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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

S. FLEMINGER, N. M. J. RUPNIAK, 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, U.K.

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Abstract—Administration of haloperidol (5 mg/kg i.p.), *cis*-flupenthixol (2.5 mg/kg i.p.) or sulpiride (2 × 100 mg/kg i.p.) daily for 21 days followed by a 3-day drug withdrawal period caused equivalent cerebral dopamine receptor supersensitivity as judged by enhanced apomorphine-induced stereotypy. These treatments also produced equivalent rises in the number of adenylate cyclase-independent dopamine receptors (D-2) in both striatal and mesolimbic tissue as assessed by specific [^{3}H]spiperone and [^{3}H]*N*,*n*-propylnorapomorphine (NPA) binding. No change in the dissociation constant (K_D) was apparent in response to neuroleptic treatment. However, only repeated administration of *cis*-flupenthixol caused an increase in the number of adenylate cyclase-linked dopamine receptors (D-1) in striatum as assessed by enhanced [^{3}H]piflutixol binding and increased dopamine-stimulated cyclic AMP formation. The dissociation constant for [^{3}H]piflutixol binding was unchanged by *cis*-flupenthixol administration. No change in D-1 receptor numbers or dopamine stimulation of adenylate cyclase occured in mesolimbic tissue. Repeated treatment with sulpiride or haloperidol was without effect on either [^{3}H]piflutixol binding to D-1 receptors or cyclic AMP formation.

In conclusion, increased apomorphine-induced stereotypy following subacute neuroleptic treatment correlates with changes in D-2 receptor numbers, but not with changes in D-1 receptors.

Multiple forms of cerebral dopamine receptors are believed to exist. A common division of dopamine receptors is into those believed to be linked to adenylate cyclase (D-1) and those which act independently of this enzyme (D-2) [1], although there is some recent evidence that suggests that D-2 receptors may in fact be linked to the inhibition of dopamine-stimulated adenylate cyclase activity [2].

Present evidence suggests that in both animals and man D-2, rather than the D-1 receptors, are responsible for the functional effects of dopamine receptor modulation [3]. The ability of neuroleptic drugs to inhibit apomorphine-induced stereotypy in rats correlates with their ability to displace [^{3}H]spiperone or [^{3}H]haloperidol from their specific binding sites on D-2 receptors. The average daily dose of neuroleptic drugs used to control schizophrenia in man correlates with their ability to displace radioactive ligands from D-2 sites, rather than with their ability to inhibit dopamine-stimulated adenylate cyclase activity (D-1). The inhibition of L-DOPA reversal of Parkinsonism by neuroleptic drugs also correlates with D-2 activity, and D-2 agonists which have no effect on or even inhibit D-1 receptors, for example bromocriptine, are effective in Parkinson's disease [4].

The importance of D-2 receptors in the action of neuroleptic drugs is apparent also in the development of supersensitivity of cerebral dopamine mechanisms in rodents. Repeated administration of neuroleptic drugs for some weeks, followed by a few days drug withdrawal, causes enhancement of apomorphine-induced stereotyped behaviour [5, 6] and increased numbers of D-2 binding sites labelled by ligands such as [^{3}H]spiperone or [^{3}H]haloperidol [7, 8]. Repeated administration of sulpiride, a selective D-2 receptor antagonist, increases apomorphine-induced stereotypy and specific [^{3}H]spiperone binding, without altering dopamine-stimulated adenylate cyclase activity [9]. Similarly, continuous chronic neuroleptic administration to rats for many months also produces an increased number of D-2 receptor sites [10].

However, repeated neuroleptic intake followed by withdrawal may cause increased stimulation of dopamine-sensitive adenylate cyclase, although this is not a consistent finding [11–13]. Continuous chronic neuroleptic intake also causes enhanced cyclic AMP formation. Indeed, it has been claimed that the dopamine receptor supersensitivity which develops in response to 6 months continuous trifluoperazine administration is due to altered D-1 receptor activity [4]. The role played by altered D-1 receptor function in the development of supersensitivity occurring in response to neuroleptic treatment remains unclear.

This study was designed to test the hypothesis that increased apomorphine-induced stereotypy occurring following subacute neuroleptic treatment is due to increased striatal D-2 receptor numbers rather than to alteration to D-1 receptors. Receptors were identified by selective labelling of D-2 receptors using the agonist ligand [^{3}H]spiperone, and the agonist

* Author to whom correspondence should be addressed.

ligand [^{3}H]*N,n*-propylnorapomorphine (NPA), and of D-1 receptors by [^{3}H]piflutixol (masking D-2 receptors with sulpiride) and measurement of dopamine-sensitive adenylate cyclase. We have used three neuroleptic drugs with differing D-1 and D-2 selectivity—*cis*-flupenthixol (D-1≡D-2), haloperidol (D-2 > D-1) and sulpiride (D-2).

We find that only D-2 receptors are altered in a manner consistent with their being responsible for the development of behavioural supersensitivity.

MATERIALS AND METHODS

Drug administration

Male Wistar rats (175 ± 15 g at the start of the experiment; Bantin & Kingman Ltd.) were housed in groups of 8 with free access to food and water under standard conditions of light (12 hr light–dark cycle) and temperature (21 ± 3°C). The animals were randomly divided in four treatment groups.

Animals were treated for 21 days with either 0.9% sodium chloride solution (saline; 0.5 ml i.p.), haloperidol (5 mg/kg i.p. in 0.15 ml vehicle; Janssen Pharmaceutica), *cis*-flupenthixol hydrochloride (2.5 mg/kg i.p. in 0.25 ml vehicle; Lundbeck, Copenhagen) or sulpiride (100 mg/kg i.p. twice daily in 0.5 ml vehicle; Delagrange, France).

Haloperidol was dissolved in a minimum quantity of glacial acetic acid, diluted to volume with water, and the pH of the solution adjusted to 5.5 by the addition of 1 N sodium hydroxide solution. Sulpiride was dissolved in a minimum quantity of 2% sulphuric acid, diluted to volume and the pH adjusted to 7.0 by the addition of 1 N sodium hydroxide solution. *cis*-Flupenthixol hydrochloride was dissolved in saline.

Following the 21-day period of drug administration animals were allowed a 3- or 4-day drug washout period prior to the start of behavioural or biochemical assessment. Animals used for biochemical measurement were not subjected to behavioural assessment. The entire experiment was performed on two separate occasions using different batches of rats from the same supplier.

Apomorphine-induced stereotypy

Stereotyped behaviour was assessed, with the animals placed in individual perspex cages (20 × 18 × 18 cm), 15 min following the administration of apomorphine hydrochloride (0.0625–1.0 mg/kg sc; MacFarlan Smith Ltd.). The following scoring system was employed: 0 = behaviour seen in normal animals following saline administration; 1 = continuous locomotor behaviour, discontinuous sniffing; 2 = discontinuous locomotor activity, continuous sniffing; 3 = discontinuous licking, gnawing or biting, accompanied by sporadic locomotor activity; 4 = continuous licking, gnawing or biting with only occasional locomotor activity.

Specific [^{3}H]*spiperone and* [^{3}H]*piflutixol binding to striatal and mesolimbic membranes*

Ligand binding assays for [^{3}H]spiperone and [^{3}H]piflutixol were carried out on the pooled tissue from 5 rats from each treatment group. Rats were killed by cervical dislocation and decapitation and the brain rapidly removed onto ice. The paired corpus striata and mesolimbic area (nucleus accumbens and tuberculum olfactorium) were dissected out into ice-cold 50 mM Tris–HCl buffer (pH 7.7). Pooled tissue samples were homogenised in 200 vol. of 50 mM Tris–HCl (pH 7.7) using an Ulta-Turrax homogeniser, and the resulting homogenate centrifuged at 45,000 *g* for 25 min using a Sorvall RC5 centrifuge. The tissue pellets were then resuspended in 200 vol. of incubation buffer (Tris–HCl 50 mM containing 120 mM NaCl, 2.5 mM $CaCl_2$, 1 mM $MgCl_2$, 5 mM KCl, 0.1% ascorbic acid, pH 7.4) and centrifuged as before, followed by final resuspension in 500 vol. incubation buffer. Tissue preparation was all carried out at 4°.

Aliquots (1.0 ml) of the washed membrane preparations were pre-incubated for 10 min on ice with either 50 μl 0.1% ascorbic acid for determination of total binding or 50 μl 0.1% ascorbic acid plus displacing agent to define non-specific binding. The assay was started by addition of the radioactive ligand.

Specific binding of [^{3}H]spiperone (16 or 21 Ci/mmole; Amersham International) was determined using concentrations between 0.05 and 1 nM (added in 50 μl 0.1% ascorbic acid). Specific binding was defined by incorporation of 3×10^{-5} M sulpiride.

Specific binding of [^{3}H]piflutixol (11.7 Ci/mmole; Lundbeck) was determined using concentrations in the range 0.08–1.8 nM (added in 50 μl 0.1% ascorbic acid). Specific binding was defined by incorporation of 10^{-6} M *cis*-flupenthixol. This technique defines the binding of [^{3}H]piflutixol to both D-1 and D-2 receptors [15–17]. In addition, [^{3}H]piflutixol binding was carried out as described above but in the presence of 3×10^{-5} M sulpiride to prevent the binding of the ligand to D-2 receptors and so define that component of binding to D-1 receptors. The characteristics of the sites labelled were determined in a previous study [18]. This work showed (a) that the number of binding sites (Bmax) in rat striatum labelled by [^{3}H]piflutixol and displaced by sulpiride (10^{-4} M) was equal to the number of binding sites labelled by [^{3}H]spiperone and defined by (+)-butaclamol (10^{-6} M). This would suggest that both ligands are labelling D-2 sites under these conditions; (b) that the K_D for [^{3}H]piflutixol binding (as defined using *cis*-flupenthixol) was not altered in the presence or absence of sulpiride, suggesting that [^{3}H]piflutixol binds to D-1 and D-2 sites with the same affinity. This conclusion is supported by the ability of unlabelled piflutixol to displace the specific binding of [^{3}H]spiperone and [^{3}H]piflutixol with the same affinity (IC_{50} 0.8 and 0.9 nM, respectively); and (c) that haloperidol displaces [^{3}H]piflutixol in a biphasic manner. The high affinity component is equal to that component of [^{3}H]piflutixol binding displaced by sulpiride (IC_{50} 6.5 nM; approximately 21% of specific binding) which compares closely with the ability of haloperidol to displace [^{3}H]spiperone (IC_{50} 5 nM). This suggests this component identifies D-2 sites. Haloperidol displaced the sulpiride insensitive component of [^{3}H]piflutixol binding with an IC_{50} value of 1700 nM, which corresponds closely to its IC_{50} value for inhibition of dopamine-stimulated adenylate cyclase activity (IC_{50} 2000 nM) [19].

Samples were incubated at 37° for either 15 min (in the case of [^{3}H]spiperone) or 25 min (in the case of [^{3}H]piflutixol). Bound ligand was separated from that free in solution by vacuum filtration over Whatman GF/C filters. Filters were rapidly washed twice with 5 ml ice-cold 50 mM Tris–HCl buffer (pH 7.7) and filter papers placed in 4 ml ES 299 scintillant (Packard). Samples were allowed to stand overnight and then counted at 45% efficiency using a Packard 460C scintillation counter.

The data from each assay was analysed by Scatchard analysis using linear regression analysis to determine the number of binding sites (Bmax; pmoles/g wet weight of tissue) and the dissociation constant (K_D; nM). The regression coefficient for [^{3}H]piflutixol or [^{3}H]spiperone binding analysed by regression analysis varied between 0.901 and 0.995.

The experiments were performed on animals from each of the two separate batches of rats used. For each batch the assay was repeated on at least three different occasions using separate tissue pools from each of the four treatment groups. Each ligand concentration was examined in triplicate. Comparison of tissue preparations from control and drug-treated animals was always carried out in parallel on the same occasion.

Specific [^{3}H]N,n-propylnorapomorphine binding to striatal membranes

In a parallel series of experiments the specific binding of [^{3}H]*N,n*-propylnorapomorphine (NPA) to striatal membranes was examined. In these experiments the following membrane preparation procedure was employed. Striatal tissue was homogenised in 50 vol. ice-cold 15 mM Tris–HCl buffer containing 1 mM ethylenediamene tetra acetic acid (Tris–EDTA buffer) (pH 7.8) using a Polytron homogeniser (setting 7 for 15 sec) and centrifuged at 40,000 *g* at 4° for 10 min using a Sorvall RC5 centrifuge. The resulting pellet was rehomogenised in 50 vol. ice-cold buffer, and incubated at 37° for 10 min and returned to ice. The membranes were washed twice more before final suspension in 100 vol. of the Tris–EDTA buffer and then placed on ice until used.

Specific binding of [^{3}H]NPA (60 Ci/mole; New England Nuclear) was carried out using concentrations between 0.05 and 2.0 nM. Specific binding was determined by incorporation of 10^{-6} M (±)-6,7-ADTN (Wellcome Research Laboratories).

Samples were incubated at 37° for 10 min after which bound ligand was separated from free by vacuum filtration over Whatman GF/B glass fibre. Each ligand concentration was examined in triplicate using two or three separate tissue pools. Bmax and K_D values again were obtained by regression analysis of Scatchard plots.

Adenylate cyclase assay

Basal and dopamine (1–1000 μM) stimulated adenylate cyclase activity were assayed according to the method of Miller *et al.* [20] using the saturation assay of Brown *et al.* [21]. After decapitation the brain was rapidly removed onto ice and the paired striata and mesolimbic areas from two animals from each group dissected out. Basal and dopamine-stimulated adenylate cyclase were determined in triplicate in the tissue homogenate from each pair of striata or mesolimbic tissue on three separate occasions.

The results for each group of animals were pooled and the amount of cyclic AMP formed over basal in the presence of 50 μM dopamine determined by linear regression analysis of the concentration response curves. This is referred to as dopamine (50 μM) stimulated cyclic AMP formation (see Results and Table 3).

Statistical analysis

Overall group differences between control and drug-treated animals were first analysed using the Kruskal–Wallis test for the non-parametric scores from apomorphine-induced stereotyped behaviour experiments; where probabilities associated with the resulting H scores were < 0.05, groups were then compared by pair-wise Mann–Whitney U-tests. A two-tailed Student's *t*-test was used for biochemical data.

RESULTS

Apomorphine-induced stereotypy

Apomorphine hydrochloride (0.0625–1.0 mg/kg s.c.) administered 15 min previously induced dose-dependent stereotyped behaviour in saline-treated animals (Fig. 1). Repeated administration for 21 days of haloperidol (5 mg/kg i.p.), *cis*-flupenthixol (2.5 mg/kg i.p.) or sulpiride (2 × 100 mg/kg i.p.) and subsequent withdrawal for 3 days, caused a shift of the dose–response curve for apomorphine-induced stereotypy to the left. Stereotyped behaviour was enhanced at doses of apomorphine between 0.0625 and 0.5 mg/kg for all drug treatment groups, with the exception of 0.0625 mg/kg apomorphine in sulpiride-pretreated animals. There was no enhancement of stereotypy induced by 1.0 mg/kg apomorphine due to the near maximal response produced by this dose in control animals.

The enhancement of stereotyped behaviour, indicative of the development of post-synaptic dopamine receptor supersensitivity, was equivalent in the three drug treatment groups.

Specific [^{3}H]spiperone and [^{3}H]piflutixol binding to striatal and mesolimbic membranes

Repeated administration of haloperidol (5 mg/kg i.p.), *cis*-flupenthixol (2.5 mg/kg i.p.) or sulpiride (2 × 100 mg/kg i.p.) for 21 days followed by drug withdrawal resulted in an increase in the number of specific binding sites (Bmax) for [^{3}H]spiperone in both striatal and mesolimbic tissue preparations compared to tissue from control animals (Fig. 2A). The three neuroleptic drugs produced equivalent rises, there was no difference between the increase in [^{3}H]spiperone binding sites in the drug-treated groups. The percentage increases in specific [^{3}H]spiperone binding in striatal and mesolimbic tissue, in response to neuroleptic treatment, were essentially the same.

In the absence of sulpiride, specific [^{3}H]piflutixol binding is to both D-1 and D-2 receptors [18, 22].

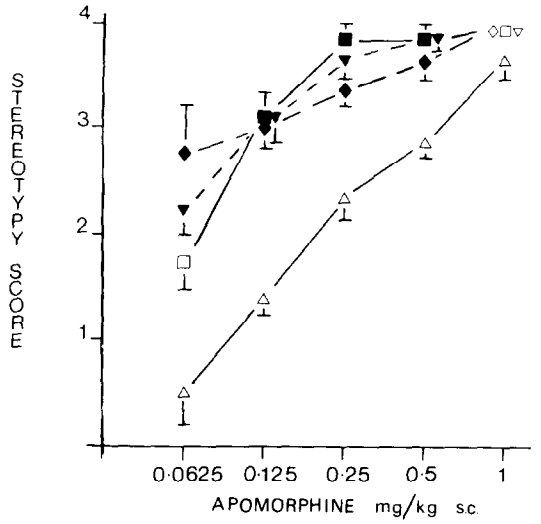

Fig. 1. Stereotyped responses induced by administration of apomorphine hydrochloride (0.0625–1.0 mg/kg s.c. 15 min previously) to animals treated with saline (—△—), haloperidol (5 mg/kg i.p.; –◇–◆–), *cis*-flupenthixol (2.5 mg/kg i.p.; --▽-▼--) or sulpiride (100 mg/kg i.p. twice daily; –□–■–) for 21 days and then withdrawn for 3 days. The results are the mean stereotypy scores (±1 S.E.M.) for six different animals from each group at each apomorphine concentration. Closed symbols represent scores significantly different from saline-treated animals as judged by Mann-Whitney U-test ($P < 0.05$). Overall group differences were first assessed using the Kruskal–Wallis analysis of variance of ranks. In cases where the probability associated with the resulting H score was <0.05, groups were then subjected to pair-wise Mann-Whitney U-tests. The following H scores and associated probabilities at each dose of apomorphine were obtained.

Dose of apomorphine (mg/kg s.c.)	H score	Probability
0.0625	9.403	<0.05
0.125	19.205	<0.001
0.25	11.373	<0.01
0.50	8.898	<0.05
1.0	2.560	<0.10

Assayed under these conditions, using *cis*-flupenthixol to define specific binding, the repeated administration of haloperidol (5 mg/kg i.p.), *cis*-flupenthixol (2.5 mg/kg i.p.) or sulpiride (2 × 100 mg/kg i.p.) for 21 days, followed by drug withdrawal, resulted in an increase in the number of [^{3}H]piflutixol binding sites in striatum (Fig. 2B). In mesolimbic tissue preparations, the number of specific [^{3}H]piflutixol binding sites was only increased in the haloperidol and *cis*-flupenthixol treated groups.

In the presence of sulpiride (3×10^{-5} M) specific binding of [^{3}H]piflutixol is to D-1 sites, as defined by *cis*-flupenthixol [18]. Under these conditions the pretreatment with *cis*-flupenthixol increased the number of specific [^{3}H]piflutixol binding sites in striatal tissue preparations. Repeated treatment with haloperidol or sulpiride was without effect. The number of specific [^{3}H]piflutixol binding sites on mesolimbic tissue preparations was not altered by repeated treatment with haloperidol, sulpiride or *cis*-flupenthixol. Repeated administration of haloperidol (5 mg/kg i.p.), *cis*-flupenthixol (2.5 mg/kg i.p.) or sulpiride (2 × 100 mg/kg i.p.) for 21 days followed by 3 or 4 days withdrawal produced no changes in the dissociation constant (K_D) for either specific [^{3}H]spiperone or [^{3}H]piflutixol binding (Table 1). There were no differences in K_D for the specific binding of [^{3}H]spiperone or [^{3}H]piflutixol in striatum or mesolimbic tissue preparations.

Specific [^{3}H]N,n-*propylnorapomorphine binding to striatal membranes*

Pretreatment of animals for 21 days, following a 3 or 4 day withdrawal period, with haloperidol (5 mg/kg i.p.), *cis*-flupenthixol (2.5 mg/kg i.p.) or sulpiride (2 × 100 mg/kg i.p.) resulted in an increase in the number of [^{3}H]NPA binding sites (Bmax) in striatal preparations (Table 2). The dissociation constant (K_D) was unaltered by drug treatment.

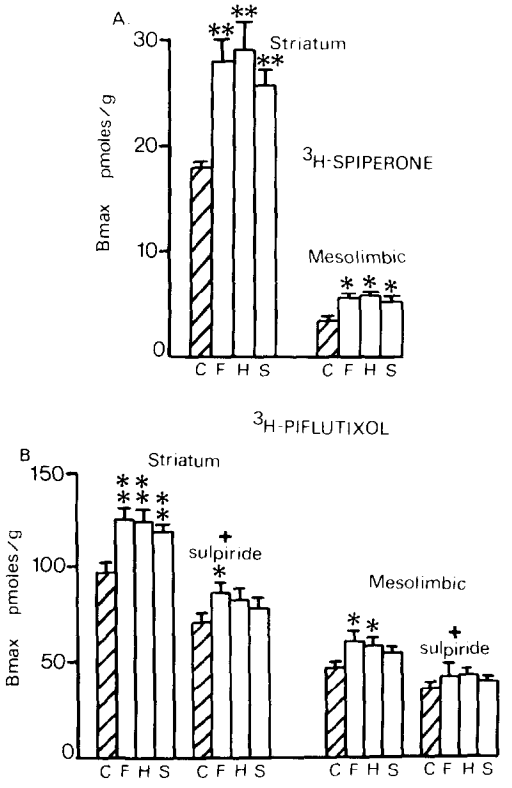

Fig. 2. The effect of repeated administration of saline (▨ C), haloperidol (5 mg/kg i.p.; H), *cis*-flupenthixol (2.5 mg/kg i.p.; F) or sulpiride (100 mg/kg i.p. twice daily; S) for 21 days followed by 3–4 days withdrawal on: (A) specific [^{3}H]spiperone binding (0.05–1 nM) in striatum and mesolimbic tissue; (B) specific [^{3}H]piflutixol binding (0.08–1.6 nM) in the presence and absence of 3×10^{-5} M sulpiride in striatum or mesolimbic tissue. The results are expressed as the mean (±1 S.E.M.) of the values obtained by Scatchard analysis of data from seven separate tissue pools for each treatment group, each ligand concentration being examined in triplicate. Specific binding of [^{3}H]spiperone was defined using 3×10^{-5} M sulpiride. Specific binding of [^{3}H]piflutixol was defined using 1 μM *cis*-flupenthixol. * $P < 0.05$, ** $P < 0.01$ compared to saline-treated animals using Student's t-test.

Table 1. The dissociation constant (K_D) for specific [^{3}H]spiperone or [^{3}H]piflutixol (in the presence and absence of 3×10^{-5} M sulpiride) binding to striatal or mesolimbic tissue preparations following repeated administration of haloperidol (5 mg/kg i.p.), *cis*-flupenthixol (2.5 mg/kg i.p.) or sulpiride (2 × 100 mg/kg i.p.) for 21 days followed by 3 or 4 days drug withdrawal compared to saline-treated controls

Treatment group	K_D (nM) [^{3}H]Spiperone Striatum	[^{3}H]Spiperone Mesolimbic	[^{3}H]Piflutixol Striatum	[^{3}H]Piflutixol Mesolimbic	[^{3}H]Piflutixol (plus sulpiride) Striatum	[^{3}H]Piflutixol (plus sulpiride) Mesolimbic
Control	0.054 ± 0.013	0.072 ± 0.023	0.40 ± 0.05	0.37 ± 0.04	0.40 ± 0.04	0.32 ± 0.03
Haloperidol	0.062 ± 0.017	0.070 ± 0.016	0.42 ± 0.09	0.33 ± 0.05	0.42 ± 0.07	0.33 ± 0.03
cis-Flupenthixol	0.068 ± 0.068	0.094 ± 0.037	0.42 ± 0.06	0.34 ± 0.05	0.42 ± 0.07	0.26 ± 0.03
Sulpiride	0.074 ± 0.21	0.068 ± 0.023	0.45 ± 0.08	0.34 ± 0.06	0.45 ± 0.08	0.31 ± 0.06

Dopamine-sensitive adenylate cyclase activity

Pretreatment of animals for 21 days with haloperidol (5 mg/kg i.p.), *cis*-flupenthixol (2.5 mg/kg i.p.) or sulpiride (2 × 100 mg/kg i.p.) produced no difference in basal cyclic AMP formation in striatal or mesolimbic homogenates (Table 3). None of the changes in basal cyclic AMP formation found in drug-treated groups were significantly different from control values. There was an impression that *cis*-flupenthixol may increase basal cyclic AMP formation by 41 and 48% in striatal and mesolimbic tissue, respectively, compared to control animals but neither were significantly different as assessed by a two-tailed Student's *t*-test. Dopamine-stimulated adenylate cyclase activity in striatum was enhanced by repeated administration of *cis*-flupenthixol but not haloperidol or sulpiride (Table 3). Drug treatment did not alter dopamine-stimulated adenylate cyclase activity in mesolimbic tissue homogenates (Table 3).

Table 2. The effect of repeated administration of haloperidol (5mg/kg i.p.), *cis*-flupenthixol (2.5 mg/kg i.p.) or sulpiride (2 × 100 mg/kg i.p.) for 21 days followed by 3 or 4 days drug withdrawal compared to saline-treated control animals, on the number of binding sites (Bmax) and the dissociation constant for specific [^{3}H]*N*,*n*,-propylnorapomorphine (0.05–2.0 nM) binding to striatal membranes

Treatment group	Experiment	[^{3}H]NPA Bmax (pmoles/g)	[^{3}H]NPA K_D (nM)
Control	1.	15.15 ± 0.44	1.00 ± 0.04
	2.	16.27 ± 0.89	1.04 ± 0.03
	3.	17.23 ± 1.02	1.03 ± 0.02
	av.	16.22 ± 0.60	1.02 ± 0.01
Haloperidol	1.	23.17 ± 1.28†	1.00 ± 0.08
	2.	25.78 ± 1.54†	1.07 ± 0.07
	3.	26.54 ± 1.89†	1.01 ± 0.03
	av.	26.16 ± 1.02*	1.03 ± 0.02
		55%	
cis-Flupenthixol	1.	22.83 ± 1.70†	0.99 ± 0.11
	2.	24.95 ± 1.49†	1.02 ± 0.03
	3.	27.29 ± 1.93†	1.07 ± 0.04
	av.	25.02 ± 1.29*	1.03 ± 0.02
		54%	
Sulpiride	1.	20.30 ± 0.96†	0.99 ± 0.07
	2.	22.16 ± 1.32†	1.05 ± 0.06
	3.	Not done	

The results are shown in general as the individual values for three different experiments. The mean values (±1 S.E.M.) are those obtained from the averaging of the individual results. Differences between groups were determined using Student's *t*-test. In the case of sulpiride only two individual experiments were performed due to a lack of animals. The individual values are shown together with the mean. Individual values were compared by regression analysis of the individual data points for each ligand concentration and comparison of the slope and intercept of the regression lines using the S.E. of the whole regression. Specific binding of [^{3}H]NPA was defined using 10^{-6} (±) M (+)-6,7-ADTN.

* $P < 0.05$ compared to mean values for control group.

† $P < 0.05$ compared to individual value for the respective control experiment.

DISCUSSION

We have confirmed the previous observation that repeated administration of neuroleptic drugs for

Table 3. Basal and dopamine (50 μM) stimulated cyclic AMP formation in striatal and mesolimbic homogenates from rats treated with haloperidol (5 mg/kg i.p.), *cis*-flupenthixol (2.5 mg/kg i.p.) or sulpiride (2 × 100 mg/kg i.p.) for 21 days and then withdrawn for 3 or 4 days compared to saline-treated controls

Treatment group	Cyclic AMP formation (pmoles/2.5 min/2 mg tissue) Basal	Dopamine (50 μM) stimulated
Striatum		
Control	46 ± 5	32 ± 5
Haloperidol	40 ± 4	37 ± 6
cis-Flupenthixol	65 ± 9	53 ± 8*
Sulpiride	32 ± 8	33 ± 8
Mesolimbic area		
Control	17 ± 6	25 ± 5
Haloperidol	16 ± 7	28 ± 8
cis-Flupenthixol	28 ± 5	28 ± 4
Sulpiride	26 ± 1	28 ± 4

* $P < 0.05$ compared to saline-treated control rats.

Results are the mean (±1 S.E.M.) of three separate determinations on the paired striata of 2 or 3 rats.

some weeks, followed by a few days drug withdrawal, results in enhanced apomorphine-induced stereotypy and increased dopamine receptor numbers, as judged by [^{3}H]spiperone binding. In particular, we have confirmed our previous observations that repeated administration of a high dose of sulpiride (100 mg/kg i.p. twice daily) increases both apomorphine-induced stereotypy and the number of [^{3}H]spiperone binding sites in rat striatum [9]. These findings contrast with the lack of effect found on repeated administration of lower doses of sulpiride [23, 24] and of high oral sulpiride intake [25].

D-2 receptors identified by [^{3}H]spiperone are labelled also by agonist ligands, such as [^{3}H]NPA, although it is debated whether these binding sites form part of the same receptor complex [26] or whether they are distinct entities [27]. The finding of enhanced numbers of [^{3}H]NPA binding sites in response to repeated neuroleptic treatment followed by drug withdrawal confirms our previous conclusion that agonist sites are altered in an identical manner to antagonist sites labelled by [^{3}H]spiperone [9]. This contrasts with the data of Goldstein *et al.* [27] who found repeated haloperidol administration not to increase [^{3}H]NPA binding.

We have utilised neuroleptic drugs in this study which possess differing affinities for D-1 and D-2 receptors. In each case the doses administered are known to cause functional antagonism of cerebral dopamine receptors. Sulpiride is a selective D-2 receptor antagonist, it has little ability to inhibit striatal dopamine-stimulated adenylate cyclase activity [29, 30] and is at least 200 times more potent in displacing the specific binding of [^{3}H]haloperidol or [^{3}H]spiperone than of [^{3}H]*cis*-flupenthixol or [^{3}H]piflutixol [22]. It is unlikely therefore that sulpiride caused inhibition of the D-1 receptor function *in vivo* in this study. Similarly, haloperidol only weakly inhibits dopamine-stimulated adenylate cyclase activity and displaces [^{3}H]haloperidol or [^{3}H]spiperone in concentrations approximately 100 times less than are required to displace [^{3}H]piflutixol or [^{3}H]*cis*-flupenthixol [3, 22]. Therefore, it is unlikely that this drug will produce marked antagonism of D-1 receptors *in vivo*. Indeed, haloperidol (5 mg/kg i.p. 2 hr before death) caused only minimal inhibition of dopamine-stimulated adenylate cyclase activity measured *in vitro* [31]. In contrast, *cis*-flupenthixol potently inhibits dopamine-stimulated adenylate cyclase activity and is equipotent in displacing [^{3}H]haloperidol or [^{3}H]spiperone and [^{3}H]*cis*-flupenthixol or [^{3}H]piflutixol from the specific binding sites on striatal preparations [22]. So, *cis*-flupenthixol is equally active at D-1 and D-2 receptors and *in vivo* should have produced inhibition of both D-1 and D-2 receptor function at the high dose used in this study.

The equivalent enhancemnt of apomorphine-induced stereotypy produced by the repeated administration of haloperidol, *cis*-flupenthixol and sulpiride was associated with equivalent increases in specific [^{3}H]spiperone and [^{3}H]NPA binding, and hence D-2 receptors in both striatal and mesolimbic tissue. This association between altered D-2 function and the development of supersensitivity was strengthened by the finding that of the three drugs administered only *cis*-flupenthixol produced an increase in specific [^{3}H]piflutixol binding to D-1 receptors (defined in the presence of sulpiride to block binding to D-2 sites), and this only occurred in striatal tissue. Only that treatment which affected D-1 receptor function increased [^{3}H]piflutixol binding but all treatments caused behavioural supersensitivity. This finding was confirmed by the change in dopamine-sensitive adenylate cyclase activity. Thus, striatal cyclic AMP formation was enhanced by repeated administration of *cis*-flupenthixol but not haloperidol or sulpiride. Again this change was selectively found in the striatum but not in mesolimbic tissue. Why *cis*-flupenthixol should show such regional specificity is not clear. Indeed, we are not aware of any evidence to show that *cis*-flupenthixol differentially alters dopamine function in the two regions or that there is any difference in the nature of the D-1 receptor population which would explain our findings. There was an impression that *cis*-flupenthixol increased basal cyclic AMP formation in both striatum and mesolimbic tissue although this was not found to be statistically significant. It is possible that *cis*-flupenthixol also effects adenylate cyclase activity at points in the pathway distant from the D-1 receptor.

Neuroleptic-induced dopamine receptor supersensitivity thus appears to be related to changes of D-2 receptor numbers. Other functional dopamine events also correlate with changes in D-2 receptors. The rate of rotation to apomorphine measured in animals with a unilateral 6-hydroxydopamine lesion of the nigro-striatal pathway correlates with increased striatal D-2 receptor numbers, as measured with [^{3}H]spiperone, but not with altered D-1 dopamine-stimulated adenylate cyclase activity [32].

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