CHANGES OF LIGAND BINDING AFTER MEMBRANE PHOSPHORYLATION

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SUMMARY

Phosphorylation of synaptic plasma brain membranes (SPM) causes a decrease of the specific binding of demethylated tricyclic antidepressants (TCA) and changes the affinity of ^{3}H-imipramine and ^{3}H-desmethylimipramine binding. The decrease of TCA binding was found also in lymphocyte membranes. In platelet membranes a decreased binding was found only with demethylated dibenzazepine derivatives. B_{max} and K_d values are also decreased in the presence of phosphatidic acid or α-glycerolphosphate.

KEY WORDS

synaptic plasma brain membranes (SPM), tricyclic antidepressants (TCA), TCA binding, lymphocyte membranes, platelet membranes, phosphorylation

INTRODUCTION

It is difficult to explain some changes in high affinity binding of tricyclic antidepressants to cell membranes, because there is not enough information available concerning the importance of the binding. A decrease of the number of ^{3}H-imipramine binding sites in platelet membranes has been reported in depressive patients /1, 2/, in schizophrenics /3/ and in Alzheimer's disease patients /4/. However, some authors and also a WHO study /5/ failed to show a decrease in the number of binding sites in platelet membranes in depressive patients. There are even some papers describing an increase /6/. In our laboratory we found an increase of ^{3}H-imipramine and ^{3}H-desmethylimipramine binding to lymphocyte membranes in depressive patients /7/. The differences found in the platelet membranes may be caused by: 1. a methodological error made during the determination of tricyclic antidepressant binding where various membrane concentrations were used; 2. clinical assessment of the patients; 3. usage of antidepressants before binding determination (some papers reported no change even after a repeated administration of tricyclic antidepressants /8/). This study addresses the factors that may influence the binding of tricyclic antidepressants. On the basis of these factors we want to explain the changes that have been found.

MATERIALS AND METHODS

Studies were carried out with synaptic plasma membranes (SPM) isolated from bovine brains according to Zukin *et al.* /9/ and lymphocyte and platelet membranes prepared from human blood after Otto and Schmidt /10/ and Garcia Sevilla *et al.* /11/. ^{3}H-imipramine, ^{3}H-desmethylimipramine and ^{3}H-didesmethylimipramine binding assays were carried out according to Raisman *et al.* /12, 13/. Chlorimipramine was used for determining high affinity non-specific ^{3}H-imipramine binding, while nortriptyline was used to determine the non-specific binding of demethylated metabolites. The membranes were phosphorylated in the presence of ATP or GTP /14/. Phosphorylation was determined in the presence of ^{32}P-ATP. Protein kinase was isolated after Miyamoto *et al.* /15/. Furthermore, the effect of phosphatidic acid and α-glycerolphosphate on ^{3}H-imipram-

ine binding in synaptic plasma membranes was investigated. Proteins were determined according to Lowry *et al.* /16/.

RESULTS

Following phosphorylation the binding of demethylated tricyclic antidepressants to the synaptic plasma membranes was decreased and the values of the binding affinities for ^{3}H-imipramine and ^{3}H-desmethylimipramine (Fig. 1) were changed. Decreased values of the binding of ^{3}H-imipramine and its demethylated metabolites were found also in lymphocyte membranes (Fig. 2). In platelet membranes the binding capacity was decreased only for ^{3}H-imipramme binding; the binding of demethylated derivatives of dibenzazepines was not decreased after phosphorylation (Fig. 3). A significant decrease in binding of methylated and demethylated tricyclic antidepressants to brain synaptic plasma membranes was found in the presence of a low concentration of phosphatidic acid or α-glycerolphosphate. K_d values were also decreased (Fig. 4).

DISCUSSION

Catecholamines and, especially, indoleamines which form a significant part of platelets might enhance the phosphorylation of these membranes. Both catecholamines and indoleamines cause an increase in the level of cAMP and diacylglycerol and also a release of Ca^{2+}. However, in depression the level of these neuromediators is decreased. Even when considering other neuromediators which are known to increase phosphorylation, the question remains as to what extent the changes in protein phosphorylation can result in changes in the binding of tricyclic antidepressants.

The results of our experiments in which membrane phosphorylation was carried out only in the presence of endogenous protein kinase did not differ from those obtained in the presence of isolated protein kinase which causes an increase in phosphorylation of membrane proteins. The binding values were identical in both experiments. Extraction of phospholipids from membranes that were phosphorylated in the presence of ^{32}P-ATP, and their chromatographic separation on a thin silica gel layer revealed the presence of phosphorylated metabolites. For this reason, we studied the effects of phosphatidic acid and α-glycerolphosphate. At low concentrations

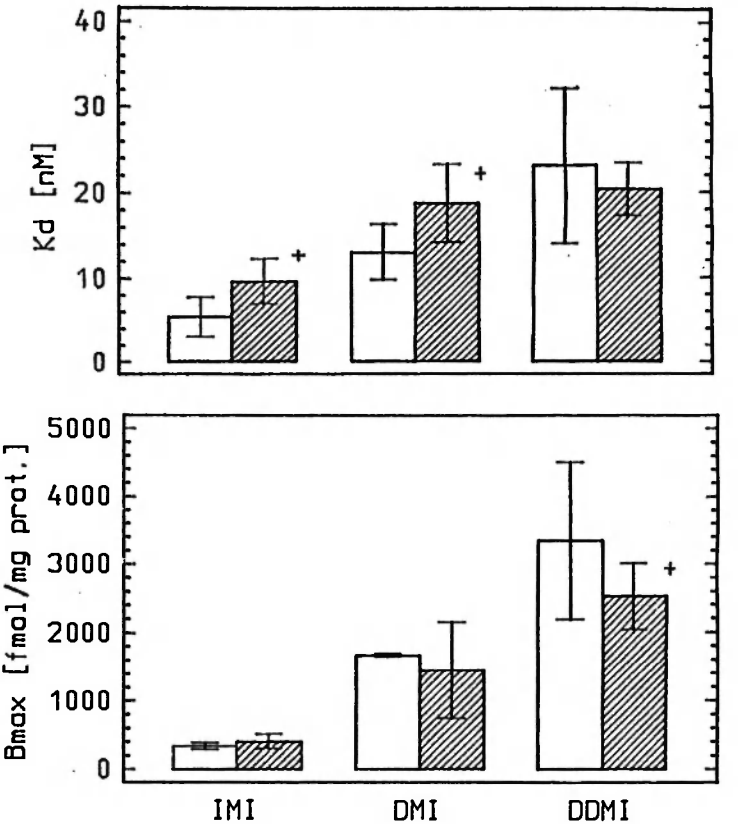

Fig. 1: Binding of ^{3}H-imipramine (IMI), ^{3}H-desmethylimipramine (DMI) and ^{3}H-didesmethyliimipramine (DDMI) in non-phosphorylated ☐ and phosphorylated with ATP ▨ brain synaptic plasma membranes. The tests were repeated 3-5 times and the results are shown as means± SEM; +P<0.05.

of these substances in the samples where the specific binding of ^{3}H-imipramine was investigated a significant decrease of ligand binding was found to occur. Lipid phosphorylation may represent a very important factor which influences the specific high affinity binding of tricyclic antidepressants in cell membranes. Phosphorylation is probably one of the regulating mechanisms which decrease binding of tricyclic antidepressants, while phosphatidylserine causes an increase in binding /17/.

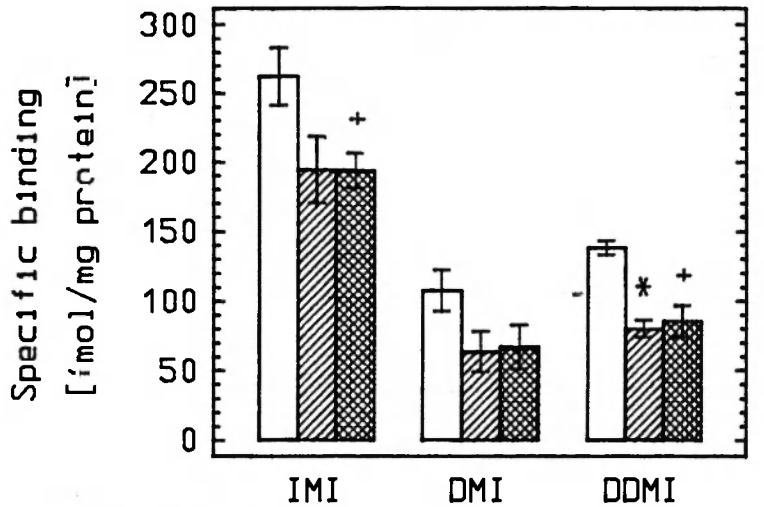

Fig. 2: Specific binding of dibenzazepines (substrate concentration 2 nM) in control ☐, and phosphorylated with ATP ▨ and GTP ▩ lymphocyte membranes; n=5, +P<0.05, *P<0.01.

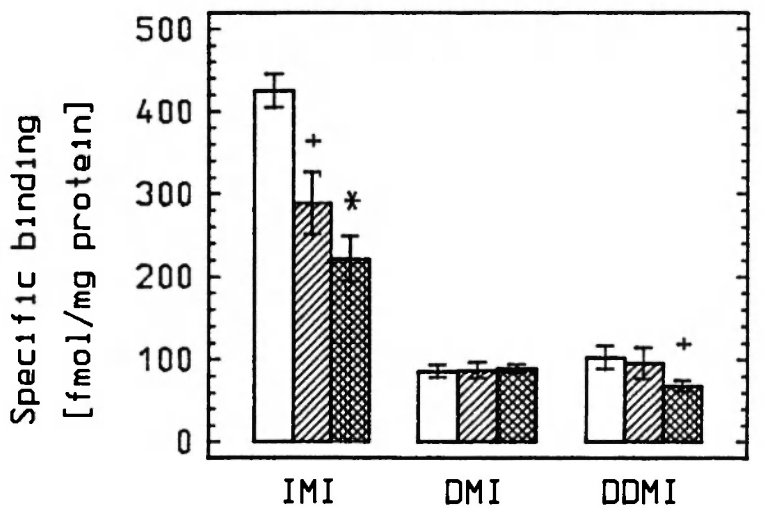

Fig. 3: Specific binding of methylated and demethylated dibenzazepines with the ligand concentration 2 nM: non-phosphorylated ☐, phosphorylated with ATP ▨ and phosphorylated with GTP ▩ platelet membranes; n=5, +P<0.05, *P<0.01.

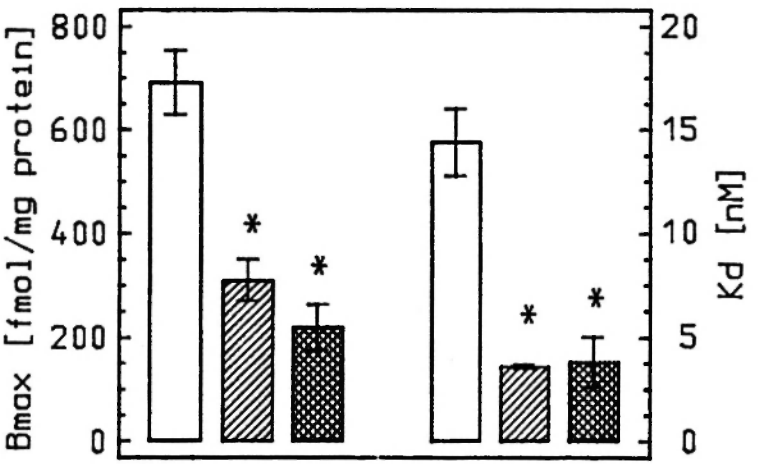

Fig. 4: Binding of ^{3}H-imipramine to synaptic plasma membranes in control samples ☐ and after addition of 10 μg. mg^{-1} protein of phosphatidic acid ▨ or α-glycerolphosphate ▩. Each test was repeated 3 times; *P < 0.01.

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