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Brief communication

Differential expression of sarcolipin protein during muscle development and cardiac pathophysiology

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Abstract

Sarcolipin (SLN) is a small molecular weight sarcoplasmic reticulum (SR) membrane protein expressed both in cardiac and skeletal muscle tissues. Recent studies using transgenic mouse models have demonstrated that SLN is an important regulator of cardiac SR Ca^{2+} ATPase 2a (SERCA2a). However, there is a paucity of information regarding the SLN protein expression in small versus larger mammals and its regulation during development and cardiac pathophysiology. Therefore, the major goal of this study was to generate an SLN specific antibody and perform detailed analyses of SLN protein expression during muscle development and in the diseased myocardium. The important findings of the present study are: (i) in small mammals, SLN expression is predominant in the atria but low in the ventricle and in skeletal muscle tissues, whereas in large mammals, SLN is quite abundant in skeletal muscle tissues than the atria, (ii) SLN and SERCA2a are co-expressed in all striated muscle tissues studied except ventricle and co-ordinately regulated during muscle development and (iii) SLN protein levels are ~3 fold upregulated in the atria of heart failure dogs and ~30% decreased in the atria of hearts prone to myocardial ischemia. In addition we found that in the phospholamban null atria, SLN protein levels are upregulated.

Keywords: Sarcolipin; SERCA2a; Phospholamban; Atria; Skeletal muscle; Heart failure; Development

1. Introduction

Sarcolipin (SLN) is a small molecular weight protein (31 amino acids) originally identified to co-purify with the skeletal muscle sarcoplasmic reticulum Ca^{2+} ATPase (SERCA) [1,2]. Subsequent studies at the mRNA level showed that SLN is expressed both in cardiac and skeletal muscle tissues of all mammals [2–5]. Within the cardiac muscle, SLN mRNA expression is more abundant in the atria compared to the ventricle [4,5]. The expression pattern of SLN mRNA is different between small and larger mammals. In rodents, SLN mRNA is abundant in the atria and expressed at low levels in the ventricle and slow skeletal muscles [4,5]. In contrast, in larger mammals including humans, SLN mRNA is more abundant in fast-twitch skeletal muscle tissues compared to the heart [2].

The importance of SLN as a regulator of SERCA pump was studied using adenoviral gene transfer [5] and transgenic mouse models [6–8]. Adenoviral mediated overexpression of N-terminally flagged SLN (NF-SLN) into ventricular myocytes resulted in decreased myocyte contractility and Ca^{2+} handling [5]. Confocal imaging analyses of ventricular myocytes overexpressing NF-SLN showed that SLN co-localizes with phospholamban (PLB) and SERCA2a in the SR membrane [5]. The functional significance of SLN in cardiac contractility either in the presence or absence of PLB was

Abbreviations: SLN, sarcolipin; SR, sarcoplasmic reticulum; SERCA, SR Ca^{2+} ATPase; PLB, phospholamban; NF-SLN, N-terminally flagged SLN; SLN-CTAb, SLN C-terminal peptide antibody.

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studied by overexpressing NF-SLN in the wildtype [6,7] as well as in the PLB knockout mouse hearts [8]. Overexpression of NF-SLN in the mouse heart inhibits the SERCA pump activity by lowering its apparent Ca^{2+} affinity, resulting in decreased Ca^{2+} transient amplitude and rates of muscle relaxation [6–8]. Additionally these studies showed that the inhibitory function of SLN is independent of PLB and is relieved upon isoproterenol treatment [6,8]. These studies suggest that SLN is an important regulator of SERCA pump activity and can mediate β-adrenergic response independent of PLB.

SLN mRNA expression is shown to be developmentally regulated [4,5] and is altered by thyroid hormones [9,10] and pathophysiological conditions [3,4,11–13]. Pressure-overloaded hypertrophy induced by transverse aortic constriction in mice or by monocrotaline administration in rats significantly decreased the expression of SLN, SERCA2a and phospholamban mRNAs in the atrium [13]. In human, decreased expression of SLN mRNA has been reported in the atria of patients with atrial fibrillation [12]. Although SLN expression is low in the ventricle, it was shown to be upregulated 50-fold higher in the hypertrophied ventricles of Nkx2−5 null mice [11]. However, most of these studies were carried out at the mRNA level and results could not be confirmed at the protein level due to the lack of an SLN specific antibody. In the present study, we generated an antibody highly specific to SLN and studied its expression during development and in cardiac pathology.

2. Materials and methods

All experiments were performed in accordance with the National Institute of Health guidelines and approved by the Institutional Laboratory Animal Care and Use Committee at The Ohio State University. Sprague–Dawley rats, B6 mice, SLN transgenic mice [6] and PLB knockout mice [14] were used for this study. Developmental studies were done in timed pregnant Sprague–Dawley rats purchased from Harlan/Taconic farms. Pacing induced heart failure canine model [15] was described previously. The myocardial infarction model susceptible or resistant to ventricular fibrillation was generated as described previously [16,17]. In brief, the anterior myocardial infarction was produced by ligation of the left anterior descending artery approximately one-third of the distance from its origin. A hydraulic occluder was also placed around the left circumflex coronary artery so that ischemia can be induced at a site distant to the previous injury. The susceptibility to ventricular fibrillation was tested by a two minute coronary occlusion during the last minute of a submaximal exercise test. The susceptible dogs had either ventricular fibrillation or ventricular tachycardia during this exercise plus ischemia test while the resistant dogs do not have arrhythmias. In this particular study, the animals were assigned to either a 10 week sedentary period or a 10 week exercise training (running on a treadmill) groups after the classification. The susceptible dogs have poorer ventricular function and an abnormal autonomic neural control as compared to the resistant animals.

2.1. Generation of SLN antibody

We utilized the 100% conserved C-terminal sequence of SLN to generate rabbit polyclonal antibody. A peptide corresponding to the last 6 residues of luminal domain (-VRSYQY) of SLN with an additional cysteine added to the C-terminus was conjugated to keyhole-limpet haemocyanin. Rabbits were immunized with these peptides and sera were collected. Sera from one of the four rabbits immunized with the above antigen recognize SLN across species. This SLN C-terminal peptide antibody (SLN-CTAb) was affinity purified and used for further studies. Specificity of the antibody was tested by Western blot analysis using bacterially expressed rat and human SLN. Generation and affinity purification of SLN antibody were carried out in Chemicon International, Inc.

2.2. Protein preparations

The following muscles were sampled in (i) rodents—atria, ventricle, soleus, quadriceps, diaphragm and tongue, (ii) rabbit—left and right atria, left and right ventricle, diaphragm, extensor digitorum longus (EDL) and soleus and (iii) dog—left and right atria, left and right ventricle, diaphragm, gastrocnemius muscle (a synergist of the soleus muscle in other species) and EDL. For developmental studies, atria, ventricle, tongue and quadriceps from rat embryos of different days of post coitum (dpc) and neonatal rats were used. Frozen tissues were homogenized in 8 volumes of homogenizing buffer containing (in mM) 50, Tris–Cl, pH 7.5, 150 NaCl, 1 $Na_2P_2O_7$, 1 benzamidine, 5 Na_3VO_4, 10 NaF and 0.5% Nonidet P-40 [6]. To express rat and human SLN in the bacteria, the coding sequence of rat or human SLN was cloned into the bacterial expression vector pET 23d (Novagen Inc). Cell growth, protein induction and protein extraction were followed precisely as described by the manufacturer's instructions. Protein concentrations were determined by the Bradford method using bovine serum albumin for the standard curve. The SR enriched microsomal fractions for rat atria and ventricles were prepared as described earlier [18].

2.3. Western blot analysis

Protein samples were analyzed by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE) and loading was normalized for Western blot analysis. The homogenates were electrophoretically separated on (8% for SERCA2a, SERCA1a and CSQ, 14% for PLB) SDS–PAGE or 16% Tricine gel (for SLN) and transferred to nitrocellulose membrane. Membranes were immunoprobed with primary antibodies [anti-rabbit SLN, 1:3000 (present study); anti-rabbit SERCA2a, 1:5000; anti-rabbit PLB, 1:3000; anti-rabbit calsequestrin (CSQ), 1:5000 [6]; anti-rabbit SERCA1a, 1:2000 (Custom made)] followed by HRP-conjugated secondary antibodies. Signals were detected by Super Signal WestDura substrate (Pierce) and quantitated by densitometry [6].