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ORIGINAL PAPER

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Intracerebral injection of phospholipase A_2 inhibits dopamine-mediated behavior in rats: possible implications for schizophrenia

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Abstract Phospholipase A_2 (PLA_2) is a key enzyme in the phospholipid metabolism. In the CNS intracellular PLA_2 plays an essential role in signal transduction by affecting both dopamine (DA) release and DA-receptor sensitivity. In schizophrenia a disordered phospholipid metabolism and increased activity of PLA_2 have been reported. In this study we investigated the effects of intracerebral PLA_2 injections on dopaminergic neurotransmission in rats using Ungerstedt's model of rotational behavior. Circling behavior induced by the DA agonist apomorphine after unilateral PLA_2 injections into the substantia nigra pars compacta was recorded. Seven and 21 days after intranigral PLA_2 injection, apomorphine induced an ipsilateral rotation indicating a long-lasting inhibition of ipsilateral nigrostriatal dopaminergic pathway by PLA_2 application. In schizophrenia a reduced dopaminergic activity in the frontal cortex has been hypothesized. Recent spectroscopy studies reported on an accelerated breakdown of membrane phospholipids in the frontal cortex from schizophrenics. The present findings suggest that increased PLA_2 activity in schizophrenia could accelerate the breakdown of membrane phospholipids and thus contribute to a hypodopaminergy in the frontal cortex of schizophrenic patients.

Key words Phospholipase A_2 · Schizophrenia · Phospholipids · Rotational behavior · Hypofrontality

Introduction

The intracellular enzyme phospholipase A_2 (PLA_2) catalyzes the hydrolysis of membrane phospholipids to release free fatty acids and cytotoxic products such as lysophosphatidylcholine. In neuronal membranes phospholipase A_2 plays an essential role in signal transduction by influencing the physicochemical properties of synaptic membranes (Farooqui et al. 1992).

In schizophrenic patients PLA_2 activity has been reported to be increased in plasma (Gattaz et al. 1987), serum (Gattaz et al. 1990; Noponen et al. 1993), and platelet membranes (Gattaz et al. 1995). In platelets of schizophrenics phosphatidylcholine concentration (substrate of PLA_2) was found to be decreased (Rotrosen and Wolkin 1987) and lysophosphatidylcholine concentration (breakdown product of PLA_2 hydrolysis) elevated (Pangerl et al. 1991). Taken together, these data suggest an accelerated breakdown of membrane phospholipids in schizophrenia.

A disordered phospholipid metabolism has also been reported in the brain of schizophrenics by means of in vivo ^{31}P-NMR spectroscopy studies. Phosphomonoesters (PME) are the precursors and phosphodiesters (PDE) are the breakdown products of membrane phospholipids in the brain. Phosphomonoesters were found to be decreased, and PDE to be elevated, in the frontal lobe of schizophrenic patients (Williamson et al. 1991; Deicken et al. 1993; Pettegrew et al. 1993) suggesting that an accelerated phospholipid breakdown may be found in some schizophrenic brains.

Because increased PLA_2 activity accelerates the phospholipid metabolism, we investigated in rats the effects of intracerebral injections of the enzyme on dopamine (DA)-mediated behavior. In vitro studies showed that increased PLA_2 activity influenced DA-receptor sensitivity (Anand-Srivastava and Johnson 1981; Oliveira et al. 1984) and DA synthesis and release (Bradford et al. 1983; Ohmichi et al. 1989). To our knowledge, there is only one in vivo investigation of the effects of intracerebral PLA_2 injections on DA-mediated behavior (Cadet et al. 1989). These authors reported apomorphine-induced ipsilateral circling behavior in rats pretreated with intranigral injections of PLA_2. However, these results need further experimental confirmation, because no control group was investigated by Cadet and collaborators. Additionally to the experiments of Cadet et al. (1989) we investigated in the present study the effects of PLA_2 at different doses, and we introduced placebo groups to control for the non-specific ef-

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fects of intranigral saline injections on behavior in rats. Cadet et al. (1989) started rotometer testing 10 days after intranigral injection of PLA_2. In our experiments testing started on the first postoperative day to assess possible acute effects. We adopted the rotational model presented by Ungerstedt and Arbuthnott (1970) because it is an easily quantifiable and well-validated approach to monitoring dopaminergic activity in vivo.

Methods

Stereotaxic surgery

Adult female Sprague Dawley rats (Charles River/Wiga, Sulzfeld) weighing 223 ± 10 g (mean ± SD) were anesthetized with 10 mg ketamine (Ketavet) s.c. per animal and 30 mg/kg pentobarbital (Nembutal) i.p. and mounted in a David Kopf stereotaxic frame with the incisor bar 5 mm above horizontal zero. The substances were injected unilaterally into the substantia nigra pars compacta (SNC) in a volume of 4 µl at a constant rate of 1 µl/min using a Hamilton microliter syringe (22 gauge). After completion of the injections, cannulas were left in place for an additional 3 min. The stereotaxic coordinates of the SNC (AP: 2.2 mm, L: 1.6 mm, DV: 2.5 mm from interaural line) were taken from the atlas of De Groot (1959) To validate stereotaxic precision nine rats received a unilateral intranigral injection of 8 µg 6-hydroxydopamine (6-OHDA, Sigma) in 4 µl of saline with 0.2 µg/µl ascorbic acid added. These animals were pretreated with 10 mg/kg desipramine (Thomae) p.o. 60 min prior to surgery to protect noradrenergic nigral neurons. With these stereotaxic coordinates the results of Ungerstedt and Arbuthnott (1970) could be replicated. Methamphetamine (5 mg/kg s.c.) induced an ipsilateral asymmetry ($P < 0.01$) with (mean ± SEM) 871 ± 69 ipsilateral and 4 ± 1 contralateral turns/h.

Experiment 1

PLA_2 isolated and purified from bovine pancreas (Sigma) with an enzymatic activity of 6.8 U/mg was unilaterally injected into the SNC in three different doses: 1 µg PLA_2 ($n = 6$), 3 µg PLA_2 ($n = 6$), and 5 µg PLA_2 ($n = 6$). 1 U hydrolyzes 1µmol L-α–phosphatidylcholine to L-α–lysophosphatidylcholine and a free fatty acid per min at pH 8.0 and 37° C. The control group ($n = 6$) received unilateral intranigral vehicle injections (4 µl of saline). Spontaneous rotational behavior was recorded for 30 min on days 1, 3, and 7 postoperatively. Seven days after stereotaxic surgery rotational behavior induced by 0.5 mg/kg apomorphine (APO) s.c. was recorded for 60 min.

Experiment 2

PLA_2 from bovine pancreas (Sigma) with a higher enzymatic activity (12 U/mg) was unilaterally injected into the SNC in two different doses: 20 µg PLA_2 ($n = 11$) and 100 µg PLA_2 ($n = 12$) dissolved in 4 µl of saline. The control group ($n = 11$) received a unilateral intranigral injection of the vehicle (4 µl of saline). Fourteen days after surgery spontaneous rotational behavior was recorded for 30 min. On day 21 postoperatively rotational behavior was monitored for 60 min after stimulation with 0.5 mg/kg APO (Sigma) s.c.

Rotational assessments

Rotational behavior was recorded automatically with a rotometer. Only complete turns (360°) in each direction were counted.

Statistics

Data were analyzed by nonparametric tests. The Mann-Whitney rank sum test was used for comparisons between different treatment groups. Rotational asymmetries (number of ipsilateral vs contralateral turns) within the same group were analyzed using Wilcoxon matched pairs signed rank test.

Results

Experiment 1

On the first postoperative day a spontaneous contralateral asymmetry was observed in all groups with low-dose intranigral PLA_2 application as well as saline-treated animals (range contralateral 34–43; ipsilateral 1–2 turns/30 min; $P < 0.001$ for all groups). The PLA_2 groups did not differ quantitatively from the saline group. The contralateral spontaneous asymmetry diminished in all treatment groups within the first postoperative week. Seven days after surgery 0.5 mg/kg APO induced an ipsilateral asymmetry only in PLA_2-treated rats. No APO-induced asymmetry was observed in saline-treated animals. Because no significant differences were found among the three PLA_2-dose groups (1 µg ipsilateral 47 ± 20, contralateral 5 ± 3; 3 µg ipsilateral 30 ± 17, contralateral 7 ± 2; 5 µg ipsilateral 48 ± 24, contralateral 22 ± 8; means ± SEM), data were pooled as presented in Fig. 1.

Experiment 2

There was no spontaneous rotational asymmetry 14 days after unilateral intranigral injection of 20 µg and 100 µg PLA_2. The administration of 0.5 mg/kg APO 21 days postoperatively caused a significant ipsilateral asymmetry in

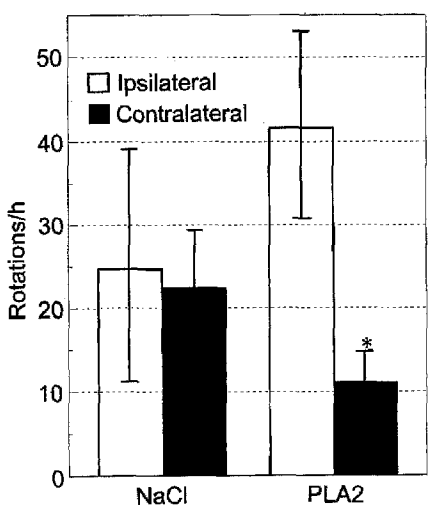

Fig. 1 Rotational behavior induced by 0.5 mg/kg apomorphine 7 days after intranigral injection of PLA_2 (pooled data 1, 3, and 5 µg) and placebo (NaCl) (means ± SEM). $P < 0.05$ compared with ipsilateral rotations

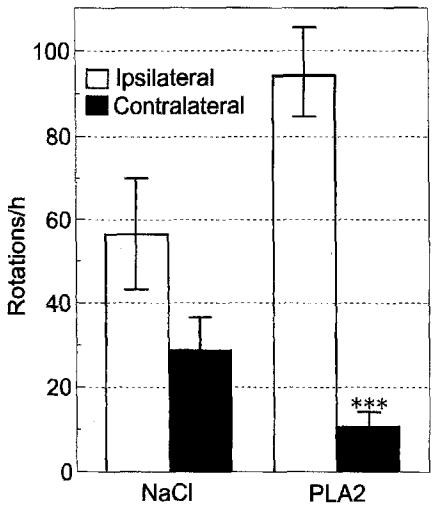

Fig. 2 Rotational behavior induced by 0.5 mg/kg apomorphine 21 days after intranigral injection of PLA_2 (pooled data 20 and 100 μg) and placebo (NaCl) (means ± SEM). $P < 0.001$ compared with ipsilateral rotations

PLA_2 pretreated rats, but not in the saline group (Fig. 2). The APO-induced ipsilateral rotation counts in this experiment did not differ between animals pretreated with 20 μg (ipsilateral 92 ± 15, contralateral 15 ± 5) and with 100 μg PLA_2 (ipsilateral 96 ± 17, contralateral 7 ± 2), but were significantly higher than the ipsilateral counts in the low-PLA_2-dose groups from experiment 1 ($P < 0.005$).

Discussion

In the rotational model used in the present study the direction of circling was ipsilateral to the hemisphere with the lower striatal dopaminergic activity (Zetterström et al. 1986; Carman et al. 1991). One day after intranigral PLA_2 *and* saline injections there was a spontaneous contralateral rotational asymmetry that diminished within the first postoperative week. It can be assumed that this acute and reversible effect resulted from a nonspecific dopaminergic stimulation caused by the fluid injections themselves.

Seven days postoperatively APO induced an ipsilateral asymmetry in rats treated with PLA_2. This effect was specific for PLA_2, because no asymmetry was induced by APO in the saline-treated animals. This asymmetry could still be observed 21 days after intranigral injection of higher doses of PLA_2. The comparison between low- and high-PLA_2-dose groups showed that the ipsilateral asymmetry increased with PLA_2 dose. However, it should be pointed out that the high- and low-dose groups were tested at different times after surgery, so that a comparison has to be seen with caution. Our findings are in line with the results of Cadet et al. (1989) who observed an APO-induced ipsilateral circling behavior 10, 17, and 24 days after intranigral injection of PLA_2. The results from these animal experiments indicate that intracerebral injection of PLA_2 reduced dopaminergic activity. This assumption is supported by the findings of Cadet et al. (1989) who reported a reduction of the striatal concentrations of DA and its metabolites DOPAC and HVA on the side of the PLA_2 injection. An inhibitory effect of PLA_2 on dopaminergic neurotransmission has also been observed by in vitro experiments in which PLA_2 inhibited the activation of DA-sensitive adenylate cyclase in striatal tissue (Anand-Srivastava and Johnson 1981) and reduced the [^{3}H]spiperone binding to DA receptors (Oliveira et al. 1984).

In our experiments it is unlikely that intranigral PLA_2 injections caused extensive lesions of dopaminergic SNC neurons. A selective dopaminergic SNC lesion induced by 6-OHDA causes *contralateral* rotation after APO (Carman et al. 1991), whereas in our PLA-treated animals the rotation induced by APO was ipsilateral. It should, however, be stressed that the effects of PLA_2 are probably not specific for DA neurons. In light of its profound effects on the membrane phospholipid bilayer, it is likely that PLA_2 will influence the function of virtually all membrane-bound receptors.

Taken together, our results and data from the literature suggest that PLA_2 inhibits DA activity in the brain. How could this effect be involved in the pathophysiology of schizophrenia? A reduced dopaminergic activity in the prefrontal cortex (hypofrontality) has been postulated in schizophrenia (Weinberger 1987). Recent ^{31}P-NMR-spectroscopy studies showed an accelerated breakdown of membrane phospholipids in the prefrontal cortex of drug-naive schizophrenics, which correlated with a deficit of frontal neuropsychological function (Williamson et al. 1991; Deicken et al. 1993; Pettegrew et al. 1993, Deicken et al. 1995). Further studies should clarify whether this acceleration is caused by increased PLA_2 activity in the brain of schizophrenics, as observed in their peripheric cells (Gattaz et al. 1994). In light of the present findings, it is conceivable that increased PLA_2 activity may be related to the postulated hypodopaminergic activity in the prefrontal system in schizophrenia. This conclusion is highly speculative and can only be understood as a preliminary working hypothesis for the development of new studies.

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