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Short communication

Effects of olanzapine and paroxetine on phospholipase D activity in the rat brain

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Abstract:
Background: Phospholipase D (PLD) plays a key role in a second messenger system producing phosphatidic acid, mediating, among others, serotonin 5-HT_2 receptor activity. The aim of the study was to evaluate a possible effect of atypical antipsychotic drug, olanzapine (OLZ), and selective serotonin reuptake inhibitor (SSRI) antidepressant, paroxetine (PX), on oleate-activated PLD activity in plasma membranes isolated from rat brain cortex.
Methods: PLD activity was determined using a fluorometric assay. Ritanserin was used to determine the 5-HT receptor mode of action.
Results: A single dose of 10 mmol/kg OLZ produced no change in rat brain cortex PLD activity, 20 mmol/kg OLZ caused a nonsignificant decrease, and long-term (21 days) administration of OLZ resulted in a 41.9% decrease in PLD activity. Single doses of PX significantly decreased PLD activity: 10 mmol/kg – by 28.6%; 20 mmol/kg – by 31.5%, and long-term (21 days) administration of PX – by 39.5%.
Conclusion: The study indicates that the 5-HT_2 receptor-mediated inhibition of oleate-activated PLD may be a common part of the mechanisms of action of OLZ and PX.

Key words:
phospholipase D (PLD), olanzapine, paroxetine, neuroleptics, antidepressants, rats

Introduction

Phospholipase D (PLD) (EC 3.1.4.4) acts on phospholipids, producing phosphatidic acid [17]. A PLD-related second messenger system was postulated in animal models, after an oleate-activated PLD was found in mammalian cells, and phosphatidic acid appeared to be a second intracellular messenger [6]. Recognition of intracellular second messengers and identification of their involvement in the mechanisms of action of psychotropic drugs represent important findings in this area, in recent years.

Two classical isoforms of PLD, PLD1 and PLD2 were reported in mammals [31]. The whole family of phospholipases D is divided, in regard to their activity, on dependent or independent from phosphatidylinositol (PI). PI-dependent PLD1 and PLD2 are inhibited *in vitro* by free fatty acids, while PI-independent phospholipases D, in order to be activated, require the presence of free unsaturated fatty acids, e.g., oleic acid [31]. The exact roles and differences between PLD isoforms still remain obscure. Hopefully, newly synthesized small molecule specific inhibitors of particular isoenzymes as well as recently developed PLD1 and PLD2 knockout mice may allow for their better understanding [29]. Although, the oleate-activated phospholipase D was discovered as the first PLD isoenzyme, its sequence, regulation, and function in the cell still remain only slightly recognized [16, 31]. Since the oleate-activated PLD is incorporated into plasmatic cell membranes, the membrane isolation preserves its activity.

PLD plays an important role in mitogenesis and cell differentiation, morphogenesis, synaptogenesis, intracellular transport and exocytosis, protein kinase activity regulation, neurodegeneration, and apoptosis [10, 13]. To date, PLD has been shown to participate in the pathogenesis of Alzheimer disease, Parkinson disease, alcoholism, brain ischemia, and brain ageing [12, 18, 25, 33]. A variety of actions performed by PLD in cellular physiology may suggest that the oleate-activated PLD is a candidate for several regulatory neuronal functions.

Antipsychotic and antidepressant agents have been used for more than half a century in the treatment of schizophrenic and depressive disorders. In schizophrenia, in which a complex pathophysiologic mechanism involves disturbances in the dopaminergic system, some antipsychotic agents are thought to act by blocking the D_{2-4} receptors. In addition, certain atypical neuroleptic agents appear to act by blocking the serotoninergic 2A (5-HT_{2A}) receptors. In depression, in which the pathophysiologic mechanism involves the monoaminergic neurotransmitters – noradrenaline, serotonin, and dopamine, some antidepressants act by blocking monoamine receptors and protein transporters of the monoamines. Long-term administration of antidepressants in animals produces downregulation of the 5-HT_2, and upregulation of the dopaminergic receptors (e.g., D_1, D_2, and D_3) [20, 22, 26]. Therefore, the 5-HT_{2A} receptors in humans play an important role in the pharmacological treatment of both schizophrenia and depression. There are only a few reports concerning the involvement of PLD in the action of the serotonin receptors 5-HT_{2A} [28].

Studies on the role of phospholipases A_2 and C in the brain have led to a better understanding of the pathomechanism of some mental diseases, and of the mechanisms of action of different antipsychotic and antidepressive drugs [8]. However, little is known about the role of phospholipase D in these processes.

There are only a few reports concerning the influence of antidepressants on PLD activity. Bobeszko et al. described the modulation of PLD activity by imipramine and amitriptyline in non-receptor mechanisms (i.e., cellular Ca^{2+} mobilization, rearrangement of membrane phospholipids and modulation of protein kinase C (PKC) [3]. To the best of our knowledge, there have been no data on the relationship between psychotropic drugs and the oleate-activated PLD. We hypothesized that influencing a novel second messenger system, related to the oleate-activated PLD, may play a role in psychotropic action. We therefore examined the influence of psychotropic drugs on oleate-activated PLD, by administering the atypical neuroleptic olanzapine (OLZ), and the antidepressant paroxetine (PX) to rats in both single-dose and long-term experiments. To determine the receptor mode of action of these agents, the experiment with single-dose administration of PX or OLZ was done, with and without pre-administration of the 5-HT_2 receptor antagonist ritanserin (RS).

Materials and Methods

Materials

Bovine serum albumin was obtained from Koch-Light (Germany). Folin-Ciocalteu reagent, sucrose, potassium sodium tartrate tetrahydrate, sodium chloride, sodium carbonate anhydrous, sodium hydroxide, and copper sulfate pentahydrate were all purchased from POCh S.A. (Gliwice, Poland), and were of analytical grade. Ethylenediaminetetraacetic acid disodium salt (EDTA disodium salt) and resorufin were obtained from Sigma (St. Louis, MO, USA). TRIZMA base and oleic acid (sodium salt) were obtained, from Sigma-Aldrich (Seelze, Germany). Ritanserin (RS) came from Research Biochemical Inc. (Natick, MA, USA). The following reagents were purchased from the Amplex Red Phospholipase D Assay Kit, A-12219 (Molecular Probes Europe BV, Leiden, The Netherlands): dimethyl sulfoxide (DMSO, anhy-

drous), L-α-phosphatidylcholine (lecithin), N-acetyl-3,7-dihydroxyphenoxazine, horseradish peroxidase, and choline oxidase (from Alcaligens sp). Olanzapine was generously donated by Eli Lilly (Basingstoke, Hampshire, UK) and paroxetine hydrochloride hemihydrate was a gift of GlaxoSmithKline (Brentford, Middlesex, UK).

Experimental animals

All experimental procedures were conducted with approval of the Ethics Committee of the Silesian Medical University. Male Wistar rats were used in both experiments. In the short-term experiment, the mean body weight was 220 ± 10 g (n = 8 per group), and in the long-term experiment, the mean body weight was 180 ± 10 g on day 1, and 230 ± 20 g, on day 21 (n = 10 per group).

Drug administration

Doses of drugs given below represent the mean doses used in the two experiments. In the short-term study, rats received a single intraperitoneal dose (1 ml/kg) of 10 or 20 mmol/kg OLZ (i.e., 3.1 or 6.2 mg/kg) or PX (i.e., 3.7 or 7.4 mg/kg). In the long-term study, rats received 2 × 10 mmol/kg of either OLZ or PX per day, for 21 days. A control group received 0.9% NaCl solution.

RS (2 mg/kg) was administered intraperitoneally, 30 min before a single dose of OLZ or PX. RS was also given to the control group of rats (not treated with a psychotropic drug).

Isolation of rat brain plasma membranes

The plasma membranes of rat brain cortex were isolated according to the method described by Jelsema [11] and modified according to Strosznajder and Strosznajder [30]. The method was previously described in details by Krzystanek et al. [14]. Briefly, decapitation was performed on each animal, after a time period equivalent to the half-life of the examined compound in rat brain (i.e., 5 h in OLZ group [1] and 1.5 h in PX group [7]). After decapitation, the rat brain was rapidly removed and put on ice. Dissected brain cortex was homogenized, and the homogenate was centrifuged for 3 min at 1,100 × g. The obtained supernatant was centrifuged for 10 min at 17,000 × g to obtain a P_2 fraction. Subsequently, the P_2 fraction underwent hypotonic shock; then it was vortexed and centrifuged for 20 min at 48,000 × g. The resulting pellet contained brain plasma membranes. The pellet was resuspended in a modified buffer (50 mM Tris-HCl, 1 mM $MgCl_2$, 1 mM EGTA, pH = 7.2) [5], and then immediately frozen at –70°C, for further experiments.

Protein determination

Plasma membranes protein content was determined using the method described by Lowry et al. [19].

Determination of PLD activity in the rat brain

Assessment of PLD activity was performed according to Zhou et al., modified to measure the activity of the oleate-activated isoenzyme in rat brain [34]. The modification was previously published by Krzystanek et al. [14]. Briefly, PLD cleaves lecithin to yield choline and phosphatidic acid. Next, choline is oxidized by choline oxidase to betaine and H_2O_2. Finally, H_2O_2 reacts with N-acetyl-3,7-dihydroxyphenoxazine in a 1 : 1 stoichiometry to produce a fluorescent product, resorufin. The latter reaction is catalyzed by horseradish peroxidase (HRP). Fluorometric measurements were made with the Ascent FL Fluoroscan, type 374 (Labsystems, Finland). Filters used in the experiments were set for excitation and emission at 560 and 590 nm, respectively. The standard reaction mixture (200 µl) contained 50 mM Tris-HCl (pH = 7.2), 1 mM $MgCl_2$, 1 mM EGTA, 100 µM N-acetyl-3,7-dihydroxyphenoxazine, 2 U/ml HRP, 0.2 U/ml choline oxidase, 0.5 mM lecithin and 4 mM sodium oleate [5]. Assays were performed at a temperature of 37°C. The amount of plasma membrane protein in a reaction volume was 25 µg, and the time of reaction was 2 min. Assessments were carried out in triplicate. The activity of oleate-activated PLD was expressed in nmol/mg/min.

Statistics

Statistical analysis was performed using the Student's *t*-test. The level considered significant was $p < 0.05$.

Results

A single dose of 10 mmol/kg OLZ produced no change in the rat brain cortex PLD activity, compared with the control group (PLD activity = 10.05 ± 2.2 nmol/

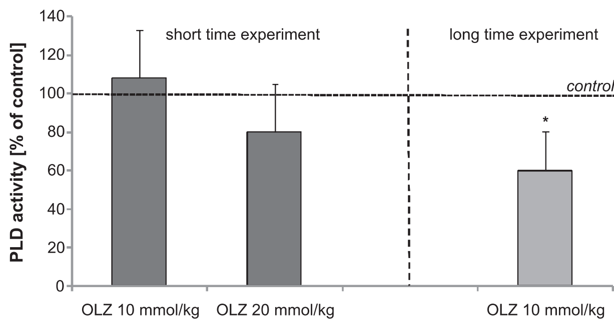

Fig. 1. Effect of a single dose (short-term study) and long-term administration (21 days, 2 doses per day) of olanzapine (OLZ) on oleate-activated phospholipase D (PLD) activity in rat brain. OLZ was given intraperitoneally (*ip*). Results are expressed as % of the control (PLD activity = 10.05 ± 2.2 nmol/mg/min) + SD for n = 8 in the short-term experiment and n = 10 in the long-term study

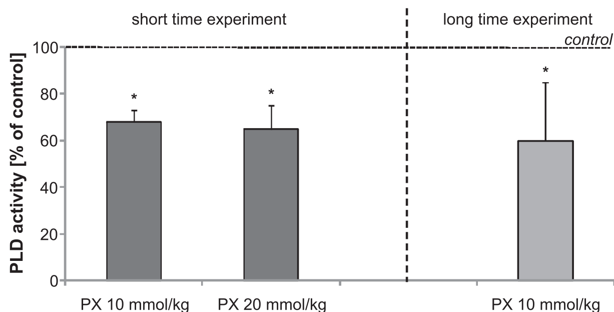

Fig. 2. Effect of a single dose (short-term study) and long-term administration (21 days, 2 doses per day) of paroxetine (PX) on oleate-activated phospholipase D (PLD) activity in rat brain. PX was given intraperitoneally (*ip*). Results are expressed as % of the control (PLD activity = 10.05 ± 2.2 nmol/mg/min) + SD for n = 8 in the short-term experiment and n = 10 in the long-term study

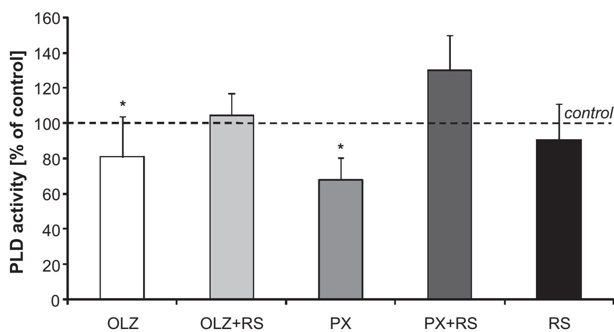

Fig. 3. Influence of ritanserin (RS, 2 mg/kg, *ip*) on olanzapine- (OLZ, 20 mmol/kg, *ip*) and paroxetine- (PX, 20 mmol/kg, *ip*) induced inhibition of oleate-activated phospholipase D (PLD) in rat brain. RS was administered 0.5 h before OLZ or PX. Results are expressed as % of the control (PLD activity = 10.05 ± 2.2 nmol/mg/min) + SD for n = 8. * $p < 0.05$ *vs.* control group

mg/min), and a dose of 20 mmol/kg OLZ produced a nonsignificant decrease of this activity (Fig. 1). However, a long-term (21 days) administration of OLZ resulted in a 41.9% decrease in the PLD activity, compared with controls ($p < 0.05$).

Single doses of PX significantly decreased the PLD activity, as compared with controls: 10 mmol/kg, by 28.6%, and 20 mmol/kg, by 31.5% ($p < 0.05$). A long-term (21 days) administration of PX significantly decreased the PLD activity, by 39.5% ($p < 0.05$) (Fig. 2).

OLZ and PX did not significantly differ in the degree of inhibition of the PLD activity, produced after a single dose, although at the 20 mmol/kg dose level, PX appeared to be a more potent inhibitor of PLD activity than OLZ. After a long-term administration, the inhibition of PLD activity was slightly (nonsignificantly) greater with OLZ (41.9% decrease) than with PX (39.5%).

Pre-administration of RS blocked the inhibition of oleate-activated PLD by both OLZ and PX (Fig. 3).

Discussion

This is the first study investigating the effect of psychotropic drugs on newly proposed second messenger system, related to oleate-activated PLD. To examine a potential mechanism of action of such agents, we have selected OLZ, because it is a modern atypical antipsychotic drug, blocking 5-HT_{2A} and D_2 receptors, and PX, since it is a high potency, selective serotonin reuptake inhibitor (SSRI) antidepressant. The main finding of our experiments is showing that both OLZ and PX decreased the activity of the oleate-activated isoform of PLD in the rat brain tissue.

Serotonin 5HT_{2A} receptors seem to be a common element of the mechanism of action of both atypical antipsychotics and SSRIs. This was confirmed in our study, in which the OLZ and PX inhibition of oleate-activated PLD activity was prevented by the 5-HT_{2A} blocking agent, ritanserin.

Our findings correspond with some of the previous reports. According to Robertson et al., serotonin induces the PLD activation, and this functional activation of PLD corresponds with ADP-ribosylation factor association with the 5-HT_{2A} [28]. An acute activation of the 5-HT_{2A} receptors may produce an in-

creased PLD activity. On the other hand, a long term activation of the 5-HT_{2A} receptors with serotonin can result in a down-regulation and a decrease of the PLD activity.

5-HT_{2A} receptor belongs to a category of G protein-coupled receptors (GPCR). After stimulation of GPCRs, the dissociation of Gα and Gβγ heterotrimeric G proteins occurs. Gα induces signaling pathways leading to stimulation of PLDs. Gβγ also activates PLCβ to indirectly activate PLD. However, Preninger et al. [27] demonstrated that the Gβγ subunit may directly inhibit PLD. Classic PKC isoforms α, β, and γ stimulate PLD1 and PLD2 activity downstream of PLD activation. Phosphorylation may modulate the activity of PLD2 in regard to specific agonist-mediated or intracellular circumstances [29]. Because the regulation of oleate-activated PLD is not fully understood, similar 5-HT_{2A} receptor mechanism could regulate the oleate-activated PLD. In our opinion this hypothesis is worth further exploration.

In our study, PX produced the PLD decrease after both short- and long-term administration, while OLZ did it mainly during the long-term study. An immediate decrease of the PLD activity after one dose of PX may be related to an unknown direct influence of PX on PLD or other factors regulating PLD activity. Therefore, a common element of action of OLZ and PX may involve a functional inhibition of PLD, during the long-term treatment.

Blocking of the 5-HT_{2A} receptors by atypical antipsychotics increases the amount of dopamine and serotonin in the limbic system and prefrontal cortex of rat brain [15]. On clinical level, such effects may be responsible for a better clinical profile of atypical antipsychotics in schizophrenia, as well as for a mood-stabilizing and lack of pro-depressive effects in affective disorders. Down-regulation of the 5-HT_{2A} receptors by some antidepressants, including SSRIs, produces increased dopaminergic firing, which seems to be decisive for the antidepressive effect [24]. Acute blocking of the 5-HT_{2A} receptors alleviates anxiety, which may also contribute to the antidepressant effect [20].

An effect of OLZ on the PLD-dependent activity of 5-HT_{2A} receptor, similar to that of the PX one, may be hypothesized as a new mechanism of action, explaining a favorable action of OLZ on different mood states. This hypothesis definitely requires further studies. However, on the clinical level, the effectiveness of OLZ in acute manic, hypomanic and mixed episodes has been well documented [6, 21]. In particular, OLZ has a significant effect on the prevention of manic relapses [2], and according to some trials, OLZ was proven effective in preventing of any kind of such relapse [32]. Combination of OLZ with antidepressant may produce a greater improvement in treatment-resistant depression, compared with antidepressant monotherapy [4]. In addition, such combination is efficacious for the treatment of major depression with psychotic features [23].

In summary, our data provide the first evidence that the atypical antipsychotics and antidepressants acting on the 5-HT_{2A} receptors may influence the membrane coupled PLD, and possibly the second messenger system related to this enzyme. We propose that the inhibition of PLD activity, and the ensuing decrease in phosphatidic acid production may be involved in the cellular mechanism of action of the atypical neuroleptic OLZ and the SSRI PX. This mechanism may contribute to the beneficial action on mood exerted by atypical neuroleptics. It may be hypothesized that the direct inhibitors of PLD could be tested as potential antidepressants.

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