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Methyl-beta-cyclodextrin Attenuates CaV2.3 Channels Modulation By NK1 Receptors

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CaV2.3 channels and neurokinin receptors type-1 (NK1R) participate in pain signaling transmission. Recently, we have shown that CaV2.3 channels, heterologously expressed in HEK293 cells, were inhibited by NK1R through Gq/11 proteins mediated signaling (Meza et al., 2007; Mol Pharmacol 71:284-293). Here, we report that such inhibitory signaling was attenuated by the treatment of HEK293 cells with the cholesterol scavenger agent methyl-beta-cyclodextrin (MbCD). Our results show that MbCD treatment (15 mM/15 min) significantly reduced (47%) inhibition of CaV2.3 channels by NKA (1 microM), a natural agonist of NK1R. Interestingly, MbCD treatment diminished also the membrane capacitance (35%), but it did not affect the CaV2.3 current density. The analysis of macroscopic current biophysical properties (i.e., steady state voltage dependent activation and inactivation, and activation and inactivation kinetics) did not show important modifications induced by MbCD treatment. Our results suggest that MbCD treatment could attenuate the NK1R signaling pathway by depleting membrane cholesterol and, according with the rafts domain hypothesis, by disrupting the signaling complex involved in the inhibitory modulation of CaV2.3 channels by NK1R.

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The Monomeric G Proteins AGS1 and Rhes Selectively Influence Gαi-dependent Signaling To Modulate N-type ($Ca_V2.2$) Calcium Channels

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Activator of G protein Signaling 1 (AGS1) and Ras homologue enriched in striatum (Rhes) define a new group of Ras-like monomeric G proteins whose signaling properties and physiological roles are just beginning to be understood. Previous results suggest that AGS1 and Rhes exhibit distinct preferences for heterotrimeric G proteins, with AGS1 selectively influencing Gαi and Rhes selectively influencing Gαs. Here, we demonstrate that AGS1 and Rhes trigger nearly identical modulation of N-type Ca^{2+} channels ($Ca_V2.2$) by selectively altering Gαi-dependent signaling. Whole-cell currents were recorded from HEK293 cells expressing $Ca_V2.2$ and Gαi- or Gαs-coupled receptors. AGS1 and Rhes reduced basal current densities and triggered tonic voltage-dependent (VD) inhibition of $Ca_V2.2$. Furthermore, each protein attenuated agonist-initiated channel inhibition through Gαi-coupled receptors without reducing channel inhibition through a Gαs-coupled receptor. The above effects of AGS1 and Rhes were blocked by pertussis toxin (PTX) or by expression of a Gβγ-sequestering peptide (masGRK3ct). Transfection with HRas, KRas2, Rap1A-G12V, Rap2B, Rheb2 or Gem failed to mimic the effects of AGS1 and Rhes on $Ca_V2.2$. Our data provide the first demonstration that AGS1 and Rhes exhibit similar if not identical signaling properties since both trigger tonic Gβγ signaling and both attenuate receptor-initiated signaling by the Gβγ subunits of PTX-sensitive G proteins. These results are consistent with the possibility that AGS1 and Rhes modulate Ca^{2+} influx through $Ca_V2.2$ channels under more physiological conditions and thereby influence Ca2+-dependent events such as neurosecretion.

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Identifying molecular mechanisms underlying PKC regulation of Cav1.2

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The regulation of Ca^{2+} influx through the phosphorylation of the L-type Ca2+ channel, $Ca_v1.2$, is important for the modulation of excitation-contraction (E-C) coupling in the heart. $Ca_v1.2$ is thought to be the target of multiple kinases that mediate the signals of both the renin-angiotensin and sympathetic nervous systems. Detailed biochemical information regarding the protein phosphorylation reactions involved in the regulation of $Ca_v1.2$ is limited. The PKC family of kinases can modulate cardiac contractility in a complex manner, such that contractility is either enhanced or depressed, and relaxation is either accelerated or slowed. We have previously reported that Ser^{1928} in the C-terminus of α_{1c} was a target for PKCα, ζ and ϵ phosphorylation. Using GST fusion proteins of all intracellular domains, we have mapped additional phosphorylation sites of several PKC isoforms. We then developed phospho-epitope specific antibodies for these phosphorylation sites to test for regulation in a heterologous expression system and cardiac myocytes. Here, we report the identification of a new PKC phosphorylation site, Ser^{1674} in C-terminus of the $Ca_v1.2$ α_{1c} subunit. Phosphorylation of this site is PKC isoform-specific, as only PKC α, βI, βII, γ, δ and θ, but not PKC ϵ, ζ and η, were able to phosphorylate this site. This site could not be phosphorylated by PKA and PKG *in vitro*. Using a phospho-epitope-specific antibodies to Ser^{1674} (pS1674) and Ser^{1928} (pS1928), we demonstrated that both sites within C-terminus are phosphorylated in HEK cells in response to PMA. Phosphorylation was inhibited with a PKC inhibitor, bisindolylmaleimide. In Langendorff-perfused rat hearts, both Ser^{1674} and Ser^{1928} were phosphorylated in response to PMA. In conclusion, we have identified a new PKC phosphorylation site within the C-terminus of α_{1c}.

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Multiple Mechanisms and Determinants Underlie Rem Inhibition of Voltage-dependent Calcium (Ca_V) Channels

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RGK GTPases potently inhibit Ca_V channels, an effect with critical (patho)physiological implications and potential biotechnology applications. Mechanisms of how RGK proteins inhibit I_{Ca} are poorly understood. Critical ambiguities surround: (1) whether inhibition occurs exclusively by either reducing the number of channels (N) at the membrane, or diminishing the activity (P_o) of channels in the membrane, and (2) the role of RGK protein sub-cellular localization. Whole-cell experiments on recombinant $Ca_V1.2$ channels indicated that Rem inhibited I_{Ca} by two distinct effects: a decrease in N, as gauged by gating charge measurements, and a reduction in effective P_o of channels at the membrane. These two effects were kinetically distinguishable. Replacing the membrane-targeting C-terminus of Rem with the PKCγ C1 domain permitted acutely inducible membrane translocation of Rem[265]-$C1_{PKCγ}$ and subsequent I_{Ca} inhibition by PdBu. Pdbu activation of Rem[265]-$C1_{PKCγ}$ acutely (seconds) inhibited I_{Ca} by selectively reducing effective P_o, while the decrease in N occurred after a longer time scale (hours). A prevailing paradigm is that membrane localization is essential for RGK GTPase inhibition of I_{Ca}. Nevertheless, a Rem C-terminus point mutant, Rem[L271G], that distributes to the nucleus and cytosol, significantly inhibits I_{Ca} leading to a suggestion that nuclear targeting represents an alternative mechanistic mode of action for Rem. However, Rem[L271G] exclusively targeted to the nucleus using a nucleus localization sequence had no impact on I_{Ca}. By contrast, an exclusively cytosolic Rem[L271G], achieved using a nucleus export sequence, essentially ablated I_{Ca}, demonstrating a clear-cut exception to the importance of membrane-targeting for RGK GTPase action on I_{Ca}. Our results reveal that the exceptional potency of Rem in inhibiting I_{Ca} is achieved via an unusual multiplicity of mechanisms and structural determinants.

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Differential Modulation Of Cardiac L-type Calcium Currents By $G\alpha_{i2}$ And $G\alpha_{i3}$

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L-type voltage dependent calcium channel (L-VDCC) activity is chronically suppressed in mouse heart overexpressing the ß$_2$-adrenoceptor (Heubach et al., 2001). We recently demonstrated that this effect is specific for ß$_2$, and not found with ß$_1$-adrenoceptor overexpression (Foerster et al., 2004). Our working hypothesis derived from single-channel analysis is that ß$_2$-adrenoceptors inhibit L-VDCC activity through activation of $G\alpha_{i3}$, but not $G\alpha_{i2}$ protein (Foerster et al., 2003). Here we examine this idea using cardiac myocytes from mice with targeted deletion of the pertussis toxin (PTX)-sensitive and highly homologous $G\alpha_i$ isoforms, $G\alpha_{i2}$ or $G\alpha_{i3}$.

Both $G\alpha_{i2}$ and $G\alpha_{i3}$ are found in cardiac tissue with $G\alpha_{i2}$ being the predominant isoform as revealed by immunoblot and ADP-ribosylation studies. Interestingly, in the absence of $G\alpha_{i2}$, steady-state protein levels of $G\alpha_{i3}$ are increased compared with wild-type levels. In myocytes from $G\alpha_{i2}$ knockout mice, whole cell L-VDCC current density was reduced, consistent with findings from previous single-channel analysis (Foerster et al., 2003). Furthermore, steady state inactivation was shifted to negative voltages and recovery from inactivation was retarded, arguing in favor for modulatory effects rather than a simple adaptation of the channel number. In contrast, myocytes from $G\alpha_{i3}$ knockout mice revealed no alteration of kinetic parameters. Cholinergic inhibition of L-VDCC current after isoprenalin stimulation was intact. However, basal current density was increased in $G\alpha_{i3}$ knockout myocytes. All genotype-related differences were ablated following incubation with PTX for 3 hrs. In conclusion, isoform-specific differential modulation of L-VDCC by $G\alpha_{i2}$ and $G\alpha_{i3}$ was confirmed at the whole-cell current level using mouse knockout models for both proteins. The role of $G\alpha_i$-isoforms in pathological adrenergic overstimulation should be further explored.