METHYLATED AND DEMETHYLATED TRICYCLIC ANTIDEPRESSANTS AND THEIR BINDING TO CELL MEMBRANES

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SUMMARY

We studied the binding of methylated and demethylated tricyclic antidepressants (TCA) to synaptic plasma brain membranes (SPM) and to lymphocyte, platelet and erythrocyte membranes. In the synaptic plasma membranes, more demethylated than methylated dibenzazepine derivatives were bound and the binding affinity was decreased. By contrast, in lymphocyte and platelet membranes more methylated derivatives were bound. Phosphatidylserine (PS) enhanced significantly the binding of methylated TCA in SPM without changing the dissociation constant (K_d). Lysophosphatidylserine did not affect the binding. PS also caused an increase of ^{3}H-imipramine binding to lymphocyte membranes but the binding to platelet membranes was not affected. PS also enhanced ^{3}H-5-HT uptake into platelets and ^{3}H-noradrenaline uptake into lymphocytes.

KEY WORDS

binding, tricyclic antidepressants (TCA), synaptic plasma brain membranes (SPM), phosphatidylserine, lymphocytes

INTRODUCTION

The high affinity specific binding of dibenzazepines was described in 1979 by Raisman *et al.* /1/ in the synaptic plasma membranes and by Briley *et al.* /2/ in platelet membranes. The first study concerning ^{3}H-imipramine binding to platelet plasma membrane vesicles was reported in 1978 by Rudnick and Talvenheimo /3/. Since then several hundred papers concerning the origin of this binding and its importance have been published. More than 70 studies concentrated on imipramine binding in depressive patients. Some authors published the results of imipramine binding studies also in other psychiatric disorders. The binding of tricyclic antidepressants is thought to be connected with neurotransmitter transport. According to this hypothesis, serotonin (5-HT) uptake is regulated by a protein which binds methylated tricyclic antidepressants (imipramine and amitriptyline), and noradrenaline uptake is regulated by a protein which binds demethylated dibenzazepines (desipramine) and dibenzcycloheptanodienes (nortriptyline). In both groups of substances the demethylation can proceed as far as didesmethyl-derivatives. No serious attention has yet been paid to these substances with regard to their binding to cell membranes.

It is important to learn how demethylation affects binding to various membranes in order to be able to clarify the connection between the binding of tricyclic antidepressants and neuromediator uptake. As the lipid part of the membrane is known to affect ligand binding, attention should also be paid to the effect of phospholipids on this binding and on 5-HT uptake.

MATERIALS AND METHODS

Synaptic plasma membranes were prepared from bovine brains using the method of Zukin *et al.* /4/. Blood cell membranes were isolated from blood (treated with EDTA) obtained from volunteers. Platelet membranes were prepared according to Garcia-Sevilla *et al.* /5/ and lymphocyte membranes according to Otto and Schmidt /6/. Erythrocyte membranes were isolated by the method of Crone *et al.* /7/.

Tricyclic antidepressants were labelled using the method described by Krulík *et al.* /8/. The specific activity was 70 Ci/mmol in ^{3}H-imipramine, 118 Ci/mmol in ^{3}H-amitriptyline, 55 Ci/mmol in

^{3}H-desmethylimipramine and 58 Ci/mmol in ^{3}H-didesmethylimipramine. The binding of these ligands was determined according to Raisman *et al.* /9, 10/. Non-specific binding was determined for ^{3}H-imipramine and ^{3}H-amitriptyline using chlorimipramine at a concentration of 5.10^{-5} mol.l^{-1} and for ^{3}H-desipramine and ^{3}H-didesmethylimipramine using 5.10^{-5} mol.l^{-1} nortriptyline. ^{3}H-5-HT uptake into platelets was determined according to Tuomisto and Tukiainen /11/ and ^{3}H-noradrenaline uptake into lymphocytes was determined according to Wood and Wyllie /12/. Protein was determined after Lowry *et al.* /13/.

RESULTS

Demethylated dibenzazepine derivatives exhibited a higher specific binding to synaptic plasma membranes than imipramine and increased K_d values (Table 1). The binding to lymphocyte membranes is decreased. In platelet membranes the binding is about 5-fold lower when demethylated derivatives are used. In erythrocyte membranes no differences have been found (Fig. 1).

TABLE 1

K_d and B_{max} values of methylated and demethylated dibenzazepine derivatives in brain synaptic plasma membranes

	IMI	DMI	DDMI
K_d [nM]	5.6±2.4	13.3±3.4	23.7±9.4
B_{max} [fmol/mg prot.]	374±65	1677±42	3355±1162

IMI - imipramine; DMI - desmethylimipramine; DDMI - didesmethylimipramine
n = 3-5.

On studying the effect of phosphatidylserine on ^{3}H-imipramine binding in the synaptic plasma membranes, we found that phosphatidylserine at a concentration of 20-80 μg/mg of protein significantly enhances the binding of these ligands in the synaptic plasma membranes, and this increase is the result of increased specific binding. The non-specific binding is not affected by phosphatidylserine (Fig. 2). Phosphatidylserine at a concentration up to 1/10 of the amount of membrane proteins enhanced both ^{3}H-imipramine and ^{3}H-amitriptyline binding. The increase of ^{3}H-desmethylimipramine binding is less pronounced (Fig. 3). In the presence of phos-

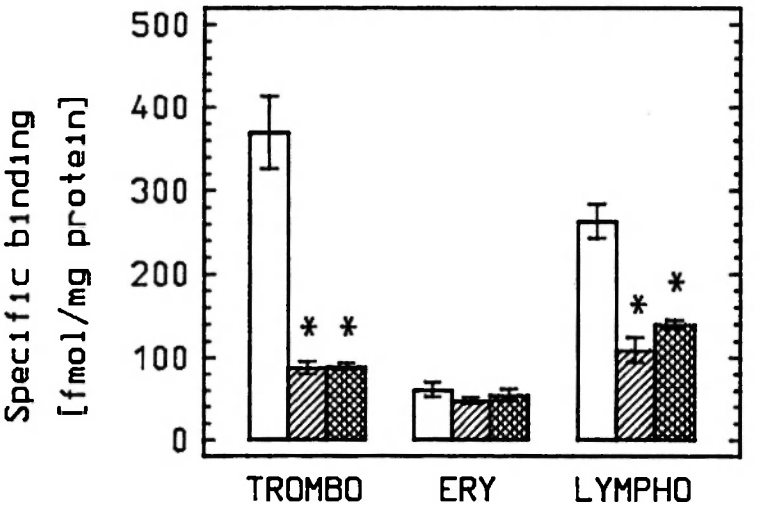

Fig. 1: Binding of ^{3}H-imipramine ☐, ^{3}H-desmethylimipramine ▨ and ^{3}H-didesmethylimipramine ▩ in platelet, erythrocyte and lymphocyte membranes. Specific binding was determined with 2 nM of the iigand. Single tests were repeated 5 times. Mean ± SEM, *P < 0.01.

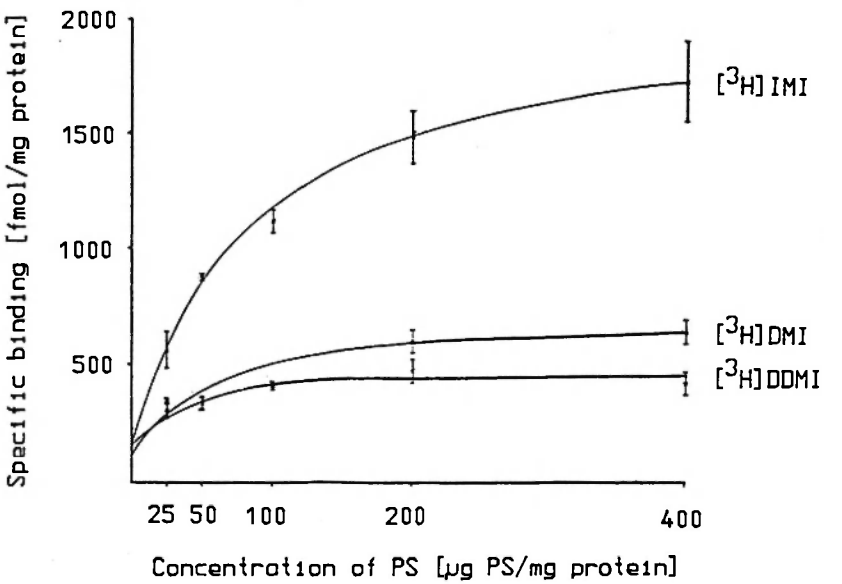

Fig. 2: The effect of various concentrations of phosphatidylserine on the binding of ^{3}H-imipramine (IMI), ^{3}H-desmethylimipramine (DMI) and ^{3}H-didesmethylimipramine (DDMI) in synaptic plasma membranes; n = 3.

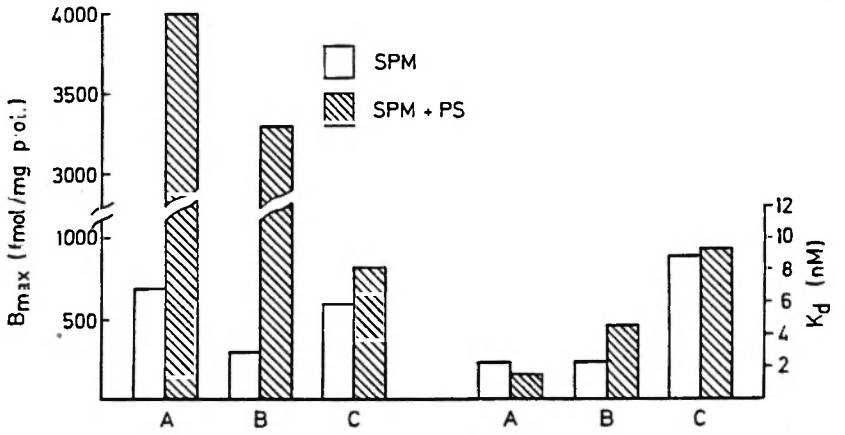

Fig. 3: The effect of phosphatidylserine at a concentration 80 μg per mg of SPM proteins on the binding of ^{3}H-imipramine (A), ^{3}H-amitriptyline (B) and ^{3}H-didesmethylimipramine (C); n = 3.

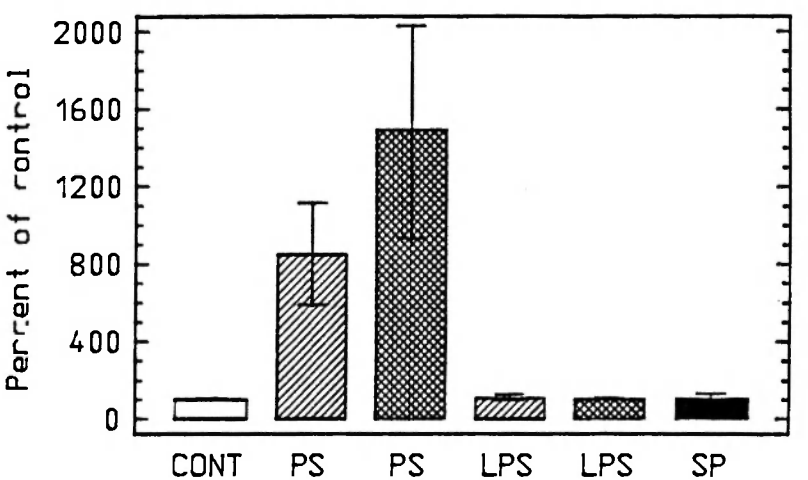

Fig. 4: The effect of phosphatidylserine (PS), lysophosphatidylserine (LPS) and serinephosphate (SP) at a concentration 80 μg.mg^{-1} protein on the binding of ^{3}H-imipramine in SPM. Concentration of the substrate was 2 nM and determinations were carried out 3 times in triplicate. Large ▨ and small ▩ liposomes were added to SPM.

phatidylserine, the binding capacity is more increased in methylated derivatives but the dissociation constant (K_d) is not affected. Lysophosphatidylserine and serinephosphate do not change the binding to synaptic plasma membranes (Fig. 4). Data on ^{3}H-imipramine binding to platelet and lymphocyte membranes in the presence of phosphatidylserine are shown in Fig. 5. Increased ^{3}H-imipramine binding occurs in both lymphocyte and synaptic plasma membranes but in platelet membranes the binding is not changed. Phosphatidylserine enhances also ^{3}H-5-HT uptake into platelets and ^{3}H-noradrenaline into lymphocytes (Fig. 6).

DISCUSSION

Studies of the binding of tricyclic antidepressants have so far concentrated primarily on ^{3}H-imipramine binding to synaptic plasma brain membranes or platelet membranes because platelets have been known to be a suitable model for studies of 5-HT transport. Although the connection of ^{3}H-imipramine binding to the receptor response has not been proved, this binding is supposed to be related to 5-HT uptake. There is a hypothesis that imipramine binds specifically to a protein closely connected with the protein transporting 5-HT through the cell membrane. However, the binding protein for imipramine has not yet been reliably isolated and identified. Data in the literature on this subject differ widely, by a factor of 100, in the determined molecular weight of this protein. These proteins are suggested to be lipoproteins /14/. The ontogenetic evolution of imipramine binding sites and of 5-HT transport is reported to be different /15/. There is also a great similarity not only between the binding of dibenzazepine and dibenzcycloheptanodienes but also in the binding of their methylated and demethylated derivatives.

In view of these facts, and also since the lipid part of the membrane has a great importance in the binding of other ligands, we studied how tricyclic antidepressant binding can be affected by phospholipids. We found that phosphatidylserine significantly increases the amount of the bound ligand both in the synaptic plasma membranes and in lymphocyte membranes. However, it does not change the ligand binding to platelet membranes and does not affect the dissociation constant of the binding. ^{3}H-noradrenaline transport into lymphocytes and ^{3}H-5-HT transport into platelets is increased. The results show a different effect on imipramine binding and ^{3}H-5-HT uptake

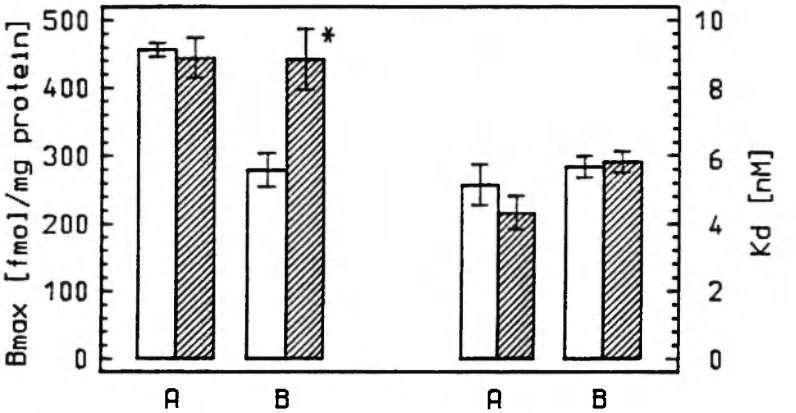

Fig. 5: B_{max} and K_d values of ^{3}H-imipramine in platelet (A) and lymphocyte (B) membranes in the control sample ☐ and in the presence of phosphatidylserine - 80 μg.mg^{-1} protein ▨ . Determinations were repeated 6 times. *P < 0.01

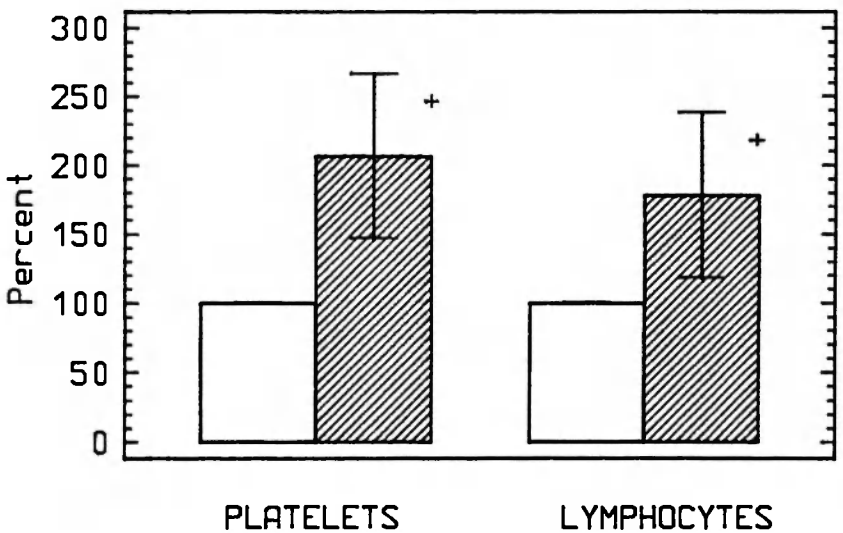

Fig. 6: Effect of phosphatidylserine ▨ (20 μg per ml of incubation medium) on ^{3}H-5-HT uptake into platelets and ^{3}H-noradrenaline uptake into lymphocytes (☐ control) at a substrate concentration of 10^{-7} M. The number of elements in sample was 2.10^7 for platelets and 2.10^6 for lymphocytes. The assays were carried out in duplicate and were repeated 5 times. + P < 0.05.

into platelets, which suggests the existence of two independent systems. A study was, therefore, carried out on model phospholipid membranes /16/.

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