FEBS Letters 587 (2013) 950–956

FEBS Letters

journal homepage: www.FEBSLetters.org

Phosphatidic acid metabolism in rat liver cell nuclei

Virginia L. Gaveglio, Susana J. Pasquaré *, Norma M. Giusto

Instituto de Investigaciones Bioquímicas de Bahía Blanca, Universidad Nacional del Sur and Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), B8000FWB Bahía Blanca, Argentina

ARTICLE INFO

Article history:
Received 19 July 2012
Revised 22 November 2012
Accepted 2 January 2013
Available online 21 February 2013

Edited by Laszlo Nagy

Keywords:
Diacylglycerol
Liver
Monoacylglycerol
Nuclei
Phosphatidic acid

ABSTRACT

The aim of the present research was to analyze the pathways for phosphatidic acid metabolism in purified nuclei from liver. Lipid phosphate phosphatase, diacylglycerol lipase, monoacylglycerol lipase and PA-phospholipase type A activities were detected. The presence of lysophosphatidic acid significantly reduced DAG production while sphingosine 1-phoshate and ceramide 1-phosphate reduced MAG formation from PA. Using different enzymatic modulators (detergents and ions) an increase in the PA metabolism by phospholipase type A was observed. Our findings evidence an active PA metabolism in purified liver nuclei which generates important lipid second messengers, and which could thus be involved in nuclear processes such as gene transcription.

1. Introduction

Many evidences suggest that phospholipids as well as their polyunsaturated fatty acids are involved in several events inside the nucleus [1]. The nuclear envelope has been identified as the primary place of nuclear lipids although a few early studies have also suggested the presence of phospholipids as a component of chromatin [2]. In eukaryotic cells, PA is a key precursor for the synthesis of major glycerophospholipids and neutral lipids as well as a major signaling lipid [3–5]. PA can be dephosphorylated by the action of phosphatidate phosphatase 1 (PAP1) which is also known as lipin [6,7]. A second type of PA phosphatase that controls the signaling pools of PA and DAG is known as either phosphatidate phosphohydrolase 2 (PAP2) or lipid phosphate phosphatase (LPP) [4].

Several nuclear enzymes involved in phospholipid metabolism have been described and characterized, and there is evidence that some might have direct regulatory roles in various aspects of nuclear function [8]. Taking into account the multitude of functions of PA and DAG in biosynthetic and signaling pathways and because little is known on PA metabolism in hepatocyte nuclei, our study aims at studying the enzymatic mechanisms through which PA-removal operates in liver nuclei.

2. Materials and methods

2.1. Materials

[2-^{3}H]glycerol (200 mCi/mmol) and Omnifluor were obtained from New England Nuclear-Dupont (Boston, MA, USA). DAPI, sphingosine 1-phosphate, ceramide 1-phosphate from bovine brain, oleoyl-L-α-lysophosphatidic acid, D-sphingosine, and non-hydroxy fatty acid ceramide from bovine brain were obtained from Sigma–Aldrich (St. Louis, MO, USA). Antibodies anti Calnexin (sc-11397) and anti LAP2 (611000) were from Santa Cruz Biotechnology, INC. and BD Transduction Laboratories, respectively. All the other chemicals used were of the highest purity available.

2.2. Purified nuclear fraction preparation

Male Wistar-strain rats were kept under constant environmental conditions and fed on a standard pellet diet. Animal handling was carried out in agreement with the standards stated in the

Abbreviations: C1P, ceramide 1-phosphate; DAG, diacylglycerol; DAGL, diacylglycerol lipase; DAPI, 4,6-diamidino-2-phenylindole; DTT, dithiotreitol; EDTA, ethylenediaminetetraacetic acid; EGTA, ethylene glycol bis (β-aminoethyl ether)-N,N,N′,N′-tetra acetic acid; G-3-P, glycerol-3-phosphate; HEPES, N-[2-hydroxyethyl]piperazine-N′-[2-ethanesulfonic acid]; LPA, lysophosphatidic acid; LPP, lipid phosphate phosphatase; MAG, monoacylglycerol; MAGL, monoacylglycerol lipase; NEM, N-ethylmaleimide; PA, phosphatidic acid; PAP1, NEM-sensitive Mg^{2+}-dependent phosphatidate phosphohydrolase; PAP2, NEM-insensitive Mg^{2+}-independent phosphatidate phosphohydrolase; PC, phosphatidylcholine; PI, phosphatidylinositol; PLA, phospholipase A; PLC, phospholipase C; S1P, sphingosine 1-phosphate; TLC, thin layer chromatography; WSP, water soluble products

* Corresponding author. Address: Instituto de Investigaciones Bioquímicas de Bahía Blanca, Centro Científico y Tecnológico CONICET Bahía Blanca and Universidad Nacional del Sur, Edificio E1, Camino La Carrindanga km 7, 8000 Bahía Blanca, Argentina. Fax: +54 291 4861200.

E-mail address: pasquare@criba.edu.ar (S.J. Pasquaré).

0014-5793/$36.00
http://dx.doi.org/10.1016/j.febslet.2013.01.074

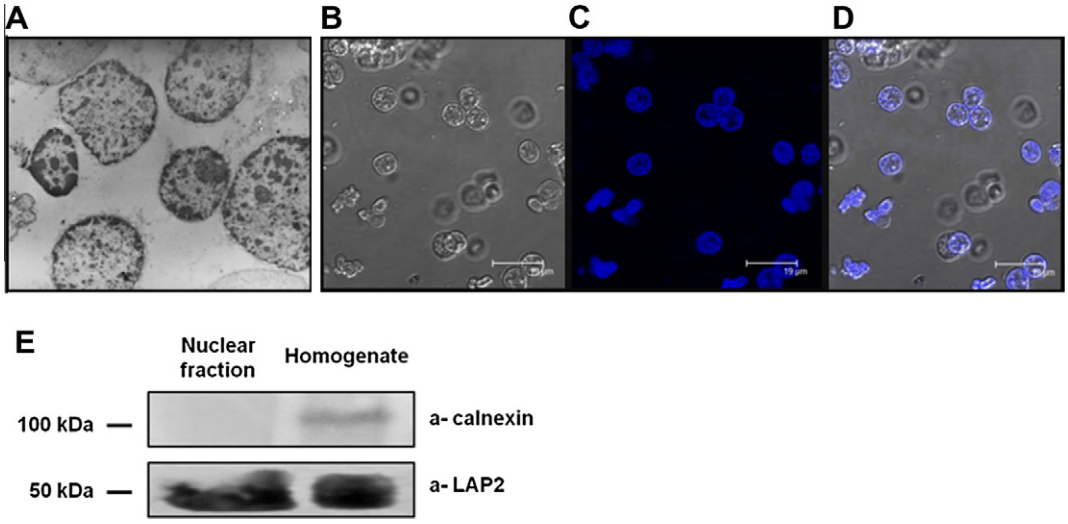

Fig. 1. Purity and integrity of nuclear preparations from rat liver. Electron micrographs of isolated nuclei prepared as described in Section 2, (A) 5000 times (scale bar 3.3 μm); (B) 600 times (scale bar 19 μm). (C) DAPI stained, isolated liver nuclei are in blue, 600 times (scale bar 19 μm), (D) merge, 600 times (scale bar 19 μm), (E) immunoblot analysis of CNX and LAP2 in homogenate and purified liver nuclei. Proteins (50 μg) were boiled in Laemmli buffer, resolved in a 10% SDS–PAGE and transferred to a PDVF membrane for further WB assays. Membranes were blocked and incubated with primary and secondary antibodies as detailed in Section 2. Immunoreactive bands were detected by enhanced chemiluminescence. Numbers on the left indicate molecular weights.

NIH Guide for the Care and Use of Laboratory Animals. Adult rats (4-month-old) were killed by decapitation and liver was immediately dissected (2–4 min after decapitation). The essential procedure for the isolation of nuclei was followed – with minor modifications – as described elsewhere [9,10]. The purified nuclear fraction was suspended in: (i) TKM to quantify proteins and DNA, (ii) 100 mM Tris–HCl, pH 8.5 to determine 5′ nucleotidase, (iii) 0.2 M Na-phosphate pH 7 to determine NADH cytochrome c reductase, (iv) the buffer adequate to the enzymatic assays, (v) cacodylate buffer for electron microscopy, (vi) paraformaldehyde (2%) and Triton-X 100 (0.1%) in phosphate buffer saline (PBS) for DAPI staining.

2.3. Evaluation of nuclear purity

The purity of nuclear preparations was assessed by transmission electron microscopy using a JEOL 100 CXII microscope operated at 80 kV, determining DNA content by diphenylamine assay [11] and measuring the activities of the marker enzymes 5′ nucleotidase [12] and NADH cytochrome c reductase [13]. Calnexin (CNX), an unglycosylated resident ER transmembrane protein, was also determined in purified nuclei [14]. Integrity was assessed by nuclear staining with DAPI.

2.4. Preparation of radioactive 1,2-diacyl-sn-glycerol-3-phosphate

Radioactive PA was obtained from [2-^{3}H]glycerol-PC ([^{3}H]PC) which was synthesized from bovine retinas incubated with [2-^{3}H]glycerol (200 mCi/mmol) as previously described [15]. [^{3}H]PA (0.1 mM, 0.1–0.2 mCi/mmol) was prepared by sonicating in buffer solution containing 5.56 mM EGTA and 5.56 mM EDTA [16].

2.5. Enzymatic assays

2.5.1. LPP assay

LPP activity was determined in an assay medium containing 50 mM Tris–maleate buffer, pH 6.5, 1 mM EDTA plus EGTA, 4.2 mM NEM, 40 μg of nuclear protein and 100 μM [^{3}H]PA, in a volume of 100 μl. When LPP activity was analyzed in the presence of LPA, S1P or C1P, the reaction was started by adding 100 μM [^{3}H]PA (prepared as was specified in Section 2.4.) simultaneously with different concentrations of unlabelled LPA, S1P or C1P (previously re-suspended in the assay buffer containing 1 mM Triton X-100) [16,17]. Radiolabeled PA was dried and re-suspended in the buffer assay and sonicated until clarity was reached.

2.5.2. PA-PLA assay

PA-PLA activity was determined as described previously [18]. Highly purified nuclei (40 μg of protein) were incubated in 100 mM Tris–HCl, pH 7.5, buffer containing 4 mM $CaCl_2$, 0.1% cholic acid and 0.2 mM of [2-^{3}H]PA in a final volume of 100 μl, at 37 °C for 1, 3, 5, 10, 15 and 30 min.

LPP and PA-PLA enzymatic assays were slowed by the addition of chloroform:methanol (2:1, v/v). Blanks were prepared in the same way as each enzyme assay except that the nuclear fraction was inactivated by heating at 100 °C or by the addition of C:M (2:1, v/v).

Lipids were extracted with chloroform:methanol (2:1, v/v) and washed with 0.2 volume of $CaCl_2$ (0.05%) [19]. LPA, PA, MAG and DAG, lipids were chromatographed by TLC on silica gel G or H using different developing solvents as was described previously [10,20]. Chromatograms were visualized by exposure to iodine vapors and scraped off for counting by liquid scintillation. The aqueous phase from Folch extraction containing radiolabel water soluble products (WSP) was concentrated to dryness and counted by liquid scintillation. Radiolabel samples were counted after the addition of 0.4 ml water and 10 ml 5% Omnifluor in toluene/Triton X-100 (4/1, v/v).

2.6. SDS–PAGE and immunoblot

SDS–PAGE was carried out using 10% gels according to Laemmli [21]. Resolved proteins were transferred to immobilon P membranes using a Mini Trans-Blot cell electro blotter (BIO-RAD Life Science Group, CA, USA) for 90 min. Membranes were blocked for 2 h with Tris–buffered saline (20 mM Tris–HCl, 150 mM NaCl) pH 7.5, containing 0.1% Tween 20 (TTBS) and 5% milk or crystalline grade bovine serum albumin (BSA). Incubations with primary antibody anti-calnexin (1:2000) were carried out at room temperature for 3 h and with anti-LAP2 (1:2000) at 4 °C overnight. Membranes were washed with TTBS and subsequently exposed to the appropiate HRP-conjugated secondary antibody (anti-rabbit or anti-mouse)