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Role Of Calmodulin On The Binding Of Alpha Actinin And C-terminus Of $Ca_V1.2$ Ca^{2+} Channel

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$Ca_v1.2$ is the most prominent L-type voltage dependent Ca^{2+} channel in heart and brain constituting ~80% of total L-type channels. L-type channel controls calcium influx and its regulation by alpha-actinin. The exact function of alpha-actinin in the cell is not clear. However, our studies have shown that alpha-actinin is critical for the activity of L-type Ca^{2+} channels as it links membrane to the cytoskeleton. We seek to understand the molecular details of how specific channel subunits or associated proteins, such as alpha-actinin and the ubiquitous Ca^{2+}-binding protein calmodulin, regulate the function of voltage-gated Ca^{2+} channels in brain and heart. In the present study, we employed biochemical and fluorescence anisotropy techniques to identify the exact binding site for alpha-actinin on the cytoplasmic C-terminal tail of the $Ca_v1.2$. Our findings indicate that the residues 1507-1733 of $alpha_1$ subunit serves as a common binding region for alpha-actinin and CaM under Ca^{2+}-depleted condition. Intriguingly, this binding is directly antagonized by Ca^{2+}/calmodulin. Furthermore, we investigated the affinity of alpha-actinin for different peptides within 1534-1697 fragments of Cav1.2 and observed affinities in the high nM range with a peptides corresponding to residues 1614-1635 and 1644-1670. We further observed that these two peptides (1614-1635 and 1644-1670) competitively bind to alpha actinin. We have also investigated the affinities of different domains of alpha-actinin for these different peptides (1588-1675 fragments) of the C-terminal tail of the Cav1.2. Thus suggesting that alpha-actinin may play a role in both the localization of $Ca_v1.2$ and its modulation by Ca^{2+}. This is a critical negative feed-back mechanism that curbs the influx of calcium, which is a potent signaling molecule inside the cell.

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Functional Properties Of The $Ca_V1.2$ Calcium Channel Activated By Calmodulin In The Absence Of $\alpha_2\delta$ Subunits

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Voltage-activated $Ca_v1.2$ calcium channels require association of the pore-forming α_{1C} subunit with accessory $Ca_v\beta$ and $\alpha_2\delta$ subunits. Binding of a single calmodulin (CaM) to α_{1C} supports Ca^{2+}-dependent inactivation (CDI). The human $Ca_v1.2$ channel is silent in the absence of $Ca_v\beta$ and/or $\alpha_2\delta$. Recently, we found that coexpression of exogenous CaM (CaM_{ex}) supports plasma-membrane targeting, gating facilitation and CDI of the channel in the absence of $Ca_v\beta$. Here we discovered that CaM_{ex} and its Ca^{2+}-insensitive mutant (CaM_{1234}) activate the $\alpha_{1C}/Ca_v\beta$ channel in the absence of $\alpha_2\delta$. Coexpression of CaM_{ex} with α_{1C} and β_{2d} in calcium-channel-free COS1 cells recovered gating of the channel and supported CDI. Voltage-dependence of activation was shifted by $\approx$ +40 mV to depolarization potentials. The calcium current reached maximum at +40 mV (20 mM Ca^{2+}) and exhibited $\approx$3 times slower activation and 5 times slower inactivation kinetics compared to the wild-type channel. Furthermore, both CaM_{ex} and CaM_{1234} accelerated recovery from inactivation and induced facilitation of the calcium current by strong depolarization prepulse, the properties absent from the human vascular/neuronal $Ca_v1.2$ channel. The data suggest a previously unknown action of CaM that in the presence of $Ca_v\beta$ translates into activation of the $\alpha_2\delta$-deficient calcium channel and alteration of its properties.

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Inhibition of L-type Ca^{2+} Channel Window Current By Steroid Hormones in Coronary Artery Smooth Muscle Cells

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Small and sustained depolarization induces window current (I_{WC}) through L-type Ca^{2+} channel that supplies Ca^{2+} for arterial contraction. Existence of I_{WC} is expected by voltage dependence of steady-state activation and inactivation. However, I_{WC} of arterial smooth muscle has little been characterized by actual current recording. Dehydroepiandrosterone (DHEA) and DHEA-sulphate (DHEA-S) are most abundant adrenal steroid hormones and epiandrosterone (EPI) is a metabolite of DHEA. We recorded $I_{Ca,L}$ and I_{WC} with 10 mM Ba^{2+} as the charge carrier, performed noise analysis and examined effects of the steroids on I_{WC} and its unitary current in bovine coronary artery smooth muscle cells. EPI and DHEA, but not DHEA-S, slightly inhibited $I_{Ca,L}$ elicited by depolarization from large negative holding potentials. EPI and DHEA but not DHEA-S shifted steady-state inactivation curve slightly to a negative direction and strongly reduced residual availability at depolarized potentials. I_{WC} elicited by staircase depolarizarion consisted of stair-duration of 20 s appeared at -40 mV and increased up to -10 mV. It decreased with further increase of depolarization. Maximal I_{WC} was $23 \pm 2\%$ (n=12) of maximal $I_{Ca,L}$. I_{WC} was associated with marked current noise. I_{WC} and the noise were strongly inhibited by EPI and DHEA, not affected by DHEA-S, and increased by Bay K 8644. Unitary current ($i_{Ca,L}$) and NP_o were obtained from mean and variance of I_{WC}. Uunitary conductance (γ) from $i_{Ca,L}$ was 17.2 pS. EPI and DHEA decreased NP_o and Bay K increased it both without affecting γ. In conclusion, EPI and DHEA inhibit L-type Ca^{2+} channel window current by accelerating inactivation and thus decreasing the number of opened channels.

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Corticotropin-releasing Factor Regulates Somatodendritic Dopamine Release By Inhibiting Voltage-dependent Ca^{2+} Channels

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Corticotrophin-releasing factor (CRF) is the main regulator of the body's stress axis. Even though it is known that CRF plays a key role in the modulation of drug addiction by acting on dopamine (DA) neurons, the mechanism of CRF action on these neurons is not clear yet. In a previous study, we showed that the application of CRF-related peptide, urocortin, reversibly inhibits T-type Ca^{2+} channels via the activation of CRF-R1 from MN9D cells, a model for DA neurons (Kim et al., 20007). Here, we tested the effect of CRF on DA neurons isolated from rat substantia nigra. Urocortin (100 nM) acutely inhibited not only low-threshold T-type Ca^{2+} current, but also high-threshold Ca^{2+} current by 50.0+4.7% via the activation of CRF-R1, similar to MN9D cells. Since Ca^{2+} influx through voltage-activated Ca^{2+} channels (VOCC) plays a pivotal role as the final signal for rapid stimulus-evoked release of neurotransmitters and hormones, we tested the effect of CRF on DA release. We found that DA neurons spontaneously released DA in resting condition. Removing extracellular Ca^{2+}, and the application of blockers for VOCC inhibited the frequency of secretion events. Activation of VOCC by stimulation with high-K^+ containing saline increased the frequency, which were sensitive to blockers for L-, and T-type Ca^{2+} channels. The application of urocortin reversibly inhibited the frequency of DA release by about 40%, while the presence of VOCC blockers prevented further inhibition by urocortin. The presence of antagonist for CRF-R or specific antagonist for CRF-R1 prevented the inhibitory effects of urocortin on DA release. These results indicate that the activation of CRF-R1 induces the decrease of cytoplasmic Ca^{2+} via the inhibition of VOCC, which causes the inhibition of somatodendritic DA release from isolated substantia nigra DA neurons.

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Rab11b GTPase Is Expressed In Ventricular Myocardium And Regulates Trafficking Of L-type Ca2+ Channels

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Alterations in the density of L-type Ca^{2+} channels (LTCCs) have been implicated in several cardiac diseases; however, the mechanisms directing the trafficking of LTCCs have not been well defined. Nearly all steps of intracellular transport are regulated by Rab GTPases, a family of over 60 proteins with varying tissue distributions. The purpose of the present study was to determine if Rab11b, a protein localized to the endocytic recycling compartment, is expressed in the heart, and if it regulates membrane trafficking of LTCCs. RT-PCR using primers specific for Rab11b yielded a 280 base pair product from cDNA prepared from purified mouse ventricular myocytes and Western blot analysis using Rab11b-specific antibody detected a single band at 28 kDa in left ventricular lysate. To test whether Rab11b regulates trafficking of LTCCs, we expressed WT Rab11b or a dominant negative GDP-locked S25N mutant fused to GFP in HEK293 cells along with the pore forming $Ca_v1.2$ and auxiliary β_{2CN4} subunits. L-type currents were recorded in 10 mM Ba^{2+} ($I_{Ba,L}$) using the whole cell ruptured patch clamp technique. Expression of GFP-Rab11bS25N increased mean peak inward $I_{Ba,L}$ from 42 ± 3 pA/pF (n=14) in GFP-expressing control cells to 68 ± 6 pA/pF (n=12), a 64.2% increase ($p<0.005$). Mean peak $I_{Ba,L}$ of GFP-Rab11bWT cells (43 ± 6 pA/pF, n=4) was not significantly different than the GFP control group. To determine whether $Ca_v1.2$ and Rab11b exist in a protein complex, HEK293 cells were transfected with HA-tagged $Ca_v1.2$, β_{2CN4}, and GFP-Rab11bWT or S25N and lysates were used for immunoprecipitation with HA antibody. Interestingly, GFP-Rab11bS25N but not GFP-Rab11bWT associated with HA-$Ca_v1.2$, suggesting Rab11b preferentially interacts with $Ca_v1.2$ in its GDP-bound form. These data demonstrate that a Rab11b-dependent pathway is important for proper maintenance of LTCC density at the surface membrane.