Novel Cation-Selective Mechanosensitive Ion Channel in the Atrial Cell Membrane

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Stretch of atrial muscle causes the release of atrial natriuretic peptide, but no stretch-sensitive membrane sensors have been clearly identified so far. The existence of an ion channel that could mediate stretch-induced Ca\(^{2+}\) influx and subsequent release of the peptide was examined in rat atrial cells. In this report, the discovery of a novel atrial ion channel whose opening probability is extremely sensitive to either positive or negative pressure (half-maximal activation pressure, \(-1.5\) mm Hg) is described. Activation of the current by pressure applied to the pipette was observed both at the whole-cell and single-channel levels. The channel was permeable to cations including Ca\(^{2+}\). The channels were clustered with six to nine channels in each cluster, and several channels tended to open simultaneously in response to a graded increase in pressure for the inwardly passing current. Hypotonic swelling also activated these channels. These results show that mechanosensitive nonselective cation channels exist in atrial cells and suggest that they could be involved in beat-to-beat regulation of the atrial contraction as well as the stretch-induced expression of proto-oncogenes and secretion of atrial natriuretic peptide via increased Ca\(^{2+}\) entry.

Key Words • atrial cells • pressure • ion channel • calcium flux • hypotonic swelling

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Stretch or pressure has been shown to change membrane ion permeability in many types of cells including animal and plant cells, and in microorganisms such as bacteria and yeast. These mechanosensitive channels have been suggested to be important for volume regulation, control of membrane potential, and cell differentiation and proliferation. In atria, distension of the muscle produced by an increased blood pressure or volume loading has been well documented to cause the release of atrial natriuretic peptide as a feedback mechanism to lower blood pressure. However, the membrane sensor that mediates the release of the peptide has not yet been identified.

Cardiac ion channels that open in response to a change in pressure have been observed in several studies. Generally, negative pressure was applied to the pipette electrode after formation of a cell-attached patch. Such application of negative pressure caused activation of a potassium-selective ion channel in moluscan ventricular cells and a nonselective cation channel in neonatal rat and chick embryo ventricular cells. More recently, the ATP-sensitive potassium channel was also reported to be activated by pressure in rat atrial cells. A local increase in intracellular [Ca\(^{2+}\)], as judged by fluo-3 fluorescence, was produced by pressing a pipette tip against the cell membrane of chick heart, indicating the presence of mechanosensitive Ca\(^{2+}\)-permeable ion channels. In canine atrial and ventricular cells, cell swelling was found to activate a Cl\(^{-}\) current. These studies clearly show that there are mechanosensitive ion channels in the heart cell membrane. In general, the half-maximal negative pressure applied to the pipette electrode was required to induce opening of ion channels was 10–20 mm Hg. This study reports the discovery and characterization of an atrial nonselective cation channel that is activated by extremely low positive or negative pressures (half-maximal pressure, \(=1.5\) mm Hg) applied to the interior of the pipette electrodes and that therefore would be likely to act as a membrane sensor to stretch signal.

Materials and Methods

Cell Preparation

Hearts from 1–2-day-old newborn rats (Sprague-Dawley) were dissociated with collagenase and trypsin. After complete anesthesia with ether, right and left atrial tissues from whole hearts were excised and placed in Ca-free Hanks' medium (Sigma Chemical Co., St. Louis, Mo.). The tissues were then cut into small pieces (<1 mm\(^3\)) with a sharp blade and placed in Hanks' balanced salt medium containing 0.05% collagenase type II and 0.03% trypsin (Worthington Biochemical Corp., Freehold, N.J.). Tissue pieces were incubated at 37°C for 10 minutes. The medium containing suspended cells was then removed and added to the same volume of 50% fetal calf serum to inhibit the enzyme activities. The remaining tissue pieces were incubated in a new enzyme solution and allowed to dissociate for another 10 minutes. This procedure was repeated five times. Dissociated cells were collected by centrifugation (1,000 rpm for 10 minutes) and placed into culture medium that consisted of Dulbecco's modified Eagle's medium (Sigma), 10% fetal calf serum, and 0.1% penicillin-streptomycin. Cells were plated onto glass coverslips and placed in a 37°C incubator containing...
5% CO₂–95% air. The cells used in this study were incubated for 20–28 hours.

Electrophysiology and Solutions

Gigaseals were formed with thin-walled borosilicate glass pipettes (Kimax) with tip resistances of 1–2 MΩ for whole-cell studies and 3–6 MΩ for single-channel studies as described by Hamill et al. A cell-attached patch was first formed, and then the cell membrane under the patch pipette was ruptured by gentle suction to form the whole cell. Capacitive transients were canceled before the measurement of the current. Currents from cell-attached patches, inside-out patches, and whole cells were recorded with a patch-clamp amplifier (Axopatch 1D, Axon Instruments, Foster City, Calif.), low-pass filtered at 10 kHz with an eight-pole Bessel filter (Frequency Devices Inc., Haverhill, Mass.), and stored on videotape (JVC) via a PCM adapter (VR-100, Instrotech, Elmont, N.Y.). The digitized data were entered into a computer (ATARI-ST) and analyzed to calculate channel open probability. Single-channel currents were filtered with a Gaussian digital filter at a bandwidth of 2 kHz (−3dB bandwidth of a Bessel filter). The open probability of the channel was calculated by integrating the current through all channels divided by the total current that would through all the channels if they were fully open.

For whole-cell experiments, the bath solution contained (mM) NaCl 135, KCl 5, CaCl₂ 1, MgCl₂ 2, and HEPES 10 (pH 7.2). The pipette solution contained (mM) KCl 140, EGTA 5, MgCl₂ 2, and HEPES 10 (pH 7.2). In experiments using cell-attached or inside-out patches, standard bath and pipette solutions contained (mM) KCl 140, EGTA 5, MgCl₂ 2, and HEPES 10 (pH 7.2). In certain experiments, K⁺ was replaced with equimolar concentrations of Na⁺, Li⁺, Cs⁺, Ca²⁺, Ba²⁺, or choline to determine cation selectivity. To determine anion selectivity, Cl⁻ was replaced with glutamate. In all ion replacement studies, liquid junction potentials at the agar-bridge bath electrode were determined separately, and all voltages were corrected for offsets that were <0.4 mV. Pressure in the pipette was changed as follows: One end of a 1-m-high manometer filled with distilled water was connected to the pipette holder, and the other end was connected to a calibrated syringe. Changes in pipette pressure could be achieved rapidly and accurately to desired levels with an automated calibrated syringe. Pressure levels measured as millimeters of H₂O were converted to millimeters of mercury. The negative pressure at the membrane patch is the pressure measured from the manometer minus the pressure due to a small amount of solution in the pipette tip (≈0.1 mm Hg). This small amount of pressure was not included in our calculations. The possible presence of other small forces in the pipette such as that produced by capillary action was not considered.

Results

Activation of the Mechanosensitive Whole-Cell Current

The whole-cell voltage-clamp technique was used first to detect the mechanosensitive ion current in atrial cells. In a physiological bath solution containing (mM) Na⁺ 135, K⁺ 5, and Ca²⁺ 1, brief applications of positive pressure in the pipette (0.7–3.6 mm Hg) elicited reversible whole-cell currents (Figure 1A). These pressure-activated currents in atrial cells were not artifacts, because the current changes elicited by similar positive-pressure applications were negligible in every cell tested when all cations in the bath were replaced by choline (n=11, Figure 1B). Because the whole-cell membrane current became unstable at positive pressure levels higher than ≈3 mm Hg, it was not possible to obtain a full pressure–current relation. Positive pressure pulses (1.4 mm Hg) of short duration (<0.3 seconds) applied repeatedly to the pipette (=2 minutes) were each associated with a rapid activation and a deactivation of the channel current without desensitization (Figure 1C). Also, little or no decrease in the channel open probability occurred during the sustained positive-pressure applications. These results show that mechanosensitive changes in ion conductance are clearly present and can be elicited by small changes in the pipette pressure in the rat atrial cell membrane.

Activation of the Mechanosensitive Single-Channel Current

The presence of mechanosensitive ion channels was further investigated at the single-channel level. In cell-
attached patches with physiological concentrations of Ca\(^{2+}\), K\(^{+}\), and Na\(^{+}\) in the pipette and bath solutions, inward single-channel currents were elicited by applying negative pressure to the inside of the pipette when the pipette potential was held at 0 mV. Initial studies using different cations and anions as charge carriers indicated that the mechanosensitive channel was permeable only to cations (see below). Therefore, single-channel currents were further studied using K\(^{+}\) as the charge carrier (140 mM K\(^{+}\) in bath and pipette). In the cell-attached form, application of negative pressure in the pipette evoked an outward current above \(-0 mV\) and an inward current below \(-0 mV\) (Figures 2A and 2B). Return of the pipette pressure to the atmospheric level resulted in closing of all channels. Channels could be reopened by application of either negative or positive pressure. The magnitude of the outward current was closely related to the amount of applied negative pressure. Also, the stochastic fluctuation of channels between open and closed states could be readily identified when the current was in the outward direction. When the current was in the inward direction, however, three to five channels tended to open and close simultaneously, and the current showed an extremely noisy behavior during the open state. Furthermore, a certain level of threshold pressure was required to open the channels when the current was in the inward direction. The threshold pressure for evoking inward currents varied between 1 and 2 mm Hg (see the current recording in Figure 2B), and no channels opened below 0.8 mm Hg. The differences in channel opening properties for the outward and inward currents were observed in cell-attached and inside-out patches and may reflect a component of voltage dependence of channel gating.

The single-channel conductance of the pressure-activated channel was 21.0 \pm 2.2 pS (mean \pm SD, n=4) in symmetrical 140 mM K\(^{+}\) (inside-out patch). Dilution of the pipette [KCl] in inside-out patches from 140 to 28 mM shifted the current–voltage curve such that the current reversed at \(-35 \pm 3 mV\) (n=3), close to the calculated equilibrium potential for K\(^{+}\). Thus, the channel passed K\(^{+}\) but excluded Cl\(^{-}\). No outward current was present when the bath K\(^{+}\) was replaced by choline. Substitution of the bath K\(^{+}\) with an equal concentration

**Figure 2.** Mechanosensitive single-channel currents in membrane patches. Panel A: Current recording from a cell-attached patch (pipette potential, \(-60 mV\)). Application of negative pressure in the pipette caused opening of ion channels. Eight channels were opened in this patch. Return of pressure to zero closed all channels. Panel B: Current recording with pipette potential held at \(+60 mV\). An experiment similar to that in panel A was performed. Panel C: Currents recorded at membrane potentials of \(+60 mV\) from three separate inside-out patches: 140 mM K\(^{+}\) (\(-0.4 mHg\)), 140 mM K\(^{+}\) plus 100 \(\mu\)M GdCl\(_3\) (\(-0.4 mHg\)), or 140 mM Ca\(^{2+}\) (\(-0.6 mHg\)) in both pipette and bath solutions. Voltage calibration is identical to that for panels A and B. Panel D: Single-channel amplitudes plotted against the membrane potential. Channel current responses to pressure were recorded in inside-out patches at various potentials. Current–voltage relations obtained in 140 mM K\(^{+}\) or Ca\(^{2+}\) in bath and pipette solutions are shown. The pipette resistances in these experiments were 4–5 M\(\Omega\). Both pipette and bath solutions contained (mM) KCl 140, MgCl\(_2\), 2, EGTA 5, and HEPES 10 (pH 7.2). EGTA was not used when K\(^{+}\) was replaced by Ca\(^{2+}\). GdCl\(_3\) was prepared just before the experiment. From the shift of the reversal potential in bi-ionic experiments in which the bath K\(^{+}\) was replaced with same concentration of Ca\(^{2+}\), the permeability ratio, \(P_{ca}/P_{k}\), calculated using the modified constant field equation,\(^{29}\) was 0.9.
of Rb⁺, Na⁺, or Cs⁺ did not measurably shift the current–voltage relation, indicating poor selectivity among monovalent cations. Substitution of the bath K⁺ with Ca²⁺ slightly reduced the conductance and shifted the reversal potential (Eᵣ) to −3.2±1.6 mV (n=3). The single-channel conductance was 17.8±2.1 pS (n=3) in symmetrical 140 mM Ca²⁺ (Figure 2D) and 17.2±2.3 pS (n=3) in 140 mM Ba²⁺. Thus, the mechanosensitive channel in the atrial cell membrane is a nonselective cation channel. The channel open probability was not altered by 100 μM GdCl₃ (Aldrich Chemical Co., Milwaukee, Wis.), which has been shown to block mechanosensitive channels in oocytes, skeletal muscle cells, and fungus (Figure 2C).⁷,¹⁶,³³

The relations between pressure and channel open probability (of outward currents such as that shown in Figure 2A) in control cells and in cells whose cytoskeleton has been disrupted by treatment with cytochalasin B are shown in Figure 3. Figure 3A shows activation of channels by negative (first two current activations) and positive-pressure (last current activation) applications in cells treated with cytochalasin B (10 μM, 6 hours). The experimental points (mean values from three cells) were fit to a theoretical curve described by the Boltzmann distribution, which relates the probability that a channel is in open or closed state to the energy available to the channel. The opening probability was calculated by integrating the current through all channels divided by the total current that would pass through all the channels if they were fully open. Data points obtained from a patch were fit by linear regression to a modified Boltzmann distribution given by Pₒ=exp[(p−pₒ]/s)]/[1+exp[(p−pₒ)/s]], where Pₒ is the opening probability, p is the negative pressure, pₒ is the pressure at which Pₒ=0.5, and s is the slope of the plot of ln[Pₒ/(1−Pₒ)] versus pressure (pₒ=1.5 mm Hg, s=0.5 mm Hg). In cells treated with 10 μM cytochalasin B for 6 hours to disrupt microfilaments, the pressure-dependent activation of channel currents was obtained similarly and was fit to the logarithmic plot (pₒ=0.8 mm Hg, s=0.4 mm Hg). Nearly maximal activation was obtained at −3.5 mm Hg, with the inflection point at −1.5 mm Hg in control cells. Similar results were obtained in 12 other patches. This sensitivity to pressure is 10-fold greater than those of other stretch-activated channels that open half maximally at 10–20 mm Hg pressure in patches obtained using similar pipette resistances.¹⁻¹⁹ Disruption of the microfilaments with cytochalasin B resulted in approximately a twofold increase in sensitivity to pressure, suggesting that the cytoskeletal elements give structural support to the membrane and reduce membrane deformation. Such an increased sensitivity to pressure after treatment with cytochalasin B has been reported previously in chick skeletal muscle cells.⁸

**Activation of Single-Channel Currents by Hyposmotic Swelling**

The high sensitivity of the atrial channel to pressure changes in the pipette suggests that these channels may also be activated by the osmotic pressure generated by cell swelling. This was tested by placing the cells in an hypotonic medium (216 mosm) to cause cell swelling. The perfusion solution was switched from a control perfusion solution (296 mosm) containing (mM) KCl 100, sucrose 80, MgCl₂, and HEPES 10 (pH 7.2) to the same solution without sucrose (216 mosm). Cell swelling was clearly visible when cells were viewed under the microscope. Cells were placed in the hypotonic solution for 3 minutes until visible swelling occurred, and then cell-attached patches were formed with pipettes containing 140 mM K⁺. Cell-attached patches from these osmotically stressed cells showed spontaneous opening of channels (Figure 4), whose activity could be further increased by applying negative pressure to the pipette. When the potassium in the pipette was replaced with choline, outward currents were clearly present, but no inward currents were observed in cell-attached patches (n=12). The similar single-channel conductance (22.3±3.5 pS in symmetrical 140 mM K⁺, n=3) measured in inside-out patches, poor discrimination among alkali cations, and exclusion of Cl⁻ all indicated that the atrial channels activated by suction and swelling were
Figure 4. Activation of the mechanosensitive channel by hypotonic swelling. Current recordings from a cell-attached patch are shown. Currents as shown were filtered at 15 Hz.

Panel A: The pipette potential was held at −90 mV to observe the outward current. Panel B: The pipette potential was held at +50 mV. Cells were placed in an hypotonic medium (216 mosm) containing (mM) KCl 100, MgCl₂ 2, and HEPES 10 (pH 7.2) or in a control medium (296 mosm) containing (mM) KCl 100, sucrose 80, MgCl₂ 2, and HEPES 10 (pH 7.2). After ≈3–5 minutes, cell-attached patches were formed. Spontaneous opening was observed in six of 16 patches of osmotically stressed cells. In these six patches, negative pressure in the pipette caused further increases in channel activity. In 10 patches that did not show channel opening, negative pressure failed to elicit any channel current. No channel opening was observed in control cells. Reversal potential was −9.3±1.6 mV as expected for a K⁺-selective channel (E_K = 58 log[K_o/K_i] = −8.5 mV). No inward current was observed when K⁺ in the pipette was replaced by choline.

The same population of nonselective cation channels. Interestingly, the opening of one or two channels in a patch containing at least six channels could be observed in hypotonically swollen cells when the current was in the inward direction, in contrast to the simultaneous opening of several channels produced by negative or positive pressure applied to the pipette. In cells that were perfused with the hypotonic solution for ≈3 minutes and then perfused with the control solution for ≈3 minutes, no cation-selective ion channels were observed in all 22 cell-attached patches. These results show clearly that the tension generated by osmotic swelling also activates the nonselective cation channel in the atrial cell membrane.

Clustered Distribution of the Mechanosensitive Ion Channel

The mechanosensitive ion channels were observed in ≈20% of the patches with pipettes of 4–5-MΩ tip resistance (45 of 230 patches). Six to nine channels were present in each successful patch, indicating that the channels were clustered. Because patches with less than six channels were rare even with pipettes with 5.0–5.5-MΩ tip resistance (two of 92 patches), the channels in a cluster probably lie within an area much smaller than 1 μm²; if it is assumed that a pipette with the tip resistance of 5 MΩ covers a membrane area of 1 μm² for a thin-walled hard glass pipette. With pipettes having larger tip diameters (<1.5 MΩ), the mechanosensitive channels were observed in 90% of the patches (18 of 20 patches). Thus, clusters of six to nine channels appear to be scattered over the atrial cell membrane. For a typical atrial cell used in this study, the whole-cell capacitance ranged from 16 to 28 pF. For an average cell (22 pF), there are approximately 2,000 channels per cell, assuming 1 μF/cm². This is about one fifth of the number of muscarinic potassium channels that are distributed relatively uniformly in these atrial cells.

Discussion

We have identified a novel nonselective cation channel that is activated by applying low pressure (0–3 mm Hg) to the pipette both at the whole-cell and single-channel levels. The single-channel current activation is thus unlikely to be an artifact of single-channel recording. Our results show that the mechanosensitive channel has kinetic properties and a mechanosensitivity that are clearly different from those of other previously identified cardiac ion channels that have been reported to be activated by changes in tension. The mechanosensitive channel is also different from the cardiac nonselective cation channel that is activated by an increase in intracellular Ca²⁺. In our studies, an increase of [Ca²⁺], up to 10 μM failed to activate the mechanosensitive channel. The high sensitivity of the mechanosensitive channel to applied pressure and the permeability of the channel to Ca²⁺ suggest that this atrial channel may be the membrane sensor that transduces the stretch signal into biochemical processes leading to such events as the release of atrial natriuretic hormone, proto-oncogene expression, and hypertrophy. These cellular events are well known to be elicited by distension of the atrial muscle produced by an increased blood pressure or by artificial stretching of the muscle.

The mechanosensitive channel discovered in this study has an interesting opening behavior. For the outward current, the open probability was closely related to the level of the applied pressure. However, for the inward current, a certain level of threshold pressure was required to open the channels. Furthermore, several channels (usually three to five) opened simultaneously when the pipette pressure reached the threshold level (≈1 mm Hg). Because the channels appear to be clustered, it was initially thought that this may be due to the opening of ion channels that are in a cluster and that these channels are simultaneously affected by pressure-induced membrane deformation. However, this is not in keeping with the finding that the outward current is increased in a graded manner in response to pressure. Nevertheless, it is possible that the applied pressure affects the inner and outer leaflets of the membrane lipids differently at different membrane potentials. The presence of a threshold pressure for activation of the channel may involve elastic structures that are connected to the channel protein or structures within the pipette tip that prevent uniform deformation of the membrane under the pressure gradient. Although a satisfactory explanation for such kinetic behavior is not apparent, the clustered distribution of the ion channels may have an important physiological role; e.g., the simultaneous opening of several channels is likely to produce a large change in Ca²⁺ or Na⁺ influx, causing a rapid change in the respective intracellular ion concentrations. A rapid increase in Ca²⁺ entry may induce
intracellular signaling processes that are different from those induced by a slow increase. It has been shown recently that different patterns of intracellular Ca\textsuperscript{2+} mobilization occur in response to activation of different receptors in *Xenopus* oocytes.\textsuperscript{38} Another interesting characteristic of the atrial mechanosensitive channel is the presence of open channel "noise" when the current is in the inward direction. Although the stochastic behavior of channel openings could be readily identified for the outward current, the same was not true for the inward current, which showed extreme current fluctuations during the open state. When many channels were open, the number of open channels was difficult to determine because of the channel noise. However, a careful examination of the current recordings at an expanded time scale showed distinct openings and closings of channels when several channels were activated above the threshold pressure level. The current–voltage relation was obtained by using current amplitude measurements from such current recordings. From the single-channel amplitude, the number of channels that opened simultaneously during a typical response to increased pressure could be determined.

The atrial channels did not exhibit adaptation (or desensitization) to a given pressure level. The lack of desensitization suggests that, as long as the membrane is stretched, influx of Ca\textsuperscript{2+} will continue to occur and modulate important signaling mechanisms in the cell, such as the release of atrial natriuretic peptide. The novel atrial channel found here is activated at relatively low pressure levels (p_{o}=1.5 \text{ mm Hg}). This sensitivity to pressure of the atrial channel can be compared with those of other mechanosensitive ion channels that open with a half-maximal activation pressure between 10 and 20 mm Hg.\textsuperscript{1,19} The high sensitivity of the atrial channel to pressure suggests that the channels could be involved in beat-to-beat regulation of the atrial function, because the atrial chamber pressure normally fluctuates between near zero and \approx 12 \text{ mm Hg}.\textsuperscript{39} If the channels behave similarly in vivo, one would expect that they would alternate between completely closed and completely open states during each cardiac cycle. The channel is permeable to Ca\textsuperscript{2+}; thus, the entry of this ion during atrial filling may help to strengthen the subsequent contraction. Increased Na\textsuperscript{+} influx would also help to augment [Ca\textsuperscript{2+}] via Na–Ca\textsuperscript{2+} exchange. Recent studies have shown that stretch stimulates proto-oncogene expression\textsuperscript{37} and augments the secretion of atrial natriuretic peptide.\textsuperscript{20,23,40} Probably involving influx of Ca\textsuperscript{2+}, the mechanosensitive atrial channel described here seems to be the most likely candidate for mediating these effects.

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