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CHRONIC TREATMENT WITH CLOZAPINE, UNLIKE HALOPERIDOL, DOES NOT INDUCE CHANGES IN STRIATAL D-2 RECEPTOR FUNCTION IN THE RAT

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Abstract—Comparison has been made of the effects on brain dopamine function of chronic administration of haloperidol or clozapine to rats for up to 12 months.

In rats treated for 1–12 months with haloperidol (1.4–1.6 mg/kg/day), purposeless chewing jaw movements emerged. These movements were only observed after 12 months' treatment with clozapine (24–27 mg/kg/day). Apomorphine-induced (0.125–0.25 mg/kg) stereotyped behaviour was inhibited during 12 months treatment with haloperidol. Clozapine treatment was without effect. After 12 months, stereotypy induced by higher doses of apomorphine (0.5–1.0 mg/kg) was enhanced in haloperidol, but not clozapine, treated rats.

B_{max} for striatal ^{3}H-spiperone binding was elevated throughout 12 months of haloperidol administration, but was not altered by clozapine treatment. B_{max} for striatal ^{3}H-NPA binding was only elevated after 12 months of haloperidol treatment; clozapine treatment was without effect. B_{max} for ^{3}H-piflutixol binding was not altered by haloperidol treatment, but was increased after 9 and 12 months of clozapine treatment. Dopamine (50 μM)-stimulated adenylate cyclase activity was inhibited after 1 month's haloperidol treatment but normal thereafter. Adenylate cyclase activity was not altered by chronic clozapine treatment. Striatal acetylcholine content was increased after 3 and 12 months of haloperidol or clozapine intake.

These findings indicate that the chronic administration of the atypical neuroleptic clozapine does not produce changes in brain dopamine function which mirror those of the typical neuroleptic haloperidol. In particular, chronic administration of clozapine, unlike haloperidol, does not appear to induce striatal D-2 receptor supersensitivity. Unexpectedly, clozapine treatment, unlike haloperidol, altered D-1 receptor function.

Chronic neuroleptic therapy for months or years is associated with the emergence of tardive dyskinesias while drug administration continues. These movements resemble involuntary movements occurring in Parkinsonian patients treated with L-DOPA and have been attributed to an overactivity of brain dopamine function [1]. In rodents treated for several weeks with classical neuroleptic agents, such as haloperidol, followed by drug withdrawal for several days, dopamine receptor supersensitivity is apparent from an exaggeration of apomorphine-induced stereotyped behaviour [2] and an increase in striatal dopamine receptor numbers (B_{max}) identified by ^{3}H-spiperone [3, 4]. Such supersensitivity also occurs when rats receive neuroleptic drugs, such as trifluoperazine, thioridazine, haloperidol or *cis*-flupenthixol, continuously for between 6 and 18 months [5–8]. These changes are accompanied by increased striatal acetylcholine concentrations, suggesting the development of enhanced inhibitory dopaminergic tone due to a functional overactivity of striatal dopamine receptors [9].

Unlike classical neuroleptic agents, clozapine is claimed not to induce tardive dyskinesia in man [10–12]. When given to rodents for several weeks, followed by drug withdrawal for some days, clozapine treatment, unlike haloperidol, may not induce an exaggeration of apomorphine-induced stereotypy or an increase in striatal ^{3}H-spiperone binding [13–18], although this finding is disputed [19–21].

In the present study we have examined the effects of *continuous* administration of therapeutically equivalent doses of clozapine (24–27 mg/kg/day) or haloperidol (1.4–1.6 mg/kg/day) to rats for up to one year on striatal dopamine function. We find that clozapine, unlike haloperidol, does not alter striatal D-2 receptor function but has some effects on D-1 receptors identified by ^{3}H-piflutixol.

MATERIALS AND METHODS

Drug administration. Male Wistar rats (205 ± 14 g at the start of the experiment; Bantin & Kingman Ltd.) were housed initially in groups of 8 under standard laboratory conditions of lighting (12 hr light/dark cycle) and temperature (21 ± 3°). Animals were randomly allocated to one of three groups in which they received as their daily drinking water for a continuous period up to 12 months either distilled water only (controls), or haloperidol solution (target dose 2 mg/kg/day) or clozapine solution (target dose 30 mg/kg/day). All animals had free access to food.

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Drug doses were based on the average daily clinical doses used in the control of schizophrenia [22], increased by an arbitrary factor of five times to offset the generally greater drug metabolising ability of the rat. Haloperidol (Janssen Pharmaceutica, Belgium) was dissolved in a minimum quantity of glacial acetic acid, and clozapine (Sandoz, U.K.) was dissolved in a minimum volume of 2 N hydrochloric acid. Drug solutions were diluted with distilled water to give stock solutions of 10 mg/ml, and the pH adjusted to between 5.5 and 6.0 using 2 N sodium hydroxide. Stock solutions were then further diluted with distilled water and presented to rats as drinking water. Behavioural and biochemical testing was carried out at 1, 3, 6, 9 and 12 months after initiation of drug treatment and occurred whilst animals continued to receive neuroleptic intake. A period of at least 3 months elapsed between behavioural testing of the same animals. Rats used for behavioural studies were not used for biochemical determinations within a single testing period.

Spontaneous chewing jaw movements. Individual rats were observed on a clean table area measuring 45 × 15 cm. Following a 2 min acclimatization period, the incidence of chewing jaw movements during a 5-min test period was recorded. Chewing was recorded only if it appeared to be purposeless, that is, if it was not directed at any specific object. Data are expressed as the mean (±1 S.E.M.) score for 8 individual animals.

Apomorphine-induced stereotyped behaviour. Stereotyped behaviour was scored in animals placed in individual perspex cages (20 × 18 × 18 cm) 15 min following administration of apomorphine hydrochloride (0.125–2.0 mg/kg s.c.; Sigma) as follows: 0 = indistinguishable from saline-treated animals; 1 = continuous locomotor activity, discontinuous sniffing: 2 = discontinuous locomotor activity, continuous sniffing; 3 = occasional locomotor activity, discontinuous licking, gnawing or biting; 4 = continuous licking, gnawing or biting; 5 = compulsive oral manipulation of faeces. Data are expressed as the mean score (±1 S.E.M.) obtained for 6–11 animals at each dose of apomorphine.

Ligand binding assays. Animals were killed by cervical dislocation and decapitation, and the brain rapidly removed onto ice. The paired corpora striata from 3 or 5 animals were dissected out and pooled in ice-cold 50 mM Tris–HCl buffer (pH 7.6) for *in vitro* determination of ^{3}H-spiperone, ^{3}H-piflutixol or ^{3}H-NPA binding.

Specific striatal ^{3}H-spiperone binding to D-2 receptors was determined according to a modification of the technique of Leysen *et al.* [23], using as the final incubation buffer 50 mM Tris–HCl (pH 7.6), containing 120 mM sodium chloride. ^{3}H-Spipereone (15.5–21.0 Ci/mmole; Amersham International) was incorporated into the incubates in a range of 6 concentrations between 0.1 and 4.0 nM. Specific binding was defined using (−)-sulpiride (10^{-5} M; SESIF). Specific binding of ^{3}H-NPA (60 Ci/mmole; New England Nuclear) was carried out using a modification [24] of the procedure employed for ^{3}H-apomorphine binding [25], incorporating EDTA (1 mM) in tissue buffers throughout the procedure to reduce nonspecific binding. A range of 6 ligand concentrations between 0.05–1.0 nM was employed, and specific binding was defined by the incorporation of (±)-ADTN (10^{-6} M; 2-amino-6,7-dihydroxy-1,2,3,4-tetrahydronaphthalene; Wellcome Research Laboratories). Binding of ^{3}H-piflutixol (11.7 Ci/mmole; Lundbeck) was determined using 5 concentrations in the range 0.08–1.3 nM (added in 50 μl 0.1% ascorbic acid). Specific binding was defined by incorporation of 10^{-6} M *cis*-flupenthixol (Lundbeck). This technique defines the binding of ^{3}H-piflutixol to both D-1 and D-2 receptors. The D-2 component of ^{3}H-piflutixol binding was, therefore, masked by the additional incorporation of (±)-sulpiride (3×10^{-5} M). All determinations were carried out in triplicate. Owing to limitations in the number of animals available for biochemical determinations, ligand binding assays were carried out on a single tissue pool at 1, 3, 6 and 9 months for ^{3}H-spiperone and ^{3}H-NPA and 1, 3 and 6 months for ^{3}H-piflutixol binding. At 9 (^{3}H-piflutixol) and 12 months (all ligands), binding was carried out on 3 separate tissue pools from each drug treatment group.

Data from each ligand binding experiment was subjected to linear regression and Eadie–Hofstee analysis to determine the number of binding sites (B_{max}; pmoles/g wet weight of tissue) and the equilibrium dissociation constant (K_D; nM).

Dopamine-stimulated adenylate cyclase activity. The ability of dopamine to stimulate striatal adenylate cyclase activity *in vitro* was determined according to the method of Miller and colleagues [26]. Three animals from each treatment group were killed by cervical dislocation and decapitation. The brain was rapidly removed and placed on ice during dissection of the paired corpora striata. The paired striata from each animal were separately homogenized in ice-cold buffer (2 mM Tris maleate, containing 2 mM EGTA, pH 7.4). Basal and dopamine hydrochloride (1–150 μM; Sigma)-stimulated adenylate cyclase activity were determined in duplicate for each individual homogenate. Data were expressed as pmoles of cyclic AMP formed/2 mg tissue/2.5 min. The linear portions of the sigmoid concentration–response curves were subjected to linear regression analysis in order to determine the increase in cyclic AMP formation over basal induced by the presence of 50 μM dopamine.

Striatal acetylcholine content. Eight animals from each drug treatment or control group were killed by cervical dislocation and decapitation and the brains rapidly removed onto ice. Owing to the weight of the animals during chronic treatment (often in excess of 500 g) it was not possible to sacrifice rats by microwave irradiation. The paired striata were dissected out and immediately frozen in liquid nitrogen. After weighing, tissue from each individual rat was separately homogenized in 1% acetic ethanol (1 ml glacial acetic acid in 99 ml absolute ethanol). Samples were stored at −70° until assay. On the day of assay, each sample was thawed and rotary evaporated to dryness at 40°. Frog Ringer (5 ml) was added to the residue and the pH adjusted where necessary to 3.0 using 0.3 N hydrochloric acid. Portions of each sample (approximately 1.0 ml) were pooled and alkalinated by the addition of a few drops of 1 N sodium

Table 1. Spontaneous chewing in rats treated for up to 12 months with haloperidol (1.4–1.6 mg/kg/day) or clozapine (24–27 mg/kg/day) compared to age-matched control animals

Duration of treatment (months)	Drug treatment and incidence of chewing movements/5 min			Kruskal–Wallis H-score
	Control	Haloperidol	Clozapine	
1	8.3 ± 1.7	27.0 ± 4.0*	12.3 ± 2.9	9.51, $P < 0.01$
3	18.0 ± 1.7	47.4 ± 7-3*	24.9 ± 4.7	10.82, $P < 0.01$
6	10.3 ± 2.1	26.0 ± 2.0*	17.9 ± 4.8	10.75, $P < 0.01$
9	17.9 ± 3.2	42.9 ± 5.5*	27.5 ± 8.4	8.00, $P < 0.05$
12	17.3 ± 3.6	59.3 ± 5.7*	41.1 ± 7.4*	13.45, $P < 0.01$

Values are mean ± 1S.E.M. for observations during a 5-min test period (N = 8). Overall group differences were determined using the Kruskal–Wallis analysis of variance of ranks. For data where H scores were associated with $P < 0.05$, groups were compared by paired Mann Whitney U-Tests.

* $P < 0.05$ vs control, Mann Whitney U-test.

hydroxide solution prior to boiling. Such alkali treatment denatures endogenous acetylcholine and ensures that any substances capable of causing muscle contraction (such as potassium) in the sample were not assayed as acetylcholine. Standard solutions of acetylcholine chloride (0.01–1.0 μg; Sigma) were mixed with 1.0 ml portions of neutralized alkali-boiled control and assayed on eserinized frog rectus abdominis muscle according to the method of Chang and Gaddum [27] and Feldberg [28]. The muscle contractions induced by known quantities of acetylcholine were compared with those caused by equal volumes of unknown tissue samples. To sensitize and stabilize the muscle preparation, 0.5 mg of Na–ATP (Sigma) was added to each sample and standard. The assay used in this way was accurate to within 10%. Results are expressed as the mean (±1 S.E.M.) acetylcholine concentration in nmol/g striatal tissue.

Statistical analysis. Non-parametric data from perioral movements and stereotypy scores were examined for overall group differences using the Kruskal–Wallis analysis of variance of ranks. In cases where the resulting H scores were associated with $P < 0.05$, data were subjected to paired Mann Whitney U-tests. Parametric data from locomotor activity and biochemical determinations were compared to control values by analysis of variance. If the resulting F ratios were associated with a probability of less than 5%, groups were then compared using two-tailed Student's *t*-test. In cases where ligand binding assays were carried out on only one tissue pool, B_{max} and K_D values for control and drug-treated rats were compared by 2-factor analysis of variance for determination of changes with drug treatment over time.

RESULTS

Drug intake and body weight

Haloperidol and clozapine stock solutions diluted in distilled water were readily acceptable to rats as drinking water over the course of the experiment. The mean daily drug intakes (±1 S.E.M.) achieved over the 12 month period were for haloperidol 1.4–1.6 mg/kg, and for clozapine, 24–27 mg/kg. The body weight of neuroleptic-treated rats had fallen slightly below that of the control group at the end of the 12 month period. Control animals weighed 512 ± 9 g compared to haloperidol-treated 477 ± 11 g, and clozapine-treated rats 481 ± 6 g ($P < 0.05$ compared to age-matched control animals, Student's *t*-test). Neuroleptic-treated animals in other respects did not differ from control rats in appearance or general health.

Spontaneous chewing jaw movements

Purposeless chewing jaw movements were approximately twice as frequent in rats treated with haloperidol (1.4–1.6 mg/kg/day) throughout the 12 months of treatment by comparison to age-matched control animals (Table 1). In rats treated with clozapine (24–27 mg/kg/day) for up to 9 months, chewing did not differ from that seen in control animals. At 12 months, clozapine treatment also resulted in increased chewing when compared to control animals (Table 1).

Apomorphine-induced stereotypy

After 1, 3, 6 or 9 months continuous administration of haloperidol, the stereotyped response to low doses of apomorphine (0.125–0.25 mg/kg s.c., 15 min previously) was inhibited (Fig. 1). After 12 months, stereotypy induced by only the lowest dose of apomorphine (0.125 mg/kg) was inhibited. Up to 9 months, there was in general little inhibition of stereotypy induced by higher doses of apormorphine (0.375–2.0 mg/kg). At 12 months, high doses of apomorphine (0.5–1.0 mg/kg) induced an exaggerated stereotyped response in haloperidol-treated rats compared to age-matched control animals (Fig. 1). In contrast, no alteration in apomorphine-induced (0.125–2.0 mg/kg) stereotyped behaviour was observed at any time during 12 months' administration of clozapine (Fig. 1).

Ligand binding assays

Using the ligand concentrations and displacing agents specified, binding data revealed evidence of saturability and linearity of plots following Eadie–Hofstee analysis. The mean correlation coefficients for each experiment are shown in the appropriate Tables for specific striatal ^{3}H-spiperone, ^{3}H-NPA or ^{3}H-piflutixol binding.

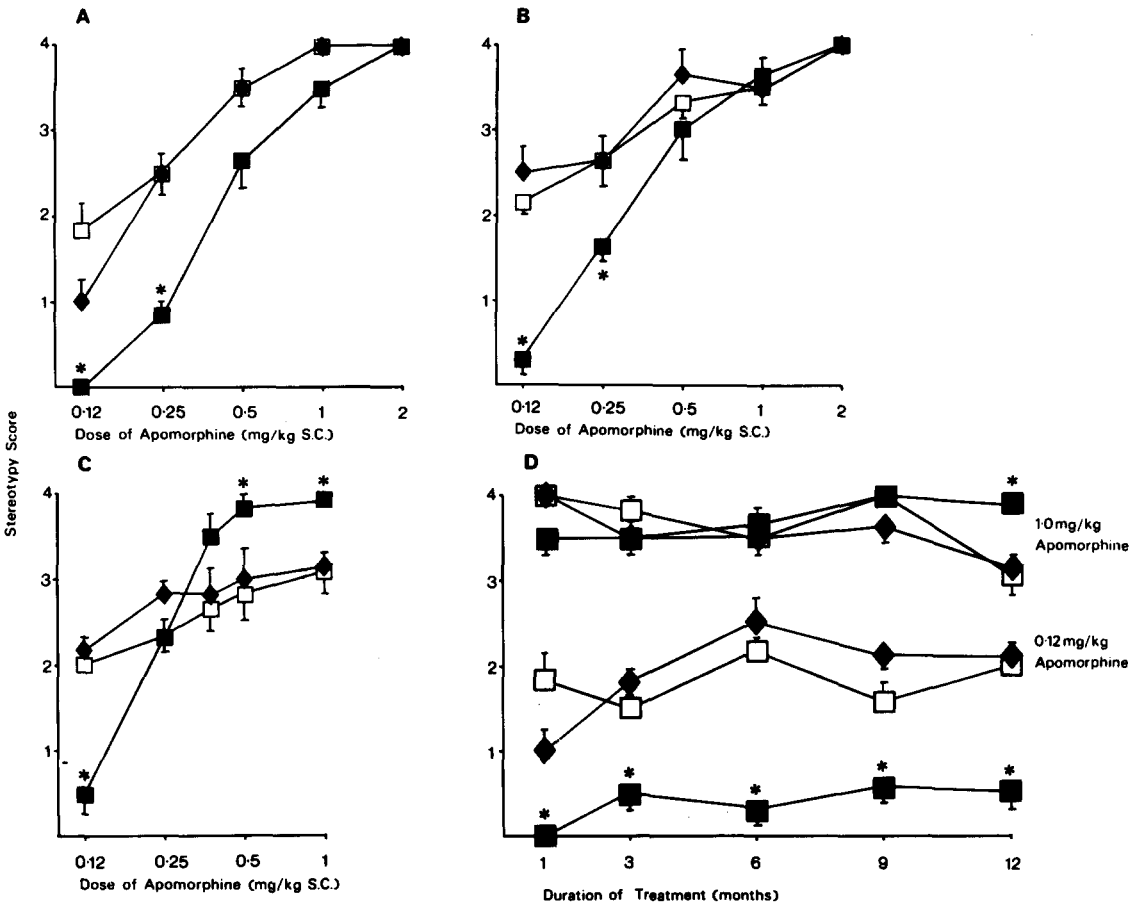

Fig. 1. Stereotyped response to apomorphine (0.125–2.0 mg/kg/sc, 15 min previously) after (A) 1 month, (B) 6 months' or (C) 12 months' continuous treatment with haloperidol (1.4–1.6 mg/kg/day) or clozapine (24–27 mg/kg/day) compared to age-matched control rats. Stereotypy induced by a low dose (0.125 mg/kg) and a high dose (1.0 mg/kg) of apomorphine over the 12 month time course are shown in (D). Results are the mean stereotypy scores ± 1 S.E.M. (N = 6–11). Overall group differences were assessed using the Kruskal–Wallis analysis of variance of ranks. The following H scores and associated probabilities at each does of apomorphine were obtained.

Dose of apomorphine (mg/kg sc)	Duration of treatment (months)				
	1	3	6	9	12
0.125	11.02, $P < 0.01$	8.55, $P < 0.005$	11.51, $P < 0.01$	10.15, $P < 0.01$	11.37, $P < 0.01$
0.25	11.37, $P < 0.01$	7.29, $P < 0.05$	6.39, $P < 0.05$	11.37, $P < 0.01$	2.84, $P > 0.05$
0.375	—	—	—	2.51, $P > 0.05$	4.01, $P > 0.05$
0.50	3.87, $P > 0.05$	7.45, $P > 0.05$	2.21, $P > 0.05$	1.18, $P > 0.05$	4.78, $P > 0.05$
1.0	3.87, $P > 0.05$	1.26, $P > 0.05$	0.32, $P > 0.05$	2.25, $P > 0.05$	9.06, $P < 0.05$
2.0	0, $P > 0.05$	3.09, $P > 0.05$	0, $P > 0.05$	—	—

In cases where the probability associated with the H score was less than 0.05, groups were compared by Mann Whitney U-tests.

* $P < 0.05$ vs age-matched control rats, Mann Whitney U-test: —□—, control; —■—, haloperidol; —◆—, clozapine.

Striatal ^{3}H-spiperone binding

Analysis of variance of the single striatal tissue pools taken from animals treated continuously with haloperidol for 1, 3, 6 and 9 months revealed an increase in the number of specific striatal ^{3}H-spiperone binding sites (B_{max}) compared to values in tissue from age-matched control animals ($F = 58.96$, $P < 0.05$; two-factor analysis of variance). B_{max} remained elevated in 3 separate tissue pools examined after 12 months' haloperidol treatment (Table 2). In contrast, administration of clozapine for up to 9 months did not alter B_{max} for striatal ^{3}H-spiperone

Table 2. The effect of continuous administration of haloperidol (1.4–1.6 mg/kg/day) or clozapine (24–27 mg/kg/day) for up to 12 months on specific H-spiperone (0.1–4.0 nM) binding to striatal membranes compared to age-matched control animals

Treatment and dose (mg/kg/day)	Duration of treatment	B_{max} (pmoles/g wet weight of tissue)	K_D (nM)	Mean correlation coefficient
Control	1	19.0	0.15	0.98
	3	18.8	0.12	0.94
	6	18.5	0.10	0.94
	9	19.7	0.17	0.98
	12	13.8 ± 1.2	0.13 ± 0.03	0.93
Haloperidol (1.4–1.6)	1	16.0	0.23	0.96
	3	26.3	0.13	0.99
	6	29.1	0.12	0.93
	9	31.7	0.24	0.99
	12	26.4 ± 2.2*	0.20 ± 0.04	0.96
Clozapine (24–27)	1	16.4	0.18	0.98
	3	16.6	0.12	0.95
	6	18.4	0.14	0.94
	9	18.8	0.19	0.99
	12	15.3 ± 0.9	0.15 ± 0.02	0.90

For data up to 9 months, the results are expressed as the mean value obtained from Eadie–Hofstee analysis of each single tissue pool, each ligand concentration being examination in triplicate. For data at 12 months, results are mean ± 1 S.E.M. values obtained from Eadie–Hofstee analysis of 3 separate pools. Data at 12 months were subjected to analysis of variance. The following F ratios and associated probabilities were obtained: B_{max}, $F = 19.52$, $P < 0.05$; K_D, $F = 1.373$, $P > 0.05$. Specific binding of ^{3}H-spiperone was defined using (−)-sulpiride (10^{-5} M).

* $P < 0.05$ vs values obtained from age-matched control animals, Student's t-test.

Table 3. The effect of continuous administration of haloperidol (1.4–1.6 mg/kg/day) or clozapine (24–27 mg/kg/day) for up to 12 months on specific ^{3}H-*N*,*n*-propylnorapomorphine (0.05–2.0 nM) binding to striatal membranes compared to age-matched control animals

Treatment and dose (mg/kg/day)	Duration of treatment	B_{max} (pmoles/g wet weight of tissue)	K_D (nM)	Mean correlation coefficient
Control	1	16.0	0.12	0.94
	3	15.6	0.23	0.91
	6	16.3	1.08	0.88
	9	10.5	1.64	0.98
	12	8.1 ± 0.3	0.78 ± 0.03	0.92
Haloperidol (1.4–1.6)	1	9.1	0.84	0.96
	3	14.8	1.06	0.98
	6	13.9	0.96	0.82
	9	16.3	1.68	0.96
	12	11.0 ± 0.3*	1.24 ± 0.05*	0.91
Clozapine (24–27)	1	14.9	1.16	0.91
	3	15.4	1.39	0.94
	6	15.0	1.08	0.94
	9	10.6	0.67	0.98
	12	7.2 ± 0.9	0.56 ± 0.04*	0.82

For this data up to 9 months, results are the mean values obtained from Eadie–Hofstee analysis of each single tissue pool, each ligand concentration being examined in triplicate. For data at 12 months, results are mean ± 1 S.E.M. values obtained from Eadie–Hofstee analysis of 3 separate tissue pools. Data at 12 months were subjected to analysis of variance. The following F ratios and associated probabilities were obtained: B_{max}, $F = 12.322$, $P < 0.05$; K_D, $F = 71.847$, $P < 0.05$.

Specific binding of ^{3}H-*N*,*n*-propylnorapomorphine was defined using (±)-ADTN (10^{-6} M).

* $P < 0.05$ vs values obtained from age-matched control animals, Student's t-test.

Table 4. The effect of continuous administration of haloperidol (1.4–1.6 mg/kg/day) or clozapine (24–27 mg/kg/day) for up to 12 months on specific ^{3}H-piflutixol (0.08–1.3 nM) binding to striatal membranes compared to age-matched control animals

Treatment and dose (mg/kg/day)	Duration of treatment (months)	B_{max} (pmoles/g wet weight of tissue)	K_D (nM)	Mean correlation coefficient
Control	1	70	0.40	0.94
	3	88	0.40	0.90
	6	83	0.41	0.99
	9	86 ± 5	0.25 ± 0.03	0.96
	12	88 ± 5	0.32 ± 0.02	0.97
Haloperidol (1.4–1.6)	1	70	0.50	0.89
	3	80	0.47	0.94
	6	77	0.36	0.89
	9	98 ± 8	0.29 ± 0.03	0.96
	12	83 ± 3	0.31 ± 0.02	0.96
Clozapine (24–27)	1	63	0.21	0.99
	3	80	0.43	0.99
	6	83	0.36	0.99
	9	112 ± 3*	0.32 ± 0.02	0.96
	12	105 ± 2*	0.35 ± 0.02	0.97

For data up to 6 months, results are the mean values obtained from Eadie–Hofstee analysis of each single tissue pool, each ligand concentration being examined in triplicate. For data at 9 and 12 months, results are the mean ± 1 S.E.M. values obtained from Eadie–Hofstee analysis of 3 separate tissue pools. Data at 9 and 12 months were subjected to analysis of variance. The following F ratios and associated probabilities were obtained: B_{max} 9 months, $F = 5.168$, $P < 0.05$; 12 months, $F = 10.303$, $P < 0.05$. K_D 9 months, $F = 1.36$, $P > 0.05$; 12 months, $F = 1.565$, $P > 0.05$.

binding ($F = 6.29$, $P > 0.05$). Similarly, in tissue taken from 3 separate pools at 12 months, there was no effect of clozapine treatment on B_{max} (Table 2). There was no effect of haloperidol or clozapine treatment for up to 9 months on K_D compared with values in age-matched control animals ($F = 4.53$, $P > 0.05$). Similarly, at 12 months K_D in haloperidol and clozapine-treated animals did not differ from that in control animals (Table 2).

Striatal ^{3}H-NPA binding

B_{max} for specific striatal ^{3}H-NPA binding in tissue from animals treated with haloperidol for up to 9 months did not differ from values in striatal preparations from control animals ($F = 0.17$, $P > 0.05$). However, after 12 months treatment, B_{max} for striatal ^{3}H-NPA binding was increased compared to control animals (Table 3). In clozapine-treated animals B_{max} for striatal ^{3}H-NPA binding decreased with duration of treatment ($F = 53.76$, $P < 0.05$). However, there was no effect of clozapine treatment on B_{max} compared with values from control tissue ($F = 3.38$, $P > 0.05$). Similarly, at 12 months, B_{max} did not differ in tissue from control or clozapine-treated animals (Table 3).

K_D for specific striatal ^{3}H-NPA binding was not affected by haloperidol treatment for up to 9 months ($F = 0.18$, $P > 0.05$); after 12 months treatment K_D was increased (Table 3). In clozapine-treated animals, K_D decreased with duration of treatment up to 9 months ($F = 46.99$, $P < 0.05$). However, there was no effect of clozapine treatment on K_D compared to values in control animals ($F = 5.99$, $P > 0.05$). At 12 months, K_D for striatal ^{3}H-NPA binding in clozapine-treated rats was decreased compared to control animals (Table 3).

Striatal ^{3}H-piflutixol binding

B_{max} for specific striatal ^{3}H-piflutixol binding in tissue from rats treated with haloperidol for 1, 3 or 6 months did not differ from values obtained from control tissue ($F = 3.77$, $P > 0.05$). In the three separate tissue pools examined at 9 and 12 months, B_{max} remained unaltered by haloperidol treatment (Table 4). In tissue taken from rats treated with clozapine, B_{max} values increased with increasing duration of treatment (F ratio = 157.75, $P < 0.05$), but did not differ from values obtained from control striatal tissue ($F = 2.99$, $P > 0.05$). However, after 9 and 12 months, B_{max} values were increased in tissue from clozapine-treated rats (Table 4).

K_D for ^{3}H-piflutixol binding was not altered during the first 6 months of treatment with either halperidol or clozapine (F ratio = 1.29, $P > 0.05$), or after 9 or 12 months treatment (Table 4).

Dopamine-stimulated striatal adenylate cyclase activity

Basal striatal adenylate cyclase activity in tissue taken from animals after 1, 3, 6, 9 or 12 months of haloperidol or clozapine treatment did not differ from values in age-matched control rats. Over the course of the 12 month period the following mean basal striatal adenylate cyclase activities were obtained: control, 71.9 ± 15.3; haloperidol-treated, 59.2 ± 17.3 and clozapine-treated, 66.3 ± 13.8 pmoles cyclic AMP formed/2.5 min/2 mg tissue ($P > 0.05$ vs control animals, Student's t-test).

Table 5. Dopamine (50 μM)-stimulated striatal adenylate cyclase activity in rats treated continuously with halperidol (1.4–1.6 mg/kg/day) or clozapine (24–27 mg/kg/day) for up to 12 months compared to age-matched control animals

Duration of treatment (months)	Cyclic AMP formation over basal in the presence of 50 μM dopamine (pmoles/2.5 min/2 mg tissue) Control	Haloperidol	Clozapine	Analysis of variance	
1	45.4 ± 3.8	31.9 ± 1.2*	50.5 ± 5.5	F = 6.08,	P < 0.05
3	30.6 ± 1.7	25.0 ± 1.9	31.2 ± 2.2	F = 2.01,	P > 0.05
6	31.8 ± 2.8	21.2 ± 1.9	28.2 ± 2.5	F = 4.95,	P > 0.05
9	27.7 ± 2.1	26.8 ± 3.2	27.2 ± 4.4	F = 0.02,	P > 0.05
12	38.3 ± 1.9	25.2 ± 4.7	51.9 ± 6.6	F = 7.674,	P < 0.05

Values are the mean ± 1 S.E.M. derived from linear regression analysis of determinations on 3 individual animals in duplicate at each dopamine concentration (1–150 μM).

Data were compared by analysis of variance in cases where the resulting F rations were associated with a probability of less than 5%, groups were subjected to Student's *t*-tests.

* $P < 0.05$ vs control animals, Student's *t*-test.

The ability of dopamine (50 μM) to stimulate striatal adenylate cyclase activity in tissue from animals treated for 1 month with haloperidol was reduced compared to tissue from age-matched control animals. Thereafter, dopamine-stimulated striatal adenylate cyclase activity did not differ from that in control animals (Table 5). In contrast, dopamine-stimulation of striatal cyclic AMP formation did not differ in tissue from clozapine-treated rats from that in control animals throughout the 12 months of drug administration (Table 5).

Striatal acetylcholine content

Striatal acetylcholine concentrations were normal at 1, 6 and 9 months of treatment with haloperidol or clozapine when compared to values from experimental and control animals, but were increased after 3 and 12 months (Table 6).

DISCUSSION

The present study was carried out in order to determine whether clozapine, a compound which is reported not to induce tardive dyskinesia [10–12] would induce striatal dopamine receptor supersensitivity as assessed during chronic continuous administration to rats.

As has been reported previously for classical neuroleptic drugs [6, 8], administration of haloperidol for 12 months induced an exaggerated stereotyped response to apomorphine, an increase in striatal ^{3}H-spiperone binding and an increase in striatal acetylcholine concentration.

Since dopamine exerts inhibitory control over acetylcholine release [30], this change may indicate an increase in spontaneous dopaminergic tone in the striatum. The increase in striatal D-2 receptor sites identified using ^{3}H-spiperone may be responsible for the increase in acetylcholine levels observed during haloperidol treatment, since inhibition of striatal acetylcholine release by dopamine appears to be mediated via D-2 rather than D-1 receptors [29]. Acetylcholine levels were also transiently increased after 3 months of haloperidol treatment. The reason for this rather surprising finding is not clear, but appears not to relate to the need to decapitate animals in this study, a procedure which is known to cause a rapid decline in acetylcholine levels. Thus, basal striatal acetylcholine content in control animals did not fall below normal levels as determined using

Table 6. Basal striatal acetylcholine content after continuous treatment with haloperidol (1.4–1.6 mg/kg/day) or clozapine (24–27 mg/kg/day) for up to 12 months compared to age-matched control rats

Duration of treatment (months)	Striatal acetylcholine content (nmoles/g tissue) Control	Haloperidol	Clozapine	Analysis of variance	
1	20.3 ± 1.8	24.8 ± 1.5	25.3 ± 1.2	F = 1.99,	P > 0.05
3	21.3 ± 1.2	29.4 ± 1.9*	28.1 ± 2.1*	F = 6.13,	P < 0.05
6	23.7 ± 1.8	24.6 ± 2.5	25.9 ± 1.7	F = 0.31,	P > 0.05
9	28.2 ± 3.6	25.3 ± 2.4	37.3 ± 4.3	F = 3.15,	P > 0.05
12	19.9 ± 2.1	35.7 ± 3.3*	29.5 ± 3.4*	F = 7.60	P < 0.05

Results are the mean ± 1 S.E.M. values obtained from 8 animals at each time point. Data were subjected to analysis of variance. In cases where the resulting F ratio was associated with a probability of less than 5% groups were compared by Student's *t*-test.

* $P < 0.05$ vs age-matched control rats, Student's *t*-test.

this procedure at 3-month time points. However, the development of behavioural supersensitivity to apomorphine only correlated with a change in striatal acetylcholine content at 12 months.

Despite the exaggerated stereotyped response produced by high dose of apomorphine following 12 months' haloperidol treatment, the effects of a low dose of apomorphine (0.125 mg/kg) remained antagonized. This finding is in agreement with previous reports [5, 7], but appears not to be caused by enduring blockade of dopamine receptors. Rather, stereotypy scores may be reduced in rats receiving chronic neuroleptic intake due to an induction of locomotor hyperactivity by apomorphine which is not seen in control animals [3].

In contrast to the persistent increase in striatal ^{3}H-spiperone binding observed during chronic haloperidol treatment, specific binding sites identified by ^{3}H-NPA were not elevated during 9 months of haloperidol treatment, but were increased only after 12 months. This finding may indicate a differential adaptation of dopamine receptor sites labelled by ^{3}H-antagonist and ^{3}H-agonist ligands during chronic neuroleptic exposure. Interpretation of these findings is complicated by the possibility of non-competitive inhibition of agonist binding due to the continued presence of haloperidol in tissue preparations at assay [25, 32]. However, examination of ^{3}H-NPA binding in haloperidol-treated rats in the present study revealed saturability, and Scatchard plots did not reveal any deviations from linearity which would be expected in the presence of non-competitive inhibition. Moreover, ^{3}H-NPA sites were increased after 12 months of treatment, despite the presence of haloperidol as at earlier examination times.

Although haloperidol is considered to act mainly at D-2 receptors, it is also capable of inhibiting dopamine-stimulated adenylate cyclase activity *in vitro* [33]. In the present study, dopamine-stimulated striatal cyclic AMP formation was only inhibited for the first month of haloperidol treatment. Tolerance to the inhibitory effect of chronic neuroleptic treatment on adenylate cyclase activity has been observed previously [6]. For the majority of the period of drug treatment, haloperidol was without effect on striatal D-1 receptor function as assessed by either adenylate cyclase activity or specific ^{3}H-piflutixol binding. Overall, therefore, haloperidol treatment resulted in an increase in striatal D-2 receptor function without altering D-1 receptors.

The effects of chronic treatment with clozapine on striatal dopamine function were strikingly different from those induced by haloperidol. Although clozapine, like haloperidol, is capable of inhibiting dopamine-stimulated adenylate cyclase activity and displacing ^{3}H-haloperidol binding from D-2 receptors *in vitro* [34], clozapine differs from other neuroleptic drugs in having a comparable or greater affinity for non-dopamine receptors. These include those for acetylcholine, serotonin, histamine and noradrenaline [35]. Indeed, the majority of ^{3}H-clozapine binding in rat brain is not associated with dopamine receptors [36, 37]. Some two thirds of ^{3}H-clozapine binding *in vitro* can be displaced by atropine, and even that component which is weakly displaced by neuroleptic drugs such as spiperone may not be associated with dopamine receptors [36]. In addition, the ability of clozapine to displace ^{3}H-spiperone binding *ex vivo* (ID_{50}) is some 180 times higher than that of haloperidol [37].

In view of the weak dopamine antagonist properties of clozapine, it is perhaps not surprising that chronic administration of this agent, unlike haloperidol, did not alter any component of stereotyped behaviour induced by apomorphine or striatal D-2 receptors identified by ^{3}H-spiperone and ^{3}H-NPA. The increase in striatal acetylcholine levels induced by chronic clozapine treatment appears likely to be due to a direct action of this drug on striatal muscarinic receptors since D-2 receptors were not altered. We have also observed an increase in striatal ^{3}H-QNB binding after 6 months' treatment with clozapine, but not haloperidol (unpublished observations). Unlike haloperidol, chronic clozapine treatment may alter striatal acetylcholine function without affecting striatal D-2 dopamine function. Similarly, the majority of studies indicate that repeated treatment with clozapine for several weeks, followed by drug withdrawal, does not alter striatal dopamine function [13–18].

If clozapine does exert dopamine antagonist activity when given chronically, it does not appear to be reflected by changes in striatal D-2 receptors. Although clozapine treatment, unlike haloperidol, did not inhibit dopamine stimulation of striatal adenylate cyclase activity, clozapine did alter D-1 receptors as indicated by an increase in sites identified by ^{3}H-piflutixol after 9 and 12 months. Dopamine-stimulated adenylate cyclase activity also appeared to be higher at 12 months, but this change failed to reach statistical significance. Interestingly, in another study with the atypical neuroleptic sulpiride, we have found that striatal adenylate cyclase activity was increased after 12 months of treatment whilst D_2 receptor function was not altered [38]. Such a selective increase in D-1 rather than D-2 receptor function would not be expected to result in behavioural supersensitivity to apomorphine [39].

If changes in striatal dopamine function are of relevance to the emergence of tardive dyskinesia, it might be expected that rats undergoing chronic neuroleptic treatment should display spontaneous dyskinesias. Rats treated chronically with a wide range of neuroleptic drugs display an increase in perioral behaviours, including chewing jaw movements [5, 40]. Throughout the 12-month period of haloperidol treatment, there was an increase in the frequency of chewing jaw movements, as has been reported previously following less prolonged treatments with this drug [41–43]. Unlike haloperidol, clozapine treatment for up to 9 months had no effect on perioral movements in rats, although these were eventually increased after 12 months of treatment. However, there are several reasons for considering that these movements do not represent an animal model of tardive dyskinesia. Firstly, whilst changes in dopamine and acetylcholine function appear to be of importance in the manifestation of these movements, they do not respond to such manipulations in a manner resembling tardive dyskinesia [43]. Moreover, in the present study, the appearance of

neuroleptic-induced chewing did not correlate with changes in striatal dopamine or acetylcholine function, suggesting that they are not associated with striatal dopamine receptor supersensitivity.

In conclusion, we have found that chronic administration of clozapine, like classical neuroleptic drugs, elevates striatal acetylcholine concentration but, unlike haloperidol, does not alter striatal D-2 receptor function. Chronic clozapine treatment also differed from haloperidol in increasing striatal D-1 receptors identified using ^{3}H-piflutixol. At present there is no known functional correlate of D-1 receptor activation, and the relevance of this change to the low incidence of tardive dyskinesia induced by clozapine remains unclear. Whilst the balance between cerebral dopamine and acetylcholine function appears to be critical to the emergence of tardive dyskinesia, a change in acetylcholine function in the absence of D-2 function might not induce this syndrome.

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