Available online at www.sciencedirect.com
SCIENCE DIRECT®
Archives of Biochemistry and Biophysics 436 (2005) 136–144
ABB
www.elsevier.com/locate/yabbi

Differential changes in phospholipase D and phosphatidate phosphohydrolase activities in ischemia–reperfusion of rat heart

Girma Asemu [a], Melissa R. Dent [b], Tushi Singal [b], Naranjan S. Dhalla [b], Paramjit S. Tappia [a,*]

[a] *Institute of Cardiovascular Sciences, St. Boniface Hospital Research Centre, Department of Human Nutritional Sciences, Faculty of Human Ecology, University of Manitoba, Winnipeg, Canada R2H 2A6*
[b] *Institute of Cardiovascular Sciences, St. Boniface Hospital Research Centre, Department of Physiology, Faculty of Medicine, University of Manitoba, Winnipeg, Canada R2H 2A6*

Received 24 November 2004, and in revised form 31 January 2005

Abstract

Phospholipase D (PLD2) produces phosphatidic acid (PA), which is converted to 1,2 diacylglycerol (DAG) by phosphatidate phosphohydrolase (PAP2). Since PA and DAG regulate Ca^{2+} movements, we examined PLD2 and PAP2 in the sarcolemma (SL) and sarcoplasmic reticular (SR) membranes from hearts subjected to ischemia and reperfusion (I-R). Although SL and SR PLD2 activities were unaltered after 30 min ischemia, 5 min reperfusion resulted in a 36% increase in SL PLD2 activity, whereas 30 min reperfusion resulted in a 30% decrease in SL PLD2 activity, as compared to the control value. SR PLD2 activity was decreased (39%) after 5 min reperfusion, but returned to control levels after 30 min reperfusion. Ischemia for 60 min resulted in depressed SL and SR PLD2 activities, characterized with reduced V_{max} and increased K_m values, which were not reversed during reperfusion. Although the SL PAP2 activity was decreased (31%) during ischemia and at 30 min reperfusion (28%), the SR PAP2 activity was unchanged after 30 min ischemia, but was decreased after 5 min reperfusion (25%) and almost completely recovered after 30 min reperfusion. A 60 min period of ischemia followed by reperfusion caused an irreversible depression of SL and SR PAP2 activities. Our results indicate that I-R induced cardiac dysfunction is associated with subcellular changes in PLD2 and PAP2 activities.

Keywords: Cardiac ischemia–reperfusion; Sarcolemma; Sarcoplasmic reticulum; Phospholipase D; Phosphatidate phosphohydrolase; Phosphatidic acid; Diacylglycerol

The hydrolysis of phosphatidylcholine (PC)[1] by phospholipase D (PLD) is known to produce phosphatidic acid (PA), which in turn is converted to *sn*-1,2 diacylglycerol (DAG) by the action of phosphatidate phosphohydrolase (PAP) [1,2]. Thus, both PLD and PAP are considered as important enzymes because they modulate the levels of both PA and DAG in the heart. Different agents such as norepinephrine, endothelin-1, and angiotensin-II have been shown to increase the formation of PA in cardiomyocytes [3,4]. The importance of PA in heart function is evident from its ability to stimulate sarcolemma (SL) and sarcoplasmic reticular (SR) Ca^{2+}-related transport systems [5,6]. Furthermore, PA was reported to increase the intracellular concentration of free Ca^{2+} in adult cardiomyocytes and augment cardiac contractile activity of the normal heart [7]. On the other hand, DAG has been shown to activate protein

* Corresponding author. Fax: +1 204 233 6723.
E-mail address: ptappia@sbrc.ca (P.S. Tappia).

[1] *Abbreviations used:* PC, phosphatidylcholine; PLD, phospholipase D; PA, phosphatidic acid; DAG, *sn*-1,2 diacylglycerol; PAP, phosphohydrolase; SL, sarcolemma; PKC, protein kinase C, PIP_2, phosphatidylinositol 4,5-bisphosphate; NEM, *N*-ethylmaleimide; I–R, ischemia–reperfusion; LV, left ventricular; LVDP, LV developed pressure; LVEDP, LV end-diastolic pressure; PEt, phosphatidylethanol; TLC, thin-layer chromatographic.

0003-9861/$ - see front matter © 2005 Elsevier Inc. All rights reserved.
doi:10.1016/j.abb.2005.02.002

kinase C (PKC) isozymes, which phosphorylate several myocardial proteins including ion channels that influence cardiac function [8].

Two mammalian PLD isozymes, PLD1 and PLD2, have been cloned [9]. PLD1 exhibits low basal activity, requires phosphatidylinositol 4,5-bisphosphate (PIP_2) for its activity, and is activated by protein kinase C (PKC) and Rho small G-protein family members [10–16]. PLD2, the major PLD isozyme in the myocardium [17], is localized to the cardiac SL membrane and also requires PIP_2 for its activity [17], but unlike PLD1, it is activated by unsaturated fatty acids such as oleate [2,10,12,16,18,19] and is insensitive to the PLD1 activating factors [20]. Likewise, two types of mammalian PAP enzymes have been identified [21]. The Mg^{2+}-dependent activity of PAP1, which is inhibited by *N*-ethylmaleimide (NEM), has been detected mainly in the cytosolic compartment of cardiomyocytes, whereas PAP2 is insensitive to NEM, inhibited by Mg^{2+}, and is localized to the SL membrane [21]. Although PLD has been localized in different subcellular membrane compartments [1,2], there is no information available in the literature regarding the status of PLD2/PAP2 in cardiac ischemia–reperfusion (I–R) and therefore the present study was undertaken to examine myocardial SL and SR PLD2 and PAP2 activities during I-R of the rat heart.

Materials and methods

Animal model and isolated perfused heart

All experimental protocols for animal studies were approved by the Animal Care Committee of the University of Manitoba, following the guidelines established by the Canadian Council on Animal Care. Heart perfusion and assessment of cardiac performance were conducted as previously described [22]. Briefly, male Sprague–Dawley rats weighing 250–300 g were anesthetized with a mixture of ketamine (60 mg/kg) and xylazine (10 mg/kg). The hearts were rapidly excised, cannulated to the Langendorff apparatus, and perfused with Krebs–Henseleit solution (37 °C) gassed with a mixture of 95% O_2 and 5% CO_2, pH 7.4, containing (in mM) 120 NaCl, 25 $NaHCO_3$, 4.7 KCl, 1.2 KH_2PO_4, 1.2 $MgSO_4$, 1.25 $CaCl_2$, and 11 glucose. The hearts were electrically stimulated at 300 beats/min (Phipps and Bird, Richmond, VA), and the perfusion rate was maintained 10 ml/min. For left ventricular (LV) pressure recordings, a water-filled small latex balloon by polythene cannula was inserted through left atrium into the left ventricle and connected to the pressure transducer (model 1050BP; Biopac System, CA, USA) for the LV systolic and diastolic pressure measurements. The LV developed pressure (LVDP) was the difference between systolic and diastolic pressures. The LV end-diastolic pressure (LVEDP) was adjusted to 10 mmHg at the beginning of the experiment and the LV pressures were differentiated to estimate the maximum rate of pressure development ($+dP/dt_{max}$) and the maximum rate of LV pressure decay ($-dP/dt_{max}$) with Acknowledge 3.71 software for Windows (Biopac System, CA, USA). All hearts were stabilized for a period of 30 min before use in this study and were maintained at a constant temperature (37 °C) throughout the experiment. The control hearts were perfused for a period of 60, 65, 90, 95, and 120 min, and because no significant difference ($P > 0.05$) with respect to each parameter was observed between these hearts, the values were grouped together. At the end of perfusion, the LV tissue was frozen immediately in liquid nitrogen and stored at −80 °C for further processing.

Isolation of cardiac subcellular membranes

LV tissue was used to prepare SL membrane fraction by the sucrose-density procedure of Pitts [23] as well as SR membranes as described elsewhere [2]. The membrane fractions were frozen in liquid N_2 and stored at −80 °C until assayed. All the above steps were carried out at 0–4 °C. Protein concentrations were determined by the Lowry method as described elsewhere [19,21]. In agreement with previous investigations [2,19,21], examination of the marker enzyme activities revealed that the SL and SR membrane preparations used in this study exhibited minimal (2–4%) cross-contamination by other subcellular organelles.

Measurement of PLD2 activity

The PLD2 activity was assayed by measuring the formation of labeled PA from 2.5 mM [^{14}C]PC (0.167 μCi/μmol) as described previously [2]. The exogenous PC substrate (12.5 mM) was prepared by combining egg PC and tracer [^{14}C]PC, which was redissolved in either water or 25 mM sodium oleate solution. The assay was carried out at 25 °C for 60 min in a final volume of 120 μl containing 50 mM of 3,3-dimethylglutaric acid, 10 mM EDTA (pH 6.5), 25 mM KF, and SL membranes (25–50 μg). Ethanol (400 mM) was included in the assay when PLD2 transphosphatidylation activity and subsequent phosphatidylethanol (PEt) formation was measured [18]. The reaction was terminated by the addition of 2 ml $CHCl_3$:CH_3OH (2:1, v/v) and the separation of the two phases was facilitated by adding 0.5 ml of 0.1 M KCl. After centrifugation, the upper phase was discarded and the lower phase was washed to remove non-lipid contaminants. For the blanks, the same procedure was conducted, except the reaction was prevented by adding SL after the $CHCl_3$/CH_3OH solution. The final lipid extract was evaporated almost to dryness under N_2, redissolved in $CHCl_3$ containing PA or PEt as a