activation of SOCE with each AP and RyR opening makes SOCE a counter-flux to fibre Ca^{2+} loss and a potential signal that transduces muscle usage patterns for gene expression.

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Role of the CaCC Channel ANO1 in Electromechanical Coupling of Murine Pulmonary Artery Smooth Muscle

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In VSMCs, Ca^{2+}-activated Cl^- channels (CaCCs) are encoded by the gene TMEM16A/Anoctamin 1 (ANO1). The mechanism by which ANO1 influence the excitability of VSMCs remains to be elucidated due to questionable pharmacology and lack of a reliable genetic knockdown mouse model of ANO1. The aim of this study was to re-evaluate the role of ANO1 in electromechanical coupling of pulmonary artery (PA) smooth muscle using newer generation ANO1 blockers and a novel smooth muscle-specific inducible ANO1 knockout mouse model (SMC-iANO1-KO). Wire myography was used to determine the vascular reactivity to 5-HT of PA from wild-type and SMC-iANO1-KO mice. Calcium imaging experiments were also carried out using SMC-iGCaMP3 mice, which genetically express the Ca^{2+} biosensor GCaMP3 in smooth muscle cells. 5-HT elicited a dose-dependent contraction (0.01-30 μM) that was similarly inhibited (~50-70%) by the ANO1 blocker $CaCC_{Inh}$-A01 (10 μM), the $Ca_V1.2$ blocker nifedipine (1 μM) or the SERCA2 pump inhibitor cyclopiazonic acid (CPA; 10 μM). Genetic ablation of ANO1 produced a reduction in 5-HT-induced tone (~ 60% at 1 μM 5-HT) that was similar to that produced by $CaCC_{inh}$A01, nifedipine or CPA. Ca^{2+} imaging experiments in the intact PA of SMC-iGCaMP3 mice revealed that 5-HT evoked spatially and temporally localized Ca^{2+} transients. These Ca^{2+} oscillations were potently inhibited by $CaCC_{Inh}$-A01 or nifedipine, and were abolished by CPA. In conclusion, 5-HT elicited highly localized Ca^{2+} oscillations that were promoted by Ca^{2+} entry through $Ca_V1.2$, most likely involving transient depolarizations evoked by ANO1 activated by a balance between oscillatory SR Ca^{2+} release through IP_3 receptors and Ca^{2+} entry through $Ca_V1.2$. We propose that the stable agonist-induced PA contraction results from the integration of stochastic and localized Ca^{2+} events supported by a microenvironment comprising ANO1, $Ca_V1.2$ and IP_3 receptors.

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Expression of Orai1 Restores Normal Sarcoplasmic Calcium Release in *Cmpt* Mice

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In our mouse model, a naturally occurring 12-bp deletion in the myostatin gene is considered responsible for the compact phenotype ($Mstn^{Cmpt\text{-}dl1Abc}$, *Cmpt*) labeled by a tremendous increase in body weight along with signs of muscle weakness, easier fatigability and decreased store operated calcium entry (SOCE, Sztretye et al. 2017). While the voltage dependence of SR calcium release was not statistically different between WT and *Cmpt* fibers (26.03 ± 1.35 mV for WT *vs.* -28.86 ± 0.77 mV for *Cmpt*), the amount of releasable calcium was significantly reduced in the latter, indicating smaller SR content. To assess the immediate role of SOCE in replenishing the SR, the evolution of intracellular calcium concentration during a train of long-lasting depolarizations to a maximally activating voltage were monitored. *Cmpt* mice exhibited a faster decline in calcium release suggesting a compromised ability to refill the SR. We found SOCE having a role in maintaining and refilling SR Ca^{2+} stores not only in repetitive tetanic stimulation, but on an immediate basis. When reconstructing the *Cmpt* fibers with venus-Orai1, we found a slight shift to more positive potentials in the voltage dependence of SR calcium release (23.57 ± 0.85 mV) When *Cmpt* cells were loaded with 50 nM TMRE fiber segments with damaged mitochondria were identified covering on average. 27.7 ± 1.7% of the fiber area (n=8). Our results favor the idea that SOCE is immediately activated upon voltage-dependent SR calcium release. By doing so it plays an important role in regulating SR calcium content both on the long run and also during a contraction-relaxation cycle. A new finding is that the *Cmpt* phenotype in mice is associated with abnormal mitochondrial function.Funded by: OTKA PD-108476, Bolyai Fellowship to MSz, GINOP-2.3.2-15-2016-00044.

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Fibroblast-Mediated Atrial Mechanical Dysfunction in HFpEF and Hypertensive Heart Disease

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Heart failure (HF) with preserved ejection fraction (HFpEF) is present in about 50% of HF patients, often related to metabolic syndrome. Atrial remodeling is common in HFpEF, associated with atrial mechanical stretch leading to changes in secretory activity (e.g. adversely altered TNF-alpha and IL-10) and independently increases mortality. We hypothesize that atrial cardiomyocyte (CM) function and its reaction to extracellular-matrix-triggers is pivotal for the manifestation and progression of atrial remodeling and mechanical dysfunction in HF.

Atrial mechanical function in-vivo was assessed using echocardiography. Excitation-contraction-coupling (ECC) was examined using Ca-imaging (Ca-transients; CaT) in atrial CM of ZFS-1 rats without (Ln; hypertension) and with metabolic syndrome (Ob; diabetes, hypertension, obesity). CaT were recorded after treatment with conditioned medium of unstressed or stressed (Flexercell system) fibroblasts isolated from Ln and Ob. CM were also exposed to TNF-alpha and IL-10 and ECC was studied.

Ob show impaired atrial function in-vivo associated with an increased diastolic Ca-content, prolonged CaT time-to-peak and Ca-removal after treatment with activated fibroblast-medium. While Ca-spark frequency was unaltered, conditioned medium significantly increased amplitude and altered time to peak, duration and width in Ob. This indicates changes of ECC due to mechanical stress in metabolic syndrome mediated by fibroblasts. In Ln however atrial in-vivo function was preserved and a shortening of Ca-removal could be observed after exposure to stressed fibroblast secretome, supporting the notion of an in-vivo compensatory phenotype. Ob showed no change in ECC when exposed to TNF-alpha. IL-10 however, had beneficial effects on ECC as it significantly accelerated Ca removal by 29% in Ob CM.

During metabolic syndrome, impaired in-vivo atrial mechanical function might be related to adversely affected CM ECC due to fibroblast secretome. In hypertensive atrial remodeling, stressed fibroblasts enhance CM Ca-removal and in-vivo atrial function is preserved.

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Surface Mechanosensors and the Fundamental Conundrum of Homeometric Regulation

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More than 100 years ago von Anrep observed the eponymous Anrep effect whereby the heart initially dilates in response to an increase in outflow resistance but over several minutes the end-diastolic volume decreases suggesting an increase in contractility. About 50 years later Sarnoff et al. made the important discovery that in the face of increased outflow resistance (afterload) the heart increased the force of contraction even at the same fiber length (preload), which is in clear contradistinction from Starling's Law of the Heart. Subsequent research supports the idea that the Anrep effect is intrinsic to the heart but the cellular mechanisms remain unclear. The conundrum is thus: Given the same starting length (precluding the Frank-Starling mechanism), how can a myocyte ''know'' it is contracting against two different resistances? We propose that on the myocyte surface, mechanosensors oriented orthogonal to the longitudinal axis enable the myocyte to detect stress (~afterload) independently of myocyte strain (~preload). During contraction the myocyte expands transversely and the extent that surface mechanosensors are compressed depends on the stiffness of its environment, which in the heart is a function of wall stress. Our model makes the surprising prediction that within a certain range, the Ca^{2+} transient will increase with increasing mechanical stiffness thus providing the cellular basis for what Sarnoff called homeometric autoregulation and the Anrep effect. This prediction was confirmed in experiments using our Cell-in-Gel system where myocytes embedded in viscoelastic hydrogel are subjected to controlled mechanical loads.