THE JOURNAL OF PHARMACOLOGY AND EXPERIMENTAL THERAPEUTICS Vol. 208, No. 3.

Selective Binding of Antipsychotics and Other Psychoactive Agents to the Calcium-Dependent Activator of Cyclic Nucleotide Phosphodiesterase[1]

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Accepted for publication December 6, 1978

ABSTRACT

Levin, Robert M. and Benjamin Weiss: *Selective binding of antipsychotics and other psychoactive agents to the calcium-dependent activator of cyclic nucleotide phosphodiesterase.* J. Pharmacol. Exp. Ther. **208:** 454–459, 1979.

We have examined the binding of several centrally active agents to the heat-stable, calcium-dependent protein activator of phosphodiesterase. Calcium-specific binding to activator was determined either by directly measuring the binding of radioactive drug to the activator during equilibrium dialysis or from the ability of the unlabeled drug to displace radiolabeled trifluoperazine from the activator. Antipsychotics of a variety of chemical classes displayed high-affinity, calcium-dependent binding similar to that originally shown for trifluoperazine. Competition studies suggest that the various chemical classes of antipsychotics bind to the same site on the activator. Scatchard analysis of the binding of pimozide, chlorpromazine and haloperidol to activator demonstrated dissociation constants of 0.8, 5 and 9 μM, respectively; the calculated number of specific binding sites was between 1 and 3 sites per molecule of activator. Antianxiety and antidepressant agents showed considerably less calcium-specific binding than did antipsychotics. A wide series of agents that affect the central nervous system, but which are devoid of antipsychotic activity, showed no calcium-specific binding. These include LSD, amphetamine, pentobarbital, morphine, histamine and dopamine. The magnitude of the calcium-dependent binding of drugs to activator correlated well with the ability of these agents to inhibit the activation of phosphodiesterase, and the relative binding of different phenothiazine derivatives paralleled their clinical antipsychotic activity. These results support our proposal that the mechanism by which antipsychotic drugs inhibit phosphodiesterase activation is by direct binding to activator. Moreover, the evidence that this calcium-dependent protein can activate several other enzyme systems, each of which is inhibited by the phenothiazine antipsychotics, suggest that the binding of antipsychotics to activator may be the mechanism by which these drugs exert several of their biochemical and pharmacological actions.

Phenothiazine antipsychotic agents inhibit several enzyme systems including the catecholamine-sensitive adenylate cyclase (Uzunov and Weiss, 1971, 1972a; Kebabian *et al.*, 1972; Miller and Iversen, 1974), activator-stimulated phosphodiesterase (Uzunov *et al.*, 1974; Weiss *et al.*, 1974; Levin and Weiss, 1976) and adenosine triphosphatase (Medzchrodsky *et al.*, 1975; Pang and Briggs, 1976; Gubitz *et al.*, 1977). Unfortunately, there has been great difficulty in establishing whether these or any other biochemical actions of antipsychotics are related to their clinical antipsychotic effects. Nevertheless, the potent action of neuroleptics on cyclic nucleotide metabolism has stimulated a great deal of interest in the relationship that may exist between the clinical effects of these compounds and the intracellular concentration of cyclic nucleotides. In an attempt to determine the reason why antipsychotics have such varied biochemical actions and in the hope of learning more about the possible relationship between schizophrenia and cyclic nucleotides, we have been studying the mechanism by which antipsychotic agents inhibit the adenylate cyclase and phosphodiesterase systems.

Received for publication June 19, 1978.

[1]This work was supported by Grant MH30096 awarded by the National Institute of Mental Health.

In previous reports we demonstrated that the phenothiazine, trifluoperazine, binds to homogenous samples of a heat-stable, calcium-dependent activator of phosphodiesterase at two distinct sets of sites: high-affinity calcium-dependent binding sites (K_d = 1 μM; 2 binding sites per molecule of activator) and low-affinity calcium-independent sites (K_d = 5 mM; approximately 24 sites) (Levin and Weiss, 1977).

Studies of the binding of trifluoperazine to a number of proteins including several calcium binding proteins showed that the calcium-dependent binding of the phenothiazine was selective for the activator (Levin and Weiss, 1977, 1978).

In this study we report that the high-affinity, calcium-dependent binding to activator is a general property of clinically effective antipsychotic compounds and that the affinity of these compounds for activator is directly related to their ability to inhibit phosphodiesterase activation.

Methods

Binding studies. The equilibrium dialysis procedure was essentially as described previously (Levin and Weiss, 1977). Briefly 500 μl of activator (33 μg/ml) were dialyzed for 18 hr at 4°C in a bath containing 20 ml of 5 mM Tris-HCl, pH 7.0, 1 mM Mg^{++} and either 0.1 mM Ca^{++} or 0.3 mM ethylenebis (oxyethylenenitrilo) tetraacetic acid (EGTA)

and various concentrations of radiolabeled drugs under study. At the end of the dialysis, the content of each dialysis bag was recovered, the radioactivity of a 200 μl aliquot was determined in a liquid scintillation spectrometer, and the concentration of drug in each sample was calculated. In these, as in previous experiments (Levin and Weiss, 1977), complete equilibrium was obtained between 6 and 8 hr of dialysis.

Penfluridol, pimozide, haloperidol, diazepam and spiroperidol were added to the baths as solutions in ethanol. The final concentration of ethanol in the baths was less than 0.5% of the volume of the buffer. This amount of ethanol did not affect either the direct binding of the drugs to activator or the displacement of one drug by another. All other pharmacological agents were added as aqueous solutions.

In studying the binding of nonradioactive agents, we measured their ability to displace radioactive [^{3}H]trifluoperazine (0.5 μM) from the activator. The labeled and unlabeled compounds were added to the baths simultaneously, and equilibrium dialysis was conducted as described above.

Phosphodiesterase activity. An activable form of phosphodiesterase was prepared from rat cerebrum by subjecting the soluble supernatant fraction of brain homogenates to polyacrylamide gel electrophoresis (Uzunov and Weiss, 1972b). Phosphodiesterase activity was measured by the luciferin-luciferase method as described previously (Weiss *et al.*, 1972).

Preparation and assay of activator. Homogenous samples of activator were prepared and generously supplied by Drs. Thomas Vanaman and Martin Watterson of Duke University. Homogeneity was confirmed by analytical gel electrophoresis. Activator activity was assessed by its ability to increase the activity of the activator-deficient phosphodiesterase prepared from rat brain. One unit of activator is defined as the amount of activator necessary to produce 50% of the maximum activation of the activator-deficient phosphodiesterase attainable under the standard experimental conditions described previously (Levin and Weiss, 1976).

Protein concentration. Protein concentration was determined by the method of Lowry *et al.* (1951).

Materials. Drug samples were generously provided as follows: trifluoperazine, trifluoperazine sulfoxide, [^{3}H]trifluoperazine (78 μCi/mg), chlorpromazine and chlorpromazine sulfoxide by Smith Kline and French Laboratories (Philadelphia, Pa.); diazepam [^{14}C]diazepam (26 μCi/mg), chlordiazepoxide and [^{14}C]chlordiazepoxide (166 μCi/mg) by Hoffmann La Roche, Inc. (Nutley, N.J.); pimozide and penfluridol by Janssen Pharmaceutica (Beerse, Belgium); [^{14}C]pimozide (30.5 μCi/mg) and [^{3}H]penfluridol (88.3 μCi/mg) by McNeil Laboratories Inc. (Fort Washington, Pa.); *cis*- and *trans*-flupenthixol by H. Lundbeck and Co. (Kobenhavn-Valby, Denmark); clozapine by Sandoz Pharmaceuticals (Hanover, N.J.); (+)and (−)-butaclamole by Ayerst Laboratories (New York, N.Y.); promethazine by Wyeth Laboratories (New York, N.Y.); amitriptyline by Merck Sharp and Dohme Research Laboratories (West Point, Pa.); desipramine by Merrell-National Laboratories (Cincinnati, Ohio); nortriptyline and [^{14}C]amitriptyline (0.3 μCi/mg) by Eli Lilly and Co. (Indianapolis, Ind.). [^{3}H]Imipramine (30 mCi/mg) was generously supplied by Drs. Alan Frazer and David Brunswick of the Veterans Administration Hospital, Philadelphia, Pa. Radiolabeled *d*-[^{3}H]amphetamine (45 mCi/mg), *d*-[^{3}H]LSD (36 mCi/mg), [^{14}C]pentobarbital (250 μCi/mg), [^{3}H]morphine (35 mCi/mg), [^{14}C]dopamine (210 μCi/mg), [^{3}H]histamine (59 mCi/mg), [^{3}H]haloperidol (259 μCi/mg) and [^{3}H]dihydroalprenolol (131 mCi/mg) were obtained from New England Nuclear Corp. (Boston, Mass.) and [^{3}H]chlorpromazine (71 mCi/mg) was from Schwarz/Mann (Orangeburg, N.Y.). Pyruvate kinase, myokinase and phosphoenolpyruvate were obtained from Boehringer Mannheim and Co. (New York, N.Y.) and firefly luciferin and luciferase from E.I. duPont de Nemours and Co. (Wilmington, Del.) Other reagents were obtained from general commercial sources.

Results

The binding of several neuroleptic agents to activator was measured in the presence of calcium or EGTA using a wide range of drug concentrations. The binding characteristics of chlorpromazine, pimozide and haloperidol to activator are shown in figures 1, 2 and 3, respectively. Scatchard analyses (Scatchard, 1949) of the data are presented in the insets. Chlorpromazine displayed two sets of binding sites; one set of high-affinity sites (K_d = 5 μM, N = 3 sites per molecule) and a second set of low-affinity sites (K_d = 130 μM, N = about 17 sites per molecule). The high-affinity binding was dependent on the presence of calcium, whereas the low-affinity sites were calcium-independent (data not shown). In the concentrations used, pimozide and haloperidol displayed only the calcium-specific, high-affinity sites. The calculated K_d values for pimozide and haloperidol were approximately 0.8 and 9 μM, respectively, and the number of calcium-dependent binding sites on the activator for each molecule was between 1 and 2. The solubility of these two agents limited the concentrations which could be utilized, perhaps preventing the low-affinity binding characteristics from being observed.

Comparison of the binding of various centrally acting drugs to activator. The binding to activator of a variety of agents that produce pharmacological effects on the central nervous system is presented in table 1. Included in this table are the I50 values for inhibiting the activation of phosphodiesterase. As can be seen, neuroleptic agents showed the highest degree of calcium-specific binding to activator. The tricyclic antidepressants and antianxiety agents displayed a lesser but still significant calcium-specific binding to activator. A variety of other centrally acting agents including *d*-LSD, pentobarbital, morphine, *d*-amphetamine and dopamine displayed no calcium-specific binding.

The degree to which these agents inhibited the activation of phosphodiesterase was directly related to their ability to bind to the activator. This is shown graphically in figure 4, which demonstrates a highly significant positive correlation (r = 0.99)

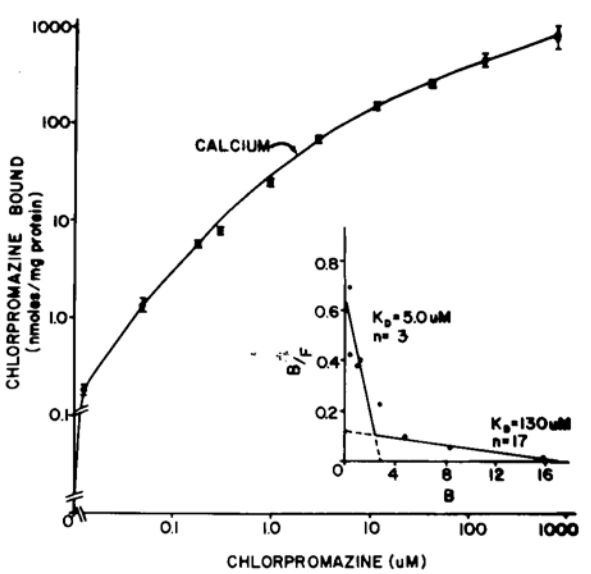

Fig. 1. Binding of chlorpromazine to activator. Equilibrium dialysis was performed in the presence of calcium (0.1 mM) as described in "Methods." [^{3}H]Chlorpromazine was added to the baths to give final concentrations of free drug ranging from 0.05 to 1000 μM. Each point represents the mean value of three samples. Vertical brackets indicate the standard error. The inset shows the data plotted according to the Scatchard equation (Scatchard, 1949). The lines of best fit were drawn by linear regression analysis. The dissociation constant (K_d) was calculated from the inverse slope of the lines and the number of binding sites (N) per molecule of activator was estimated from the x-intercept. B, moles of chlorpromazine bound per mole of activator; F, concentrations of free chlorpromazine at equilibrium.

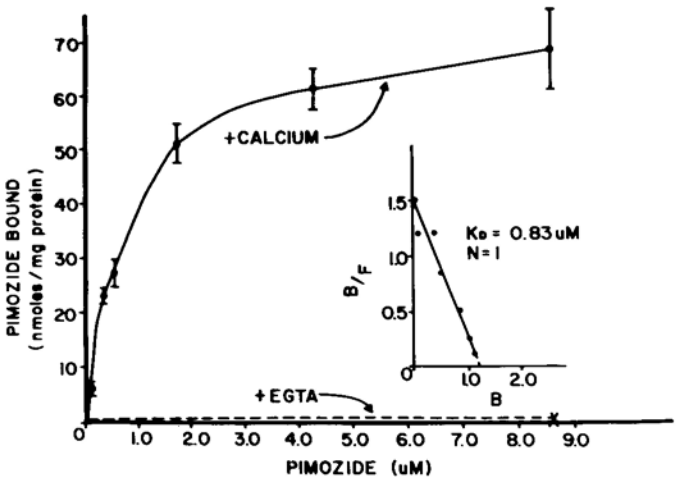

Fig. 2. Binding of pimozide to activator. Equilibrium dialysis was performed in the presence of either calcium (0.1 mM) or EGTA (0.3 mM) as described in "Methods." [^{14}C]Pimozide was added to the baths to give final concentrations of free drug ranging from 0.2 to 9.0 μM. Each point represents the mean value of three samples. Vertical brackets indicate the standard error. The inset shows the data plotted according to the Scatchard equation. *B*, moles of pimozide bound per mole of activator; *F*, concentrations of free pimozide at equilibrium.

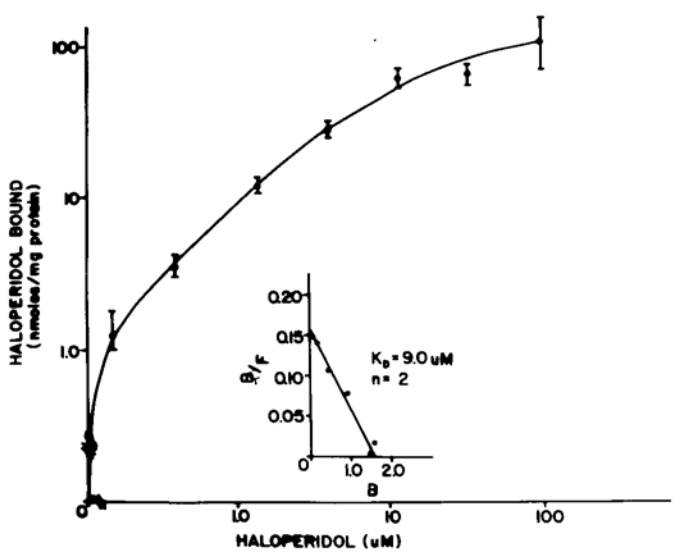

Fig. 3. Binding of haloperidol to activator. Equilibrium dialysis was performed in the presence of calcium (0.1 mM) as described in "Methods". [^{3}H]Haloperidol was added to the baths to give final concentrations of free drug ranging from 0.15 to 100 μM. Each point represents the mean value of three samples. Vertical brackets indicate the standard error. The inset shows the data plotted according to the Scatchard equation. *B*, moles of haloperidol bound per mole of activator; *F*, concentrations of free haloperidol at equilibrium.

between these two parameters. The pharmacological agents that displayed no calcium-specific binding to activator also showed no specific inhibition of the activation of phosphodiesterase.

Competition of penfluridol and pimozide for the [^{3}H] trifluoperazine binding site. To determine whether different chemical classes of antipsychotic agents compete for the same binding sites on the activator, we studied the effect of the butyrophenone, penfluridol, and the diphenylbutylamine, pimozide, on the calcium-specific binding of [^{3}H]trifluoperazine to activator.

Penfluridol caused a concentration-dependent shift in the binding curve for [^{3}H]trifluoperazine to activator. At low concentrations of trifluoperazine, penfluridol produced a marked displacement of trifluoperazine from the activator. However, at high concentrations of trifluoperazine, penfluridol failed to displace the phenothiazine significantly. These data, when plotted as reciprocals (fig. 5), suggest that penfluridol is a competitive inhibitor of trifluoperazine binding; linear regression analysis of

TABLE 1

Comparison of the binding of various drugs to activator with their ability to inhibit the activation of phosphodiesterase

Equilibrium dialysis was performed as described in "Methods". Labeled drugs were added to the bath to give a final concentration of 1 μM, and the binding of these agents in the absence or presence of 0.1 mM calcium was determined. The I50 value is the concentration of drug required to produce a 50% inhibition of the activation of phosphodiesterase. Each value is the mean of three to six experiments ± standard error. N.S. = no significant binding was detectable.

Drug	Calcium-Specific Binding	Calcium-Independent Binding	Inhibition of Phosphodiesterase Activation (I50)
	nmol drug bound/mg protein		
Penfluridol	105 ± 11	1.0 ± 0.5	2.5 μM
Pimozide	65 ± 5	<0.2	7 μM[a]
Trifluoperazine	56 ± 6	2.3 ± 1.4	10 μM[a]
Chlorpromazine	23 ± 1	1.4 ± 0.4	42 μM[a]
Haloperidol	13 ± 1	<0.2	60 μM
Amitriptyline	9.0 ± 0.7	1.0 ± 0.5	130 μM[a]
Imipramine	6.8 ± 0.6	1.6 ± 0.2	125 μM
Chlordiazepoxide	5.5 ± 0.2	<0.2	320 μM[a]
Diazepam	4.7 ± 0.1	<0.2	140 μM
Dihydroalprenolol	1.5 ± 0.2	<0.2	880 μM
d-Amphetamine	N.S.	<0.2	>10 mM
d-LSD	N.S.	1.3 ± 0.2	>2.5 mM
Pentobarbital	N.S.	0.8 ± 0.4	>10 mM
Morphine	N.S.	<0.2	>4 mM
Histamine	N.S.	<0.2	>10 mM
Dopamine	N.S.	<0.2	>10 mM

[a] Taken from Levin and Weiss (1976).

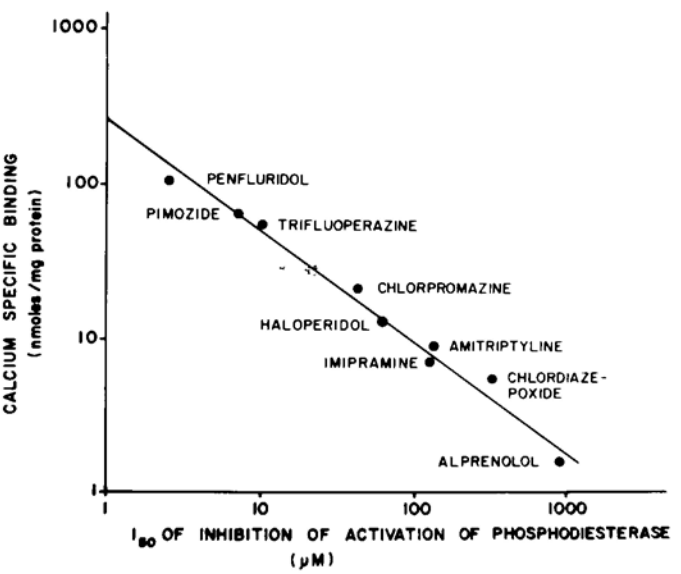

Fig. 4. Correlation of calcium-specific binding of neurotropic agents to activator with their ability to inhibit the activation of phosphodiesterase. The data from table 1 are plotted with the calcium-specific binding (nanomoles per milligram of protein) on the ordinate and I50 (micromolar concentration) for phosphodiesterase inhibition on the abscissa. The line of best fit was determined by regression analysis after logarithmic transformation. The correlation coefficient was 0.99.

the points shows that all three lines intersect on the ordinate, indicating that penfluridol does not change the maximum binding of trifluoperazine to activator. However, there was a shift in the point of intersection with the abscissa suggesting a reduction in the apparent affinity of trifluoperazine for the activator binding sites. Analysis of the binding of [^{3}H]trifluoperazine in the presence of pimozide demonstrated that pimozide, like penfluridol, also competed for the trifluoperazine binding site on activator (data not shown).

Displacement of labeled psychoactive compounds by various agents. Figure 6 shows that the ability of phenothiazine derivatives to displace trifluoperazine from the activator correlated with their clinical antipsychotic activity. Trifluoperazine was more potent than chlorpromazine which was more potent than either of the sulfoxide metabolites.

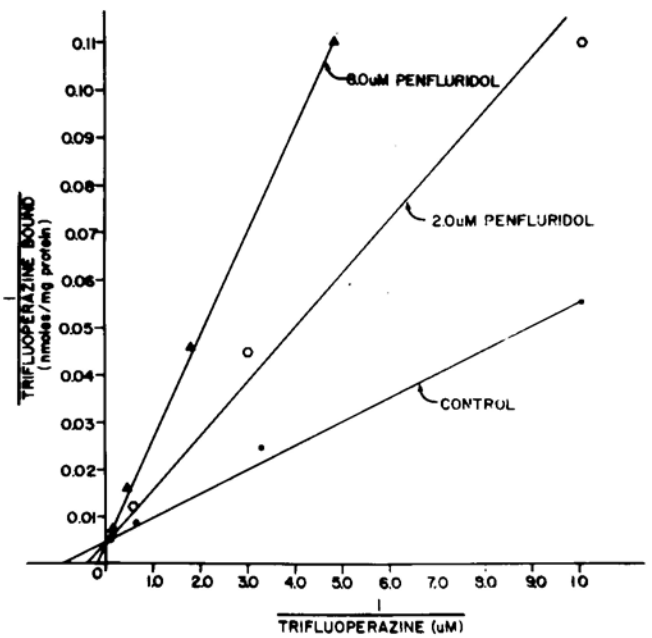

Fig. 5. Binding of trifluoperazine to activator in the presence of penfluridol. Equilibrium dialysis was performed as described in "Methods." [^{3}H]Trifluoperazine was added to baths containing 0, 2.0 or 8.0 μM penfluridol. The free trifluoperazine concentrations ranged from 0.1 to 40 μM. The data are plotted as the reciprocals of the bound *vs.* free trifluoperazine. Each point is the mean of three determinations. Lines are fitted by linear regression analysis.

Table 2 shows the concentration of these phenothiazines and several other agents required to displace 50% of the labeled trifluoperazine from the activator (I50). As can be seen, the antipsychotic agents, trifluoperazine, flupenthixol, clozapine, chlorpromazine, pimozide and penfluridol, all displaced [^{3}H] trifluoperazine from the activator with I50 values of less than 20 μM. The phenothiazines which have little or no antipsychotic activity such as promethazine, trifluoperazine sulfoxide, and chlorpromazine sulfoxide were significantly less potent than the antipsychotics in displacing [^{3}H]trifluoperazine. The antianxiety agent, chlordiazepoxide and the antidepressants, amitriptyline and nortriptyline, displayed moderate potency at displacing [^{3}H]trifluoperazine. Agents which were relatively ineffective in displacing [^{3}H]trifluoperazine from activator included the *alpha* adrenergic blocking agent, phentolamine, the phosphodiesterase inhibitors, theophylline and papaverine, and the cholinomimetic agent, methacholine. Table 2 also shows that radiolabeled pimozide and penfluridol can be displaced by relatively low concentrations of trifluoperazine but not by low concentrations of its sulfoxide metabolites.

Stereospecificity of the displacement of [^{3}H]trifluoperazine by butaclamole and flupenthixol. The two stereoisomers utilized in these studies were (+)and (−)-butaclamole, and *cis*- and *trans*-flupenthixol. From table 2, it can be seen that although (+)-butaclamole was approximately 5 times more effective than the (−)-isomer at displacing [^{3}H]trifluoperazine from its binding site on activator, little stereospecificity was observed for flupenthixol. That is, both forms of flupenthixol were about equally effective at displacing [^{3}H]trifluoperazine. Studies on the effects of *cis*- and *trans*-flupenthixol on the activation of phosphodiesterase demonstrated that both isomers of the drug were potent inhibitors of the activated form of phosphodiesterase. The limited solubility of butaclamole prevented us from determining the effect of this agent on phosphodiesterase activity.

Discussion

One approach to understanding the etiology or pathogenesis of mental illness is to study the biochemical actions of known antipsychotic drugs and to learn the specific sites and mechanisms by which they elicit their actions. Previous studies showed that antipsychotic agents selectively inhibit the catecholamine-sensitive adenylate cyclase and the activator-sensi-

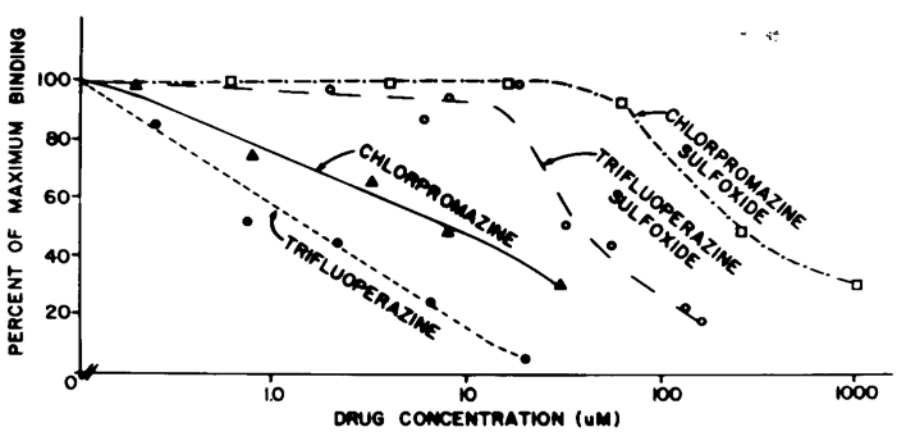

Fig. 6. Displacement of [^{3}H]trifluoperazine from activator by a series of phenothiazines. Equilibrium dialysis was performed as described in "Methods." The binding of [^{3}H]trifluoperazine (0.5 μM) to activator was measured in the presence of several concentrations of the drugs under study.

TABLE 2

Displacement of labeled psychoactive compounds from activator by various agents

Equilibrium dialysis in the presence of calcium was performed as described in "Methods." Dialysis baths contained 0.5 μM [^{3}H]trifluoperazine, [^{3}H]pimozide or [^{3}H]penfluridol. The binding of the labeled compounds was measured in the presence of several concentrations of the drugs under study. The concentration of unlabeled drug which displaced 50% of the labeled compound (I50) is given for each drug. All binding experiments were run in triplicate.

Displacement of	Displaced by	IC50
		μM
[^{3}H]Trifluoperazine	Trifluoperazine (TFP)	1.5
[^{3}H]Trifluoperazine	*trans*-Flupenthixol	2.5
[^{3}H]Trifluoperazine	*cis*-Flupenthixol	4.0
[^{3}H]Trifluoperazine	Chlorpromazine (CPZ)	8.0
[^{3}H]Trifluoperazine	Clozapine	20
[^{3}H]Trifluoperazine	TFP-sulfoxide	45
[^{3}H]Trifluoperazine	Promethazine	60
[^{3}H]Trifluoperazine	(+)-Butaclamole	75
[^{3}H]Trifluoperazine	Nortriptyline	80
[^{3}H]Trifluoperazine	Chlordiazepoxide	90
[^{3}H]Trifluoperazine	Amitriptyline	100
[^{3}H]Trifluoperazine	CPZ-sulfoxide	250
[^{3}H]Trifluoperazine	Phentolamine	350
[^{3}H]Trifluoperazine	(−)-Butaclamole	350
[^{3}H]Trifluoperazine	Papaverine	>250
[^{3}H]Trifluoperazine	Theophylline	>1000
[^{3}H]Trifluoperazine	Methacholine	>1000
[^{3}H]Pimozide	Trifluoperazine	1.0
[^{3}H]Pimozide	TFP-sulfoxide	35
[^{3}H]Penfluridol	Trifluoperazine	7.5
[^{3}H]Penfluridol	TFP-sulfoxide	65

tive cyclic nucleotide phosphodiesterase. More recently, we demonstrated that phenothiazine antipsychotics inhibit the activation of phosphodiesterase by binding to the calcium-dependent activator of phosphodiesterase. In this report we present further evidence that the mechanism by which antipsychotic agents inhibit the activation of phosphodiesterase is by direct calcium-dependent binding to the heat-stable, calcium-dependent protein activator of cyclic nucleotide phosphodiesterase.

Several antipsychotic compounds including the phenothiazines, trifluoperazine and chlorpromazine, the diphenylbutylamine, pimozide, the butyrophenones, haloperidol and penfluridol, and the dibenzodiazepine, clozapine, displayed similar binding characteristics; that is, they all demonstrated high-affinity, saturable, calcium-dependent binding to activator. In fact, all antipsychotic agents examined exhibited this calcium-dependent binding to activator, and the degree of binding to activator directly correlated with their ability to inhibit the activated form of phosphodiesterase. The antidepressant agents, amitriptyline and imipramine, and the antianxiety agents, chlordiazepoxide and diazepam, showed significantly less calcium-dependent binding to activator and were less potent inhibitors of activated phosphodiesterase. A series of other agents which affect the central nervous system but which have little or no antipsychotic activity, including lysergic acid diethylamide, amphetamine, pentobarbital, phentolamine, theophylline, methacholine and dopamine, showed no calcium-dependent binding to activator nor did they selectively inhibit the activation of phosphodiesterase. Thus, these studies showed an excellent correlation between the ability of drugs to block the activation of phosphodiesterase and their ability to bind to the activator, and showed a good qualitative relationship between clinical antipsychotic activity and binding to the activator. That is, if one considers the various pharmacological agents known to affect central nervous system activity one finds that the compounds studied that have been shown to be clinically potent antipsychotic agents (Gorden, 1967; Miller *et al.*, 1974) bind to activator to a greater degree than do those pharmacological agents that have little or no clinical antipsychotic activity (table 1). It should be noted, however, that there is also a good correlation between the ability of drugs to produce extrapyramidal effects (Clement-Cormier *et al.*, 1974) and their ability to bind to activator. Whether there will be a better correlation between binding and antipsychotic activity or between binding and extrapyramidal effects is still unclear.

The relative ability of pharmacological agents to displace [^{3}H]trifluoperazine from activator is also, in general, consistent with the concept that the affinity of these agents for activator may be related to their antipsychotic activity. However, unlike other *in vitro* actions of antipsychotics such as their blockade of the rise of cyclic adenosine 3′:5′-monophosphate induced by norepinephrine (Uzunov and Weiss, 1972a), their blockade of dopamine-sensitive adenylate cyclase (Miller *et al.*, 1974; Clement-Cormier *et al.*, 1974) or their displacement of the binding of dopamine (Burt *et al.*, 1975), we have been unable to demonstrate marked stereospecificity for the binding to activator. A possible explanation for this lack of stereospecificity is that during its purification and isolation, the activator may have undergone sufficient conformational changes to lose its stereospecificity. Studies of the calcium-specific binding of antipsychotics to cruder membrane preparations may help resolve this question.

In relating the *in vitro* and *in vivo* actions of drugs it is of interest to compare their affinity constants *in vitro* with the concentration of drug required to produce a pharmacological effect *in vivo*. In this regard, Maickel *et al.* (1974) correlated the concentration of chlorpromazine in rat brain with the behavioral effects elicited by this drug during chronic administration. They found that behavioral effects were evident only when the concentration of chlorpromazine in brain was at least 15 μmol per kg of brain. In the present studies, we found that the K_d value for the binding of chlorpromazine to activator was 5 μM. Thus, at least in the rat, the concentration of chlorpromazine in brain required to produce a behavioral effect *in vivo* is similar to the affinity constant for the binding of chlorpromazine to the activator protein *in vitro*.

As mentioned previously, phenothiazine antipsychotic agents have been shown to inhibit several intracellular enzyme systems including phosphodiesterase, adenylate cyclase and adenosine triphosphatase. Although we have no direct evidence to show that the mechanism by which neuroleptic agents inhibit enzymes other than phosphodiesterase is by interacting with the calcium-dependent activator, the recent reports showing that activator can also activate adenylate cyclase (Brostrom *et al.*, 1975, 1976; Cheung *et al.*, 1975; Lynch *et al.*, 1977) and calcium-dependent adenosine triphosphatase (Gopinath and Vincenzi, 1977; Jarrett and Penniston, 1977) suggest the possibility that the phenothiazines are inhibiting all these enzymes by a common mechanism.

In conclusion, the characteristics of the binding of neuroleptic agents to activator can be summarized as follows. These agents bind to activator at two distinct sets of binding sites; one set of high-affinity, saturable, calcium-dependent sites, and a second set of low-affinity, calcium-independent sites. All neuroleptic agents examined displayed this high-affinity, calcium-dependent binding to activator. Antianxiety and antidepressant com-

pounds show considerably less calcium-specific binding than the neuroleptic agents. The potency with which these drugs inhibit activated phosphodiesterase shows a direct correlation with the degree of calcium-specific binding. The binding of these pharmacological compounds to activator is specific for activator and is not a general property of a wide variety of other proteins including several calcium binding proteins.

Acknowledgments

We acknowledge with thanks the excellent technical assistance of Ms. Becky Simon.

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