uncoupling between the S4S5 linker and the S6 gate. We used two different chimeric approaches to further study the structural determinants of Kv4 channel closed-state inactivation: First, chimeric swapping of S4S5 linker and distal S6 sequences between N-terminally truncated Kv4.2*delta*2-40 A-type channels and non-inactivating *Shaker*IR channels; Second, chimeric insertion of the Kv4.3 cytoplasmic C-terminus or the Kv4.3 T1S1 linker in Kv4.1. The first approach was pursued to possibly prevent inactivation in Kv4.2*delta*2-40 or introduce inactivation in *Shaker*IR. The second approach was pursued to possibly transfer slow Kv4.3 inactivation kinetics to Kv4.1. By two-electrode voltage-clamp on cRNA-injected *Xenopus* oocytes and kinetic analysis of the recorded currents we found that *Shaker* sequences slowed Kv4.2*delta*2-40 inactivation, and that Kv4.2 sequences introduced a novel form of inactivation in *Shaker*IR. Furthermore, we found that, rather than Kv4.3 C-terminal sequences, chimeric introduction of Kv4.3 T1S1 linker sequences made Kv4.1 channels inactivate slower. Our data confirm a model of temporary uncoubling between S4S5 and S6 as a mechanism involved in closed-state inactivation. Furthermore, our data suggest that the T1S1 linker region plays a role in closed-state inactivation.

2704-Pos

Differential Integration of DPLP Protein Variants Regulates Inactivation Kinetics of Neuronal A-Type Current

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Voltage-sensitive potassium channel complexes consisting of Kv4 pore-forming subunits and modulatory subunits mediate the neuronal subthreshold A-type current (I_{SA}), a regulator of membrane excitability and action potential firing patterns. Both the KChIP (Kv channel-interacting proteins) and DPLP (dipeptidyl peptidase-like protein) modulatory subunits are expressed as N-terminal variants, and two DPLP variants (DPP10a, DPP6a) possess the unusual property of inducing similar fast inactivation kinetics of Kv4 channels in reconstitution studies. To investigate whether their effects are similar on native I_{SA}, we characterized I_{SA} from DPP10a-expressing cortical layer II/III pyramidal neurons (CtxPN) and DPP6a-expressing cerebellar granule neurons (CbGN) in rat brain slices. Surprisingly, CtxPN I_{SA} and CbGN I_{SA} differ significantly in their inactivation kinetics. CtxPN I_{SA} undergo mono-exponential decay (τ = ~8 ms); CbGN I_{SA}, bi-exponential decay (at +36 mV: τ-1 = ~11 ms, τ-2 = ~120 ms). While CtxPN I_{SA} resembles heterologously expressed Kv4.2+KChIP3a+DPP10a current, CbGN I_{SA} is not recapitulated by the Kv4.2+KChIP3a+DPP6a current. Since CbGN reportedly also express robust levels of other DPP6 variants competing for Kv4 channels, we quantitated the levels of different DPP6 isoforms by qRT-PCR and determined their percentage contributions: DPP6a (DPP6-E) = ~32%, DPP6b (DPP6-K) = ~42%, DPP6c (DPP6-L) = ~8%, and DPP6d (DPP6-S) = ~18%. Since DPP6a and DPP6b variants together constitute the majority of the CbGN DPP6 species, we performed coexpression studies to investigate whether CbGN I_{SA} is mediated by ternary complex channels with these DPP6 variants. We propose that relative ratios of DPLP auxiliary subunit isoforms likely contribute to the variability of I_{SA} inactivation kinetics between neuronal populations.

2705-Pos

Pyridine Nucleotide Dependence of Kv Beta - Induced Kv Inactivation: Role of Kv Alpha C-Terminus

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Binding of ancillary β-subunits (Kvβ) to the N-terminal T1 domain of Kv1 and Kv4 regulates channel function and localization. The β subunits of Kv channels belong to the aldo-keto reductase superfamily (AKR6). These proteins bind NAD(P)(H) with high affinity, but the mechanisms by which nucleotides regulate channel gating are unclear. Herein we report that when coexpressed with Kv1.5 in COS-7 cells, Kvβ3 shifts the half-activation potential and imparts inactivation to slowly inactivating Kv1.5 current. Addition of NAD(P)H to the patch pipette increased rate and extent of inactivation, whereas $NAD(P)^+$ reduced inactivation. These results conform to a model assuming that NAD(P)(H) binding regulates rate and extent of inactivation synergistically by altering the number of Kvβ monomers involved in inactivation. Deletion of 56 C-terminal amino acids of Kv1.5 (KvΔC56) did not significantly affect Kv association with Kvβ or Kvβ-mediated inactivation. KvΔC56 did not, however, respond to changes in intracellular pyridine nucleotide concentration when co-expressed with Kvβ3 and neither NAPDH nor $NADP^+$ altered rate or extent of inactivation. Glutathione-S-transferase (GST) fusion protein containing peptides from the last 38 (Ile565-Leu602) and 60 (Arg543-Leu602), but not 19 (Asp584-Leu602), amino acids of Kv1.5 C-terminus precipitated Kvβ2 and Kvβ3 in pull-down assays from lysates of transformed bacteria. The C-terminal peptide (GST-C60) also precipitated Kvβ1 and Kvβ2 from mouse brain extracts. The GST-C60 construct did not bind to apoKvβ2, and it displayed higher affinity for Kvβ2:NADPH than for the Kvβ2:$NADP^+$ binary complex. These results suggest that nucleotide binding provides an efficient mechanism to adjust potassium flux in response to metabolic changes. The C-terminal domain of Kv1.5 from Arg543-Asp584 interacts with Kvβ and this interaction may be involved in sensing different conformational states of Kvβ bound to either reduced or oxidized pyridine nucleotides.

2706-Pos

Depenedence of Ependence 6β-Acetoxyl-7α-Hydroxyroyleanone Block of Kv1.2 Channels on C-Type Inactivation

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Voltage-gated K^+ (Kv) channels repolarize excitable cells by providing a pathway for K^+ efflux. Kv channels activate when the membrane is depolarized, and subsequently exhibit slow inactivation (C-type inactivation) during continuous depolarization. A selective pharmacological agent targeting C-type inactivation is so far lacking. Here we reported that 6β-acetoxy-7α-hydroxyroyleanone (AHR), a diterpenoid compound isolated from *Taiwania cryptomerioides* Hayata, could selectively modify C-type inactivation of Kv1.2 channels. Extracellular, but not intracellular, AHR (50 μM) dramatically speeded up the slow decay of Kv currents and left-shifted the steady-state inactivation curve. AHR blocked steady-state Kv currents with an IC_{50} of 17.7 μM and the effects of AHR were completely reversible. AHR did not affect at all the kinetics and voltage-dependence of Kv1.2 channel activation. The degree of block of Kv currents by AHR was independent of the intracellular K^+ concentration. In addition, effect of AHR was much attenuated in a Kv1.2 V370G mutant defective in C-type inactivation. Furthermore, ATP-sensitive K^+ (K_{ATP}) channel, which does not display C-type inactivation, was not affected by AHR. Therefore, block of Kv1.2 channel by AHR did not appear to involve direct occlusion of the outer pore but may depend on the C-type inactivation gate. AHR could thus be a pharmacological tool targeting the C-type inactivation gate of Kv channels.

Ligand-gated Channels-Glutamate Receptors

2707-Pos

TARP Modulation of AMPA Receptor Pharmacology: Polyamine Block and Competitive Antagonism

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External polyamines have been a valuable tool as pharmacological markers of Ca^{2+}-permeable AMPARs (CP-AMPARs). However, recent work in the developing retina has revealed a population of CP-AMPARs which are unexpectedly insensitive to external polyamines. Because TARPs attenuate internal polyamine block, we hypothesized that TARP association with CP-AMPARs would also diminish external polyamine block, and thus display the phenotype observed in the retina. Similarly, TARPs have also been reported to reduce the potency of another useful pharmacological tool: the competitive antagonist CNQX. We therefore examined the impact of TARPs on CP-AMPARs for both of these antagonists using excised patches. We found that TARPs did attenuate internal block, however external block was not substantially reduced. Indeed, the rate of onset of block was actually accelerated. Moreover, TARPs do not significantly reduce CNQX inhibition of peak responses. In the presence of TARPs, CNQX has been reported to