(CSI). By combining fast changes of $[H^+]$ or $[Ni^{2+}]$ with test depolarizations, the kinetics of OSI and CSI were studied to assess the possible kinship of closed-inactivated and open-inactivated states. At −80 mV, the mean onset time constant of CSI (τ_{CSI}) in nominally K^+ -free medium was 3.1, 1.2 and 0.18 s at pH 6.9, 6.4 and 5.4, respectively; τ_{OSI}, measured at 50 mV, and at the same pHs, was 1.2, 0.6 and 0.16 s. With 0.1 mM Ni^{2+}, τ_{CSI} and τ_{OSI} were 7.5 s and 1.8 s; in 10 mM Ni^{2+} these values decreased to 0.6 and 0.4 s, respectively. Following CSI or OSI, either at pH 5.4 or in 0.1 mM Ni^{2+}, recovery in control solution (pH 7.4, Ni^{2+}-free) was biphasic, and the recovery time constants were comparable to those fitted to recovery following OSI in control solution. However, the relative weight of the fast and slow components depended on the ligand used. Following OSI and CSI induced at low pH_o, recovery was dominated by the faster process (τ approx. 1s); with Ni^{2+}, recovery occurred mainly by the slower process (τ approx.10 s). Thus, Ni^{2+} and H^+ cause a concentration-dependent increase of CSI and OSI, with τ_{CSI} approaching τ_{OSI} at high concentrations. Inactivation at low pH_o, either from the open- or the closed state, is to a state that is also visited at pH 7.4 and from which recovery is relatively rapid. The latter conclusion also applies for Ni^{2+}, except that the inactivated state is predominantly that from which recovery is slow.

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Low External pH_o Induces Closed-state And Enhances Open-state Inactivation Of *Shaker*IR Channels Expressed In A Mammalian Cell Line

Yen May Cheng, Steven J. Kehl.
University of British Columbia, Vancouver, BC, Canada.

In fast-inactivation removed *Shaker* (*Shaker*IR) channels, external acidification reduces peak current. Previous studies have suggested that this is due to the low pH_o-induced acceleration of P/C-type inactivation from the open state (OSI). However, recent fluorimetric studies of *Shaker*IR channels suggested that reduced channel availability due to a closed-state inactivation (CSI) process may also be involved, although the time-dependence of this process is unclear. To determine the relationship, if any, between OSI and CSI, we examined the pH_o- and time-dependence of both OSI and CSI of *Shaker*IR channels expressed in an HEK cell line. Consistent with previous findings in *Xenopus* oocytes, both peak current and the time constant of OSI (τ_{OSI}) at +50 mV decreased with reductions in pH_o; the pK_as for these relationships were 4.5 and 5.2, respectively. Thus, at pH_o 4.5, peak current was ~41% of that seen at control pH_o 7.4, while τ_{OSI} decreased from ~1.3 s to ~140 ms. The time constant of CSI (τ_{CSI}) at pH_o 4.5, estimated by monitoring the decrease in the peak current evoked by a pulse to +50 mV after a variable exposure time to pH 4.5 solution at −80 mV, was ~200 ms. Measurements of τ_{CSI} for comparison with τ_{OSI} at other pH_o values are currently in progress.

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Addition of a Charged Residue at Position 363 Focuses the Electric Field Sensed by S4 During Activation of *Shaker* K-channels

Vivian M. Gonzalez Perez, Katherine Stack, Katica Boric, Tania Estevez, David Naranjo.
Centro de Neurociencia de Valparaiso, Valparaiso, Chile.

Voltage-gated potassium channels contain a voltage sensor domain in each of its four subunits that confers exquisite sensibility to the trasmembrane electric field. The fourth transmembrane segment (S4) of a *Shaker* K-channel subunit has seven highly conserved basic amino acids, periodically spaced every two hydrophobic residues. The four outermost arginine residues (R1-R4) in S4 move through most of the electric field upon depolarization. This charge movement (12-13 e0 per channel) is tightly coupled to the conformational change leading the opening of the potassium conduction pathway. Any residue located within the R1-R4 limits would be dragged through the electric field, and if charged would contribute to the charge movement. Surprisingly, when we replaced V363 (located between R1 and R2) by either an arginine or an aspartate, both charge adding mutations decreased the effective valence of opening by 50%. This reduction in effective valence was not attributable to an uncoupling between charge movement and channel opening because the Q-V curve remained preceding the G-V curve. To test if the added charges promote a remodeling of the electric field sensed by S4, we assessed the state-dependency of external accessibility of R1 or R2 in the presence of the additional charge. Then we tested the accessibility to methanethiosulfonate derivates in R1C/V363R, R1C/V363D, R2C/V363R or R2C/V363D double-mutants. We measured the rates of cysteine modification within a ten-fold change in the overall open probability for each mutant. Unlike the R1C control, the modification rates were not state-dependent for R1C/V363X double-mutants, while they remained state-dependent for R2C/V363X double-mutants as the R2C control. These results suggest that in channels carrying a charged residue at position 363, R362 remains outside the field during voltage dependent activation.

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Gating Differs Between an Antarctic and a Tropical K_v1 Channel Because of RNA Editing

Sandra C. Garrett-Rodrigues, Joshua J.C. Rosenthal.
University of Puerto Rico, San Juan, PR, USA.

In squid, shaker-like potassium channel mRNA's are extensively edited by adenosine deamination. Because A-to-I RNA editing tends to recode for smaller amino acids, it could create more flexible proteins and be involved in cold adaptation. In this study we compare editing patterns and their functional consequences for a K_v1 K^+ channel mRNA from two species of octopus: *Pareledone sp*., from the extreme cold of Antarctica and *O. vulgaris* from tropical inshore waters of Puerto Rico. From each species, the same K_v1 gene was cloned and sequenced. An editing map was created by sequencing 50 cDNA clones and identifying sites with A/G variation. At the genomic level, the channels were nearly identical, differing by four amino acids. By contrast, their editing patterns differed substantially. In total there were 13 non-silent editing sites, with five sites unique to one species or the other. At four sites editing percentages differed by more than 50% between species: N40S and S54G are edited more in *O. vulgaris* while N105G and I321V are edited more in *Pareledone*. We tested the eletrophysiological effects of differences at both the genomic and RNA editing levels. Channels were expressed in *Xenopus* oocytes and their kinetics of activation, deactivation, and inactivation, along with their voltage-dependence, were compared. The two genomically encoded channels were nearly identical, although the activation kinetics were slightly faster for the Antarctic channel. Individual editing sites, however, changed multiple parameters. The ''tropical'' edits N40S and S54G, both in the tetramerization domain, slowed activation and accelerated inactivation. The ''Antarctic'' edit I321V shifted the voltage dependence of activation by +12 mV and more than doubled the rate of deactivation. I321V, which is in the S5 helix, appears to be a candidate for cold-adaptation.

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Closed-State Inactivation in Kv4.3 Splice Forms is Differentially Modulated by Protein Kinase C

Chang Xie[1], Vladimir Bondarenko[2], Michael J. Morales[1], **Harold C. Strauss[1]**.
[1]UB, SUNY, School of Medicine, Buffalo, NY, USA, [2]Georgia State University, Atlanta, GA, USA.

Kv4.3, with its complex open- and closed-state inactivation (CSI) characteristics, is a primary contributor to early cardiac repolarization. The two alternatively-spliced forms of Kv4.3 (L and S) differ by the presence of a 19 amino acid exon 81 amino acids downstream from the sixth transmembrane segment. The two isoforms are reported to be kinetically similar, however, the longer form has a unique PKC phosphorylation site. To test the possibility that inactivation is differentially regulated by phosphorylation, we stimulated PKC in *Xenopus* oocytes expressing Kv4.3 isoforms and examined their inactivation properties. There was no difference in open-state inactivation, there were profound differences in CSI; in Kv4.3-S, PMA (10nM) reduced the extent of CSI from 0.53 ± 0.06 to 0.69 ± 0.05 after 14.4 s at −50 mV. In contrast, CSI in Kv4.3-L increased from 0.71 ± 0.05 to 0.51 ± 0.03 under the same conditions. Mutation of the unique putative PKC phosphorylation site in Kv4.3-L eliminated its isoform-specific behavior. This effect was independent of the intervention used to increase PKC activity; identical results were obtained with either PMA or injected purified PKC. Our data demonstrate that Kv4.3 can be differentially regulated by PKC, and that the carboxy terminus of Kv4.3 plays an important role in regulation of CSI.

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Inverse Modes of Coupling in Leak and Voltage-activated K^+ Channel Pore Gates Underlie their Distinct Roles in Electrical Signaling

Yuval Ben-Abu[1], Yufeng Zhou[2], Noam Zilberberg[1], **Ofer Yifrach[1]**.
[1]Ben-Gurion University of the Negev, Beer-Sheva, Israel, [2]Yale University, New haven, CT, USA.

Voltage-activated (Kv) and leak (K_{2P}) potassium channels play key, yet distinct roles in electrical signaling in the nervous system. Here, we examined how differences in the operation of the activation and slow inactivation pore gates of Kv and K_{2P} channels underlie their unique roles in electrical signaling. We report that (1) leak potassium channels possess a lower activation gate, (2) the activation gate is an important determinant controlling the conformational stability of the K^+ channel pore, (3) the lower activation and upper slow inactivation gates of leak channels cross-talk and (4) in contrast to Kv channels, where the two pore gates are negatively-coupled these two gates are positively-coupled in K_{2P} channels. Our results thus demonstrate how basic thermodynamic properties of the K^+ channel pore, particularly conformational stability and coupling between the pore gates, underlie the specialized roles of Kv and K_{2P} channel families in electrical signaling.