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ADP-ribosyl cyclase couples to cyclic AMP signaling in the cardiomyocytes

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Abstract

ADP-ribosyl cyclase (ADPR-cyclase) produces a Ca^{2+}-mobilizing second messenger cyclic ADP-ribose (cADPR) from β-NAD^+. In this study, we examined the molecular basis of which β-adrenergic receptor (βAR) stimulation induces cADPR formation and characterized cardiac ADPR-cyclase. The results revealed that isoproterenol-mediated increase of $[Ca^{2+}]_i$ in rat cardiomyocytes was blocked by pretreatment with a cADPR antagonistic derivative 8-Br-cADPR, a PKA inhibitor H89 or high concentration of ryanodine. Moreover, incubation of ventricular lysates with isoproterenol, forskolin or cAMP resulted in activation of ADPR-cyclase that was inhibited by pretreatment with H89. Supporting the observations, the cADPR antagonist and H89 blocked 8-CPT-cAMP, a cell-permeant cAMP analog-induced increase in $[Ca^{2+}]_i$ but not cGMP-mediated increase. Characterization of partially purified cardiac ADPR-cyclase showed a molecular mass of approximately 42 kDa and no cross-activity with CD38 antibodies, and the enzyme activity was inhibited by Zn^{2+} but not dithiothreitol. Microinjection of the enzyme into rat cardiomyocytes increased the level of $[Ca^{2+}]_i$ in a concentration-dependent manner. The enzyme-mediated increase of $[Ca^{2+}]_i$ was blocked by the cADPR antagonist. These findings suggest that βAR-mediated regulation of $[Ca^{2+}]_i$ in rat cardiomyocytes is primed by activation of cardiac ADPR-cyclase via cAMP/PKA signaling and that cardiac ADPR-cyclase differs from CD38 in biochemical and immunological properties.

Keywords: Cyclic ADP-ribose; ADP-ribosyl cyclase; Calcium signaling; β-Adrenergic receptor; Cardiomyocytes

ADP-ribosyl cyclase (ADPR-cyclase) is a bifunctional enzyme which produces cyclic ADP-ribose (cADPR) from β-NAD^+ and hydrolyzes it [1]. A prototype of mammalian ADPR-cyclases is a lymphocyte antigen CD38. This enzyme is widely distributed and plays a critical role in regulation of intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) via cADPR production. The ability of cADPR to mobilize Ca^{2+} is as powerful as inositol 1,4,5-trisphosphate (IP_3), but distinct from the IP_3 signaling mechanism [2,3]. The cADPR-mediated Ca^{2+} signaling plays a role in insulin secretion [4,5], cell proliferation [6], excitation–contraction coupling [7], cell migration [8,9], and the effects of nitric oxide [10]. In addition, a number of recent studies have indicated that a Zn^{2+}-sensitive ADPR-cyclase(s), which differs from CD38 or *Aplysia californica* ADPR-cyclase, is present in seminal fluid, brain, arterial smooth muscle cells, and bone marrow cells [11–14]. It is also observed that

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ADPR-cyclase activity is not abolished completely in mouse brain and heart by gene disruption of CD38 [9]. These findings indicate that other member(s) of ADPR-cyclase family may exist.

Accumulating evidence suggests that production of cADPR is regulated by a variety of cell surface heterotrimeric G-protein-coupled receptors (GPCR) [15]. These receptors include muscarinic acetylcholine receptors in adrenal chromaffin cells, coronary artery smooth muscle, and pancreatic acinar cells [16–18], angiotensin II receptor in cardiomyocytes and afferent arterioles from rat kidney [19,20], and β-adrenergic receptor (βAR) in rat pulmonary artery smooth muscle cells and the membrane preparation from rat ventricle [21,22]. Very recently, we have demonstrated activation of CD38 via cGMP/protein kinase G (PKG) by stimulation of interleukine (IL) 8 receptor in lymphokine-activated killer cells [8]. These findings suggest that CD38 and/or ADPR-cyclase function as an effector molecule in GPCR signaling pathways.

Sympathetic nerve excitation stimulates βAR in cardiomyocytes by releasing adrenaline, leading to Ca^{2+}-induced Ca^{2+} release [23]. It has also been observed that direct application of cADPR to cardiomyocytes potentiates Ca^{2+} transient and sparks, including Ca^{2+} release during excitation–contraction coupling via either activation of ryanodine receptor and/or increase sarcoplasmic reticulum (SR) Ca^{2+} content [24,25]. However, the molecular basis of the cardiac ADPR-cyclase activation in βAR signaling pathways and cADPR-mediated increase of Ca^{2+} in the heart remain elusive.

In this study, we have investigated βAR-mediated regulation of the cardiac ADPR-cyclase and characterized the enzyme. We here report that cardiac ADPR-cyclase is activated via cAMP/PKA signaling, resulting in increase of $[Ca^{2+}]_i$ and that the enzyme activity is inhibited by Zn^{2+} but not dithiothreitol (DTT), indicating the existence of a new member of ADPR-cyclase in rat heart.

Materials and methods

Materials. 8-Br-cADPR, 2,3-butanedione monoxime (BDM), bovine serum albumin, collagen Type IV, etheno-nicotinamide adenine dinucleotide (ε-NAD^+), H89, nicotineamide guanine dinucleotide (NGD^+), protease inhibitors (leupeptin, aprotinin, soybean trypsin inhibitor, and cystatin), protease Type IV and X, taurine, and trichloroacetic acid (TCA) were purchased from Sigma–Aldrich Chemical, Collagenase Type II was from Worthington Biochemical (Freehold, NJ), and Fluo 3-AM and Fura 2-AM were from Molecular Probes (Eugene, Oregon). 8-CPT-cAMP, and 8-pCPT-cGMP were obtained from Calbiochem (Darmstadt, Germany). MEM was from Gibco-BRL (Grand Island, NY). Ryanodine was obtained from Alomone labs (Jerusalem, Israel).

Isolation of cardiomyocytes. Cardiomyocytes were isolated from Sprague–Dawley rats, weighing 150–200 g, by the method as described previously [26]. Briefly, hearts were rapidly excised, cannulated, and subjected to retrograde perfusion on a Langendorff apparatus at 37 °C with Ca^{2+}-free Krebs–Hanseleit (KH) buffer (10 mM Hepes, 118 mM NaCl, 4.7 mM KCl, 1.2 mM $MgSO_4$, 1.2 mM KH_2PO_4, 25 mM $NaHCO_3$, 10 mM pyruvate, 11 mM dextrose, and 1 mM $CaCl_2$, pH 7.3) for 5 min and then with KH buffer containing 10 mM of 2.3-butanedione monoxime (BDM), 5 mM taurine, 0.075 % collagenase Type II, and 0.08 mg/ml protease Type IV and X for 7 min, and washed with KH buffer containing 0.2 mM Ca^{2+}. The left ventricle was removed, chopped into small pieces, and incubated in a glass conical flask at 37 °C for 10 min with shaking. The undigested tissue was removed through a 200-μm mesh nylon filter. The Ca^{2+} concentration in the cell preparation was gradually increased up to 1 mM. Isolated myocytes were pelleted by centrifugation at 60*g* for 2 min at room temperature and resuspended in a stabilizing buffer (pH, 7.4) containing 20 mM Hepes, 137 mM NaCl, 4.9 mM KCl, 1.2 mM $MgSO_4$, 15 mM glucose, and 10 mM BDM. The cell preparation was kept in the stabilizing buffer containing 1% bovine serum albumin at room temperature about 1.5 h and then was washed three times with MEM at 37 °C. Cardiomyocytes were seeded on the confocal dish coated with collagen (50 μg/ml) and incubated in the humidified 5% CO_2 incubator for 4 h.

Partial purification of ADPR-cyclase. Rat heart membrane was prepared from Sprague–Dawley rats weighing 200–250 g. The hearts removed from rats were washed with ice-cold phosphate-buffered saline, minced in a lysis buffer (30 mM Tris–HCl buffer, pH 7.3, protease inhibitors, 1 mM EDTA, 1 mM EGTA, 250 mM sucrose, and protease inhibitors) and homogenized with a Teflon glass homogenizer in an ice bath. The homogenate was centrifuged at 4 °C for 10 min at 1000*g* to remove unbroken cells and nuclei. Membrane-rich pellets were obtained by centrifugation at 100,000*g* for 1 h at 4 °C. The pellets were suspended and solubilized 1% Triton X-100 in the lysis buffer containing 100 mM NaCl at 4 °C for 1 h. After centrifugation at 100,000*g* for 1 h at 4 °C, the extract was used to purify ADPR-cyclase. The ADPR-cyclase was not able to bind Mono Q-Sepharose, heparin–agarose, CM–Sepharose, and Mono S-Sepharose in the presence of 50 mM NaCl. Therefore, these resins were used for the negative purification. The flow-through fraction was subjected to sequential chromatography using phenyl–Sepharose, Sephacryl 200, and Cibacron blue 3GA-agarose. Although >4000-fold purification was achieved, the final preparation ADPR-cyclase contained >10 proteins visualized by Coomassie brilliant blue staining. This partially purified ADPR-cyclase was used to characterize the enzyme. Detailed procedure will be presented elsewhere.

In-gel activity measurement of ADPR-cyclase. Partially purified ADPR-cyclases from various tissues were separated on non-reducing SDS–PAGE gel (11%) according to the method described by Ziegler et al. [27]. After electrophoresis, the gel was washed in a solution containing 50 mM Tris–HCl (pH 7.3) and 0.3% Triton X-100 for 30 min. The gel was washed with 50 mM Tris–HCl (pH 7.3) containing 0.1% Triton X-100 and incubated with 150 μM ε-NAD at room temperature for 15–30 min. ADPR-cyclase was then visualized with a UV transilluminator. The apparent molecular mass of a protein corresponding to a fluorescent band was estimated by marking the band with a needle and comparing its position to those of marker proteins after staining of the gel with Coomassie brilliant blue. The bands were sliced and subjected to reducing SDS–PAGE gel (11%). The molecular mass was not changed. In addition, the putative ADPR-cyclase determined by in-gel assay was subjected to MALDI-TOF analysis but neither CD38 nor CD38-like enzyme was detected.

Assay for ADPR-cyclase activity. Specific ADPR-cyclase activity was determined by measuring cyclic GDP-ribose (cGDPR) fluorometrically using NGD^+ as a substrate [28]. Since cGDPR is resistant to hydrolysis, this procedure was demonstrated to be highly sensitive and convenient for the measurement of ADPR-cyclase activity. For βAR-mediated ADPR-cyclase activation, heart homogenate in 25 mM