals. There was no difference in basal enzyme activity in the absence of dopamine in tissue from control or drug-treated animals. The following mean basal activities (± 1 SEM), expressed as pmoles cyclic AMP formed/2.5 min/2 mg tissue, were obtained: control, 43 ± 14; haloperidol-treated, 33 ± 8; sulpiride-treated, 40 ± 8, and clozapine-treated, 33 ± 7 ($P < 0.05$ by comparison to control animals, Student's *t*-test). In the presence of dopamine (50 μM), cyclic AMP formation was approximately doubled in all groups (Table 3).

The increase in cyclic AMP formation induced by 50 μM dopamine appeared reduced after 1 week of haloperidol treatment, although this failed to reach statistical significance (Table 3). Dopamine-stimulated striatal cyclic AMP formation in tissue from animals treated with haloperidol, sulpiride, or clozapine did not differ from that in control animals after 1, 2, or 4 weeks of treatment, or following drug withdrawal (Table 3).

Table 3. Striatal dopamine (50 μM)-stimulated adenylate cyclase activity after 1, 2, and 4 weeks continuous administration and subsequent withdrawal of haloperidol (1.7–1.9 mg/kg/day), sulpiride (112–126 mg/kg/day) or clozapine (30–35 mg/kg/day) compared to age-matched control animals

Treatment	Duration of treatment (weeks)	Dopamine (50 μM)-stimulated cyclic AMP formation over basal (pmoles/2.5 min/2 mg tissue)
Control	1	29.5 ± 3.0
	2	25.2 ± 4.9
	4	27.9 ± 3.0
	Withdrawal	44.7 ± 1.8
Haloperidol	1	17.7 ± 3.5
	2	26.2 ± 3.5
	4	27.2 ± 2.8
	Withdrawal	32.4 ± 7.8
Sulpiride	1	37.1 ± 7.8
	2	36.8 ± 3.8
	4	27.0 ± 4.4
	Withdrawal	40.0 ± 5.8
Clozapine	1	31.2 ± 1.6
	2	33.9 ± 8.7
	4	26.3 ± 5.5
	Withdrawal	32.7 ± 4.3

* $P < 0.05$ vs control animals, Student's t-test, $n = 3$

Discussion

Many previous studies have shown that repeated administration of haloperidol for a few weeks, when followed by drug withdrawal, induces behavioural supersensitivity to apomorphine and increases the number of ^{3}H-spiperone binding sites in the striatum (Tarsy and Baldessarini 1974; Burt et al. 1977; Jenner et al. 1982). The present study was designed to investigate whether the atypical neuroleptic agents sulpiride and clozapine induce dopamine receptor supersensitivity as classical neuroleptic compounds do, when given in clinically equivalent dose ratios.

We found that withdrawal from continuous oral administration of haloperidol induced behavioural supersensitivity to apomorphine, and that the number of ^{3}H-spiperone binding sites was increased even during the course of continuous haloperidol administration. This indicates that the increased number of ^{3}H-spiperone binding sites occurs as a consequence of haloperidol administration independently of a period of drug withdrawal. As we have reported previously, those sites identified by ^{3}H-NPA were also increased in number following haloperidol withdrawal (Jenner et al. 1982). although there was no change in B_{max} for ^{3}H-NPA binding whilst drug administration continued. This contrasts with the findings of Goldstein et al. (1980), who reported no increase in ^{3}H-NPA binding following withdrawal from haloperidol treatment.

These data might be taken to indicate that agonist and antagonist binding sites do not respond to continuous neuroleptic treatment in an identical manner. The presence of neuroleptic drug in the striatal preparations, however, may complicate the interpretation of these findings. Whilst antagonist drugs displace antagonist ligands (such as ^{3}H-spiperone) in a competitive manner, the displacement of agonist ligands (such as ^{3}H-NPA) by antagonists is not competitive, but occurs by a mixed-type inhibition (Sibley and Creese 1980; Leysen and Gommeren 1981). Hence, B_{max} values measured for ^{3}H-NPA binding in the presence of continuing drug treatment may be only an apparent value, and may be lower than values measured in the absence of the neuroleptic drug. Such non-competitive inhibition of ^{3}H-NPA binding might be expected to cause non-linearity of Scatchard plots (Leysen and Gommeren 1981). However, even in animals receiving neuroleptic treatment, regression analysis did not reveal any deviation from linearity, suggesting that neuroleptic present in the tissue may not have reduced ^{3}H-NPA binding through non-competitive inhibition.

The ability of apomorphine to induce stereotyped behaviour was reduced in animals still receiving haloperidol, and was exaggerated only following drug withdrawal, at a time when ^{3}H-NPA binding was increased.

Repeated treatment and subsequent withdrawal of haloperidol in the present study failed to alter striatal dopamine-stimulated adenylate cyclase activity, a finding in agreement with previous studies (von Voigtlander et al. 1975; Rotrosen et al. 1975; Heal et al. 1976; Jenner et al. 1982), although others have observed an enhancement of enzyme activity following such treatment (Gnegy et al. 1977).

In contrast to haloperidol, sulpiride treatment did not increase the number of striatal ^{3}H-spiperone binding sites either during treatment or following withdrawal. It should be pointed out, however, that in two previous studies from this department, we have found that a *high* dose of sulpiride (2 × 100 mg/kg) given as an IP bolus for 21 days did increase ^{3}H-spiperone binding following drug withdrawal (Jenner et al. 1982; Fleminger et al. 1983). In contrast, the present study employed PO administration of sulpiride, the drug being ingested freely over a period of several hours. When given orally in man, the bioavailability of sulpiride may be as low as 25% (Weisel et al. 1980). The differences between our previous and the present findings may, therefore, relate to relatively low blood and brain levels of sulpiride achieved by PO rather than IP dosage. Consistent with this proposal, those investigators who failed to observe an increase in ^{3}H-spiperone binding sites either administered sulpiride PO (Bannet et al. 1980) or administered a relatively low dose (20 mg/kg IP) (Trabucchi et al. 1980). However, we were able to detect the presence of sulpiride in brain tissue from rats treated with this drug. Although (±)-sulpiride is some 120 times less potent than haloperidol in displacing ^{3}H-spiperone binding in vitro (Creese et al. 1979a), the free brain drug levels of sulpiride achieved in this study were 400 times higher than those of haloperidol. Under these conditions, therefore, it is rather surprising that the administration of haloperidol, but not sulpiride, altered the number of striatal ^{3}H-spiperone binding sites. Moreover, these observations indicate that the differential effects of haloperidol or sulpiride treatment on striatal dopamine receptors may not be attributable to the poor brain penetration of sulpiride, since the dose of this compound was only 50 times higher than that of haloperidol.

Unlike haloperidol, sulpiride treatment failed to alter stereotyped behaviour induced by apomorphine while animals were receiving the drug, but once treatment was withdrawn, like haloperidol-treated rats, stereotyped

behaviour was exaggerated, as as been observed previously (Costall et al. 1978; Jenner et al. 1982; Fleminger et al. 1983). The failure of sulpiride to antagonise apomorphine-induced stereotypy during drug treatment may be attributable to insufficient receptor occupation by this compound. This interpretation is consistent with the ability of sulpiride to induce dopamine receptor supersensitivity when given in high doses, but is questionable for several reasons. Firstly, sulpiride is a drug which on acute administration, even in near toxic doses, does not inhibit stereotyped behaviour (Costall and Naylor 1975), yet on withdrawal, sulpiride treatment enhances this behaviour. Secondly such enhancement occurred in the absence of any apparent change in the number of ^{3}H-spiperone binding sites. Thirdly, the free brain drug levels of sulpiride detected in this study were some 400 times higher than those of haloperidol; under these conditions both compounds would be expected to occupy dopamine receptor sites as judged from their in vitro potencies in displacing ^{3}H-spiperone binding (Creese et al. 1979a). Rather, the behavioural effects of sulpiride appear to correlate more closely with sites identified by ^{3}H-NPA, which were mostly unaltered during sulpiride treatment, but were increased following its withdrawal. This finding is in contrast to the currently held view that the functional effects of neuroleptic drugs correlate with changes in sites labelled by neuroleptic ligands. It is not clear why sulpiride treatment should have caused a selective increase in ^{3}H-NPA and not ^{3}H-spiperone sites. In vitro binding assays do not indicate a selectivity of sulpiride at ^{3}H-NPA rather than ^{3}H-spiperone binding sites. However, it is known that the binding site for ^{3}H-sulpiride differs from that labelled by other groups of neuroleptic drugs; there is an absolute dependency on the presence of sodium ions for specific binding (Theodorou et al. 1980). One possible explanation may be that the ^{3}H-sulpiride site is more closely related to the ^{3}H-NPA site than the ^{3}H-spiperone site. This possibility is suggested indirectly by the differentiation of agonist and antagonist binding sites according to their sensitivity to guanyl nucleotides (Creese et al. 1978, 1979b). Guanosine triphosphate is thought to be involved in the coupling between D-1 receptors and adenylate cyclase. Although sulpiride is an antagonist of dopamine receptors not linked to adenylate cyclase, this compound may modulate cyclic AMP formation in striatal prisms (Stoof and Kebabian 1981). Such modulating processes in other neurotransmitter systems may be sodium-dependent. Potentially, therefore, both sulpiride and NPA may be able, indirectly, to modulate the activity of adenylate cyclase-linked receptors. However, in the present study, sulpiride treatment, like haloperidol, did not alter the ability of dopamine to stimulate adenylate cyclase activity either during treatment or following withdrawal.

Administration of clozapine to rats, unlike haloperidol or sulpiride, did not alter any parameter of dopamine function examined, whether during drug treatment or after drug withdrawal. This is in agreement with many previous studies showing that dopamine receptor supersensitivity is not induced by repeated clozapine treatment (Sayers et al. 1975; Gnegy et al. 1977; Kobayashi et al. 1978; Racagni et al. 1980; Seeger et al. 1982). In two other studies, however, clozapine treatment increased both the stereotyped response to apomorphine and the number of striatal ^{3}H-spiperone binding sites (Smith and Davis 1976; Allikmets et al. 1981). The reason for the discrepancies in these studies is not clear. In previous studies, even when the same dose of clozapine was used (20 mg/kg/day IP) for the same duration (3 weeks), opposite findings have been obtained in independent studies (Allikmets et al. 1981; Seeger et al. 1982). The failure of clozapine to alter striatal dopamine function in the present study appears unlikely to be due to insufficient dosage levels, since the brain concentration of clozapine achieved was nearly 2,000 times higher than that for haloperidol, and yet clozapine is only 60 times less potent than haloperidol in displacing ^{3}H-spiperone binding in vitro (Creese et al. 1979a). As for sulpiride, it would appear that the atypical actions of clozapine are not attributable to poor brain penetration by this compound since it was administered in a dose only 15 times higher than that of haloperidol.

Whilst clozapine does possess demonstrable dopamine receptor antagonist acitivity, as evidenced by the elevation of cerebral dopamine turnover (Anden and Stock 1973; Bartholini 1967), in vitro inhibition of adenylate cyclase activity (Clement-Cormier et al. 1974), and ^{3}H-neuroleptic binding (Creese et al. 1979a), and the induction of catalepsy (Costall and Naylor 1975), such activity is weak by comparison to other neuroleptic drugs, and may not be sufficient to cause striatal dopamine receptor supersensitivity. Only a small proportion of ^{3}H-clozapine binding is to cerebral dopamine receptors (Burki 1980) and, in particular, the high anticholinergic activity of this drug is believed to protect against the emergence of striatal dopamine receptor supersensitivity (Miller and Hiley 1974; Snyder et al. 1974).

In conclusion, we have found that repeated administration of clinically relevant doses of haloperidol, sulpiride, and clozapine affect striatal dopamine function in different ways and that these findings cannot be attributed to differences in the brain levels of these compounds. Our observations may be of importance with respect to the low incidence of extrapyramidal side-effects induced by sulpiride and clozapine in man.

Acknowledgements. This study was supported by the Medical Research Council and the Research Funds of King's College Hospital and the Bethlem Royal and Maudsley Hospitals. M.D.H. is an MRC Scholar.

References

Alberts JL (1983) Survey presented at Symposium on Sulpiride, Paris

Allikmets LH, Zarkovsky AM, Nurk AM (1981) Changes in catalepsy and receptor sensitivity following chronic neuroleptic treatment. Eur J Pharmacol 75: 145–147

Anden NE, Stock G (1973) Effect of clozapine on the turnover of dopamine in the corpus striatum and in the limbic system. J Pharm Pharmacol 25: 346–348

Bannet J, Gillis S, Ebstein RP, Belmaker RH (1980) The effect of chronic sulpiride on striatal spiperone binding. Int Pharmacopsychiatry 15: 334–337

Bartholini G (1976) Differential effect of neuroleptic drugs on dopamine turnover in the extrapyramidal and limbic system. J Pharm Pharmacol 28: 429–433

Burki HR (1980) Inhibition of ^{3}H-clozapine binding in rat brain after oral administration of neuroleptics. Life Sci 26: 2187–2193

Burt DR, Creese I, Snyder SH (1977) Antipsychotic drugs: chronic treatment elevates dopamine receptor binding in brain. Science 196: 326–328

Clement-Cormier YC, Kebabian JW, Petzold GL, Greengard P (1974) Dopamine-sensitive adenylate cyclase in mammalian brain: a possible site of action of antipsychotic drugs. Proc Natl Acad Sci USA 71: 1113–1117

Costall B, Naylor RJ (1975) Detection of the neuroleptic properties of clozapine, sulpiride and thioridazine. Psychopharmacology 43: 69–74

Costall B, Naylor RJ, Owen RT (1978) Behavioural correlates of modified dopaminergic/anticholinergic responses following chronic treatment with neuroleptic agents of differing activity spectra. Eur J Pharmacol 48: 29–36

Creese I, Prosser T, Snyder SH (1978) Dopamine receptor binding: specificity, localisation and regulation by ions and guanyl nucleotides. Life Sci 23: 495–500

Creese I, Stewart K, Snyder SH (1979a) Species variations in dopamine receptor binding. Eur J Pharmacol 60: 55–66

Creese I, Usdin T, Snyder SH (1979b) Guanine nucleotides distinguish between two dopamine receptors. Nature 278: 577–578

Fleminger S, Rupniak NMJ, Hall MD, Jenner P, Marsden CD (1983) Changes in apomorphine-induced stereotypy as a result of subacute neuroleptic treatment correlates with increased D-2 receptors, but not with increases in D-1 receptors. Biochem Pharmacol 32: 2921–2927

Fuxe K, Ogren S-O, Hall H, Agnati LF, Anderson K, Kohler C, Schwartz R (1980) Effect of chronic treatment with l-sulpiride and haloperidol on central monoaminergic mechanisms. In: Cattabeni F, Racagni G, Spano PF, Costa E (eds) Long-term effects of neuroleptics. Raven Press, New York, Adv Biochem Psychopharmacol 24: 193–197

Gerlach J, Koppelhuis P, Helweg E, Monrad A (1974) Clozapine and haloperidol in a single blind cross-over trial: therapeutic and biochemical aspects in the treatment of schizophrenia. Acta Psychiatr Scand 50: 410–424

Gnegy M, Uzunov P, Costa E (1977) Participation of an endogenous Ca^{++}-binding protein activator in the development of drug-induced supersensitivity of striatal dopamine receptors. J Pharmacol Exp Ther 202: 558–564

Goldstein M, Lew JY, Asaro T, Ueta K (1980) Alterations in dopamine receptors: effect of lesion and haloperidol treatment. Commun Psychopharmacol 4: 21–25

Hall MD, Jenner P, Marsden CD (1981) Characteristics of in vitro binding of ^{3}H-N,n-propylnorapomorphine to rat striatal preparations. Neurosci Lett Suppl 7: 363

Heal DJ, Green AR, Boullin DG, Grahame-Smith DG (1976) Single and repeated administration of neuroleptic drugs to rats: effects on striatal dopamine-sensitive adenylate cyclase and locomotor activity produced by tranylcypromine and L-tryptophan or L-dopa. Psychopharmacology 49: 287–300

Jenner P, Hall MD, Murugaiah K, Rupniak N, Theodorou A, Marsden CD (1982) Repeated administration of sulpiride for three weeks produces behavioural and biochemical evidence for cerebral dopamine receptor supersensitivity. Biochem Pharmacol 31: 325–328

Kobayashi RM, Fields JZ, Hruska RE, Beaumont K, Yamamura HI (1978) Brain neurotransmitter receptors and chronic antipsychotic drug treatment: a model for tardive dyskinesia. In: Usdin E (ed) Animal models in psychiatry. Pergamon Press, New York, pp 405–409

Leysen JE, Gommeren W (1981) Optimal conditions for ^{3}H-apomorphine binding and anomalous equilibrium binding of ^{3}H-apomorphine and ^{3}H-spiperone to rat striatal membranes: involvement of surface phenomena versus multiple binding sites. J Neurochem 36: 201–219

Leysen JE, Gommeren W, Laduron PM (1978) Spiperone: a ligand of choice for neuroleptic receptors. I. Kinetics and characteristics of in vitro binding. Biochem Pharmacol 27: 307–316

Martindale (1982) The Extra Pharmacopoeia 28th Edition, EF Reynolds (ed). Pharmaceutical Press, London

Matz R, Rick W, Oh D, Thompson H, Gershon S (1974) Clozapine – a potential antipsychotic agent without extrapyramidal manifestations. Current Therapeutic Research 16: 687–695

Miller R, Hiley CR (1974) Anti-muscarinic properties of neuroleptics and drug-induced parkinsonism. Nature 248: 596–597

Miller RJ, Horn AS, Iversen LL (1974) The action of neuroleptic drugs on dopamine stimulated adenosine cyclic 3′,5′-monophosphate production in rat neostriatum and limbic forebrain. Mol Pharmacol 10: 759–766

Racagni G, Bruno F, Bugatti A, Parenti M, Apud JA, Santini V, Carenzi G, Groppetti A, Cattabeni F (1980) Behavioural and biochemical correlates after haloperidol and clozapine long-term treatment. In: Catabeni G, Racagni G, Spano PF, Costa E (eds) Long-term effects of neuroleptics. Raven Press, New York. Adv Biochem Psychopharmacol 24: 45–52

Rotrosen J, Friedman E, Gershon S (1975) Striatal adenylate cyclase activity following reserpine and chronic chlorpromazine administration in rats. Life Sci 17: 563–568

Sayers AC, Burki HR, Ruch W, Asper H (1975) Neuroleptic-induced hypersensitivity of striatal dopamine receptors in the rat as a model of tardive dyskinesias. Effects of clozapine, haloperidol, loxapine and chlorpromazine. Psychopharmacology 41: 97–104

Seeger TF, Thal L, Gardner EL (1982) Behavioural and biochemical aspects of neuroleptic-induced dopaminergic supersensitivity: studies with chronic clozapine and haloperidol. Psychopharmacology 76: 182–187

Sibley DR, Creese I (1980) Pseudo non-competitive agonist interactions with dopamine receptors. Eur J Pharmacol 65: 131–133

Simpson GM, Varga E (1974) Clozapine – a new antipsychotic agent. Current Therapeutic Research 16: 679–686

Smith RC, Davis JM (1976) Behavioural evidence for supersensitivity after chronic administration of haloperidol, clozapine and thioridazine. Life Sci 19: 725–732

Snyder SH, Greenberg D, Yamamura HI (1974) Antischizophrenic drugs and brain cholinergic receptors. Affinity for muscarinic sites predicts extrapyramidal effects. Arch Gen Psychiatry 31: 58–61

Stoof JC, Kebabian JW (1981) Opposing roles for D1 and D2 dopamine receptors in efflux of cylic AMP from rat neostriatum. Nature 294: 366–368

Tarsy D, Baldessarini RJ (1974) Behavioural supersensitivity to apomorphine following chronic treatment with drugs which interfere with synaptic function of catecholamines. Neuropharmacology 13: 927–940

Theodorou AE, Hall MD, Jenner P, Marsden CD (1980) Cation regulation differentiates specific binding of ^{3}H-sulpiride and ^{3}H-spiperone to rat striatal preparations. J Pharm Pharmacol 32: 441–444

Titeler M, Seeman P (1980) Radioreceptor labeling of pre- and post-synaptic dopamine receptors. In: Cattabeni F, Racagni G, Spano PF, Costa E (eds) Long-term effects of neuroleptics. Raven Press, New York. Adv Biochem Psychopharmacol 24: 159–166

Trabucchi M, Memo M, Battaini F, Regiani A, Spano PF (1980) Effect of long-term treatment with haloperidol and sulpiride on different types of dopaminergic receptors. In: Cattabeni F, Racagni G, Spano PF, Costa E (eds) Long-term effects of neuroleptics. Raven Press, New York. Adv Biochem Psychopharmacol 24: 275–282

von Voigtlander PF, Losey EG, Triezenberg HJ (1975) Increased sensitivity to dopaminergic agents after chronic neuroleptic treatment. J Pharmacol Exp Ther 193: 88–94

Wiesel F-A, Alfredsson G, Ehrnebo M, Sedvall G (1980) The pharmacokinetics of intravenous and oral sulpiride in healthy human subjects. Eur J Clin Pharmacol 17: 385–391

Received November 3, 1982; Final version July 3, 1984