Proc. Nat. Acad. Sci. USA
Vol. 71, No. 7, pp. 2915–2918, July 1974

Antipsychotic Drugs and Dopamine-Stimulated Adenylate Cyclase Prepared from Corpus Striatum of Rat Brain

(neuroleptic drugs/drug analogs)

M. KAROBATH AND H. LEITICH

Department of Experimental Psychiatry, Psychiatrische Universitätsklinik, Universität Wien, Austria

Communicated by Seymour S. Kety, May 6, 1974

ABSTRACT **Antipsychotic drugs and their clinically impotent congeners were examined as inhibitors of dopamine-sensitive adenylate cyclase (EC 4.6.1.1) in cell-free membrane preparations of the caudate-putamen of rat brain. Of 12 neuroleptic drugs with reported antipsychotic efficacy, all inhibit stimulation of adenylate cyclase by 40 μM dopamine at micromolar concentrations. Among 14 other structurally related drugs that are not clinically effective as antipsychotic agents, 12 were almost ineffective while two drugs were moderate inhibitors of dopamine-sensitive adenylate cyclase.**

Phenothiazines and other structurally analogous drugs with antipsychotic action and neuroleptic drugs of the butyrophenone and diphenylpiperidine classes are reported to enhance 3,4-dihydroxyphenylethylamine (dopamine) turnover in the brain (1–4), possibly as a consequence of their blockade of dopamine receptors. This enhancement of dopamine turnover in the basal ganglia by neuroleptic agents has been suggested to be characteristic only for the subgroup of phenothiazines with antipsychotic action since promethazine and methdilazine, drugs lacking antipsychotic activity, did not have this effect (1, 2, 4). These observations (for review see ref. 4), and the coincidence of "parkinsonian" side effects in patients during therapy with neuroleptic drugs, have led to the speculation that the effects of antipsychotic drugs on dopamine metabolism in the brain might be specifically related to their psychiatric and neurological effects in patients. Until recently, only indirect *in vivo* methods existed to evaluate the effectiveness of drugs as dopamine-receptor blockers, notably the rate of rise of homovanillic acid, the main metabolite of dopamine, in brain and cerebrospinal fluid. The recent discovery of a dopamine-sensitive adenylate cyclase (EC 4.6.1.1) in homogenates of corpus striatum prepared from rat brain and its similarity or close relationship with the dopamine receptor (5) has prompted the present study. We attempted to learn whether drugs with antipsychotic action would inhibit stimulation of dopamine-sensitive adenylate cyclase and if their clinically ineffective structural analogs would fail to inhibit stimulation by dopamine *in vitro*.

MATERIALS AND METHODS

Materials. [α-^{32}P]ATP and [8-^{3}H]3′:5′-cyclic AMP (cAMP) were obtained from the Radiochemical Center, Amersham, and used without further purification. The drugs were obtained from several sources.*

Methods. Dopamine-sensitive adenylate cyclase was assayed with cell-free homogenates from rat corpus striatum (caudate-putamen) obtained from the brains of Sprague–Dawley rats weighing 200–250 g, as described (5). Rats were killed by decapitation, and the striatum was rapidly dissected and homogenized in a glass Elvehjem-type homogenizer with a motor-driven Teflon pestle in 25 volumes (v/w) of a hypotonic medium that contained 2 mM Tris-maleate buffer (pH 7.4) and 2 mM EGTA. To each incubation tube 0.010 ml of the homogenate was added. The standard incubation medium (final volume, 0.05 ml) contained in mM: Tris-maleate buffer (pH 7.3), 80; [α-^{32}P]ATP, 0.5 (6 to 9 $\times$ 10^5 cpm); $MgSO_4$, 2; theophylline, 10; EGTA, 0.4; unlabeled cAMP, 0.15; plus test substances. Reactions were initiated by addition of ATP and conducted for 150 sec in a shaking water bath at 30°. The reaction was terminated by placing the tubes in a boiling water bath for 2 min; [8-^{3}H]cAMP (1 $\times$ 10^4 cpm) was added as an internal standard to permit estimation of the recovery of cAMP after column chromatography. cAMP was then isolated by chromatography on neutral aluminum oxide columns (6). Radioactivity was determined by liquid scintillation spectrometry. Recovery of [^{3}H]cAMP was 90–100%. Blank values were obtained by incubation of the complete

Abbreviation: dopamine; 3,4-dihydroxyphenylethylamine.

Correspondence to: M. Karobath, Department of Experimental Psychiatry, Psychiatrische Universitätsklinik, Lazarettgasse 14, A-1090 Wien, Austria.

* Drugs were generously contributed as follows: chlorpromazine·HCl, triflupromazine·HCl, trifluoperazine·2 HCl, and trimeprazine-tartrate, Smith Kline & French, Philadelphia, Pa.; droperidol, Janssen Pharmaceuticals Ltd. N. V. Beerse, Belgium; diethazine·HCl, fentethazine·HCl, and chlorpromazine-sulfoxide·HCl, Phone-Poulenc, Vitry s/Seine, France; thiethylperazine, mesoridazine, and thioridazine·HCl, Sandoz Pharmaceuticals, Hanover, N.J.; ethopropazine, Warner Chilcott Labs., Morris Plains, N.J.; haloperidol, McNeil Labs., Evansville, Ind.; pyrathiazine·HCl, Upjohn Pharmaceuticals, Kalamazoo, Mich.; fluphenazine·2 HCl, E. R. Squibb & Sons, New Brunswick, N.J.; chlorprothixene and chlordiazepoxide, Roche Laboratories, Nutley, N.J.; mepazine·HCl, Chemische Fabrik Promonta, Hamburg, Germany; clozapine, Wander A.G. Basel, Switzerland; methdilazine·HCl, Mead Johnson Labs., Evansville, Ind.; promethazine·HCl, Wyeth Labs., Philadelphia, Pa.; benztropine-mesylate, Merck Sharp and Dohme, West Point, Pa.; desmethylimipramine·HCl, Lakeside Laboratories, Milwaukee, Wis.; imipramine·HCl, Ciba-Geigy, A.G., Basel, Switzerland; diphenhydramine·HCl, Parke-Davis & Co., Detroit, Mich.; and thiothixene, Roering Division, Pfizer & Co., New York, N.Y.

TABLE 1. *Effect of antipsychotic drugs on dopamine-stimulated adenylate cyclase from rat brain striatum*

Concentration of drugs (μM) →	% Inhibition of stimulation by 40 μM dopamine ± SEM		
	0.3	1	3
Trifluprom azine	52.0 ± 3.9	93.9 ± 1.8	102.3 ± 3.3
Fluphenazine	42.5 ± 4.2	94.6 ± 0.0	113.9 ± 7.9
Trimeprazine	41.1 ± 1.6	71.1 ± 5.6	102.4 ± 2.4
Mesoridazine	37.4 ± 2.3	81.5 ± 3.1	95.1 ± 2.3
Trifluoperazine	30.3 ± 0.9	67.4 ± 3.1	93.7 ± 1.5
Thioridazine	29.7 ± 2.3	69.6 ± 2.9	98.5 ± 2.1
Chlorpromazine	29.5 ± 3.3	66.6 ± 2.1	88.6 ± 3.9
Chlorprothixene	53.2 ± 1.4	85.4 ± 2.2	99.3 ± 0.9
Thiothixene	5.7 ± 1.2	39.1 ± 2.1	67.7 ± 1.1
Haloperidol	39.6 ± 3.6	71.1 ± 6.6	96.2 ± 2.7
Droperidol	13.2 ± 3.4	23.2 ± 3.5	49.6 ± 2.3
Clozapine	8.6 ± 4.6	43.2 ± 7.7	70.5 ± 1.6

Concentration of blocking agents are expressed in μM. Values for the per cent inhibition of the response to 40 μM dopamine are the means ± SEM (n = 4 replications); three or more experiments were performed with each drug. Average values for [^{32}P]-cAMP formed in control incubations from 10 different preparations were 7.00 ± 0.45 (mean ± SEM, n = 10) pmol.

reaction mixture with boiled homogenates. Blank values were less than 0.015% of the counts per min of the added [α-^{32}P]-ATP.

In some control experiments the purity of the ^{32}P-labeled product eluted from the aluminum oxide columns was confirmed by chromatography on Dowex-50 or Dowex-1 columns and by precipitation by $ZnSO_4$ and $Ba(OH)_2$ (7). In these experiments the enzymatically formed ^{32}P-labeled product behaved like authentic [^{3}H]cAMP.

Stimulation of dopamine-sensitive adenylate cyclase by dopamine varied somewhat in different preparations, from 80 to 110% of basal activity in the absence of dopamine. For example, in 10 preparations the mean stimulation (±SD) by 40 μM dopamine was 95 ± 14.3% above basal activity. This is similar to the stimulation described for the same system when the formation of unlabeled cAMP was measured (5). Since the stimulation of the cyclase varied in different experiments and so that the results obtained in different preparations could be compared, results are expressed as per cent inhibition of stimulation by dopamine. Apparent inhibition by more than 100% indicates that a drug inhibited activity of the enzyme to values below the basal activity estimated without added dopamine.

RESULTS

We examined 12 drugs, which are reported to have antipsychotic effects, for their capacity to inhibit the stimulation of adenylate cyclase in homogenates from rat striatum. The inhibition by these agents of the response to 40 μM dopamine is shown in Table 1. All of these antipsychotic drugs were effective inhibitors of dopamine-stimulated adenylate cyclase. Among the phenothiazines, fluphenazine and trifluprom azine were the most potent drugs. High potency of fluphenazine has also been reported in a dopamine-stimulated adenylate cyclase in bovine retina, where it was about five times more potent than haloperidol or chlorpromazine (8). Mesoridazine, thioridazine, and trimeprazine were comparable to chlorpromazine as inhibitors of dopamine-stimulated adenylate cyclase. Chlorpromazine and haloperidol had in our experiments similar potency, which is consistent with earlier findings of Kebabian *et al.* (5), who also studied these two neuroleptic drugs. Chlorprothixene, a thioxanthene, was as potent as fluphenazine (Table 1). Droperidol, a butyrophenone, was a relatively weak inhibitor of dopamine-stimulated adenylate cyclase, in contrast to haloperidol. Clozapine, a drug reported to be antipsychotic (9), although lacking neuroleptic activity in animals (10), was a relatively weak inhibitor of dopamine-stimulated adenylate cyclase.

TABLE 2. *Effect of drugs with poor antipsychotic activity on dopamine-stimulated adenylate cyclase activity from rat brain striatum*

Concentration of drug (μM) →	% Inhibition of stimulation by 40 μM dopamine ± SEM	
	1	10
Thiethylperazine	18.7 ± 4.9	58.7 ± 5.4
Methdilazine	3.8 ± 2.3	55.0 ± 1.3
Fenethazine	3.6 ± 3.5	37.8 ± 5.5
Promethazine	4.2 ± 4.6	35.5 ± 1.6
Diethazine	6.0 ± 2.6	22.8 ± 5.8
Ethopropazine	7.8 ± 0.1	21.8 ± 2.4
Mepazine	0.7 ± 2.6	10.5 ± 4.9
Chlorpromazine-sulfoxide	0.7 ± 7.4	6.1 ± 3.6
Pyrathiazine	3.2 ± 2.9	8.3 ± 2.3
Diphenhydramine	0.0 ± 2.1	0.0 ± 2.5
Imipramine	6.3 ± 1.3	29.3 ± 4.2
Desmethylimipramine	N.D.	25.3 ± 2.1
Benztropine-mesylate	0.0 ± 1.9	12.6 ± 2.0
Chlordiazepoxide	4.0 ± 6.9	1.6 ± 2.7

Concentrations of drugs are expressed in μM. Values for the percent inhibition of the response to 40 μM dopamine are means ± SEM (n = 4 replications); three or more determinations have been made for each drug; *N.D.*, not determined.

We also examined drugs with poor or no antipsychotic activity for their potency as inhibitors of stimulation of adenylate cyclase by 40 μM dopamine. In contrast to the antipsychotic drugs, we observed either less potent inhibition of dopamine stimulation or failure to inhibit the response to dopamine (Table 2). Thiethylperazine and methdilazine were the most potent drugs of this series, but were still less potent than the antipsychotic drugs.

DISCUSSION

The results indicate that all of the 12 antipsychotic drugs that were examined are potent inhibitors of dopamine-stimulated adenylate cyclase. They belonged to four different chemical groups, including phenothiazines, thioxanthenes, butyrophenones, and a tricyclic dibenzoazepine (clozapine). It seems, therefore, unlikely that the potent inhibition of dopamine-stimulated adenylate cyclase by these drugs is a mere coincidence; it seems likely that antipsychotic drugs are inhibitors of dopamine-stimulated adenylate cyclase in brain tissue. In other experiments, compounds with clinical use as antihistaminic, anti-Parkinson, tranquilizing, antipruritic, antiemetic, antianxiety, and antidepressant drugs, but not as antipsychotic drugs, were also examined as inhibitors of dopamine-stimulated adenylate cyclase. Most of them were phenothiazines and structurally similar to those pheno-

thiazines with antipsychotic activity. These compounds either failed to inhibit dopamine-stimulated adenylate cyclase or they were less potent inhibitors than their antipsychotic congeners. Thus, there is a good correlation between antipsychotic activity and ability to block dopamine-stimulated adenylate cyclase responses *in vitro*.

The effects of antipsychotic drugs on cAMP formation in homogenates are unlikely to be due to varying inhibition of phosphodiesterase activity, since the disappearance of cAMP in our assay conditions, even without addition of an excess of the exogenous unlabeled cAMP, has been reported to be less than 10% (5). Moreover, the concentrations of antipsychotic drugs that inhibited dopamine-stimulated adenylate cyclase were generally 100 times lower than those that are required to inhibit cAMP-phosphodiesterase in brain tissue (11). We studied dopamine-stimulated adenylate cyclase in homogenates of striatal tissue prepared by homogenization in a hypotonic medium to disrupt compartments enclosed by cellular and subcellular membranes. Thus, effects of drugs on amine-uptake mechanisms, which might influence the results with more complex tissue such as brain minces or slices, are unlikely to affect our results, since dopamine uptake does not occur in hypotonic homogenates (12).

With few exceptions, there is a good correlation between the effects on dopamine-stimulated adenylate cyclase *in vitro* and the effects of antipsychotic drugs on the turnover of dopamine in the striatum *in vivo*. Thus, the antipsychotic drugs and potent inhibitors of the response of adenylate cyclase to dopamine (Table 1), chlorpromazine (1, 2, 4, 13, 14), fluphenazine (13), haloperidol (2, 13), and chlorprothixene (2, 13), are all reported to increase dopamine turnover in basal ganglia. Trimeprazine in low doses exerts antipruritic effects (15). It was used in a recent study as nonantipsychotic "control" phenothiazine, and produced a marked increase of dopamine turnover in basal ganglia (4). However, in higher doses, trimeprazine has antipsychotic properties (16–18), and it was a potent inhibitor of dopamine-stimulated adenylate cyclase (Table 1). Conversely, the nonantipsychotic drugs and weak inhibitors of dopamine-stimulated adenylate cyclase (Table 2), promethazine (1, 2, 4) and methdilazine (4), are reported not to enhance dopamine turnover *in vivo*. The butyrophenones gave evidence of inhibitory potency far less than their clinical potency or *in vivo* neuroleptic activity would predict (10, 19). Droperidol was particularly weak, and was about as potent as thiethylperazine, a drug considered to be "poorly antipsychotic" (Tables 1 and 2). These results are consistent with the previous report of relatively weak activity of haloperidol in very similar experiments by Kebabian *et al.* (5). The results would thus appear to suggest either that the actions *in vivo* of the butyrophenones are not well represented by our measurements *in vitro*, or that the behavioral and antipsychotic effects of these agents are not simply dependent on the blockade of dopamine receptors. Many other reasons might be given for this discrepancy, since different classes of drugs might have a different distribution and metabolism *in vivo* and since dopamine-sensitive adenylate cyclase might not completely represent the dopamine receptor or might have different properties in other brain areas such as the limbic system. Further studies of the potency and actions of these agents *in vivo* and *in vitro* are clearly indicated. Two other exceptional drugs are clozapine and thioridazine. Thioridazine enhances dopamine turnover *in vivo* in the basal ganglia of rabbits, mice, and cats after single doses but not after chronic administration (20); there is less effect in rats (14) and none in monkeys (4). Clozapine increases levels of homovanillic acid in striatal tissue only at high doses (21). Differences between clozapine or thioridazine and other antipsychotic drugs on extrapyramidal control of movement have also been observed. Clozapine is reported to have antipsychotic properties (9), but is not a neuroleptic drug and does not produce catalepsy in animals (10). Higher doses of thioridazine are necessary to produce catalepsy in animals when it is compared with other neuroleptic drugs, including other phenothiazines (10, 14) or butyrophenones (10). Thioridazine is less potent than chlorpromazine in supressing metamphetamine-induced locomotor behavior in rats with unilateral lesions of the anterior portion of the substantia nigra (22). The relatively weak actions of these two agents upon presumably striatal motor functions in animals appear to correspond with the low incidence of extrapyramidal side effects in patients treated with thioridazine (23) or clozapine (9). These experimental and clinical observations have been used to argue against the concept that the blockade of dopamine receptors might underly the therapeutic antipsychotic effects of thioridazine (22) and clozapine (10). However, we find that in homogenates of rat brain striatum, the antipsychotic drug thioridazine is almost as potent an inhibitor of dopamine-sensitive adenylate cyclase as the antipsychotic neuroleptic drug chlorpromazine, suggesting that both drugs are potent inhibitors of dopamine receptors (Table 1). While clozapine was weaker in its antagonism of the effect of dopamine than thioridazine, it was active (Table 1). Thus, other pharmacological effects of thioridazine and clozapine unrelated to their actions on the dopamine receptors in the striatum might be responsible for antipsychotic activity, together with the relative lack of effects on the extrapyramidal system. One difference between neuroleptic drugs and clozapine is the strong anticholinergic potency of clozapine (10). While thioridazine has been reported to have somewhat stronger anticholinergic potency than chlorpromazine (24–26), as estimated in several preparations of peripheral tissues, the potency of these agents against central muscarinic receptors is apparently unknown.

In summary, the present results have shown that antipsychotic and neuroleptic drugs are potent inhibitors of dopamine-sensitive adenylate cyclase, suggesting that blockade of dopamine receptors in the central nervous system might be involved in the mechanism of action of antipsychotic drugs. However, there appears to be a discrepancy between the potency of the butyrophenones *in vivo* and their action on dopamine-sensitive adenylate cyclase *in vitro*.

The help of R. J. Baldessarini during the preparation of this manuscript is gratefully acknowledged. This work was supported by Fonds zur Förderung der wissenschaftlichen Forschung in Österreich, Projekt 1606.

1. Carlsson, A. & Lindqvist, M. (1963) *Acta Pharmacol. Toxicol.* **20**, 140–144.
2. Nybäck, H., Borzecki, Z. & Sedvall, G. (1968) *Eur. J. Pharmacol.* **4**, 395–403.
3. O'Keeffe, R., Sharman, D. F. & Vogt, M. (1970) *Brit. J. Pharmacol.* **38**, 287–307.
4. Mathysse, S. (1972) *Fed. Proc.* **32**, 200–205.
5. Kebabian, J. W., Petzold, G. L. & Greengard, P. (1972) *Proc. Nat. Acad. Sci. USA* **69**, 2145–2149.
6. Ramachandran, J. (1971) *Anal. Biochem.* **43**, 227–239.

7. Krishna, G., Weiss, B. & Brodie B. B. (1968) *J. Pharmacol. Exp. Ther.* **163**, 379–385.
8. Brown, J. H. & Makmann, M. H. (1973) *J. Neurochem.* **21**, 477–479.
9. Angst, J., Bente, D., Berner, P., Heimann, H., Helmchen, H. & Hippius, H. (1971) *Pharmacopsychiatry* **4**, 201–211.
10. Stille, G. & Hippius, H. (1971) *Pharmacopsychiatry* **4**, 182–191.
11. Beer, B., Chasin, M., Clody, D. E., Vogel, J. R. & Horvitz, Z. P. *Science* **176**, 428–430.
12. Harris, J. E. & Baldessarini, R. J. (1973) *Neuropharmacology* **12**, 669–679.
13. Andén, N.-E., Roos, B. E. & Werdinius, B. (1964) *Life Sci.* **3**, 149–158.
14. Bernheimer, H. & Hornykiewicz, O. (1965) *Arch. Exp. Pathol. Pharmacol.* **251**, 135.
15. Bell, B. T., Viek, P. & Santangelo, S. C. (1960) *J. Amer. Med. Ass.* **174**, 1976–1977.
16. Fernandez-Zoila, A. (1961) *Semaine Hôpitaux Paris* **37**, 1355–1357.
17. Naviau, Y., Benoit, Y., Fouché, F. & Serret, J. (1960) *Ann. Med. Psychol.* **119 I**, 579–585.
18. Schenker, E. & Herbst, H. (1963) *Progr. Drug Res.* **5**, 269–627.
19. Janssen, A. J., Niemegeers, C. J. E. & Schellekens, K. H. L. (1965) *Drug. Res.* **15**, 104–117.
20. Roos, B. E. (1965) *J. Pharm. Pharmacol.* **17**, 820–821.
21. Bartholini, G., Haefely, W., Jalfre, M., Keller, H. H. & Pletscher, A. (1972) *Brit. J. Pharmacol.* **46**, 736–740.
22. Crow, T. J. & Gillbe C. (1973) *Nature New Biol.* **245**, 27–28.
23. Shader, R. I. & Di Mascio, A. (1970) *Psychotropic Drug Side Effects* (Williams & Wilkins Co. Baltimore, Md.).
24. Ban, T. (1969) in *Pyschopharmacology* (Williams & Wilkins Co. Baltimore, Md.), p. 208.
25. Taeschler, M. & Cerletti, A. (1958) *Schweiz. Med. Wochenschr.* **88**, 1216–1220.
26. Harley, T. J., Flesher, A. M. & Raymond, K. (1960) *Arch. Int. Pharmacodyn.* **124**, 455–460.