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Are stretch-sensitive channels in molluscan cells and elsewhere physiological mechanotransducers?

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Abstract. Single-channel recordings of dozens of cell types, including invertebrate (molluscan) and vertebrate heart cells, reveal stretch-sensitive ion channels. The physiological roles of these channels are undoubtedly diverse but it is usually assumed that the roles they play are related to the channels' mechanosensitive gating. Whether this assumption is valid remains to be seen. Attempts to connect the single-channel observations with the mechanical aspects of physiological or developmental processes are discussed. In the case of molluscan cells, recent work suggests that their stretch channels have physiological functions unrelated to mechanosensitive gating.
Key words. Stretch; mechanosensitive; ion channel; physiological role.

Introduction

Ion channels sensitive to tension may be important for cytomechanics and tissue mechanics, topics germane to the control of circulation in invertebrates. I will discuss stretch-activated (SA) ion channels, but the focus will be broader than their possible involvement in circulatory control; my purpose is to ask, "How good is the evidence implicating SA ion channels in mechanotransduction in any physiological process?"

About two years after the initial single-channel study of stretch-activated (SA) channels, which were nonselective cation (SACat) channels of vertebrate skeletal muscle[20,46], SA K^+ (SAK) channels were described. They were found in molluscan heart[8,50], and being K^+-selective rather than nonselective, they constituted a second general type of SA channel. Given that molluscan heart is both mechanically active and stretch-sensitive[26], it was tempting, having found SAK channels uniformly distributed in the ventricular cells at $\sim 1\ \mu m^{-2}$, to speculate about special cardiac functions which they might serve. Subsequently, however, we showed that SAK channels in molluscs are not restricted to heart cells; essentially identical channels are ubiquitous in the neurons of gastropods[3,40]. Insect somatic muscle, too, has comparable SAK channels[19,61], and SAK channels have been described in diverse vertebrate cell types[41].

In fact, SA channels of various selectivities have now turned up in most cell types (bacteria, yeast, plant, animal)[41,47] and suggestions about their possible functions have been legion. Membrane tension induced by suction during single-channel recording does not affect most channel types (see, for example, Zagotta et al.[61]). The effect of tension on the 'open probability' of SA channels, however, is so striking that it is natural to presume that the channels act as mechanosensor/effectors. A continual mechanical threat to all cells, colloidal osmotic stress, is exacerbated by osmotic perturbations. Hence, an evolutionarily ancient SA channel-based mechanism for swelling-activated electrolyte loss (using the channels both as sensor and effector in the simplest case) would make sense a priori[31,48] and help account for the phenomenon of hyposmotic-shock-induced increased permeability to ions[9].

This is an appealing notion, but to date, no experimental evidence conclusively links stretch channel activity to osmoregulation or to any other cell physiological process. At the outset we need to deal with the reductionist's

perennial question: does the microscopic behaviour of the system reflect its macroscopic behaviour? More particularly, does mechanosensitive channel behaviour, as seen in patches under single-channel recording conditions, reflect in vivo channel behaviour? The lack of a spectrum of pharmacological tools hinders progress. Another factor is the problem of stretching a membrane in intact cells in a calibrated, reproducible manner.

In this overview, I discuss experimental approaches that are being used in attempts to connect stretch channel activity to physiological processes. Some of our own findings are unsettling, since they suggest[21,39] that the stretch-sensitivity of the SAK channels in molluscan neurons may be experimentally-induced. In an earlier review[41] I worried academically about 'red herrings'; presently I feel compelled to take the part of the devil's advocate even more seriously. I will begin by summarizing our exploratory findings (five lines of enquiry, mostly on molluscan cells), then will consider the work of others. What criteria should we use to establish that a particular channel is a physiological mechanotransducer? Surely it is not enough to show that a channel is stretch-sensitive at the single channel level and that it resides in a cell which might be able to make use of such a channel. The concluding section is an outline of steps that could be taken to verify that a putative mechanotransducer channel performs a mechanosensitive physiological (or developmental) task.

SA channel physiology: mostly molluscs

1. Hypothesis

Molluscan SAK channels alleviate the effects of hyposmotic shock by producing a regulatory volume decrease (RVD) which is abolished by SAK channel blockers. Test: *Lymnaea* heart, neuron and kidney cells were subjected to osmotic shock in the absence and presence of quinidine, a blocker of heart and neuron[8,51] SAK channels[43]. Result: None of the cell types exhibited RVD under control (i.e. without quinidine) conditions. Quinidine slightly increased the extent of swelling in heart cells, but the effect could have been due to the blockade of chronically-open channels. With no RVD to act as an indicator of volume-activated conductances during control swelling, the finding is neutral with respect to the question of stretch-activation of the channels. The possibility remains that the channels activate, limiting swelling and preventing cell lysis, without producing RVD. Quinidine considerably increased swelling in kidney cells, but we do not know if these cells exhibit SAK channel activity.

2. Hypothesis

Molluscan heart SAK channels are activated by cell swelling. Test: *Lymnaea* heart pieces were loaded with $^{86}Rb^+$, a radioactive K^+ analogue which permeates the SAK channel[49], and $^{86}Rb^+$ efflux was followed during osmotic shock in the absence and presence of quinidine[42]. Result: Hyposmotic-induced (quinidine-sensitive) increases in $^{86}Rb^+$ efflux were consistent with the hypothesis, but would also be explained if the osmotically swollen cells were slightly more susceptible to lysis (which would masquerade as transmembrane efflux) rather than more $K^+(Rb^+)$-permeable (due to swelling-activated SAK channels). Further experiments along this line with increased time resolution at the onset of the hyposmotic shock, and with a monitor of the integrity of the heart cells throughout the efflux are needed. Greater precision is possible in radioisotope studies of osmotic perturbation if cells suitable for Coulter counter monitoring of cell size and number are used; SA channel-bearing cells like neuroblastoma or hepatoma might be good candidates for this approach.

3. Hypothesis

SACat channels in eggs and embryos are activated during cell cleavage (see Medina and Bregestovski[35]) and/or morphogenetic movements and have a regulatory role in early embryogensis. Test: *Xenopus* embryos were exposed to gadolinium, a lanthanide which blocks *Xenopus* oocyte and myotube SACat channels[20], and dechorionated *Boltenia* (tunicate) embryos (which have SACat channels of unknown pharmacology[38]) were exposed to a cocktail of putative mechanosensitive channel blockers (gadolinium, curare, gallamine). Tunicate larvae can develop in the dechorionated condition, but *Xenopus* larvae collapse without their vitelline, which was therefore left intact. The vitelline layer of *Xenopus* is permeable to gadolinium's sister-lanthanide, lanthanum. Nevertheless, the vitelline layer may have slowed the access of gadolinium to the plasma membrane, so we used the blocker in large excess[56]. Result: No effects were observed in *Xenopus* which could reasonably be attributed to gadolinium blockade of SACat channels. No developmental abnormalities were apparent in the tunicate larvae. We also tested the possibility that continuous osmotic stress during development would bring the channels into play, by having tunicate larvae develop in 50% seawater with the drug cocktail. Development was unaffected. What do these findings mean? They cannot be taken as evidence that SA channels are not activated during embryogenesis. In the case of *Boltenia*, it may be that the drugs we used did not block the SA channels. But in the case of *Xenopus*, for which gadolinium is known to be effective, the results mean that either SACat channels are not activated by the tensions associated with cell cleavage or morphogenetic processes like gastrulation, or they mean that even if the SACat channels activate, this is irrelevant to early embryogenesis. It is worth noting that *Xenopus* oocytes inflated under voltage-clamp conditions do not exhibit tension-induced macroscopic non-selective current (Eduardo Perozo, personal communication).

4. Hypothesis

Outgrowth of snail neuron growth cones is reported to be responsive to mechanical stimuli[37] and to Ca^{2+} influx via

voltage sensitive Ca^{2+} channels[27]. *Mechanosensitive K^+ currents which affect membrane potential and hence Ca^{2+} influx therefore have a role in controlling outgrowth* (see Morris and Sigurdson[40]). Test: The first step in testing this hypothesis was to demonstrate that the SA and stretch-inactivated K^+ channels seen by single-channel recording in *Lymnaea* growth cones contributed to membrane currents, as measured by macroscopic methods. Molluscan growth cones offered some important advantages for this undertaking. We could use isolated growth cones, a preparation that can be properly voltage-clamped by whole-cell or perforated-patch recording. Perforated-patch recording was ideal because it causes a minimum of mechanical disruption to the cytoplasm; clearly this is a desirable condition for studying a mechanosensitive channel in its physiological context. The K^+-selectivity of the molluscan SA channels also afforded us the opportunity to look for a mechanosensitive current component that reversed at E_K. In preparations with SACat channels, by contrast, there is always a problem distinguishing between leak current and current through MS channels. We used a variety of different methods to stretch the neuronal membrane, in each case, increasing the intensity of the stretch to an endpoint of membrane rupture[39]. Result: our technique allowed us to measure mechanosensitive K^+ currents with high resolution, but most cells did not exhibit such currents. The few cells that did produced minute currents (several picoamps over the whole growth cone) at extreme (almost rupture-producing) stimulus conditions. Thus, a preparation that seemingly had both 'the motive and the means' for producing macroscopic mechanosensitive currents was essentially unresponsive to mechanical stimuli when studied at a less reductionist level than that of single-channel recording. Our tentative explanation for the disparity is that disruption of the patch[53-54] in single-channel recording configurations renders the K^+ channels more mechanosensitive than they are in vivo. More recently, whole-cell mechanosensitive currents from yeast spheroplasts have been shown to be in accord with single-channel data for the same preparation[21]; saturating macroscopic currents were obtained, the magnitude of these currents was as expected from the density of channels in single-channel studies, and both the macroscopic and single-channel currents were gadolinium-sensitive. This is an important finding but it still falls short of providing a link to the physiological level, since spheroplasts are yeast cells with their cell walls digested away and (under whole-cell clamp) their cytoplasm washed away. It is probably worth recalling that the yeast spheroplast is little bigger than an outside-out patch; in snail neurons SAK channel activity was observed when blowing into outside-out patches but not isolated growth cones or cells[39]. It is fortunate that the yeast channel is gadolinium-sensitive and that yeast does not express other channel types with which gadolinium might cross-react[21,22]. This should make it fruitful to seek gadolinium-sensitive aspects of fungal physiology (thigmotropic aspects of hyphaeal outgrowth? gravity sensing? osmoregulation?) around which mutants could be sought.

5. Hypothesis

Molluscan SAK channels are evidently not physiological stretch transducers; since they are ubiquitous and therefore presumably physiologically important, perhaps they are S-like receptor-mediated channels. Test: *Lymnaea* SAK channels are very similar to another molluscan neuron K^+ channel, the 'S' (serotonin) channel of *Aplysia* sensory neurons. S channels are regulated by serotonin[4] and FMRFamide[5,45], which close and activate the channels respectively. We asked whether the S channel is also a SAK channel[58,59]. Result: Single-channel recordings of identified *Aplysia* sensory neurons revealed a K^+ channel that is stimulated by FMRFamide, inhibited by serotonin and activated by stretch. We have concluded that the S channel is a SAK channel. Other (unidentified) *Aplysia* neurons also had SA channels, but we do not know if they are 'S-like' receptor-mediated channels. Nevertheless, SAK channels seem to be ubiquitous in *Aplysia* neurons as they are in terrestrial[3] and aquatic[40] snail neurons. We now need to determine whether SAK channels in other cells (preferably homogeneous populations or identified cells) are also S-like receptor-mediated channels. To do this, we are returning to the heart ventricle cells of *Lymnaea*. Serotonin is an excitatory transmitter for molluscan heart, as it is for *Aplysia* mechanosensory neurons. Serotonin reduces $^{86}Rb^+$ efflux from *Lymnaea* heart[42] and so probably inhibits a K^+ channel; we intend to determine if the heart SAK channel is an S-like receptor-mediated channel, sensitive to serotonin or other neurotransmitters.

Stretch channels and osmoregulation

In many preparations, SA channels in cell-attached patches activate when cells swell during an osmotic shock[1,13]. (See also Morris[41] for earlier examples.) On the face of it, this would seem to be a tidy example of physiologically-induced stretch activation of the channels. Several issues, however, need examining. One is the partial mechanical isolation of the gigaohm-sealed patch, an inherent problem in the cell-attached configuration when studying mechanosensitive channels. If hyposmotic activation is a direct stretch effect (rather than a chemically-mediated one), tension is presumably conveyed through the cytoskeleton to the patched membrane. A separate issue is whether the activation is general or occurs only in the patch because patching has rendered a class of otherwise refractory channels hypermechanosensitive, as we suspect for molluscan SAK channels[39]. Fortunately, various workers have begun to use macroscopic recording methods[23,39] so that currents from non-patched membrane are measured during osmotic perturbations to the cell[28,39]. Hyposmotic solutions can

evidently produce swelling in cells under whole-cell clamp[15, 57, 60]. If this constitutes true osmotic swelling, it is somewhat unexpected; it requires that the cell membrane's water permeability exceed that of the open pipette tip and/or that the cell membrane be sufficiently compliant to make it easier for water (entering by osmosis) to swell the cell than to push solution back up the pipette. Moreover, a fundamental question about the degree to which swelling in the whole-cell configuration is strictly osmotic (i.e. like blowing up a balloon with a fixed amount of rubber) is raised by a finding[15] in lymphocytes. When swelling under whole-cell clamp, these cells show an increased membrane area (measured via capacitance measurements) that is correlated with increased Cl^- conductance. Here, the hyposmotic solution may increase the cell's Cl^- conductance by promoting (chemically? mechanically?) fusion of channel-bearing vesicles to the plasma membrane. In these circumstances, cell 'swelling' need not be associated with increased membrane tension. As an aside, it seems likely that when membrane area expands by vesicle fusion, reorganization of cortical cytoskeleton is inevitable and this may affect the mechanosensitivity of some channels (see refs[14, 30, 39]). Even without fusion, any restructuring of membrane that originally formed invaginations or evaginations should affect cortical cytoskeleton; this may occur in GH_3 cells which exhibit whole-cell macroscopic currents when irreversibly inflated[39].

A whole-cell study of a swelling-activated Cl^- conductance has also been undertaken in canine heart cells, but the preliminary report[57] does not indicate whether membrane area increased along with conductance as it did in lymphocytes; it is too early to conclude that a SA channel is responsible for the swelling-activated Cl^- conductance increase in heart.

For neuroblastoma cells, a three channel model has been proposed for volume regulation[60]. In the model, SACat channels mediate volume regulation at the initial swelling-detection stage by depolarizing the cell, thus activating voltage-dependent K^+ and anion conductances which act as a path for osmolyte loss[12, 60]. Data from neuroblastoma are promising. Perforated-patch recordings of cells swelling and undergoing RVD are consistent with the three-channel model. Interestingly, swelling, but not RVD, occurs under whole-cell recording conditions. Gadolinium, which blocks the SACat channels, abolishes the RVD[60]. If perforated-patch recordings were made which could demonstrate, at the macroscopic level, a mechanosensitive, gadolinium-sensitive, nonselective conductance, it would do much to establish a mechanosensor role for the SACat channel in this preparation.

Bacterial SA channels[33, 34] carry enormous single-channel currents and are correspondingly nonselective. Pharmacological evidence hints at a role for these channels in allowing *E. coli* to cope with hyposmotic shock[17]. The channels are apparently gadolinium-sensitive, and in a giant liposome preparation, gadolinium blocks the loss of organic molecules (ATP, lactose) which are otherwise released by osmotic swelling. However, since an issue as basic as the cellular location of the SA channel (i.e., the outer or inner membrane or spanning both) still needs to be settled, it is not surprising that the cellular physiology also remains to be determined.

Mechanoreceptors

The cell body and primary dendrites of the crustacean stretch receptor neuron have SA channels[11]. As I have discussed previously[41], it is tempting but premature to conclude that they represent the mechanism which operates in the distal regions of the modified mechanosensory dendrites. Function-by-association (i.e. presence in a mechanoreceptor cell) is part of the evidence favouring their candidacy (having the right selectivity is also persuasive), but, countering this, SA channels which are not likely to represent the primary transducer have now been seen in two other types of mechanoreceptor cell[25, 58]. Moreover, it was recently found that SA channels of similar selectivity to those in the crustacean stretch receptor neuron (i.e. SACat) are ubiquitous in central neurons of the leech[44]; it is not yet known if central neurons of crustaceans have SA channels. Crustacean stretch receptor neurons are peripherally located but, because of an extensive surround of connective tissue, are extremely difficult to work with using patch electrodes[11]; it is therefore encouraging that a procedure has recently been developed to isolate and patch these cells in culture[18].

Stretch channels and stretch-excitation

Amphibian smooth muscle and mammalian endothelium are examples of cells with stretch-excited physiological functions[23, 29, 31a]; both have SA channels which might be implicated. Given the potential importance of mechanosensitive currents to endothelial cell function[6], it is disappointing that the single-channel report of SA channel activity has not been followed up at the whole-cell level. In smooth muscle, the obstacles to making whole-cell recordings of mechanically-activated currents would seem daunting, but since a preliminary report indicates that one group has succeeded[10], progress may be forthcoming in this area. Caveats remain, however, even when whole-cell mechanosensitive currents are observed; GH_3 cells, as mentioned above, exhibit nonselective macroscopic mechanosensitive currents[39], but only when the cells are inflated enough to distend them irreversibly. These currents may simply represent leak, but even assuming they are channel-mediated, they remain suspect because of the disrupted state of the cytoskeleton.

On chick cardiac myocytes, a sharp indentation of the surface of SACat channel-bearing membrane induces contractile activity and a gadolinium-sensitive rise in intracellular Ca^{2+} [52]. If this observation were repeated, but

with current measurements and under conditions in which the cortical cytoplasm is not likely to be damaged, it could make a strong case for channel-mediated stretch-excitation.

Stretch channels and pathology

We have suggested that the mechanosensitive patch-behaviour of stretch channels may be conditioned by disruption of the cortical cytoskeleton [39]. Aspirating membrane into a pipette tip should produce a broad-spectrum disruption; a more discrete disruption is expected in cells which are defective in that they lack one of the elements of cortical cytoskeleton. It is intriguing that dystrophic muscle, which by virtue of an *mdx* mutation lacks the cytoskeletal element dystrophin, has stretch channels with an abnormality. These channels show stretch-inactivation, a mode not seen in normal fibers, which only exhibit stretch-activation [14]. If these abnormally stretch-sensitive cation channels provide the Ca^{2+} leak path of dystrophic muscle, understanding the molecular basis of this pathophysiology may help us understand what SA channels normally do.

In addition to the murine *mdx* mutant, mutants of *E. coli* which show stretch channel abnormalities are being studied. The lipoprotein mutant JE5505 (which lacks Braun's lipoprotein, a link between cell wall peptidoglycan and the outer membrane), whose SA channels have reduced mechanosensitivity and are not activated by amphipaths [30], promises to be a powerful tool for attempting to correlate cellular physiology with single-channel studies.

Whether the cytoskeletal element, actin, has any effect on SA channel gating in eukaryotic cells is unclear. The original report [20] that cytochalasin D, a drug which promotes actin depolymerization, augments the stretch-sensitivity of SA channels has been retracted [54] on the grounds that the effect was not repeatable with the more actin-specific form, cytochalasin B. We used cytochalasin B on snail growth cones (where it depolymerizes actin within minutes) but saw no evidence of an effect on SA channels [39]. A preliminary report on a SA Cl^- channel in rabbit renal cells [55] states, however, that dihydrocytochalasin B produced an ATP-sensitive increase the channel's open probability. Pharmacological and genetic ablation of cytoskeletal elements may eventually be supplemented with molecular approaches like the use of antisense oligonucleotides to knock out specific cytoskeletal elements or membrane components.

Receptor-mediated channels and stretch

Of those channels whose primary role it is to modulate membrane conductance in response to chemical messages received at remote receptors, the *Aplysia* S channel is, perhaps, the best characterized. It may not be the only 'modulator' (or, for want of a better name, 'receptor-mediated') channel that is stretch-sensitive in the patch. Fish embryos have a SAK channel [35] whose activity, as monitored in the patch, oscillates with the cell cycle. Patch recordings of the channel indicate that it may be regulated during the cell cycle by cyclic-AMP dependent phosphorylation [36]. Whether this constitutes a modulation of a genuine physiological mechanosensor/effector is too early to say; there have been no macroscopic recordings demonstrating mechanically-induced current. If these channels prove to be mechanotransducers, this will doubtless be a fascinating area to watch.

Another case which links SA channels with receptor-mediated channels has recently been reported in rat hepatoma cells. ATP, probably acting via P_2-purinergic receptors, activates a channel [2] which is thought to correspond to the SACat channel of this preparation [1]. More tenuously, FMRFamide-activated divalent cation channels in *Lymnaea* heart cells, though not characterized as SA channels, are excessively active for seconds to minutes after seal formation, prompting the suggestion that "suction, applied to the pipette during patch formation, transiently stimulates these channels" [7].

Should more examples of SA channels which are also primarily receptor-mediated channels come to light, it would be worth asking what feature(s) of such channels or of their connections to the local environment predispose them to mechanosensitivity in the patch. It might be, for example, that the stretch-sensitive 'dystrophy' channel [14] is a receptor-mediated channel run amok in the absence of dystrophin, rather than a malfunctioning mechanotransducer channel.

Criteria for putative mechanotransducer channels

Stretch channels are everywhere. Do they all have physiological roles which require a mechanical stimulus? Do the SA Na^+ channels which are seen in *Lymnaea* heart cells only when there is a zero-Ca^{2+} solution in the pipette [16] really care about membrane tension? Are the SA Cl^- channels of land snail neurons which are only evident in excised patches [3] physiological mechanotransducers? My suspicion is that the mechanosensitivity of some stretch channels is an adventitious feature brought on by the mechanical bludgeoning the membrane suffers during recording procedures. Our evidence does not support the hypothesis that neuronal SAK channels normally detect membrane tension [39]. It is, however, abundantly clear from macroscopic voltage clamp studies that in hair cells [24] and in ciliates [32], mechanosensitive channels of some kind underlie the mechanotransduction process. It seems reasonable to expect that some of the SA channels evident in single-channel studies are also mechanotransducers. What criteria need to be satisfied to establish that a SA (or stretch-inactivated) channel performs a mechanical cellular task? The list below seems reasonably comprehensive. (Note that, with appropriate adjustments for gating modality, all the items could be checked off for

ligand-gated and voltage-gated channels whose physiological roles are established [e.g. nicotinic acetylcholine channel, tetrodotoxin-sensitive Na^+ channel]).

1) Demonstrate and characterize the mechanosensitive channel at the single-channel level.

2) Find a blocker for the channel and establish its specificity. Alternatively (or preferably, in addition) obtain a version of the organism (a mutant or a cell whose protein expression has been tinkered with appropriately) in which the stretch channel has abnormal single-channel properties, and explore the mutant (or tinkered) versus wildtype properties.

3) Show that some physiological or developmental aspect of the cell is mechanosensitive.

4) Show that the mechanosensitive cellular function is specifically impaired by the channel blocker (or by the mutation or molecular tinkering).

5) Obtain macroscopic recordings (preferably perforated patch as well as whole-cell configurations, since the former is gentler on the cytoskeleton) of currents with the following characteristics which correspond to the single-channel recordings:

- selectivity,
- pharmacology,
- noise characteristics consistent with the single-channel kinetics and amplitude,
- saturation with large mechanical stimuli (to demonstrate that the current path is constituted of a finite number of channels; particularly important for non-selective conductances),
- currents which saturate with large stimuli and have the expected magnitude (see Gustin et al.[21] for the only available example),
- expected mechanosensitive current density variations (with cell type or cell region) if these are predicted from other findings or from the cellular process in question, and
- stretch sensitivity.

Exhausting though it is to even imagine satisfying all these criteria, the criteria are less than exhaustive. They ignore the multiple stretch channel types seen in many cells. They are clearly not applicable to mechanoreceptor membrane which, because it consists of fine processes, is non-patchable. The issue, however, is not one of finding the mechanism underlying mechanotransduction. The issue is that we need to determine what if any mechanical role the SA channels seen at the single-channel level perform in situ. If most of these criteria could be met for even one SA channel, it would greatly strengthen the case that other SA channels are implicated in the mechanical aspects of cellular regulation.

Epilogue

After writing this account, I learned of a paper in press which meets many of the criteria listed here. The fungal germling of the bean rust can sense minute topographical features, a trick it uses to find stomatal openings on the leaf surface. Kung and colleagues[62] have shown that germ tube protoplasts have SA channels which are evident both in the patch and whole-cell recordings. Saturation is readily obtained in whole-cell recordings. Gadolinium blocks both single-channel and whole-cell currents in protoplasts and, in vivo, inhibits germ tube growth and differentiation. It is not clear how the large conductance stretch channels would be used by the growing tip, but calculations suggest that membrane tensions large enough to activate the channels might be experienced. Given the enormous conductance of the channels (600 pS) and their ability to pass Ca^{2+}, they are presumably used with great care!

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