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Comparison of the Effects of Neuroleptic Drugs on Pre- and Postsynaptic Dopaminergic Mechanisms in the Rat Striatum

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SUMMARY

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Neuroleptic drugs of various chemical classes were compared as inhibitors of the postsynaptic dopamine-sensitive adenylate cyclase in rat striatum and for their ability to influence a variety of presynaptic mechanisms in dopaminergic nerve terminals in striatum. Sixteen chemical analogues of the butyrophenone haloperidol were tested on the dopamine-sensitive adenylate cyclase, and the results showed a good correlation between inhibitory potency in this system and the known effects of these compounds as apomorphine antagonists *in vivo*. In intact synaptosome preparations from rat striatum apomorphine was a potent inhibitor (IC_{50} = 0.2 μM) of the conversion of tritiated tyrosine to catechols. Other dopamine-mimetic drugs [epinine, dopamine, 2-amino-6,7-dihydroxy-1,2,3,4-tetrahydronaphthalene (ADTN)] also had similar inhibitory effects, although the *alpha* adrenoceptor agonist phenylephrine and the *beta* agonist isoprenaline were also inhibitory at higher concentrations. The inhibitory actions of dopamine, epinine, ADTN, and noradrenaline were significantly reduced by addition of the dopamine uptake inhibitor benztropine, suggesting that they act at least in part by inhibition of intrasynaptosomal tyrosine hydroxylase after uptake into dopaminergic terminals. The effects of apomorphine, however, were unaffected by benztropine, suggesting a direct action on presynaptic "autoreceptors" at dopaminergic terminals. All the compounds tested were at least 50 times less potent as inhibitors of free tyrosine hydroxylase in detergent-containing striatal homogenates. The inhibitory effects of apomorphine on synaptosomal catechol synthesis were partially reversed by various neuroleptic drugs, and this appeared to be due to a competitive interaction between the neuroleptic drugs and apomorphine at presynaptic receptor sites. The neuroleptics, however, also tended themselves to inhibit catechol synthesis when added alone. Haloperidol, spiroperidol, and pimozide were particularly potent in reversing the presynaptic actions of apomorphine on catechol formation, being active at concentrations between 10 and 100 nM. Neuroleptics also had some activity as inhibitors of [^{3}H]dopamine uptake and as dopamine releasers in striatal synaptosomes. They also antagonized the evoked release of [^{3}H]dopamine elicited by protoveratrine. None of these effects, however, occurred at very low drug concentrations, and the butyrophenones were no more potent than chlorpromazine. It is concluded that neuroleptics possess actions on both pre- and postsynaptic sites in the striatum, but that the postsynaptic blocking action on dopamine receptors is likely to be most crucial for the clinical activity of these drugs.

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INTRODUCTION

There is considerable evidence that neuroleptic drugs act as antagonists at postsynaptic dopamine receptor sites in brain (1). Several laboratories have recently used a specific dopamine-sensitive adenylate cyclase in homogenates of the dopamine-rich neostriatum to assess such dopamine antagonist effects (2–4). Neuroleptic drugs of various chemical classes are effective inhibitors of the dopamine-stimulated adenylate cyclase, and we and others have found a good correlation between the results obtained in this system and neuroleptic potency *in vivo* for such drugs (5). The butyrophenone neuroleptics, however, behave somewhat anomalously in this test. Although drugs such as haloperidol, spiroperidol, and pimozide are effective inhibitors of the dopamine-sensitive adenylate cyclase, their potencies *in vitro* are not greater than that of chlorpromazine, whereas from data *in vivo* one would expect them to be considerably more potent than chlorpromazine (6, 7).

In addition to their postsynaptic actions, neuroleptic drugs can also exert various effects on presynaptic mechanisms in dopaminergic neurons. Thus neuroleptics increase the rate of turnover of dopamine in the intact brain, and this effect is only partially abolished by procedures that prevent increases in impulse traffic in dopaminergic neurons (8–10), suggesting that the drugs may act at least in part on presynaptic mechanisms. Recent findings suggest that presynaptic receptor sites which can respond to external dopamine or apomorphine may exist at dopaminergic nerve terminals, and that when these receptors are activated dopamine biosynthesis is inhibited (8, 11). Experiments both *in vivo* and *in vitro* indicate that neuroleptic drugs can act as antagonists at such presynaptic dopamine receptors, in addition to their postsynaptic actions (12–16). Seeman and his colleagues (17–19) have also reported that neuroleptics have a variety of other presynaptic actions on dopamine terminals: a dopamine-releasing effect, inhibition of dopamine uptake sites, and an ability to block stimulus-evoked release of the transmitter. They have suggested that the activities of neuroleptics in blocking transmitter release correlate well with clinical antipsychotic potencies of these drugs (19).

In the present studies we examined some of the presynaptic effects of various neuroleptic agents to determine which, if any, of these actions might correlate best with neuroleptic activity. In addition, the inhibitory effects of a larger group of butyrophenones were assayed on the dopamine-sensitive adenylate cyclase, in order to determine whether the dopamine-blocking potencies *in vitro* of individual members of this series of compounds could be shown to correlate with their dopamine antagonist properties *in vivo*. We conclude that such a correlation does exist among the butyrophenones, and that although some neuroleptic drugs possess potent actions on presynaptic dopaminergic mechanisms, these actions are less likely to be crucial in determining neuroleptic activity than the postsynaptic dopamine antagonist properties of the same molecules.

METHODS

Animals and dissection. Male Sprague-Dawley rats (200–250 g) were used for all studies. Striatal tissue was dissected as described previously (20).

Dopamine-sensitive adenylate cyclase. Drugs were tested as inhibitors of the dopamine-sensitive adenylate cyclase in homogenates of rat striatum as described previously (2). The concentration of dopamine was constant at 100 μM, and each drug was tested at four different concentrations, each involving quintuplicate estimations. From these results an IC_{50} value for each drug was determined graphically by log probit analysis, and K_i values were calculated as previously described (2, 3).

Effects of drugs on synaptosomal synthesis of labeled catechols. The method used was similar to that described by others (15, 16, 21). Rat striatal tissue was homogenized in 40 volumes of ice-cold 0.3 M sucrose, using a glass-Teflon homogenizer. The homogenate was centrifuged at 1,000 × g for 5 min, and the resulting supernatant was centrifuged at 10,000 × g for 20 min in a refrigerated centrifuge. The

tubes were carefully drained, and the P2 pellet (crude synaptosomal-mitochondrial fraction) was resuspended in 0.3 M sucrose, 1 ml/100 mg of original striatal tissue, wet weight. Aliquots of this crude synaptosomal preparation were used for subsequent incubation, and sufficient material was prepared to provide all the samples for each experiment from one suspension. Ten-microliter aliquots of the synaptosomal suspension (approximately 40 μg of protein) were added to tubes containing 50 μl of Krebs phosphate solution and test drugs and incubated at 37° for 10 min; 10 μl of Krebs solution containing 2.5 μCi of L-[^{3}H]tyrosine and nonradioactive L-tyrosine (final incubation concentration, 5 μM) were then added, and incubation was continued for a further 10 min. Labeled catechol impurities were removed from the [^{3}H]tyrosine solution immediately before use by shaking with a small amount of alumina (Woelm, neutral, activity grade 1) in 5 mM Tris-HCl buffer, pH 8.6. At the end of the incubation the tubes were removed to an ice bath, and 200 μl of 0.4 N perchloric acid containing carrier L-dopa (2 μg/ml) were added. Labeled catechols were then isolated by adsorption on alumina, eluted with acid, and measured by scintillation counting as described previously (22). Each drug examined was tested at several different concentrations, with at least duplicate estimations at each concentration. All experiments included blank samples (with no added tissue suspension) and control incubations of tissue with no added drugs.

In some experiments drug effects were assessed on cell-free tyrosine hydroxylase in rat striatum. In these experiments striatal tissue was homogenized in 20 volumes of ice-cold 5 mM Tris-HCl buffer, pH 6.5, containing 0.1% (v/v) Triton X-100, and 10-μl aliquots of this homogenate were used for tyrosine hydroxylase assay as described by Hendry and Iversen (22), using added reduced dihydrobiopterin (1 mM) as cofactor and 5 μM L-[^{3}H]tyrosine as substrate.

Dopamine uptake. Effects of drugs on [^{3}H]dopamine uptake in crude synaptosomal preparations from rat striatum were assessed by a previously described procedure (3). Briefly, this consisted of incubating aliquots of striatal sucrose homogenates with test drugs and tritiated dopamine (50 nM) in Krebs phosphate solution, followed by harvesting of labeled synaptosomes by membrane filtration.

Effects of drugs on release of [^{3}H]dopamine from striatal synaptosomes. Striatal tissue was homogenized in 10 volumes of ice-cold 0.3 M sucrose using a glass-Teflon homogenizer. After centrifugation at 1000 × g for 5 min, an aliquot of the supernatant was added to 10 volumes of Krebs phosphate solution containing [^{3}H]dopamine (500 nM) and incubated at 37° for 10 min, with shaking. Aliquots (0.2 ml) of the labeled synaptosome suspension were then added to warmed 10-ml beakers containing 2.0 ml of Krebs phosphate with or without test drugs, and incubation was continued for a further 5 min. The beakers were then removed to an ice bath, and samples were rapidly filtered through 25-mm-diameter Sartorius membrane filters, 0.45-μm pore size. The filters were washed twice with 10 ml of cold NaCl solution and counted in scintillation vials after addition of 2 ml of ethoxyethanol and 10 ml of 0.4% butyl-PBD (Ciba) in toluene. In each experiment blank samples were included, with no added tissue, and the resulting filter blank values were subtracted. Incubations of tissue in medium with no added test drugs were used in each experiment as controls to assess drug effects. Each drug was tested at several concentrations, and each concentration involved triplicate estimations.

Materials. [*ethylamine*-1,2-^{3}H]Dopamine hydrochloride (specific activity, 2.4 Ci/mmole), [8-^{3}H]adenosine cyclic 3′,5′-monophosphate (specific activity, 27.5 Ci/mmole), and L-[*side chain*,2,3-^{3}H]tyrosine (specific activity, 22.0 Ci/mmole) were obtained from the Radiochemical Centre. Protoveratrines A and B were obtained from K & K Laboratories, Plainview, N. Y. We are grateful for the following gifts of drugs: fluphenazine, Squibb; chlorpromazine, promazine, promethazine, and trifluoperazine, May & Baker, Ltd.; α and β forms of flupenthixol and clopenthixol,

Lundbeck, Ltd.; pimozide, haloperidol, and other butyrophenones, Janssen Pharmaceutica; (+) and (−) enantiomers of butaclamol, Ayerst Laboratories, Inc.; and (−)-apomorphine HCl, MacFarlan-Smith, Ltd.

Most of the butyrophenones used were supplied as water-soluble hydrochloride salts, and stock solutions were prepared by dissolving the drugs in warm distilled water. In other cases butyrophenone drugs were dissolved in 0.01 N hydrochloric acid, and the resulting stock solutions were diluted at least 100-fold in the final assay tubes.

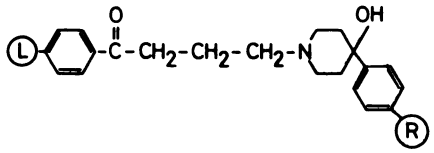
RESULTS

Adenylate cyclase. Previous results have shown haloperidol and spiroperidol to be effective as inhibitors of the dopamine-stimulated adenylate cyclase in rat striatal homogenates, although the potencies of these agents in the test system *in vitro* were not as high as expected from data *in vivo* (2, 3). In the present studies we confirmed these findings and examined the effects of 16 other butyrophenones, related structurally to haloperidol; the results are summarized in Table 1. These substances were made available from the research laboratories of Janssen Pharmaceutica, and were tested on a blind basis. The biochemical results were subsequently compared with data *in vivo* on the effects of these drugs as apomorphine antagonists in dogs; these data were obtained by Dr. C. Niemegeers and were kindly made available by Drs. P. Laduron and P. Janssen (Table 1). In general there was a good agreement with the findings *in vivo*, those

TABLE 1

Inhibition of dopamine-stimulated adenylate cyclase in rat striatum by butyrophenones

Basal enzyme activity was 29.6 ± 7.5 pmoles of cyclic AMP per 2 mg (wet weight) per 2.5 min, and in the presence of 100 μM dopamine, 60.7 ± 10.9 pmoles of cyclic AMP per 2 mg (wet weight) per 2.5 min. K_i values were calculated as described by Miller *et al.* (2), on the assumption that all drugs acted as competitive inhibitors, as shown previously for haloperidol and spiroperidol (24).



L	R		Name	IC_{50}	K_i	ED_{50}[a]
				μM	μM	mg/kg
F	Br		Bromperidol	0.3	0.013	0.018
F	OCH_3		R 15316	1.5	0.068	0.03
F	Cl		Haloperidol	2.0	0.091	0.02
F	F		R 1610	2.3	0.10	0.07
F	H		R 1589	3.8	0.17	0.1
H	Br		R 11872	5.0	0.19	0.022
F	CH_3		R 1658	9	0.41	0.045
H	OCH_3		R 2839	25	1.1	0.06
Cl	Cl		R 1670	25	1.1	1.5
H	Cl		R 1532	68	3.1	0.06
H	CH_3		R 1639	56	2.5	0.05
H	H		R 1484	80	3.6	0.4
Cl	CH_3		R 8446	>10	>4.5	0.03
Cl	F		R 1673	>100	>45	>2.0
Cl	H		R 1519	>100	>45	>5.0
Cl	Br		R 11530	>100	>45	2.5
F	Cl	$N\text{-}CH_3I$	R 2388	>100	>45	>5.0

[a] Apomorphine test in dogs; data provided by Dr. P. Laduron, Janssen Pharmaceutica. Data were recorded 30 min after the administration of each neuroleptic drug.

drugs that were the most potent apomorphine antagonists *in vivo* being also most effective as dopamine antagonists *in vitro*.

Synaptosomal synthesis of labeled catechols. Previous studies have shown that the conversion of labeled tyrosine to catechols can be measured in synaptosome suspensions or in brain slices incubated *in vitro* in the absence of added pteridine cofactor (12, 15, 21, 25, 26). Christiansen and Squires (15) and Goldstein *et al.* (25) have also shown that low concentrations of apomorphine will inhibit the hydroxylation of tyrosine in such intact tissue preparations, and that this inhibitory effect can be partially reversed by neuroleptic drugs.

Using the resuspended striatal P2 pellet as a source of synaptosomes, we were able to confirm these findings. The conversion of [^{3}H]tyrosine to labeled catechols was readily measureable (tissue values were more than 10 times blank values after 10 min of incubation) and continued linearly with time for at least 30 min. Apomorphine caused a concentration-dependent inhibition of the conversion of tyrosine to catechols, with an IC_{50} (concentration causing 50% inhibition) of 200 nM. The inhibition of catechol synthesis by apomorphine in intact synaptosomes does not seem to be due to a direct inhibitory effect of the drug on the enzyme tyrosine hydroxylase, since much higher concentrations of apomorphine were needed (IC_{50} = 11 μM) to inhibit the enzyme in detergent-containing homogenates of striatal tissue, in which the enzyme is not occluded (Fig. 1). The inhibitory effects of apomorphine were similar if estimated after incubations of 5-, 10-, or 15-min duration, and were not affected by prior treatment of the animals with reserpine (5 mg/kg intraperitoneally 18 hr previously). Similar inhibitory effects were seen when dopamine and various dopamine analogues were added to intact synaptosome suspensions (Fig. 2); in each case much higher concentrations were needed to inhibit free tyrosine hydroxylase (Table 2). However, in intact striatal synaptosomes, dopamine and some of its analogues are rapidly concentrated within the cytoplasm of dopaminergic terminals by a high-affinity membrane uptake system and may thus attain

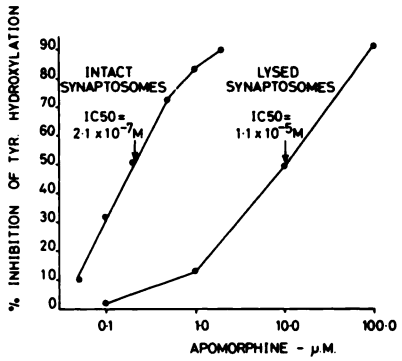


FIG. 1. *Inhibition of tyrosine hydroxylation by apomorphine in intact rat striatal synaptosomes and in detergent-containing homogenates of striatum (lysed synaptosomes)*

Increasing concentrations of apomorphine were added to each system, and the conversion of [^{3}H]tyrosine to labeled catechols was determined after a 10-min incubation at 37° as described under METHODS. The results are expressed as percentage inhibition of tyrosine hydroxylation in the presence of apomorphine, relative to controls with no added drug. Each point represents the mean of three determinations. Control values for intact synaptosomes were 0.105 pmole of labeled catechols per minute per milligram of tissue, and for lysed synaptosomes, 6.3 pmoles/min/mg of tissue.

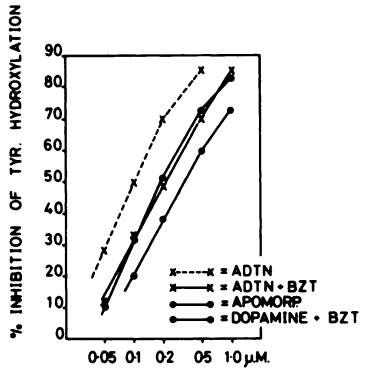


FIG. 2. *Inhibition of tyrosine hydroxylation in striatal synaptosomes by added dopamine-like drugs*

Each drug was tested by addition to striatal synaptosome preparations in the presence and absence of the uptake inhibitor benztropine (BZT) (2 μM) or [^{3}H]tyrosine (5 μM). Labeled catechol formation was measured after a 10-min incubation at 37°. Each point is the mean of three determinations, and results are expressed as percentage inhibition relative to controls with or without benztropine added. Control values in the absence of benztropine were 0.115 pmole of labeled catechols per minute per milligram of tissue; controls with added benztropine consistently yielded somewhat higher values, 0.132 pmole/min/mg of tissue.

TABLE 2

Inhibition of formation of catechols by dopamine and related compounds in rat striatum

Compounds were tested as inhibitors of the conversion of [^{3}H]tyrosine to catechols in intact striatal synaptosomes incubated in Krebs phosphate solution for 10 min at 37°. Each compound was tested at least four concentrations, in duplicate, and the IC_{50} values were estimated graphically (see Fig. 2). The same compounds were also tested on intact synaptosomes in the presence of the dopamine uptake inhibitor benztropine (2 μM) and by addition to homogenates of striatum in 5 mM Tris buffer, pH 6.5, containing 0.1% Triton X-100, 1 mM dihydrobiopterin, and 5 μM [^{3}H]tyrosine ("detergent homogenate") to assess their direct inhibitory effects on the activity of tyrosine hydroxylase (see Fig. 1).

Compound	IC_{50} for tyrosine hydroxylation		
	Intact synaptosomes	Intact synaptosomes + 2 μM benztropine	Detergent homogenate
	μM	μM	μM
Dopamine	0.25	0.34	68.0
(±)-α-Methyldopamine	0.06	0.20	140.0
Apomorphine	0.21	0.20	11.0
ADTN[a]	0.10	0.20	150.0
Epinine	0.16	0.25	
(−)-noradrenaline	0.34	1.50	180.0
(+)-noradrenaline	0.17	1.40	200.0

[a] See the text, footnote 3.

much higher concentrations in the cytoplasm than those added to the medium. Inhibition of the dopamine uptake mechamism by benztropine (27) (2 μM) led to a decrease in the potency of all the compounds tested in the intact synaptosome system (Fig. 2 and Table 2), except for apomorphine, whose inhibitory potency was unaffected (Table 2). This suggests that at least part of the actions of the other compounds may be due to direct inhibitory effects on intrasynaptosomal tyrosine hydroxylase following the active accumulation of these compounds in the cytoplasm of the synaptosomes. Benztropine alone at a concentration of 2 μM caused a consistent stimulation of catechol synthesis by 15–25% above normal control values. This effect was evident only at benztropine concentrations of 1 μM or more, and did not increase at higher concentrations. The inhibitory potency of the compounds tested was still high in the presence of benztropine, however, suggesting that they also exert some action on receptor sites located externally on the presynaptic terminal membrane. In the presence of benztropine, the compounds apomorphine, (±)-α-methyldopamine, ADTN,[3] and epinine were approximately equipotent, and slightly more potent than dopamine; (+)- and (−)-noradrenaline were equipotent and approximately 5 times less potent than dopamine (Table 2).

The ability of various neuroleptic drugs to reverse the inhibition of catechol synthesis caused by apomorphine was examined. In these experiments a constant concentration of 200 nM apomorphine was used, which by itself caused approximately 50% inhibition of tyrosine conversion. Various concentrations of each neuroleptic drug were then tested by addition to the synaptosomal system in both the presence and absence of apomorphine. Since most of the drugs tested caused an inhibition of catechol synthesis when added alone, the reversal of the apomorphine effect was difficult to assess precisely. However, if the effect of apomorphine was assessed in the presence of a neuroleptic drug by comparison with the control value obtained in incubations with that drug alone at the same concentration, a consistent series of results could be obtained. The results obtained when the concentration-response curve for apomorphine was determined in the presence and absence of a fixed concentration of haloperidol or α-flupenthixol (Fig. 3) showed a parallel shift to the right of the response curve for apomorphine in the presence of these neuroleptics, suggesting a competitive interaction between the drugs and apomorphine. The apomorphine-blocking effects of α-flupenthixol were similar when estimated after incubations of 5-, 10-, or 15-min duration, suggesting a rapid interaction between the two drugs. Haloperidol or chlorpromazine had no direct inhibitory effects on tyrosine hydroxylase activity in detergent-containing homogenates of striatal tissue when

[3] The abbreviation used is: ADTN, 2-amino-6,7-dihydroxy-1,2,3,4-tetrahydronaphthalene.

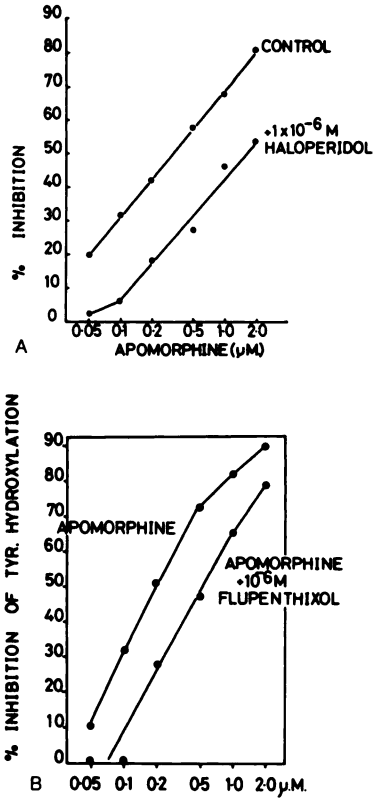


FIG. 3. *Concentration-effect curves for inhibition of tyrosine hydroxylation by apomorphine in striatal synaptosomes in the presence and absence of neuroleptic drugs: 1 μM haloperidol (A) and 1 μM α-flupenthixol (B)*

Each point is the mean of three determinations, and values are expressed as percentage inhibition relative to controls incubated without added drug ("control") or with the test concentration of haloperidol or flupenthixol. Control values (n = 4) were: for experiment A in the absence of haloperidol, 0.085 pmole/min/mg, and with haloperidol, 0.042 pmole/min/mg; for experiment B, in the absence of added flupenthixol, 0.090 pmole/min/mg, and in the presence of flupenthixol, 0.076 pmole/min/mg.

tested at a concentration of 5μM. Table 3 summarizes the results obtained with 18 neuroleptic drugs tested in the synaptosome system. The results are presented as IC_{50} values for drug-induced inhibition of tyrosine hydroxylation, from experiments in which drugs were added alone, and as EC_{25} values, representing the drug concentrations needed to cause a 25% reversal of the inhibition caused by apomorphine (Fig. 4). The latter measure was considered more reliable than the more usual EC_{50} value, since several neuroleptic drugs (e.g., chlorpromazine) failed to produce more than 50% reversal of the apomorphine effect even when tested at high concentrations, and most of the drugs tested failed to produce 100% reversal. All the drugs tested caused some inhibition of tyrosine hydroxylation when added alone, and most were able to cause some reversal of the inhibitory effects of apomorphine. In some cases (pimozide, spiroperidol, and haloperidol) the drugs were very potent apomorphine antagonists, with EC_{25} values for apomorphine reversal of less than 100 nM.

TABLE 3

Effects of neuroleptic drugs on catechol formation in intact striatal synaptosomes and on inhibitory effects of apomorphine

Drugs were tested as inhibitors of the conversion of [^{3}H]tyrosine to labeled catechols in intact striatal synaptosomes incubated in Krebs solution for 10 min at 37°; each drug was tested at three to five concentrations, in duplicate, and IC_{50} values were determined graphically. Each drug was also tested in the presence of 200 nM apomorphine, to determine its ability to reverse the inhibition caused by this concentration of apomorphine; the drug concentration required to cause 25% reversal of the apomorphine effect (EC_{25}) was determined graphically (see Fig. 4). Structures of numbered compounds are given in Table 1. Data on inhibition of dopamine-sensitive adenylate cyclase are from previous publications (see ref. 5) and from Table 1.

Drug	IC_{50} for tyrosine hydroxylation	EC_{25} for reversal of apomorphine inhibition	K_i for dopamine-sensitive adenylate cyclase
	μM	μM	μM
α-Flupenthixol	>10.0	0.15	0.001
β-Flupenthixol	>10.0	5.50	>1.00
Trifluoperazine	>10.0	0.30	0.019
Chlorpromazine	10.0	0.50	0.048
(+)-Butaclamol	2.0	0.20	0.0088
(−)-Butaclamol	18.0	1.00	>1.00
Pimozide	2.0	0.055	0.14
Haloperidol	1.0	0.016	0.08
Spiroperidol	>10.0	0.035	0.095
R 1639	6.5	0.24	2.50
R 1484	1.2	1.00	3.60
R 1658	7.0	>10.00	0.41
R 2839	16.0	0.50	1.10
R 1589	2.5	0.35	0.17
R 1610	2.5	0.40	0.10
R 1673	2.5	4.00	>45.00

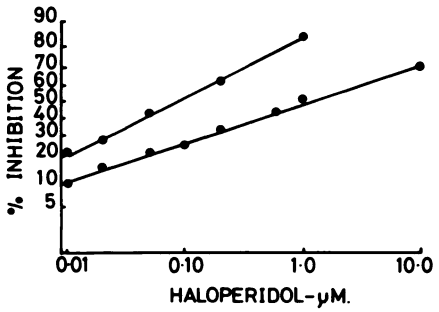


FIG. 4. *Concentration-effect curves for haloperidol as an inhibitor of tyrosine hydroxylation in intact striatal synaptosomes (○) and as an antagonist of inhibitory efect of apomorphine (0.2 μM) on tyrosine hydroxylation in this preparation (●)*

Each point is the mean of three determinations, and values are expressed as percentage inhibition relative to controls with no added drugs (○) or percentage inhibition of the apomorphine effect (●), taking into account the inhibitory effect of haloperidol when added alone at each concentration. Results are plotted on log probit graph paper to allow determination of IC_{50} and EC_{25} values (see Table 3).

Various other substances were tested for their possible effects on catechol synthesis in the striatal synaptosomal system (Table 4). The cholinergic agonists carbamylcholine (carbachol) and oxotremorine and the indirectly acting sympathomimetic amphetamine had no significant effects on tyrosine hydroxylation at concentrations up to 10 μM. The *alpha* adrenoceptor agonist phenylephrine and the *beta* agonist isoprenaline, however, both caused about 50% inhibition of catechol synthesis at 10 μM.

Inhibition of dopamine uptake. Nine drugs were tested as inhibitors of [^{3}H]dopamine uptake in striatal synaptosome preparations. All the drugs were active as uptake inhibitors, with IC_{50} values in the range 1.5 μM (α-clopenthixol) to 16 μM (promazine and promethazine) (Table 5). The inhibition of dopamine uptake caused by two drugs, chlorpromazine and α-flupenthixol, appeared to be of a simple competitive type when this was examined by kinetic analysis (27). There was no correlation, however, between neuroleptic activity and ability to inhibit dopamine uptake, since the potent neuroleptic phenothiazine fluphenazine was only marginally more potent than the inactive compounds promazine and promethazine, and the active neuroleptics α-clopenthixol and α-flupenthixol were only slightly more potent as uptake inhibitors than their β isomers, which are very much less active as neuroleptics (5).

Effects of drugs on dopamine release from synaptosomes. Seeman and his colleagues (17–19) reported that various neuroleptic drugs can cause a release of dopamine from striatal synaptosomes and are also able to block the release of labeled dopamine evoked by electrical stimulation from striatal slices *in vitro*. They found that the potent butyrophenones haloperidol and spiroperidol were particularly powerful inhibitors of the stimulus-evoked release of dopamine (19). In the present studies we used striatal synaptosomes rather than slices, and studied drug effects

TABLE 4

Effects of various substances on catechol formation in rat striatal synaptosomes

Compounds were tested on tyrosine hydroxylation in striatal synaptosomes by measuring formation of labeled catechols from [^{3}H]tyrosine after a 10-min incubation at 37°. Each value is the mean of two or three determinations, and is expressed as percentage of control values in the absence of added drugs. Control values (n = 4) were 0.13 pmole of labeled catechols per minute per milligram of tissue.

Compound	Concentration	[^{3}H]catechols formed
	μM	% control
(±)-Isoprenaline	1	117.5
	10	54.6
(±)-Amphetamine	0.1	94.6
	1	98.5
	10	100.3
(−)-Phenylephrine	0.1	98.6
	1	77.7
	10	49.7
Carbachol	0.1	90.8
	1	96.5
	10	91.2
Oxotremorine	0.1	96.1
	1	89.5
	10	85.5

TABLE 5

Inhibition of [^{3}H]dopamine uptake in rat striatal synaptosomes by neuroleptic drugs

Drug effects on [^{3}H]dopamine uptake in homogenates of rat striatum were determined after 5 min of incubation at 37°. Each drug was tested at three concentrations, with quadruplicate estimations, and IC_{50} values were determined graphically. Data for inhibition of dopamine-sensitive adenylate cyclase are from previous publications (see ref. 5).

Drug	IC_{50} for [^{3}H]dopamine uptake	K_i for dopamine-sensitive adenylate cyclase
	μM	μM
Promazine	16.0	>1.00
Promethazine	16.0	>1.00
Fluphenazine	11.0	0.0043
α-Flupenthixol	3.5	0.001
β-Flupenthixol	5.8	>1.00
Trifluoperazine	9.0	0.019
Chlorpromazine	10.0	0.048
α-Clopenthixol	1.5	0.016
β-Clopenthixol	3.0	>1.00

both on the spontaneous efflux of labeled dopamine and on the stimulated efflux caused by addition of the depolarizing alkaloid protoveratrine. Protoveratrine has been shown to cause a tetrodotoxin-sensitive depolarization of nerve terminals in brain tissue incubated *in vitro* (28). The experimental system involved loading striatal synaptosomes by incubation with [^{3}H]dopamine, followed by a 10-fold dilution in nonlabeled medium and further incubation for a short period (5 min) before harvesting labeled synaptosomes by membrane filtration. During this 5-min period the further uptake of labeled dopamine still present in the medium was greatly reduced by the dilution, and very little (less than 10%) of the labeled catecholamine initially accumulated was lost by spontaneous efflux. Very similar results were obtained in experiments in which labeled synaptosomes were harvested by centrifugation after the initial labeling period and resuspended in nonradioactive medium, as described by Seeman and Lee (17).

Protoveratrine caused a marked concentration-dependent acceleration of the loss of labeled dopamine from synaptosomes. After a 5-min exposure to 35 μM protoveratrine the treated synaptosomes contained approximately 50% of the labeled dopamine present in control tissues incubated without drug. Neuroleptic drugs by themselves also caused increases in the efflux of [^{3}H]dopamine, and the results obtained are in good agreement with those reported previously by Seeman and Lee (17). The four drugs tested were also able to antagonize the releasing effects of 35 μM protoveratrine, although this effect, as in the previous studies with reversal of the apomorphine inhibition of tyrosine hydroxylation, was difficult to assess because of the releasing effect of the neuroleptics when added alone. Using the effects of the neuroleptic drugs alone as a control, however, a dose-dependent antagonism of the releasing action of protoveratrine could be detected (Fig. 5 and Table 6). Unlike Seeman and his colleagues (19), however, we did not find that haloperidol or spiroperidol was especially potent in this respect. The dopamine-releasing effect of chlorpromazine (2 μM) was not affected by the simultaneous presence of apomorphine (2 μM), which caused no release of [^{3}H]dopamine when added alone at this concentration. Apomorphine (2 μM) also had no effect on the blockade of protoveratrine-induced dopamine release by chlorpromazine (2 μM).

DISCUSSION

The present results extend our previous findings on the inhibitory effects of neuroleptic drugs on the dopamine-sensitive adenylate cyclase in rat striatum, and compare the effects of these drugs on this postsynaptic model with their actions on various biochemical mechanisms present in the presynaptic dopamine terminals in this area of brain. It appears to be valid to consider the dopamine-sensitive adenylate cyclase as a postsynaptic model, since the activity of this system is unaffected, or even increased, in striatal tissue after surgically or chemically induced lesions of the dopaminergic nigrostriatal pathway (29, 30). There was a fair correlation between dopamine antagonist properties in the adenylate cyclase system and actions

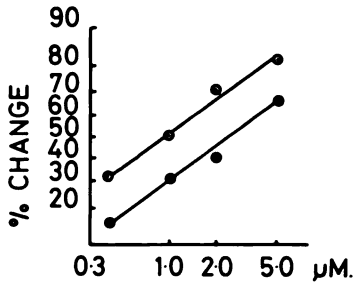


FIG. 5. *Concentration-effect curves for chlorpromazine on release of [^{3}H]dopamine from rat striatal synaptosomes (●) and antagonism of dopamine-releasing effects of protoveratrine (35 μM) in the same preparation (⊙)*

Synaptosomes were labeled by incubation with [^{3}H]dopamine (0.5 μM) in Krebs phosphate solution for 10 min, and aliquots of the labeled synaptosomes were then diluted 10-fold in Krebs solution containing various concentrations of chlorpromazine with or without added protoveratrine. After 5 min of incubation at 37°, labeled synaptosomes were harvested by filtration. Each point is the mean of three determinations, and results are expressed as percentage change in [^{3}H]dopamine content relative to controls with no added drugs (●) or as percentage change in the releasing effect of protoveratrine in the presence of chlorpromazine (⊙), taking into account the release induced by chlorpromazine itself at each drug concentration. Control values with no added drug (n = 6) were 22.5 pmoles of [^{3}H]dopamine per sample (equivalent to 2 mg of striatal tissue). Protoveratrine alone (35 μM) caused a 55.0% release of [^{3}H]dopamine during the 5-min incubation (n = 4).

in vivo as apomorphine antagonists for the 16 analogues of haloperidol tested. Since apomorphine antagonism *in vivo* is a good predictive test for neuroleptic activity, the dopamine-sensitive adenylate cyclase again seems to be a reliable predictor of neuroleptic activity in this chemical class, although all the butyrophenones remained less potent in the system *in vitro* than predicted from their high potencies *in vivo*. We have suggested possible reasons that might explain this anomaly elsewhere (5).

In agreement with previous reports (15), we found that apomorphine was a very potent inhibitor of the conversion of tyrosine to catechols in intact striatal synaptosomes. When dopamine uptake sites were inhibited with benztropine, to eliminate effects due to accumulation of drugs within synaptosomes, it was clear that dopamine and various related compounds also possess potent inhibitory effects on catechol synthesis in this presynaptic model. The specificity of this response was similar to that found previously for activation of the postsynaptic dopamine-sensitive adenylate cyclase in rat striatum, in that dopamine, epinine, ADTN, and apomorphine were approximately equipotent and (−)-noradrenaline was less potent than dopamine (5). However, the present results on the presynaptic model show that two compounds acted on the tyrosine hydroxylation system that were only very weak activators of the adenylate cyclase: (+)-noradrenaline and (±)-α-methyldopamine. In addition, apomorphine behaved as a simple dopamine agonist in the present studies, whereas on the adenylate cyclase it has mixed agonist and antagonist properties (5). The presynaptic "autoreceptors" for dopamine on dopaminergic terminals may thus differ slightly in their pharmacological specificity from postsynaptic dopamine receptor sites. This question is difficult to answer on the basis of the present results, however, since in addition to

TABLE 6

Dopamine-releasing effects of neuroleptic drugs in striatal synaptosomes and blockade of releasing effect of protoveratrine

Drugs were tested for their ability to cause an increased efflux of [^{3}H]dopamine from striatal synaptosomes previously labeled by exposure to the labeled amine in the absence of added drug, and for their ability to antagonize the release of [^{3}H]dopamine evoked by addition of protoveratrine (35 μM) to the incubation medium. Each drug was tested at three or four concentrations, with triplicate estimations at each point, and EC_{50} and IC_{50} values were determined graphically (see Fig. 6).

Drug	EC_{50} for [^{3}H]dopamine release	IC_{50} for blockade of protoveratrine release of [^{3}H]dopamine
	μM	μM
α-Flupenthixol	7.0	4.0
Chlorpromazine	2.6	0.9
Haloperidol	4.0	1.9
Spiroperidol	5.0	2.0

dopamine receptors other categories of presynaptic receptors may be present on dopaminergic terminals; we found, for example, that high concentrations of phenylephrine and isoprenaline also had some inhibitory effects on catechol synthesis although cholinergic agonists were without effect. The existence of autoreceptors on dopamine terminals may be of relevance for the normal regulation of dopamine turnover and release in such terminals by an "autoinhibition" mechanism, similar to that described recently in noradrenergic nerve terminals (31). The potent effects of apomorphine on such receptors may also be relevant to some of the "paradoxical" behavioral effects elicited by low doses of apomorphine *in vivo* (8). The mechanism whereby apomorphine and other agonists for presynaptic receptor sites exert their inhibitory effects on the conversion of tyrosine to catechol remains obscure. Recent findings suggest that tyrosine hydroxylase in nerve terminals can be activated by a cyclic AMP-dependent mechanism (11, 13, 32, 33).

Neuroleptic drugs could at least partially antagonize the inhibitory effects of apomorphine, as reported previously (15, 16). It was difficult to make precise estimates of drug potencies, however, because the neuroleptics themselves also caused an inhibition of synaptosomal catechol synthesis. Nevertheless, a competitive interaction appeared to exist between, for example, haloperidol or α-flupenthixol and apomorphine at presynaptic sites (Fig. 3). Haloperidol, pimozide, and spiroperidol were particularly potent apomorphine antagonists in this system, and these presynaptic effects were more potent than those found for the same drugs on the dopamine-sensitive adenylate cyclase (2, 3). The potent neuroleptics α-flupenthixol and (+)-butaclamol each possess isomeric forms with little or no neuroleptic activity [β-flupenthixol and (−)-butaclamol]; α- and β-flupenthixol and the (+) and (−) enantiomers of butaclamol differ by more than three orders of magnitude in their ability to inhibit the dopamine-sensitive adenylate cyclase in rat striatum (2, 5). In their ability to reverse apomorphine presynaptically, however, the isomeric forms of butaclamol and flupenthixol differed by only 5- and 37-fold respectively. It is difficult to know how relevant these findings may be to the pharmacological profile of the neuroleptic drugs. Certain drugs, notably the butyrophenones and pimozide, do appear to be particularly active as antagonists at presynaptic dopamine receptors, and this may suggest that subtle differences in the agonist and antagonist specificity of such sites exist when compared with the postsynaptic dopamine receptors in the central nervous system. It seems unlikely, however, that blockade of presynaptic dopamine receptors contributes importantly to the antipsychotic actions of the neuroleptic drugs, although such blockade may well contribute to the increased rate of dopamine turnover caused by many neuroleptics, by removing a normal local autoinhibitory process at dopamine nerve terminals (13). It seems unlikely, however, to contribute to the clinical effects, since *inhibition* of dopamine synthesis with α-methyl-*p*-tyrosine has been shown to potentiate the antipsychotic actions of the neuroleptic drug thioridazine (8).

In addition to their effects on pre- and postsynaptic dopamine receptors, we confirmed that neuroleptic drugs also affect dopamine uptake and release in striatal synaptosomes and antagonize the evoked release of dopamine from such particles. However, none of these effects was elicited by drug concentrations as low as those found to affect pre- and postsynaptic receptor mechanisms, and we did not find the butyrophenones to be particularly potent by comparison with chlorpromazine. It seems likely that these effects can be attributed to nonspecific membrane actions of the neuroleptics, perhaps related to their local anesthetic properties (18), or to weak interactions of these drugs with other dopamine recognition sites in presynaptic dopaminergic nerve terminals. The findings reported by Seeman and his colleagues (19) on the blocking effects of neuroleptics on stimulus-evoked release of dopamine from striatal terminals could, of course, be of considerable relevance in offering an alternative mechanism to ex-

plain the depressant effects of these drugs on dopaminergic neurotransmission in the central nervous system. Using a synaptosomal model and protoveratrine-evoked release, however, we were unable to find any particularly powerful blocking effect in the butyrophenone drugs by comparison with chlorpromazine. The synaptosome model, however, may not be as suitable for such studies as the electrically stimulated slice preparation used by Seeman and Lee (19).

We conclude from the present findings that the actions of neuroleptic drugs on dopamine-stimulated adenylate cyclase in rat striatum are good predictors of neuroleptic activity, although many of these drugs also possess potent actions on presynaptic mechanisms in dopaminergic terminals.

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