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A Nonselective Cation Channel Activated by Membrane Deformation in Oocytes of the Ascidian *Boltenia villosa*

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Summary. Cell-attached patch clamp recordings from unfertilized oocytes of the ascidian *Boltenia villosa* reveal an ion channel which is activated by mechanical deformation of the membrane. These channels are seen when suction is applied to the patch pipette, but not in the absence of suction or during voltage steps. The estimated density of these stretch-activated channels is about $1.5/\mu m^2$, a figure equal to or greater than the density of known voltage-dependent channels in the oocyte. Ion substitution experiments done with combined whole-cell and attached patch recording, so absolute potentials are known, indicate that the channel passes Na^+, Ca^{2+} and K^+, but not Cl^-. The channel has at least two open and two closed states, with the rate constant that leaves the longer-lived closed state being the primary site of stretch sensitivity. External Ca^{2+} concentration affects channel kinetics: at low calcium levels, long openings predominate, whereas at high calcium virtually all openings are to the short-lived open state. In multiple channel patches, the response to a step change in suction is highly phasic, with channel open probability decreasing over several hundred milliseconds to a nonzero steady-state level after an initial rapid increase. This channel may play a role in the physiological response of cells of the early embryo to the membrane strains associated with morphogenetic events.

Key Words stretch-activated channel · calcium · oocyte · development · patch clamp · tunicate

Introduction

A recently reported class of ion channels opens in response to mechanical deformation of the plasma membrane. Such "stretch activated" channels were first discovered in chick skeletal muscle (Guharay & Sachs, 1984) and subsequently have been described in a wide variety of cells (*see* Sachs, 1986, for review). Most stretch-activated channels seem to be rather nonselective cation channels (*see*, e.g., Lansman, Hallam & Rink, 1987), although some are K^+ (Sigurdson et al., 1987) or Cl^- (Martinac et al., 1987) selective. No clear physiological function for these channels has been demonstrated, although roles in mechanoreception (Guharay & Sachs, 1984), volume regulation (Christensen, 1987) and vascular reflexes (Lansman et al., 1987; Lansman, 1988; Olesen, Clapham & Davies, 1988) have been suggested. In choroid plexus Christensen (1987) has shown that physiologically effective amounts of Ca^{2+} can enter cells through stretch-activated channels during osmotically-induced membrane deformation.

The existence of stretch-activated channels in oocytes (Methfessel et al., 1986) raises particularly interesting questions about their role in the transduction of mechanical events in the early embryo into transmembrane ion fluxes or changes in membrane potential. Dramatic changes in cell shape occur in the early embryo during a number of developmental events, most prominently cleavage cycles, gastrulation and neural tube closure. The presence of stretch-activated channels in cells at these stages could provide a mechanism for the physiological response of cells to the membrane strains generated during such events.

We have begun to study these questions in unfertilized oocytes of the ascidian *Boltenia villosa*. Ascidians offer several experimental advantages for this type of study, among them well-characterized cell lineages that are determined early in development, embryos that are experimentally manipulable, and oocytes and blastomeres that are accessible to patch clamp techniques without enzymatic treatments (*see* Block & Moody, 1987). In the experiments described here, we have demonstrated the existence of stretch-activated channels in the unfertilized oocyte and have characterized their ion permeability, the dynamics of their response to membrane stretch, and the effects of external divalent ions on channel kinetics.

Materials and Methods

Methods for the collection and maintenance of animals and the preparation of oocytes were as described in Block and Moody

(1987). Oocytes were dechorionated manually, using electrolytically sharpened tungsten needles. No enzyme treatments were used. Dechorionated oocytes were sufficiently clean so that seal resistances >5 GΩ were obtained almost without fail.

Solutions

Artificial seawater (ASW) had the following composition (mM): NaCl, 400; KCl, 10; $CaCl_2$, 10; $MgCl_2$, 50; HEPES, 10; pH 8.0. Modifications of this basic solution for testing channel selectivity and kinetics are as follows: low Cl^-: 400 mM Na gluconate, 10 mM K gluconate replaced NaCl and KCl; high K: 400 mM KCl, 10 NaCl replaced 400 mM NaCl, 10 KCl, with 1 mM $BaCl_2$ added to block inwardly rectifying K channels, which become activated at negative potentials in high K solutions (*see* Hagiwara & Takahashi, 1974; Hagiwara et al., 1978); 0 Na, 0 Ca: 400 choline chloride, 10 $MgCl_2$ replaced 400 NaCl, 10 $CaCl_2$, with KOH used to titrate the buffer; 200 Ca, 0 Na: 200 $CaCl_2$, 100 choline Cl replaced 400 NaCl; for experiments studying the kinetic effects of elevated $CaCl_2$, $CaCl_2$ replaced NaCl isosmotically, with $MgCl_2$ held constant at 5 mM. The pipette solution for whole cell recording had the following composition (mM): KCl, 400; NaCl, 10; EGTA, 10; HEPES, 20; pH 7.3.

Electrical Recordings

All experiments studying the permeability characteristics of the channel, in which it was important to know the absolute membrane potential, were done with two pipettes: one in the whole-cell mode to control internal potential, and one in the cell-attached mode to record single-channel events. Other experiments, such as those examining the response of the channel to prolonged suction application or the effects of altered ion composition on channel kinetics, were done with only the single, cell-attached patch pipette. Internal potential could be accurately estimated in these cases from the known current-voltage relation of the channel, and the amplitudes of single channel currents could be used to show that internal voltage was constant. These estimates indicated that with an attached patch pipette in place, the internal potential of the oocyte was between −40 and −60 mV, even though microelectrode impalements give resting potentials of −70 to −80 mV (Hice & Moody, 1988). The decline in internal potential may occur during seal formation, when numerous stretch-activated channels would be expected to be active (*see* Lansman et al., 1987). The very high membrane resistance of *Boltenia* oocytes in the potential range −40 to −60 mV (*see* Block & Moody, 1987; Hice & Moody, 1988) probably contributes to the depolarizing effect of seal formation. Potential across the patch membrane was controlled by voltage commands applied to either, or both pipettes, as indicated.

Pipettes were pulled from borosilicate micropipette glass using a two-stage pull, to a diameter of 1–5 μm, and then fire polished immediately before use. Pipette resistances were 0.5–6 MΩ in seawater. Pipettes for single-channel recording were coated with Polystyrene Q-dope (GC Electronics, Rockford, IL) before fire polishing. Several attached patch recordings were often made sequentially during a single whole-cell recording. Seals were made by gentle application of suction to the pipette. The whole-cell configuration was achieved by transient application of greater suction, and membrane rupture was monitored electrically, as a large increase in capacitance (total C_M = about 0.6 nF for the oocyte, diam. 130 μm), and visually, as the entry of yolk granules into the pipette lumen. Series resistance compensation was used in the whole-cell recording. Further details about the adequacy of voltage control and the electrical properties of these cells under whole-cell clamp can be found in Block and Moody (1987). Experiments examining the effects of changes in external ion composition were done on separate patches.

Vacuum to activate the stretch-sensitive channels was applied using a value in line with a manometer connected to the building vacuum supply. The connection to the supply was led through several traps to damp small pressure fluctuations. In some experiments, vacuum was measured with a pressure transducer placed in parallel with the vacuum line in close proximity to the patch electrode holder.

Data were recorded on FM magnetic tape or were digitized and stored on a laboratory microcomputer. On replay, data were filtered with an 8-pole Bessel characteristic at the frequencies indicated. All experiments were carried out at 9–12°C. Channel analysis was performed using commercially available software (PCLAMP, Axon Instruments, Burlingame, CA).

Results

Recording of Stretch-Activated Channels in the Oocyte Membrane

In about 90% of attached patches in unfertilized *Boltenia* oocytes, application of gentle suction (10–40 cm H_2O) to the pipette resulted in the opening of ion channels not seen either in the absence of suction or during application of voltage pulses to the patch membrane. Figure 1 shows some examples of the activity of stretch-activated channels. The response in Fig. 1*A* is from a large patch (4 μm diam.) in which six channels were simultaneously active at the level of suction shown. The amplitude histogram from this trace in Fig. 1*B* shows six evenly-spaced open levels, indicating that even in this fairly large patch, there is only one population of stretch-activated channel, as distinguished by conductance. Figure 1*C* shows the response to a 530-msec application of suction in a smaller diameter patch which showed at least two active channels. Figure 1*D* shows a continuous 3-sec segment of channel activity from a third patch during a 1-min application of suction. Most long records showed two classes of events: brief openings interspersed with longer duration events, both of which are seen in this record. In general, the stretch-activated channels opened immediately on suction application and, if still open at the end of the pulse of suction (*see below*), closed immediately on release of suction (e.g., Fig. 1*C*). Channel openings were rarely seen in the absence of applied suction. Channel amplitude did not vary with the amount of applied suction. The stretch-activated channels could not be opened by changes in membrane potential independent of suction. Na channels and inwardly

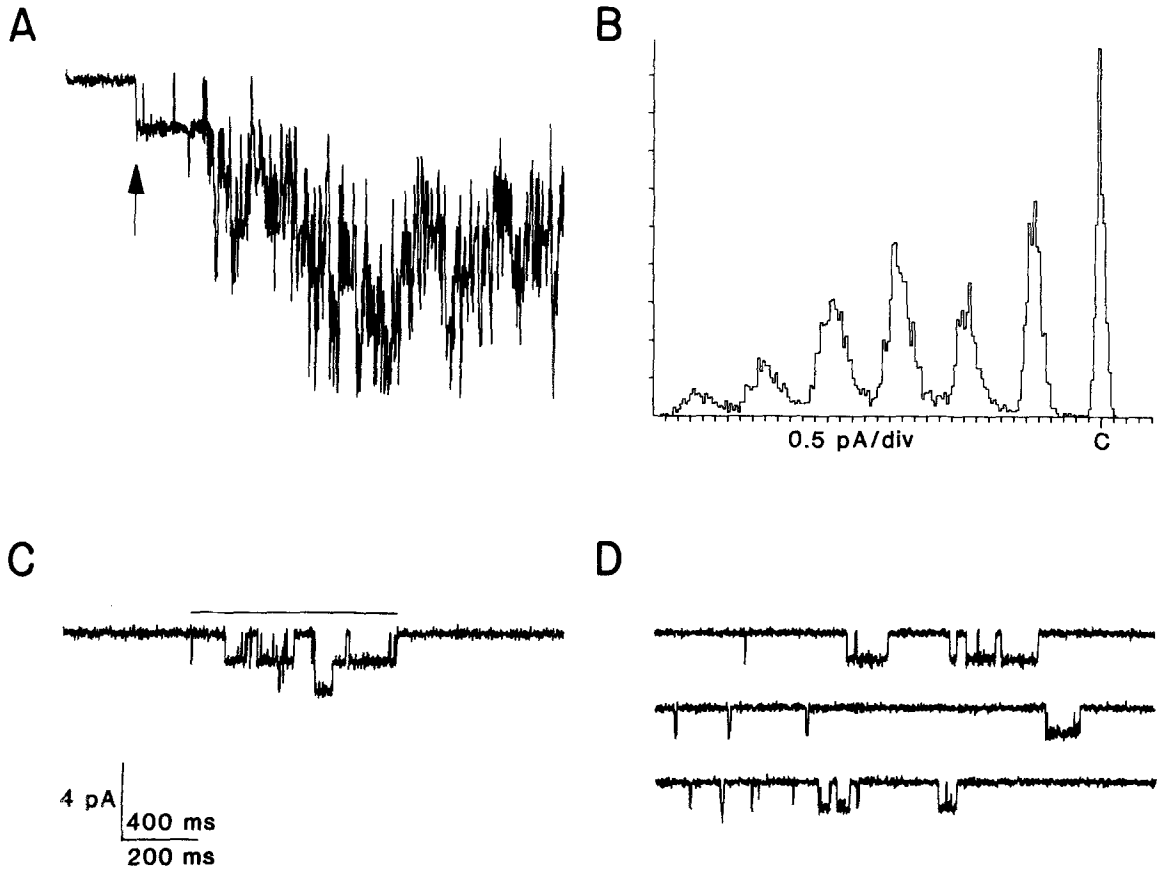

Fig. 1. Cell-attached patch recordings of stretch-activated channels in the unfertilized *Boltenia* oocyte. (*A*) Response to suction in a large patch containing at least six active channels. Suction was applied at the arrow and was held constant throughout. Patch diam., ca. 3 μm; suction, 30 cm H_2O. (*B*) Amplitude histogram of data points in the record of panel *A*. Points were binned at 0.1 pA increments; the horizontal scale of the histogram is in 0.5-pA divisions. The closed, or zero-current, level is to the right. (*C*) Response to a 530-msec pulse of suction in a patch showing at least two active channels. The bar above the trace indicates the period of suction application. (*D*) A continuous 6-sec segment from a 3-min application of suction to another patch. This record illustrates the long and short openings commonly seen in such records. The vertical calibration bar applies to all records. The 200-msec time bar applies to *C* and *D*, 400 msec to *A*. All records were filtered at 1 kHz

rectifying K channels were recorded in some patches and could be clearly distinguished from the stretch-activated channel.

The average density of stretch-activated channels was estimated to be 1–1.5/μm^2, corresponding to roughly 5×10^4 channels per oocyte, based on recordings from large patches (e.g., Fig. 1*A*). This figure may underestimate the true density, because the levels of suction needed to activate all the available channels often caused patch instability and seal breakdown. This value is five- to 10-fold higher than the density of voltage-dependent Na channels in the oocyte, and approximately 50-fold higher than the density of Ca channels (*unpublished data*). We saw no indications that the channels were clustered in particular regions of the cell.

In order to rule out the possibility that partial, transient breakdown of the seal caused by suction pulses might contribute currents which could be mistaken for single channels in small patches or for simultaneous opening of several channels in larger patches, we performed the following experiment. Oocytes were voltage-clamped in the whole-cell mode with one pipette, and a second pipette was used to record single-channel currents from a cell-attached patch. Patches were chosen that contained 5–10 stretch-activated channels. Pulses of suction were applied to the patch, and current was recorded from both the patch pipette and, at high gain, from the whole-cell pipette. To achieve sufficiently low noise in the whole-cell recording from such large cells (total capacitance = 0.6 nF), the internal voltage was held at −60 mV, a region of minimum conductance in the *I-V* relation (Block & Moody, 1987), no series resistance compensation was used (holding current at this potential was <100 pA), and records were filtered at 60 Hz. An additional 100-mV hyperpolarization was applied to the patch pipette

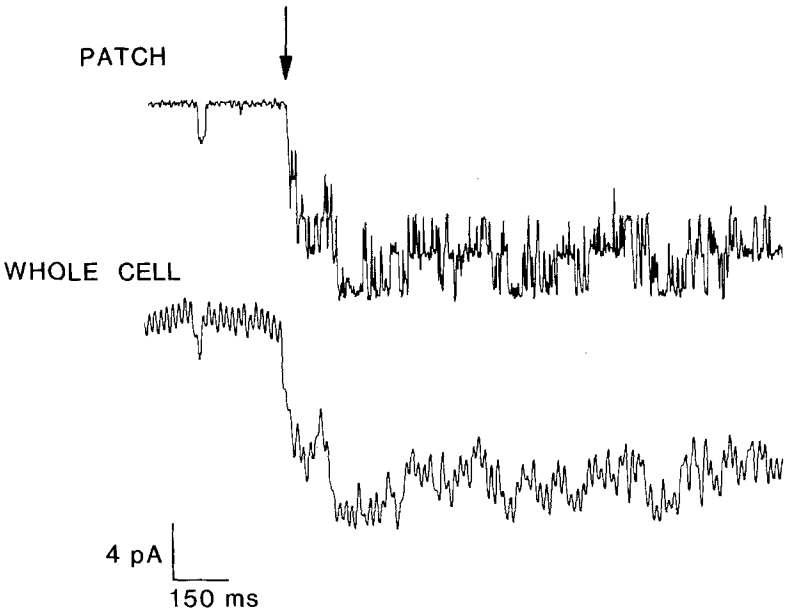

Fig. 2. Simultaneous attached patch and whole-cell recording of single channel currents resulting from application of suction to the patch pipette, demonstrating that stretch-activated currents cross the patch membrane and not the seal resistance (*see* text). Arrow indicates suction onset. The cell was held at −60 mV, with an additional −100 mV applied to the patch. The whole-cell record was filtered at 60 Hz. The scale applies to both records. A single opening can be discerned in both records approximately 200 msec before the onset of suction, as can several of the longer-lived transitions during suction application

to increase single-channel current to approximately 3 pA. The result of one such experiment is shown in Fig. 2. The patch record shows a single-channel opening before application of suction, five superimposed openings within 200 msec of suction onset, and then transitions between five, four, and three open channels over the next 1 sec of continued suction. The whole-cell current exactly matched the patch current in amplitude, and longer-lasting transitions between the number of active channels in the patch—including the single opening preceding the suction pulse—could clearly be resolved in the whole-cell recording. Thus all of the current leaving the patch pipette exists across the cell membrane and cannot be explained as leakage of current across the seal resistance in response to suction.

Permeability Characteristics of the Stretch-Activated Channel

All experiments concerning channel permeability characteristics were performed using a second pipette in the whole-cell configuration so that absolute potentials across the patch membrane were known (*see* Materials and Methods). Current-voltage relations for the stretch-activated channel were determined either by activating the channel with suction pulses at different holding potentials or by applying a ramp voltage command in the presence and absence of applied suction (voltages controlled by the whole-cell pipette). Figure 3*A* shows a record from the latter type of experiment. In this patch, depolarizing ramps were applied between −200 and +85 mV absolute potential, and a level of suction was used that activated two channels. The open-channel current reversed at −10 mV and showed considerable inward rectification at negative potentials. Figure 3*B* shows superimposed *I-V* relations for three patches determined from measuring single-channel amplitudes at various holding potentials. The chord conductance of the channel at −100 mV is 11 pS. As is apparent from Fig. 3*B*, conductances and reversal potentials were very consistent between patches.

Ion substitution experiments were carried out to determine the ion selectivity of the stretch-activated channel. Figure 4A shows the effect on the *I-V* relation of the channel of changing the Cl^- concentration bathing the external face of the patch in both attached and excised patches. Decreasing the external (pipette lumen) Cl^- concentration from 530 to 120 mM ($n = 4$; records taken with this solution were corrected for a +7-mV junction potential measured between the pipette and bath) had almost no effect on either the conductance or reversal potential of the channel in either attached or excised patches, indicating that the channel is cation selective. Excising the patch so that both faces were exposed to ASW resulted in an approximate doubling of the channel conductance (to 21 pS at −100 mV) and shifted the reversal potential about 10 mV in the positive direction. Since intracellular $[K^+]$ in these cells is probably near 180 mM (*see* Hagiwara & Yoshii, 1979), excising the patch into ASW effectively substitutes 400 mM Na^+ for 200 mM K^+ on the cytoplasmic face. The increased conductance and relatively unchanged reversal potential thus implies that the channel discriminates poorly between Na^+

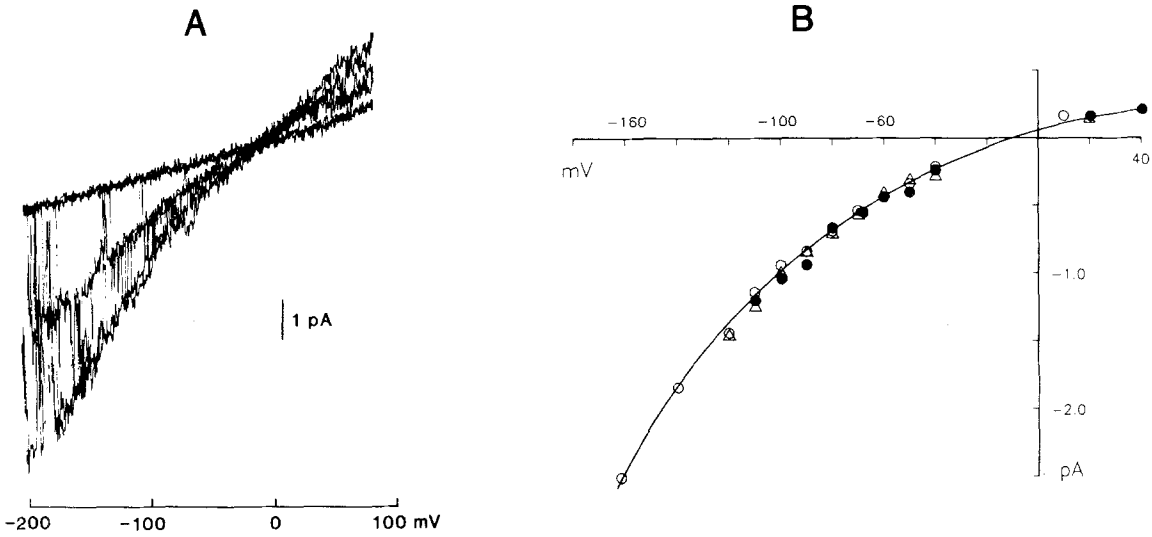

Fig. 3. Open channel *I-V* relations for the stretch-activated channel. All experiments were done with combined whole-cell and attached patch recording (*see* Materials and Methods). (*A*) Current records resulting from the application of the depolarizing ramp voltage to the whole-cell pipette (patch pipette voltage held constant at bath potential), from −200 to +85 absolute potential, in the absence (quiet trace with smallest slope) and presence of suction. Two channels were active in the patch. Two traces in the absence of suction and four in the presence of suction are superimposed. The reversal potential is −8 mV. (*B*) *I-V* relations from three patches determined by applying suction at various holding potentials (potentials applied to the whole-cell pipette with patch pipette held at bath potential). Horizontal axis is in absolute potential. The conductance at −100 mV is 11 pS, and the reversal potential is −10 mV

and K^+. This was confirmed in two experiments which showed that substituting 400 mM K^+ for 400 mM Na^+ on the external face of the patch (pipette lumen) had almost no effect on the *I-V* relation of the channel (*not shown*). We did not test whether the channel displayed more selectivity among monovalent cations when external Ca^{2+} concentration was reduced to micromolar concentrations, as has been reported for the stretch-activated channel in lens epithelium (Cooper et al., 1986).

We tested the Ca^{2+} permeability of the stretch-activated channel in four experiments using high Ca^{2+} (200 Ca, 0 Na (choline)) ASW (Fig. 4*B*). In attached patches, channel conductance was relatively unaffected at negative potentials with Ca^{2+} as the primary charge carrier, but at potentials positive to about −70 mV the *I-V* relation showed more pronounced rectification and the extrapolated reversal potential was shifted to more positive voltages (*compare* to Fig. 4*A*, attached, ASW *I-V*). The actual shift in reversal potential was difficult to determine because of the substantial rectification and short channel open times at elevated Ca^{2+} concentrations (*see below*). In patches excised into ASW this substitution had little effect on either conductance or reversal potential. This is expected if Ca^{2+} and Na^+ are about equally permeant in the channel, since the ASW into which the patch is excised contains approximately the same level of Ca^{2+} as the oocyte cytoplasm contains Na^+ (Hagiwara & Yoshii, 1979). Figure 4*B* also shows the effects of removing both Na^+ (choline replacement) and Ca^{2+} (Mg^{2+} replacement). In attached patches the *I-V* relation in 0 Na, 0 Ca external solution was somewhat linearized and the reversal potential shifted to more negative values. The extrapolated reversal potential for this patch was −38 mV. The K^+ equilibrium potential under these ionic conditions is approximately −61 mV. This discrepancy could be due to either choline or Mg permeation through the channel (*see* Christensen, 1987).

The above results indicate that the stretch-activated channel in the unfertilized *Boltenia* oocyte behaves as a rather nonspecific cation channel, which excludes anions but passes Na^+, K^+, and Ca^{2+} with approximately equal ease. This is in close agreement with the results of Lansman et al. (1987) working on aortic endothelial cells, who showed approximately equal permeation of Na^+ and K^+ through the channel, with Ca^{2+} slightly more permeable than either.

Kinetic Analysis of the Response of the Channel to Suction

We performed a basic kinetic analysis of the stretch-activated channel to determine the basis for the increased open probability at increased levels of membrane deformation.

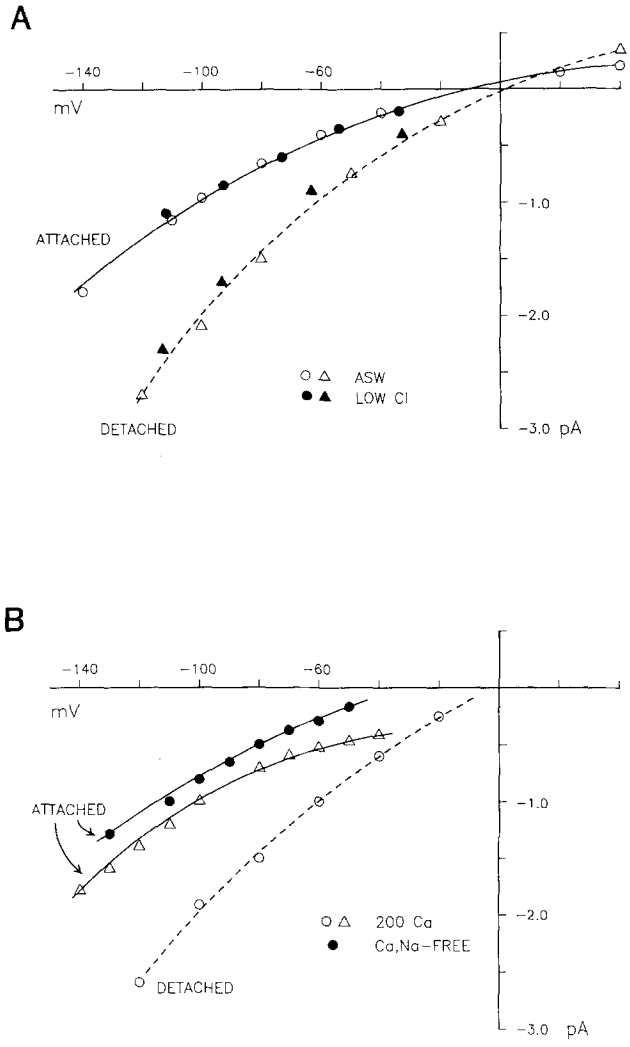

Fig. 4. Current-voltage relations for the stretch-activated channel under various ion conditions. All attached patch experiments were done with combined whole-cell and patch recording (*see* Materials and Methods). (*A*) Comparison of normal (530 mM) and reduced (120 mM) Cl^- in the external (pipette) solution in attached and excised patches (different patches for different solutions). Patches were detached into ASW (*see* Materials and Methods for composition). (*B*) *I-V* relations for attached and detached patches in 200 Ca external solution and for an attached patch in 0 Na, 0 Ca external solution. In the attached recordings for *A* and *B*, potential is controlled by the whole-cell pipette. The attached and detached recordings in each solution are from the same patch

Frequency distributions of open times were determined in 10 prolonged applications of suction in four patches; in eight of these runs, sufficient numbers of openings occurred to allow reasonable fits to the open time histograms. In six of these eight patches the histograms of open times were best fit by the sum of two exponentials, with time constants in the range of 2–5 and 16–30 msec. These two time constants correspond to the brief and long openings seen in most long records (*see* Fig. 1*D*). In two patches, a single exponential with a time constant near 10 msec provided an adequate fit. Figure 5 compares open times from a single patch at two levels of suction, the higher level increasing the open probability, measured over a 2-min continuous suction application, by approximately threefold. The time constants describing the two open states were essentially unaffected by suction. A third application of lower suction was analyzed for this patch and gave similar time constants (*data not shown*). A second patch, which showed only a single time constant to the open time histogram, was similarly analyzed at four increasing levels of suction and gave time constants of 10.6, 10.04, 10.6 and 12.5 msec. Similar results were obtained in several other patches, which were analyzed less completely.

It was difficult to analyze closed intervals, since in patches with low or moderate open probabilities and no overlapping openings, it was not clear whether more than one channel was active in the patch. Since long closed intervals could easily be bounded by the openings of two different channels, closed time histograms in such cases do not yield meaningful rate constants. We determined closed time histograms in several patches in which long records were obtained with few or no overlapping events to obtain a qualitative estimate of the effects of suction on closed intervals. Inspection of records indicated that there were at least two closed states, representing the brief flickers within the longer openings and the long intervals between openings, respectively. The high open probability at high suction levels seemed to be primarily the result of a shortening of the longer closed interval or intervals, and this was borne out by the analysis. Figure 5 shows closed time histograms for the same patch at the same two levels of suction. The sum of two exponentials provided a reasonable fit to both sets of data. The longer of the two time constants was greatly reduced by increased suction, whereas no consistent effect was seen on the shorter time constant. In patches in which several levels of suction were used, the longer closed time constant decreased monotonically with increasing suction. Thus membrane deformation appears to increase channel open probability primarily by shortening the long closed intervals between channel openings. This agrees with previous findings for the stretch-activated K channel in *Lymnaea* cardiac cells (Sigurdson et al., 1987).

Effects of External Calcium Levels on Channel Kinetics

In studying the permeation of calcium through the stretch-activated channel, it became clear that external concentration of calcium had pronounced effects on channel kinetics. We studied this effect in

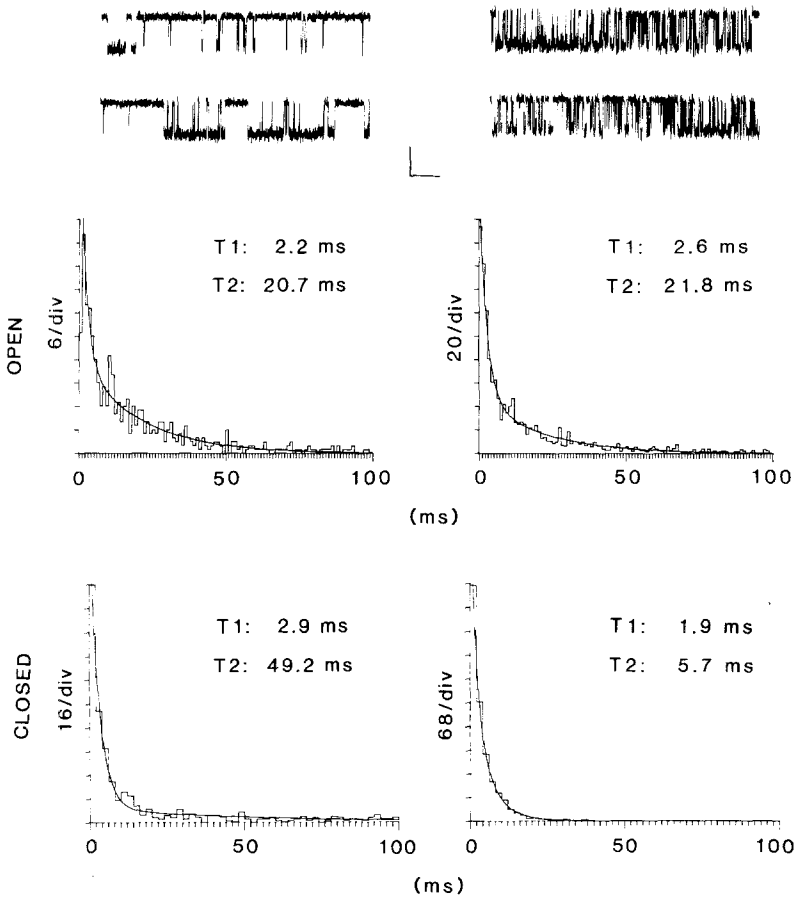

Fig. 5. Sample records and open and closed time distributions from a single cell-attached patch at two levels of suction. The horizontal axes for all histograms are the same. The open time distributions are binned at 1-msec intervals, the closed time distributions at 2 msec. Although the higher suction caused a dramatic increase in channel open probability, the open time distributions were fit at both suction levels by the sum of two exponentials with similar time constants. In this patch, the relative contribution of the longer lasting open state to the distribution was higher at lower suction, but this was not consistently observed. The closed time distributions at both suction levels are also fit by the sum of two exponentials, but increased suction shortens the longer time constant. Attached patch recordings without whole-cell. Patch diam. ca. 1.5 μm; suction levels, 40 and 60 cm H_2O. Voltage held at −60 mV relative to rest. Calibration bars for the current traces: 1.5 pA, 150 msec

10 patches at four different levels of external Ca^{2+}. Figure 6 illustrates results from three of these recordings. Channel activity in three different patches was recorded during a 3-min application of suction at external calcium concentrations of 0, 50 and 315 mM (Mg^{2+} held constant at 5 mM). At nominally zero calcium concentration, long openings dominate the recordings; as calcium concentration is raised, shorter openings become increasingly predominant. The effect does not seem to be a simple one. At low Ca, there appears to be only a single, long-lived open state (4 patches; time constants: 71.1, 53, 21 and 15 msec). Transitions to the short-lived open state are not seen. Note that in two of the patches, the single time constant in 0 Ca approximated the longer time constant determined from openings recorded at more physiological Ca levels (10 mM; time constants 16–30 msec), although in the patch shown in Fig. 6, it was clearly longer. (It is possible that a third, long-lived open state exists in normal Ca levels, but is poorly resolved in the distributions, and that only transitions into this state are seen at very low Ca levels.) At Ca levels of 10–50 mM, the distributions are similar to those in ASW (10 Ca, 50 Mg). The longer openings are interrupted more frequently by brief closings, which could be interpreted as blocking events, but the records do not divide into recognizable bursts. At very high Ca levels (Fig. 6*A*), only short openings are seen, and the open time histograms can be described by a single exponential in the same range (2–5 msec) as that of the short-lived open state in ASW (Fig. 5). It appears that Ca may both promote transitions into the short-lived open state and block or facilitate closure of the long-lived open state. Clearly, more work is needed to understand these effects.

Dynamic Response to Suction Onset in Multiple-Channel Patches

We performed a series of experiments on multiple-channel patches, in which between two and 20 channels could be opened simultaneously at the onset of step changes in suction (10–60 cm H_2O), to study the dynamics of the increase in channel activity with increased suction. In these experiments, suction was monitored simultaneously using a force transducer placed at the entrance of the vacuum line to the patch pipette holder, and only records in which the rise time of suction onset was less than 100 msec were used. In the large majority of such vacuum applications (20/25), the response of the channel population was highly phasic. Maximum

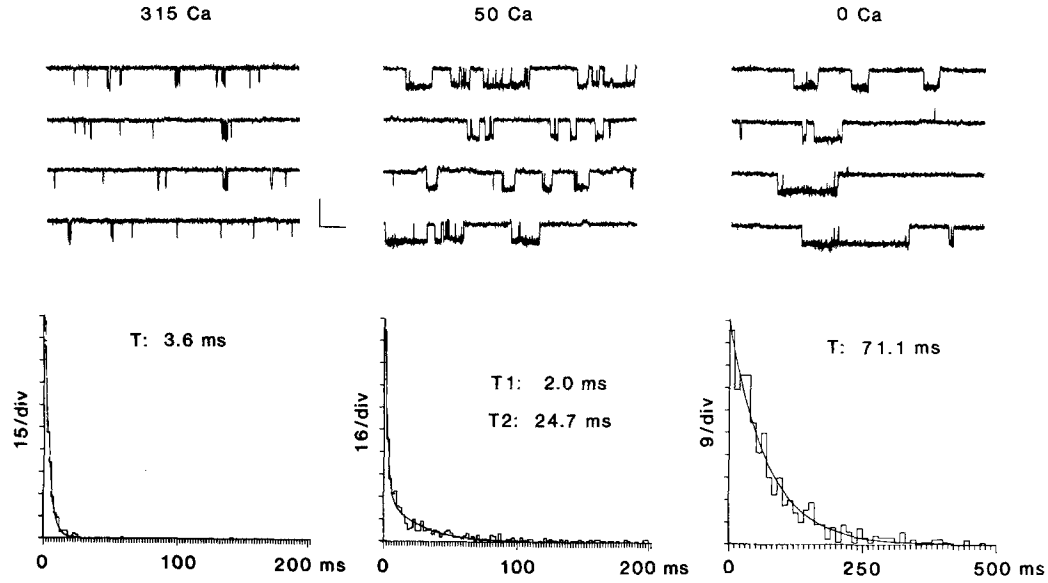

Fig. 6. Effect of external Ca concentration on channel kinetics. Representative records from three patches at three levels of external Ca^{2+} (Mg^{2+} constant at 5 mM). Open time histograms are shown, binned at 2-msec intervals in 50 and 315 mM Ca^{2+}, and at 10 msec in 0 Ca^{2+}. Rebinning the 0 Ca^{2+} distribution at 0.5 msec over the first 20 msec did not reveal a shorter time constant. Attached patch recordings without whole-cell. Voltage held at −60 mV relative to rest. Calibration bars: 1.5 pA, 200 msec

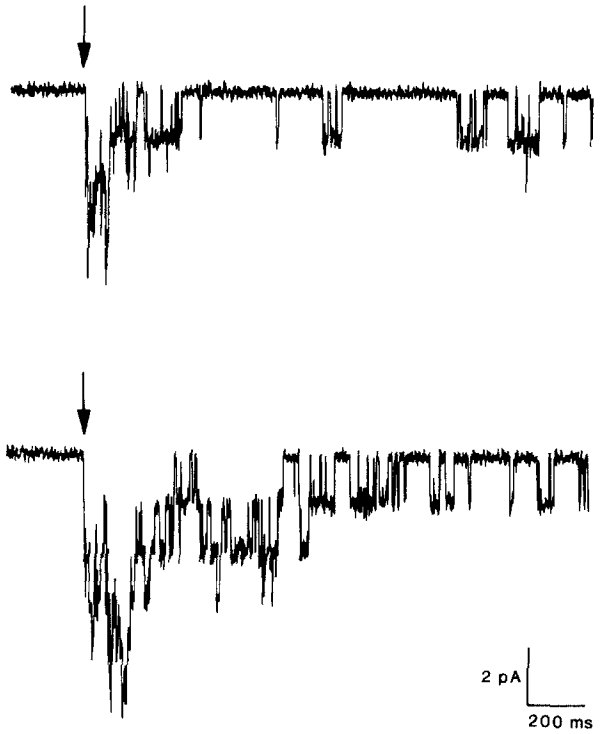

Fig. 7. Phasic response of stretch-activated channels to step onsets of suction. Representative records from two different patches. Attached patch without whole-cell. Voltage held at −60 mV relative to rest. Arrows indicate onset of suction, which was monitored by a pressure transducer; onset time of suction was less than 100 msec in all cases, and suction remained constant for the duration of the application. *See* text. Patch diam. ca. 2 μm (top), 3 μm (bottom). Suction: 30 cm H_2O (top), 40 cm H_2O (bottom)

channel activity was seen immediately after the onset of vacuum and declined rapidly to a low steady-state level within 200 msec to 1 sec. Figure 7 shows two examples of this type of response. The steady-state level of channel activity represented about 10–50% of the peak activity and was maintained at an approximately constant level throughout the remainder of the vacuum application (1–3 min). Following a 2-min vacuum application, the previous peak response to a second, identical suction was not regained even after a 1-min interval. This phasic response was not as obvious in patches containing a small number of channels, especially at large suction levels which can effectively saturate the channel response. The transient response of the stretch-activated channel to step changes in suction may have important functional implications in terms of predicting effective physiological stimuli for channel opening. Possible molecular substrates of the transient nature of the response are discussed below.

Discussion

We have described a stretch-activated (s.a.) ion channel in the unfertilized oocyte of the ascidian *Boltenia villosa*. This channel is a relatively nonselective cation channel, passing Na^+, K^+ and Ca^{2+} ions. It exists in the membrane at a density comparable to or higher than voltage-dependent channels

that we have previously described in this cell (Block & Moody, 1987). The channel has at least two open and two closed states, with transitions out of the long-lived closed state being the primary site of stretch sensitivity. The kinetics of the channel were also affected by the external concentration of calcium ions, such that as the level of calcium was increased, short openings became increasingly predominant. In multi-channel patches, the channel population showed a phasic response to a step onset of suction, with open probability declining within 100–800 msec to a nonzero steady-state level.

The properties of the *Boltenia* s.a. channel are very similar to those reported in a variety of other preparations. Calcium permeation has been found in s.a. channels in endothelial cells (Lansman et al., 1987) and in choroid plexus epithelium (Christensen, 1987). Effects of high calcium levels on channel kinetics are also apparent in records from these two preparations (*see* Lansman et al., 1987, Fig. 3; Christensen, 1987, Fig. 1). Evidence for two open states of the channel has been reported in endothelium (Lansman et al., 1987), frog oocytes (Methfessel et al., 1986), and for the s.a. K-selective channel in molluscan cardiac muscle (Sigurdson et al., 1987). It also appears that stretch sensitivity of the transition out of the long-lived closed state may be a general property, independent of the ion selectivity [*compare* the present results with Guharay and Sachs (1984) and Sigurdson et al. (1987)]. The apparent density of the s.a. channels in *Boltenia* oocytes is similar to that reported in amphibian oocytes (Methfessel et al., 1986), choroid plexus epithelium (Christensen, 1987) and molluscan heart cells (Sigurdson et al., 1987). This is particularly interesting since the densities of voltage-dependent channels in these tissues varies widely. Thus the s.a. channel exists at low density relative to other ion channels in many tissues, but at similar or higher density than other channels in the *Boltenia* oocyte.

The rapid decline of channel open probability following a step change in vacuum has not been reported in other cells, although decrement in channel activity with repeated applications of suction has been described (Cooper et al., 1986). A simple mechanical model that puts an elastic channel in series with a viscoelastic membrane predicts that the strain on the channel should increase instantaneously to a maintained level following a step change in vacuum (*see*, e.g., Rand, 1964; Hochmuth & Waugh, 1987). However, the relaxation of strain on the channel could be explained by assuming that there is a second viscoelastic element in parallel with the channel whose strain increases on applied stress at a rate faster than the element in series with the channel. In this way, strain on the channel could be relieved after the initial increase even while strain on the entire membrane-channel complex continues to increase. The strain-relieving element in parallel with the channel might be cytoskeletal, since the steady-state response of the s.a. channel to vacuum is greatly increased by cytochalasin, which disrupts cytoskeletal elements (Guharay & Sachs, 1984). In this model, the dynamic response and sensitivity of the s.a. channel would be governed by the mechanical parameters of both the lipid membrane and the cytoskeletal network associated with the channel.

The presence of s.a. channels in ascidian oocytes at a density similar to or greater than several voltage-activated channels suggests that they may participate in events in early development which involve mechanical strain on the membrane or contractions of the cortical cytoskeleton. The single channel current at −70 mV, the normal resting potential of the oocyte (Hice & Moody, 1988), is about 0.5 pA. The deactivation of the inwardly rectifying K current as the oocyte is depolarized from −70 to −60 mV yields an extremely high steady-state input resistance, often greater than 1 GΩ, at voltages positive to −60 mV (Block & Moody, 1987). Thus only about 30–60 pA of current is required to bring the oocyte membrane to threshold for action potential generation, which involves both Na and Ca entry through voltage-dependent channels (Block & Moody, 1987; Hice & Moody, 1988). This current represents the activation of about 100 s.a. channels, or less than 1% of the estimated population in the oocyte. The activation of these channels could thus admit calcium ions to the cytoplasm by virtue of their Ca permeability and because the resulting potential change activates voltage-sensitive Ca channels.

There are several points in early embryogenesis when membrane strains might be sufficient to activate the s.a. channel. Ooplasmic segregation, which immediately follows fertilization in ascidians, is mediated by contraction of cortical actin filaments (Jeffery & Meier, 1983) and is accompanied by visible shape changes in the oocyte and often dramatic movements of membrane-adherent particles or supernumerary sperm over the egg surface (Sawada & Osanai, 1981). Sufficient stimulus for s.a. channel activity might also occur during cytokinesis. Aside from the mechanical strains involved in the formation of the cleavage furrow itself, the cyclical extension and retraction of microvilli with cleavage cycles in ascidians (Satoh & Deno, 1984) might reflect underlying cytoskeletal activity with which s.a. channels might be involved. Finally the tissue movements and cell shape changes that characterize gastrulation (*see* Gerhart & Keller, 1986) certainly result in substantial mechanical strains that

might effectively activate the population of s.a. channels in the membrane. Interestingly, one of the major mechanical models of epithelial infolding during morphogenesis is based in part on the assumption that mechanical strain above a certain threshold in embryonic cells triggers active contraction of cytoskeletal elements, possibly through intracellular release or influx of calcium ions; calculations of the propagation of this contraction from a single deformed cell can closely mimic a number of morphogenetic movements, such as sea urchin gastrulation and amphibian neural tube closure (Odell et al., 1981). Direct tests of whether s.a. channels can provide a physiological mechanism behind the assumptions of this type of model may prove difficult because the mechanical constraints placed on the membrane aspirated into the patch pipette itself are likely to alter the response of that membrane to events over the remainder of the cell surface. We are at present working at solutions to this problem and examining the activity of s.a. channels at various stages of early embryogenesis.

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Note Added in Proof

Adaptation of channel open probability following a step change in vacuum has recently been reported in fibroblasts and yeast cells (Stockbridge, L.L., French, A.S., 1988, *Biophys J.* **54:**187; Gustin, S., Zhou, X.-L., Martinac, B., Kung, C., 1988, *Science* **242:**762).