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Age-dependent suppression of SERCA2a mRNA in pediatric atrial myocardium

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Abstract

Differential expression of sarcoplasmic reticulum Ca^{2+}-ATPase (SERCA2a) and phospholamban (PLB) has been shown in heart failure and atrial arrhythmias. We investigated the influence of volume overload and age on their expression in pediatric atrial myocardium. Right atrial specimens from 18 children with volume overloaded right atrium (VO) and 12 patients without overload were studied. Each group was further divided into patients less than and older than 12 months of age. Only in the younger patients SERCA2a was significantly reduced in the VO group. In younger patients PLB mRNA level tended to be lower in VO. The PLB:SERCA protein ratio was significantly reduced in the VO group. Age itself did not influence the SERCA2a and PLB expression, if the hemodynamic overload was not taken into account. This study is the first to show a combined influence of volume overload and age on atrial SERCA2a expression.

Keywords: Congenital heart disease; Age; Sarcoplasmic reticulum; SERCA; Phospholamban

Congenital abnormalities of the heart occur in about 8 per 1000 live births, making them one of the most common types of congenital malformations [1]. Volume overload of the right atrium is a feature of several congenital cardiac defects, most frequently it is found in atrial septal defects (ASDs). If not corrected, this clinically benign condition during childhood leads to increased morbidity and mortality in adults, mainly due to atrial arrhythmias [2]. It has been shown that Ca^{2+} handling proteins and ion-channels of the cardiomyocyte are key components of the molecular remodeling process [3], characterized by elevated cytosolic Ca^{2+} levels, due to an impaired Ca^{2+} sequestration by the sarcoplasmic reticulum (SR) [4,5]. The cardiac isoform of sarcoplasmic reticulum calcium-ATPase (SERCA2a) is the main regulator of cytosolic calcium, which is not only determining the electrical, but also the contractile, properties of the myocardium. Phospholamban (PLB) is the main regulator of SERCA2a activity, inhibiting it if PLB is dephosphorylated [6].

Studies in animal models dealing with the developmental regulation found increasing expression of SERCA2a mRNA and protein after birth in different species [7–9]. Accordingly, the calcium uptake of the SR has also been found to be higher in the adult compared with neonatal hearts [10,11]. In human myocardium no developmental change has been found on the mRNA level, however the SERCA2a protein increased with development [12]. Regarding phospholamban, the regulator of SERCA2a, its mRNA and protein levels increased from neonatal to adult stage in mouse [9], in

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rabbit mRNA remained unchanged after birth [8], only the protein level increased [13], whereas in the rat PLB mRNA and protein were unchanged [14].

Due to the developmental regulation and the species specificity of the investigated proteins, results from former studies cannot be adopted to the situation in childhood. This study aimed to look for the influence of volume overload and age on the expression of SERCA2a and PLB in pediatric atrial myocardium.

Materials and methods

Patients. A total of 30 patients undergoing open heart surgery were studied. In all patients the surgical procedure required a right atriotomy, and tissue samples of 20–50 mg of the right atrial free wall were obtained during the surgery. The age distribution in the group with right atrial volume overload (mean age 38 ± 46 months) and the group without overload (mean age 50 ± 51 months) did not differ significantly (data not shown). According to the echocardiographic diagnosis the patients were assigned to a group having an atrial septal defect with mild right atrial volume overload (VO) and a group having no shunt across the atrial septum, hence without volume overload (NO) of the right atrium. Moreover, groups were sub-divided into younger (0–12 months of age) and older (12–165 months of age) patients (Table 1). Pre- and post-operatively all patients were in sinus rhythm. All parents of the patients gave written informed consent. The study protocol has been approved by the Local Ethics Committee (Request-Nr. 168/2000). The investigation conforms with the principles outlined in the Declaration of Helsinki.

RT-PCR. Quantitative RT-PCR (reverse transcription-polymerase chain reaction) was used to measure mRNA expression of SERCA2a and PLB. Atrial myocardium, removed during corrective surgery, was immediately snap-frozen in liquid nitrogen and stored at −80 °C until further processing. Ten milligrams of right atrial myocardium was used for extraction of total cellular RNA. Frozen samples were homogenized with a mortar and pestle, and the lysate was passed 10 times through a 20-G needle fitted to a syringe. Dnase I treatment was performed. RNA extraction was performed with the RNeasy Mini-Kit (Qiagen) using the protocol for heart tissue, including proteinase K digestion. The amount of isolated RNA was determined using the RiboGreen dye (Molecular Probes, Eugene, OR) in a fluorescent assay, specifically measuring RNA concentration according to the manufacturer's instructions. For quantitative one-step RT-PCR an OneStep RT-PCR Kit (Qiagen, Hilden, Germany) was used. Two microliters (approx. 20 ng) of total RNA were reverse transcribed and directly amplified in the glass capillary of a LightCycler system (F. Hoffmann-La Roche AG, Basel, Switzerland). Online fluorescence monitoring was performed by adding SYBR-Green I. The final reaction volume was 20 μl and final reaction concentrations were as follows: 1× OneStep RT-PCR Buffer, 400 μM of each dNTP, 0.6 μM of each primer, 7.5 U RNase inhibitor, freshly prepared SYBR-Green I, 1:2105 diluted in H_2O (v/v) (Sigma–Aldrich, St. Louis, MO), and 1 μl of the OneStep RT-PCR Enzyme Mix. LightCycler conditions were as follows: reverse transcription for 30 min at 50 °C, initial RT inactivation and polymerase activation step for 15 min at 95 °C, PCR rapid cycling for 40 cycles: denaturation at 95 °C for 30 s, annealing at 58 °C for 30 s, elongation at 72 °C for 15 s, and final melting curve analysis from 58 to 95 °C with a slope of 0.1 °C/s. Fluorescence was measured at the end of the elongation phase. LightCycler fluorescence settings were F1 Gain 5 and Channel setting F1.

Primer design. The primer pairs were designed to discriminate between the different isoforms. Each primer pair contains at least one exon-boundary spanning primer, in order to prevent amplification of contaminating genomic DNA. The calculated annealing temperatures were identical for both primer pairs. Building of primer dimers was excluded using software OLIGO 6.1 (MedProbe, Norway). The size of the amplicons was 204 bp for PLB and 188 bp for SERCA2a, respectively (Table 2).

PCR product characterization. In addition to the quantitative analysis of PCR product amplification and melting curve analysis using LightCycler system, RT-PCR products were analyzed by PAGE and silver staining to ensure specificity of the amplification (Fig. 1). Reagent samples without template were included to identify contamination. Additional confirmation of product identity was performed by sequencing of forward and reverse strands of several products with the PRISM Ready Reaction Dye Deoxy Terminator Sequencing Kit (Perkin–Elmer/ABI, Huenenberg, Switzerland) using an ABI 373 DNA Sequencer.

Table 1
Age distribution of the patients

	NO < 12 months	VO < 12 months	NO > 12 months	VO > 12 months
n	6	7	6	11
Mean age	8	5	65	80
SD	3	7	53	45

NO, not volume overloaded atrium; VO, volume overloaded atrium.

Table 2
Nucleotide sequence of the primers used in the study

Gene		Product length
PLB		
Fwd	TCACAGCTGCCAAGGCTACC	204
Rev	TAGATTCTGTAGCTTTTGACGTGC	
SERCA2a		
Fwd	AGCGGTTACTCCAGTATTGCAG	188
Rev	CTGTCCATGTCACTCCACTTCC	

Fwd, forward primer; PLB, phospholamban; rev, reverse primer; and SERCA2a, cardiac sarcoplasmic reticulum calcium adenosine triphosphatase.

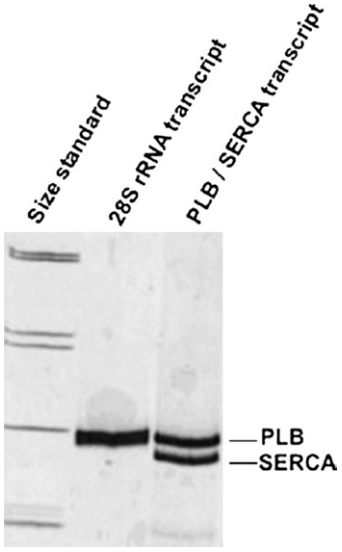

Fig. 1. Polyacrylamide gel electrophoresis of PCR products (28S rRNA, PLB, and SERCA).