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INTERACTION OF PSYCHOTROPIC DRUGS WITH PHOSPHOLIPIDS

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Abstract—The interactions of psychotropic drugs with phospholipids, including lysophosphatidylcholine, phosphatidylinositol, and lysophosphatidylserine, were studied using calmodulin-dependent cyclic nucleotide phosphodiesterase partially purified from the cortex of hog brain. All the compounds used inhibited both calmodulin- and phospholipid-stimulated phosphodiesterase activity but not the basal activity. Fluphenazine was confirmed by kinetic analysis to be a competitive inhibitor, with both calmodulin and phospholipid. Using fluphenazine–Sepharose affinity chromatography, it was demonstrated that fluphenazine did not interact with the enzyme. The potencies of antipsychotics such as fluphenazine in inhibiting the various phospholipid-dependent activations decreased in the following order: lysophosphatidylcholine-, phosphatidylinositol-, and lysophosphatidylserine-dependent activation. On the other hand, antidepressant drugs exhibited similar inhibitory potencies towards lysophosphatidylcholine- and phosphatidylinositol-dependent activation. Antipsychotic and antidepressant drugs appear to have different characteristics with regard to lipid–drug interaction.

Levin and Weiss [1] reported that, in the presence of Ca^{2+}, antipsychotic drugs could bind to calmodulin, a Ca^{2+}-dependent regulatory protein [2, 3], and thereby inhibit calmodulin-stimulated cyclic nucleotide phosphodiesterase (3′,5′-cyclic-nucleotide 5′-nucleotidohydrolase, EC 3.1.4.17) activity. This inhibition appears to be related to the binding of drugs to hydrophobic regions of calmodulin which are exposed by Ca^{2+}-induced conformational changes [4, 5]. Subsequently, the antipsychotic drug fluphenazine was shown to inhibit lysophosphatidylcholine-dependent activation of phosphodiesterase, in a competitive fashion with lysophosphatidylcholine [6]. This suggested that antipsychotic drugs may be capable of binding to phospholipids as well as to calmodulin.

Recently reported data suggested that there are interactions between antipsychotics and phospholipid [7, 8], and there is increasing evidence that lipids may modulate the activities of membrane-bound enzymes [9–11]. Mori *et al.* [7] have shown that chlorpromazine and dibucaine inhibit Ca^{2+}-activated, phospholipid-dependent protein kinase by competitively inhibiting the interaction of phospholipid with the enzyme. However, the question remained as to whether there is a selective interaction between psychotropic drugs and various phospholipids.

We examined several psychotropic drugs as possible inhibitors of calmodulin- or phospholipid-stimulated phosphodiesterase activity and our results indicate that individual psychotropic drugs do have a different affinity for lysophosphatidylcholine, phosphatidylinositol, and lysophosphatidylserine respectively. We also present evidence that the affinity of fluphenazine for lysophosphatidylcholine is comparable to the affinity for calmodulin.

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MATERIALS AND METHODS

Chemicals. Bovine brain phosphatidylcholine, egg yolk lysophosphatidylcholine, bovine brain phosphatidylserine, bovine brain lysophosphatidylserine, bovine liver phosphatidylethanolamine, bovine liver lysophosphatidylethanolamine, soybean phosphatidylinositol, and firefly lantern extract were obtained from the Sigma Chemical Co., St. Louis, MO, U.S.A. Pyruvate kinase, myokinase, and phosphoenolpyruvate were purchased from Boehringer Mannheim-Yamanouchi, Tokyo, Japan. Epoxy-activated Sepharose 6B was purchased from Pharmacia Fine Chemicals Ltd., Uppsala, Sweden. The following psychotropic drugs were donated: trifluoperazine dimaleate, fluphenazine dimaleate, chlorpromazine hydrochloride, and chlorprothixene hydrochloride (Yoshitomi Pharmaceutical Co., Osaka, Japan), desipramine hydrochloride (Fujisawa Pharmaceutical Co., Osaka, Japan), and amitriptyline hydrochloride (Yamanouchi Pharmaceutical Co., Tokyo, Japan). Other reagents were of the purest grade commercially available.

Preparation of calmodulin-dependent cyclic nucleotide phosphodiesterase. Calmodulin-deficient phosphodiesterase was prepared by the method of Teo *et al.* [12]. A fresh hog brain cortex was homogenized in 0.1 M Tris–HCl buffer (pH 7.5) con-

taining 0.2 mM EGTA,* 0.05 mM indomethacin, and 200 mg/l of trypsin inhibitor, and the homogenate was centrifuged at 105,000 *g* for 90 min. The 0–55% ammonium sulfate fraction of the supernatant was dialyzed against 0.02 M Tris–HCl buffer (pH 7.5) containing 0.2 mM EGTA and 0.08 M NaCl. The dialyzed fluid was applied to a DEAE-cellulose column and eluted with a linear gradient of 0.08 to 0.4 M NaCl in the same buffer. Fractions containing calmodulin-sensitive phosphodiesterase activity were combined and stored frozen at −80°.

Preparation of calmodulin. Calmodulin was prepared from the cortex of hog brain by the method of Yazawa and Yagi [13] and showed a single band upon 10% SDS–polyacrylamide gel electrophoresis.

Enzyme assay. Phosphodiesterase activity was measured by a minor modification [14] of the method of Weiss *et al.* [15]. The reaction was carried out in 0.5 ml of mixture containing 80 mM Tris–HCl (pH 7.5), 2 mM $MgCl_2$, 2 mM dithiothreitol, 0.5 mM $CaCl_2$ or EGTA, 20 μg of bovine serum albumin, 0.84 μg of calmodulin-depleted phosphodiesterase, and 10 μM cAMP. Incubations were conducted for 15 min at 30° and were terminated by boiling for 2 min. 5′-AMP produced was then converted to ATP by the second stage incubation (3 hr at 37°): the reaction was carried out by adding 0.5 ml of mixture containing 100 mM Tris–HCl (pH 7.5), 2 mM $MgCl_2$, 10 mM dithiothreitol, 50 μg bovine serum albumin, 1 mM EGTA, 20 mM KCl, 0.4 mM phosphoenolpyruvate, 10^{-8} M ATP, 10 μg pyruvate kinase, and 5 μg myokinase. ATP thus produced was determined using the luciferin–luciferase system and the ATP photometer model 2000 (SAI Co., U.S.A.).

For fluphenazine–Sepharose affinity chromatography, phosphodiesterase activity was measured by the method of Sharma and Wang [16]. The standard reaction mixture contained 50 mM Tris–HCl (pH 7.5), 1 mM $MgCl_2$, 0.5 mM $CaCl_2$, 1 mM dithiothreitol, 1 mM cAMP, and 20 μg calmodulin. Reactions were carried out for 15 min at 30°.

Fluphenazine–Sepharose affinity chromatography. Fluphenazine was covalently crosslinked to epoxy-activated Sepharose 6B by the procedure recommended by the manufacturer (Pharmacia). A mixture of partially purified calmodulin-dependent phosphodiesterase (6.8 mg) and calmodulin (0.5 mg) was slowly applied to a fluphenazine–Sepharose column (1.5 × 6 cm), which had been equilibrated previously in buffer C [50 mM Tris–HCl (pH 7.5)/2 mM $MgCl_2$/4 mM 2-mercaptoethanol/0.2 mM $CaCl_2$/0.3 M NaCl]. The column was washed with the same buffer until no further protein was eluted. The buffer was then changed to buffer E [same composition as buffer C, but containing EGTA (2 mM) instead of Ca^{2+} (0.2 mM)].

RESULTS

It was reported previously that cyclic nucleotide phosphodiesterase, which normally requires calmodulin and Ca^{2+} for full activity, was activated by lysophosphatidylcholine and phosphatidylinositol, even in the absence of Ca^{2+} [6]. The effects of calmodulin and various phospholipids were tested on the activity of a partially purified phosphodiesterase which had been deactivated by the removal of calmodulin (Fig. 1). Phosphodiesterase activity was examined with 10 μM cAMP as substrate in an incubation mixture containing either EGTA–phospholipid or Ca^{2+}–calmodulin. In accord with the results of Wolff and Brostrom [6], lysophosphatidylcholine and phosphatidylinositol were effective as Ca^{2+}-independent activators of phosphodiesterase, while phosphatidylcholine, phosphatidylserine, phosphatidylethanolamine, and lysophosphatidylethanolamine were not. In addition, we found that phosphodiesterase was activated by lysophosphatidylserine as well as by calmodulin. Figure 1 shows

* Abbreviations: EGTA, [ethylenebis(oxoethylenenitrilo)] tetraacetic acid; cAMP, adenosine 3′,5′-monophosphate; K_i, inhibitor constant; IC_{50}, drug concentration necessary to produce 50% inhibition of the maximally stimulated enzyme activity; and SDS, sodium dodecylsulfate.

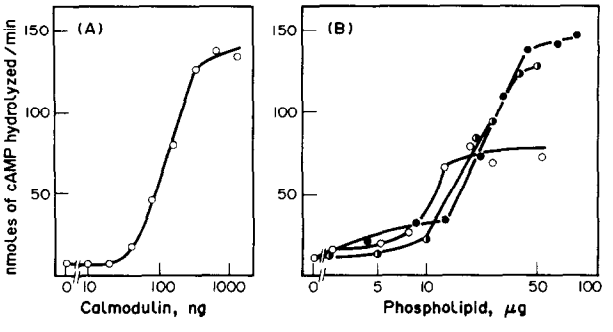

Fig. 1. Calmodulin and phospholipid concentration dependence of phosphodiesterase activation. Phosphodiesterase activity was assayed under standard conditions described in Materials and Methods. All reactions were performed in 0.5 ml of standard reaction mixture containing 80 mM Tris–HCl (pH 7.5), 10 μM cAMP, 0.84 μg of calmodulin-depleted phosphodiesterase, 0.5 mM $CaCl_2$ (A) or 0.5 mM EGTA (B), and activator as indicated. (A) Calmodulin concentration dependence of phosphodiesterase activation. (B) Phospholipid concentration dependence of phosphodiesterase activation. Key: (○) lysophosphatidylcholine; (◑) lysophosphatidylserine; and (●) phosphatidylinositol.

that the minimum concentration of activator necessary to maximally activate phosphodiesterase was 0.32, 18, 37, and 43 μg/tube for calmodulin, lysophosphatidylcholine, lysophosphatidylserine, and phosphatidylinositol respectively.

In the presence of each activator at the minimum concentration, phosphodiesterase activity was measured at various concentrations of the psychotropic drug. Among a large number of psychotropic drugs, the following six were selected simply because these have often been used to modify various cellular processes [17, 18]: trifluoperazine, fluphenazine, chlorpromazine, chlorprothixene, amitriptyline, and desipramine.

The results in these experiments are summarized in Table 1, and, as an example, the curves obtained with fluphenazine are shown in Fig. 2. As can be seen in this figure, fluphenazine had little or no effect on the basal activity but did inhibit to variable extents calmodulin- or phospholipid-dependent activation. Table 1 shows that all the psychotropic drugs investigated inhibited not only calmodulin- but various phospholipid-stimulated phosphodiesterase activities. In the case of antipsychotic drugs such as trifluoperazine, fluphenazine, chlorprothixene, and chlorpromazine, the IC_{50} value for lysophosphatidylcholine-dependent activation was 2.5 to 4 times lower than that for phosphatidylinositol-dependent activation. By contrast, the IC_{50} values of antidepressant drugs such as amitriptyline and desipramine for lysophosphatidylcholine-dependent activation were similar to that for phosphatidylinositol-dependent activation.

Next, experiments were performed to explore the mode of inhibitory action of these drugs. The inhibition of phosphodiesterase activity by fluphenazine was determined at different activator concentrations. The results in Fig. 3 suggested that fluphenazine was a competitive inhibitor with both calmodulin and lysophosphatidylcholine. Similar results were obtained with phosphatidylinositol and lysophosphatidylserine (data not shown). In addition to these results, concentrations of psychotropic drugs that

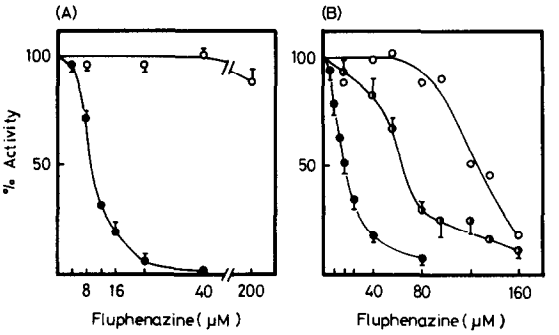

Fig. 2. Inhibition of various activator-stimulated phosphodiesterase activities by fluphenazine. Phosphodiesterase activity was measured at a series of fluphenazine concentrations. Each point except for lysophosphatidylserine is the mean ± S.E.M. of duplicate determinations of activity. Data are expressed as the increment of activity in the presence of activator except for the basal activity. The reaction was carried out in 0.5 ml of standard reaction mixture in the presence of 0.5 mM EGTA (unactivated) (A, ○); 0.5 mM $CaCl_2$ containing 0.32 μg calmodulin (A, ●); 0.5 mM EGTA containing 18 μg lysophosphatidylcholine (B, ●), 37 μg lysophosphatidylserine (B, ○), and 43 μg of phosphatidylinositol (B, ◑).

almost completely inhibited the activation of phosphodiesterase did not inhibit the enzyme activity in its unactivated state (Table 1).

To further demonstrate that psychotropic drugs did not interact with the enzyme, we used fluphenazine–Sepharose affinity chromatography. A mixture of phosphodiesterase and calmodulin was applied to a fluphenazine–Sepharose column (Fig. 4). None of the applied phosphodiesterase activity was absorbed by fluphenazine–Sepharose in the presence of Ca^{2+}. Similar results were obtained when the amount of applied enzyme was varied over a wide range, indicating that the column was not simply overloaded. More than 90% of the applied calmodulin activity was bound to fluphenazine–Sepharose in the presence of Ca^{2+}; it was eluted with excess EGTA. These results suggested that phosphodiester-

Table 1. Effects of psychotropic drugs on activated and unactivated phosphodiesterase*

	IC_{50} (μM)				
			Phospholipid		
Drug	Unactivated	Calmodulin	LPC	LPS	PI
Trifluoperazine	>200	7 ± 1	20 ± 5	110	49 ± 9
Fluphenazine	>200	11 ± 0	17 ± 1	132	67 ± 1
Chlorprothixene	>200	17 ± 0	17 ± 3	114	45 ± 5
Chlorpromazine	>200	24 ± 0	24 ± 5	121 ± 12	64 ± 1
Amitriptyline	>200	90 ± 13	73 ± 16	>200	103 ± 14
Desipramine	>200	107 ± 4	82 ± 18	166	81 ± 1

* Phosphodiesterase activity was measured as described in the legends to Figs. 1 and 2. The IC_{50} (unactivated) values were estimated as the concentration of drug required to induce a 50% reduction of phosphodiesterase activity in the presence of 0.5 mM EGTA. The IC_{50} (activated) values were calculated as the concentration necessary to produce 50% inhibition of activator-stimulated phosphodiesterase in the presence of 0.5 mM Ca^{2+} (calmodulin) or 0.5 mM EGTA (phospholipid). Abbreviations: LPC, lysophosphatidylcholine; LPS, lysophosphatidylserine; and PI, phosphatidylinositol. Each IC_{50} value except for lysophosphatidylserine represents the mean ± S.E.M. of duplicate determinations of activity.

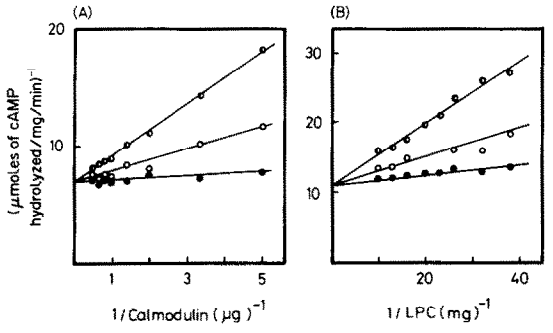

Fig. 3. Double-reciprocal plots of the calmodulin- and lysophosphatidylcholine-dependent phosphodiesterase activations in the presence of fluphenazine. Phosphodiesterase activity was measured in a standard reaction mixture containing the indicated concentrations of added activator in the absence (A and B, ●) and presence of 10 μM (A and B, ○), 15 μM (A, ◐), and 30 μM (B, ◐) fluphenazine. LPC, lysophosphatidylcholine. Other conditions were as described under Materials and Methods.

ase activity was inhibited by direct binding of psychotropic drug to activator. Therefore the IC_{50} values in Table 1 can be regarded as reflecting the interaction between psychotropic drug and activator.

The $1/IC_{50}$ values do not, however, express the drug–activator affinity since different amounts of activator were used in the reaction mixture. To investigate the affinity of fluphenazine for each activator, the apparent K_i values were calculated from the data of Figs. 1 and 3 and of similar experiments with lysophosphatidylserine and phosphatidylinositol (Table 2). The results in Table 2 mean that the affinity of fluphenazine for lysophosphatidylcholine was comparable to that for calmodulin.

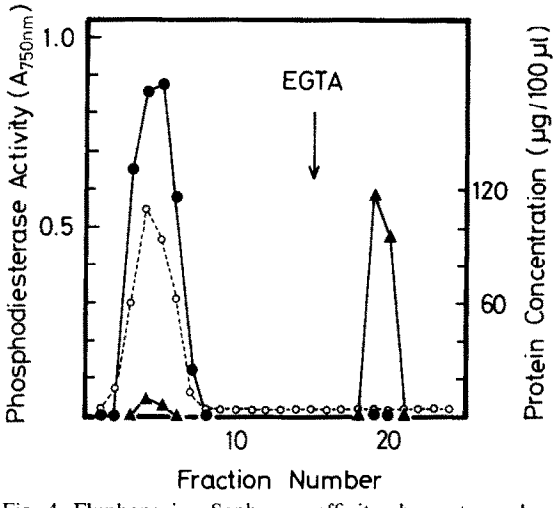

Fig. 4. Fluphenazine–Sepharose affinity chromatography. A mixture of calmodulin-dependent phosphodiesterase and calmodulin was chromatographed on a fluphenazine–Sepharose column. Samples were applied and washed in buffer C, containing 0.2 mM Ca^{2+}. At the point indicated by the arrow, the buffer was changed to buffer E, containing EGTA (2 mM). The protein concentration was determined by the dye binding method [19] (○---○). Phosphodiesterase activity was measured in the presence of 0.5 mM Ca^{2+} with 20 μg calmodulin (●—●). For the assay of the calmodulin activity, an aliquot of the fraction was boiled for 2 min and used to activate calmodulin-dependent phosphodiesterase (▲—▲). Other conditions were as described under Materials and Methods.

Table 2. Apparent K_i values of fluphenazine for calmodulin- and phospholipid-stimulated phosphodiesterase activity*

	K_i (μM)			
		Phospholipid		
Drug	Calmodulin	LPC	LPS	PI
Fluphenazine	11	10	72	28

* These apparent K_i values were calculated from the data in Figs. 1 and 3 and in similar experiments on lysophosphatidylserine and phosphatidylinositol. Abbreviations: LPC, lysophosphatidylcholine; LPS, lysophosphatidylserine; and PI, phosphatidylinositol.

DISCUSSION

Recently, it has been reported that the activities of several enzymes, for example adenylate cyclase [20], (Ca^{2+}-Mg^{2+})-ATPase [11], or plasma membrane ATPase of yeast [21], may be dependent on the presence of certain specific phospholipids. Wolff and Brostrom [6] reported that lysophosphatidylcholine and phosphatidylinositol were identified as Ca^{2+}-independent activators of cyclic nucleotide phosphodiesterase shown to be regulated *in vitro* by Ca^{2+} and calmodulin. In addition to these phospholipids, we found that calmodulin-dependent phosphodiesterase was also activated by lysophosphatidylserine. Acidic phospholipids such as phosphatidylinositol and lysophosphatidylserine activated phosphodiesterase as well as calmodulin, whereas neutral phospholipid lysophosphatidylcholine activated the enzyme only to a certain extent. However, the difference in extent of activation cannot be merely explained by differences in the polar head group since phosphatidylcholine and phosphatidylserine had no effect on the activation of phosphodiesterase. The fatty acid composition of lysophosphatidylcholine may possibly influence the ability of lysophosphatidylcholine to activate phosphodiesterase [21].

Levin and Weiss [22] reported that antipsychotic, and other psychoactive, drugs bind to calmodulin, with high affinities and in a Ca^{2+}-dependent fashion. In our present work, we found that psychotropic drugs can interact not only with calmodulin but also with phospholipids and that the affinity for phospholipids is rather high. The affinity of fluphenazine for lysophosphatidylcholine was similar to that for calmodulin (Table 2). It has been shown recently that calmodulin undergoes a conformational change upon binding with Ca^{2+} and that this change exposes a hydrophobic region of calmodulin [5, 23]. It is quite possible that lipophilic psychotropic drugs bind to this hydrophobic region rendering calmodulin incapable of activating phosphodiesterase [4, 5].

Among the several phospholipids investigated, phosphodiesterase was activated only by lysophos-

phatidylcholine, phosphatidylinositol, and lysophosphatidylserine; therefore, it is unclear whether psychotropic drugs do interact with other phospholipids including phosphatidylcholine and phosphatidylserine. However, taking into account the importance of the hydrophobic interactions in drug–activator interactions, it is conceivable that psychotropic drugs may also bind to other phospholipids with a high affinity.

Antipsychotic and antidepressant drugs may have different characteristics in the lipid–drug interaction. As can be seen in Table 1, antipsychotic drugs exhibited a higher inhibitory potency for calmodulin- and lysophosphatidylcholine-dependent activation than did antidepressant drugs. Significant distinctions between the two classes of drugs were not apparent with regard to the potencies required to inhibit phosphatidylinositol- and lysophosphatidylserine-dependent activations. The results in Table 1 suggest that the order of affinities of antipsychotic drugs for each phospholipid was lysophosphatidylcholine > phosphatidylinositol > lysophosphatidylserine. On the other hand, the affinities of antidepressant drugs for lysophosphatidylcholine were similar to those for phosphatidylinositol, and amitriptyline interacted little with lysophosphatidylserine.

Recently, it was shown that chronic amitriptyline treatment produces a dose- and time-dependent decrease in ligand binding to 5-HT_2 and beta-adrenergic receptors [24], and that antipsychotic drugs nonselectively inhibit binding of dopamine to the D_1 and D_2 receptors [25]. These pharmacological effects may be related to the ability of psychotropic drugs to interact with membrane phospholipid and, perhaps, to induce changes in the biological functions of cell membranes.

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