J. Membrane Biol. 127, 205–214 (1992)

The Journal of
Membrane Biology

Stretch Activation of the *Aplysia* S-Channel

David H. Vandorpe and Catherine E. Morris
Department of Biology, University of Ottawa, and Neurosciences, Loeb Institute, Ottawa Civic Hospital, Ottawa, Ontario, Canada, K1Y 4E9

Summary. The S-channel, a receptor-mediated K^+ channel of *Aplysia* sensory neurons which functions in neuromodulation, bears a strong resemblance to the ubiquitous stretch-activated channels of snail neurons. Snail neuron stretch channels are stretch sensitive only in the patch, not at the macroscopic level, a situation which leaves open the question of their physiological role. If S-channels resemble snail stretch channels because both belong to the same general class of channels, the S-channel, too, should display stretch sensitivity in the patch. We show, using single-channel recording, that the S-channel can be activated by stretch. Furthermore, we show that *Aplysia* neurons in general have stretch-activated K^+ channels. We suggest that the stretch-sensitive K^+ channels of molluscan neurons and other preparations (e.g., *Drosophila* muscle, snail heart) are S-like channels, i.e., receptor-mediated channels which adventitiously exhibit mechanosensitivity in the patch.

Key Words K^+ channel · S-channel · stretch activation · receptor mediated

Introduction

Stretch-activated K^+ (SAK) channels are a major channel type in many cells, including snail neurons, snail heart cells, *Drosophila* muscle and fish embryo cells [4, 21, 32, 33, 37]. Given the mechanosensitivity of these channels under single-channel recording conditions, it has been thought that their physiological roles relate to mechanotransduction. The fact that snail neurons and growth cones subjected to mechanical stimuli during whole-cell and perforated-patch voltage clamp produce almost no macroscopic mechanosensitive K^+ currents has, however, seriously undermined the idea that they are mechanotransducers [16, 24]. It has also re-opened the question of the channels' real functions.

If mechanosensitivity is not the physiological function of SAK channels but, rather, a trait conferred by disruptions in the patch environment, how do these channels normally function? Two modes of gating can be ruled out: voltage- and Ca^{2+}-dependent gating [4, 33, 37]. None of the features of the channels, on the other hand, would rule out receptor-mediated neuromodulation as their function. Receptor-mediated K^+ channels in molluscan neurons have not, however, been tested for patch-induced stretch sensitivity. An obvious candidate to test is the best-established molluscan receptor-mediated K^+ channel (a channel very similar to snail neuron SAK channels)—the *Aplysia* sensory neuron S-channel [30]. We show that the S-channel, whose identity was confirmed by a range of criteria including insensitivity to extracellular tetraethylammonium, activation by FMRFamide and inactivation by 5-hydroxytryptamine, is mechanosensitive under single-channel recording conditions.

Materials and Methods

Aplysia californica from Marinus (Long Beach, CA) were housed at 15°C in aerated artificial seawater (Forty Fathoms, Marine Enterprises, Baltimore, MD). Animals (50–200 g) were injected with about ~50% of their weight of isotonic $MgCl_2$ and pleural and pedal ganglia were removed to sterile seawater/L-15 culture medium [19]. Ganglia were digested in 1% protease (Sigma, St. Louis, MO) type IX for 4–10 hr at room temperature, with gentle mechanical agitation. The pleural and pedal ganglia were then pinned to a Sylgard dish and desheathed. The ventrocaudal cluster of the pleural ganglion was identified according to Walters et al. [35]. Under a stereomicroscope, cell bodies were teased free by hand, using a long-shanked micropipette; 5–10 per dish were transferred by macropipette to culture medium supplemented with ~15% hemolymph in culture dishes with a glass coverslip insert. Culture medium consisted of L-15 medium (GIBCO, Grand Island, NY), and sufficient concentrated saline stock solution to bring the salinity to that of normal *Aplysia* saline [19]; 50 μg/ml gentamycin sulfate was added to the final solution. Cells were used 1–3 days in culture.

Initial cell-attached recordings were made with normal *Aplysia* saline (460 mM NaCl, 10 mM KCl, 55 mM $MgCl_2$, 10 mM HEPES, 11 mM $CaCl_2$, pH 7.6 adjusted with 1 N NaOH) in both pipette and bath. Most experiments, however, were done with 10

mM TEA (*see below*) and normal saline or high K^+ salines (as noted) in the pipette. Only cell bodies were patched.

Patch pipettes of bubble number ~5 [12] were made from borosilicate glass (Corning 7052 (i.d. 0.80 mm, o.d. 1.65 mm) or N51A (i.d. 1.15 mm, o.d. 1.65 mm) Garner Glass, Claremont, CA) using a List L/M-3P-A (Darmstadt, FRG) pipette puller. They were coated with Sylgard 184 (Dow Corning, Midland, MI) and fire-polished. Gigaohm seals were occasionally obtained spontaneously as the pipette touched the cell but usually required transient suction to the pipette. The pipette holder had a sideport which was connected to a pressure transducer (Biotek Instruments, Winooski, VT) to control and monitor pressure. For *I/V* relations, suction was applied briefly at various voltages to confirm the stretch sensitivity of the currents.

For current/voltage (*I/V*) relations of cell-attached patches, the membrane voltage was taken to be $V_m = (V_{rest} - V_p)$, where V_p is the pipette holding potential and V_{rest} is the resting membrane potential of the cell. V_{rest} was assumed to be -45 mV [1, 35]. High K^+ bath solution was not used to zero the membrane potential because this treatment may have additional consequences, including cell swelling and modification of signal transduction processes, either of which might affect the channels we are studying (*see* [23] and [24] for comments on the ambiguous nature of cell swelling-induced channel activation).

To eliminate the possibility that S-channels could be confused with Ca^{2+}-activated K^+ channels, we routinely used TEA in the pipette. The K_d for TEA block of *Aplysia* Ca^{2+}-activated K^+ channels is 0.4 mM; 10 mM completely abolishes this conductance [17]. At +60 mV, 10 mM TEA reduces the apparent single-channel current of the S-channel by only 10% [29]. Shuster et al. [28] report that in pleural ganglion sensory cells, 0.3 mM is the K_d for TEA block of Ca^{2+}-activated K^+ channels, whereas S-channels are relatively insensitive to 100 mM external TEA.

In some experiments, 5-hydroxytryptamine (5-HT or serotonin) hydrochloride (Sigma) or FMRFamide (Phe-met-arg-phe-amide) (Sigma) was superfused over the cell in normal saline by macropipette. After a 5 min control period, 5-HT was applied. NP_{open} was determined 7 ± 1 min (n = 12) after exposure to 5-HT. FMRFamide experiments were conducted in the same way except that the NP_{open} determination was made 3 ± 1 min (n = 5) after drug treatment, in keeping with the relatively quick action of FMRFamide noted by Belardetti et al. [6]. Apart from the transient suction required to form the seal, patches in these experiments were not mechanically stimulated until the effects of tne neurotransmitter had been determined.

Means are reported with their standard error; two-tailed t-tests were used to determine if means came from the same population, and paired one-tailed t-tests were used to determine if experimental means were larger or smaller than control means (*see* section on 5-HT and FMRFamide).

All experiments were carried out at room temperature (19–23°C).

Channel currents were recorded using an Axopatch 1D (Axon Instruments, Foster City, CA) and stored on videotape following pulse code modulation (PCM-1, Medical Systems Corporation, Greenvale, NY). Currents were analyzed by replaying the tape through an 8-pole Bessel filter (Frequency Devices, Haverhill, MA) connected to a microcomputer (Akran 386, Ottawa, Ont.) via a Labmaster interface (Axon Instruments, Claremont, CA) and displayed on a digital storage oscilloscope (Tektronix, Beaverton, OR) with an output to a digital plotter. Unless otherwise stated, records were sampled at 5 kHz and filtered at 1 kHz. Analysis was performed using a single channel analysis program (pCLAMP 5.5, Axon Instruments, Claremont, CA). Channel activity was measured as the time-averaged current divided by the single channel current; this is expressed as the product NP_{open}, where N is the number of functional channels in the patch and P_{open} is the fraction of time spent open.

For two Table entries on *Lymnaea* neuron SAK channels, intracellular Ba^{2+} and TEA were tested on inside-out patches. Cells were as previously described [32]. Ba^{2+} was tested with *Lymnaea* saline (as before) in the pipette, K^+ saline in the bath (50 mM KCl, 1 mM $CaCl_2$, 5 mM HEPES, pH 7.6), then 1 mM $BaCl_2$ in K^+ saline perfused with a macropipette. For TEA, a K^+ solution (64 mM KCl, 5 mM HEPES, 2 mM $MgCl_2$, pH 7.6) was used in the pipette and bath; 10 mM TEA in this solution was perfused over patches. Ba^{2-} was a "slow blocker" [36] of SAK channels; NP_{open} for SA (-50 mm Hg, -40 mV) events decreased to 15 ± 5% of control (n = 3, $P < 0.04$). Mechanosensitivity was not abolished by Ba^{2+}. 10 mM TEA did not affect SAK channel amplitude (77 pS without, 78 pS with TEA) or NP_{open} (NP_{open} was 0.23 ± 0.14 without and 0.27 ± 0.18 with TEA, -50 mm Hg, -40 mV, n = 3).

Results

Stretch-induced Channel Activity in Patches from Sensory Neurons

SA channels were abundant in *Aplysia* sensory neurons, with 1–4 channels in every patch. The response to stretch of an initially quiescent cell-attached patch is shown in Fig. 1*A* and *B*. Immediately upon application, suction-induced channel activity was observable as "flickery bursts." The figure illustrates that deactivation was slower than activation; after release of suction, several minutes were often required for return to baseline. Similar behavior (i.e., immediate activation upon stimulation, slower decay of NP_{open} upon release) is commonplace for SAK channels in snail neurons and heart cells [25, 33]. Short (~1 sec) pulses usually produced much briefer periods of activation.

The response of cell-attached patches to a range of pressures is shown in Fig. 1*C*. The position of the stretch-activation curve along the pressure axis varied considerably among patches, but in all cases NP_{open} increased as a steep function of pressure. Rupture of the patch occurred before saturation was observed. Comparable x-axis variability is seen in stretch-activation curves from snail cells [4, 32, 33] where, too, patches often rupture before saturating pressures can be applied.

SA Events in the Presence or Absence of Intracellular Ca^{2+}

Although most experiments were conducted on cell-attached patches, we confirmed that stretch-induced activity of these SA channels could also be obtained in excised patches, and that the stretch-induced ac-

Table. Comparison of *Lymnaea* and *Aplysia* SAK channels and *Aplysia* S-channels

	Lymnaea SAK	*Aplysia* SAK[d]	*Aplysia* S
Permeability	$P_K >> P_{Na}$[c]	$P_K >> P_{Na}$	$P_K >> P_{Na}$[a,i]
Voltage dependence	None in physiological range[f]	Not apparent	Weak or absent[a,i]
Cell-attached conductance (physiological solutions)	~44 pS[c]	50 ± 3 pS	55 ± 6 pS[a]
External TEA	K_d ~ 10 mM[f]	$K_d >>$ 10 mM	K_d ~ 90 mM[b]
Internal TEA	No effect (10 mM)[d]	—	K_d ~ 40 mM[b]
External Ba^{2-}	No effect (50 mM)[f]	—	No effect (10 mM)[b]
Internal Ba^{2+}	Slow block[d]	—	Slow block[b]
Internal Ca^{2+}	Does not activate[c]	Does not activate	Does not activate[a]
Apamin	No effect (1 μM)[h]	—	No effect (10 μM)[b]
4-Aminopyridine	No effect (10 mM)[g]	—	No effect (10 mM)[b]
Density	4–8/patch[c]	1–4/patch	Up to 5[a]
Distribution (neuronal)	Circumeosophageal ganglia cells[c]	Pleural sensory cells and others	Pleural and abdominal sensory cells[a,e]
Stretch activated	Cell-attached & excised patches[c]	Cell-attached & excised patches	—
5-HT	—	Decreases NP_{open}	Decreases NP_{open}[a]
FMRFamide	—	Increases NP_{open}	Increases NP_{open}[e]

(References: [a] [30]; [b] [29]; [c] [32]; [d] present study; [e] [6]; [f] [31]; [g] [9]; [h] [33]; [i] [28]).

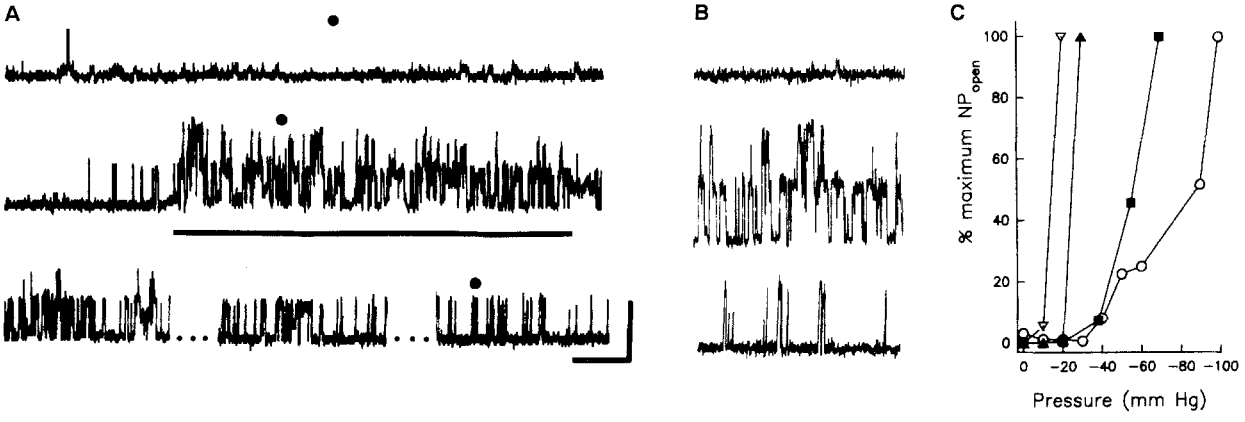

Fig. 1. Suction-induced channel activity in sensory neurons. (*A*) Continuous trace (except for 20 and 34 sec interruptions at dotted lines) showing the effect of suction. During the bar, −40 mm Hg was applied; the activity immediately prior to this occurred as suction was increased from 0 mm Hg. Higher resolution traces from times indicated by large dots are shown in *B*. Cell-attached patch, with high K^+ solution and 10 mM TEA in the pipette, V_m = +55 mV. Filtered at 500 Hz in *A*, 1 kHz in *B*. Scale: 10 pA, 500 msec (*A*); 5 pA, 100 msec (*B*). (*C*) The effect of negative pressure on SA channel NP_{open} in four different patches from *Aplysia* sensory neuron cell bodies. For comparison among patches, the ordinate is normalized to the maximum value of NP_{open} in each patch (these were, left to right, 0.78, 1.5, 0.86, and 0.67).

tivity occurred whether Ca^{2+} was present or absent at the intracellular membrane face (Fig. 2). Since it was important, in identifying the channel, to show that the Ca^{2+}-independent stretch-sensitive channel in excised patches pass K^+ in preference to Na^+ and Cl^- [4], the solution change which deleted intracellular Ca^{2+} also provided for a change in the $[K^+]$ and $[Na^+]$ gradients. The change in the shape of the *I/V* relation is consistent with a K^+-selective SA channel, and rules out Na^+ and Cl^- as the charge carriers. The K^+ selectivity of the SA channel is dealt with in more detail in the next section, for cell-attached patches.

Conductance and Selectivity of the SA Channel

To avoid possible interference from Ca^{2+}-activated channels which are present in these neurons [28], 10 mM tetraethylammonium (TEA; *see* Materials and

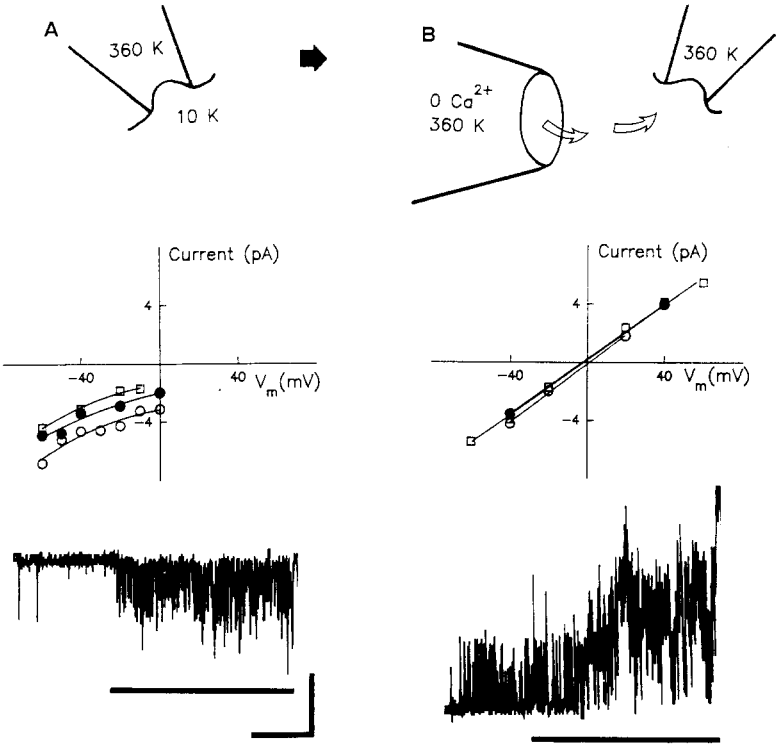

Fig. 2. Stretch-induced activity and intracellular Ca^{2+}. *I/V* relations for SA channels in three excised patches (open squares, open circles, filled circles) in salines with (*A*) and without (*B*) Ca^{2+} at the intracellular face. In *A*, K^+ was available only in the pipette and only inward SA currents were evident (sample trace: −50 mm Hg applied during bar, −40 mV, filled circle patch), whereas in *B*, K^+ was available on both sides and both inward and outward SA currents were seen (−50 mm Hg, +40 mV, same patch; flowing perfusion saline partially activated channels even before suction was applied). The lines in *A* were fit by eye; the conductance in *B* was 96 ± 2 pS. In *A*, bath was normal saline; pipette solutions were (in mM): 360 KCl, 10 TEA, 10 NaCl, 2 $MgCl_2$, 1 $CaCl_2$, 20 HEPES, 272 sucrose, pH 7.4 (adjusted with KOH). "0 Ca^{2+}" had 0 mM Ca^{2+} and 10 mM EGTA. Scale: 5 pA, 500 msec.

Methods) was included in the pipette in almost all experiments. The exception, shown in Fig. 3*A* and *B* was designed to compare the SA channel unitary conductance in normal saline to that reported for the S-channel in normal saline (with no TEA).

I/V relations of SA events under two different $[K^+]$ gradients are shown in Fig. 3*A* with representative traces of the stretch-sensitive single-channel events at various membrane potentials in Fig. 3*B* and *C*. Outward, but never inward SA current was observed when the major pipette cation was Na^+, whereas bidirectional SA currents were seen when $[K^+]$ was high bilaterally (Fig. 3*B* and *C*). The slope conductance for SA current in normal saline was 50 ± 3 pS, in good agreement with the conductance (55 ± 6 pS) reported by Siegelbaum et al. [30] for S-channels in cell-attached patches (artificial seawater in the pipette) from abdominal ganglion sensory neurons. *I/V* data for S-channels from pleural ganglion sensory neuron growth cones (*see* Belardetti et al. [7]; part of their Fig. 4*C* is displayed here in Fig. 3*A*), when fit over the nearly-ohmic range (−20 to +60 mV) yield 54 pS. Current amplitudes for SA events at given potentials fell within the range reported for S-channels (e.g., whereas the curve reproduced from Belardetti et al. [7] lies slightly below our average curve, that from another report by the same group [6] would lie above it.)

Figure 3*A* gives *I/V* relations of five cell-attached patches when all of the Na^+ in normal saline was substituted in the pipette with K^+ (giving 470 mM K^+, plus 10 mM TEA); representative events are shown in Fig. 3*C*. The *I/V* relation shifted to the right and the reversal potential (7.6 ± 3.2 mV) was near 6.7 mV, the calculated reversal potential assuming an intracellular $[K^+]$ of 360 mM. Since the depolarizing shift and the appearance of inward SA currents occurred with no change in the $[Cl^-]$ gradient, the SA channel must be largely K^+ selective. The slope conductance of the *I/V* relation (data for inward and outward currents are included in this slope, as shown in Fig. 3*A*) in high K^+ was 91 ± 7 pS, comparable to the ~90 pS conductance of the S-channel measured from outward currents, using inside-out patches with 360 mM K^+ (no TEA) in the bath and artificial seawater in pipette [28].

Voltage Effects on SAK Channel Activity

We found no consistent or pronounced effects of voltage on the steady-state NP_{open} of the SAK channel. With normal saline in the pipette (no TEA), three cell-attached patches at membrane potentials 80 mV apart (−25 and +55 mV) gave steady-state NP_{open} ratios (+55 mV/−25 mV) for SAK channels of 0.9, 1.5, and 5.1. With high K^+ (and TEA) in the pipette, NP_{open} ratios (+55 mV/−25 mV) in three patches were 0.5, 0.6 and 1.5 (0 mm Hg). For two excised patches tested 50 mV apart (conditions as in

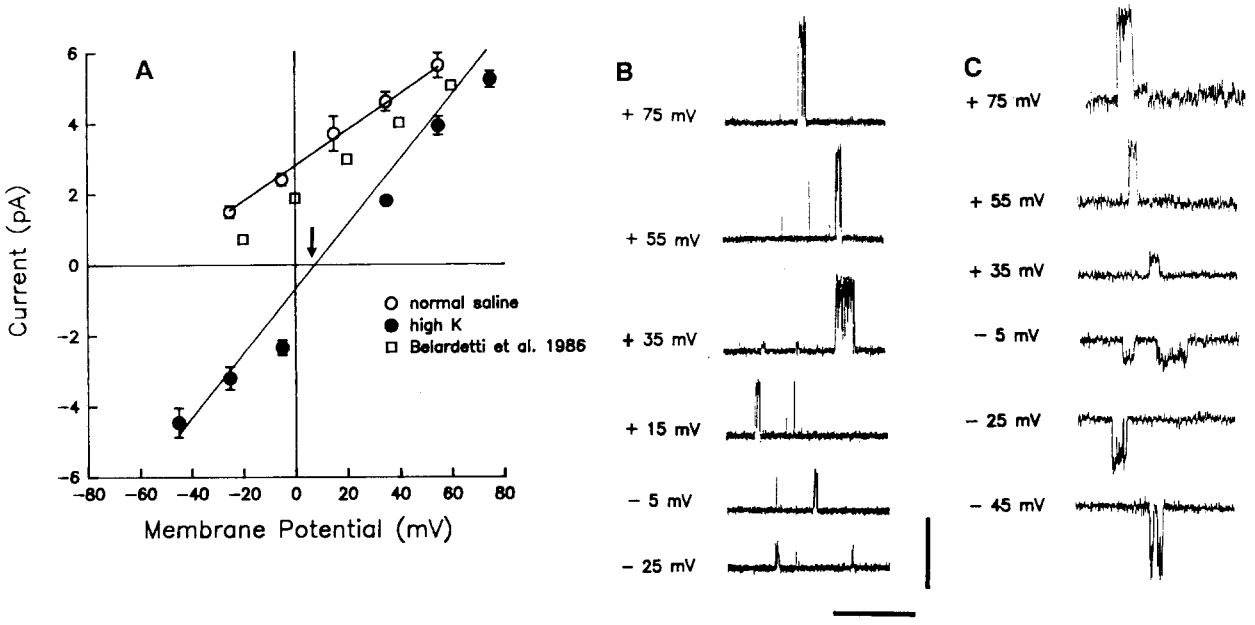

Fig. 3. *I/V* relations for SA channels from sensory neurons. (*A*) Normal saline in the bath and normal or high K^+/TEA saline in the pipette; cell-attached patches. Conductances given in the text were determined as the mean of slopes for individual experiments. (*I/V* data from Belardetti et al. [7] are also plotted (*see* text)). The slopes passed through zero current at -56 ± 2 mV for normal saline ($n = 3$); the reversal potential for high K^+ was 7.6 ± 3.2 mV ($n = 5$). The arrow indicates the calculated reversal potential. Representative traces for normal and high K^+ are shown in *B* and *C*, respectively. Filtered at 1 kHz. Scale: 4 pA, 250 msec (*B*); 4 pA, 100 msec (*C*).

Fig. 2*A*), with TEA present, the ratios (0 mV/−50 mV) were 2.0 and 1.0. For the S-channel, Shuster et al. [28] re-examined the variable (and seldom large) effects of voltage on gating. Patch configuration is a factor (cell-attached patches have the least voltage-dependent S-channels) as is the duration of the sojourn at a given potential. Ensemble S-channel currents from patches given ~0.5 sec depolarizing steps are consistently ohmic; long steady-state measurements (tens of seconds) are required for effects of voltage to reveal themselves, and even then they are not consistent.

On the basis of the properties described to this point, namely, TEA insensitivity, conductance, selectivity for K^+, insensitivity to intracellular Ca^{2+}, no consistent effect of membrane potential on gating, and low homogeneous membrane density, these sensory neuron SAK channels are indistinguishable from S-channels.

Effects of 5-HT and FMRFamide on SAK Channel NP_{open}

In *Aplysia* sensory neurons, S-channels are inactivated via a 5-HT receptor-mediated cyclic-AMP cascade [7, 30] and are activated via a FMRFamide receptor-mediated arachidonic acid cascade [6, 10, 11]. Using concentrations previously shown to be effective on pleural ganglion mechanosensory cell S-channels, we tested the effects of 5-HT and FMRFamide on NP_{open} of sensory neuron SAK channels. To provide a parallel with the Belardetti et al. [6, 7] work on the S-channel, with which we wished to compare the SAK channel, we used patches whose baseline NP_{open} was relatively high to determine the effects of 5-HT and patches in which it was relatively low to study FMRFamide. Stretch activated channels in both types of patches.

In 10 of 12 patches, 100 μM 5-HT caused a decrease in channel activity, as illustrated in Fig. 4. NP_{open} fell 7.3-fold ($0.30 \pm 0.13 \rightarrow 0.041 \pm 0.017$, $n = 12$, $P < 0.05$). A decrease in NP_{open} (of similar magnitude—roughly sixfold, ~0.6 → 0.1, judging from five quantitated patches) in 11 of 11 patches was reported using 100 μM 5-HT for pleural sensory neuron S-channels [7]. In abdominal sensory neurons, 5-HT reduced S-channel activity in 10 of 13 cells [30]. S-channels are kinetically complex; they have low and high P_{open} states, both of which are 5-HT sensitive [28, 30]. Some of the patches in which we studied stretch/5-HT sensitive channels appeared to have channels in both states.

The peptide, FMRFamide, increased channel NP_{open} in five of five patches ($0.0082 \pm 0.0044 \rightarrow 0.049 \pm 0.010$, $n = 5$, $P < 0.001$), as illustrated in

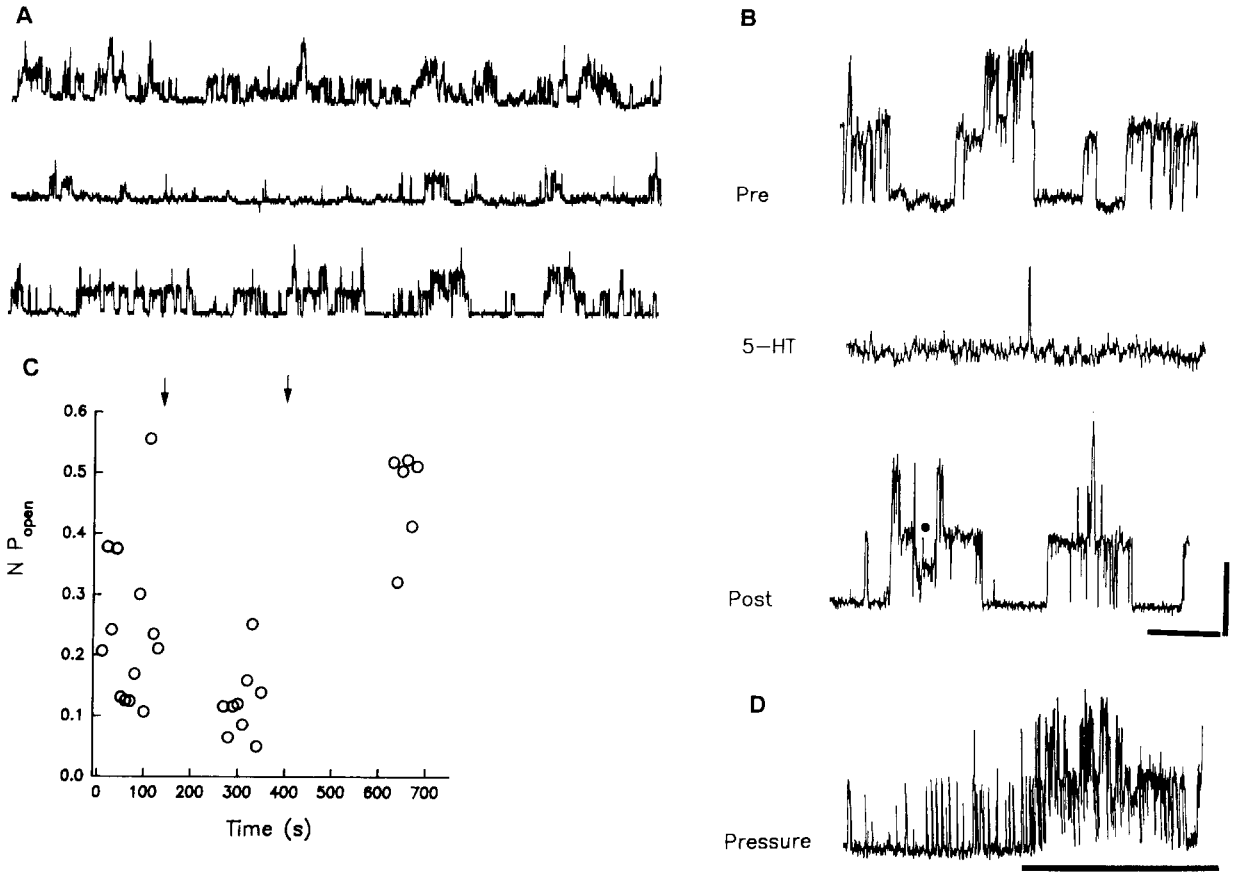

Fig. 4. Actions of 5-HT and of stretch. (*A*) Channel activity before application of 100 μmol 5-HT to the cell (top), 4 min after start of application (middle), and 5 min after the end of application. (*B*) Higher resolution recordings from the same patch (a subconductance is indicated by large dot). (*C*) NP_{open} record (10 sec intervals) for the same patch. Arrows indicate time of application and removal of drug pipette. (*D*) After recovery from 5-HT, −60 mm Hg (applied continuously during bar) increased NP_{open} in the same patch. Normal saline and TEA in the pipette, V_m = +55 mV. Filtered at 1 kHz. Scale: 11 pA, 550 msec (*A*); 4 pA, 100 msec (*B*); 4 pA, 1 sec (*D*).

Fig. 5. The 6.0-fold FMRFamide-induced increase was of the same magnitude as the fivefold increase (seven of eight patches; 0.03 → 0.16) reported by Belardetti et al. [6] for pleural sensory neuron S-channels. Stretch/5-HT and stretch/FMRFamide channels in a given patch were (apart from an occasional subconductance event (*see* Fig. 4*B*) typical of S-channels (e.g., [30]) uniform in their conductance level, as shown in Figs. 4*D* and 5*D*. Pairs of all-points current histograms (Fig. 6*A*) illustrate that in a given patch, channels activated by stretch at the end of the experiment had the same current amplitude as spontaneously active channels that were closed by 5-HT. A comparable all-points histogram for FMRFamide (Fig. 6*B*) illustrates that, in a given patch, FMRFamide activates channels with the same amplitude as channels subsequently activated by stretch. In other trials, we noted that SA events with the same amplitude as S-channel currents could be induced while the actions of FMRFamide or 5-HT are in effect (*not shown*). If stretch, on the one hand, and the second messengers of FMRFamide and 5-HT, on the other, acted on fundamentally different channel populations, we would not expect to consistently observe the same amplitudes when comparing stretch-activated and neurotransmitter-modulated events in a given patch.

These results show that the SAK channel of *Aplysia* sensory neurons and a receptor-mediated channel, the S-channel, are indistinguishable.

SAK Channels in other *Aplysia* Neurons

Preliminary data from abdominal ganglion neurons of *Aplysia* revealed SA events (outward currents, probably carried by K^+ [5], and Lin et al. [19] reported SA channels in medial cells of the pleural ganglion. We tested eight unidentified neurons from the pedal ganglion; all had SA channels (Fig. 7). The channels exhibited responses to stretch comparable to those of the S-channel and of snail neuron SAK

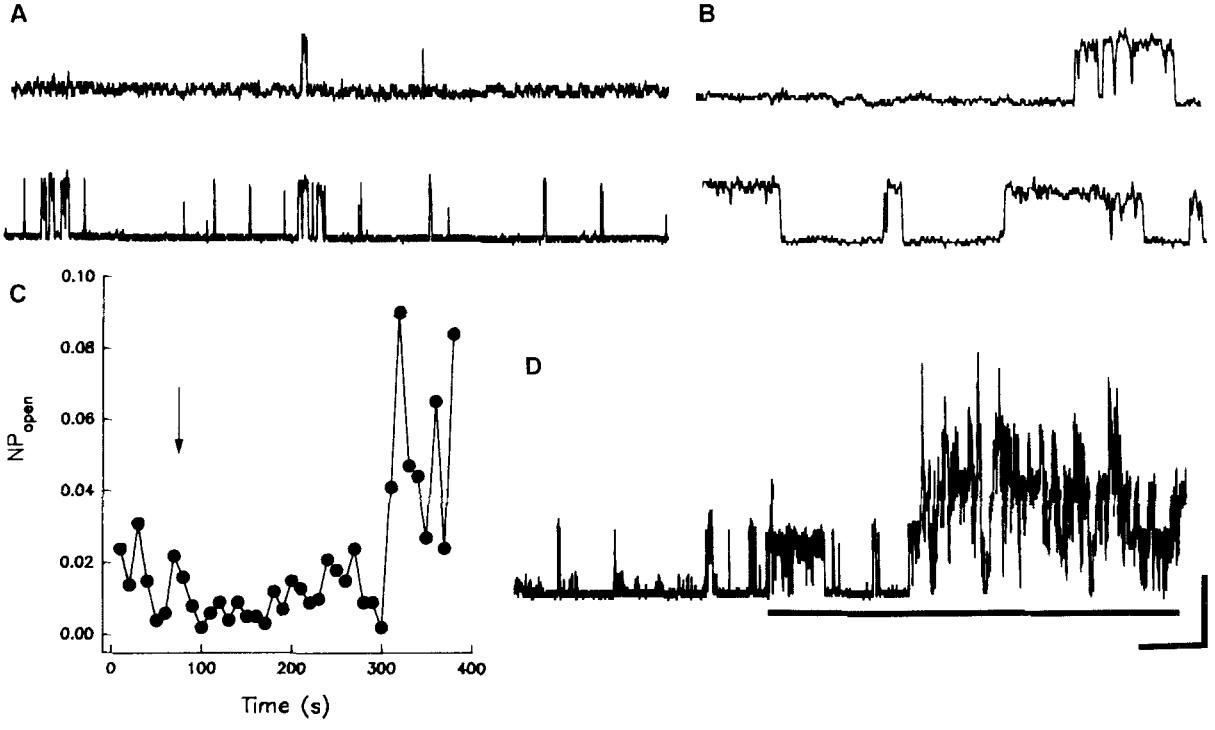

Fig. 5. Actions of FMRFamide and of stretch. (*A*) Channel activity before application of 20 μM FMRFamide (top) and about 3 min after start of application (bottom). (*B*) Higher resolution recordings from the same patch before (top) and during (bottom) FMRFamide. (*C*) NP_{open} record (10 sec intervals) for the same patch. FMRFamide was added at the arrow. (*D*) Stretch activation (same patch, following FMRFamide treatment) induced by −40 mm Hg (applied continuously during bar). Normal saline and TEA in the pipette. V_m = +55 mV. Filtered at 1 kHz. Scale: 5 pA, 500 msec (*A*); 5 pA, 20 msec (*B*); 5 pA, 500 msec (*D*).

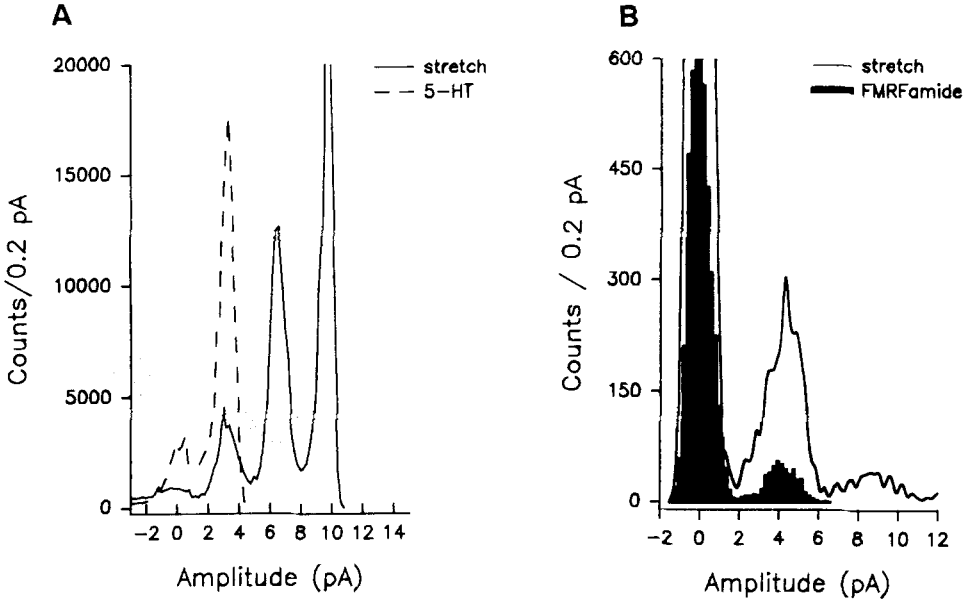

Fig. 6. All-points current histograms illustrating 5-HT/stretch and FMRFamide/stretch effects. (*A*) 5-HT-sensitive channel activity immediately prior to 5-HT application (hence, before NP_{open} was reduced by 5-HT) and during −60 mm Hg suction-induced activity. (*B*) FMRFamide-induced channel activity and −60 mm Hg suction-induced activity. In both patches, normal saline and TEA in the pipette, V_m = +55 mV.

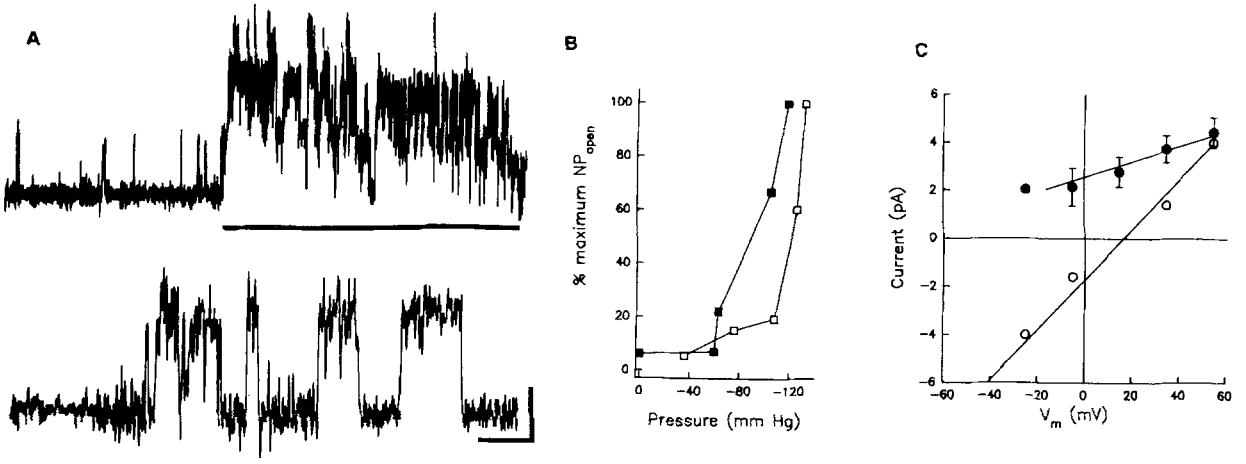

Fig. 7. SAK channels from patches on unidentified pedal ganglion neurons (cell-attached). (*A*) Example of SA single-channel activity (−60 mm Hg at bar; V_m = +25 mV; normal saline and TEA in the pipette) and below it, higher resolution trace of the events from the same record prior to stretch. Scale: top, 2 pA, 500 msec; bottom, 1 pA, 20 msec. (*B*) Effect of stretch on open probability (both patches were at V_m = +35 mV, assuming, for simplicity, that V_{rest} = −45 mV, with normal saline, TEA in pipette; NP_{open} maxima were 0.74 and 1.3). (*C*) *I/V* relations for normal saline and TEA (filled circle; n = 3; conductance, 42 ± 2 pS, not significantly different from S-channel ($P > 0.07$)) of 470 mM K^+ and TEA (as in Fig. 3; open circle; n = 1; conductance 104 pS) in the pipette.

channels [5, 32]. With normal saline/TEA in the pipette, only outward SA currents were observed; K^+ selectivity was demonstrated by using high K^+ in the pipette, in which case outward and inward SA events were seen and the *I/V* relation shifted to the right ~70 mV (Fig. 7*C*). The fast flickery bursts of pedal ganglion SAK channels resembled those of the S-channel and of other SAK channels [5, 32, 33]. Tests on four bag cell neurons revealed similar outward-only SA currents of ~50 pS (cell-attached, normal saline in pipette).

SAK channels thus appear to be ubiquituous in *Aplysia* neurons, as they are in snail neurons, snail heart cells and *Drosophila* muscle. The possibility that all these SAK channels are, like the S-channel, responsible for receptor-mediated modulation, should now be investigated.

Discussion

Relation of the S-Channel to Other SAK Channels

We have shown that *Aplysia* sensory neurons have SAK channels indistinguishable from 5-HT and FMRFamide receptor-mediated channels known as S-channels. We emphatically do not imply that the S-channel is this sensory cell's physiological mechanotransducer; though peripheral terminals of the sensory neurons pass excitatory mechanoreceptor current in vivo, K^+ selectivity disqualifies the S-channel for this job. No mechanical functions have, to our knowledge, been suggested for the S-channel, but the possibility that S-channels sometimes act in a mechanosensitive mode is not ruled out by the present study. The implication of our finding is, however, of a different nature. The implication is that as in snail neuron SAK channels [24], patching induces mechanosensitivity in a class of channels which, as we have now shown, includes the S-channel. The effects of stretch on gating are presumably an adventitious feature which arises from special but unknown interactions of the channel with its membrane environment. Assuming the S-channel is not the only channel of its type, stretch should provide a convenient way of revealing previously overlooked S-like receptor-mediated K^+ channels. The SA channels seen in other *Aplysia* neurons, for example, are likely to be receptor-mediated channels with as yet unknown first messengers. As the Table emphasizes, there are many congruences between the *Aplysia* SAK/S-channel and the "original" SAK channels of the snail, *Lymnaea*, which proved insensitive to stretch at the macroscopic level [24]. Both can be described as mechanosensitive K^+-selective, Ca^{2+}-insensitive channels of low (compared, for example, to endplate or node of Ranvier channels) homogeneous membrane density, which are insensitive to apamin and 4-aminopyridine, insensitive to TEA in the sub-millimolar range, sensitive to internal but not external Ba^{2+}, and which exhibit little voltage-dependence. Similar SAK channels, also with no known mechanosensitive function, are found elsewhere (e.g., *Drosophila* [15, 37].

Patch-mechanosensitivity is confined to a subset of the total population of K^+ channels in a given cell type, as shown with particular clarity in *Drosophila* muscle. Zagotta et al. [37] and Gorczyca and Wu [15] studied seven classes of K^+ channels in *Drosophila* myocytes and myoblasts. Only one class is activated by stretch. In light of our findings, the possibility that the voltage- and Ca^{2+}-independent, stretch-sensitive subset of K^+ channels in this insect are S-like receptor-mediated channels should be examined. The cAMP-dependent action of octopamine on insect muscle [13] might, for example, involve S-like channels. Using *Drosophila* to pursue the molecular biology of an S-like channel would be substantially easier than using *Aplysia* or most other preparations.

Patch Tension and the Variability of S-Channel Activity

S-channel activity is inherently variable [28, 30]. In cell-attached patches, much of this variablity is probably due to uncontrolled fluctuations in regulatory agents (e.g., arachidonic acid metabolites [26], but our findings suggest that part of the variance could arise from uncontrolled membrane tension in the patch [23]. Morris and Sigurdson [25] demonstrated that residual tension of about −10 mm Hg in pipettes nominally at 0 mm Hg can markedly affect the activity of stretch-sensitive channels. In *Aplysia*, channels in some patches (*see* Fig. 2) were activated at applied pressures of as little as −10 mm Hg. Sigurdson et al. [33] noted that in excised patches, flow from a perfusion pipette will sometimes increase the activity of SAK channels. We noted the same effect (Fig. 2*B*) in excised *Aplysia* patches. It is also possible that baseline ("control") levels of S-channel activation are affected by a third inadvertent source of stretch activation. Sokabe and Sachs [34] show that, surprisingly, active contractions can occur in both cell-attached and excised patches, and point out that "these forces could have important consequences for mechanically-sensitive . . . channels."

SA Channels which Modulate Membrane Conductance

The S-channel functions primarily to modulate membrane conductance in response to second messengers. Do any other conductance-modulator channels exhibit mechanosensitivity in the patch? Several lines of evidence suggest that they do. On rat hepatoma cells, ATP, acting as first messenger via P_2-purinergic receptors, activates a channel characterized previously as a SA (and voltage-independent) nonselective cation channel [2, 3]. In early teleost embryos, SAK channels activate cyclically during cell cleavages and the activity of this SAK channel may be regulated through cAMP-dependent phosphorylation [22]. A FMRFamide-activated divalent cation channel in snail heart is thought to be transiently activated by the suction required for seal formation [8].

Effects of Stretch

Mechanosensitivity of SA channels may be artifactual (as in molluscan SAK channels), physiological (at mechanosensory terminals) or, as in muscular dystrophy, pathological [14]. Even where artifactual, mechanosensitivity must reflect special but unknown interactions of the affected channels with their immediate surround. There is a death of information about the structure of S-like channels and about how they interact mechanically with other membrane components. Likewise, we are ignorant of what makes any channel, including S-like channels, stretch sensitive [23]. The state of the cortical cytoskeleton seems to be important (*see* Sachs [27]; mutations which delete elements of the cytoskeleton alter stretch channels [14, 18]. In a recent model proposed by Martinac et al. [20] and based on the effects of amphipaths, a determining factor in mechanosensitive gating is local curvature in the lipid bilayer. In the context of this model which emphasizes the lipid/channel interface, a simple point can be reiterated: both stretch and certain membrane lipid metabolites activate the S-channel [11]. Perhaps when the membrane/channel is mechanically stressed, ordinary membrane lipids are able to mimic the agonist action of these special lipid metabolites at the channel's interface with the bilayer.

We thank C. Miller for a critical reading of the manuscript. This work was supported by NSERC, Canada; the laboratory is a participant in the Canadian Networks of Centers of Excellence (Neural Regeneration and Functional Recovery).

References

1. Baxter, D.A., Byrne, J.H. 1990. Differential effects of cAMP and serotonin on membrane current, action potential duration, and excitability in somata of pleural sensory neurons of *Aplysia*. *J. Neurophysiol.* **64:**978–990
2. Bear, C.E. 1990. A nonselective cation channel in rat liver cells is activated by membrane stretch. *Am. J. Physiol.* **258:**C421–C428
3. Bear, C.E., Li, C. 1991. Calcium permeable channels in rat hepatoma cells are activated by extracellular nucleotides. *Am. J. Physiol.* **261:**C1018–C1024

4. Bedard, E., Morris, C.E. 1992. Channels activated by stretch in neurons of the helix snail. *Can. J. Physiol. Pharmacol.* **70:**207–213
5. Bedard, E., Sigurdson, W.J., Morris, C.E. 1988. Stretch-activated (SA) channels in the neurons of *Cepaea* (Helicideae) and *Lymnaea*. *Biophys. J.* **53:**154*a*
6. Belardetti, F., Kandel, E.R., Siegelbaum, S.A. 1987. Neuronal inhibition by the peptide FMRFamide involves opening of S K channels. *Nature* **325:**153–156
7. Belardetti, F., Schacher, S., Kandel, E.R., Siegelbaum, S.A. 1986. The growth cones of *Aplysia* sensory neurons: Modulation by serotonin of action potential duration and single channel currents. *Proc. Natl. Acad. Sci. USA* **83:**7094–7098
8. Brezden, B.L., Benjamin, P.R., Gardner, D.R. 1991. FMRFamide activates a divalent cation-conducting channel in heart muscle cells of the snail *Lymnaea stagnalis*. *J. Physiol.* **443:**727–738
9. Brezden, B.L., Gardner, D.R., Morris, C.E. 1986. A potassium-selective channel in isolated *Lymnaea stagnalis* heart muscle cells. *J. Exp. Biol.* **123:**175–189
10. Brezina, V., Eckert, R., Erxleben, C. 1987. Modulation of potassium conductances by an endogenous neuropeptide in neurones of *Aplysia californica*. *J. Physiol.* **382:**267–290
11. Buttner, N., Siegelbaum, S.A., Volterra, A. 1989. Direct modulation of *Aplysia* S-K^+ channels by a 12-lipoxygenase metabolite of arachidonic acid. *Nature* **342:**553–555
12. Corey, D.P., Stevens, C.F. 1983. Science and technology of patch-recording electrodes. *In:* Single-Channel Recording. pp. 53–68. B. Sakmann and E. Neher, editors. Plenum, New York
13. Evans, P.D., Swales, L.S., Whim, M.D. 1988. Second messenger systems in insects. *In:* Neurotox'88: Molecular Basis of Drug & Pesticide Action. pp. 225–234. G.G. Lunt, editor. Exerpta Medica, Amsterdam
14. Franco, A., Jr., Lansman, J.B. 1990. Calcium entry through stretch-inactivated ion channels in *mdx* myotubes. *Nature* **344:**670–673
15. Gorczyca, M.G., Wu, C.-F. 1991. Single-channel K^+ currents in *Drosophila* muscle and their pharmacological block. *J. Membrane Biol.* **121:**237–248
16. Gustin, M., Sachs, F., Sigurdson, W.J., Rudnikin, A., Bowman, C., Morris, C.E., Horn, R. 1991. Technical comments: Single-channel mechanosensitive currents. *Science* **253:**800–802
17. Hermann, A., Gorman, A.L.F. 1981. Effects of tetraethylammonium on potassium currents in a molluscan neuron. *J. Gen. Physiol.* **78:**87–110
18. Kubalski, A., Martinac, B., Adler, J., Kung, C. 1991. Altered properties of the mechanosensitive ion channel in a lipoprotein mutant of *Escherichia coli*. *Biophys. J.* **59:**455*a*
19. Lin, S.S., Dagan, D., Levitan, I.B. 1989. Concanavalin A modulates a potassium channel in cultured *Aplysia* neurons. *Neuron* **3:**95–102
20. Martinac, B., Adler, J., Kung, C. 1990. Mechanosensitive ion channels of *E. coli* activated by amphipaths. *Nature* **348:**261–263
21. Medina, I.R., Bregestovski, P.D. 1988. Stretch-activated ion channels modulate the resting membrane potential during early embryogenesis. *Proc. R. Soc. London B* **235:**95–102
22. Medina, I.R., Bregestovski, P.D. 1991. Sensitivity of stretch-activated K^+ channels changes during cell-cleavage cycle and may be regulated by cAMP-dependent protein kinase. *Proc. R. Soc. London B* **245:**159–164
23. Morris, C.E. 1990. Mechanosensitive ion channels. *J. Membrane Biol.* **113:**93–107
24. Morris, C.E., Horn, R. 1991. Failure to elicit neuronal macroscopic mechanosensitive currents anticipated by single channel studies. *Science* **251:**1246–1249
25. Morris, C.E., Sigurdson, W. 1989. Stretch-inactivated ion channels coexist with stretch-activated ion channels. *Science* **243:**807–809
26. Piomelli, D., Volterra, A., Dale, N., Siegelbaum, S.A., Kandel, E.R., Schwartz, J.H., Belardetti, F. 1987. Lipoxygenase metabolites of arachidonic acid as second messengers for presynaptic inhibition of *Aplysia* sensory cells. *Nature* **328:**38–43
27. Sachs, F. 1990. Stretch-sensitive ion channels. *Sem. Neurosci.* **2:**49–57
28. Shuster, M.J., Camardo, J.S., Siegelbaum, S.A. 1991. Comparison of the serotonin-sensitive and Ca^{2+}-activated K^+ channels in *Aplysia* sensory neurons. *J. Physiol.* **440:**601–621
29. Shuster, M.J., Siegelbaum, S.A. 1987. Pharmacological characterization of the serotonin-sensitive potassium channel of *Aplysia* sensory neurons. *J. Gen. Physiol.* **90:**587–608
30. Siegelbaum, S.A., Camardo, J.S., Kandel, E.R. 1982. Serotonin and cyclic AMP close single K channels in *Aplysia* sensory neurons. *Nature* **299:**413–417
31. Sigurdson, W.J. 1990. Mechanosensitive ion channels in the freshwater snail *Lymnaea stagnalis*. Ph.D. Thesis, University of Ottawa
32. Sigurdson, W.J., Morris, C.E. 1989. Stretch-activated ion channels in growth cones of snail neurons. *J. Neurosci.* **9:**2801–2808
33. Sigurdson, W.J., Morris, C.E., Brezden, B.L., Gardner, D.R. 1987. Stretch-activation of a K channel in molluscan heart cells. *J. Exp. Biol.* **127:**191–209
34. Sokabe, M., Sachs, F. 1990. The structure and dynamics of patch-clamped membranes: A study using differential interference contrast light microscopy. *J. Cell Biol.* **111:**599–606
35. Walters, E.T., Byrne, J.H., Carew, T.J., Kandel, E.R. 1983. Mechanoafferent neurons innervating tail of *Aplysia*. I. Response properties and synaptic connections. *J. Neurophysiol.* **50:**1522–1542
36. Yellen, G. 1984. Ionic permeation and blockade in Ca-activated K channels of bovine chromaffin cells. *J. Gen. Physiol.* **84:**157–186
37. Zagotta, W.N., Brainard, M.S., Aldrich, R.W. 1988. Single-channel analysis of four distinct classes of potassium channels in *Drosophila* muscle. *J. Neurosci.* **8:**4765–4779

Received 18 September 1991; revised 18 December 1991