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The canonical transient receptor potential channel 1 is an essential structural component of the mechanosensitive calcium permeable channel in vertebrate cells

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The mechanosensitive cation channel (MscCa) transduces membrane stretch into cation (Na^+, K^+, Ca^{2+} and Mg^{2+}) flux across the cell membrane, and is implicated in cell volume regulation, cell locomotion, muscle dystrophy and cardiac arrhythmias (Hamill & Martinac, 2001). However, the membrane protein(s) forming the MscCa in vertebrates remains unknown. Here we use an identification strategy based on detergent-solubilizing of frog oocyte membrane proteins followed by liposome reconstitution and evaluation by patch-clamp (Sukharev *et al.*, 1993; Maroto *et al.*, 2005). The oocyte was chosen because it expresses the prototypical MscCa ($\geq 10^7$ MscCa/oocyte) that is preserved in cytoskeleton-deficient membrane vesicles (Zhang *et al.*, 2000). We identified a membrane protein fraction that reconstituted high MscCa activity and showed an abundance of an 80 kDa protein identified immunologically as the canonical transient receptor potential channel 1 (TRPC1) (Wes *et al.*, 1995; Brereton *et al.*, 2000). Heterologous expression of the human TRPC1 resulted in a > 1000% increase in MscCa patch density, whereas injection of a TRPC1-specific antisense RNA abolished endogenous MscCa activity. hTRPC1 transfection of CHO-K1 cells also significantly increased MscCa expression. These observations indicate that TRPC1 is a component of the vertebrate MscCa, which like various prokaryotic Ms channels (Martinac & Kloda, 2004), is gated by tension developed in the lipid bilayer.

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Sensing pressure with K_{2P} channels

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The K_{2P} channels are highly conserved from *C. elegans* to humans. They are structurally distinct from other K^+ channel family members, with four transmembrane segments and 2P domains in tandem. K_{2P} channels are homo- or hetero-dimers that play a dominant role in cell electrogenesis, controlling the resting membrane potential and the action potential duration.

The K_{2P} channel TREK-1 is predominantly expressed in the central and peripheral nervous system, with a particularly strong expression during early development. TREK-1 is activated by membrane stretch as well as cell swelling. Mechanical force is transmitted directly to the channel via the lipid bilayer. Moreover, intracellular acidosis strongly sensitizes TREK-1 to membrane stretch, leading to channel opening at atmospheric pressure.

TREK-1 is reversibly opened by polyunsaturated fatty acids, including arachidonic acid (AA). Activation of TREK-1 by AA in the excised patch configuration indicates that the effect is direct by interacting either with the channel protein or by partitioning into the lipid bilayer. Additionally, TREK-1 channel activity is reversibly stimulated by volatile general anaesthetics including halothane.

The recent invalidation of TREK-1 in a mouse model demonstrates that this K^+ channel is important for neuroprotection against epilepsy and ischemia. Furthermore, TREK-1 -/- mice are also more resistant to volatile general anaesthetics, indicating a key role for TREK-1 in the mechanism of general anaesthesia.

Mutagenesis studies have demonstrated that the cytosolic carboxy terminal domain of TREK-1 plays a key role in TREK-1 gating. Protonation of a key residue in this region, E306, leads to channel activation. Interaction of the carboxy terminal domain of TREK-1 with the inner leaflet phospholipids including PIP_2 is critical for channel activity and is controlled by a cluster of cationic residues. Conversely, down-modulation of TREK-1 is achieved by receptor- coupled protein kinase A phosphorylation of residue S333.

In conclusion, the TREK channels are polymodal K^+ channels that integrate multiple physical and chemical stimuli.

Varieties of mechanotransduction: the cytoskeletal stress fibre as a force transmitter and a mechanosensor

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It has been established that bacterial mechanosensitive (MS) channels are activated directly by stress in the membrane. However, whether eukaryote MS channels need some other accessory proteins, typically cytoskeletons, for their activation has been a pending problem. Theoretically the cytoskeleton would be a more efficient force transmitter than the membrane owing to its larger elastic modulus. It is likely that higher organisms have utilized such a device to increase the sensitivity of their MS channels. However, no direct evidence has been provided to show the role of cytoskeleton in MS channel activation.

We have developed two methods by which we can stretch the actin-based cytoskeleton (stress fibres) while monitoring MS channel activities either by Ca^{2+} influx or whole cell currents measurements in cultured endothelial cells (HUVECs). In one method a fibronectin coated glass bead, which is attached on the apical cell surface and connected to the basal focal adhesions *via* stress fibres, was mechanically moved to stretch the attached stress fibres. In the other method, phalloidin coated beads microinjected into the cell where they attached to stess fibres. One of these was pulled with laser tweezers to stretch the attached stress fibre. In either way, we could consistently record stretch activated currents and Ca^{2+} transients that originated from the activation of cation selective MS channels carried by HUVECs. The force required for a single MS channel activation was estimated as low as 1-2 pN. Ultra fast near field Ca^{2+} imaging resolved the Ca^{2+} influx spots across individual MS channels near basal focal adhesions. Simultaneous imaging of integrin molecules indicated that MS channels are located near integrin molecules as close as a few hundred nm. Forces originally created by membrane deformation and transmitted through a stress fibre/integrin complex seem to activate MS channels.

MS channels serve as a typical fast mechanosensor, however, they are not the only mechanosensor in the cell. Turning to slowly going mechanotransduction, we can see another world of mechanosensors. Endothelial cells *in situ* exhibit a spindle-like shape, aligning their long axis running along the vessel. They lose this characteristic shape when cultured in dish. However, they recover their original shape and alignment when subjected to uniaxial cyclic stretch (20% at 1Hz) that mimics circumferential cyclic stretch in the vessel. In other words, cells can detect the direction of applied forces and convert this information into their morphology. We found that Ca^{2+} influx *via* MS channels was indispensable for this mechanically induced cell shape change, but that the Ca^{2+} signal by itself could not indicate the direction of the force to the cell. As the stress fibres in the stretch axis were preferentially disrupted by cyclic stretch in a few minutes and then reorganized perpendicular to the stretch axis, we suspected that the stress fibre, particularly its major component actin fibre, might be a force direction sensor. To test this hypothesis in a more direct way, we examined the dynamics of single actin fibres in response to mechanical stretch. Surprisingly, relaxing but not stretching an actin fibre caused its rapid depolymerization with an aid of cofilin, a soluble actin-depolymerizing factor. In living cells disruption of stress fibres caused by actin fibre depolymerization activates several downstream signal molecules around the focal adhesion, which eventually leads to a cell shape change. In this sense, the stress fibre (actin fibre) is eligible as a mechanosensor.

In conclusion, the stress fibre serves as a force transmitter in fast mechnotransduction, and acts as a mechanosensor with direction sensitivity in slow mechanotransduction.

Role of tryptophan residues in ion channel function

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(Introduced by David Adams)

The linear peptide gramicidin forms prototypical ion channels specific for monovalent cations and has been extensively used to study the organization, dynamics and function of membrane spanning channels. The role of tryptophan residues is a key issue in ion permeation by gramicidin (Becker *et al.*, 1991). We have monitored the organization and dynamics of gramicidin tryptophans in various types of microheterogenous molecular assemblies using wavelength-selective fluorescence and other approaches (Rawat *et al.*, 2004; Kelkar & Chattopadhyay, 2005). Taken together, these results provide comprehensive information on the dynamics of the functionally important tryptophan residues of gramicidin. Experiments using synthetic analogues of gramicidin containing single tryptophan residues further help to delineate the crucial role of tryptophan in maintaining the ion conducting conformation of gramicidin.

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