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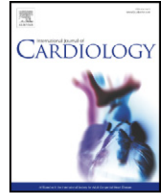

Modulation of cardiac contractility by serine/threonine protein phosphatase type 5

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Background: Protein phosphatase 5 (PP5) a serine/threonine phosphatase is ubiquitously expressed in mammalian tissues including the heart, but its physiological role in the heart is still unknown. Therefore, we used a transgenic mouse model to get a first insight into the cardiac role of PP5.
Methods and results: We generated transgenic mice with cardiac myocyte specific overexpression of PP5. Successful overexpression of PP5 was demonstrated by Western blotting, immunohistochemistry and enhanced arachidonic acid-stimulated protein phosphatase activity in transgenic hearts. Cardiac function was examined on the level of isolated cardiac myocytes, isolated organs and in intact animals. Whereas Ca^{2+} transients and cell shortening remained unchanged, L-type Ca^{2+} currents were decreased in isolated cardiac myocytes from transgenic mice. Ventricular contractility was reduced in isolated perfused hearts under basal conditions and after β-adrenergic stimulation. In intact animals, echocardiography revealed increased left ventricular diameters and decreased contractility and invasively measured hemodynamic performance by left ventricular catheterization demonstrated a reduced response to β-adrenergic stimulation in transgenic mice compared to wild type.
Conclusions: Overexpression of PP5 affected contractility and β-adrenergic signaling in the hearts of transgenic mice. Taken together, these findings are indicative of a regulatory role of PP5 in cardiac function.
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1. Introduction

Protein phosphatase 5 (PP5), a serine/threonine phosphatase, is highly conserved among species and is expressed in all eukaryotic cells examined so far. The catalytic domain is structurally related to that of PP1, PP2A and PP2B with approximately 40% identity in amino acid sequence [1]. PP5 is sensitive to inhibition by okadaic acid, microcystin, cantharidin, tautomycin, and calyculin A as it is known for PP1 and PP2A (reviewed in Ref. [2]). A unique feature of PP5 is the existence of catalytic, regulatory and subcellular targeting functions in one polypeptide. This is in contrast to all other protein phosphatases. Regulatory and targeting functions are mediated by three N-terminal tetratricopeptide repeat (TPR) motifs. This TPR domain is responsible for protein–protein interactions [3]. PP5 was initially identified through interaction with the atrial natriuretic peptide (ANP) receptor of the heart via its TPR domain [4]. Compared to other protein phosphatases, PP5 remained undiscovered for a long time due to its low phosphatase activity under normal conditions [5]. This may result from an inhibitory interaction between the C-terminus and the N-terminal TPR domain [6]. Removal of this TPR domain by partial proteolysis *in vitro* activated phosphatase activity up to 50-fold [5]. Skinner and co-workers as well as Chen and Cohen demonstrated that PP5 can be activated by polyunsaturated fatty acids e.g. arachidonic acid (AA) through binding to the TPR domain [5,7]. Later, it was postulated that fatty acyl-CoA esters and heat shock protein 90 (hsp90) might serve as physiological activators [8].

PP5 can interact with hsp90 via its TPR domains and PP5 is a major component in the glucocorticoid receptor (GR) — hsp90 complex [9] modulating GR phosphorylation, GR-induced transcriptional activity, and GR transfer into the nucleus [10–12]. Furthermore, it was shown that PP5 acts as a negative regulator of estrogen receptor (ER) activity at several ER-responsive genes [13]. Other biological functions may include regulation of cellular proliferation, migration, differentiation, electrolyte balance, apoptosis, survival, and DNA damage repair (reviewed in Refs. [14,15]). Recent results with PP5-deficient mice revealed that PP5 is not an essential phosphatase but an important regulator of cellular functions [16]. PP5-deficient mice were viable and no growth defects were noted but the DNA damage checkpoint

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pathway was impaired in fibroblasts of these mice [16]. However, cardiac contractility of PP5-deficient mice was not investigated.

In general, little is known about cardiac functions of PP5. But it was demonstrated that PP5 acts as a physiological inhibitor of apoptosis signal-regulating kinase 1 (ASK1), which is ubiquitously expressed in eukaryotic cells including the heart and which plays an important role in stress-induced cellular responses including cardiac contractility, hypertrophy and remodeling [17–19]. Therefore, it appears that PP5 is also an important regulator of cardiac myocyte functions. This initiated us to confirm the role of PP5 especially in the heart by generating transgenic mice with cardiac myocyte specific over-expression of PP5. Here, we report on the characterization of PP5 transgenic mice and demonstrate that PP5 may serve as modulator of cardiac function.

2. Materials and methods

2.1. Human cardiac tissue

Nonfailing ventricular probes were obtained from prospective organ donors whose hearts could not be used. The study protocol conforms to the ethical guidelines of the 1975 Declaration of Helsinki and is in accordance with the guidelines from the local ethics committee and patients gave written informed consent.

2.2. Transgenic mice

The investigation conforms with the *Guide for the Care and Use of Laboratory Animals* published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1996). Approval was granted by the local ethics review board (approval reference number 42502-02-691 MLU). The cDNA of rat PP5 along with its 3′ untranslated region was generously provided by M. Chinkers [4]. The transgene, composed of the α-myosin heavy chain promoter, the entire protein coding region for rat PP5 (and 483 base pairs of 3′ untranslated sequence), and the SV40 polyadenylation signal sequence was isolated from the parent plasmid and used for microinjection of fertilized mouse eggs (CD1). Transgene-positive mice were identified by Southern-blotting and PCR assay of tail genomic DNA. Two founder lineages were established and used for further studies. Western blotting, protein phosphatase measurements and immunocytochemical analyses were performed as described elsewhere [20–23].

2.3. Ca^{2+} transients and shortening of isolated cardiomyocytes

Isolation of ventricular myocytes from PP5-transgenic and wild type mice, measurement of Ca^{2+} transients (Indo-1 fluorescence), and measurement of cell shortening (video edge detection system) were performed as described previously [24]. Cardiomyocytes were stimulated at 0.5 Hz.

2.4. Whole-cell L-type Ca^{2+} current (I_{Ca})

Single cardiomyocytes were studied using the whole-cell patch-clamp technique as described previously [24]. Recordings were performed under conditions that suppress Na^+ and K^+ currents. Ca^{2+} currents were elicited by voltage steps from a holding potential of −40 mV to a test potential of +10 mV for 200 ms, applied every 10 s. Cell capacitance and I_{Ca} were recorded with an L/M-PC amplifier (LIST-Electronic, Darmstadt, Germany) according to standard protocols.

2.5. Work-performing heart preparations

Work-performing heart preparations were utilized as described previously [24]. Mice were anesthetized intraperitoneally with 2.0 g/kg body weight urethane and treated with 1.5 units of heparin. The hearts were removed from the opened chest, immediately attached by the aorta to a 20-gauge cannula, and perfused retrogradely with oxygenized Krebs–Henseleit buffer (37.4 °C) containing (mmol/L) NaCl 118, $NaHCO_3$ 25, Na-EDTA 0.5, KCl 4.7, KH_2PO_4 1.2, $MgSO_4$ 1.2, $CaCl_2$ 2.5, and glucose 11. During the short period of retrograde perfusion, the pulmonary vein was cannulated. The perfusion of the heart was then changed to an anterograde mode. The venous return (preload) and the aortic pressure (afterload) were adjusted to 5 mL/min cardiac output and 50 mm Hg, respectively. Heart rate, aortic pressure, left intraventricular pressure (systolic, diastolic, and enddiastolic), and atrial pressure were measured and monitored continuously. The first derivative of left intraventricular pressure (+dP/dt and −dP/dt) was calculated with a computer program (PowerLab; ADInstruments, Spechbach, Germany).

2.6. Left ventricular catheterization

Left ventricular catheterization was performed in closed-chest mice anesthetized with tribromoethanol (15 µL/g body weight of a 2.5% solution). Through the right carotid artery a 1.8 F polyethylene catheter (Micromed, Houston, TX, USA) was inserted into the left ventricle. Analog pressure signals were obtained using a pressure transducer TXD-310 (Micromed), recorded and digitized at a sampling rate of 1000 Hz with the PowerLab system (ADInstruments, Spechbach, Germany). The left jugular vein was cannulated for intravenous access. Increasing doses of the β-adrenoceptor agonist dobutamine were administered via the left jugular vein using an automated syringe pump (B. Braun Melsungen, Germany). Heart rate, blood pressure and the first derivative of left intraventricular pressure (+dP/dt and −dP/dt) were measured continuously [20].

2.7. Echocardiography and Doppler studies

Transthoracic echocardiographic measurements were performed on mice, which were anesthetized intraperitoneally with a mixture of ketamine S (25 mg/kg) and xylazine (10 mg/kg) allowing spontaneous breathing. All measurements were made using a commercially available echocardiographic system (Hewlett-Packard Sonos 5500) equipped with a 15 MHz linear transducer for two-dimensional and M-mode imaging and a 12 MHz transducer for Doppler measurements. The parasternal long and short axes were obtained. Five heartbeats for every parameter were analyzed. The fractional shortening of the heart was calculated from the M-mode left ventricular (LV) diameters as (LVEDD − LVESD) / LVEDD × 100, where LVEDD was the enddiastolic diameter and LVESD was the endsystolic diameter. In addition, Doppler flow measurements of aortic and mitral flows were performed. The analysis of data was performed by two observers, who were blinded to the mouse lineage [20].

2.8. Statistics

Data are presented as mean ± SEM. Comparisons between groups were evaluated using Student's *t* test or two-way ANOVA followed by Bonferroni's post-hoc test for multiple group comparisons, with significance imparted at the $p<0.05$ level.

3. Results

3.1. PP5 in the human heart

Western blotting analyses of ventricular samples (100 µg protein per lane) from human hearts revealed high expression of PP5 (Fig. 1A). To further characterize the role of PP5 in the heart, a transgenic mouse model with cardiac myocyte restricted overexpression of PP5 was generated.

3.2. Transgenic mice

Generation of PP5 transgenic mice (TG) resulted in two lineages with different amounts of cDNA integrated into the genome. We found gene dose dependent effects of the transgene, more precisely, all effects were more pronounced in TG mice with higher PP5 protein levels based on the higher amount of integrated cDNA. Therefore, all data shown here were from the high expression TG lineage. PP5 protein expression in the hearts from 10 week old mice was elevated 4.5-fold in TG compared to WT (Fig. 1A and B). Protein phosphatase activity in heart homogenates was analyzed with [^{32}P]-labeled casein as substrate. To mainly measure PP5 activity, 200 µmol/L arachidonic acid (AA) was used to activate PP5. As other phosphatases e.g. PP2C are also stimulated by AA divalent cations like Mg^{2+} were omitted in the assay to reduce their activity. In both, WT and TG homogenates protein phosphatase activity was stimulated by AA, but stimulation was much more effective in TG compared to WT (Fig. 1B). To visualize PP5 expression in the myocardium, we carried out immunostaining of PP5 in ventricular sections. Fig. 1C and D demonstrates localization and increased expression of PP5 in cardiac myocytes of 10 week old TG mice. The green signal, specific for PP5, was prominent in the cytosol (Fig. 1C) and in the nucleus (Fig. 1D). We did not detect any fibrosis in TG hearts using appropriate staining or other microscopic changes in morphology using hematoxylin and eosin staining (data not shown). Older (six months) TG mice, developed mild cardiac hypertrophy demonstrated by an elevated relative heart weight (5.6 ± 0.1 for WT, $n = 29$; vs. 6.1 ± 0.2 for TG, $n = 37$, $p<0.05$; body weights were not different: WT 35.2 ± 0.8 g; TG 35.8 ± 0.8 g), but morphology and cardiomyocyte diameters (15.3 ± 0.4 µm for WT, $n = 26$ cells of 3 mice; 14.4 ± 0.3 µm for TG, $n = 52$ cells of 3 mice) remained unchanged.

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