sparks, single channel and whole-cell BK currents were measured in cerebral artery myocytes. Expression of BK channel alpha and beta-1 subunits and RyR2 was examined by RT-PCR. The effects of blockers for BK channels (paxilline, 1 μM) and RyRs (ryanodine, 10 μM) were examined on diameter of isolated cerebral arteries. Ca^{2+} spark frequency, but not amplitude, was decreased ~50% following SAH. This decrease in Ca^{2+} spark frequency corresponded to a reduction in the number of functional Ca^{2+} spark sites and decreased RyR2 expression in myocytes from SAH animals. A similar reduction in the frequency of transient BK currents was observed following SAH, although the properties and expression of BK channels were similar between groups. Inhibition of this vasodilatory pathway by paxilline or ryanodine induced constriction of control arteries, which was greatly diminished following SAH. These data suggest decreased Ca^{2+} spark frequency in cerebral myocytes following SAH is due to decreased RyR2 expression and a reduction in functional spark sites. The resulting decrease in BK currents leads to an enhanced cerebral artery constriction that may contribute to the development of neurological deficits following SAH. (Supported by AHA 0725837T, 0725841T, NIH R01 HL078983, R01 HL44455, and the Totman Medical Research Trust).

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Mitochondrial Modulation Of Spontaneous Ca^{2+} Oscillations In Portal Vein Smooth Muscle Myocytes

Marnie L. Olson[1], Susan Chalmers[1], Nicolaos Avlonitis[2], Megan N. Stanton-Humphreys[3], Stuart J. Conway[3], John G. McCarron[1].
[1]University of Strathclyde, Glasgow, United Kingdom, [2]University of St Andrews, St Andrews, United Kingdom, [3]University of Oxford, Oxford, United Kingdom.

Transient increases in cytosolic Ca^{2+} concentration ($[Ca^{2+}]_c$) regulate many smooth muscle activities including contraction, transcription, growth and apoptosis. The onset of some processes involve sarcoplasmic reticulum Ca^{2+} release triggered by either inositol 1,4,5-trisphosphate ($InsP_3$) or occurring spontaneously. Mitochondrial Ca^{2+} uptake influences the spatio-temporal features of spontaneous and $InsP_3$-mediated Ca^{2+} waves. For example the magnitude of phenylephrine-evoked Ca^{2+} waves was decreased when mitochondrial membrane potential ($\Delta\Psi_M$) was depolarized. How individual mitochondria interact to regulate global Ca^{2+} signals is unresolved. Mitochondria may act as a series of separate entities or as a continuum to shape the amplitude, propagation speed and frequency of these Ca^{2+} release events. We examined how mitochondria affect spontaneous Ca^{2+} oscillations by either inhibiting mitochondrial Ca^{2+} uptake in small isolated regions or throughout the entire cell. Mitochondrial Ca^{2+} uptake was inhibited throughout the entire cell by depolarizing the $\Delta\Psi_M$ using the protonophore CCCP to dissipate the driving force for Ca^{2+} uptake. Alternatively, mitochondrial Ca^{2+} uptake was inhibited in small, restricted regions by locally photolyzing a caged mitochondrial uncoupler (caged AG10), which we have synthesised. Photolysis of caged AG10, in a small region depolarizes $\Delta\Psi_M$ in that area alone leaving the $\Delta\Psi_M$ of the remaining mitochondrial complement intact. Spontaneous Ca^{2+} oscillations were observed in about 25% of the cells examined. When $\Delta\Psi_M$ was depolarized throughout the cell oscillations were inhibited. In contrast, depolarization of $\Delta\Psi_M$ in a small isolated region of the cell decreased the amplitude but increased the frequency of Ca^{2+} oscillations. These results indicate that mitochondria act as independent entities but their activities throughout coordinate to regulate Ca^{2+} release. Supported by the Wellcome Trust, British Heart Foundation, BBSRC and Leverhulme Trust.

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Dopamine and Pancreatic Islet Function

Alessandro Ustione, David W. Piston.
Department of Molecular Physiology and Biophysics, Vanderbilt University, Nashville, TN, USA.

Pancreatic islets are spheroidal clusters of electrically and chemically connected endocrine cells, whose function plays a key role in glucose homeostasis. Islet β-cells secrete insulin in response to increased blood glucose concentration, but their response is modulated by other stimuli (such as glucagon, insulin, acetylcholine, epinephrine, etc.). The application of stimulatory glucose concentrations (>6 mM) to isolated pancreatic islets produces synchronized oscillations in intracellular $[Ca^{2+}]$. During these $[Ca^{2+}]$ oscillations, the entire pancreatic islet secretes insulin in a pulsatile manner.

We are exploring the role of endogenous production of dopamine by β-cells in the regulation of $[Ca^{2+}]$ oscillations and insulin secretion. Using fluorescent Ca^{2+} indicators, micro-fluidic devices, and confocal microscopy, we have measured the period of $[Ca^{2+}]$ oscillations in isolated intact islets. Treating the islets with the dopamine precursor, L-DOPA (3,4-Dihydroxy-L-phenylalanine), or a selective antagonist of D2 dopamine receptor, Raclopride, causes a decrease and an increase in the frequency of $[Ca^{2+}]$ oscillations. Parallel experiments were performed using islets from genetically modified mice that do not express the dopamine transporter, and it was found that their $[Ca^{2+}]$ oscillations are also slower than those from wild type mice. Hence, we propose that this autocrine dopaminergic system is part of the mechanism that regulates $[Ca^{2+}]$ oscillations.

The oscillation period depends on the islet electrical and metabolic activity. To begin understanding the potential mechanisms by which dopamine is exerting its function in the β-cell, we are using computer modeling to simulate the islet activity and reproduce the data and to gather information on the main pathway involved in the observed dopaminergic effect.

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Interactions With Imidazole Side Groups As A Mechanism To Block The Alzheimer'S Aβ Peptide-induced Intracellular Calcium Increase

Nelson J. Arispe, Juan C. Diaz, Michael Flora.
Uniformed Services University, Bethesda, MD, USA.

We have proposed the formation of Aβ ion channels as a mechanism to explain the intracellular calcium increase which occurs after cells are exposed to the cytotoxic Alzheimer's Aβ peptide. We developed highly effective and specific histidine-related Aβ channel blockers which prevent the Aβ-induced intracellular calcium response. Substitution experiments showed that histidine is essential for the blocking process. We hypothesized that the blocking efficiency of these compounds was related to imidazole side chains in the histidine residues. We rationalized that the resonance structure of the imidazole ring would be attracted to a full or partial positively charged form of the Histidine in the Aβ subunits of the Aβ channels. This investigation studied the intracellular calcium increase which occurs after cells are exposed to the Aβ peptide. The role of the imidazole side chains in mechanism of action was determined using histidine-related Aβ channel blockers in which the imidazole side chains were methylated. Additionally, we studied these compounds when modified by amidation and acetylation of the carboxyl and amine end groups, respectively. Our results showed that the efficacy to prevent the Aβ-induced intracellular calcium increase, and the capacity to protect cells from the toxic action of Aβ, is completely abolished when the imidazole side chains of the histidine-related Aβ channel blockers are methylated. On the other hand, the efficacy of the Aβ channel blockers is significantly improved when the ends of the molecules are capped leaving the imidazole side chain as the sole group available for interaction. We conclude that aromatic interactions between the imidazole side chains in the histidine-related blockers, and the charged form of the His residues in the Aβ channels, constitutes a blocking mechanism for the Aβ peptide-induced intracellular calcium increase.

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Tightly Regulated Store-operated Ca^{2+} Entry In Healthy And Dystrophic Skeletal Muscle

Bradley S. Launikonis[1], D. George Stephenson[2], Joshua N. Edwards[1], Robyn M. Murphy[2], Oliver Friedrich[1].
[1]University of Queensland, Brisbane, Australia, [2]La Trobe University, Melbourne, Australia.

Store-operated Ca^{2+} entry (SOCE) is a mechanism that allows the entry of extracellular Ca^{2+} upon depletion of the internal stores. This mechanism has been described in skeletal muscle and the two main molecular players, Stim1 and Orai1, have been identified. Previous work, mainly on myotubes, suggested that SOCE may become deregulated in dystrophic skeletal muscle and result in cellular Ca^{2+} overload. The final result of such a process would be activation of proteolytic enzymes, cell necrosis and/or apoptosis. To examine the regulation of SOCE in healthy and dystrophic muscle we examined the biochemistry and physiology of skeletal muscle from wild-type (wt) and mdx mice (8-20 weeks old). Western blotting of single fibres showed that Stim1 and Orai1 were expressed at 2-3 times higher levels in mdx compared with wt muscle (normalized to total myosin). Consistent with this, enzymatically isolated interossei fibres loaded with fluo-4AM and depleted of Ca^{2+} in a solution containing 0 Ca^{2+}, 20 μM cyclopiazonic acid and 10 mM caffeine showed a 3-fold higher rate of Ca^{2+} entry into the depleted mdx fibres compared to wt upon re-addition of 2 mM Ca^{2+}. However, this does not imply Ca^{2+} overload will occur via SOCE in dystrophic cells if deactivation is unaffected. To study SOCE kinetics, skinned fibres with t-system trapped fluo-5N were bathed in an internal solution with rhod-2 and continuously imaged in xyt mode on a confocal microscope. Intracellular Ca^{2+} release was induced by lowering cytoplasmic $[Mg^{2+}]$. Transient t-system Ca^{2+}-depletion and reuptake was preceded by transient SR Ca^{2+}-release and reuptake in fibres from both mdx and wt mice. This indicates robust activation and deactivation mechanisms of SOCE in both wt and dystrophic muscle which prevent not only depletion but also overloading of the internal Ca^{2+}-stores.