Acidic phospholipids inhibit the phospholipase D activity of rat brain neuronal nuclei

J.N. Kanfer[a,*], D.G. McCartney[a], I.N. Singh[a], L. Freysz[b]

[a]*Department of Biochemistry and Molecular Biology, University of Manitoba, Winnipeg, Manitoba R3E 0W3, Canada*
[b]*Centre de Neurochimie, Strasbourg 67084, France*

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Abstract An oleate dependent form of phospholipase D is present in rat brain neuronal nuclei and both the hydrolytic and transphosphatidylation activities measured. Several acidic phospholipids were found to inhibit this activity in a dose dependent manner. The IC_{50} values varied from 3.5 μM for PIP_2 to 200 μM for phosphatidic acid. The hydrolysis of PIP_2 by phospholipase C would be expected to result in the disinhibition of the oleate dependent phospholipase D activity.

Key words: Phospholipase D; Neuronal nuclei; Phosphatidic acid; Phosphatidylinositol-4,5-diphosphate; Acidic phospholipids

1. Introduction

There are two distinct forms of PLDs in mammalian tissues which hydrolyze PtdCho or phosphatidylethanolamine. The original description of the existence of a mammalian PLD focused upon the isoform that is an integral membrane protein [1,2] and required the presence of an unsaturated fatty acid such as sodium oleate for in vitro measurements [3]. Another isoform of PLD requires a small molecular weight ARF like GTP binding protein and PIP_2 for in vitro measurements [4–9]. Therefore, it seemed of interest to determine if PIP_2 and other acidic lipids could influence this neuronal PLD activity. We have characterized the properties of rat neuronal nuclear PLD [10]. This form requires the presence of sodium oleate for its measurement and is inhibited by both GTPγS or ATPγS. Therefore, it seemed of interest to determine if PIP_2 and other acidic lipids could influence this neuronal PLD activity. We have found that this neuronal nuclear PLD activity is inhibited by acidic phospholipids.

2. Materials and methods

Neuronal nuclei were isolated from rat brains according to a published procedure [11]. Protein content was quantitated by a classical method [12]. The incubations for the PLD activity measurements routinely contained 50 mM dimethylglutarate pH 6.5, a substrate cosonicate of 0.75 mM [^{14}C]PtdCho and 1.2 mM sodium oleate, 10 mM EDTA, 25 mM NaF and 20 μg nuclear protein. In the absence of ethanol the quantity of [^{14}C]PtdA produced is a measure of the hydrolytic activity of PLD. In the presence of 0.3 M ethanol the sum of [^{14}C]PtdA plus [^{14}C]PtdEtOH produced is a measure of the transphosphatidylation activity of PLD [13,14]. The various acidic phospholipids tested were prepared as a co-sonicate with the [^{14}C]PtdCho and sodium oleate. The reaction was terminated after 1 h at 37°C and processed as previously described [15]. All incubations were in duplicate and each experiment was performed on at least 3 separate occasions. The hydrolytic activity of PLD is calculated as nmoles of PtdA formed in the absence of ethanol and transphosphatidylation activity expressed as nmol of PtdA + PtdEtOH formed in the presence of ethanol/mg protein/h ± S.D.

1,2-[^{14}C]Dioleoyl PtdCho (spec. act. = 114 mCi/mmol) was purchased from Amersham Life Sciences, Oakville, Ontario, Canada, and mixed with authentic carrier to provide a final specific activity of 2800–3000 dpm/nmol. PIP_2 was obtained from Fluka Chemika-BioChemika, Ronkonkoma, NY, USA. PtdSer, Ptdglycerol, cardiolipin and PtdA were obtained from Serdary Research Laboratories, London, Ontario, Canada and phosphatidylethanol was obtained from Avanti Polar Lipids Inc., Alabaster, AL, USA. Silica gel 60 TLC plates were obtained from Merck, Darmstadt, Germany. Retinoic acid, cholesterol sulfate, dehydroisoandrosterone-3-sulfate, bis(2-ethylhexyl)hydrogen phosphate, 5-prenen-3β-ol-20-one-sulfate, taurocholate and cholate were from Sigma-Aldrich Canada, Mississauga, Ontario, Canada. All other routine reagents were from the usual commercial suppliers.

*Corresponding author. Fax: (1) (204) 789 3500.

Abbreviations: PLD, phospholipase D; PtdCho, phosphatidylcholine; PtdSer, phosphatidylserine; Ptdglycerol, phosphatidylglycerol; PtdEtOH, phosphatidylethanol; PtdA, phosphatidic acid; PIP_2, phosphatidylinositol-4,5-diphosphate; PtdIns, phosphatidylinositol; PLC, phospholipase C; ARF, ADP ribosylation factor.

3. Results

3.1. Inhibition by PtdA

PtdA is one of the products of PLD hydrolysis of PtdCho and, therefore, a potential inhibitor of PLD activity due to product inhibition. A dose dependent inhibition by PtdA of both the hydrolytic activity, measured as PtdA formation and transphosphatidylation activity, measured as PtdA + PtdEtOH formation, was observed (Fig. 1).

3.2. Inhibition by PtdSer, Ptdglycerol and cardiolipin

In order to learn if this inhibition by PtdA was a reflection of product inhibition and to establish the specificity, selected acidic phospholipids were tested. A dose dependent inhibition by PtdSer, Ptdglycerol and cardiolipin on the hydrolytic and the transphosphatidylation activity of the neuronal nuclear PLD is shown in Fig. 2.

3.3. Inhibition by PIP_2

The presence of PIP_2 is necessary for the in vitro assay of the small molecular weight GTP binding protein dependent PLD activity [4–9]. It was, therefore, surprising to find that there was a dose dependent inhibition of the oleate dependent PLD activity of neuronal nuclei as shown in Fig. 3.

3.4. IC_{50}s

The IC_{50} values of PtdA, PtdSer, Ptdglycerol, cardiolipin and PIP_2 for the inhibition of the neuronal nuclear PLD activity is presented in Table 1. It is apparent that there are considerable differences in the inhibitory effectiveness of these acidic phospholipids. PIP_2 is the most inhibitory and PtdA is the least inhibitory.

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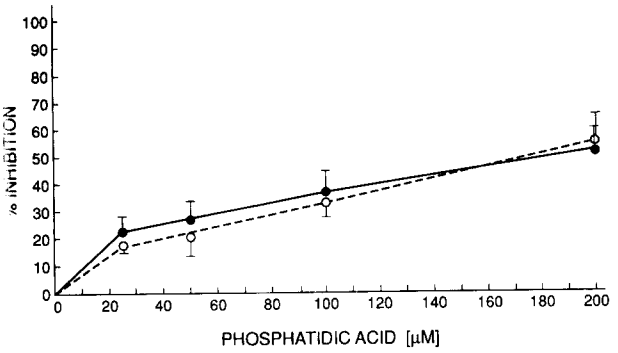

Fig. 1. Inhibition of rat brain neuronal nuclear PLD activity by increasing concentrations of PtdA. The experimental details are described in the text. The closed circles represent PtdA formation and the open circles represents the sum of PtdA + PtdEtOH formation.

3.5. *Other anionic amphiphiles*

There was no inhibition of PLD activity by several anionic amphiphiles including retinoic acid, cholesterol sulfate, dehydroisoandrosterone-3-sulfate, bis(2-ethylhexyl) hydrogen phosphate, 5-prenen-3β-ol-20-one-sulfate, taurocholate or cholate.

4. Discussion

The presence of several acidic phospholipids in incubation mixtures containing 0.75 mM PtdCho and 1.2 mM sodium oleate inhibited the activity of rat brain neuronal nuclear PLD. The inhibition by PtdA was expected based upon the general principle of product inhibition. The basis for the inhibition by the other acidic phospholipids is not readily understood. It cannot be due to the negative charge per se since these phospholipids were added as their sodium salts and there is routinely present many fold greater amounts of sodium oleate in the incubation mixtures. The inhibitory activity of acidic phospholipids upon enzymatic reactions has been observed. A 34 000 fold purified bovine testicular diacylglycerol kinase was inhibited by PtdSer [16]. The activity of a 3500 fold purified human lymphoblastic *N*-acetylglucosamine-1-phosphotransferase was inhibited by PtdSer, Ptdglycerol and PtdA [17]. The PtdIns specific PLC activity of pancreatic islet cells was inhibited by PtdSer [18]. A soluble PtdA phosphohydrolase from rat liver was inhibited by Ptdglycerol, PtdSer and PtdIns [19]. A partially purified CDP-diacylglycerol synthetase from bovine brain was inhibited by cardiolipin [20]. Rat brain hexokinase activity was inhibited by PtdIns, PtdSer and cardiolipin [21]. Therefore, a diversity of enzymes are inhibited by acidic phospholipids with little specificity.

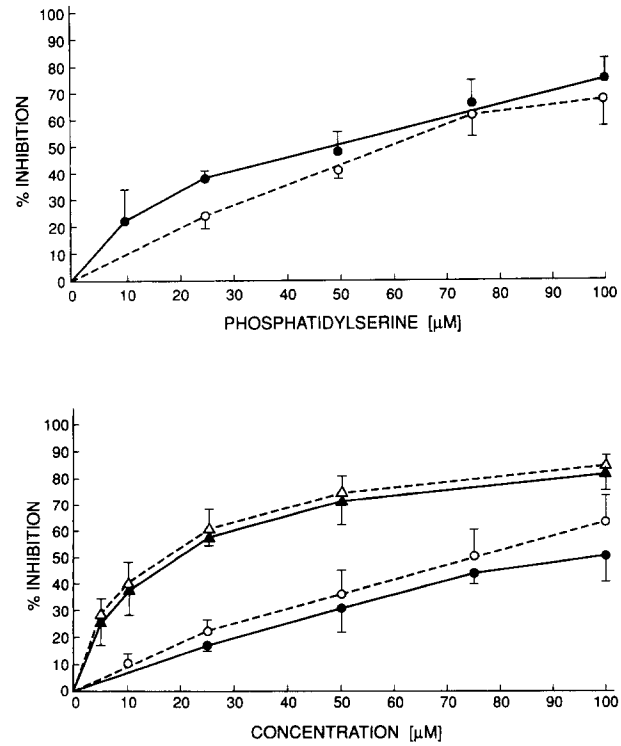

Fig. 2. Inhibition of rat brain neuronal nuclear PLD activity by increasing concentrations of PtdSer (upper panel), (lower panel) Ptdglycerol (circles) and cardiolipin (triangles). The experimental details are described in the text. The closed symbols represent PtdA formation and the open symbols represents the sum of PtdA + PtdEtOH formation.

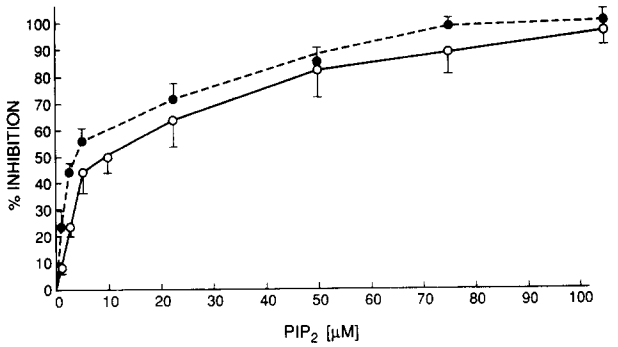

Fig. 3. Inhibition of rat brain neuronal nuclear PLD activity by increasing concentrations of PIP_2. The experimental details are described in the text. The open circles represent PtdA formation, and the closed circles represent the sum of PtdAS + PtdEtOH formation.

The IC_{50} values for the 5 acidic phospholipids employed in these studies ranged from 3.5 μM to 200 μM. The most potent was PIP_2 at 0.4 to 0.8 mole% ratio compared to PtdCho present. This is a range of PIP_2 present in mammalian tissues and reported to stimulate the ARF dependent PLD [22]. It, therefore, is reasonable to speculate that any stimulation of the hydrolysis of PIP_2 by PIP_2-PLC would result in reduced ARF dependent PLD activity and in increased oleate dependent PLD activity. It has been suggested that PIP_2 acts as a cofactor for ARF interactions with proteins [23,24]. Hydrolysis would reduce the quantity of PIP_2 available as cofactor for these ARF interactions. The reduction of PIP_2 content would be expected to disinhibit the oleate dependent PLD. This reciprocal relationship would provide a control mechanism for allowing either the small molecular weight G protein dependent PLD or the oleate dependent PLD linked processes to proceed. Agonist stimulated PLD activity subsequent to PIP_2-PLC activation has been reported [25–27]. The hydrolysis of PIP_2 by PLC produces a diglyceride directly, the hydrolysis of PtdCho by PLD produces a PtdA which is hydrolysed by a PtdA phosphatase to diglyceride. The receptor mediated biphasic diglyceride formation with differences in their time of appearance, duration and molecular species have been observed. These investigations have identified PIP_2 as the source of the rapidly appearing short duration diglyceride. PtdCho is

Table 1
The IC_{50} values for inhibition of rat brain neuronal nuclear PLD by PtdSer, PtdA, Ptdglycerol, cardiolipin and PIP_2

	IC_{50} of acidic phospholipids (μM)	
	PtdA (−EtOH)	PtdA + PtdEtOH (+ EtOH)[a]
PtdSer	49	60
PtdA	200	180
Ptdglycerol	97	75
Cardiolipin	20	17
PIP_2	6	3.5

The details are provided in the text.
[a]0.3 M EtOH was present in these incubation mixtures.

the source of the later appearing, sustained duration diglyceride [28].

This phenomenon supports the speculation that the hydrolysis of PIP_2 by PLC releases the PIP_2 inhibition of the oleate dependent PLD.

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