Mechanosensitivity is mediated directly by the lipid membrane in TRAAK and TREK1 K⁺ channels

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Mechanosensitive ion channels underlie neuronal responses to physical forces in the sensation of touch, hearing, and other mechanical stimuli. The fundamental basis of force transduction in eukaryotic mechanosensitive ion channels is unknown. Are mechanical forces transmitted directly from membrane to channel as in prokaryotic mechanosensors or are they mediated through macromolecular tethers attached to the channel? Here we show in cells that the K⁺ channel TRAAK (K2P4.1) is responsive to mechanical forces similar to the ion channel Piezo1 and that mechanical activation of TRAAK can electrically counter Piezo1 activation. We then show that the biological origins of force transduction in TRAAK and TREK1 (K2P2.1) two-pore domain K⁺ (K₂P) channels come from the lipid membrane, not from attached tethers. These findings extend the "force-from-lipid" principle established for prokaryotic mechanosensitive channels MscL and MscS to these eukaryotic mechanosensitive K⁺ channels.

Significance

Mechanical force opens mechanosensitive ion channels in the cellular membrane to produce electrical signals that underlie sensation of touch, hearing, and other mechanical stimuli. An unanswered question is: How are mechanical forces transmitted to eukaryotic mechanosensitive channels in the membrane? We show that two mechanosensitive ion channels in eukaryotes, TRAAK (K2P4.1) and TREK1 (K2P2.1), are directly opened by mechanical force through the lipid membrane in the absence of all other cellular components. This finding extends the "force-from-lipid" paradigm established in bacterial channels to TRAAK and TREK1, eukaryotic mechanosensors.

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Mechanosensation encompasses the host of processes that cells have evolved to sense and respond to mechanical forces ubiquitous in biology.Mechanosensation underlies our sense of touch, hearing, and balance as well as our ability to regulate blood and osmotic pressures. Despite their broad importance, the molecules involved in mechanosensation have been largely difficult to identify and characterize. Mechanosensitive ion channels are cells’ fastest mechanosensors and translate mechanical forces into cellular electrical signals to produce rapid neuronal responses to mechanical stimuli. Whereas the list of eukaryotic ion channels implicated in mechanosensation continues to grow (1–7), a fundamental question remains: How do these channels sense force?

Mechanical force gating of ion channels can in principle occur either directly through the lipid bilayer or through accessory tether-forming proteins (8). Lipid bilayer-mediated gating can occur if a force induces tension in the cellular membrane, which can provide a tension-dependent energy difference between closed and open conformations. Tether-mediated gating can occur if a mechanical stimulus is transmitted along accessory proteins or other macromolecular structures (cytoskeletal or extracellular matrix) that are attached to the channel (8, 9). Only the bacterial mechanosensitive channels MscL and MscS have been demonstrated rigorously to undergo lipid bilayer-mediated gating via membrane tension (10). Using a reconstituted system of purified channel protein in defined lipids, these channels were mechanically activated by membrane tension induced with pressure applied to the patch pipette (11–14). Difficulties in high level expression, purification, and reconstitution have precluded such an analysis of mechanosensitivity in eukaryotic ion channels with the same rigor as applied to MscL and MscS (15, 16). Whereas mechanosensitivity of eukaryotic channels has been demonstrated by poking cell membranes under whole-cell voltage clamp and by pressure activation of channels in patches excised from cells or membrane blebs from cells (17), these experiments have not distinguished a direct membrane-mediated mechanism from other mechanisms that would rely on additional macromolecular components inescapably present in the cell-based assays.

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stimulation of N2A cells under current clamp by cell poking resulted in strong membrane depolarization and an action potential-like spike in membrane voltage at sufficient stimulus intensity (Fig. 1E). However, expression of TRAAK in N2A cells caused them to respond differently to mechanical force: instead of a large depolarization, a small biphasic change in membrane potential was observed, an initial hyperpolarization, and then a depolarization (Fig. 1F). This electrical behavior is consistent with underlying and opposing mechanosensitive activities of TRAAK and Piezo1, each with slightly different gating kinetics. Based on these results, we conclude that TRAAK and Piezo1 are mechanically activated by similar perturbations of the cell membrane.

Fig. 2 shows results from the cell-poking assay applied to CHO cells expressing representative members (at least one) from each of the major branches of the K2P channel family [TRAAK, TREK1 (K2P2.1), TREK2 (K2P10.1), TWIK2 (K2P6.1), TASK3 (K2P9.1), THIK1 (K2P13.1), TRESK (K2P18.1), TASK2 (K2P5.1), and TALK1 (K2P16.1)] (Fig. 2 A–J). Only small nonselective, nonmechanosensitive currents are observed in control cells (Fig. 2K).

Whereas the channels tested all expressed K+ selective current, only TRAAK, TREK1, and TREK2 are mechanosensitive in this assay. Thus, mechanosensitivity is not a general property of all K2P channels. Some aspect of TRAAK and TREK channels enable them to sense mechanical forces in the cell-poking assay.

Biophysical Origins of Mechanosensitivity in TRAAK and TREK1. If we eliminate every cellular component other than a lipid bilayer and a channel still exhibits mechanosensitive gating, then gating forces must be mediated by the lipid bilayer. Following this line of reasoning, human TRAAK and zebrafish TREK1 were heterologously expressed in the yeast Pichia pastoris, purified to homogeneity in detergent micelles (Figs. 3A and B and 4A and B), and reconstituted into phosphatidylcholine lipids. Proteoliposomes were induced to form membrane blisters from which gigaseals were readily formed, excised in the “inside-out” configuration, and studied under voltage clamp. Macroscopic currents were recorded from patches of TRAAK or TREK1 proteoliposomes that were progressively larger with increasing protein:l lipid ratio reconstitutions (Figs. 3C and 4C). These currents were potassium selective as they reversed direction near EK (Figs. 3C and 4C). Activation of TRAAK and TREK1 was essentially voltage- and time-independent and currents were rapidly flickering and nonactivating. TRAAK currents were nonrectifying, whereas TREK1 currents were outwardly rectifying with respect to the ionic asymmetry across the membrane (Figs. 3C and 4C). These properties of the reconstituted channels are consistent with those of the channels expressed in cells (25, 32).

To determine whether TRAAK and TREK1 are gated by solely membrane-mediated mechanical forces (i.e., in the absence of other cellular components), we applied pressure through the recording pipette to proteoliposome patches held at a constant voltage. For both channels, application of pressure elicited a rapid, transient increase in current that peaked approximately coincident with the pressure peak (3–10 ms after pressure onset) and decayed while pressure was maintained (Figs. 3D and 4D). Both positive and negative (relative to atmospheric) pressures activated channels similarly in the reconstituted membrane, as one would expect in a system of reconstituted channels in which the channels are oriented randomly in the membrane (Fig. 4 J–L). We note, however, that in cell membranes in which the channels are oriented uniformly in one direction, the gating response is also symmetric with respect to pressure application (Fig. 3 J–L).

Increasing steps of pressure applied to the same reconstituted proteoliposome patch elicited progressively larger currents (Figs. 3D and 4D). At high pressures, TRAAK was activated 3.5 ± 0.2-fold and TREK1 was activated 7.0 ± 0.7-fold above the basal current level (mean ± SEM, −50 mmHg, Vh = 0 mV, n = 6 TRAAK patches, n = 7 TREK1 patches). A particularly stable gradient of K+ (EK = −59 mV) (Fig. 1C). Larger hyperpolarizations (toward EK) occurred after injecting current to depolarize the cell before poking or by increasing probe displacement at a given membrane potential (Fig. 1 C and D). Nontransfected control CHO cells displayed small nonselective currents and no mechanosensitive current induced by probe displacement (Fig. 2K).

We further examined whether the hyperpolarizing currents through TRAAK elicited by mechanical force could counteract depolarizing currents in a system with a well-characterized mechanosensitive response. The mouse Neuro2A (N2A) neuroblastoma cell line contains nonselective cation mechanosensitive currents due to endogenous expression of Piezo1 (2). Mechanical
A proteoliposome patch of TREK1 was activated greater than 10-fold by the highest pressure tested (10.5-fold, −80 mmHg, Vh = 0 mV, Fig. 4D). To test whether the mechanically evoked current is potassium selective, a single pressure step was applied while holding at different voltages (Figs. 3E and 4E). Both the basal and mechanically stimulated current reversed direction close to Ec (Figs. 3F and E and 4E and F), indicating that the evoked currents are due to increased TRAAK or TREK1 activity. Control patches of lipid alone produced negligible (~5 pA at 100 mV) currents even at the highest attainable pressure steps before patch rupture (n = 8, Fig. 3M). Perfusion of the polyunsaturated fatty acid arachidonic acid, a known activator of TRAAK (33) and TREK1 (20), onto proteoliposome patches, gave qualitatively similar activation of K⁺ selective currents (1.5 ± 0.1-fold activation of TRAAK and 2.4 ± 0.2-fold activation of TREK1, mean ± SEM, Vh = 0 mV, n = 3 TRAAK patches, n = 5 TREK1 patches, Figs. 3 G–I and 4 G–I).

We observed that immediately upon release of applied pressure to patches from TRAAK or TREK1 proteoliposomes, current level was transiently lower than the average basal current observed before or several hundred milliseconds after the pressure step (Figs. 3D and E and 4D and E). This phenomenon can be explained if TRAAK and TREK1 channels have a higher open probability at higher membrane tensions. Applied pressure, by altering membrane tension, is expected to influence the reversible exchange of lipid molecules between the membrane patch and the surface of the glass pipette. Specifically, increased pressure, and thus tension, will cause lipid to flow from the glass electrode to the patch. Then, when pressure is released, the patch will have excess lipid (and excess area), which will result in a transiently reduced tension until the excess lipid runs back onto the glass surface and an equilibrium value of tension at zero pressure is restored.

Implicit in the above description comes the idea that TRAAK and TREK1 channels are to some degree basally activated by nonzero tension that occurs in an unpressurized gigaseal patch. This tension has been estimated to be ∼0.5–4 mN/m (34). Data in Figs. 3 and 4 suggest that TRAAK and TREK1 might have different “thresholds” for mechanical activation. Resting currents from TRAAK were approximately twofold higher than those from TREK1 reconstituted at the same protein-to-lipid ratio (e.g., 2.1-fold higher in 1:20 protein:lipid ratio patches at 100 mV, Figs. 3C and 4C). But TREK1 was activated by both pressure and arachidonic acid to a greater extent over its baseline than TRAAK. These observations are consistent with TRAAK compared with TREK1 having a lower threshold for tension activation (i.e., it begins to activate at lower tension values).
Fig. 3. TRAAK mechanosensitivity is mediated directly by the lipid membrane. (A) Elution profile of purified human TRAAK from a Superdex 200 size exclusion column. TRAAK runs as a single monomer-disperse peak. The void volume of the column is indicated. (B) Coomassie stained SDS/PAGE with increasing amounts of purified TRAAK loaded in successive lanes (Left). Western blot of purified TRAAK using anti-human TRAAK antibody 13E9 primary antibody (Right) (29). TRAAK runs as a mixture of monomeric and dimeric species on SDS/PAGE. (C) Current-voltage relationships recorded from patches excised from liposomes reconstituted with varying protein:lipid ratios (weight:weight). Mock reconstitution was prepared with buffer in place of purified TRAAK. Currents plotted are the mean ± SEM of 1 s of recording at each holding potential (1:20, n = 8 patches; 1:100, n = 8 patches; 1:1,000, n = 6; mock, n = 8 patches). All recordings were performed in a 10-fold [K⁺] gradient (internal 200 mM K⁺, 0 mM Na⁺ and external 180 mM Na⁺, 20 mM K⁺) in C- and M and internal 150 mM K⁺, 0 mM Na⁺ and external 135 mM Na⁺, 15 mM K⁺ in J-L and presented in physiological convention; positive currents indicate K⁺ flow from the high [K⁺] (intracellular) to low [K⁺] (extracellular) side. (D) Current response to pressure steps applied to a patch excised from TRAAK proteoliposomes. Increasing steps of pressure (Lower) were applied every 5 s. Currents recorded during each pressure step (Upper) are vertically offset for clarity. Dashed red lines beneath each current trace indicate the zero current level. Holding potential (Vh) = 0 mV, holding pressure (Ph) = 0 mmHg. (E) Currents (Upper) recorded from a patch excised from TRAAK proteoliposomes during a voltage step protocol (Vh = −50 mV, ΔV = 10 mV, every 40 mV shown). During each voltage step, a pressure step of −50 mmHg was applied through the pipette (Lower). (Inset) Magnified time scale of the traces at the time of pressure onset. (F) Current-voltage relationship of data from the experiment shown in E at 10-mV increments. The average current before the pressure step and the peak current during the pressure step at each voltage are plotted. (G and H) Currents recorded from a patch excised from TRAAK proteoliposomes during a voltage step protocol (Vh = −50 mV, −100 to +100 mV, ΔV = 10 mV, every 40 mV shown) before (G) and after (H) perfusion of 50 μM arachidonic acid. (I) Current-voltage relationship of data from the experiment shown in G and H at 10-mV increments (n = 3 sequential recordings, mean ± SEM). (J and K) Current response to (J) negative and (K) positive pressure protocols applied to the same patch excised from a TRAAK-expressing CHO cell (Ph = 0 mmHg, 0 to ±70 mmHg, ΔP = 10 mmHg, 0, ±30, ±50 mmHg shown). Increasing steps of pressure (Lower) were applied every 5 s. Currents recorded during each pressure step (Upper) are vertically offset for clarity. Holding potential (Vh) = 0 mV, holding pressure (Ph) = 0 mmHg. (L) Current-pressure relationship of data from the experiment shown in J and K at 10-mmHg increments. The average current with no pressure and the peak current during each pressure step are plotted against the absolute value of applied pressure. (M) Currents (Upper) recorded from a patch excised from mock-reconstituted liposomes during a voltage step protocol (Vh = −50 mV, −100 to +100 mV, ΔV = 10 mV, every 40 mV shown). While holding at each voltage, a pressure step of −50 mmHg was applied through the pipette (Lower). Scale is the same as in E for comparison.

Discussion

The fundamental observation here is that TRAAK and TREK1 channels are mechanically gated by the lipid bilayer in the absence of all other cellular components. Thus, mechanical gating in these channels does not have its origin in a tether-mediated mechanism. The forces must be transmitted bilayer to channel. Mechanical gating through membrane forces has already been established for the prokaryotic channels MscL and MscS (10, 35). For the case of MscL and MscS, physically plausible models have been proposed to explain how increased membrane tension favors channel opening by energetically favoring protein conformations associated with a greater cross-sectional area and repositioning of helices with respect to the plane of the membrane (10, 36–38). At this point the data are insufficient to propose a physical model to explain how membrane tension controls TRAAK and TREK1 gating. It would seem to us that structural differences between TRAAK/TREK and the nonmechanosensitive K₂P channels (Fig. 2) should offer first hints toward a physical model (25). The crystal structures of TRAAK and TWIK1 provide a framework for future mechanistic investigation (25, 26, 39). However, from the results presented here, we conclude with certainty that TRAAK and TREK extend the force-from-lipid paradigm for mechanosensitivity to a class of eukaryotic mechanosensitive channels.

The data presented here are in direct contrast to a recent report that reconstituted TREK1 channels are insensitive to negative pipette pressure and inhibited by positive pressure (16). In the present study, TRAAK and TREK1 channels are activated by positive and negative pipette pressure. In reconstituted proteoliposomes, a symmetric response is expected because channels reconstitute randomly (Fig. 4 J–L). Further, in cells where channels are oriented uniformly in one direction, we still observe that both positive and negative pressures activate TRAAK channels (Fig. 3 J–L), as is observed in bacterial mechanosensitive channels (40). Symmetric activation in cells (where channels are uniformly oriented) is consistent with the idea that lateral membrane tension controls channel gating (41). Persistence of mechanosensitive gating in reconstituted proteoliposomes, where only lipid and...
channel are present, leaves no other explanation than direct activation of the channel through the lipid bilayer.

It is informative to compare the apparent threshold for mechanical gating in the prokaryotic channels and TRAAK/TREK1. MscL and MscS begin to open at membrane tensions of ~9.0 and 5.0 mN/m, respectively (12, 14, 42). These values come from measurements of channel activation in pressurized membrane patches in which membrane curvature was measured, allowing the determination of tension using Laplace’s law. In contrast to MscL and MscS, TRAAK and TREK1 channels have a low, but nonzero, open probability near zero tension (as in a whole-cell assay opens channels by increasing membrane tension in localized regions of the cell membrane). We think that, whereas membranes of certain cells in the nervous system must to convert force into an electrical signal. From a biophysicist’s perspective, however, the cell-poking assay is very qualitative. Not only is it difficult to quantify how much force is being applied to a channel or elements surrounding the channel, but the assay does not distinguish between a tether-mediated and a membrane tension-mediated mechanism. The best-known application of the cell-poking assay is the version from auditory physiology, in which hair cell stereocilia are displaced to evoke an electrical signal (31). These experiments, along with specific molecular anatomical features of the stereocilia, underlie the famous tip-link tether-mediated transduction channel model (43, 44), which, interestingly, has a more recent version in which the tether has been proposed to “tent” (i.e., exert tension on) the membrane surrounding the transduction channel (45). In the case of TRAAK and TREK channels it is clear that mechanosensitivity persists in the absence of potential tethers (Figs. 3 and 4). This leads us to suspect that at least for TRAAK and TREK channels, the cell-poking assay opens channels by increasing membrane tension in localized regions of the cell membrane. We think that, whereas membrane tension is the direct mediator of force, the cytoskeleton,
possibly other macromolecular components, and the discontinuous mosaic nature of cell membranes will likely play an important indirect role by influencing which regions of the cell membrane experience changes in tension when forces are applied to a cell.

Methods

TRAAD and TREK1 were heterologously expressed in P. pastoris and purified in detergent before reconstitution in phosphorylcholine lipids from soybean. Proteoliposomes blisters for patch recording were generated by dehydration and rehydration. Cell poking was accomplished with a glass probe mounted to a piezo-driven actuator. Pressure application to patches was performed with a high-speed pressure clamp. Cellular electrophysiological recordings were made from transfected CHO-K1 and N2A cells. Detailed materials and methods are presented in SI Methods.

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Cloning of Homo sapiens TRAAK (K2P4.1) (UniProt Q9NYG8-2) and heterologous expression in Pichia pastoris was previously described (1). The identical construct used in the structural work was used in this study (1, 2). Human TRAAK is C-terminally truncated (by 119 amino acids), incorporates two mutations to remove N-linked glycosylation sites (N104Q/N108Q), and is expressed as a C-terminal PreScission protease-cleavable EGFP-10x His fusion protein. Purified human TRAAK (aa 1–384; UniProt Q7Z418), TASK2 (aa 1–404; UniProt Q9NYG8-2), TREK1 (K2P2.1) (National Center for Biotechnology Information reference sequence XP_688186.3) was expressed in Pichia pastoris (aa 1–419; UniProt Q9NYG8-2), TREK1 (K2P2.1) (UniProt Q9NPC2), THIK1 (aa 1–499; UniProt O95279), TALK1 (aa 1–538; UniProt P57789), TASK3 (aa 1–538; UniProt Q9NPC2), THIK1 (aa 1–408; UniProt B5T1L8), TRESK (aa 1–384; UniProt Q7Z418), TASK2 (aa 1–499; UniProt Q95279), TALK1 (aa 1–309; UniProt Q96T5S), and Rattus norvegicus TWIK2 (aa 1–313; UniProt G3V8R8) were codon optimized for eukaryotic expression, synthesized (GeneWiz), and cloned into the same modified PICZ-B vector (Invitrogen) as TRAAK. The C terminus was truncated to aa 322 and predicted glycosylation sites were mutated (N95Q, N122Q) by PCR. This C-terminal truncation has been shown to retain the functional properties of full-length TREK1 (3, 4). Purified D. rerio TREK1 (aa 1–322; N95Q, N122Q)–SNS-LEVLFO/GP-EGFP-H10 is referred to as TREK1 in the text for clarity.

Frozen Pichia cells expressing TRAAK or TREK1 were disrupted by milling (Retsch model MM301) for five times at 3 min at 25 Hz. All subsequent purification steps were carried out at 4 °C. Cell powder was added to lysis buffer (50 mM Tris pH 8.0, 150 mM KCl, 60 mM decyl-β-D-maltoside (DM; Affymetrix), 0.1 mg/mL DNase 1, 1 μg/mL pepstatin, 1 μg/mL leupeptin, 1 μg/mL aprotinin, 10 μg/mL soy trypsin inhibitor, 1 mM benzamidine, and 1 mM phenylmethylsulfonyl fluoride added immediately before use) at a ratio of 1 g cell pellet/4 mL lysis buffer. Membranes were extracted for 3 h with gentle stirring followed by centrifugation at 35,000 ×g for 45 min. Cobalt resin (Clontech) was added to the supernatant (1 mL resin/5 g cell pellet) and stirred gently for 3 h. Resin was collected on a column and serially washed and eluted in buffer (50 mM Tris pH 8.0, 150 mM KCl, 6 mM DM) with 10 mM, 30 mM, and 300 mM imidazole pH 8.0. EDTA pH 8.0 (1 mM final) and PreScission protease (1:50 wt:wt) were added to the elution before incubation with gentle rocking overnight. Cleaved protein was concentrated [50 kDa molecular weight cutoff (MWCO)] and applied to a Superdex 200 column (GE Healthcare) equilibrated in size exclusion chromatography (SEC) buffer (20 mM Tris pH 8.0, 150 mM KCl, 1 mM EDTA, 4 mM n-decyl-β-D-maltopyranoside(DM)). Pure TRAAK or TREK1 was concentrated (50 kDa MWCO) to 2 mg/mL for reconstitution. Samples were analyzed by SDS-PAGE [12.5% (wt/vol) gels; Bio-Rad] and either stained by Coomassie blue or transferred to PVDF for Western blotting. PAGE [12.5% (wt/vol) gels; Bio-Rad] and either stained by silver staining or transferred to PVDF for Western blotting. SDS-PAGE and Western blotting were performed as described (5). Purified proteins were concentrated (50 kDa) and dried under vacuum at 37 °C. One day before recording, an aliquot was thawed at room temperature in a water bath and pipetted in 4–6 equal size drops to a 14-mm glass coverslip inside a 35-mm Petri dish (Mattek; P35G-1.5–14-C). Proteoliposomes were dried under vacuum at 37 °C. The next day, 5 mL bath solution (DR buffer containing 50 mM Hepes, 200 mM KCl pH 7.2) was added to the lipids and the solution was bath sonicated until transparent. TRAAK or TREK1 (or SEC buffer for empty reconstitution control) was added to 4 mg lipids in DR buffer at varying protein-to-lipid ratios in a 15-mL Falcon tube. The final volume of the reconstitution was brought to 4 mL with DR buffer, layered with argon, and rotated at room temperature for 1 h. Approximately 500 mg blotted dry Biobeads-SM2 adsorbents (washed by rotating in methanol for 30 min three times, water for 30 min three times, and DR buffer for 30 min three times) was then added and the reconstitution was rotated at room temperature for 3 additional hours. Two-milliliter aliquots of the reconstitution were spun in single tubes in a RPS55 rotor at 200 krpm for 1 h at 4 °C. Pelleted proteoliposomes were resuspended in 80 μL DR buffer, flash frozen in liquid nitrogen in 20-μL aliquots, and stored at −80 °C for future use.

One day before recording, an aliquot was thawed at room temperature in a water bath and pipetted in 4–6 equal size drops to a 14-mm glass coverslip inside a 35-mm Petri dish (Mattek; P35G-1.5–14-C). Proteoliposomes were dried under vacuum at room temperature for 6 h then rehydrated with 20 μL DR buffer inside a 15-cm Petri dish lined with wet filter paper overnight at 4 °C (8–24 h). The next day, 5 mL bath solution (DR buffer + 40 mM MgCl2) was added to each dish before recording. Blisters were visible immediately and were competent to form high resistance seals for at least 12 h.

Constructs for Mammalian Cell Expression. Genes for full-length H. sapiens’ TRAAK (aa 1–419; UniProt Q9NYG8-2), TREK1 (aa 1–426; UniProt Q90569), TREK2 (aa 1–538; UniProt P57789), TASK3 (aa 1–374; UniProt Q9NPC2), THIK1 (aa 1–408; UniProt B5T1L8), TRESK (aa 1–384; UniProt Q7Z418), TASK2 (aa 1–499; UniProt Q95279), TALK1 (aa 1–309; UniProt Q96T5S), and Rattus norvegicus TWIK2 (aa 1–313; UniProt G3V8R8) were codon optimized for eukaryotic expression, synthesized (GeneWiz), and cloned into the EcoRI/XhoI sites of a modified pCEH vector to generate C-terminally EGFP-tagged constructs (K2P-SNSAV-DAGLVPGRSAAA-EGFP-H10) for transient transfection.

Cell Culture. CHO-K1 cells (ATCC) were used as no significant endogenous mechanosensitive current was observed under the conditions used in experiments presented here (Fig. 2K). Cells were cultured in DMEM-F12 (Gibco) with 10% FBS, 2 mM l-glutamine, 100 units/mL penicillin, and 100 μg/mL streptomycin. Cells were plated in 35-mm plastic dishes or on poly-d-lysine–coated glass coverslips (BD BioCoat) ~24 h before transfection with FugeneHD (Promega) following the manufacturer’s protocol and patched after 48 h. Neuro-2A cells (ATCC) were cultured in DMEM (Gibco) with 10% FBS, 2 mM l-glutamine, 100 units/mL penicillin, and 100 μg/mL streptomycin. Cells were transfected with Lipofectamine 2000 (Invitrogen) following the manufacturer’s protocol, incubated overnight, split onto poly-d-lysine–coated glass coverslips (BD BioCoat), and patched within 72 h. Immediately before recording, media was replaced by bath solution.

Electrophysiology. Pipettes of borosilicate glass (Sutter Instruments; BF150-86-10) were pulled to 3–5 MΩ resistance when filled with recording solution. Recordings were obtained with an Axopatch 200B amplifier (Molecular Devices) using standard whole-cell.

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Supporting Information
patch clamp and excised inside-out patch techniques. Current clamp experiments were performed on the I-fast mode. Recordings were filtered at 1 kHz and digitized at 10 kHz (Digidata 1440A; Molecular Devices) for storage on a computer hard disk. All recordings were performed at room temperature. Pressure application through patch pipettes was performed with a high-speed pressure clamp (ALA Scientific) controlled through the Clampex software. Pressure application velocity was set to the maximum rate of 8.3 mmHg/msec. Glass probes for whole-cell mechanical stimulation were made from borosilicate glass pipettes fire polished until sealed with a tip diameter ~3–4 μm. The probe was mounted to piezo-driven actuator driven by a controller/amplifier (P-601/E-625; Physik Instrumente) controlled through Clampex software. After formation of a whole-cell seal, the probe was positioned at 60° to the cell ~2 μm away from the membrane. Probe displacement velocity was set to the maximum rate of 1 μm/ms.

All recordings were performed in a 10-fold concentration gradient of K+ except cellular recordings from Neuro-2A cells, which were performed in a 30-fold [K+] gradient. For recordings from proteoliposome blisters, pipette solution was 5 mM Hepes, 180 mM NaCl, 20 mM KCl, pH 7.2 (adjusted with NaOH) and bath solution was 5 mM Hepes, 200 mM KCl, 40 mM MgCl2, pH 7.2 (adjusted with KOH). For recordings from CHO-K1 cells, pipette solution was 10 mM Hepes, 150 mM KCl, 3 mM MgCl2, 5 mM EGTA, pH 7.2 (adjusted with KOH) and bath solution was 10 mM Hepes, 15 mM KCl, 135 mM NaCl, 3 mM MgCl2, 1 mM CaCl2, pH 7.3 (adjusted with NaOH). For recordings from Neuro-2a cells, pipette solution was 10 mM Hepes, 150 mM K-Gluconate, 2 mM MgCl2, 5 mM EGTA, pH 7.4 (adjusted with KOH) and bath solution was 10 mM Hepes, 147 mM NaCl, 5 mM KCl, 2 mM MgCl2, 1 mM CaCl2, pH 7.4 (adjusted with NaOH). Arachidonic acid (Sigma; A9673) was aliquoted at 50 mg/mL in ethanol and stored under argon at −20 °C until immediately before use. Perfusion with arachidonic acid at 50 μM in bath solution (+AA) or bath solution with the same concentration of ethanol (before AA) was accomplished using a nitrogen pressurized perfusion system (ALA Scientific). All recordings are presented in physiological convention: Positive currents indicate K+ flux from the high [K+] (intracellular) to low [K+] (extracellular) side.