TRPC Channels

TRPC Channels Mediate the Nonselective Cation Current and Store-Operated Calcium Influx in Human Atrial Myocytes

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Little information is available in the literature regarding transient receptor potential (TRP) channels in human atrial myocytes. The present study was designed to investigate whether TRPC channels would mediate the nonselective cation current reported previously and store-operated Ca\(^{2+}\) entry channels (SOCs) in human atrial myocytes using approaches of whole-cell patch-clamp, RT-PCR, Western blot analysis, confocal microscopy, and co-immunoprecipitation. It was found that the nonselective cation current was recorded under K\(^+\)-free conditions in human atrial myocytes, and the current was inhibited by the nonselective TRP channel blocker La\(^{3+}\). The TRPC1 channel activator thapsigargin activated the current, and the effect was suppressed by La\(^{3+}\) and prevented by pipette inclusion of anti-TRPC1 antibody. In addition, confocal microscopic experiment revealed intracellular Ca\(^{2+}\) transient mediated by SOCs in human atrial myocytes, which was inhibited or prevented by La\(^{3+}\). The mRNAs and proteins of STIM1 and Orai1 (components of SOCs), were abundantly expressed in human atria. Co-immunoprecipitation analysis demonstrated an interaction of TRPC1 with STIM1 and/or Orai1. Interestingly, protein expression of TRPC1 and STIM1, but not Orai1, was upregulated in human atria with atrial fibrillation. Our results indicate that novel information that TRPC1 channels not only mediate the nonselective cation current, but also is a component of SOCs in human atria. The upregulation of TRPC1 and STIM1 in human atria with atrial fibrillation may suggest that TRPC1 channels and SOCs are likely involved in the atrial electrical and/or structure remodeling in patients with atrial fibrillation.

TRPC3 Expression Modulates Store-Operated Currents in RBL-2H3 Cells

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TRPC3 was repeatedly discussed as a potential component of store-operated signaling pathways and is expressed in mast cells, which display the classical Orai1-mediated CRAC conductance. To elucidate if TRPC3 interferes with this store-operated conductance, we overexpressed TRPC3 in RBL-2H3 mast cells and characterized currents activated by passive store depletion (20mM Ca\(^{2+}\)). TRPC3 overexpression diminished the inwardly rectifying CRAC current component significantly when free intracellular Mg\(^{2+}\) was kept at a level of 1.6 mM, and this inhibitory effect was more pronounced when free intracellular Mg\(^{2+}\) was elevated to 8 mM. Moreover, store depletion-induced currents were completely abolished with expression of a pore-dead mutant of TRPC3 (E630K). By contrast, the potential STIM1-binding deficient mutant, E697K/E698K lacked effects on CRAC current amplitude. Importantly, at low (1.6 mM) intracellular Mg\(^{2+}\), we observed a reduced Ca\(^{2+}\) selectivity of the store depletion-activated conductance with appearance of a clearly outward rectifying I-V relation. This nonselective conductance was absent at high (8 mM) Mg\(^{2+}\), eliminated by co-expression of the permeation deficient Orai1 E106Q mutation and displayed sensitivity to the TRPC3 blocker Pyr3 (3 μM) as well as to the TRPM7 inhibitor NDGA (10 μM). Biophysical and pharmacological features of the nonselective conductance, which was activated by store depletion at low (physiological) intracellular Mg\(^{2+}\) levels, favor the concept of generation of a unique membrane conductance by TRPC3 overexpression in RBL cells. Our findings suggest a complex interaction of TRPC3 with Orai1 and probably also TRPM7 in plasma membrane microdomains of mast cells. Supported by FWF, DK-MCD

TRPC3 Overexpression Promotes AngiotensinII-Induced Cardiac Dysfunction

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TRPC3 was recently suggested as a player in the development of cardiac hypertrophy. Little is known about the direct proarrhythmogenic role for TRPC3. Here we examined the involvement of TRPC3 in cardiac actions of AngiotensinII (AngII), a pathophysiologically relevant mediator and activator of GPCR/Gq/TRPC3 signaling, using a TRPC3 transgenic mouse model. A comparison of AngII actions in the isolated Langendorff perfused heart of TRPC3+/- (N=5) and wild-type (WT) mice (N=5) revealed that TRPC3 overexpression strongly promoted the impairment of cardiac functions by AngII. Administration of AngII (100nM) reduced left ventricular pressure (LVP) within 2 min to 64 %, +dp/dt to 50 % and -dp/dt to 55 % of control in TRPC3+/- hearts, while these functions remained largely unaffected in WT hearts. Simultaneously ECG recordings demonstrated AngII-induced episodes of acute arrhythmogenicity with ventricular ectopies in all TRPC3+/- hearts (N=6), whereas rhythm of WT hearts (N=6) remained unaffected. Changes in Ca\(^{2+}\) transients and sarcomere shortening were analyzed in isolated ventricular myocytes. AngII (100nM) induced a rise in the diastolic Ca\(^{2+}\) level, which was accompanied by irregular contractions in TRPC3 overexpressing but not in WT myocytes. Our results demonstrate that AngII modulation of cardiac functions is strictly dependent on TRPC3 expression and suggest a key role of TRPC3 channels in AngII-mediated arrhythmogenicity. Supported by FWF, DK-MCD

TRPC Channel Blockers but not TRPC6 Blockers Inhibit Background Calcium Influx and Modulate CalciumTransients in Mouse Muscle Fibers

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We have recently shown that the cation channels TRPC3 and C6 are expressed in mouse skeletal muscle. Both mRNAs could be detected by RT-PCR and immunohistochemical staining revealed the presence of TRPC6 and in part TRPC3 in the sarcolemma of mouse muscle fibers. OAG, an activator of TRPC3/C6 and C7, stimulated background Ca\(^{2+}\) influx, supporting the hypothesis of functional expression of TRPC3 and/or C6 in skeletal muscle. TRPC6 could be pharmacologically activated, however, it does not seem to contribute to background Ca\(^{2+}\) influx, since the specific TRPC6 blocker ML-9 was ineffective. Here we studied whether the unspecific TRPC channel blocker 2-APB and/or the specific TRPC3 blocker Pyr3 affect muscular Ca\(^{2+}\) homeostasis. To investigate divalent cation influx we used single intersseus muscle fibers and applied the Mg\(^{2+}\)-chelate technique. Quench of Fura-2 fluorescence was recorded in the presence of 0.5 mM Mg\(^{2+}\) (excitation at 360 nm). Changes of cytoplasmic Ca\(^{2+}\) were measured using Fura-2 and alternate excitation at 340 and 380 nm. 2-APB inhibited background Ca\(^{2+}\) influx by more then 50% (n=44, p<0.01). The half time of decay of KC1 induced calcium transients was as well significantly influenced by 2-APB (control vs. 2-APB: 3.61 ± 0.18 s vs. 3.18 ± 0.15 s; n=27, 31; p<0.05). The application of Pyr3 resulted in a marked inhibition of Fura-2 quench rate (control vs. Pyr3: 6.4 ± 0.6 vs. 2.5 ± 0.4 %/min; n=46, 46; p<0.01). We conclude that both channels, TRPC3 and TRPC6 are functional in the sarcolemma of isolated mouse muscle fibers. However, TRPC6 has no resting activity, but TRPC3 contributes substantially to the background Ca\(^{2+}\) influx of muscle fibers.

A Self-Limiting Regulation of TRPC3 C6 C7 Channels Linked with Pl(4,5)P2-Diacylglycerol Signaling

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