whether Cav1.2 channels are mechanosensitive and the possible role of integrins in this process, patch clamp methods were used to investigate the properties of either native or heterologously expressed Cav1.2 cannels by stretch of single cells plated onto a flexible substrate. Thin silicone membranes were coated with either fibronectin (FN) or poly-l-lysine (PLL) to assess integrin-dependent and -independent responses, respectively, and stretched using two blunt micropipettes driven in equal and opposite directions by piezoelectric translators. Graded stretch to 130% of resting cell length induced graded increases in Cav1.2 current (up to 63%) in HEK 293 cells expressing the neuronal channel isoform (Cav1.2c). The increase in current was ~2-fold greater for cells adhering to FN than for cells on PLL. On FN, 130% longitudinal stretch of primary VSM cells induced ~50% increases in Cav1.2 current. However, the magnitude of stretch-activated Cav1.2 current was the same on FN or PLL for cells expressing a Cav1.2 construct containing two C-terminal mutations (Y2122F/S1901A) to prevent phosphorylation by PKA and c-Src, or for cells expressing a Cav1.2 construct with the C-terminus truncated. Our results suggest that the Cav1.2 channel can be potentiated by membrane stretch, with one component due to intrinsic mechanosensitivity of the channel and a second component due to signaling through an integrin-dependent process.

1682-Pos

Dissecting the Molecular Mechanism of How Force Activates Yeast TRP Channel TRPY1

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Though clear for the prokaryotic mechanosensitive (MS) MscL and MscS, how mechanical force activates eukaryotic MS channels remains poorly understood. Several members of transient receptor potential (TRP) channel family are apparently involved in animal mechanosensations and are therefore promising MS-channel candidates. The yeast homolog, TRPY1, expressed in the yeast vacuolar membrane, can clearly be gated by hyper-osmotic shock *in vivo* and by directly stretching excised vacuolar membrane patches under patch clamp. Here, we investigated the structure-function relationship of TRPY1 by mutagenesis, aiming at dissecting how force is sensed by TRPY1 and how force opens the channel gate. TRP channels share general organization with well-studied voltage-gated potassium channels, being tetramers with each subunit consisting of six transmembrane helices (S1-S6) and N- and C-terminal cytoplamic domains. We found that the C-terminal cytoplasmic domains of TRPY1 harbor Ca^{2+} binding motifs, which confer TRPY1 Ca^{2+} activation. We demonstrated that the Ca^{2+} activation and the force activation are synergistic and the two gating mechanisms act in parallel. We proposed that force is perceived by the transmembrane domains. Our further in-depth analyses showed that strategic insertions of long peptide linkers before S4-S5 linker and after S6 can surprisingly yield functional channels with largely intact mechanosensitivity, highlighting the crucial roles of the pore module in TRPY1 mechanosensitivity. Together with our detailed scanning mutagenesis, we will discuss possible molecular mechanisms on how force activates TRPY1.

1683-Pos

Inactivation of the Bacterial Mechanosensitive Channel MscL Involves Flexible Transmembrane Helices and a 'Dry' Gate

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MscL, a mechanosensitive channel of large conductance, is an emergency release valve residing in the cytoplasmic membrane of E. coli. Under osmotic shock, when membrane tension approaches the lytic limit of ~10 mN/m, MscL opens a 3 nm pore relieving osmotic stress. The conformational transition in the pentameric complex was previously envisioned as a tilting iris-like motion of tightly coupled pairs of the centrally located TM1 and peripheral TM2 helices. Wetting the hydrophobic constriction formed by the rings of L19 and V23 was identified as is the rate-limiting step over a barrier of more than 50 kT. While adaptation of MscL was reported, the channel was generally considered non-inactivating. Special pressure protocols involving prolonged conditioning steps and short saturating test pulses revealed that after a 30 s exposure to half-saturating pressure (p0.5) in spheroplast patches, about 20% of MscL population reversibly inactivates. The channels return to the resting state within 1 s upon pressure release. Introduction of a flexible double glycine motif (A91G/I92G) in TM2 dramatically increased the rate of inactivation resulting in a 90% silent channel population after a 10 s step to p0.5. Single-channel traces revealed a split of concerted 70 pA opening transitions into a staircase of irregular ~7 pA substates in the double glycine mutant. The additional hydrophilic substitution in the constriction (V23T/A91G/I92G) pre-hydrates the pore, reduces p0.5 by ~55% and, while generating multiple substates, completely abolishes inactivation. In extrapolated-motion simulations TM2 kinks at A91G/I92G and its C-terminal end separates from TM1. We propose that in WT MscL TM1s and TM2s are tightly coupled, whereas in the double glycine mutant the unsupported TM1s may reform the tight hydrophobic seal independent of the positions of TM2s thus creating a tension-insensitive non-conductive state.

1684-Pos

Caveolin and Cholesterol Control of Mechanosensitive Channels in Muscle

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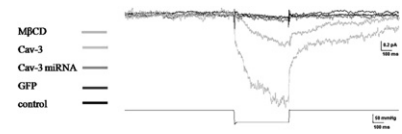
In Duchene muscular dystrophy the loss of the cytoskeletal element dystrophin modifies the localization of sarcolemma components and the stress distribution to those components. There is a change in the distribution of caveolae and dysregulation of mechanosensitive ion channels (MSCs) both of which may contribute to the elevated Ca2+ levels present in dystrophic myotubes. Caveolae are curved cholesterol rich membrane structures associated with dystrophin that contain many signaling molecules. We have shown that overexpression of Caveolin-3 in mouse myotubes increases the incidence of MSCs in patches and average MSC current. However, it has no effect on their kinetics of activation. Cholesterol depletion by MβCD produces an even larger increase in MSC incidence and average patch current, and also significantly decreases the relaxation rate of the membrane as observed by membrane capacitance changes. We have used miRNA against TRP channels (TRPC1, TRPC4, TRPC6, TRPV2) that have been reported to mechanosensitive in various systems to try and determine the identity of the channel revealed by cholesterol depletion. Average patch current and immunofluorescence staining has been used to determine the level of knockdown of the individual TRP channel subunits.



1685-Pos

Clustering and Functional Interaction of MscL Channels

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Mechanosensitive channels allow bacteria to respond to osmotic stress by opening a nanometer size pore in the cellular membrane. While the underlying mechanism has been studied intensively on the basis of individual channels, the work described here sheds light on the behavior of an ensemble of mechanosensitive channels of large conductance (MscL) in the membrane. Evaluating the spatial distribution of MscL channels in the bilayer using patch clamp, fluorescence, neutron scattering and reflection techniques, as well as atomic force microscopy and mathematical modeling, MscL was found to form clusters under a wide range of conditions. Within the cluster, MscL is closely packed, but still active and mechanosensitive. The channel activity, however, is modulated by the presence of neighbouring proteins, indicating functional protein-protein interactions. Collectively, the results demonstrate a potential functional role for self-assembly of MscL in the membrane.

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1686-Pos

A Kinetic Characterization of the Human Erythrocyte Mechano-Activated K^+ Channel Inactivation Process

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Human erythrocyte (hRBC) shows a live span of 120 days, as these cells lack nucleus and organelles a question arises: which is the subjacent molecular process to this tightly controlled programme cell death (the biological clock)? It has been proposed that the increased Ca^{2+} concentration characteristic of the senescent cells is due to a mechanical stress at the microcirculation level. Using the Patch Clamp Technique, we had characterized a mechano-activated K^+