3619-Pos

Interaction of the Calcium Channel α_2/δ_1 Subunit with an ATP Synthase in the Plasma Membrane of Muscle Cells

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The calcium channel α_2/δ_1 subunit is expressed prior to $Ca_v1.1$ and is localized at the leading edges of young skeletal myotubes, suggesting that it may interact with other cellular components. To address this issue, multi protein complexes were isolated from skeletal muscle of P0 mice and analyzed with blue native PAGE and Western blots. These studies showed that the α_2/δ_1 subunit is found in a large complex of ~670 kDa, which was also present in cultures of skeletal myotubes and C2C12 cells. This complex was isolated and further analyzed with mass spectroscopy. Mascot search results identified the β subunit of the ATP synthase (ATP5β) in the ~670 kDa complex, which is normally present in the mitochondrial membrane. However, the presence of ATP5β and α_2/δ_1 subunit in the plasma membrane was confirmed by biotin labeling of membrane proteins. Immunocytochemistry analysis of skeletal myotubes in culture showed co-localization of α_2/δ_1 subunit and ATP5β in some cells. To determine a functional significance of the interaction between α_2/δ_1 subunit and ATP5β, calcium transients were electrically evoked and recorded from myotubes using confocal microscopy. Exposure of myotubes to an ATP5β monoclonal antibody resulted in a significantly faster decay of calcium transients after a train of stimuli. This effect was less pronounced when myotubes were stimulated with a single pulse. These experiments provide the first direct evidence suggesting that the α_2/δ_1 subunit may be able to form complexes with proteins other than the Cav1.1 and suggest new signaling roles of α_2/δ_1 in muscle cells. In addition, they show that a molecule involved in the synthesis of ATP in the inner mitochondrial membrane can be also localized in the plasma membrane. Supported by the Muscular Dystrophy Association.

3620-Pos

Activity-Dependent Cav1.2 Cluster Surface Expression in Insulin-Secreting Cells

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Background
L-type Calcium channels evoke insulin secretion, and are particularly important for an initial rapid burst of hormone secretion in both rodents and human. The magnitude of this secretory burst depends on the number of Cav1.2 in plasma membrane (PM). However, the regulation of Cav1.2 dynamics in the PM has previously not been elucidadted.
Methods
4D confocal imaging performed in Cav1.2-GFP transfected INS-1 cells. The distance (R) between geometrical cell center and each Cav1.2 cluster, was determined for assessment of Cav1.2 surface dynamics. Flourescence Recovery After Photobleaching (FRAP) and Fluctuation Correlation Spectroscopy (FCS) were used for quantifying Cav1.2 kinetics in PM.
Results
Stimulation with 20mM glucose or 70mM KCl for >15min lead to Cav1.2 cluster internalization as revealed by 4D confocal imaging. After 30min, the average R value decreased from 7.093 ± 0.176 μm and 6.214 ± 0.1082 μm to 5.76 ± 0.202 μm and 5.23 ± 0.156 μm, respectively ($P<0.0001$ and 0.001). The internalized Cav1.2 clusters co-localized with EEA-1, an early endosome marker. Cav1.2 internalization was also detectable by FCS. Stimulation with glucose decreased the number of Cav1.2 clusters in the PM by ~ 40%. In addition, after glucose stimulation Cav1.2 clusters were loss mobile and diffusion times increased from 0.4 ± 0.2s to 0.6 ± 0.2s.
eIF3e (eukaryotic translation initiation factor 3, subunit E) has been implicated in the regulation of activity-dependent Cav1.2 surface expression in neurons. Silencing of eIF3e counteracted Cav1.2 cluster internalization. FRAP experiments demonstrated that ablation of eIF3e increased the time constant of Cav1.2 recovery from 0.79s to 1.50s under resting conditions and from 0.21s to 0.63s in stimulated cells. These effects were associated with reduced insulin secretion, slower cell proliferation and increased apoptosis rates.
Conclusion
Cav1.2 cluster surface distribution in insulin-secreting cells is activity-dependent. Cav1.2 traffic is regulated by eIF3e and affects both cell function and survival.

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Vascular Cav1.2 Expression is Increased in Atherosclerosis

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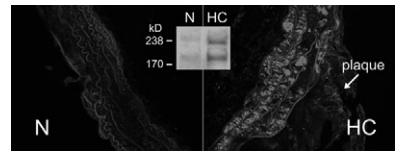
Vascular Ca^{2+} channels play an important role in the pathogenesis of hypertension, but little is known about their role in atherosclerosis. We studied the expression and function of $Ca_V1.2$ in aorta from LDLR -/- mice fed normal or high cholesterol (HC) diet for 26 weeks. HC diet markedly increased serum triglyceride, total cholesterol and phopholipid levels and generated atherosclerotic plaques, but had no significant effect on blood pressure. Immunohistochemistry (Figure) and Western blot analysis (inset) revealed that aortic $Ca_V1.2$ expression was significantly upregulated in mice fed HC diet compared to normal (N) diet. The majority of the $Ca_V1.2$ expression was in vascular smooth muscle cells (VSMCs), rather than atherosclerotic plaques. Despite overexpression of $Ca_V1.2$ subunits, aortic rings from the HC mice had a diminished constrictor response to $Ca_V1.2$ channel activator FPL64176, perhaps a result of reduced expression of the contractile protein α-actin. Inclusion of 1 mg/kg/d rosuvastatin or amlodipine in the HC diet ameliorated all observed changes. These data suggest that during atherosclerosis: (1) VSMCs develop a phenotype switch involving upregulation of $Ca_V1.2$ subunits but downregulation of contractile protein α-actin, and therefore reduced contractility; (2) $Ca_V1.2$ channels may be involved in atherosclerosis.



3622-Pos

Expression of Calcium Channel Subunits in Mesenchymal Stem Cells Undergoing Muscle Differentiation

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Mesenchymal stem cells (MSC) have the potential to generate multiple lineages including muscle. We examined expression of the $Ca_v1.1$ and α_2/δ_1 subunits of the L-type calcium channel and muscle-specific genes in Lin^- bone marrow MSC (BM-MSC) isolated from mouse. BM-MSC were initially cultured in Mesencult media with serum (StemCell Technologies). Multiple passaged and adherent in culture BM-MSC were enriched for c-kit^+ and Sca-1^+ and treated for myogenic differentiation in low serum (2% FBS) media. Total RNA was extracted from cells at different times after differentiation. RT-PCR demonstrated the presence of $Ca_v1.1$ and α_2/δ_1 subunits message after 15 days of differentiation. However, the relative amount of α_2/δ_1 subunit message was consistently larger than for the $Ca_v1.1$ subunit from day 15 to day 26. In addition, several isoforms of the α_2/δ_1 subunit were detected during the same time span. To determine the lineage of BM-MSC differentiation, we examined genes specific for muscle. At day 15 cells expressed myogenin and the cardiac isoforms of troponins T and I, and GATA4. Because cells showed a robust expression of α_2/δ_1 subunit mRNA, we used the α_2/δ_1 subunit monoclonal antibody 20A to sort cells using flow cytometry. Sorted cells cultured for an additional 30 days contracted with electrical stimulation. Cultures from sorted cells showed a similar relationship between $Ca_v1.1$ and α_2/δ_1 subunit as found in unsorted cultures. Cardiac TnT was also detected in these cells. These results show that cardiac and skeletal muscle genes are co-expressed in the population of BM-MSC at early stages of differentiation into muscle cells and suggest that further differentiation into cardiac or skeletal muscle depends on other local factors, such as neighboring cells or extracellular cues.