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Short communication

The expression and cellular localization of phospholipase D1 in the rodent retina

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Abstract

Phospholipase D (PLD) is one of the intracellular signal transduction enzymes and plays an important role in a variety of cellular functions. We investigated the expression and cellular localization of the PLD isozyme PLD1 in the rodent retina. Western blot analysis showed the presence of PLD1 at the protein level in the rat, mouse and guinea pig retinas. PLD1 immunoreactivity was localized in all Müller cells. Thus, PLD1 protein appears to be important in the functions of these cells in the rodent retina. © 2001 Published by Elsevier Science B.V.

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Phospholipase D (PLD), which catalyzes the hydrolysis of phosphatidylcholine to phosphatidic acid and choline, has been suggested to play an important role in the receptor-mediated signal pathway to cell proliferation [2,3,15], apoptosis [9,16,23], cell differentiation [13], and membrane trafficking and secretory events [6,12,21], possibly including neurotransmitter release [5]. Recently, two mammalian isoforms of PLD, PLD1 and PLD2, have been characterized at the molecular level. Although the functional role of PLD in cells is still poorly understood, it has been shown that PLD1 is expressed in certain neuron and glial cells in some parts of the rat brain [11]. However, no information concerning cell-specific expression of PLD is available in the mammalian retina. Here, we have carried out the first investigation of the expression and the cellular localization of PLD1 in the rat, mouse and guinea pig retinas.

Adult rats, mice, and guinea pigs were used. The animals were maintained on a daily cycle, 12 h in dim light and 12 h in darkness. They were deeply anesthetized by intraperitoneal injection of 4% chloral hydrate (1 ml/100 g body weight). The eyeballs were enucleated and the animals killed by an overdose of 4% chloral hydrate. The anterior segments of the eyeballs were removed, and the retinas were carefully dissected. For Western blot analysis, retinal tissues were quickly dissected on an ice-cold plate, frozen on dry ice, and stored at −70°C. Western blot analysis was performed on extracts of retinal tissue which was homogenized in ice-cold extraction buffer (20 mM HEPES, pH 7.2, 1% Triton X-100, 1% deoxycholate, 0.1% SDS, 150 mM NaCl, 10 μg/ml leupeptin, 10 μg/ml aprotinin, 1 mM phenylmethylsulfonyl fluoride). Aliquots of tissue samples corresponding to 50 μg of total protein were run on 10% SDS–PAGE gel and blotted onto a nitrocellulose membrane. Immunostaining of blotted proteins was carried out using an anti-PLD1 antiserum raised in rabbit using the C-terminal peptide of PLD1 corre-

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sponding to amino acid residues 1063–1074 of the sequence of rat brain PLD1: TKEAIVPMEVWT [10]. Immunoreactive bands were visualized by chemiluminescence using horseradish peroxidase-conjugated anti-rabbit IgG and enhanced chemiluminescence (ECL; Amersham, Arlinton Heights, IL, USA). Specificity of the immune reaction was verified by immunoprecipitation and immunoblot analysis using anti-PLD1 antibody (2.5 μg) that had been preabsorbed overnight at 40°C with its specific immunopeptide (25 μg). For PLD1 immunoreactivity, the retinas were fixed by immersion in 4% paraformaldehyde–0.2% picric acid in 0.1 M phosphate buffer, pH 7.4, for 2–3 h. Subsequently, they were transferred to 30% sucrose in phosphate buffer for 24–48 h. They were then frozen in liquid nitrogen, thawed, and rinsed in 0.01 M phosphate buffered saline (PBS), pH 7.4. Immunostaining was performed by fluorescence histochemistry. Fifty-micrometer thick vibratome sections were incubated in 10% normal goat serum (NGS) and 1% Triton X-100 in PBS for 1 h at room temperature to block non-specific binding sites, followed by incubation in the affinity purified anti-PLD1 antiserum in PBS containing 0.5% Triton X-100 for 24 h at 4°C. After being washed in PBS for 45 min (3×15 min), the retinas were incubated for 12 h in rhodamine-conjugated goat anti-rabbit IgG (Jackson; diluted 1:100) at room temperature; the incubation medium was PBS containing 0.5% Triton X-100 and 1% NGS. The sections were then rinsed in two changes of PBS and cover-slipped.

To identify the presence of PLD1 in the rodent retina at the protein level, we performed immunoblot analysis. As shown in Fig. 1, anti-PLD1 antibody specifically recognized approximately 120 kDa of the PLD1 protein in the extracts of all species examined in this study, suggesting the expression of PLD1 in the rodent retina at the protein level. In addition, the blots were incubated with anti-PLD1 antibody that had been preabsorbed with antigen (Fig. 1). Anti-PLD1 antibody recognition of the protein was specifically blocked, indicating the specificity of the antibody.

To identify the cellular localization of PLD1, we performed immunocytochemistry. PLD1 immunoreactivity was observed in somata located in the middle of the inner nuclear layer (INL) and in the radial fibers running through the whole retinal layer (Fig. 2A, C and E). These PLD1 immunoreactive cells in the INL were further characterized

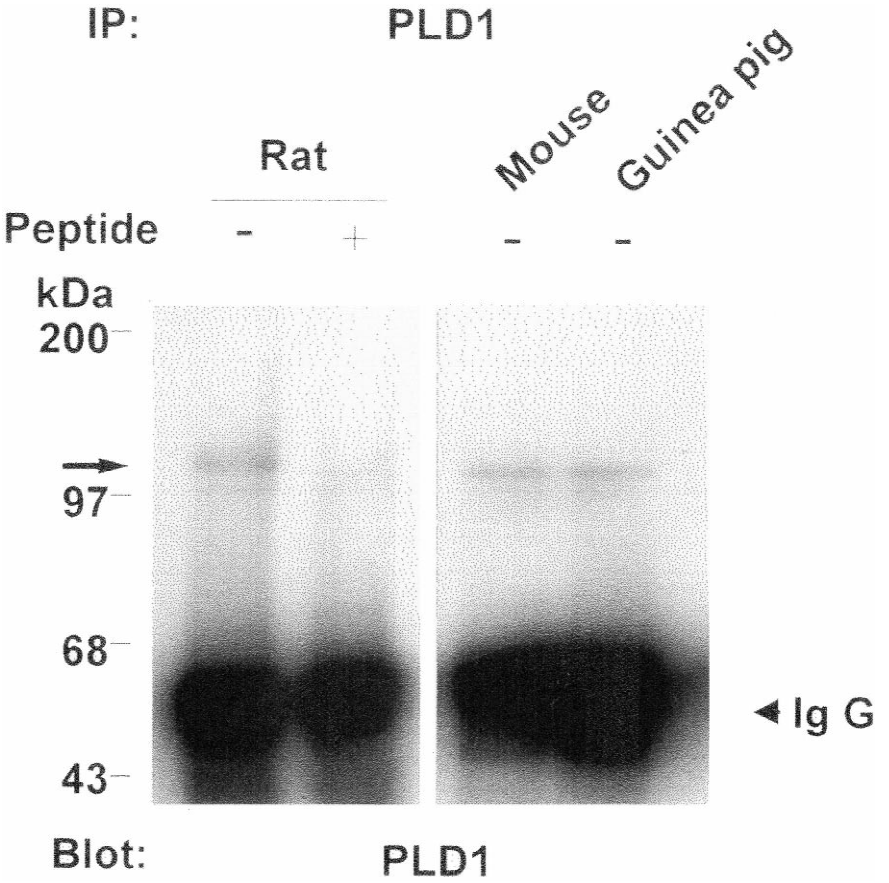

Fig. 1. Western blot analysis of PLD1 in the rat, mouse and guinea pig retinas. Position of PLD1 protein is indicated by arrow. Extracts of rat retina were prepared, and the lysates were immunoprecipitated using anti-PLD1 antibody preincubated in the absence (−) or presence (+) of its specific immunopeptide (25 μg).

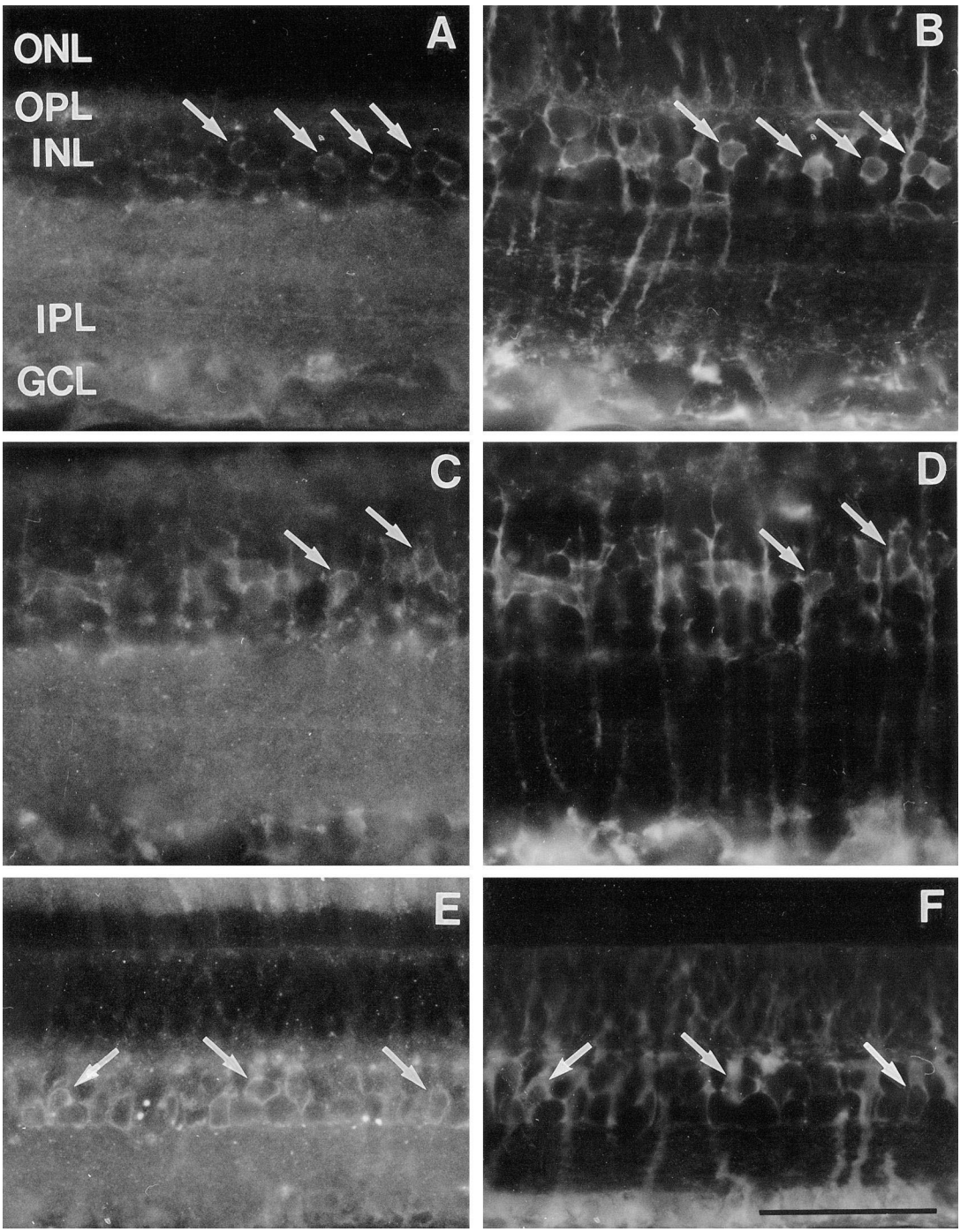

Fig. 2. Photomicrographs of sections through the rat (A, B), mouse (C, D) and guinea pig (E, F) retinas, showing double labeling. Rhodamine-labeled, PLD1-immunoreactive cells (arrows in A, C, E) are also labeled with anti-glutamine synthase antisera (arrows in B, D, F). Calibration bar: 50 μm.

by double labeling with anti-PLD1 and anti-glutamine synthase (GS) antisera, a specific marker for Müller cells in the mammalian retina [14]. The sections were incubated with a mixture of a rabbit antiserum against PLD1 and a mouse monoclonal antibody against GS (Chemicon, Temencula, CA, USA) diluted 1:500 in PBS for 10–12 h at 4°C. After washing in PBS for 45 min (3×15 min), the sections were incubated with a mixture of FITC-conjugated goat anti-mouse IgG (Jackson; diluted 1:100) and rhodamine-conjugated goat anti-rabbit IgG (Jackson; diluted 1:100) in PBS containing 0.5% Triton X-100 and 1% NGS for 2 h at room temperature. The sections were then

rinsed in two changes of PBS and cover-slipped. Fig. 2 shows examples of a vertical section processed for immunoreactivity to PLD1 (A, C, E) or GS (B, D, F). Comparison of the two figures shown in A, C, E and B, D, F, respectively, reveals that PLD1 immunoreactive cells also show GS immunoreactivity. All cells in the present study showed GS immunoreactivity. In order to identify whether astrocytes are labeled, we immunostained retinal wholemounts with anti-PLD1 antiserum. No immunoreactivity was seen in the astrocytes located in the ganglion cell layer (data not shown). These results clearly indicate that PLD1 is expressed in the somata and radial processes of the Müller cells in the rodent retina.

This study characterizes the expression of PLD1 and its cellular localization in the rodent retina. PLD1 was clearly localized to somata and radial fibers of Müller cells. In the mammalian retina, there are at least two distinct types of neuroglial cells, namely Müller cells and astrocytes. Astrocytes are confined to the nerve fiber layer and ganglion cell layer forming the retinal–blood barrier, whereas the Müller cells are the major supportive glia for neurons in the retina [8,20]. It is generally accepted that the Müller cells largely clear the extracellular glutamate via a glutamate transporter, GLAST-1 [18,19], and synthesize glutamine from glutamate via glutamine synthase (GS) in the mammalian retina. Thus, PLD1 might be involved in the glutamate receptor or transporter mediated signal transduction pathway for the GS activation in the retina as in the case of hippocampal slices where it induces breakdown of membrane phospholipids [3]. This hypothesis is supported by the present results that PLD1 expression appeared in two radial processes each in the outer nuclear layer and the inner half of the inner retina, in which there is glutamatergic neurotransmission.

In addition, like protoplasmic astrocytes in other brain regions, Müller cells participate in reactive gliosis via up-regulation of glial fibrillary acidic protein or ciliary neurotrophic factor induced by a variety of injury stimuli, including ischemia [1,4,10]. Recent studies have demonstrated that PLD1 is expressed in astrocytes in certain regions of the brain [11], and activated in astrocytes by extracellular signals [22], and have raised the possibility of PLD being involved in astrocyte proliferation [7,17]. Taken together, the expression of PLD1 may suggest a specialized role for the function of Müller cells in the rodent retina. However, the actual function of PLD1 remains to be elucidated in further studies.

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