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Dysferlin Stabilizes Excitation-Contraction Coupling in Murine Skeletal Myofibers

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Voltage-induced Ca^{2+}-transients (VICT) in dysferlin-null (A/J) but not control (A/WySnJ) myofibers are reduced after osmotic shock injury (OSI). Diltiazem (10μM) prevented this loss (Kerr et al., PNAS, 2013). We compared the effects of blockers of L-type Ca^{2+}-channels (diltiazem, nifedipine, verapamil) and RyR1 (dantrolene, tetracaine) on VICT in A/WySnJ and A/J FDB myofibers in culture. We also examined A/J fibers expressing N-terminal Venus chimaeras of wild type (WT) or mutant (R1331L) dysferlin, introduced 1 week earlier by electroporation. Isolated myofibers were loaded with Rhod2-AM Ca^{2+}-sensing dye, and Ca^{2+}-transients were monitored by confocal microscopy during 1 Hz voltage pulses. Diltiazem inhibited VICT at the same half maximal concentrations (~60μM) in both A/J and A/WySnJ, and actually increased the amplitude of VICT in A/WySnJ but not A/J fibers by ~20% at 10μM. Verapamil and nifedipine were more potent inhibitors of VICT in dysferlin-null fibers and neither drug increased the amplitude of VICT in A/WySnJ. Dantrolene and tetracaine did not increase VICT amplitude but showed similar half maximal inhibitory concentrations in WT and dysferlin-null fibers. A/J myofibers expressing WT dysferlin interacted with diltiazem like A/WySnJ fibers, with no change in diltiazem affinity but an increase in amplitude at 10μM. Myofibers expressing dysferlin-R1331L were indistinguishable from A/J fibers in their response to diltiazem, showing no change in affinity and no increase in VICT amplitude. Neither verapamil nor nifedipine protected A/J fibers from the loss of VICT after OSI. Remarkably, OSI induced Ca^{2+} waves and spontaneous Ca^{2+} transients in A/J fibers expressing dysferlin-R1331L but not WT dysferlin. These events were inhibited by diltiazem (10μM). These results suggest that dysferlin stabilizes the excitation-contraction coupling (ECC) machinery of myofibers and that this interaction can be mimicked specifically by diltiazem.

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Role of ZIP7 in Regulation of Cytosolic Free Zn^{2+} Level in Mammalian Cardiomyocytes

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Zinc (as Zn^{2+}), increasingly recognized as an important trace element, has physiological role in mammalian cell function. Resting intracellular free Zn^{2+} level ($[Zn^{2+}]i$) can be greatly increased by thiol-reactive oxidants or high glucose that contributes to oxidant-induced alterations in EC-coupling of cardiomyocytes. Since Zn^{2+} is important in cellular physiology, very little is known how $[Zn^{2+}]i$ is controlled and stored intracellularly in cardiomyocytes. Therefore, in the present study we aimed to investigate the role of ZIP7 on regulation of $[Zn^{2+}]i$ in cardiomycoytes. We first used newly-developed genetically-encoded FRET-based sensors to monitor cytosolic (CYS), mitochondrial (MitC) and endoplasmic reticulum (ER) $[Zn^{2+}]$. Our data showed that $[Zn^{2+}]$ levels in these compartments were about 0.5, 5.0 and 0.2 nM, respectively in isolated left ventricular cardiomyocytes. Since expression but not location of ZIP7 has been shown in the heart tissue, inhere, we tested whether Zn^{2+} transport from ER into cytosol by a transporter ZIP7 thereby responsible for cytosolic/S(E)R Zn^{2+} homeostasis in cardiomyocytes under pathological condition. Our data have shown that both protein level and expression of ZIP7 increased in either cardiomyocytes isolated from streptozotocin-induced diabetic rat heart or high glucose (25mM; 24-hours) incubated H9c2 cells. Our immunofluorescence analysis also demonstrated that ZIP7 is localised in ER but not in Golgi apparatus in these cells. Furthermore, ZIP7 knocked-down in cardiomycoytes induced a significant reduction of cytosolic $[Zn^{2+}]$. All experimental data showed that ER is a Zn^{2+} pool and ZIP7 has a crucial role in regulation of cytosolic Zn^{2+} in mammalian cardiomyocytes (Supported by TUBITAK SBAG-113S466, COST Action TD1304, EFSD Fellowships).

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Phosphodiesterase Type-1 Regulates Transient BK Currents and Contractility of Human Urinary Bladder Smooth Muscle

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Large conductance Ca^{2+}-activated K^+ (BK) channels are critical regulators of urinary bladder smooth muscle (UBSM) function. We aimed to investigate phosphodiesterase type 1 (PDE1) interactions with BK channels in human UBSM to determine the mechanism by which PDE1 regulates human urinary bladder function. A combined electrophysiological, functional, and pharmacological approach was applied using human UBSM specimens obtained from open bladder surgeries. The perforated whole cell patch-clamp technique was used to record transient BK currents (TBKCs) and the cell membrane potential in freshly-isolated human UBSM cells in combination with the selective PDE1 inhibitor, 8-methoxymethyl-3-isobutyl-1-methylxanthine (8MM-IBMX). Isometric tension recordings were used to measure spontaneous phasic and electrical field stimulation (EFS)-induced contractions in human UBSM isolated strips. Selective pharmacological inhibition of PDE1 with 8MM-IBMX (10 μM) increased TBKC activity in human UBSM cells, which was abolished by subsequent inhibition of protein kinase A (PKA) with H-89 (10 μM). The stimulatory effect of 8MM-IBMX on TBKCs was reversed upon activation of muscarinic acetylcholine receptors with carbachol (1 μM). 8MM-IBMX (10 μM) hyperpolarized the UBSM cell membrane potential, an effect blocked by PKA inhibition. 8MM-IBMX significantly decreased spontaneous phasic and nerve-evoked contractions of human UBSM isolated strips.

The results reveal that pharmacological inhibition of PDE1 attenuates human UBSM excitability and contractility by activating BK channels via a PKA-dependent mechanism. The data also suggest interactions between PDE1 and muscarinic signaling pathways in human UBSM. Overall, our data provide mechanistic evidence that pharmacological inhibition of PDE1 can be a useful therapeutic approach for the treatment of overactive bladder syndrome by attenuating UBSM excitability and contractility. These investigations are an important step in validating PDE1 as a potential novel therapeutic target for overactive bladder.

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Effects of *Prunus Mume* Siebold & Zucc. On the Pacemaking Activities of Interstitial Cells of Cajal in Murine Small Intestine

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Interstitial cells of Cajal (ICCs) are the pacemaker cells in the gastrointestinal (GI) tract and play an important role in the regulation of GI motility. The authors investigated the effects of a plum (*Prunus mume* Siebold & Zucc.) on the pacemaker potentials of cultured ICCs from mouse small intestine. The effects of a methanolic extract of *Prunus mume* (m-PM) on ICC pacemaking activities were examined using the whole-cell patch clamp technique. ICC pacemaker membrane potentials were depolarized by m-PM in a concentration dependent manner in current clamp mode. 4-DAMP (a muscarinic M_3 receptor antagonist) blocked m-PM-induced pacemaker depolarizations, whereas methoctramine (a muscarinic M_2 receptor antagonist) did not. When GDP-β-S (1 mM) was in the pipette solution, m-PM induced slight pacemaker depolarization. Pretreatment with Ca^{2+}-free solution or thapsigargin (a Ca^{2+}-ATPase inhibitor in endoplasmic reticulum) abolished the generation of pacemaker currents and suppressed m-PM-induced pacemaker membrane depolarization. Also, pretreatments with PD98059, SB203580, or JNK II inhibitor blocked m-PM-induced ICC depolarizations. Furthermore, m-PM inhibited transient receptor potential melastatin (TRPM) 7 channels, but did not affect Ca^{2+}-activated Cl^- channels. These results suggest that m-PM modulates pacemaker potentials through muscarinic M_3 receptor via G-protein and external and internal Ca^{2+} in a mitogen-activated protein kinase and TRPM7 dependent manner. In addition, they also indicate that the methanoic extract of *Prunus mume* Siebold and Zucc. offers an excellent basis for the development of a novel gastroprokinetic agent.

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Molecular Interactions of slc26a6 and CFTR in Cardiomyocytes

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We have recently identified and cloned cardiac isoforms of a solute carrier, slc26a6. Importantly, we demonstrated that slc26a6 mediated electrogenic Cl^-/oxalate and Cl^-/HCO_3^- exchanges in both atrial and ventricular myocytes.