channel which shows a sigmoid dependence of *Po* on applied pressure, a mean conductance of 17pS, and is Ca^{2+} modulated (*140mM*KCl, *10mM*NaCl, *1mM*$CaCl_2$, *pH*7.0)(HEMKCA)(1,2), and had proposed a new hypothesis for the senescent process of hRBC with this channel as the responsible for the molecular clock. This channel presented an inactivation process producing an exponential decay of *Po* (τ=4.55 ± 1.95min). Here we present a complete kinetically characterization of this inactivation process: intriguingly, this process seemed to begin just when a voltage step is applied and ionic current started, suggesting that the activation process is necessary but not sufficient to allow the inactivation development. We had characterized the burst mode activity of this channel (17.43 ± 17.15 events/burst, 264.27 ± 291.3ms burst duration and 15.67 ± 7.1ms intraburst interval). The decay in *Po* produced by the inactivation seemed to be the effect of decay in the number of events per burst, probably related to burst duration decay, with no effect in intrabursts variables like intraburst intervals and intraburst mean duration. We present a complete kinetic model for this channel with two independent kinetic branches: one for the non-inactivated mode and the other for the inactivation pathway. This inactivation mechanism is presented as a molecular damper (security system) in our new hypothesis for the hRBC senescence.

(1)(2005) Biophys. J.88(1):593
(2)(2008) Biophys. J.91(1):1101
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1687-Pos

Deletion Analysis of the Mechanosensitive TREK-1 Channel

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TREK-1, the first functionally characterized mechanosensitive K channel from the two-pore family (K2P) is involved in protective regulation of resting potential in CNS neurons and many other tissues. The structural basis of TREK-1 sensitivity to stretch and other factors such as arachidonic acid (AA) and anesthetics remains unknown. Attempts to use existing K channel structures as templates for TREK-1 modeling have identified several motifs that are not present in canonical K channels, which include divergent cytoplasmic N- and C-termini, and a characteristic 50-residue extracellular loop in the first homologous repeat. To characterize functional roles of these domains, we analyzed TREK-1 deletion and cysteine mutants in patch-clamp experiments. In response to steps of suction, the control TREK-1-EGFP fusion protein expressed in HEK-293 cells produced transient currents in cell-attached patches and non-inactivating sustained currents upon patch excision. Responses in both configurations were augmented by AA. Deletion of the extracellular loop (Δ76-124) reduced functional surface expression of channels and increased background activity, but the activation by tension augmented by AA was fully retained. Further deletion of the C-terminal end (Δ76-124, Δ334-411) produced no additional effect. In an attempt to generate cysteine-free version of the channel, we additionally mutated two cysteines in the transmembrane domain. C219A did not compromise channel activity, whereas C159A/S was essentially inactive. Experiments in the presence of mercaptoethanol suggested that none of these cysteines form functionally-important disulfides. The functional deletion mutant without C219 is now topologically closer to other K channels and makes an amenable system for homology modeling and testing by disulfide cross-linking.

1688-Pos

Hot Or Hot? Differentiating the Effects of Heat and Capsaicin on the TRPV1 Channel

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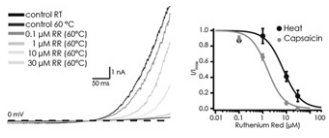
The temperature-sensitive ion channel family is receiving increasing attention as a potential contributor to pain states. In particular, ion channels activated by noxious heat, for example the TRPV1 channel, could provide a novel target for the treatment of chronic pain.

Using an automated patch clamp system, TRPV1 receptors expressed in CHO cells were activated using either noxious heat or the ligand capsaicin. Antagonists can be used to block either the heat- or capsaicin-activated TRPV1 response. Since blocking the heat response of TRPV1 can have undesirable effects on core body temperature in animals and humans, being able to discriminate between antagonising the ligand-activated vs. the heat-activated response of TRPV1 channels may be important for discovering novel compounds with a reduced side-effect profile. Data will be shown of TRPV1 currents activated by increasing temperature and block of the heat-activated response by ruthenium red. Data will also be presented showing different antagonist profiles for ligand- and heat-activation of TRPV1.



1689-Pos

Heat and Capsaicin Activate TRPV1 Channels to Different Open States:

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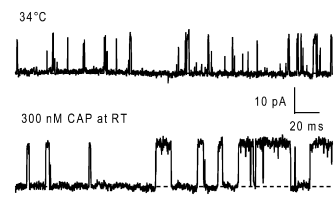
Both heat and capsaicin can strongly activate TRPV1 channels. However, the underlying mechanism by which the activation gate responds to these two distinct stimuli is unknown.

We use single-channel recordings of the wildtype and mutant TRPV1 channels with large unitary conductance to compare channel activation driven by each of the gating modalities. As shown in the figure, the heat-induced openings (top trace) exhibit much shorter open times than those of ligand-induced channel openings (bottom trace). In addition, we observe that at saturating concentrations capsaicin can only partially open TRPV1 channels. Raising temperature in the presence of capsaicin leads to an increase in open probability.



These results demonstrate that heat and capsaicin gate the channel to different open states, suggesting that they do not share the same activation pathway.

1690-Pos

Study Heat-Induced TRP Channel Activation Using Near-Infrared Laser As a Heat Source

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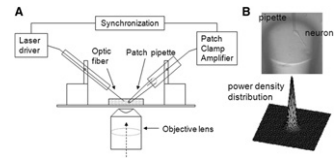
Rapid and reliable temperature change is required for the study of temperature-dependent channel gating mechanisms. We use continuous near-infrared (CNI) laser as a heat source to rapidly increase the local temperature at a cell or a cell-free patch membrane containing temperature-sensitive TRP channels. The photothermal effects of laser irradiation can be characterized by transient changes in fluorophore emission, liquid junction potential, and function of membrane proteins. The CNI laser-based method is advantageous over perfusion-based heating methods mainly in speed but also in its reproducible temperature raising profile and minimal interference with perfused solutions containing channel agonists/antagonists.



1691-Pos

The Proximal C-Terminal Region of TRPV1 Controls Phosphoinositide Selectivity

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The lipid messenger PIP2 is a critical modulator of multiple membrane proteins, including TRPV1 ion channels. We have previously reported that PIP2 is an important cofactor for activation of TRPV1. A critical question to elucidate the molecular mechanism of activation is where in the channel is the binding site for PIP2. Here we report that a single amino-acid mutation, located in a region of the C-terminus proximal to the transmembrane domains of TRPV1, inverts selectivity of these channels for phosphoinositides by making PI(4)P a stronger activator than PIP2. An *in vitro* FRET-based binding assay shows this proximal site is capable of binding PIP2. In addition, the distal C-terminal region, previously proposed as a candidate site for PIP2 binding, is not required for PIP2 regulation. We also addressed a recent report suggesting that an integral membrane protein called Pirt acts as the PIP2 sensor for regulation of TRPV1. Pirt expression did not appear to alter TRPV1 apparent affinity for PIP2. In summary, these results implicate the C-terminus proximal site as a PIP2 interacting domain. More importantly, PIP2 binding to this proximal site is central to TRPV1 activation.