

## EFFECT OF ANTIDEPRESSANTS ON THE PHOSPHOLIPASE A<sub>2</sub> ACTIVITY IN PLASMA MEMBRANES OF THE RAT BRAIN CORTEX

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The aim of the present study was to establish whether antidepressants (ADs) of potentially different chemical structure and mechanisms of action affected the phospholipase A<sub>2</sub> (PLA<sub>2</sub>) activity in plasma membranes of the rat brain cortex. It was decided to evaluate the influence of imipramine (IMI), amitriptyline (AMI), fluvoxamine (FLU), mianserin (MIA) and tianeptine (TIA) on PLA<sub>2</sub> activity after an acute and long-term (4 weeks) drug administration. To study the time-related effects of FLU on PLA<sub>2</sub> activity, animals were treated for 1, 7, 14 and 28 days. The experiments were performed on male Wistar rats. The PLA<sub>2</sub> activity was determined by the method of Strosznajder and Strosznajder as well as Jelsema with slight modifications. It was shown that ADs significantly changed the PLA<sub>2</sub> activity in plasma membranes of the rat brain cortex and the effects depended on the dose, time of administration and the structure of the drug. Tricyclic ADs, both classic (IMI and AMI) as well as atypical (e.g. TIA) inhibited PLA<sub>2</sub> activity. It seems that FLU was the only antidepressant, which induced either inhibition or activation of PLA<sub>2</sub> depending on time of administration. It may be suggested that PLA<sub>2</sub> appears to be a common target for drugs showing quite different mechanisms of action.

**Key words:** *phospholipase A<sub>2</sub>, antidepressants, rats, brain cortex, plasma membranes*

*Abbreviations:* AA – arachidonic acid, ADs – antidepressants, AMI – amitriptyline,  $[Ca^{2+}]_i$  – intracellular  $Ca^{2+}$ , DMI – desipramine, DOC – sodium deoxycholate, FLU – fluvoxamine, IMI – imipramine, MAP – mitogen-activated protein, MIA – mianserin, PC – phosphatidylcholine,  $PLA_2$  – phospholipase A<sub>2</sub>, PLC – phospholipase C, PKA – protein kinase A, PKC – protein kinase C, SSRI – selective serotonin reuptake inhibitors, TCAs – tricyclic antidepressants, TIA – tianeptine

## INTRODUCTION

Phospholipase A<sub>2</sub> ( $PLA_2$ , E.C.3.1.1.4) catalyzes hydrolysis of the ester bond at the *sn*-2 position of 1,2-diacyl-*sn*-3-phosphoglycerides, thereby regulating the release of free fatty acids, in particular arachidonic acid (AA), and lysophospholipids [8, 32]. AA has been shown to have biological effects of its own. Moreover, AA is metabolized through several pathways (e.g. cyclooxygenases, lipoxygenases, cytochrome P-450s) into a variety of bioactive products [15]. Phospholipases A<sub>2</sub> are divided into broad classes of isoenzymes on the basis of their requirement for  $Ca^{2+}$  in *in vitro* activity assays [13]. The main members of the superfamily of  $PLA_2$  are: the secretory  $PLA_2$  (s $PLA_2$ ); type IV  $PLA_2$ , also known as cytosolic  $PLA_2$  (c $PLA_2$ ); and  $Ca^{2+}$ -independent  $PLA_2$  (i $PLA_2$ ) [22]. s $PLA_2$  isozymes were distinguished by their low molecular mass (14–18 kDa), obligatory dependence upon millimolar concentrations of  $Ca^{2+}$  for catalytic activity and a low selectivity for phospholipids with different polar heads [54]. Among s $PLA_2$  isoenzymes, group II A is the most widely distributed isoform in humans and can be released by neurons and astrocytes after activation [28]. c $PLA_2$  family of 80 kDa enzymes hydrolyzes selectively AA esterified at *sn*-2 position of phospholipids, and does not absolutely require  $Ca^{2+}$  for hydrolysis, although nanomolar concentrations of  $Ca^{2+}$  dramatically augment *in vivo* c $PLA_2$  activity [29]. c $PLA_2$  activity is regulated by biochemical mechanism that involves both docking to cell membranes for access to phospholipid substrate and phosphorylation-dependent activation [22]. The  $Ca^{2+}$ -dependent binding of c $PLA_2$  to the membrane is due to C2 domain located at the N terminus of the enzyme, reproducing a sequence of other enzymes involved in the intracellular signal transduction, e.g. phospholipase C (PLC) and protein kinase C (PKC) [19].

Moreover, c $PLA_2$  contains the primary consensus sequence for phosphorylation by mitogen activated protein (MAP) kinases [22]. Finally, a third group of isoenzymes identified as  $Ca^{2+}$ -independent in *in vitro* activity assays (i.e. the i $PLA_2$  of 80 kDa) could be distinguished by their exquisite sensitivity to inhibition by (E)-6-(bromomethylene)-3-(1-naphthalenyl)-2H-tetrahydropyran-2-one (BEL) [21]. The i $PLA_2$  has recently been found to be the dominant  $PLA_2$  activity in the cytosolic fraction of the rat brain, although there are no data available as yet about its functional role [59]. Neuronal functions of  $PLA_2$  include its modulatory effect on physico-chemical properties of cell membrane through effect on phospholipid turnover, on the release of neurotransmitters from synaptosomes, and on the activity of other membrane enzymes, ion channels and receptor sensitivity [37].

The changes in  $PLA_2$  activity have been reported to be implicated in the pathogenesis of numerous diseases, e.g. rheumatoid arthritis, psoriasis and myocardial infarction. Additionally in mental disorders, e.g. schizophrenia and Alzheimer's disease, aberrations in c $PLA_2$  activity have been found [17, 18]. So far, there have been no direct measurements of  $PLA_2$  activity in depressed patients. However, there is also some experimental evidence associating disturbances in  $PLA_2$  activity with the onset of depression. Results of numerous biochemical studies indirectly indicate an increase in  $PLA_2$  activity in this disorder. For example, the increased fluidity of hydrophobic core of erythrocyte membranes, higher concentration of free fatty acids in serum and increased ratio of AA to eicosapentaenoic acid in red blood cells correlated positively with clinical symptoms of depression [1]. In addition, the accelerated breakdown of neuronal membranes and decreased phosphatidylcholine (PC) concentration in platelets and erythrocytes were observed in suicide victims [44]. The disturbances in lipid-protein interactions induced by the enzyme could probably be a predisposing factor in affective illness. The increased  $PLA_2$  activity may account for accelerated phospholipid turnover, excessive fusion of synaptic vesicles with presynaptic membranes, enhanced release of neurotransmitters as well as the alterations of some endogenous modulators of enzyme activity [23].

Yet, mechanisms through which the antidepressants (ADs) exert the therapeutic effects are diverse and not fully recognized. ADs may act on one or

more different transporters, receptors, or at intracellular level. The convergence of different effects could also be of some importance. The common result of long-term treatment with different classes of ADs is an enhancement of synaptic neurotransmission in certain monoaminergic pathways. A few attempts have been made to correlate the pharmacological effects of ADs to the changes in PLA<sub>2</sub> activity in experimental animals [4, 7, 31, 34]. However, there is increasing evidence that ADs may also have effects on phospholipid metabolism. Therefore, we decided to investigate if and in what way ADs with potentially different chemical structure and mechanisms of action affect PLA<sub>2</sub> activity in plasma membranes of the rat brain cortex.

We decided to evaluate the effect of the following ADs on PLA<sub>2</sub> activity in plasma membranes of the rat cerebral cortex after an acute and long-term (4 weeks) drug administration: imipramine (IMI), amitriptyline (AMI), fluvoxamine (FLU), mianserin (MIA) and tianeptine (TIA). To study the time-related effects of FLU on PLA<sub>2</sub> activity, a time course of these changes was investigated by treating the animals for various periods: 1, 7, 14 and 28 days. Such experimental design was necessary because of different pattern of FLU influence on enzyme activity observed in preliminary study.

## MATERIALS and METHODS

### Animals

Male Wistar rats weighing approximately 180 ± 20 g at the start of the experiments were housed six per cage. The proper temperature, humidity and light cycle were maintained, and all rats had free access to food and water. The Ethics Committee of the Medical University of Silesia approved the study.

### Drugs

The rats received *ip* injections of the following ADs: IMI (Polpharma, Poland; 10 mg kg<sup>-1</sup> at a single dose or once a day for 28 days and 10 mg kg<sup>-1</sup> twice a day for 28 days); AMI (Polfa, Poland; 10 mg kg<sup>-1</sup> at a single dose and once a day for 28 days and 10 mg kg<sup>-1</sup> twice a day for 28 days); MIA (Sigma, USA; 10 mg kg<sup>-1</sup> at a single dose and 10 mg kg<sup>-1</sup> once a day for 28 days); FLU (Solvay Pharma, Netherlands; 10 mg kg<sup>-1</sup> and 20 mg kg<sup>-1</sup> at a single dose and 10 mg kg<sup>-1</sup> twice a day for 7,

14 and 28 days); TIA (Servier, France; 10 mg kg<sup>-1</sup> at a single dose and 10 mg kg<sup>-1</sup> twice a day for 28 days). Control groups in acute and chronic experiments received 0.9% saline by the same route. The drugs were dissolved in saline and administered in a volume of 5 ml kg<sup>-1</sup>. Each treatment group comprised 8 animals. The doses of drugs used and time intervals from drug administration to animal sacrifice were chosen according to literature data and preliminary results obtained in our laboratory [4, 10, 14, 31, 48, 57].

### Isolation of plasma membranes

Plasma membranes of the rat brain cortex were isolated according to the method described by Strosznajder and Strosznajder [53]. The brain was rapidly removed and put on ice. The dissected cortex hemispheres were homogenized in an ice-cold Potter-Elvehjem homogenizer in a medium containing 0.32 M sucrose, 10 mM Tris-HCl (pH 7.4) and 1 mM EDTA. The homogenate (10% w/v) was centrifuged for 3 min at 1100 × g. The resulting supernatant was centrifuged for 10 min at 17000 × g to yield a crude mitochondrial fraction (P2). Subsequently, the pellet (P2) was dispersed in 1 mM Tris-HCl buffer (pH 7.4) for hypotonic shock, then vigorously vortexed and centrifuged for 20 min at 48000 × g. The resulting pellet, further referred to as brain plasma membranes, was gently resuspended in 10 mM Tris-HCl buffer (pH 7.4), immediately frozen and used for further analysis.

### PLA<sub>2</sub> activity assay

The brain plasma membranes were used as a source of the enzyme in PLA<sub>2</sub> assay according to Jelsema [26] with slight modifications [53]. In brief, a mixture of 1-stearoyl-2-[1-<sup>14</sup>C]-arachidonyl-L- $\alpha$ -phosphatidylinositol (spec. act. 20–50 mCi mM<sup>-1</sup>, NEN, DuPont) and unlabeled phosphatidylinositol (Sigma) was dried under nitrogen, and after addition of 0.01% sodium deoxycholate (DOC) and 0.01 M Tris-HCl buffer (pH 7.8), the residue was solubilized by vigorous vortexing for 2 min. The assays were performed in the incubational mixture (200  $\mu$ l) containing 2.5 × 10<sup>4</sup> dpm of radioactive and 25 nmol of unlabeled phosphatidylinositol, 0.01% DOC and 2 mM CaCl<sub>2</sub> in 10 mM Tris-HCl buffer (pH 7.8). The reaction was initiated by the addition of 200  $\mu$ g of proteins of brain plasma membranes. The incubation was carried out at 37°C for 15 min in shaking water bath. Control

tubes without enzyme protein were always included. Reaction was terminated by the addition of 3 ml of isopropyl alcohol : n-heptane : 0.5 M H<sub>2</sub>SO<sub>4</sub> (40:10:1, v/v/v) and vigorous vortexing at room temperature. Following the addition of 1.5 ml of n-heptane and 1.0 ml of H<sub>2</sub>O, the samples were vortexed and centrifuged for 10 min at 1000 × g to extract the fatty acids. The enzymatically released [<sup>14</sup>C]arachidonate was separated from untransformed substrate by modification of the procedure described by Antonis [3]. Untransformed radiolabeled substrate remaining in the fatty acid-rich upper phase was removed by the addition of 150 mg of silica gel (20–200 mesh, Fisher) to 1 ml aliquots of the upper phase. The samples were vortexed, centrifuged (1000 × g for 10 min) and radioactivity was measured in Beckmann LS 6000 IC scintillation counter. To make up for loss of [<sup>14</sup>C]arachidonate during lipid extraction, [<sup>3</sup>H]arachidonate (5 × 10<sup>3</sup> dpm) was added to the same sample prior to extraction. The radioactivity was corrected for recovery of [<sup>3</sup>H]arachidonate. The PLA<sub>2</sub> activity was expressed as nM of arachidonate released per min per mg of protein and then presented as a percent of the control values. The protein content was determined by the method of Lowry et al. [30] using bovine serum albumin as a standard.

#### Statistical analysis

The data were analyzed using one-way analysis of variance (ANOVA) followed by the Bonferroni

post-hoc test. A *p* value less than 0.05 was considered as statistically significant. All data were expressed as means ± SD.

## RESULTS

### Influence of single administration of ADs on PLA<sub>2</sub> activity

A significant decrease in PLA<sub>2</sub> activity by 16.8% occurred after single administration of AMI (10 mg kg<sup>-1</sup>). Similarly, a single dose of IMI (10 mg kg<sup>-1</sup>) and TIA (10 mg kg<sup>-1</sup>) slowed AA release in comparison with the control values by 18% and 9.4%, respectively.

However, MIA administration at this dose significantly increased PLA<sub>2</sub> activity by 13.5%. The substantial increase in AA release stimulated both by 10 mg kg<sup>-1</sup> and 20 mg kg<sup>-1</sup> FLU indicated a significant increase in PLA<sub>2</sub> activity by 7% and 11% respectively (Fig.1).

### Influence of 28-day administration of ADs on PLA<sub>2</sub> activity

Administration of AMI at doses of 10 mg kg<sup>-1</sup> and 20 mg kg<sup>-1</sup> to rats for 4 weeks significantly decreased PLA<sub>2</sub> activity in the isolated cortical plasma membranes by 8.8% and 9.1%, respectively, in comparison with the control groups. Similarly, a decrease in AA release after treatment with

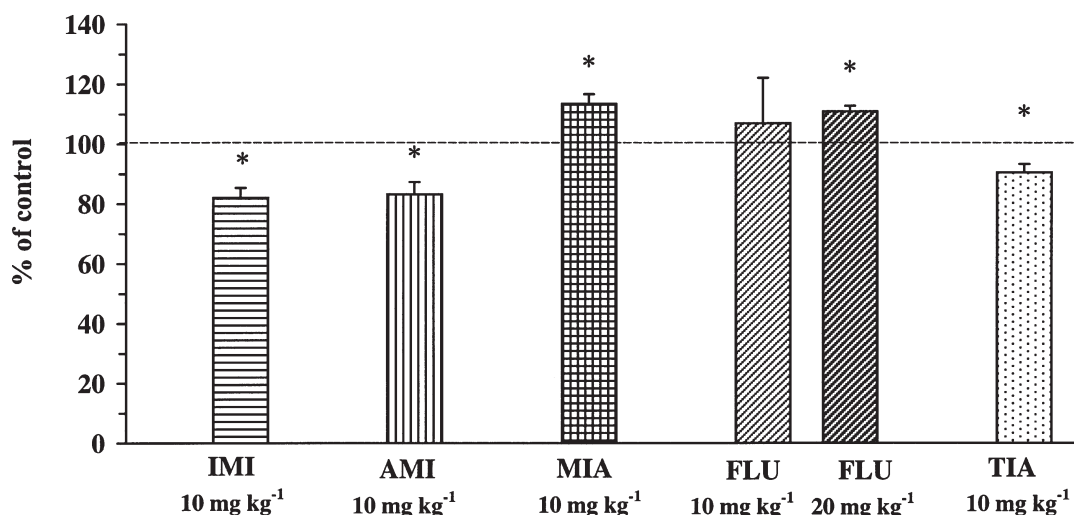


Fig. 1. Effect of acute treatment with antidepressants on PLA<sub>2</sub> activity in the isolated plasma membranes of the rat brain cortex. Data were analyzed using one-way ANOVA followed by the Bonferroni post-hoc test. The results are shown as a percentage in relation to the control value. Data are means ± SD (n = 8); \* *p* < 0.05

TIA (10 mg kg<sup>-1</sup> twice a day) by 8.5% was observed. The biggest decrease in PLA<sub>2</sub> activity was induced by the treatment with IMI at a dose of 10 mg kg<sup>-1</sup> both once and twice a day by 14.5% and 17.2%, respectively. An insignificant decrease in enzyme activity occurred after long-term administration of FLU (10 mg kg<sup>-1</sup> twice a day).

On the other hand, MIA (10 mg kg<sup>-1</sup> once a day) significantly increased PLA<sub>2</sub> activity by 23.4% in comparison with the control values (Fig. 2).

### Influence of FLU on PLA<sub>2</sub> activity

Figure 3 shows the influence of FLU administration for 1, 7, 14 and 28 days on PLA<sub>2</sub> activity. A substantial increase in AA release was stimulated by one-week administration of FLU at a dose of 10 mg kg<sup>-1</sup> twice a day (by 34.7%). Also after treatment with a single dose of FLU (20 mg kg<sup>-1</sup>), higher activity of PLA<sub>2</sub> (by 11%) was observed. However, the treatment with FLU (10 mg kg<sup>-1</sup>)

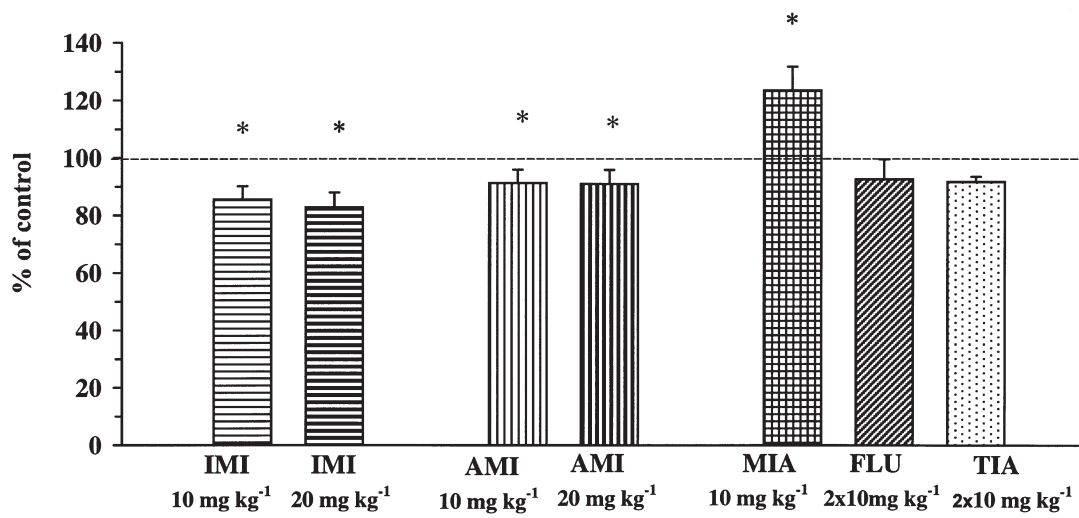


Fig. 2. Effect of long-term treatment with antidepressants on PLA<sub>2</sub> activity in the isolated plasma membranes of the rat brain cortex. Data were analyzed using one-way ANOVA followed by the Bonferroni post-hoc test. The results are shown as a percentage in relation to the control value. Data are means ± SD (n = 8); \* p < 0.05

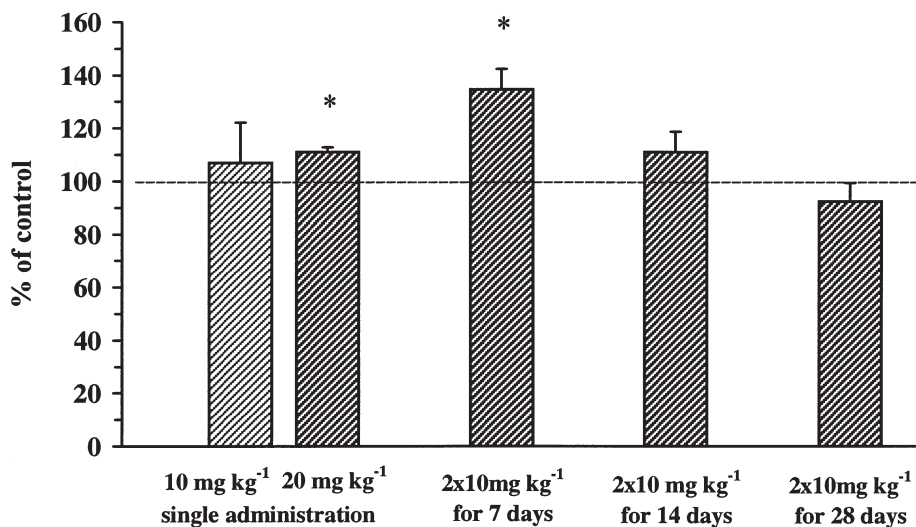


Fig. 3. Effect of treatment with FLU on PLA<sub>2</sub> activity in the isolated plasma membranes of the rat brain cortex. Data were analyzed using one-way ANOVA followed by the Bonferroni post-hoc test. The results are shown as a percentage in relation to the control value. Data are means ± SD (n = 8); \* p < 0.05

once as well as for 2 or 4 weeks did not significantly change the enzyme activity in comparison with the control.

## DISCUSSION

PLA<sub>2</sub> activity in plasma membranes of the rat cerebral cortex isolated from the control group stayed in the range from 0.32 to 0.49 nM mg of protein<sup>-1</sup> min<sup>-1</sup> and was similar to the results obtained with the nervous tissue by other investigators using radiometric method [53]. Crucial problem in the study of the mechanism of action of ADs seems to be the correlation between the observed biochemical effect and the time course of antidepressant action [45]. Adaptive changes induced by ADs in postreceptor signaling mechanism could contribute to their therapeutic effect observed after about 3–4 weeks of treatment [46]. For this reason we decided to administer the chosen ADs for 4 weeks.

The initial hypothesis on the action of ADs suggested that the therapeutic effects were related to the changes in monoamine transporters, metabolic enzymes and receptors [50]. Evidences indicate that several sites in postreceptor signaling cascades are involved in the mechanism of action of ADs. It was shown that the long-term modulation of neurotransmission, possibly responsible for the therapeutic effect of ADs, is related to receptor-coupled G proteins expression and activity, neuronal compartment-specific protein kinase activation, and gene expression (through the changes in the activation of transcription factors, neurotrophic factors or corticosteroid receptors) [33, 42, 45]. The other assumption is that therapeutic effect of ADs can be accounted for by their influence on signal transduction, immune function and receptor-mediated events, which may be related to disturbances in phospholipid metabolism [58].

Inhibitory effects of IMI on PLA<sub>2</sub> and PLC activity were established in lysosomes from the rat hepatocytes. IMI exhibits the same IC<sub>50</sub> value for PLA<sub>2</sub> as chlorphentermine, a well-known amphiphilic drug and also as do other agents such as chloroquine, amantadine, chlorpromazine and propranolol [25]. Inhibition of phospholipid degradation caused by various agents with an amphiphilic structure, among them IMI, was observed also in macrophages of rat lung and in cultured human fibroblasts [16].

Tricyclic antidepressants (TCAs) belong to cationic amphiphilic drugs. TCAs contain three 5- or 6-membered rings forming a bulky lipophilic structure and a hydrophilic side-chain consisting of a short carbon chain with a positively charged amino group at its end [58]. These cationic amphiphilic drugs interact with cellular membranes. Several underlying mechanisms may be responsible for the induction of phospholipid disturbance. It has been suggested that the TCAs bind to phospholipids and such binding may alter the phospholipid suitability as a substrate for phospholipases. This may result in a decreased breakdown of phospholipids, a process that occurs continuously within the cell. Moreover, TCAs or thereof metabolites may also inhibit phospholipases directly. Both of these mechanisms might result in phospholipidosis, i.e. intracellular accumulation of various phospholipids [58].

Physicochemical features of lipid bilayer are changed after drug incorporation, as manifested by a decrease in temperature of phase transition from gel to fluid-crystalline condition and membrane fluidization [20]. Vigo et al. [56] showed that PLA<sub>2</sub> hydrolyzed PC with optimal efficiency at phase transition temperature. Above or below this point, the enzyme activity was markedly decreased. Substances altering plasma membranes fluidity probably inhibit PLA<sub>2</sub> activity [12].

A phenomenon called “flip-flop” affects cell membrane fluidity and is connected with two-stage methylation of membrane phospholipids [24]. At the first stage catalyzed by phospholipido-N-methyltransferase I, methyl group is inserted into phosphatidylethanolamine. The second stage, methylation of phosphatidyl-N-methylethanolamine to PC, occurs only in the presence of phospholipido-N-methyltransferase II [36] and leads to successive migration of PC from internal to external membrane layer during methylation processes [24]. Accumulation of products of the first stage of methylation affects the fluidization process, and accumulation of PC leads to stiffness of cell plasma membranes. Melzacka et al. [36] proved that a single IMI administration intensified the synthesis of phosphatidyl-N-methylethanolamine and phosphatidyl-N-dimethylethanolamine in the rat cortical plasma membranes, simultaneously inhibiting generation of PC. On the other hand, long-term IMI administration caused only a decrease in PC amount. So, IMI given at a single dose produced more significant effect on membrane fluidity than after

chronic administration. In the present study, we have observed similar inhibition of PLA<sub>2</sub> activity in isolated cortical plasma membranes after IMI in acute and chronic experiment (Fig. 1 and 2).

Besides the possible effect of IMI and AMI on membrane fluidity, other mechanisms by which PLA<sub>2</sub> could be involved in the neurobiology of depression might operate. Most if not all receptors, which couple to PLC, also couple to PLA<sub>2</sub> [6]. For example, activation of receptors of the 5-HT<sub>2</sub> family (5-HT<sub>2A</sub>, 5-HT<sub>2B</sub> and 5-HT<sub>2C</sub>) leads to the activation of both PLC and PLA<sub>2</sub> [55]. It has been shown that long-term administration of IMI alters the density and sensitivity of 5-HT<sub>2</sub> receptors [10]. Thus, it is tempting to speculate that desensitization of 5-HT<sub>2</sub> receptors after IMI administration may be responsible for fastening one of possible pathways of PLA<sub>2</sub> activation. IMI has also affinities for  $\alpha_1$ -adrenergic, muscarinic and histamine H<sub>1</sub> receptors and a weak affinity for  $\alpha_2$ -adrenoceptors [10]. Some of these receptors may also affect PLA<sub>2</sub> activity and AA turnover [40]. That is why the decreased PLA<sub>2</sub> activity observed in our study may also be due to the effect of IMI on other neurotransmitter systems.

Previous research has shown that therapeutic efficacy of TCAs was associated with desensitization of  $\beta$ -adrenoceptors [49]. Dijcks et al. [14] have reported that stimulation of the rat hippocampal slices with  $\beta$ -receptor agonist isoprenaline resulted in the decreased  $\beta$ -adrenergic sensitivity. This effect was also observed after a single administration of desipramine (DMI), a metabolite of IMI. However, in *in vitro* study on C6 glioma cell cultures, the observed  $\beta$ -receptor down-regulation after single administration of DMI required the presence of PLA<sub>2</sub> [34]. A direct, postsynaptic mechanism of this phenomenon has been suggested.

The changes in intracellular calcium homeostasis and signaling may also play a significant role in antidepressant effect [45]. It was shown that incubation of neuronal cultures with ADs decreased the N-methyl-D-aspartate (NMDA)- or K<sup>+</sup>-induced intracellular Ca<sup>2+</sup> levels ([Ca<sup>2+</sup>]<sub>i</sub>) [11, 51]. Some of these effects were observed after monoamine reuptake inactivation, suggesting a direct effect of ADs on calcium fluxes. Similar effect was observed also in Chinese hamster ovary (CHO) cells exposed to 5-HT<sub>2</sub> receptor agonists and TCAs [2]. However, Shimizu et al. [51] observed transient increase in

[Ca<sup>2+</sup>]<sub>i</sub> concentration in neuronal cultures treated with ADs. The authors suggested that the mobilization of Ca<sup>2+</sup> from endoplasmic reticulum was up-regulated and dependent on the stimulation of receptors by inositol triphosphate. To establish a clearer correlation between the changes in calcium homeostasis and PLA<sub>2</sub> activity, direct [Ca<sup>2+</sup>]<sub>i</sub> measurements would be necessary.

PLA<sub>2</sub> is under control of phosphorylation by several protein kinases (e.g. PKC, PKA, MAP kinases) [39]. PKC activity is probably necessary to couple the phosphorylation cascade and cPLA<sub>2</sub> activation, as PKC activity seems necessary for the phosphorylation of both p42-MAP kinase and JUN N-terminal kinase (also known as stress-activated kinase), both enzymes being located upstream in the cascade that leads to cPLA<sub>2</sub> phosphorylation [22]. However, still little is known about the action of ADs on the changes in activity and expression of protein kinases in the cells. Long-term administration of fluoxetine or DMI was found to decrease significantly the PKC activity in the cerebral cortex and hippocampus [35]. Furthermore, PKC was implicated in the desensitization of 5-HT<sub>2A</sub> receptors induced by the agonist 1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane, a finding suggesting the involvement of this kinase in receptor mediated effects of ADs [47]. Moyer et al. [38] showed a decrease in the soluble PKA activity in the pineal gland of rats subjected to acute or long-term treatment with DMI. In the rat frontal cortex, Nestler et al. [41] found also a decrease in PKA activity in the cytosol and its increase in the nuclear fraction, after long-term administration of IMI, tranylcypromine or electroconvulsive shock. These observations indirectly confirm the results obtained in our study, indicating the presence of an inhibitory effect of IMI on PLA<sub>2</sub> activity in the rat cortical membranes.

We observed weaker inhibition of the PLA<sub>2</sub> activity after long-term AMI administration in comparison with IMI. It is known that AMI is a several times stronger  $\alpha_1$ -adrenergic and muscarinic receptor antagonist than IMI. Supersensitization of  $\alpha_1$  and M receptors opens one of the pathways leading to PLA<sub>2</sub> activation. It is possible that membrane mechanisms are involved in the attenuation of inhibitory effect on PLA<sub>2</sub> observed after long-term treatment with AMI. The primary mechanism of action of principal TCAs is based on nonselective inhibition of NA, 5-HT, and dopamine reuptake [45]. Stockert et al. [52] used radiolabeled IMI

( $^3\text{H}$ IMI), which bound specifically with protein carrier for 5-HT and found reduction of a number of  $^3\text{H}$ IMI binding sites in synaptosomes from the rat cerebral cortex after long-term TCA administration. Moreover, preincubation of synaptosomes with PLA<sub>2</sub> or AA increased a number of  $^3\text{H}$ IMI binding sites and suggested antagonistic effect of PLA<sub>2</sub> and TCAs on 5-HT carrier activity. The effects on PLA<sub>2</sub> activity after once a day IMI and AMI administration for 4 weeks were weaker in comparison with twice daily schedule. It is probably related to the lowered drug concentrations in blood and less intense membrane and receptor adaptive changes.

Acute MIA administration (10 mg kg<sup>-1</sup>) increased PLA<sub>2</sub> by 13.5%, and long-term treatment intensified this effect to 23.4% (Fig. 1 and 2). There are no data about possible MIA complexes with plasma membrane phospholipids. MIA has a tetracyclic structure and no features characteristic of amphiphilic drugs. The results of a freeze-fracture studies have demonstrated that chronic administration of MIA decreased cholesterol concentration in the rat cerebral cortex cell membranes leading to their increased fluidization [4]. Whereas MIA antagonized  $\alpha_2$ -adrenergic autoreceptors, it was shown that its metabolites inhibited 5-HT reuptake [10]. Sallés et al. [48] found that 14-day administration of MIA increased  $\alpha_1$ -adrenoceptor density in the rat brain cortex. Moreover, an increased phosphatidylinositol metabolism was observed after stimulation by noradrenaline. These findings were in line with our results that showed increased PLA<sub>2</sub> activity after long-term treatment with MIA.

The newer generation of ADs, the so-called selective serotonin reuptake inhibitors (SSRI) have molecular structures, which differ completely from those of the TCA drugs. It has been proposed that a reduction in the responsiveness of both the 5-HT<sub>1A</sub> and 5-HT<sub>1B</sub> receptor systems is involved in the mechanism of SSRI action [9]. We have chosen fluvoxamine belonging to SSRI group. Bel and Artigas [5] using *in vivo* microdialysis have demonstrated the increase in 5-HT release 30 min after FLU administration. The observed effect was directly proportional to the used drug dose and stayed constant till the end of 4-hour experiment. In our study, a single administration of 10 mg kg<sup>-1</sup> or 20 mg kg<sup>-1</sup> of FLU increased PLA<sub>2</sub> activity by 7% and 11%, respectively. The animals were decapitated

3 h after FLU administration. An increase in PLA<sub>2</sub> activity after acute treatment with FLU may be explained by the amplified stimulation of 5-HT<sub>2</sub> receptors by 5-HT, which leads in turn, to the activation of PLA<sub>2</sub> [55]. The biggest increase in PLA<sub>2</sub> activity (by 34.7%) was induced by one-week administration of FLU (10 mg kg<sup>-1</sup> twice a day). Perez et al. [43] observed intensified phosphorylation of microtubule-associated protein 2 (MAP-2) after 5-day administration of FLU (but not after 12 or 21 days). MAP-2 is a major substrate for PKA and is involved in the regulation of microtubule assembly. The increased Ca<sup>2+</sup>/CaM-dependent protein kinase II phosphorylation in the rat hippocampal synaptosomes was also reported following long-term FLU administration [45]. These results suggest that FLU can be implicated in the process of cellular phosphorylation of proteins, including PLA<sub>2</sub>. No significant change in PLA<sub>2</sub> activity was observed after acute (10 mg kg<sup>-1</sup>) or subchronic and chronic (10 mg kg<sup>-1</sup> twice a day for 14 or 28 days) treatment with the drug (Fig. 1 and 3). Such results could be due to the desensitization of 5-HT<sub>2</sub> receptors indirectly activating the enzyme. Besides, it can be suggested that, at least for this drug, the action on PLA<sub>2</sub> is linked to monoamine transporter blockade and not to structural properties of the compound.

During the past decade, ADs with molecular structure and mechanism of action different from TCA have been introduced into clinical practice. TIA, however, has the molecular structure similar to the classic TCA. This structurally tricyclic compound contains a middle dibenzotiazepine ring with atoms of sulphur and nitrogen. A side-chain of the drug containing carboxyl group at its end, is longer than in the most of TCAs. A mechanism of TIA action is based on the stimulation of neuronal uptake of 5-HT [57]. Moreover, no affinity of TIA for the  $\alpha$ - and  $\beta$ -adrenergic, dopamine, serotonin, GABA, muscarinic or histaminic receptors and also no its effects on the MAO-A and B activity were found [27]. Presynaptic action of TIA leads to the reduction of 5-HT concentration in the synaptic cleft. Therefore, it is unlikely that the reduced PLA<sub>2</sub> activity after TIA administration in our experiment is a direct effect of 5-HT on the postsynaptic 5-HT<sub>2</sub> receptors activating PLA<sub>2</sub>.

Our study have shown that long-term administration of TCAs, like IMI and AMI, caused a decrease in PLA<sub>2</sub> activity. Results of the studies with

TIA treatment seem to confirm the association of tricyclic structure of the drugs containing side-chain, with their potential ability to inhibit PLA<sub>2</sub> activity. TIA (10 mg kg<sup>-1</sup>) induced a decrease in PLA<sub>2</sub> activity after acute and long-term treatment by 9.4% and 15.8%, respectively (Fig. 1 and 2). These results strengthened the association between antidepressant effect and PLA<sub>2</sub> inactivation. The change in PLA<sub>2</sub> activity is a stable, long-lasting modification, because it is induced by long-term AD treatment, and it is measurable after the time necessary to prepare the rat cortical membranes.

In conclusion, ADs significantly change the PLA<sub>2</sub> activity in plasma membranes of the rat brain cortex. The effects depend on the drug structure, dose and time of administration. TCAs, classic (IMI and AMI) as well as atypical (e.g. TIA) inhibit PLA<sub>2</sub> activity. In contrast, FLU seems to be an example of antidepressants, which can induce inhibition as well as activation of PLA<sub>2</sub> depending on time of administration. It may be suggested that PLA<sub>2</sub> appears to be a common target for drugs acting through quite different mechanisms.

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