Urocortin 2 Protects Against Pacing-Induced Alternans via Phosphorylation of Phospholamban in Cardiac Myocytes from Normal and Failing Hearts

Stefanie Walther, Joshua N. Edwards, Florentina Pluteanu, Susanne Renz, Kurt Schmidt, Burkert Pieske, Jens Koeksaemper, Lothar A. Blatter

Molecular Biophysics and Physiology, Rush University Medical Center, Chicago, IL, USA, Institute of Pharmacology and Clinical Pharmacy, Philippus-University of Marburg, Marburg, Germany, Department of Cardiology and Pneumology, University Medicine Goettingen, Goettingen, Germany, Institute of Pharmaceutical Sciences, Pharmacology and Toxicology, Karl-Franzens-University of Graz, Graz, Austria, Division of Cardiology, Medical University of Graz, Graz, Austria.

Cardiac alternans is a high risk indicator for cardiac arrhythmias, stroke and sudden cardiac death. The cardioactive peptide Urocortin 2 (Ucn2) exhibits beneficial effects in normal and failing hearts, and elicits PKA-dependent positive inotropic and lusitropic effects in normal myocytes. Thus, we investigated if Ucn2 protects against pacing-induced alternans and elucidated the underlying mechanism.

Experiments were performed on single rabbit atrial and ventricular myocytes from normal and failing hearts. Chronic heart failure was induced by combined pressure and volume overload through aortic banding. Ucn2 administration increased the pacing frequency until stable Ca alternans occurred at room temperature. Global Ca transients were measured with the fluorescent Ca indicators Fluo-4 or Indo-1 and monitored simultaneously with mechanical alternans (sarcomere length). In some experiments, cytosolic Ca alternans and intra-SR Ca alternans were simultaneously recorded with the Ca indicators Rhod-2 and Fluo-5N, respectively.

The average alternans ratio (AR = 1-(small-amplitude/large-amplitude)) in atrial myocytes was 0.79, and in normal and failing ventricular myocytes the ARs were 0.69 and 0.64, respectively. Ucn2 (100 nM) completely abolished Ca and mechanical alternans (within 2-3 min) in atrial and ventricular myocytes from normal and failing hearts. An increased sarcoplasmic reticulum (SR) Ca content, together with an enhanced SR Ca release flux, suggested that Ucn2 normalized alternans through effects on SR Ca ATPase (SERCA). Ucn2 increased significantly the level of cyclic adenosine monophosphate (cAMP) in normal cells (~12-fold), and enhanced phosphorylation of phospholamban (PLB) at Ser16 in normal myocytes (~10-fold) and to a lesser extent (~5-fold) in failing myocytes. These data demonstrate that Ucn2 rescues alternans presumably via increased SERCA activity in atrial and ventricular myocytes and thus protects normal and failing hearts from proarrhythmic alternans.

NOS1AP Modulates Intracellular Ca2⁺ in Cardiac Myocytes and is Up-Regulated in Dystrophic Cardiomyopathy

Adriana V. Treuer, Daniel R. Gonzalez

Universidad de Talca, Talca, Chile.

NOS1AP gene (nitric oxide synthase 1-adaptor protein) is strongly associated with abnormalities in the QT interval of the electrocardiogram and with sudden cardiac death. To determine the role of NOS1AP in the physiology of the cardiac myocyte, we assessed the impact of silencing NOS1AP, using siRNA, on [Ca²⁺]ᵣ transients in neonatal cardiomyocytes. In addition, we examined the co-localization of NOS1AP with cardiac ion channels, and finally, evaluated the expression of NOS1AP in a mouse model of dystrophic cardiomyopathy.

Using siRNA, NOS1AP levels were reduced to ~30% of the control levels (p<0.05). NOS1AP silencing in cardiac myocytes reduced significantly the amplitude of electrically evoked calcium transients (p<0.05) and the degree of S-nitrosylation of the cells (p<0.05). Using confocal microscopy, we evaluated NOS1AP subcellular location and interactions with other proteins by co-localization analysis. NOS1AP showed a high degree of co-localization with the L-type calcium channel and the inwardly rectifying potassium channel Kir3.1, a low degree of co-localization with the ryanodine receptor (RyR2) and alpha-sarcomeric actin and no co-localization with connexin 43, suggesting functionally relevant interactions with the ion channels that regulate the action potential duration.

Finally, using immunofluorescence and Western blotting, we observed that in mice with dystrophic cardiomyopathy, NOS1AP was significantly up-regulated (p<0.05).

These results suggest for a role of NOS1AP on cardiac arrhythmias, acting on the L-type calcium channel, and potassium channels, probably through S-nitrosylation.