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Effect of neuroleptics on phospholipase A_2 activity in the brain of rats

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Abstract The effect of neuroleptics on phospholipase A_2 (PLA_2) activity in rat brain plasma membranes was studied. Chlorpromazine (10 mg/kg), fluphenazine (5 mg/kg), thioridazine (5 mg/kg), trifluoperazine (5 mg/kg), haloperidol (2 mg/kg), and sulpiride (100 mg/kg) were administered to rats intraperitoneally as a single dose or long-term treatment (4 weeks). The PLA_2 activity was determined 24, 48, and 72 h after the last injection of a drug. The enzyme activity was decreased after a single or 4-week administration of chlorpromazine, trifluoperazine, haloperidol, and sulpiride. Fluphenazine and thioridazine caused an increase of PLA_2 activity in rat brain both after a single dose and long-term administration. For the first time it was shown that neuroleptics cannot only inhibit but also increase, PLA_2 activity. Elucidation of this fact requires further studies.

Key words Neuroleptic treatment · Rats · Phospholipase A_2 · Brain plasma membranes

Introduction

Phospholipases represent a widespread and abundant class of enzymes in biological systems (Dennis 1983). Phospholipase A_2 (PLA_2) is a ubiquitous enzyme that cleaves fatty acids from the sn-2 position of phospholipids. Intracellular existence of PLA_2 have been found in all cells investigated thus far. In the brain the enzyme is present in cell membranes, microsomes, and cytosol (Farooqui et al. 1992; Woelk et al. 1981, 1978).

The PLA_2 plays an important role in phospholipid turnover of cell membranes, which, in turn, affects membrane fluidity and function (Van den Bosch 1980). Moreover, changes in membrane lipids induced by PLA_2 action are important factors in signal transduction by generating lipid second messengers (Anand-Srivastava and Johnson 1981; Mallorga et al. 1980; Oliveira et al. 1984).

Elevated PLA_2 activity has been reported in serum, plasma, and platelets from schizophrenic patients as compared with healthy and psychiatric controls, as well as a reduction of the enzyme activity to controls' levels after therapy with haloperidol (Gattaz et al. 1987, 1990, 1995; Noponen et al. 1993).

Based on these findings, we have examined the effects of short- or long-term treatment with different neuroleptics on PLA_2 activity in brain of rats.

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Materials and methods

Animals and drug administration

Experiments were performed on male Wistar rats bred in the Central Experimental Animal Farm of the Silesian Academy of Medicine. The initial body weight was 170±10 g. The rats were fed standard diet as pellets and water ad libitum, and housed eight per cage (58 × 38 × 20 cm) under optimal environmental conditions (22° C, 55–60% relative humidity, 12-h day cycle).

The drugs were administered in a single dose or for 4 weeks. Treatment was carried out daily (6 days a week) by intraperitoneal administration (ip), using one dose of drug: fluphenazine hydrochloride (5 mg/kg, Polfa), thioridazine hydrochloride (5 mg/kg, Polfa), trifluoperazine dihydrochloride (5 mg/kg, Sigma), chlorpromazine hydrochloride (10 mg/kg, Polfa), haloperidol (2 mg/kg, Polfa), sulpiride (100 mg/kg, Sigma). All doses of neuroleptics are expressed as a free base. The drugs were dissolved in 0.9% NaCl solution, except haloperidol and sulpiride, which were dissolved in a minimum amount of 20% acetic acid and 1 *N* sulfuric acid, respectively, and the pH was adjusted to pH 7.0 with 1 *N* sodium hydroxide. Control animals were given the vehicle only by the same route. Either drugs or solvents were applied in a volume of 2 ml/kg. Each treatment group comprised 8 animals.

At the established time the rats were decapitated 24, 48, 72 h after the last injection of neuroleptics, except haloperidol, where animals were given an additional 96 h.

Isolation of plasma membranes

The brain was rapidly removed and put on ice. Dissected cortex hemispheres were homogenized in ice-cold Potter-Elvehjem homogenizer in a medium containing 0.32 *M* sucrose, 10 n*M* Tris-HCl buffer (pH 7.4) and 1 m*M* ethylenediaminetetraacetate (EDTA). The plasma membranes of rat brain cortex were isolated according to the method described by Strosznajder and Strosznajder (1989). 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 (P_2).

Subsequently, the pellet was dispersed in 1 m*M* 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 a brain plasma membrane, was gently resuspended in 10 m*M* Tris-HCl buffer (pH 7.4), immediately frozen, and used for further estimation.

Phospholipase A_2 assay

The brain plasma membranes were used as a source of enzyme for PLA_2 assay according to Jelsema (1987) with slight modification (Strosznajder and Strosznajder 1989). In brief, a mixture of L-α-1-stearoyl-2-[1-^{14}C]-arachidonyl phosphatidylinositol (spec. act. 48 mCi/mmol, NEN, DuPont) and unlabelled 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) was solubilized by vigorous vortexing for 2 min. The assays were performed in incubational mixture (200 µl) containing 2.5×10^4 dpm radioactive and 25 nmol unlabelled phosphatidylinositol, 0.01% DOC, and 2 m*M* $CaCl_2$ in 10 m*M* Tris-HCl buffer (pH 7.8).

The reaction was started by the addition of 200 µg proteins of brain plasma membranes, and the incubation was carried out at 37° C for 15 min in water bath shaker. Control tubes without enzyme protein were always included. Reaction was stopped by the addition of 3 ml Dole's reagent (isopropyl alcohol : n-heptan : 1 N H_2SO_4, 40 : 10 : 1, v/v/v) and vigorous vortexing in room temperature. Following addition of 1.5 ml of n-heptane and 1 ml of H_2O, samples were vortexed and centrifuged for 10 min at 1000 g to extract the fatty acids. The enzymatically released [^{14}C]arachidonate was separated from unreacted substrate by modification of procedure described by Antonius (1965). Unreacted radiolabelled substrate remaining in the fatty acid-rich upper phase was removed by addition of 150 mg silica gel (20–200 mesh, Fisher) to 1.5 ml aliquots of upper phase. Samples were vortexed, centrifuged (1000 g for 10 min), and radioactivity was measured in Beckman LS 6000 IC scintillation counter.

To make up for loss of [^{14}C]arachidonate released during lipid extraction, [^{3}H]-arachidonate (5×10^3 dpm) was added to the same sample prior to extraction. The value for [^{14}C]arachidonate was corrected for recovery of [^{3}H]arachidonate. The PLA_2 activity was expressed as nmole of [^{14}C] arachidonate release per min/mg protein, and then following as a percent of control value. The protein content was determined by the method of Lowry et al. (1951) using bovine serum albumin as a standard. The Student's *t*-test was used for statistical evaluation of data.

Results

In the first experiment we estimated PLA_2 activity in brain plasma membranes of rats treated with a single dose of neuroleptics. The results are shown in Fig. 1. After thioridazine and fluphenazine administration, PLA_2 activity was significantly increased 24 and 48 h after single injection. On the contrary, trifluoperazine, chlorpromazine, and sulpiride decreased PLA_2 activity in brain plasma membrane up to 48 h after injection. A single dose of haloperidol decreased the enzyme's activity by 33% in brain plasma membrane of rats 24 h after drug injection. After 72 h of haloperidol administration, PLA_2 activity was still diminished approximately 10% in comparison with controls.

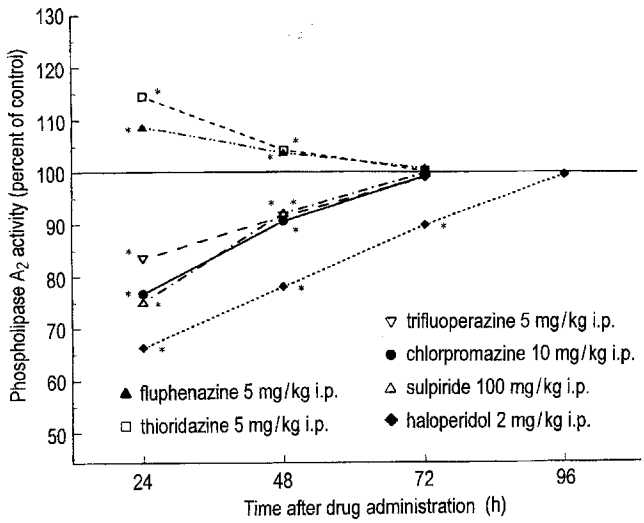

Fig. 1 Phospholipase A_2 activity in brain plasma membranes of rats treated with a single dose of neuroleptics. Results are expressed as the percent of control values and represent the mean of 8 rats ($P < 0.001$)

The changes in PLA_2 activity in brain plasma membranes of rats after long-term treatment with neuroleptics are shown in Fig. 2. A marked increase (27–30%) in PLA_2 activity of brain plasma membranes was measured after

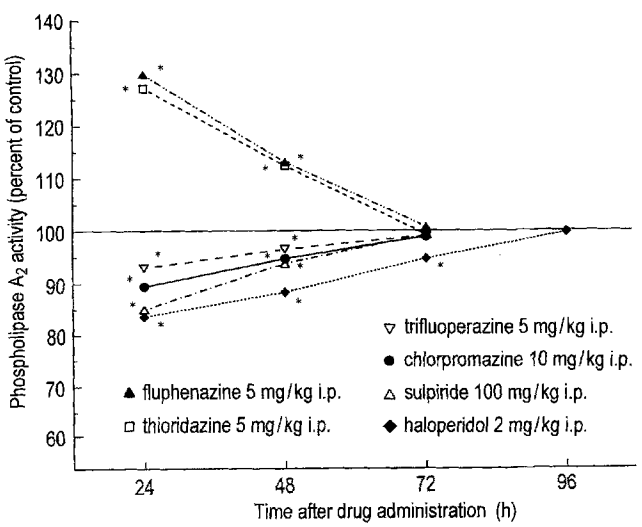

Fig. 2 Phospholipase A_2 activity in brain plasma membranes of rats after long-term treatment (4 weeks) of rats with neuroleptics. Results are expressed as the percent of control values and represent the mean of 8 rats; $P < 0.001$

24 and 48 h of fluphenazine and thioridazine administration. The chronic treatment with haloperidol, sulpiride, chlorpromazine, and trifluoperazine diminished PLA_2 activity from 16.3 to 7% after 24 h of the last injection. Haloperidol diminished the enzyme activity (5.4%) up to 72 h after long-term drug treatment in comparison with control. Control mean values for PLA_2 activity in brain plasma membranes of rats in short- and long-term experiments were 14394 ± 117 and 14935 ± 127 dpm/mg protein/15 min, respectively.

Discussion

Gattaz et al. (1987, 1990, 1994) found a marked difference between PLA_2 activity with significantly higher levels in plasma, serum, and platelets of drug-free schizophrenics than healthy controls or nonschizophrenic psychiatric patients. Also, higher levels of lysophosphatidylcholine, a toxic product of phosphatidylcholine metabolism by PLA_2 were observed in platelets of schizophrenics in Gattaz's laboratory (Pangerl et al. 1991). Noponen et al. (1993) also found increased PLA_2 activity in the serum of schizophrenic patients, whereas Albers et al. (1993) did not confirm these results in a small sample of patients.

Several studies have indicated that various typical neuroleptics inhibit PLA_2 activity in vitro and in vivo (Aarsman et al. 1985; Kunze et al. 1974; Schroder et al. 1981; Taniguchi et al. 1988; Vanderhoek and Feinstein 1979). Also, Gattaz et al. (1987, 1990, 1995) observed decreased PLA_2 activity in the serum and platelets of schizophrenic patients treated with haloperidol.

In the present study both atypical (sulpiride) and typical neuroleptics (chlorpromazine, thioridazine, trifluoperazine, fluphenazine, and haloperidol) were administered to rats either as a single dose or long-term treatment. The doses corresponded to those generally used in psychopharmacological studies. In order to prevent any immediate effect of the drugs on PLA_2 activity in cerebral plasma membranes of rats, the studies were carried out every 24 h during 3 days. Post-haloperidol PLA_2 activity was determined every 24 h during 4 days after the last administration of the drug considering the fact that its biological half-life is longer when compared with other neuroleptics. Most neuroleptics were found to have decreased PLA_2 activity in the brain of rats both after a single dose and long-term administration. The findings have been observed by other authors (Gattaz et al. 1987, 1990, 1995) and suggest some interdependence between PLA_2 activity and dopaminergic neurotransmission. Cadet et al. (1989) reported a reduction of the striatal concentrations of dopamine (DA) and its metabolites, DOPAC and HVA, at the site of PLA_2 injection. An inhibitiory effect of PLA_2 on dopaminergic neurotransmission has also been described by Anand-Srivastava and Johnson (1981), who observed the inhibition of activated DA-sensitive adenylate cyclase in striatal tissue by PLA_2. Oliveira et al. (1984) found that PLA_2 diminished /^{3}H/-spiperone binding to DA receptors. The changes in DA-receptor function can result not only from hydrolysis of membrane phospholipids, but also from alteration of lipid environment by lysophospholipids, the toxic end products of phospholipid hydrolysis. Sun (1985) indicated that the formation of lysophospholipids might alter the microenvironment and the physicochemical properties of synaptosomal membranes, and thus directly change the release of neurotransmitters by exocytosis.

Long-term administration of chlorpromazine, trifluoperazine, haloperidol, and sulpiride also inhibited PLA_2 activity, but to an evidently lesser degree than single doses of the drugs. The reason for this phenomenon seems to be drug tolerance acquired in the course of long-term administration of the neuroleptics.

For the first time, however, it was shown that some neuroleptics (e.g., fluphenazine, thioridazine) increased PLA_2 activity in the brain of rats. The effect is more pronounced after long-term administration of the drugs when compared with the enzyme activity after single doses.

Considering the previously mentioned results, it is controversial to account for the decreased PLA_2 activity by interaction of neuroleptics with cell membranes that protect them against the influence of PLA_2 probably through interference with the substrate-enzyme interface or by some alteration in the physical structure of the lipid bilayer in the way that promotes the desorption of the bound enzyme (Chang et al. 1987; Jain et al. 1989). The mechanism of receptor-mediated PLA_2 regulation is not well understood. It has, however, been proposed that calcium ions, lipocortins, protein kinase C, neuropeptides, and G-proteins may be involved in the regulation of enzyme activity (Farooqui et al. 1992). On the other hand, the difference in metabolic sulfoxidation pathways of chlorpromazine and thioridazine in the liver (Traficante et al. 1979) may convert activation as well as deactivation of PLA_2. This hypothesis is, however, highly speculative and would have to be proved.

In conclusion, we showed for the first time that neuroleptics might not only inhibit, but also increase, PLA_2 activity in plasma membranes of rat brain. It is, however, noteworthy that in our study the effects of neuroleptics on the enzyme activity were completely reversible 3–4 days after neuroleptic withdrawal. Therefore, it is unlikely that the reported increases in PLA_2 activity in schizophrenia (Gattaz et al. 1987, 1990, 1995; Noponen et al. 1993) are an artifact of previous neuroleptic treatment, because in these studies all patients were at least 1 week drug-free. Moreover, and most important, in the studies by Gattaz and collaborators an increased PLA_2 activity was also observed in *drug-naive* schizophrenics, indicating that a disordered phospholipid metabolism in schizophrenia is not a result of previous neuroleptic treatment. Further experiments are warranted to clarify the possible role of PLA_2 in the pathophysiology of schizophrenia and the relationship between the effects of neuroleptics on enzyme activity and on psychotic symptoms.

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