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Impaired Intracellular Calcium Handling in Atrial Cardiomyocytes from Rats Selected for Low Aerobic Capacity and Metabolic Syndrom

Anne Berit Johnsen, Marcia Alvez, Guri Kaurstad, Natale Rolim, Ulrik Wisløff, Morten A. Høydal.
Department of Circulation and Medical Imaging, University of Science and Technology, Trondheim, Norway.

Background

Atrial fibrillation (AF) is the most common arrhythmia and is often associated with altered Ca^{2+} handling. Evidence points to low aerobic capacity as predictor of cardiovascular disease and AF. The hypothesis of the present study was that rats, genetically selected on the basis of low running capacity has impaired Ca^{2+} handling compared to rats selected for high running capacity.

Method

Low capacity runners (LCR) (n=5) develop characteristics of the metabolic syndrome, and high capacity runners (n=5) develop a healthy and athletic phenotype. The exercise capacity was measured by VO_{2max}. Ca^{2+} handling were measured in isolated Fura-2/AM loaded atrial cardiomyocytes.

Results

VO_{2max} was 30% lower in LCR than HCR. Cardiomyocyte function was significantly depressed in LCR; fractional shortening was 52% lower and time to 50% relenghtening was 42% prolonged. We observed no differences in peak systolic and diastolic Ca^{2+}, as well as no significant difference in amplitude of Ca^{2+} transient between the two groups. This indicate reduced Ca^{2+}sensitivity in cardiomyocytes from LCR. SR Ca^{2+} load, assessed by caffeine-induced Ca^{2+} release, was not different between groups. Time to 50% Ca^{2+} transient decay was prolonged by 58% in LCR. We found no difference in Ca^{2+} decay between the two groups during caffeine induced Ca^{2+} transient, reflecting unaltered NCX function; neither did we find any changes in the plasma membrane Ca^{2+} exchanger (PMCA). The impaired diastolic Ca^{2+}removal is therefore mainly attributed to reduced SERCA function in LCR (39%). Diastolic SR Ca^{2+} leak was increased in LCR compared to HCR.

Conclusion

Our data demonstrate differences in atrial Ca^{2+}handling between rats selected for high and low aerobic capacity and we suggest that impaired Ca^{2+} handling in LCR may be an important factor in initiation of AF.

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Role of the Putative NAADP Receptor, Two-Pore Channel 2, in Ventricular Myocyte Responses to Isoproterenol

Laura C. Elson, William D. Owen, Arie R. Gafson, John Parrington, Antony Galione, Derek A. Terrar, Stevan Rakovic.
University of Oxford, Oxford, United Kingdom.

NAADP is a potent endogenous Ca^{2+}-mobilizing molecule. It modulates signalling in several different cell types, including atrial[1] and ventricular[2] cardiomyocytes, by releasing Ca^{2+} from a lysosome-related acidic compartment distinct from the endo/sarcoplasmic reticulum. A recent study has suggested that the molecular target of NAADP is the two-pore (Ca^{2+}) channel, TPC2[3]. Here, we examine the role of TPC2 in ventricular myocyte responses to the β-adrenoceptor agonist isoproterenol, which has been demonstrated to utilize NAADP as a second messenger in these cells[2]. Ventricular myocytes isolated from transgenic mice with absence of TPC2 expression ($TPC2^{-/-}$) and corresponding wild-type (WT) control mice were electrically field-stimulated (3 ms pulses; 36°C). Cell contractions were measured using an edge detection system. Ca^{2+} transients were imaged using Nipkow spinning-disk confocal microscopy (fluo-5F-AM). There were no significant differences in contraction or Ca^{2+} transient amplitudes under control conditions. However, isoproterenol (3 nM) elicited significantly smaller increases in $TPC2^{-/-}$myocyte contraction amplitude when compared to WT myocytes (165 ± 57 % vs. 688 ± 114 % following 2 min application, n=6,4, $p<0.01$). The increases in Ca^{2+} transient amplitudes elicited by isoproterenol (3 nM) were also significantly smaller in $TPC2^{-/-}$ myocytes compared to those seen in WT myocytes (58 ± 17 % vs. 186 ± 37 %, n=6,5, $p<0.05$). There were no significant differences in contraction or Ca^{2+} transient amplitudes following washout of isoproterenol. We conclude that TPC2 is important in mediating the actions of isoproterenol in ventricular myocytes. These data also support the hypotheses that β-adrenoceptors utilize NAADP as an intracellular messenger, and that TPC2 is important in mediating NAADP actions in mammalian cells.

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Biphasic Effect of Reactive Oxygen Species on Skeletal Muscle Sarcolemmal Ca^{2+} Influx

George G. Rodney, Luke Michaelson, Guoli Shi, Chris W. Ward.
Univerisity of Maryland School of Nursing, Baltimore, MD, USA.

In striated muscle, sarcolemmal Ca^{2+} influx (SCI) serves to maintain the store of Ca^{2+} within the sarcoplasmic reticulum during repetitive contractions. Furthermore, dysregulated SCI is suggested to underscore the altered Ca^{2+} homeostasis implicated in the pathogenic progression of muscular dystrophy. Despite its importance, we have little insight into the regulation of SCI in health and disease. In this study we investigated the role of oxidative stress on SCI. We report that in wild-type myofibers, oxidation has a biphasic effect on SCI as estimated via the Mn^{2+} quench technique. At low [H_2O_2] (<50μM), basal SCI was enhanced (+ 70%) while at higher [H_2O_2] (200μM) SCI was decreased (−48%). This biphasic effect of oxidation was also evident during long K^+ depolarization's in BTS paralyzed myofibers where low [H_2O_2] enhanced (+82%) while high [H_2O_2] decreased (−15%) SCI. Pre-incubation of myofibers with reduced glutathione diminished the oxidation induced alterations in SCI, allowing us to conclude that oxidation modulates SCI in wild-type myofibers. Myofibers from the *mdx* mouse exhibited an exuberant basal SCI compared to wild-type muscle (+873%). Pre-incubation of *mdx*fibers with reduced glutathione decreased SCI back toward wild-type, suggesting that the exuberant SCI in *mdx* myofibers may be a potential therapeutic target via. redox modulation. Taken together, these data support a hypothesis in which low levels of ROS stimulate SCI while higher levels are inhibitory. Ongoing studies are addressing the role of ROS production during strenuous exercise as a dynamic regulator of SCI in health and disease.

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The Purinergic Agonist, ATP, Inhibits IP_3-Evoked Ca^{2+} Release in Smooth Muscle

Debbi MacMillan, Charles Kennedy, John G. McCarron.
University of Strathclyde, Glasgow, United Kingdom.

Adenosine 5-triphosphate (ATP) mediates a variety of biological functions following nerve-evoked release via activation of either G-protein coupled P2Y- or ligand-gated P2X- receptors. In various smooth muscle types, ATP, acting via P2Y receptors, acts as an inhibitory neurotransmitter. The underlying mechanism(s) remain unclear, but have been proposed to involve Ca^{2+} release from the internal store and subsequent activation of Ca^{2+}-activated potassium channels (K_{Ca}) to cause hyperpolarisation. Clearly, this proposal requires Ca^{2+} release from the internal store. In the present study ATP induced reproducible transient increases in the intracellular Ca^{2+}concentration in only approximately 10% of voltage-clamped single smooth muscle cells and failed to evoke Ca^{2+} release in the remainder. These results do not support activation of K_{Ca} as the major mechanism underlying inhibition of smooth muscle activity. Interestingly, ATP and its metabolite, adenosine 5-diphosphate, each inhibited inositol 1,4,5-trisphosphate (IP_3)-evoked Ca^{2+}release in cells that did not show a Ca^{2+} increase in response to purinergic stimulation. The inhibitory effect of ATP on IP_3-evoked Ca^{2+} release was blocked by the $P2Y_1$-selective receptor antagonist MRS2179. In conclusion, the present study demonstrates, for the first time, an inhibitory effect of purinergic activation via $P2Y_1$ receptors on IP_3-evoked Ca^{2+} release in smooth muscle.

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AKAP150 is Required for NFATC3 Transcriptional Regulation of Kv2.1 and BK Channel Expression in Smooth Muscle

Madeline Nieves-Cintron, Manuel F. Navedo, John D. Scott, Luis F. Santana.
University of Washington, Seattle, WA, USA.

In smooth muscle, activation of the Ca^{2+}-sensitive phosphatase calcineurin dephosphorylates the transcription factor nuclear factor of activated T cells c3 (NFATc3). Upon de-phosphorylation, NFATc3 translocates into the nucleus of arterial smooth muscle cells where it modulates the expression of multiple genes including Kv2.1 and the α and β1 subunits of large conductance K^+ (BK) channels. Recent work by our group, suggested that L-type Ca^{2+} channels, the A-kinase anchoring protein 150 (AKAP150), calcineurin, and PKCα form a signaling triad that controls Ca^{2+} influx into these cells. Ca^{2+} influx via L-type Ca^{2+}channels is necessary for NFATc3 activation in smooth muscle cells during angiotensin II signaling. In this study, we tested the hypothesis that AKAP150 is required for the down regulation of Kv2.1 and BK channel α and