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Stretch-Activated Ion Channels in Growth Cones of Snail Neurons

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Using single-channel recording, we show that neurons contain ion channels sensitive to membrane tension. Neurons isolated from the snail, *Lymnaea stagnalis*, actively rearborized in culture yielding cell bodies and growth cones suitable for patch clamping. All neurons contained, in both their soma and growth cones (at a density of ~1–2 μm^{-2}), stretch-activated channels highly selective for K^+. The presence of this mechanosensitive channel in the motile region of the neuron, a region characterized by insertion of new membrane—the growth cone—is of particular interest. Under physiological conditions, the channel was permeable to K^+, but not to Na^+ or Cl^-. Its conductance to K^+ under these conditions was ~44 pS. Channel activation was steeply dependent on membrane tension, showing thresholds at between −50 to −100 mm Hg (suction was applied through the recording pipette). Kinetic analysis indicated that the stretch-dependent increase in the channel's open probability was related to a long closed state rather than to one of the open states. Given the importance of Ca^{2+} in the regulation of growth cone motility, we speculate that this stretch-activated K^+ channel could play a role in neurite elongation by a tension-dependent modulation of membrane voltage which in turn would act on voltage-gated Ca^{2+} channels.

The idea that cells which are not specialized mechanosensors should possess stretch-sensitive ion channels still seems novel, but following the first observation of such channels in skeletal muscle (Guharay and Sachs, 1984), similar channels have been described in cells as diverse as bacteria, yeast, plant protoplasts, lens epithelium, smooth muscle, endothelial cells (reviewed by Kullberg, 1987; Sachs, 1988), and teleost embryos (Medina and Bregestovski, 1988).

Here we report that molluscan neurons contain stretch-activated channels. These channels occurred in the cell body, but more interestingly yet, in the mechanically active part of the cell, the growth cone. They were ubiquitous rather than confined to identified neurons. Isolated in culture, *Lymnaea* neurons actively put out new processes with growth cones that, like those in other gastropod neurons (Siegelbaum et al., 1986), are large enough for single-channel recording.

Neurite elongation and cell locomotion are related processes, probably using the common underlying mechanisms of exocytosis and actin–myosin contractions (Lackie, 1986; Bray, 1987; Kater et al., 1988). Models of these processes therefore require that Ca^{2+} concentrations be controlled within narrow limits (Kater et al., 1988). Snail neurons have been instrumental in showing how neurotransmitters and electrical activity, through indirect effects on intracellular Ca^{2+}, control growth cone motility. Given the strong precedent for regulation of neuronal Ca^{2+} levels by K^+ channels (Siegelbaum et al., 1986), stretch-activated channels that are K^+ selective would naturally be of special interest in growth cones. Growth cone motility, by definition, is a mechanical process, so that neurite membranes should experience tension changes during growth. Stretch-sensitive channels in growth cones could provide a link between this membrane tension and—via membrane voltage—transmembrane Ca^{2+} fluxes.

Preliminary communications describing stretch-activated channels in snail neurons (Sigurdson et al., 1987b; Bedard et al., 1988) and in neuroblastoma (Falke and Misler, 1988) have appeared.

Materials and Methods

Lymnaea stagnalis were collected locally or purchased from Blades Biological (Edenbridge, England). Sterile isotonic normal saline (NS) (50 mM NaCl, 1.6 mM KCl, 3.5 mM $CaCl_2$, 2.0 mM $MgCl_2$, 5 mM HEPES, 5 mM glucose, pH 7.6, adjusted with NaOH) supplemented with penicillin (500 IU/ml) and streptomycin (500 μg/ml) was used as the culture medium. Pipette solutions were either antibiotic-free NS or 50 mM KCl saline (50 mM KCl, 1 mM $CaCl_2$, 5 mM HEPES, pH 7.6). A low Ca^{2+} saline (buffered to 10^{-8} M Ca^{2+}; 60 mM KCl, 2 mM $MgCl_2$, 0.2 mM $CaCl_2$, 2.2 mM EGTA, 5 mM HEPES, pH 7.6) was used to perfuse inside-out patches in some experiments. Neurons from the circumoesophageal ganglia were isolated by first loosening the connective tissue sheath in 0.25% protease (Sigma) (40 min agitation) followed by mechanical dispersion. Neurons from individual ganglionic masses were then dispersed directly onto coverslips (recording chamber bottoms). Chambers were detergent-washed, rinsed thoroughly with distilled water, surface-sterilized with 70% ethanol, and then dried in a 60°C oven for at least 2 hr before use. Cell adhesion and spreading on the glass substratum was generally good provided the amount of cellular debris was minimized during dispersal.

Standard single-channel recording techniques were employed (Hamill et al., 1981) to record from the neuron cell membrane. Patch electrodes were pulled (Kopf 750 pipette puller) from Corning 7052 capillary tubes (Garner Glass Co., Claremont, CA; i.d. = 0.8 mm, o.d. = 1.65 mm), fire-polished, and filled with NS, with NS plus 1 mM quinidine (Sigma), or 50 mM KCl saline. Pipette tip openings were estimated to be a maximum of 2 μm in diameter prior to fire-polishing and had a mean bubble number of 4 ± 0.6, n = 5 (Corey and Stevens, 1983). Suction applied to the patch pipette through the sidearm of the electrode holder was monitored by a Bio-Tek pressure transducer (Bio-Tek Instruments, Burlington, VT).

Single-channel currents were recorded using a patch-clamp amplifier (Axon Instruments, Burlingame, CA), filtered at 10 kHz, and stored on video tape using a PCM-1 (Medical Systems, Greenvale, NY) digitizing unit. For analysis of current amplitudes, data were filtered at 1 or 2 kHz, displayed on a digital storage oscilloscope and the amplitudes of at least 10 individual events directly measured from the display. For analysis of open probabilities [P(O)] and for mean open and closed times, data were filtered at 2 kHz and digitized at 0.1 msec/point (5

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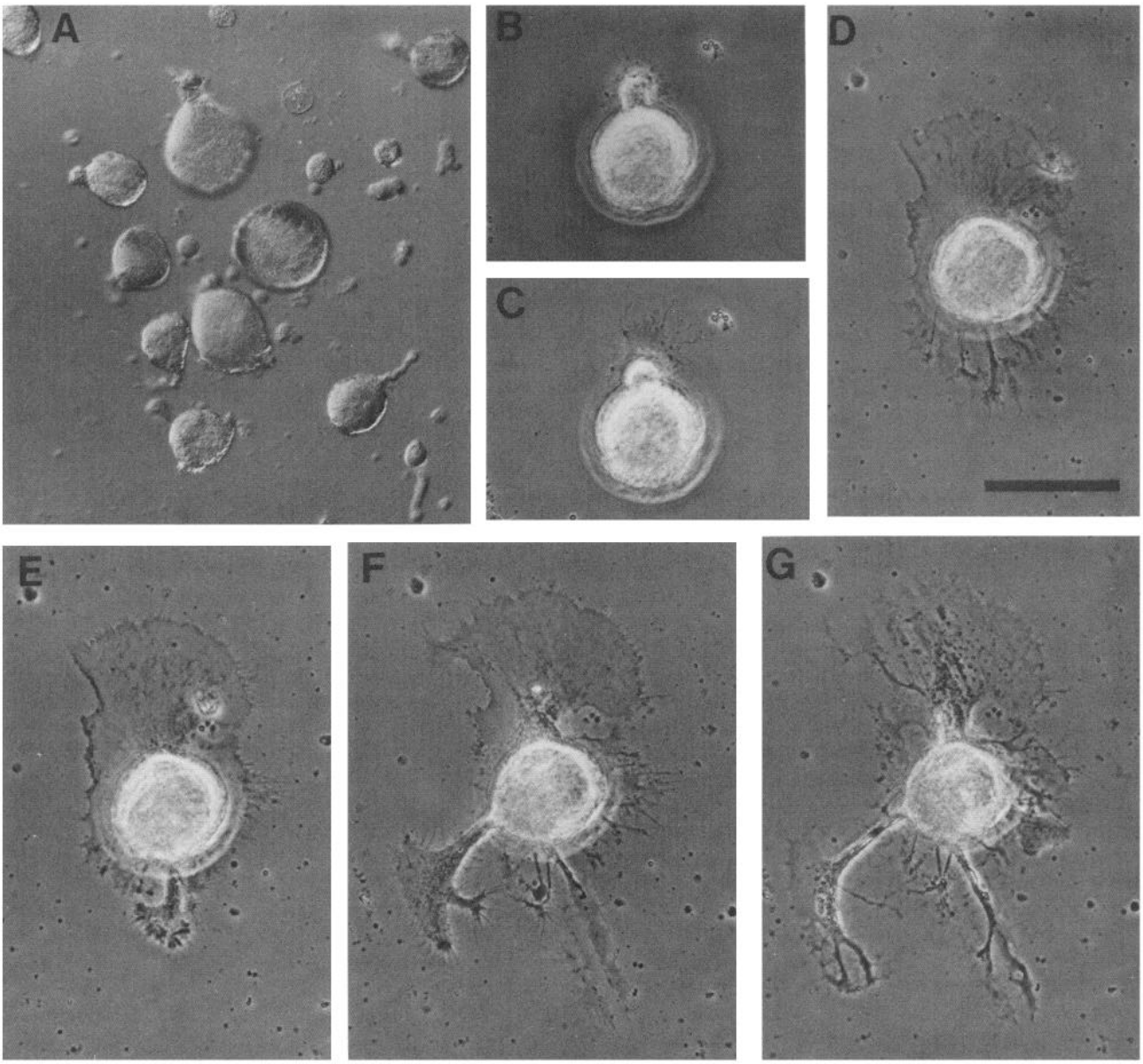

Figure 1. Timecourse of neurite growth. *A,* Freshly isolated unidentified *Lymnaea* neurons. DIC optics; scale bar, 106 μm. *B–G,* Neurite development of one cell from isolation until day 3. *B,* 20 min. *C,* 1 hr. *D,* 21.5 hr. *E,* 24 hr. *F,* 45.5 hr. *G,* 68 hr. Phase contrast optics; scale bar, 54 μm.

times the filter cutoff frequency). An event-detection program run on a DEC 11/23 computer produced an idealized record of the open and closed times, thus allowing determination of the proportion of time the channels spent open. When intense channel activity precluded accurate event detection, an index of $P(O)$ was determined by integrating the current record over a known time interval and dividing by the single-channel current. The true number (n) of channels in the patch is unknown, hence, in both approaches, $P(O)$ is reported as (Sigurdson et al., 1987a):

$$\text{Index } P(O) = N_1P_1 + N_2P_2 + \ldots + N_nP_n,$$

where N_i is the number of channels simultaneously open and P_i the proportion of time spent with N_i channels open. Dividing index $P(O)$ by n, were it known, would give $P(O)$, the open probability for a single channel.

Curve-fitting procedures used to determine time constants associated with the open and closed times have been described previously (Sigurdson et al., 1987a).

For current/voltage (I/V) relations of cell-attached patches, the membrane voltage (V_m) was taken to be

$$V_m = V_{rest} - V_p,$$

where V_p is the pipette holding potential and V_{rest} the assumed resting potential of the cell. V_{rest}, obtained from intracellular measurements, was -50 ± 1.9 mV (SEM, $n = 32$).

Photomicrographs were made using an Olympus IMT-2 inverted microscope employing phase-contrast or Nomarski differential interference contrast (DIC) optics. Cells were cultured, and all recordings made at room temperature (18–23°C).

Results

Rearborization of the isolated neurons

Isolation disrupted the *in vivo* structure of neurons. Freshly dissociated, neurons consisted only of a cell body, sometimes

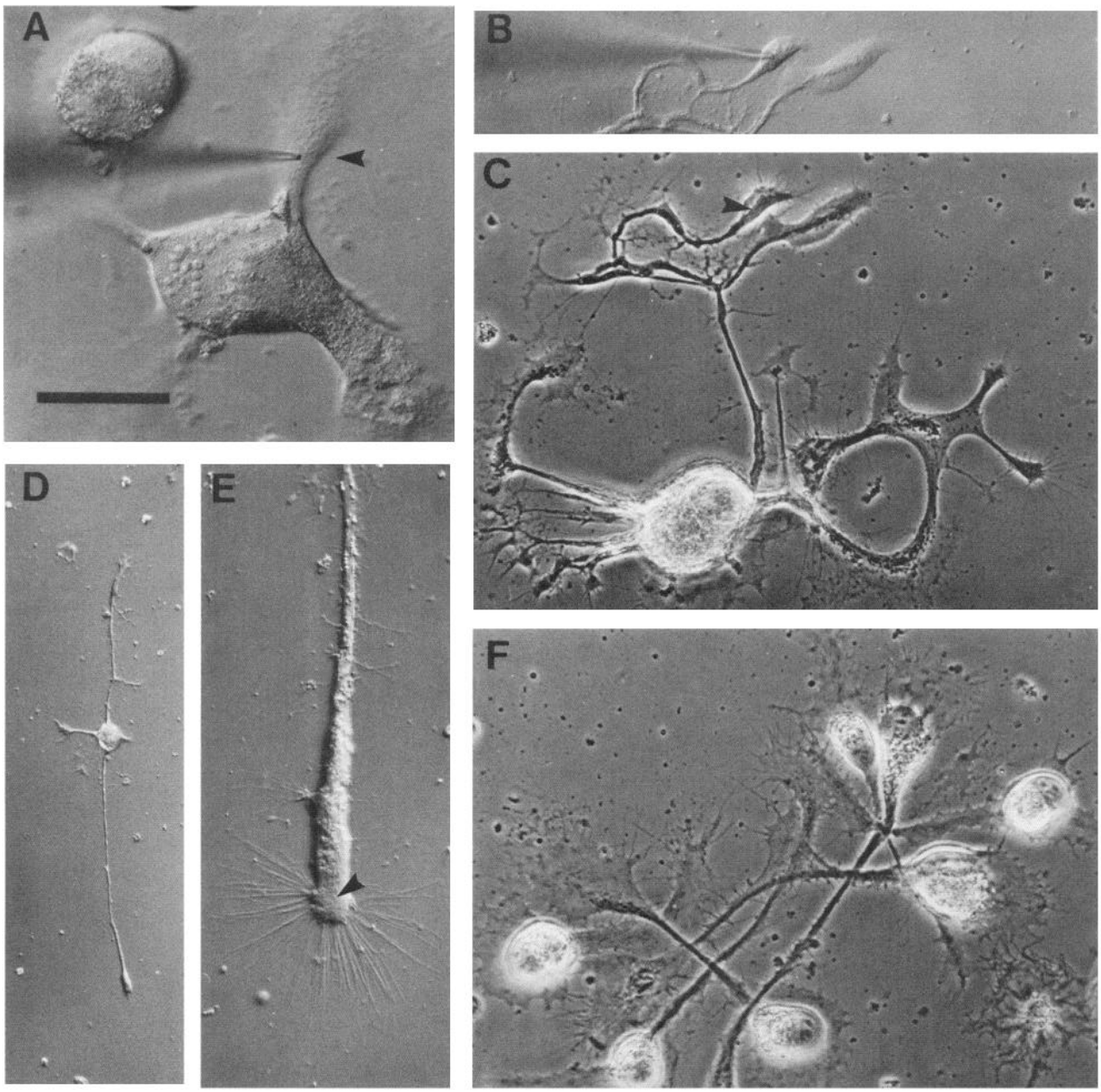

Figure 2. *Lymnaea* neurons several days in culture. *A,* Neurons 5 d after isolation. Note patch pipette (*arrowhead*) located on a process close to the cell body. SAK channels were observed during recordings made at this point. *B,* Patch pipette on growth cone from the cell in *C.* Recordings from this patch exhibited SAK channels. *C,* Day 3 cell with numerous neurites terminated by growth cones with lamellae and microspikes. *Arrowhead* marks the location of the patch pipette pictured in *B. D,* A very long (>720 μm) day 3 neuron. Patch recordings from the growth cone (lower part of plate) and the cell body both showed SAK channels (see Fig. 3). *E,* Higher magnification view of the patched growth cone (with numerous microspikes) in *D.* Patch location indicated by *arrowhead. F,* Day 5 neurons showing extensive neurite development with overlapping of processes. As in other cells, growth cones are well-developed, many containing large veil areas with protruding microspikes. DIC optics for *A, C, D, E*; Phase contrast optics for *B, F.* Scale bar for *A, B, C, F,* 54 μm; for *D,* 208 μm; for *E,* 36 μm.

bearing a short axon stump (Fig. 1 *A, B*). For our purposes, this was advantageous, because the subsequent rearborization provided an opportunity to record from membrane that, we could assume, was newly elaborated. To illustrate some of the dynamics of regrowth of isolated *Lymnaea* neurons under the minimal culture conditions used (cf. Kater and Mattson, 1988), Figure 1, *B–G,* follows a cell for 68 hr postdissociation. The axon remnant (Fig. 1*B*) became the first attachment site 20 min after isolation. In the next hour, a lamella appeared (Fig. 1*C*), then spread extensively. The cell became firmly attached (Fig. 1*D,* 21.5 hr), as indicated by its stability during jarring movements of the culture dish. Over the same period, small neurites with microspikes began to extend from the opposite side of the soma (Fig. 1*D*). One of these neurites rapidly broadened into a growth cone (Fig. 1*E,* 24 hr), which extended for ~70 μm before retracting (not shown). By 46 hr, another neurite had formed and produced a much larger growth cone (Fig. 1*F*), which continued to elongate and branch (Fig. 1*G,* 68 hr). Meanwhile the neurite

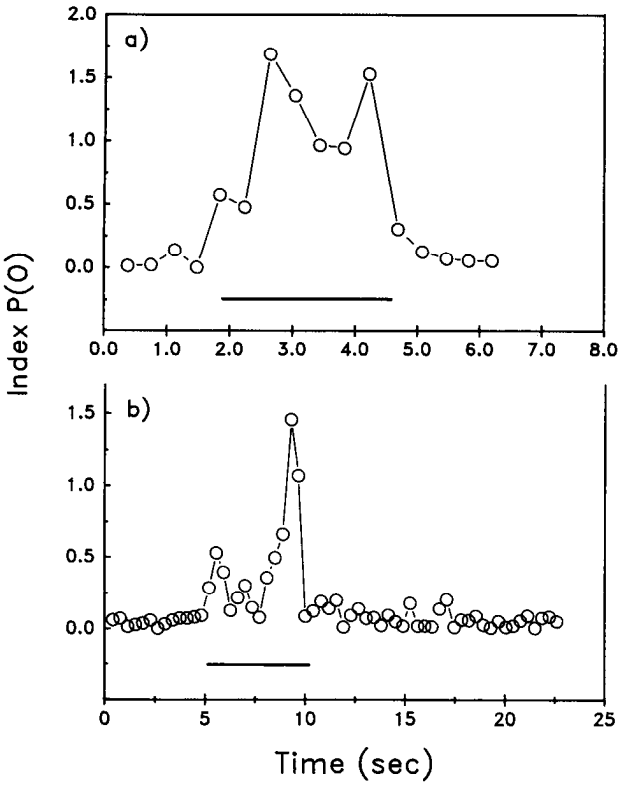

Figure 3. Stretch increases the open probability of SAK channels from the cell body and growth cone. *a*, Cell body and *b*, growth cone of the neuron pictured in Figure 2*D*. Bars indicate suction of ~ −50 to −70 mm Hg, which was submaximal for this patch. V_m, 70 mV for both patches.

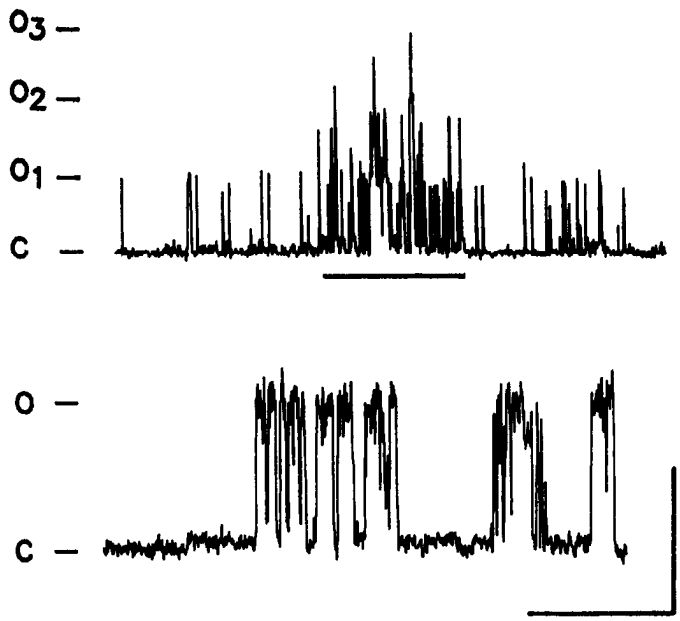

Figure 4. Recordings of SAK channel activity. *Upper trace,* Suction (bar, ~ −70 mm Hg) produces simultaneous activation of up to 3 channels. *C* indicates the zero current (all channels closed) baseline, O_1–O_3 indicate successive open levels. *Lower trace,* Higher resolution recording showing the bursty nature of SAK channel openings. Upper trace filtered at 1 kHz; V_m, 30 mV; scale, 5 pA and 2.5 sec. Lower trace, 2 kHz; V_m = 70 mV; scale, 5 pA and 50 msec.

(Fig. 1*E*) that had retracted, reextended. Throughout the neuron's regrowth process, the large lamella established from the axon remnant was relatively stable, with major neurite and growth cone formation occurring on the opposite side of the soma.

Most, but not all isolated cells attached and began spreading within minutes. Figure 2*A* shows 2 cells 5 d in culture, one which merely persisted, another which rearborized. The gamut of rearborization patterns was wide. At one extreme (Fig. 2, *B, C*), cell bodies had multiple neurites with subbranches. Some cells were dominated by 2 long neurites (Fig. 2, *D, E*), whereas others were essentially monopolar (Fig. 2*F*). As the figures show, neurites were terminated by growth cones from which extended microspikes (Fig. 2, *B, E*) and/or lamellae (Fig. 2, *B, F*). Recordings were made from cell bodies and growth cones in neurons of all morphological types.

Stretch-activated K^+ (SAK) channel distribution

Stretch-activation of the (SAK) channel was observed in cell-attached and excised (inside-out) patches. Except were specifically noted data were obtained from cell-attached patches using pipettes containing antibiotic-free NS.

SAK channels were ubiquitous. They were observed in each of 6 freshly isolated neurons tested at 0.3–2.0 hr postdissociation, as well as in the cell bodies of all older neurons tested. They were observed in broad dilations near the cell body (Fig. 2*A*) in older, spread-out cells. They were observed in regions of extensive neurite and growth cone formation (Fig. 2, *B, C*). Figure 2*D* shows an actively advancing growth cone approximately 370 μm from the cell body; SAK channels were recorded from both the cell body and growth cone of this cell (Fig. 3, *a, b*). This growth cone (Fig. 2*E*) and that in Figure 2, *B, C*, approach the minimum size from which single-channel recordings were successfully made. Patch recordings were not made on lamellae or microspikes, but note that growth cone recordings were obtained only a few micrometers from these structures (see Fig. 2, *B, C, E*), where the cell thickens slightly.

Growth cone SAK channel stretch sensitivity

SAK channel openings generally occurred in bursts as illustrated in the lower trace of Figure 4. A typical response to suction is demonstrated in the upper trace, where, on this time scale, individual channel openings (outward current) appear as spikes. During suction, with the increased membrane tension, the rate of opening and the probability of multiple open channels (up to 3 channels in this case) increased. Upon release, activity subsided to the presuction level. Other channels recorded from the growth cones displayed no stretch-activation under the range of recording conditions (i.e., pressure and voltage excursions and various salines) described in this paper. We have, however, recently observed a low-conductance K^+ channel *in*activated by stretch (Morris and Sigurdson, 1989); it occurs in growth cones as well as cell bodies.

The lower trace of Figure 4 contains several low-conductance events that appear as small outward current shifts from the baseline. Such events were routinely excluded from the quantitative analyses described below.

Figure 5*a* shows single-channel recordings with no applied suction, as well as suction sufficient to begin activating the channels. Such data were used to construct the curve shown in Figure 5*b*. The open probability increased steeply beyond a "threshold"

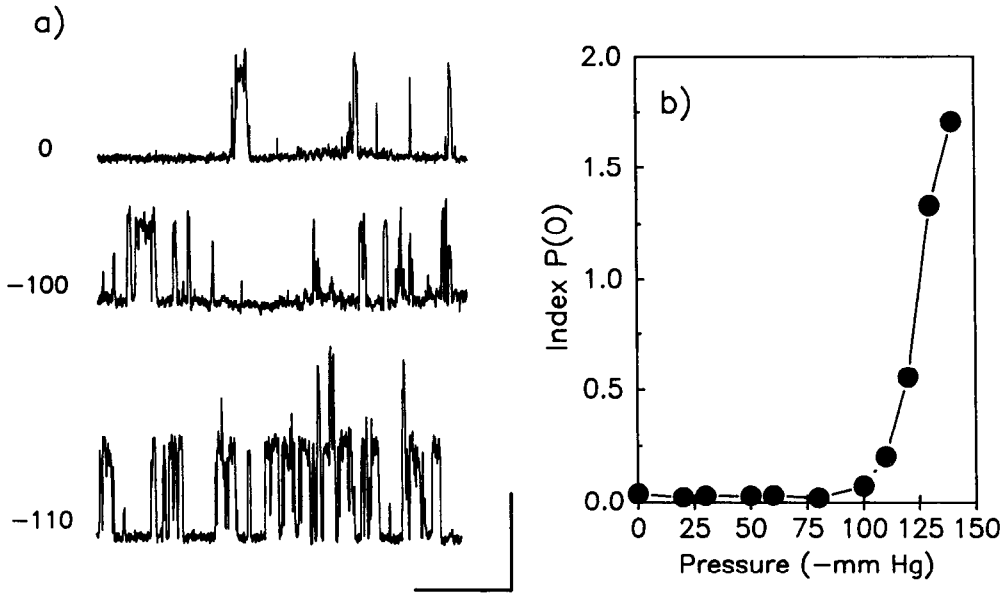

Figure 5. Effect of pressure on SAK channel activity, *a*, Recordings at indicated suction (mm Hg) applied by the pressure transducer. Note that channel open probability appears to increase by a reduction of closed intervals, yielding more openings per unit time. V_m, 70 mV. Scale, 5 pA and 50 msec. *b*, Open probability data for the same patch shown in *a*.

pressure. Thresholds in the range of −50 to −100 mm Hg were observed for various patches. Usually $P(O)$ curves are expected to saturate at higher pressures when all channels are recruited (see Sigurdson et al., 1987b), but in the example shown in Figure 5*b*, the membrane ruptured at −140 mm Hg. The index $P(O)$ at this highest experimental pressure is a reflection of the average channel activity seen over a period of about 15 sec but is deceptively low as an indicator of the number of SAK channels present. As many as 8 channels (as opposed to the average of 1.8) were open simultaneously at some points, indicating that at least 8 channels must have been present in this patch. In general, patches from growth cones had similar SAK channel density to those from cell bodies (~4–8/patch; corresponding to a density of ~1–2/μm^2 (see Guharay and Sachs, 1984; Sigurdson et al., 1987a)).

The SAK channel of *Lymnaea* heart cells is insensitive to intracellular Ca^{2+} (Sigurdson et al., 1987a), as is the neuronal cell body SAK channel (W. J. Sigurdson and C. E. Morris, unpublished observations). Ca^{2+} insensitivity of growth cone SAK channels was confirmed using inside-out patches (50 mM KCl saline in pipette) which contained both Ca^{2+}-activated K^+ channels (Lux et al., 1981) and SAK channels. When these patches were perfused with a low Ca^{2+} solution (not shown) which silenced the Ca^{2+}-activated K^+ channels, the SAK channels remained activatable by suction.

More detailed kinetic analysis of the recordings used to generate Figure 5*b* was possible for 0, −100, −110, and −120 mm Hg. This allowed us to ask which of the kinetic parameters contributes to the stretch-induced increase in $P(O)$. At all pressures, open-time distributions were best fit to a sum of 2 exponentials (Fig. 6), while closed times (histogram not shown) were best fit to a sum of 3 exponentials (see Table 1). The number of open and closed times and the values of their constants (mean dwell times) are in good accord with those reported for *Lymnaea* heart SAK channels (Sigurdson et al., 1987a). In principle, increased open times and/or decreased closed times could be responsible for increased $P(O)$. A pronounced effect of increasing tension is the progressive decrease of the longest closed time. This coincides with what has been observed for other stretch-sensitive channels, where a between-bursts closed time is most affected by stretch (Guharay and Sachs, 1984; Sigurdson et al., 1987a).

SAK I/V *relations*

I/V relations of the stretch-sensitive channel in growth cones were determined under near-physiological ionic conditions (cell-attached, NS in pipette and bath) as well as with high-K^+ saline (Fig. 7). For each voltage, suction was applied to activate the channels and thus unambiguously distinguish the SAK channels from stretch-insensitive channels. With NS in the pipette, the zero-current potential for stretch-activated currents was near the resting potential of the cell. With 50 mM KCl saline in the pipette, the zero-current potential shifted approximately 50 mV to the right, a shift that would be in accord with the altered K^+ equilibrium potential. As expected for a K^+-selective channel, when excised patches were exposed to symmetrical NS and suction was applied, we observed no channel activity. Con-

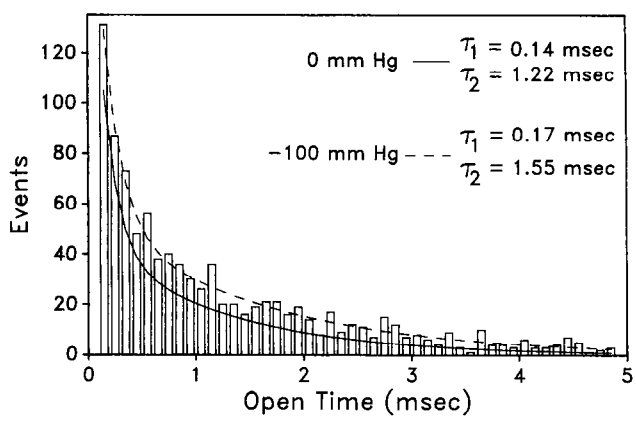

Figure 6. Open time histogram of growth cone SAK channels. Superimposed on the histogram (for −100 mm Hg) are best fit curves for a sum of 2 exponentials to the 0 mm Hg data (*solid line*; histogram not shown; total number of events was 788) and to the −100 mm Hg data (*dashed line*; total number of events was 1168). Error bounds for the indicated open time constants are given in Table 1.

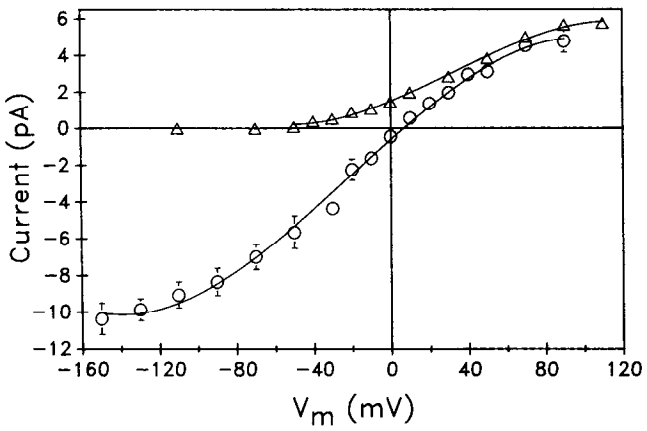

Figure 7. *I/V* relations for growth cone SAK channels. *Triangles,* NS in pipette; *circles,* 50 mM KCl saline in pipette. Each curve (lines fit by eye) incorporates data from 4 patches; error bars are standard errors and, where not shown, are contained within the symbols. The steepest portions of the NS and 50 mM KCl curves correspond to slope conductances of 44 pS and 80 pS, respectively.

versely, excised patches exposed to 50 mM KCl in the pipette with NS in the bath (symmetrical Cl^-, asymmetrical K^+ and Na^+) demonstrated stretch-activated inward current, but not outward current. This is consistent with a channel permeable to K^+ and not to Na^+ or Cl^-. Nonlinearities in single-K^+ channel *I/V* relations, such as those seen in Figure 7 (except for the nonlinearity at the foot of the NS curve, which is predicted by electrodiffusion for a K^+ channel under asymmetric K^+), can generally be attributed to effects of voltage on ion permeation through a multi-ion channel (Yellen, 1984a, 1987) and to apparent attenuation produced by high-frequency flickering at large polarization (Yellen, 1984b).

Quinidine blocks SAK channels

The K^+ channel blocker, quinidine, blocks cell body SAK channels in a dose-dependent manner (Sigurdson et al., 1987b); 1 mM produces a flickery block (Yellen, 1984b) or, in some cases, a full block of SAK channel currents. For growth cone SAK channels, this concentration of quinidine induced flickery block in 3 of 4 patches. The fourth patch was silent.

Discussion

It has been demonstrated that growing neurites, like fibroblastic cells, experience varying degrees of tension associated with their motility (Dembo and Harris, 1981; Bray, 1987; Letourneau et al., 1987). Accordingly, some models of neurite elongation and neurogenesis incorporate tension as a regulatory element (Bray, 1984; Gordon and Brodland, 1987; Letourneau et al., 1987; Bray and White, 1988; though see Goldberg and Burmeister, 1986, 1988). Yet despite the clearly mechanical nature of neurite motility, no mechanism has been identified by which tension could be sensed. Stretch-activated channels would seem to be ideally suited for this role.

Although the mechanism by which stretch-sensitive channels transduce membrane tension is still speculative, there is evidence that the channels are tied into the underlying cortical cytoskeleton. In particular, Guharay and Sachs (1984) showed that cytochalasin increased the stretch-sensitivity of SA channels in skeletal muscle. Their interpretation (Sachs, 1988) was that actin filaments normally relieve tension on the membrane cytoskeletal elements which directly apply tension to the channels. These unknown elements may involve spectrin/ankyrin; the density of Na^+ channels and glutamate receptors has recently been shown to be controlled by anchorage to these proteins (Siman et al., 1985; Srinivasan et al., 1988). For stretch-activated channels, one could envisage that such connections were specialized to render the channels sensitive to membrane tension.

A question that we wished to address by recording from growth cones was whether newly incorporated membrane would contain stretch-sensitive channels. If the channel's tension-sensing elements were not in place, then even if the channel were present, stretch would not activate it. Incorporation of plasmalemma by exocytosis is believed to take place in the main body of the growth cone (Tosney and Wessells, 1983; Goldberg and Burmeister, 1986). Since we found SAK channels in all growth cones examined, the simplest conclusion is that newly incorporated membrane contains the channel and its tension-sensing element(s) as an already functional unit, or at least in a state that allows rapid acquisition of full function.

If SAK channels play a role in regulation of neurite extension, the membrane tensions exerted during growth cone movement should match the range of tensions required for activation. Traction forces produced by fibroblast locomotion can exceed 10^{-3}

Table 1. Kinetic analysis of growth cone SAK channels[a]

Pressure (mm Hg)	Index *P*(O)	Open times		Closed times[b]		
		τ_1 (msec)	τ_2 (msec)	τ_1 (msec)	τ_2 (msec)	τ_3 (msec)
0	0.038	0.14 ± 0.03	1.22 ± 0.12	0.3 ± 0.05	1.29 ± 0.32	57.2 ± 8.5 (57.9)
−100	0.073	0.17 ± 0.02	1.55 ± 0.09	0.18 ± 0.02	1.46 ± 0.12	37.2 ± 4.5 (40.6)
−110	0.203	—	—	—	—	(20.0)
−120	0.555	—	—	—	—	(6.8)

[a] Same patch as used for Figure 4. N.B. Ratios of *P*(O)s relative to 0 mm Hg are 1.92, 5.34 and 14.5 for −100, −110 and −120 mm Hg, respectively.

[b] Beyond −100 mm Hg the records contained too many multiple openings for generating the histograms needed for curve fitting. Instead, an approximation to τ_3 was obtained by averaging the closed (zero current) intervals greater than 2 msec and subtracting this cutoff time from the average (Colquhoun and Sigworth, 1983, equation 50). Note that when both approaches are used where *P*(O) is low (0 and −100 mm Hg), the estimated average (given in brackets) falls within the error bounds of the values obtained by fitting.

dyn/μm (Harris et al., 1980), while forces needed to move particles over the cell surface are much smaller, 10^{-8} dyn/μm (Dembo and Harris, 1981). The experimental tensions applied to the growth cone membrane in this study fall within this range (6.7×10^{-4} dyn/μm for -100 mm Hg in a typical patch, based on the calculations of Guharay and Sachs, 1984), but we cannot as yet relate this tension to traction and/or other forces experienced by growth cones. Evidence that physiologically relevant tensions affect stretch-sensitive channels comes from a recent report on teleost embryos (Medina and Bregestovski, 1988), in which tensions generated during cytokinesis activate SAK channels.

The potential role of any channel is constrained by its ion selectivity: K^+ channels typically hyperpolarize, whereas (nonselective) cation or Na^+ channels depolarize the membrane. The *I/V* relations show that the growth cone stretch-activated channel is essentially identical to that in the soma, namely, K^+ selective (Sigurdson et al., 1987b). Vertebrate SAK channels have been identified (Sackin, 1987, 1989; Ding et al., 1988; Medina and Bregestovski, 1988), but the most-studied vertebrate stretch-sensitive channel is a cation channel that allows K^+, Na^+ (Cooper et al., 1986; Sachs, 1987) and Ca^{2+} (Christensen, 1987; Kirber et al., 1988) to permeate. Activation of either class of channel could profoundly affect local intracellular Ca^{2+} concentrations (Lipscombe et al., 1988). The cation channels would act to increase Ca^{2+}, indirectly by depolarizing the membrane and directly by passing Ca^{2+} ions. Activation of K^+ channels, on the other hand, would stabilize the membrane potential near E_K, thereby decreasing influx of Ca^{2+} through its voltage-gated channels. Motile growth cones in *Helisoma* have recently been shown to have higher free Ca^{2+} levels than those that have stopped growing (Cohan et al., 1987). This Ca^{2+}-driven motility could employ tension-sensitive K^+ channels as a negative-feedback mechanism.

In a recent summary scheme of "known and suspected pathways of Ca^{++} regulation in the growth cone," Kater et al. (1988) feature a variety of ion channels. SA channels can probably now be added to this scheme under the heading, "suspected." Much of the work that led to the present picture of Ca^{2+} regulation was done on *Helisoma,* a snail of the same suborder (Basommatophora) as *Lymnaea.* Since we have shown that SAK channels also occur in neurons of a more distantly related gastropod (*Cepaea nemoralis,* from the same family as *Helix* in the Suborder Stylommatophora) (Bedard et al., 1988), we feel justified in presuming that the stretch-activated channels are widely distributed in pulmonate snails. It would be surprising if they were not found in *Helisoma* neurons.

The presence of mechanosensitive integral membrane proteins in the growth cone (see also Morris and Sigurdson, 1989) raises the possibility that additional (perhaps electrically silent) proteins might likewise possess mechanotransduction machinery, giving them access to information about growth cone motility that could help them govern the pace and/or direction of neurite elongation.

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