Activation of mechanosensitive currents in traumatized membrane

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Wan, Xiaodong, Peter J. Uranka, and Catherine E. Morris. Activation of mechanosensitive currents in traumatized membrane. Am. J. Physiol. 276 (Cell Physiol. 45): C318–C327, 1999.—Mechanosensitive (MS) channels, ones whose open probability varies with membrane tension in patch recordings, are diverse and ubiquitous, yet many are remarkably insensitive to mechanical stimuli in situ. Failure to elicit mechanocurrents from cells with abundant MS channels suggests that, in situ, the channels are protected from mechanical stimuli. To establish what conditions affect MS channel gating, we monitored Lymnaea neuron stretch-activated K (SAK) channels in cell-attached patches after diverse treatments. Mechanosensitivity was gauged by rapidity of onset and extent of channel activation during a step pressure applied to a “naive” patch. The following treatments enhanced mechanosensitivity: actin depolymerization (cytochalasin B), N-ethylmaleimide, an inhibitor of ATPases including myosin, elevated Ca (using A-23187), and osmotic swelling (acutely and after 24 h). Osmotic shrinking decreased mechanosensitivity. A unifying interpretation is that traumatized cortical cytoskeleton cannot prevent transmission of mechanical stimuli to plasma membrane channels. Mechanoprotection and capricious mechanosensitivity are impediments to doing efforts with MS channels. We demonstrate a potpourri of endogenous MS currents from L-M(TK-1) fibroblasts; others had reported these cells to be MS current null and hence to be suitable for expressing putative MS channels. Ischemia; actin; snail neuron; fibroblast

DIVERSE MECHANOSENSITIVE (MS) ion channels are widely found by applying suction during patch-clamp recording, but direct evidence for in situ mechanical gating of the channels is rare (42). MS channels in Xenopus oocytes, in Escherichia coli, and in molluscan neurons are examples. For these, when whole cell currents can be elicited mechanically, it is only at near-rupture tensions (1, 16, 32, 50, 51).

Cytoskeletal integrity in situ vs. in patches might explain the general discrepancy between patch and whole-cell MS channel behavior. For molluscan neuron stretch-activated K (SAK) channels, the stimulus history of the patch, neuronal age in culture, and cytochalasin D all affect channel responses (45). In skeletal muscle treated with cytochalasin D, stretch-activated cation channels are hypermechanosensitive (14). In the case of N-methyl-D-aspartate type glutamate channels (38), cytoskeletal damage might be the reason that general cytoplasmic “rundown” coincides with “runup” tension; actin; snail neuron; fibroblast

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A poor understanding of the variable mechanosensitivity of MS channel complicates efforts to clone and functionally express eukaryotic MS channels. A "null" cell type for heterologous expression of putative MS channels is required, and L-M(TK−) cells have been said to lack endogenous MS currents (24). The implication is that MS currents elicited in L-M(TK−) cells transfected with putative MS channel cDNA arise from recombinant channels. In our hands, as demonstrated here, L-M(TK−) cells were not MS current null. We caution that unintentionally greater trauma delivered to experimental patches (vs. controls) may facilitate the activation of endogenous MS channels in these patches.

METHODS
Molluscan Neurons

Cell preparation. Central neurons were prepared from mature Lymnaea stagnalis as described previously (43, 57) and cultured in normal saline (NS; in mM: 55 NaCl, 1.6 KCl, 2 MgCl₂, 3.5 CaCl₂, and 5 HEPES-NaOH adjusted to pH 7.6) with 5 mM glucose and 0.1 mg/ml gentamicin sulfate on plastic culture dishes. Cells were kept 4-6 days in culture before patch-clamp recording.

Patch-clamp recordings. For snail neurons, cell-attached recordings were made with NS in the bath and a high-K solution in the pipette [in mM: 55 KCl, 2 MgCl₂, 1 tetrathylammonium (TEA) chloride, and 5 HEPES-KOH adjusted to pH 7.6]. TEA was included to select for SAK over other K channels, particularly Ca-activated K channels; −50 mM extracellular TEA diminishes single SAK channel currents by only 50% (47).

Pipettes were made from borosilicate glass (ID 1.15 mm, OD 1.65 mm; N51A, Garner Glass, Claremont, CA) on a List L/M-3P-A (Darmstadt, Germany) pipette puller, coated with Sylgard 184 (Dow Corning, Midland, MI) and fire polished. Tip outside diameter was 2–3 µm before fire polishing and 1.5–2.5 µm afterward, with a resistance of 2–4 MΩ. To make a seal, −5 mmHg positive pressure was maintained until the pipette touched the membrane. On release of this pressure, gigahm seals occasionally formed spontaneously, but sealing usually required a gentle blow. When seals did not form with <10 mmHg applied for <10 s (defined as a gentle seal), patches were discarded, and so too were patches exhibiting stretch-inactivated channel activity (35).

Hoffman modulation optics and, as noted, epifluorescence microscopy were used. A ×40 objective was used in both cases, and VHS video recordings were made via a charge-coupled device camera. Subsequently, a frame grabber was used to capture individual video frames.

Mechanical stimulus. Step changes of pipette pressure were achieved as described previously (45) using two pressure transducers (Biotek Instruments, Winooski, VT), one to establish pressure and the other (modified so its output was suitable for an analog-to-digital converter) to measure the pressure acting on the patch membrane. The standard pressure used for "first hits" was −80 mmHg. In some cases, as noted, other pressures were used. Manual valves initiated and terminated pressure steps (see Fig. 1A and 1B in Ref. 45), the durations of which varied according to the response. Once channel activity had plateaued for ~1 s or had reached a maximum and begun to fall, a step was terminated.

Analysis. Channel currents were recorded using an Axopatch 1D (Axon Instruments, Foster City, CA) connected to a microcomputer via a TL-1 interface (Axon Instruments). pCLAMP (Axon Instruments) was used to digitize and export records to SigmaPlot 5.1 (Systat Software, San Jose, CA) for analysis. Current, voltage, and pressure were sampled simultaneously at 9-ms intervals. Ideally, first latency is used to describe delayed channel responses. However, SAK channels often have a nonzero resting activity that contributes sporadic events to the delay period (from initiation of the pressure step to the onset of stimulated activity). Therefore, we quantified delay using a running average of current as previously described (45). Responses to first hits (the very first pressure stimulus delivered to a patch other than the small pressure used for seal formation) were used for all patches unless indicated. The stimulus duration varied, depending on the delay. Once channel activity started, the stimulus was continued for several seconds, as described next.

To obtain an index of channel activation (activation index), the above-baseline current record was integrated from the end of the delay to the end of the stimulus or, if activation showed an obvious peak during the sustained stimulus, to that peak. This resulting charge (current × time) was divided by the pressure × time, giving an activation index in units of picoamperes per millimeters of Hg.

Values are given as means ± SE. Paired and unpaired t-tests or nonparametric tests were carried out as indicated, and differences were considered significant when P < 0.05.

L-M(TK−) Cells

Cell preparation. L-M(TK−) cells from the American Type Culture Collection (ATCC; CCL-1.3; passage 19) were grown in DMEM (GIBCO) with 4.5 g/l glucose and 10% fetal bovine serum (FBS). Calcium phosphate-mediated transfection of cells with DNA coding for a green fluorescent protein (GFP; pcSNucGFP DNA kindly provided by Adrian Salic) (18, 53) was carried out as follows. Cells were plated 24 h before transfection at 2 × 10⁵ cells·100-mm-diameter plate⁻¹·10 ml medium⁻¹. pcSNucGFP DNA (10 µg prepared via Qiagen midiprep kit) in 450 µl sterile water was mixed with 50 µl of 2.5 M CaCl₂; 500 µl of 2× HEPES-buffered saline (280 mM NaCl, 1.5 mM Na₂HPO₄, and 50 mM HEPES, pH 7.1) were added dropwise to the DNA-Ca solution. Immediately, the DNA precipitate was removed and added dropwise to the cells. After 18 h, the medium was replaced with fresh medium. After 24 h, cells were trypsinized (0.5% trypsin in PBS + 5 mM EDTA) and replated at lower density in 35-mm culture plates. Records were made once coexpression of green fluorescent protein was evident by epifluorescence.

Patch recording. The bath and pipette saline was (in mM) 150 NaCl, 2 KCl, 2 CaCl₂, 1 MgCl₂, 5 HEPES, and 1 glucose (pH 7.3 and osmolarity 312 mosM). Pipettes for cell-attached patch recordings and pressure stimuli were as described above for Lymnaea. Recordings were made at room temperature. Cells expressing green fluorescent protein were located by epifluorescence illumination, but recordings were made under Hoffman modulation optics.

RESULTS

Operational Definition of Mechanosensitivity

Because pipettes and gigahm seals vary, nominally identical mechanical stimuli differ from patch to patch and over time in a given patch. Characterizing MS channel sensitivity (ease of activation) by a Boltzmann distribution (open probability vs. membrane tension) therefore has limited utility. Here "mechanosensitivity" is used qualitatively: if, in response to a standard step stimulus, channels activate more or less readily than
under control conditions, mechanosensitivity increases or decreases, respectively. The two measures of mechanosensitivity used are the delay to the start of activation and extent of activation after the delay (“activation index”). Because delay increases from day 1 to day 4 (45), we used exclusively cells from days 4–6, thus minimizing variance in controls and maximizing latitude for detecting changes.

Choosing a Stimulus Protocol

As previously noted (45), SAK channels were ubiquitous in Lymnaea neurons. With NS in the bath and high-K saline in the pipette and pipette voltage of ~60 mV, TEA-insensitive inward single channel currents of 4–5 pA were obtained with suction. Naive patches challenged with sufficiently large first-hit stimuli (see sample traces from two cells; Fig. 1, A) exhibited activation of SAK channels, but, notably, activation began after a substantial delay. Delays are not seen in nongentle patches, which, instead, show immediate and sustained activation (e.g., Ref. 45). First-hit stimuli of ~30–40 mmHg occasionally yielded activation after delays of 5–10 s (e.g., Fig. 1A, bottom) but usually produced delays of tens of seconds, making data storage and handling onerous and raising concerns about what elicited activity. Consequently, larger first hits were used for the experiments below.

The duration of delay depended on stimulus intensity (Fig. 1B) and patch history (Fig. 2). As shown in Fig. 1B, first hits at ~60 mmHg yielded delays more than twice as long (significant) as first hits at ~80 mmHg, although activation index (Fig. 1B) was not significantly different at these two pressures (unpaired t-tests). The delays were impressive; channels were oblivious to ~60 mmHg for ~10 s and to ~80 mmHg for ~3 s. For subsequent experiments, we chose to use ~80 mmHg on the grounds that the delay was neither impractically long nor too short to accurately detect any decreases and that activation was not near saturation levels so that increases would be readily detectable. Near saturation, four to eight channels were generally seen per patch.

Attempts to Recover From Exercising Patch

Repeated stimulation (“exercise”) affected SAK channels. Figure 2A shows sample traces for an exercised patch. The delay and activation index for this example are plotted in Fig. 2B, along with the summary data for 16 patches. The first and second hits were separated by 10 s. After this interval, delay fell and the activation index increased (significant by paired t-tests; Fig. 2B). Although channels were always active during the last part of the first hit, they did not reactivate immediately when the second-hit step was applied. A measurable delay, albeit a reduced one, was obtained with the second hit. Thus during the 10-s interstimulus interval,
some process occurred that partially restored the patch to its initial large-delay state.

Given a considerably longer interstimulus interval than 10 s, would patches recover more fully from stimulation-induced changes? To test this, we followed the second hit with a 10-min rest and then restimulated (third hit). After the rest, neither mechanosensitivity parameter was significantly different than at the second hit. Thus, although the patch features responsible for the rapid (<10 s) partial recovery that occurred between the first and second hits had not deteriorated, the stimulus-induced change (damage?) that prevented full recovery was not, evidently, rectified during 10 min of rest. Attempts to provide a longer (30 min) rest period before the third hit were unsuccessful because gigahm seals deteriorated.

Location of Patch

If gentle seals on Lymnaea neurons (see METHODS) formed tens of microns into the pipette, as can happen (49), then, even before a first hit, major cytoplasmic trauma may have been incurred. With gentle seals and first hits at ~80 mmHg, we could not see patches via Hoffman optics, presumably because they were obscured by the cell. Given more extreme stimuli (~120 mmHg), patches often relocated deeper into the pipette as in Fig. 3, A and B, where they were detectable. Such relocated patches held firm when even higher suctions were applied, right until rupture. On release of pressure, the patches flattened, as in Fig. 3C.

To confirm that gentle patches were indeed nearer the pipette tip, we used fluorescence. With Lucifer yellow (1 mM) in the pipette (not shown), the patch position (i.e., the fluorescence front) did not detectably change between first touching the cell and formation of a gentle gigahm seal or with a first hit to ~80 mmHg. With overstimulation (as in Fig. 3, A and B), however, the front moved as nonfluorescent cytoplasm displaced pipette solution. In another test, Lucifer yellow (1 mg/ml) was dialyzed into the cytoplasm by whole cell recording (Fig. 3 D–G). Once fluorescent, neurons were repatched (gentle seal, cell attached) at a new location and, despite low signal, the patch location was evident near the pipette tip and did not relocate during a ~80 mmHg hit.

Thus, in experiments below involving gentle seals and ~80 mmHg first-hit stimuli, but viewed by Hoffman optics, patches were evidently obscured because they formed stable seals close to the pipette tip.

Effects of Cytochalasin B and N-ethylmaleimide

In the following experiments, cells were exposed to agent before gigahm seal formation, and agents were present until experiments ended. All the data were...
obtained from first hits that were applied within ~10 s of obtaining a gentle seal.

Pretreatment with 25 µM cytochalasin B (1–2 h) significantly decreased the first-hit delay and increased the activation index (Fig. 4, top). Controls for the vehicle, 0.5% DMSO, showed no effect on either mechanosensitivity parameter.

Pretreatment (20–30 min) of cells with the sulfhydryl reagent N-ethylmaleimide (NEM; 1 mM) also had a major impact on mechanosensitivity; delay fell to 4% of control values, and activation index increased 3.8-fold. NEM impairs many proteins, but visual inspection of single-channel events for amplitudes and flickeriness (Fig. 4, bottom) revealed no apparent inhibitory effect of NEM pretreatment on SAK channel permeation characteristics. Clearly, NEM did not prevent the channels from activating in response to mechanical stimuli. Thus NEM, which chemically disrupts many cellular functions by abolishing ATPase and GTPase activities, unequivocally enhanced rather than diminished the mechanosensitivity of SAK channels.

Effects of Hyposmotic and Hyperosmotic Conditions

Under perforated patch, swelling Lymnaea neurons develop a TEA-insensitive K conductance that may be mediated by SAK channels (57). Whether these or the many other swelling-activated channels reported in the literature gate by membrane tension is a major unresolved issue, so it is particularly interesting to determine whether osmotic perturbations affect channel mechanosensitivity.

To test the effect of swelling, neurons were exposed to 0.6× normal osmolarity (some NaCl removed from NS) for 15 min before patching. Although this is physiologically extreme, Lymnaea neurons not only withstand but remain healthy (reaborize processes) after far more extreme and prolonged exposures (36, 57). Gentle

seals were obtained on swollen neurons, and then ~80 mmHg first hits were done. Delay was only 11% of the control value, and, after the shortened delay, a significantly greater level of channel activity was obtained, as indicated by the 4.7-fold increase in the activation index (Fig. 5).

An additional experiment showed that for both the parameters the swelling-induced changes were persistent (Fig. 5). Neurons were exposed for 15 min to 0.6× NS, returned to NS for 24 h, and then tested. As expected (41), the swollen neurons reshrank and made transient vacuole-like dilations when switched back to NS. Although neurons appeared as healthy and as well arborized as neurons in unperturbed control dishes when patched 24 h later, their plasma membranes were evidently mechanically different, since delay and activation had not recovered.

Neurons were also tested after exposure to hyperosmotic solution (1.9× normal; sucrose added to NS) for 15 min. The cells were clearly shrunken before formation of gentle seals. Hyperosmia almost doubled the first-hit delay (Fig. 5) but had no effect on activation index. Thus, in shrunken cells, the channels were able to avoid the stimulus for an extended period, but, once felt, the stimulus was as effective as in controls.

Effects of Elevated Intracellular Ca

Because many physiological and pathological events elevate intracellular Ca, we tested whether elevated Ca affects SAK channel mechanosensitivity. Intracellular Ca in cultured Lymnaea neurons bathed in NS is ~0.1 µM (21). We chose to clamp Lymnaea neuron cytoplasmic Ca to a level between rest (~0.1 µM) and 1.0 µM [attained in squid axons during a train of action potentials (2)]. Ca at 0.5 µM is more than sufficient to activate the actin-depolymerizing enzyme gelsolin (39), so neurons were incubated in saline containing 0.5 µM free Ca (NS but with Ca 1.9 mM and EGTA 2 mM, yielding 0.5 µM free Ca) and 20 µM A-23187, a Ca ionophore (final concentration 0.3% in DMSO). When incubated for >5 min in this solution, Lymnaea neurons swelled and developed spherical blebs. We therefore patched the cells as rapidly as possible after adding the ionophore solution (by ~2 min), before blebbing

Fig. 4. Top: effects of cytochalasin-B (Cyt-B), vehicle (DMSO), and N-ethylmaleimide (NEM). Bottom: sample trace of currents activated during a first hit after NEM treatment.

Fig. 5. Effects of high intracellular Ca and of osmotic perturbations on mechanosensitivity parameters.
began. Figure 5 shows the results from first hits to 14 cells. The delay of channel activation was decreased to 16% of controls, and the activation index increased sevenfold. Thus elevated Ca strongly increased SAK channel mechanosensitivity.

MS Currents in Fibroblasts

Figure 6 illustrates a collection of MS currents (plus an example of unusual spontaneous activity) elicited from L-M(TK –) cells using cell-attached patches and gentle seals. The tabulated data (Table 1) represent first-hit responses from experiments whose aim had been to express a putatively mechanosensitive cation channel subunit (designated P4L). The activation data were scored blindly by C. E. Morris (i.e., without knowledge of whether the currents were obtained from transfected or untransfected cells and hence without knowledge of whether MS channels were “expected” or not). We were rigorous in ensuring that control and experimental patches had the same patch histories. All cells had been subjected to the transfection procedures (see METHODS) for GFP, and some were also cotransfected with cDNA encoding the putative MS channel monomer P4L. Cells lacking GFP fluorescence were assumed to be nontransfected (see traces for nonfluorescent cells in Fig. 6).

There was a dismaying variability in the mechanocurrents elicited, and the variations showed no consistent correlation with transfection status. Although a greater proportion of MS-current-positive patches was observed among the transfected cells, we judged it nearly impossible to ascertain whether there might be “signal” hidden in the high background. As it was not our goal to study the endogenous MS channels of L-M(TK –) cells, we characterized the currents no further. It is worth pointing out that we turned to L-M(TK –) cells because, in our hands, HEK-293 cells were even more prone than L-M(TK –) cells to exhibit endogenous MS currents.

DISCUSSION

Equivocal Mechanosensitivity of SAK Channels

The designation “MS channels” applies to Lymnaea SAK channels because in exercised patches, small suction of, say, –20 mmHg (e.g., Refs. 46, 47) routinely increase the open probability manyfold. Alluding to the importance of exercise for channel sensitivity is an
informal version of formally showing that responsiveness increases with successive identical stimuli.

Results presented here demonstrate that even at the most fundamental quantitative level (i.e., scoring 0 or 1 for presence or absence of MS channels in the cells) there can be problems. With a small change in our stimulus protocol, it would have been quite legitimate to decide that Lymnaea neurons are “MS channel null”. Assume that a gentle seal was obtained and then a large first hit of, say, – 60 mmHg for 2 s was applied. No channels would have been activated by this substantial mechanical stimulus. The standard protocol that we arrived at (–80 mmHg for as many seconds as was required to see activation) was chosen empirically because, bluntly put, it was sufficiently brutal to routinely activate the channels. Thus, although the molluscan SAK channels are certainly mechanosusceptible, they must be regarded as poor mechanotransducers unless, in vivo, unknown factors intervene and boost their mechanosensitivity. Admittedly, such channels might serve to signal that a dangerously high membrane tension had developed (3, 34), but even that signal might come too late to be of use.

Delay, Activation Index, and Membrane Mechanical Properties

Because pressure steps had rise times in the 100-ms range, delays of several seconds were a feature not of the stimulator but of the membrane patch. On a typical healthy patch of control membrane, therefore, a large mechanical stimulus (first hit) failed for several seconds to activate MS channels. The implication is that stimulus energy was dissipated for those several seconds before affecting channel gating. This suggests that significant mechanical forces were felt by channels only when the cortical skeleton had become distended, disorganized, or damaged.

Overall, then, treatments that sped activation on a first hit also yielded a greater extent of activation; this is consistent with the idea that, as for MS channels from E. coli (51), tension applied to naked channels in the bilayer sufficed to elicit MS gating in mechanosusceptible channels.

SAK channel responses to multiple stimuli can be viewed as involving a combination of elasticity, viscoelasticity, and plasticity. The elastic component was the fundamental tension-induced channel activation (see Ref. 42). Viscoelasticity was seen in the fact that second-hit delay, although reduced, was not zero but instead remained on the order of seconds. Between hits (time frame 10 s), partial viscoelastic recovery may occur as a result of corrective adjustments of the cortical actin cytoskeleton (12, 39) and, more speculatively for neurons, of the skeleton (see Ref. 54 for mechanical effects on the spectrin skeleton in erythrocytes). Finally, plasticity (strain) is evident in the failure of delay to relengthen even partially toward its control (first-hit) level after a 10-min rest. Activation index, too, was also unchanged after 10 min, indicating that the fundamental mechanotransduction machinery did not deteriorate as the patch became more traumatized. This is consistent with the idea that the unadorned channel in the bilayer is all that is required for a mechanosusceptible SAK channel (see Ref. 13).

Cortical Cytoskeleton

Cytochalasins interfere with actin polymerization, resulting in a net loss of filamentous actin. They promote activation of many channels (26, 27, 37, 43), suggesting a broad regulatory role for filamentous actin on membrane proteins.

Like cytochalasin D (45), cytochalasin B greatly enhanced SAK channel mechanosensitivity. In Lymnaea growth cones (whole cell recording), cytochalasin B does not facilitate activation of the channels (33). Even with the B form, therefore, there continues to be a discrepancy between whole cell and patch effects of cytochalasins on channel mechanosensitivity. The present finding confirms that “adequate” mechanical stimuli are more readily delivered to channels in patches than to channels in situ. Cytochalasin-resistant elements of the membrane skeleton may be more fragile in patches than in situ and therefore less able to provide any mechanoprotection. For bacterial MS channels, the channel protein alone (linked to no network) is the basic mechanosensitive unit (51); if naked SAK channels too are mechanosensitive, then, in situ, even damaged membrane skeleton may protect channels from mechanical loads.

Although NEM inhibits many proteins, there can be no suggestion that the NEM-induced increase in mechanosensitivity observed here relates to any inhibition of channel function. There were no obvious effects of NEM on single-SAK channel properties and, supplied intra-cellularly (6), NEM (0.3 mM) does not block hair cell mechanotransducer channels. External NEM (1 mM, as used here) abolishes cytoplasmic and filopodial motility in Lymnaea neurons and renders them osmotically fragile (41, 57), effects likely to involve actomyosin. The profound enhancement of SAK channel mechanosensitivity by 1 mM NEM probably depended on disruption of disulfide-bearing enzymes, including myosin (23), in the cortical cytoskeleton.

The actions of cytochalasin and NEM suggest that the “tonus” of the cortical cytoskeleton normally protects ion channels from unacceptable mechanical loads. If dynamic actomyosin cross bridges contribute to the load-bearing strength of the membrane, then increased osmotic fragility (57) and increased SAK channel mechanosensitivity are both predictable outcomes of NEM or cytochalasin treatment.

Elevated Intracellular Ca

Clamping intracellular Ca at 0.5 µM (see Ref. 21 for an account of fura 2 measurements of intracellular Ca under conditions similar to those used here) produced a liquefied unhealthy-looking cytoplasm, and its effect on SAK channel mechanosensitivity was similar to cytochalasin: unequivocally increased mechanosensitivity. Ca activates the actin-depolymerizing enzyme gelsolin (40) plus various Ca-dependent proteases, including ones.
that cleave the spectrin skeleton (31). Clearly, SAK channel mechanosensitivity is not dependent on a well-organized cortical cytoskeleton. Interestingly, volume-sensitive Cl currents in astrocytes are potentiated by both intracellular Ca and cytochalasin (26). In cells in which Ca-permeant MS channels appear to be mechanotransducers, such as in baroreceptors (52), it should be interesting to determine whether mechanosensitivity is Ca enhanced. Assuming that persistent pressure changes are of primary interest, auto-upregulation of mechanoresponsiveness by the Ca-permeant MS channels might enhance low-pass mechanosensory filtering by baroreceptors.

Osmotic Perturbations

Swelling and shrinking both alter cortical architecture, but only swelling, which disorganizes actin filaments (7, 19), increased SAK channel mechanosensitivity. In this case, the change was not secondary to Ca effects, since in Lymnaea neurons 50% dilution has a negligible effect on intracellular Ca (20). For swelling (as for cytochalasin, NEM, and high intracellular Ca) the simplest explanation for enhanced SAK channel mechanosensitivity is a nonspecific degradation of the submembrane shock absorber. In 50% medium, membrane tension rises approximately threefold above controls (9) and gradually (over ~20 min) a macroscopic K current develops (57). Whether tension is what eventually activates the SAK channels in swollen neurons is unresolved. In fact, what stimulates any “volume-activated” conductance (see Refs. 26, 27) is an open question. Nevertheless, it is intriguing to wonder whether the mechanosensitivity of volume-activated channel increases when hyposmia impairs the cortical shock absorber.

Hyposmia, which causes F-actin to reorganize at membranes in Lymnaea neurons (20), may increase delay by augmenting the robustness of the cortical cytoskeleton.

“Osmomodulation” of MS channel mechanosensitivity might be able to tune osmoreception by MS channels. Consider stretch-activated channels supporting a net osmolyte efflux: osmomodulation by swelling would augment the loss of osmolytes, favoring regulatory volume decrease. Or, for stretch-inactivated channels permeable to depolarizing ions (e.g., Ref. 4), osmomodulation during swelling (increased ease of stretch-inactivation) would facilitate stretch-induced repolarization, thereby bolstering the sensory response.

Overall, then, treatments that sped activation on a first hit also yielded a greater extent of activation; this is consistent with the idea that, as for MS channels from E. coli (51), tension applied to naked channels in the bilayer sufficed to elicit MS gating in mechanosensitive channels.

Persistent Effects

The enhanced SAK channel mechanosensitivity produced by 15 min of hyposmia persisted in neurons patched 24 h later. Given the evident health of the rearborizing neurons at 24 h, one cannot necessarily infer long-term damage to the cortical cytoskeleton. The persistent effect may, instead, reflect the dynamism of the cytoskeleton as the neurons remodeled (see Ref. 30) after the perturbation.

If a 15-min bout of osmotic swelling is a mechanical trauma yielding persistent posttrauma effects, so too is isolation of neurons from the central nervous system: SAK channels in neurons 24 h postisolation are significantly more mechanosensitive than those 4–6 days postisolation (45). In a cellular model of mechanical brain trauma (59), nonlethal (31%) stretching of neurons for 50 ms yields, after some minutes, sustained (>6 h) protein kinase C-dependent changes in channel properties. Clearly, caution is needed when extrapolating about channels from acutely isolated (read, “recently traumatized”) neurons, and MS channels are no exception.

Hyperosmia, Neuroprotection, and Ischemia

Hyperosmia and reshrinkage of previously swollen cells (including Lymnaea neurons) both cause cortical F-actin to rapidly organize (7, 19, 21), and so in neurons exposed to hyperosmia the cortical cytoskeleton should be strengthened. Because disrupters of F-actin increase MS channel mechanosensitivity (Refs. 14, 45, and the present study), “enhancers” of F-actin like hyperosmia should decrease MS channel mechanosensitivity. In line with this, the F-actin stabilizing reagent phalloidin inhibited whole cell mechanosensitive currents from putative baroreceptor neurons in which the MS current is thought to arise from channels residing in the soma (8).

The mild but significant reduction of SAK channel responsiveness with hyperosmia (delay almost doubled) may have clinical resonances, since hyperosmotic media are broadly neuroprotective in the ischemic brain (22). Glutamate channel activity (38) and voltage-gated Ca channel activity (25) can both be enhanced by membrane stretch. Because excessive activity of such channels spells excitotoxic damage in stroke, a trauma accompanied by neuronal swelling and by degradation of the spectrin skeleton (31), the neuroprotection afforded by hyperosmia might occur in part because it counteracts spurious MS channel activity that is only of significance after loss of membrane mechanoprotection. Although any clinically applied hyperosmia would be of more modest magnitude than that used here, duration might compensate.

L-M(TK- ) Cell Currents

In our hands, mechanostimulation of L-M(TK- ) cell patches elicited currents in about one-half of control patches. By contrast, Kizer et al. (24) reported no mechanically activated currents in 30 of 30 control patches of the same cell line, whereas cells transfected with a recombinant Na channel yielded MS currents in 23 of 79 patches tested. Because several pharmacological and permeability characteristics of the MS currents reported by Kizer et al. (24) are unlike recombinant Na
channels elsewhere, the identity of the MS current-carrying entity is somewhat uncertain. Details of stimulus history (see Ref. 16) could be a source of discrepancy between our results (frequent observation of endogenous MS currents) and those of Kizer et al. (24). Where MS channel activity was expected (i.e., in cells transfected for the putative MS channels), patches may have been inadvertently exercised until MS currents appeared. In control patches, there may have been a ready acceptance of a “no-MS-events” result after a single attempt. Thereafter, any endogenous channel activity elicited in more vigorously exercised (traumatized) transfected cells would be misconstrued as recombinant channel events.

Trauma and Mechanotransduction

A recurrent finding was that trauma facilitated mechanosensitivity in channels that are mechanosensitive but not normally used for mechanotransduction. The native channel microenvironment evidently provides a mechanoprotective shock absorber. Even in auditory hair cells, cytoskeletal mechanoprotection seems to prevail, relaxed only on the single axis along which minute vibrational stimuli are detected; in that axis, moreover, actomyosin-based mechanoprotection of the channel provides for adaptation (11). Conceivably, there may be cases in which trauma-induced enhancement of MS channel mechanosensitivity is used physiologically. Hypermechanosensitivity of somatic sensory fields following trauma (e.g., in Aplysia; see Ref. 10) might, for instance, partly depend on trauma-induced lowering of the threshold for nociceptive MS membrane proteins.

NOTE ADDED IN PROOF

Patel et al. (A. J. Patel, E. Honoré, F. Mainiac, F. Lesage, M. Fink, F. Duprat, and M. Lazdunski. A mammalian two pore domain mechano-gated S-like K+ channel. EMBO J. 17: 4283–4290, 1998) recently showed that a cloned mammalian K channel, TREK-1, is mechanosensitive and a member of the wider family to which the stretch-activated K channel described here belongs. They also showed that TREK-1 can be activated by amphiathanin crenators, strong evidence that mechanical energy is conveyed to the channel directly via the bilayer. This is fully consistent with our contention that membrane skeleton is mechanoprotective for the channel.

This work was supported by Grant ST2716 from the Heart and Stroke Foundation of Ontario and by equipment and research grants from the Natural Sciences and Engineering Council, Canada. Address for reprint requests: C. E. Morris, Loeb Health Research Institute, Ottawa Hospital, 725 Parkdale Ave., Ottawa, ON, Canada K1Y 4E9.

Received 13 May 1998; accepted in final form 4 November 1998.

REFERENCES


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