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Characterization of the Stretch-activated Chloride Channel in Isolated Human Atrial Myocytes

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Abstract: Macroscopic and unitary currents through stretch-activated Cl^- channels were examined in isolated human atrial myocytes using whole-cell, excised outside-out and inside-out configurations of the patch-clamp technique. When K^+ and Ca^{2+} conductances were blocked and the intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) was reduced, application of positive pressure via the pipette activated membrane currents under whole-cell voltage-clamp conditions. The reversal potential of the current shifted by 60 mV per 10-fold change in the external Cl^- concentration, indicating that the current was Cl^- selective. The current was inhibited by bath application of 4,4′-diisothiocyanatostilbene-2,2′-disulfonic acid (DIDS) and 9-anthracenecarboxylic acid (9-AC). β-Adrenergic stimulation failed to activate a Cl^- current. In single channel recordings from outside-out patches, positive pressure in the pipette activated the unitary current with half-maximal activation of 14.7 mm Hg at +40 mV. The current-voltage relationship of single channel activity obtained in inside-out patches was linear in symmetrical Cl^- solution with the averaged slope conductance of 8.6 ± 0.7 pS (mean ± SD, $n = 10$). The reversal potential shift of the channel by changing Cl^- concentration was consistent with a Cl^- selective channel. The open time distribution was best described by a single exponential function with mean open lifetime of 80.4 ± 9.6 msec ($n = 9$), while at least two exponentials were required to fit the closed time distributions with a time constant for the fast component of 11.5 ± 2.2 msec ($n = 9$) and that for the slow component of 170.2 ± 21.8 msec ($n = 9$). Major changes in the single channel activity in response to pressure were caused by changes in the interburst interval. Single channel activity was inhibited by DIDS and 9-AC in a manner similar to whole-cell configuration. These results suggest that membrane stretch induced by applying pressure via the pipette activated a Cl^- current in human atrial myocytes. The current was sensitive to Cl^- channel blockers and exhibited membrane voltage-independent bursting opening without sensitive to β-adrenergic stimulation.

Key words: Patch-clamp technique — Stretch-activated Cl^- channel — Human atrial myocytes

Introduction

In a variety of cell types, electrical response can be caused by cell membrane stretch. The membrane stretch-activated ion channels have been described in erythrocytes (Hamill, 1983), skeletal muscle (Guharay & Sachs, 1984), lens epithelia (Cooper et al., 1986), vascular endothelium cells (Lansman, Hallam & Rink, 1987), *E. coli* (Martinac et al., 1987), choroid plexus epithelium (Christensen, 1978), renal cells (Sackin, 1987), smooth muscle cells (Kirber, Walsh & Singer, 1988), liver cells (Bear, 1990) and fungus (Zhou et al., 1991). In cardiac preparations, mechanical stretch-activated K^+ channels have been documented in Lymnaea stagnalis heart (Brezden, Gardner & Morris, 1985), rat atrium (Kim, 1992) and molluscan ventricle (Sigurdson et al., 1987). Cell swelling activated Cl^- channels have been described in rabbit atrial myocytes (Hagiwara et al., 1992; Duan, Fermini & Nattel, 1995), canine cardiac cells (Tseng, 1992; Sorota, 1992), cultured chick heart cells (Zhang et al., 1993).

Recently, the patch-clamp technique has been applied to examine membrane ion conductances in isolated

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human heart cells and electrophysiological characteristics have been reported in this and other laboratories (Heidbuchel, Vereecke & Carmeliet, 1990; Beuckelmann, Nabauer & Erdmann, 1993; Koumi et al., 1994; Koumi, Backer & Arentzen, 1995*a;* Koumi et al., 1995*b*). In addition, whole-cell swelling-induced Cl^- currents were examined in human atrial and ventricular myocytes (Oz & Sorota, 1995; Sakai et al., 1995). These two reports have demonstrated that cell swelling induced by perfusing hypotonic solution caused activation of Cl^- conductances. In contrast to these detailed studies on human heart Cl^- currents, Cl^- channel kinetics evoked by direct membrane stretch has not yet been fully analyzed. In the present study, we have characterized the membrane stretch-activated Cl^- channels in human atrial myocytes using whole-cell and single channel recording techniques.

Materials and Methods

Human Cardiac Specimens

Adult human atrial specimens were obtained from patients undergoing cardiac surgery. Institutional and National Institutes of Health guidelines for human experimentation were followed in obtaining surgical specimens and informed consent was obtained from all subjects. A total of 24 patients were studied (31–67 years; median age 52 years). Eighteen patients were male and six female. Twenty-one patients underwent surgery for ischemic heart disease and three for valvular heart disease. All patients did not have symptomatic heart failure without elevation of mean right atrial pressure (4.1 ± 1.2 mm Hg, $n = 24$, all patients < 5 mm Hg) and without atrial enlargement as diagnosed by ultrasound cardiography. All patients were in sinus rhythm. All specimens were obtained from the right atrial appendage. The administration of cardiac drugs was stopped 48 hr before surgery. Immediately after surgical explantation of patient or donor heart, the specimen was placed in a chilled transport solution and carried to the laboratory within one hour.

Isolation of Atrial Myocytes

Human atrial myocytes were isolated by an enzymatic dissociation method identical to that described previously (Koumi et al., 1995*a,b*). Specimens were minced into small pieces using a fine razor and washed three times, 7 min each time, in oxygenated Ca^{2+}-free Tyrode's solution. The tissues were then incubated in oxygenated Ca^{2+}-free Tyrode's solution containing 300–350 U/ml collagenase (Sigma, Type V, St. Louis, MO), 0.5 U/ml protease (Sigma, Type XXIV) and 1 mg/ml of bovine serum albumin (Sigma) at 37°C, and gently stirred with a magnetic stirring bar until isolated myocytes appeared (~40 min). The tissue was then strained through a 200 μm nylon mesh to collect the individual myocytes. Myocytes were stored at room temperature in a modified Kraftbruhe (KB) medium (Isenberg & Klockner, 1982). The residual nondigested tissue was reincubated in enzyme-containing solution for an additional 10 min and isolated myocytes collected in the similar manner as above. This process was repeated until viable myocytes could no longer be isolated. Isolated cells were stored in KB solution at room temperature. Only Ca^{2+}-tolerant, clearly-striated, rod-shaped cells without any blebs were studied.

Solutions and Chemicals

The transport solution for human specimens contained (in mM): NaCl 27, KCl 20, $MgCl_2$ 1.5, N-2-hydroxyethylpiperazine-N′-2-ethanesulfonic acid (HEPES) 5.0, glucose 274 (pH = 7.0). The Tyrode's solution contained (in mM): NaCl 140.0, KCl 5.4, $CaCl_2$ 1.8, $MgCl_2$ 0.5, HEPES 5.0, glucose 5.0 (pH = 7.4 with NaOH). Ca^{2+}-free Tyrode's solution was made by omitting $CaCl_2$ from the normal Tyrode's solution. The modified KB solution had the following composition (in mM): KCl 25, KH_2PO_4 10, KOH 116, glutamic acid 80, taurine 10, oxalic acid 14, HEPES 10, glucose 11 (pH = 7.0 with KOH). The external solution used during whole-cell recordings contained (in mM): NaCl 140, $CaCl_2$ 1.8, $MgCl_2$ 2.0, glucose 10, HEPES 5.0 (pH = 7.4). The external solution used during different Cl^- concentrations ($[Cl^-]$) measurements contained (in mM): at $[Cl^-] = 150$ mM; NaCl 142.4, $CaCl_2$ 1.8, $MgCl_2$ 2.0, glucose 10, HEPES 5.0 (pH = 7.4). Low $[Cl^-]$ solutions were prepared by replacing NaCl with Na-glutamate, for example at $[Cl^-] = 75$ mM, NaCl was replaced with NaCl 67.4 and Na-glutamate 75 mM in order to minimize the osmotic change. The external solution used during the outside-out patch recordings contained (in mM): Na-glutamate 110, CsCl 30, $CaSO_4$ 1.8, $MgSO_4$ 2.0, glucose 10, HEPES 5.0 (pH = 7.4). The composition of internal solution used for the whole-cell and outside-out recordings was (in mM): Cs-aspartate 100, tetraethylammonium (TEA)-aspartate 40, $MgSO_4$ 1.0, TEA-Cl 30, Na_2-ATP 5.0, ethyleneglycol-bis(β-aminoethylether)-N,N′-tetraacetic acid (EGTA) 5.0, HEPES 5.0, pH 7.2 with TEA-OH. In excised inside-out patch recordings, pipette solution contained (in mM): Cs-aspartate 120, CsCl 30, HEPES 5.0 (pH = 7.4). The internal solution for outside-out patch recordings was used as the bath solution (cytosolic side). Experiments with different Cl^- concentrations (pipette $[Cl^-]$) were performed by replacing Cs-aspartate with CsCl in the pipette, for example at $[Cl^-] = 75$ mM, Cs-aspartate 75 mM and CsCl 75 mM (pH = 7.4). The recording chamber (0.18 ml) was continuously perfused with bath solution at the perfusion rate of 5 ml/min.

Nifedipine, 4,4′-diisothiocyanatostilbene-2,2′-disulfonic acid (DIDS) and 9-anthracenecarboxylic acid (9-AC) were applied to the external solution. The synthetic peptide inhibitor of cAMP-dependent protein kinase (PKA), 5-24 amide, was prepared as described previously (Cheng et al., 1986) and added to the pipette solution during whole-cell recordings. All chemicals were purchased from Sigma Chemical, St. Louis, MO.

Electrical Recordings

Membrane currents were recorded using the patch-clamp technique (Hamill et al., 1981; Koumi & Wasserstrom, 1994) and an Axopatch-1C amplifier (Axon Instruments, Foster City, CA). The pipettes were pulled in two stages from hard glass tubing (Narishige Scientific Instruments Laboratories, Tokyo, Japan) using a vertical microelectrode puller (Type PE-2, Narishige Scientific Instruments Laboratories, Tokyo, Japan) and then fire-polished using a microforge (Model MF-83, Narishige Scientific Instruments Laboratories, Tokyo, Japan). Electrodes had resistances of 1.5–2.5 MΩ for whole-cell recording and 2.5–3.5 MΩ for single-channel recording when filled with appropriate standard internal solutions. Inverted voltage-clamp pulses were applied to the bath through an Ag-AgCl pellet-KCl agar bridge with the pipette potential maintained at ground level. The feedback resistance of the head stage was 500 MΩ for recording whole-cell current and 50 GΩ for recording single channel current. The pipette holder tubing was connected to a mercury manometer with valving to allow either application of positive, negative pressure or equilibration to atmospheric pressure.

Single-channel currents were monitored with a storage oscilloscope (Type 5113, Tektronix, Beaverton, OR) and were stored continuously on digital audiotape (R-60DM, MAXELL, Tokyo, Japan) using a PCM data recording system (RD-100T, TEAC, Tokyo, Japan). The recorded single channel events were reproduced and filtered off-line with a cutoff frequency of 2 kHz through an eight-pole low-pass Bessel filter (48 dB/octave, Model 902-LPF, Frequency Devices, Haverhill, MA), digitized with 14-bit resolution at a sample rate of 10 kHz. The data were analyzed on a computer (PC-9801, NEC, Tokyo, Japan) using locally written analysis programs that are based on the half-amplitude threshold analysis method of Colquhoun and Sigworth (1983). The measurements derived from the channel transitions were collected into histograms to allow an analysis of single channel kinetics. Mean dwell times were determined from the sum of exponential fits to the distributions of open and closed times recorded from patches with only one channel. When sufficiently long (~20 min) continuous recording was achieved, active number of channels (N) in the patch can be predicted by using the equation (Colquhoun & Hawkes, 1983):

$$P \approx \exp[\{-T(N-1/N)m_o\}/m_s^2] \quad (1)$$

where T is the length of the observed record, m_o is the mean open lifetime, and m_s is the mean closed time. It is considered that the patch has only one functional channel when the predicted probability achieves 99.9%. When multiple channel activities are observed in a patch (more than two channels open simultaneously), ''an averaging technique'' was used to obtain the channel open probability using the equation:

$$I = NP_{o^i} \quad (2)$$

where I is the averaged total channel current amplitude, N is the number of channels, P_o is the channel open state probability and i is the unitary amplitude of the single channel current. The unitary current amplitude (i) can be obtained by directly measuring the individual channel current magnitude. The average total channel current amplitude (I) can be obtained by calculating the average level of channel open events. When sufficiently long recording was achieved, P_o can be obtained from Eq. 2. The temperature of the bath was maintained at 37 ± 0.5°C using a Peltier thermo-electrical device.

Data Analysis

All curve fitting was performed with a nonlinear least-square algorithm on a computer (PC-9801, NEC, Tokyo, Japan) using custom software. The results are expressed as mean ± SD. Differences between sample means were determined using Student's t test or one-way analysis of variance. A P value <0.05 was considered statistically significant.

Results

Whole-Cell Cl^- Currents

To characterize the macroscopic current induced by membrane stretch in human atrial myocytes, positive pressure (5.0–20.0 mm Hg) was applied to the cell interior via the patch pipette. Figure 1*A* shows examples of whole-cell membrane current families obtained before and after application of positive pressure (20 mm Hg) recorded from an isolated human atrial myocyte at 37°C.

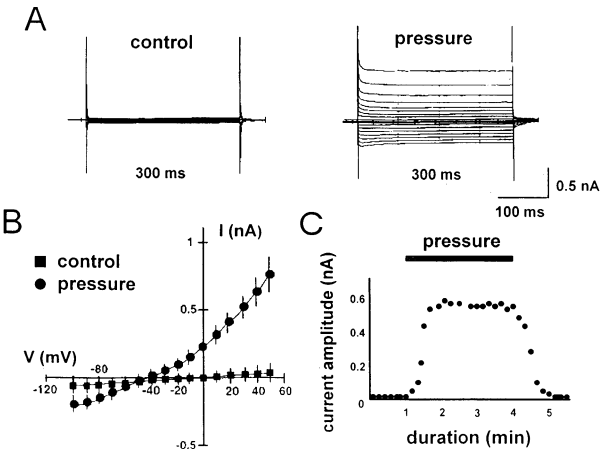

Fig. 1. The stretch-activated whole-cell current in isolated human atrial myocytes. (*A*) Representative families of whole-cell membrane currents recorded in control condition (left Panel) and during application of 20.0 mm Hg positive pressure via the pipette (right Panel) in K^+-free solution at 37°C. The voltage-clamp pulse protocol used to obtain the recordings; 300 msec duration pulses to test potentials ranging from −100 to +50 mV in 10-mV increments were applied from a holding potential of −40 mV. Current and time calibration are shown in the lower corner. (*B*) The averaged current-voltage (*I-V*) relationships in the control condition (squares) and during application of positive pressure (circles). Each point was measured at the end of the test pulse. The vertical bars through each point represent the SD ($n = 12$ for each). (*C*) Time course of pressure-induced change in the whole-cell current. Time course of changes in membrane current elicited by a 300-msec voltage-clamp pulse to +40 mV from a holding potential of −40 mV. Positive pressure (20 mm Hg) was applied for 3 min via the pipette.

Three hundred msec duration voltage-clamp steps were delivered ranging between −100 and +50 mV from a holding potential of −40 mV. K^+ conductances were blocked by replacement of pipette K^+ with Cs^+, adding TEA to the internal solution and omitting K^+ from external solution. Nifedipine (5 μM) was added to the external solution in order to block the Ca^{2+} current. Figure 1*B* shows averaged current-voltage (*I-V*) relationships from 12 different cells before and during application of positive pressure. The slope conductance measured at 0 mV was 0.8 ± 1.4 nS ($n = 12$) in the control and 9.0 ± 2.1 nS ($n = 12$) during application of pressure. The reversal potential of the pressure-induced current was ~−40 mV, which is compatible for a Cl^--selective channel with external Cl^- concentration ($[Cl^-]_o$) of 147.6 mM (internal Cl^- concentration $[Cl^-]_i = 30$ mM). This type of current was observed in 105 out of 129 cells (81.4%). Application of negative pressure caused no significant event ($n = 10$). Figure 1*C* illustrates the time course of the response to positive pressure as well as the reversibility of the reaction to pressure. The current turned on with a sigmoidal shape and saturated within 1 min following application of positive pressure. Release of pressure deactivated the current.

The ionic selectivity of this current was estimated by

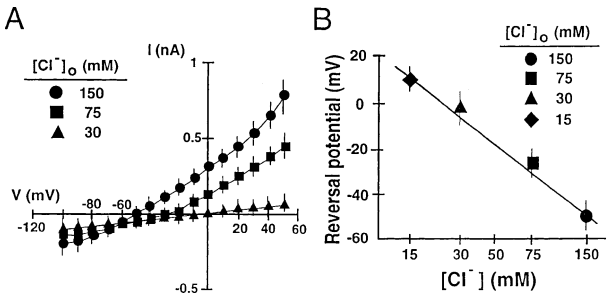

Fig. 2. Reversal potential measurements for the current. *A* The averaged current-voltage (*I-V*) relationships in different $[Cl^-]$ external solutions. $[Cl^-]$ in the pipette solution was 30 mM. After obtaining the *I-V* curve in 150 mM $[Cl^-]$ external solution (circles), it was switched to 75 mM $[Cl^-]$ (squares) and 30 mM $[Cl^-]$ (triangles). The vertical bars through each point represents the SD ($n = 8$ for each). (*B*) The reversal potentials were plotted semilogarithmically as a function of the extracellular Cl^- concentrations ($[Cl^-]_o$). $[Cl^-]_o$ was changed to 150 (circle), 75 (square), 30 (triangle) and 15 mM (diamond). The vertical bar through each point represents the SD ($n = 8$ for each).

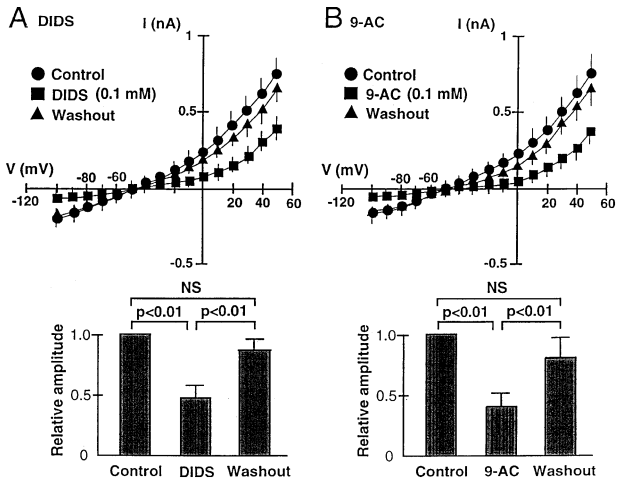

Fig. 3. Pharmacological modification of the whole-cell current. Effects of bath application of 4,4′-diisothiocyanatostilbene-2,2′-disulfonic acid (DIDS) and 9-anthracenecarboxylic acid (9-AC) on the current. (*A*) (Top panel) Effect of bath application of DIDS (0.1 mM) on the current. Following the measurement of the current in the absence of DIDS, DIDS (0.1 mM) was applied to the external solution. The averaged *I-V* relationships of the current in control (circles), during exposure to (squares) and after washout of DIDS (triangles) are shown. The vertical bar through each point represents the *SD* ($n = 6$). (Bottom panel) Relative amplitude of currents during exposure to and after washout of DIDS (0.1 mM) measured at +40 mV and normalized by the control in six different cells. The vertical bar through each bar represents the SD. (*B*) (Top panel): Effect of bath application of 9-AC (0.1 mM) on the current. After the measurement of the current, 9-AC (0.1 mM) was applied to the external solution. The averaged *I-V* relationships of the current in control (circles), during exposure to (squares) and after washout of 9-AC (triangles). The vertical bar through each point represents the SD ($n = 6$). (Bottom panel) Relative amplitude of currents during exposure to and after washout of DIDS (0.1 mM) measured at +40 mV and normalized by the control in six different cells. The current amplitude between the control and washout was not significantly different (NS).

changing $[Cl^-]_o$. $[Cl^-]$ in the pipette solution was 30 mM. Figure 2*A* shows the averaged *I-V* relationships in different $[Cl^-]_o$. The current was activated by application of positive pressure of 20.0 mm Hg. Decreasing $[Cl^-]_o$ reduced the conductance and shifted the reversal potential to less negative membrane potentials. The averaged reversal potential at $[Cl^-]_o = 150$ mM was -46.0 ± 4.4 mV ($n = 9$). The reversal potential was shifted to -23.9 ± 2.5 mV ($n = 8$) and -0.1 ± 2.2 mV ($n = 8$) when $[Cl^-]_o$ was decreased to 75 and 30 mM, respectively. The averaged reversal potential at $[Cl^-]_o = 15$ mM was 9.8 ± 2.9 mV ($n = 8$). The averaged reversal potential was plotted in the logarithm of the $[Cl^-]_o$ in Fig. 2*B*. The best fit to data points (mean ± SD) was obtained with a line having a slope of 60 mV per 10-fold change in $[Cl^-]_o$. These results indicate that this positive pressure-induced membrane current is highly selective for Cl^- ions.

In contrast to positive pressure, β-adrenergic stimulation with isoprenaline (1 μM) did not activate the current even if GTP (100 μM) was included in the pipette solution ($n = 12$). In addition, neither bath application of forskolin (10 μM) nor internal application (to the pipette solution) of cAMP (10 μM) activated the current ($n = 8$ for each, *not shown*). Loading the myocytes with selective cAMP-dependent protein kinase (PKA) inhibitor, 5–24 amide (50 μM), did not prevent activation of the current by positive pressure ($n = 8$). After activation of the channel by positive pressure, isoprenaline could not enhance the amplitude of the current. These results suggest that the PKA cannot activate the Cl^- current and β-adrenergic stimulation-induced Cl^- current is not present in human atrial myocytes.

Pharmacological Modification of the Current

DIDS, known to inhibit the Cl^- channel, was applied to the bath solution to test the effect on this current (Fig. 3*A*). Initially, application of positive pressure (20.0 mm Hg) activated the current (control). DIDS (0.1 mM) reversibly inhibited the positive pressure-activated current. The averaged current amplitude at +40 mV was inhibited to $47.1 \pm 5.6\%$ of control ($n = 6$, $P < 0.001$) during exposure to DIDS and recovered to nearly the control level after washout of DIDS. The averaged *I-V* relationships before, during exposure to and after washout of DIDS are shown in Fig. 3*A*. Inhibition was observed at all voltages tested. Inhibition was significant in outward currents than inward currents, although it did not reach statistical significance. Effect of 9-anthracenecarboxylic acid (9-AC) on the Cl^- current was also tested. Figure

3*B* shows an example of the effect of externally applied 9-AC (0.1 mM) on the Cl^- current. Following exposure to 9-AC, the current was reduced. The current at +40 mV was reduced to 43.9 ± 5.0% (n = 6) of control during application of 9-AC. Following washout of 9-AC, the current magnitude was recovered to nearly the control level.

Single Cl^- Channel Current in Outside-out Patches

To gain further insight into the channel gating characteristics and modification of the channel by pressure, we studied the characteristics of single Cl^- channels in the outside-out patch recording. Figure 4*A* illustrates examples of individual channel activity recorded at a holding potential (HP) at +40 mV at 37°C. Before applying positive pressure (0 mm Hg), no channel activity was observed. By applying positive pressure via the pipette (>4.5 mm Hg), unitary channel activity was observed. Channel openings occurred in bursts separated by variable interburst intervals. Individual burst duration increased and interburst period decreased with increasing pressure (4.5–20.0 mm Hg); Channel open state probability (P_o) increased from 0.03 ± 0.02 (n = 7) at 4.5 mm Hg to 0.94 ± 0.08 (n = 6) at 20.0 mm Hg (P < 0.001). After release of pressure, the channel closed. Figure 4*B* illustrates the normalized P_o of the channel plotted as a function of positive pressure. Data points were fitted by a least-square routine to a modified Boltzmann distribution:

$$P_o = [\exp\{(p - p_{1/2})/k\}]/[1 + \exp\{(p - p_{1/2})/k\}] \quad (3)$$

where p is the positive pressure in the pipette, $p_{1/2}$ is the pressure at half-maximum channel activation and k is the slope factor. The half-maximum channel activation ($p_{1/2}$) was at 14.7 mm Hg. The channel had same activation range as macroscopic currents. In addition, consistent with the results obtained in whole-cell measurement, bath application of isoprenaline (1 μM), forskolin (10 μM) and dibutyryl cAMP (5 mM) could not induce channel openings (n = 4 for each, not shown) in outside-out patches. Similar channel activation was present in the inside-out patch recording. Application of negative pressure to the pipette caused channel opening in a manner similar to positive pressure-induced channel opening in outside-out patches. In inside-out patches, bath application (cytosolic side of membrane) of catalytic subunit of PKA (1 μM) also failed to activate the channel (10 out of 10 cells).

Characteristics of the Cl^- Channel in Inside-out Patches

Figure 5*A* illustrates examples of individual channel activity induced by applying 20.0 mm Hg negative pressure

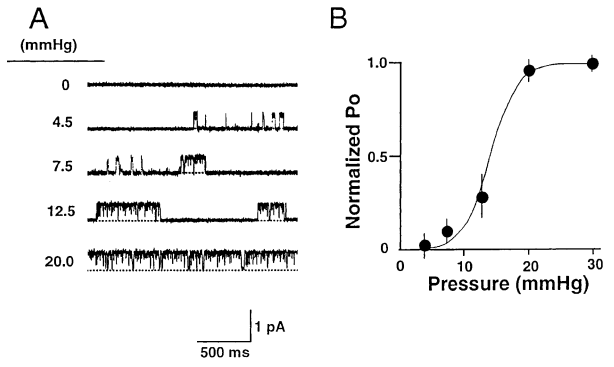

Fig. 4. Pressure-dependent nature of the unitary Cl^- current measured in outside-out patch mode single channel recordings. (*A*) Original recordings of single channel activity of the current at +40 mV. Pressure in the pipette was changed from atmospheric (0 mm Hg) to 20.0 mm Hg. Pressure is indicated to the left of each current trace. No channel activity was detected at 0 mm Hg. Progressive increase in single-channel open state probability (P_o) with increasing positive pressure in the pipette. The dotted line running in each trace indicates the closed state (baseline level). Currents were low pass filtered at 2 kHz. (*B*) Pressure-dependent change of channel open probability (P_o) of the current. The graph shows the relationship between normalized P_o of the channel and positive pressure (n = 6–8).

in the pipette recorded in excised inside-out membrane patches. The inside-out patch configuration can effectively eliminate cytoplasmic regulation via second messengers. Channel activity was measured at a voltage range between −80 and +80 mV at 37°C. The channel did not exhibit any time-dependent differences in P_o at any potential. The unitary current amplitude was influenced by the membrane voltage. Current traces became flat at around 0 mV, which was the Cl^- equilibrium potential (E_{Cl}). Figure 5*B* shows the *I-V* relationship. The *I-V* curve was linear and the averaged sloped conductance at 0 mV was 8.6 ± 0.7 pS (n = 10). Single channel activity induced by applying 20.0 mm Hg negative pressure in the pipette was measured in different $[Cl^-]_o$ external solutions in excised inside-out patch configurations. In this experimental mode, $[Cl^-]_o$ indicates pipette Cl^- concentration and $[Cl^-]_i$ indicates Cl^- concentration in the bath solution. Pipette Cl^- concentration ($[Cl^-]_o$) was changed under the condition where $[Cl^-]_i$ was fixed at 30 mM. Figure 6 summarizes the result. The averaged reversal potential of the current shifted by 60 mV per 10-fold change in $[Cl^-]_o$. The result suggests that the observed single channel current activated by negative pressure is conducted through Cl^- channels.

Figure 7 shows the distribution of open and closed times of the channel at +40 mV. Open time distribution was best described by a single exponential function with a mean open lifetime of 80.4 ± 9.6 msec (n = 9). At least two exponentials were required to fit the closed time distributions with a time constant for the fast com-

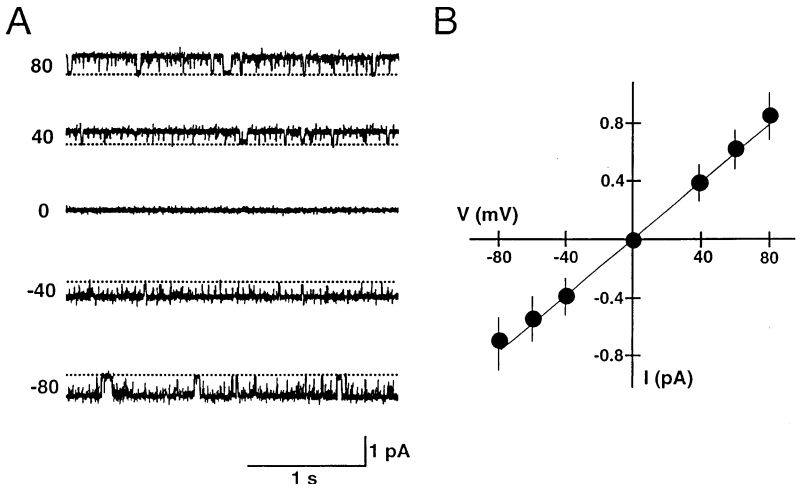

Fig. 5. Voltage-dependence of the unitary Cl^- current measured by inside-out patch single channel recordings. (*A*) Original recordings of single channel activity of the stretch-activated channel in the inside-out patch recording. Negative pressure (20.0 mm Hg) was applied via the pipette. Channel activity exhibited slow bursting kinetics. Currents were low pass filtered at 2 kHz. (*B*) The averaged *I-V* relationships of the stretch-activated current. The vertical bar through each bar represents the SD.

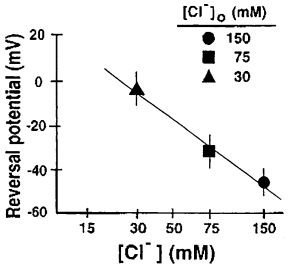

Fig. 6. Reversal potential measurements for single Cl^- channel currents in inside-out patch recordings. The reversal potentials were plotted semilogarithmically as a function of the extracellular Cl^- concentrations ($[Cl^-]_o$) in excised inside-out patch configurations. In the inside-out mode, $[Cl^-]_o$ means pipette $[Cl^-]$. Bath solution ($[Cl^-]_i$) was kept at 30 mM. $[Cl^-]_o$ was changed to 150 (circle), 75 (square) and 30 mM (triangle). The averaged reversal potential at $[Cl^-]_o$ = 150 mM, 75 mM and 30 mM was −45.5 ± 4.0 mV (n = 6), −27.8 ± 4.9 mV (n = 6) and −0.2 ± 3.1 mV (n = 6), respectively. The vertical bar through each point represents the SD (n = 6 for each).

ponent of 11.5 ± 2.2 msec (n = 9) and that for the slow component of 170.2 ± 21.8 msec (n = 9). The time constants for open and closed time distributions were measured as a function of membrane voltage. Table 1 summarizes the result. These time constants measured as a function of membrane voltage did not exhibit significant voltage dependence. Table 2 summarizes the change in gating kinetics of the channel at different stimulating pressure. P_o increased with pressure with decreasing the interburst interval (τcs).

Effects of DIDS and 9-AC on the Cl^- Channel in Outside-Out Patches

Modification of the pressure-induced unitary Cl^- channel current by DIDS was estimated using excised outside-out patch recordings. Figure 8*A* shows an example of the current recording. The channel was activated by applying positive pressure (15.5 mm Hg) in the pipette. Bath applications of DIDS (0.1 mM) inhibited channel activity. P_o was inhibited to 48.6 ± 6.0% (n = 5) of control ($P < 0.01$).

Inhibition of the channel by 9-AC was also evaluated (Fig. 8*B*). Similar to DIDS, 9-AC (0.1 mM) inhibited channel activity evoked by application of positive pressure. P_o was inhibited to 42.8 ± 9.1% (n = 5) of control ($P < 0.01$). Single channel currents were blocked by DIDS or 9-AC with the same potency as the whole-cell currents (*see* Fig. 3).

Discussion

The major findings in this study are as follows: (i) Membrane stretch induced by applying pressure via the pipette could activate a Cl^- current in isolated human atrial myocytes; (ii) The current was inhibited by external application of DIDS and 9-AC; (iii) Single channel open state probability progressively increased with increasing pressure in the pipette and no channel activity was observed in normal atmospheric pressure; (iv) Single channel activity had Cl^- selectivity and sensitivity to DIDS and 9-AC similar to whole-cell currents, indicating that whole-cell and single channel currents are carried by the same channels; (v) Channel activity exhibited bursting opening and the increase in channel activity was caused mainly by decrease in the interburst intervals; and (vi) β-Adrenergic stimulation failed to activate a Cl^- current.

Relation to Previous Studies

The cell membrane stretch-activated ion channels have been demonstrated in the heart (Brezden et al., 1985; Sigurdson et al., 1987; Kim, 1992). These previous studies describe stretch activation of K^+ selective channels.

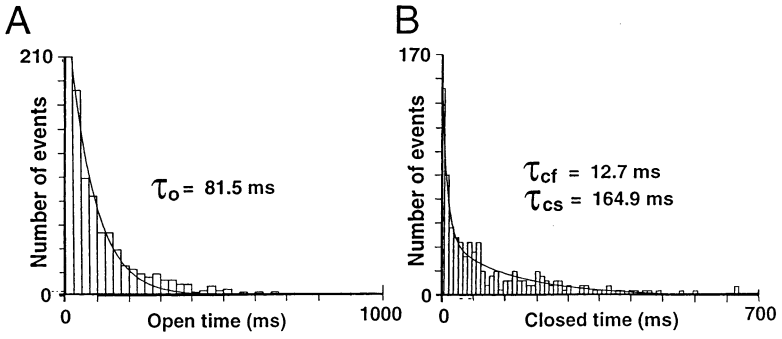

Fig. 7. Dwell time histograms of single Cl^- channel currents in inside-out patch recordings. (*A*) Histogram of open times recorded from a representative single channel activated by 20.0 mm Hg negative pressure at +40 mV. This patch never exhibited more than a single channel during 20 min of continuous recording. Open time distributions were formed from the recording with low-pass filtering at 2 kHz. Open state lifetimes were distributed according to a single exponential function with a time constant (τ_o) of 81.5 msec. (*B*) Closed times histogram determined at the same condition as *Panel A.* At least two exponentials were required to fit the closed time distributions with time constants of 12.7 msec for the fast component (τ_{cf}) and 164.9 msec for the slow component (τ_{cs}).

Table 1. Single channel gating parameters at different holding voltages

HP	τ_o	τ_{cf}	τ_{cs}
−80	75.5 ± 8.1	14.6 ± 3.3	157.1 ± 30.5
−40	79.6 ± 8.6	13.8 ± 2.4	160.9 ± 20.9
+40	80.4 ± 9.6	11.5 ± 2.2	170.2 ± 21.8
+80	79.9 ± 11.5	13.5 ± 2.9	161.7 ± 28.4

All parameters were measured in inside-out patch single channel recordings. HP, holding potential (mV); τ_o, time constant for open time distributions (msec); τ_{cf}, time constant for the fast component of closed time distributions (msec); τ_{cs}, time constant for the slow component of closed time distributions (msec). Values are presented as mean ± SD for mean values of 9 different cells.

Table 2. Single channel gating parameters at different stimulating pressure

P	T_o	T_{cf}	T_{cs}	P_O
12.5	72.8 ± 8.1	9.9 ± 1.7	761.0 ± 93.8[a]	0.40 ± .06[b]
15.0	74.4 ± 8.2	9.7 ± 1.9	498.1 ± 57.0[a]	0.54 ± .06
17.5	79.1 ± 9.0	10.4 ± 1.9	247.5 ± 26.6[a]	0.87 ± .07[c]
20.0	80.4 ± 9.6	11.5 ± 2.2	170.2 ± 21.8[a]	0.95 ± .07

All parameters were measured in inside-out patch single channel recordings at +40 mV. *P*, stimulating pressure (mm Hg); τ_o, time constant for open time distributions (msec); τ_{cf}, time constant for the fast component of closed time distributions (msec); τ_{cs}, time constant for the slow component of closed time distributions (msec); P_o, channel open probability. Values are presented as mean ± SD for mean values of 10 different cells. [a] $P < 0.01$ different from other groups, [b] $P < 0.01$ different from 17.5 and 20.0 mm Hg, [c] $P < 0.01$ different from 20.0 mm Hg.

Cl^- channel characteristics have been initially described in guinea-pig and rabbit ventricular myocytes (Bahinski et al., 1989; Harvey & Hume, 1989) that are similar to the channel observed in the epithelial cystic fibrosis transmembrane-conductance regulator (CFTR). The cardiac variant of CFTR ($CFTR_{cardiac}$) has different kinetic properties from our described channel; the *I-V* curve exhibited outward-rectification in symmetrical Cl^- concentrations; and single channel activity exhibited slow gating kinetics with mean open lifetime was 485 msec (Ehara & Matsuura, 1993). Different from these characteristics, our described stretch-activated Cl^- channel exhibited an almost linear *I-V* relationship. Our described channel exhibited gating kinetics relatively faster than $CFTR_{cardiac}$. Recently, Duan and Nattel (1994) have described single Cl^- channel activity in rabbit atrial cells. The channel exhibited double exponential open time distributions with time constants of approximately 43 msec and 988 msec which is different from our described channel.

The information on the electrophysiological properties of stretch-activated Cl^- channel activity is limited. Recently, swelling-induced Cl^- channels have been characterized in cardiac preparations (Sorota, 1992; Tseng, 1992; Oz & Sorota, 1995; Duan et al., 1995). Our described stretch-activated Cl^- channel is different from these previously documented swelling-induced Cl^- channels in the sensitivity to β-adrenergic stimulation. β-Adrenergic receptor stimulation by catecholamines has been shown to enhance swelling-induced Cl^- channels. α-Adrenergic stimulation also modifies swelling-induced Cl^- channels in rabbit atrial myocytes (Duan et al., 1995). Our described channel was not enhanced by β-adrenergic stimulation. In contrast, there are some similarities between the stretch-activated Cl^- channel in rabbit sino-atrial and atrial cells reported by Hagiwara et al. (1992) and our described channel. The whole-cell stretch-activated Cl^- current in rabbit atrial myocytes is time-independent with slope conductance of 12 nS and insensitive to β-adrenergic stimulation by catecholamines. Swelling-induced Cl^- currents having similar properties have been reported in human atrial myocytes (Sakai et al., 1995). Our described channel may have some overlapping components with the swelling-induced Cl^- currents. Although these reports have not shown

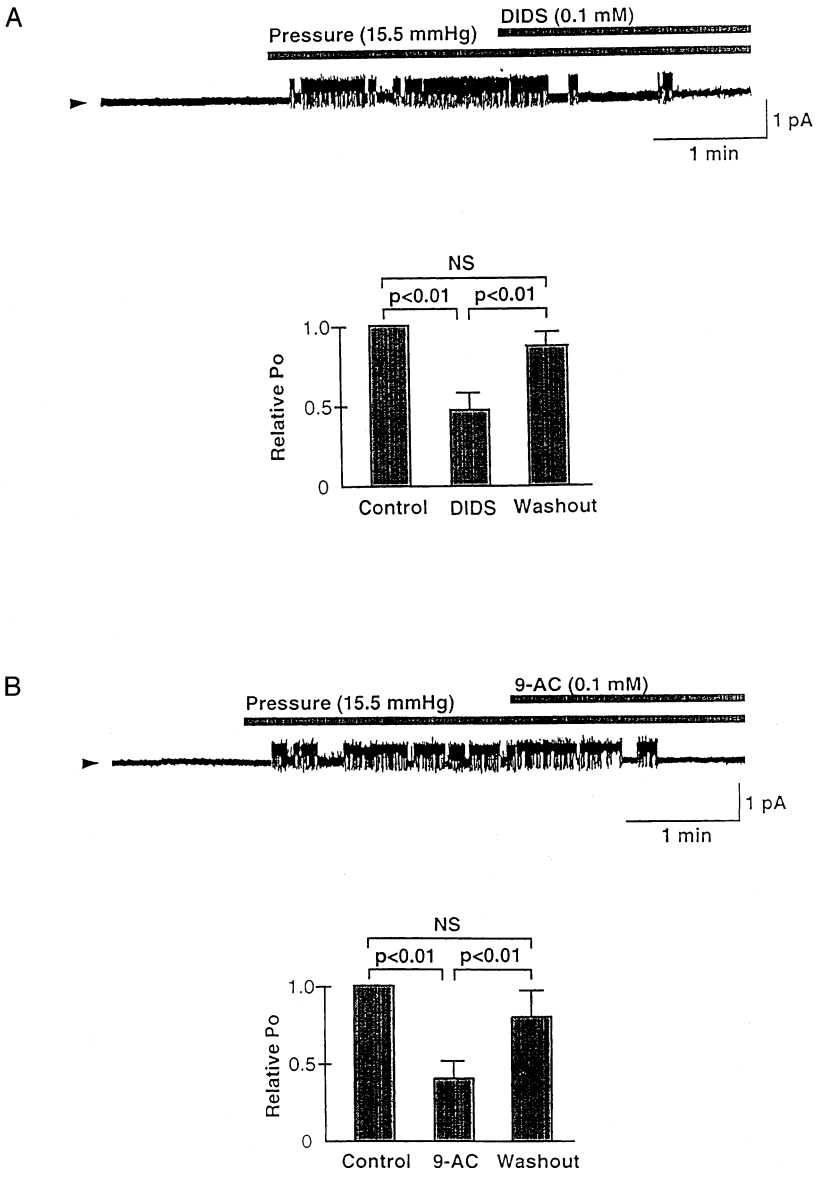

Fig. 8. Effect of DIDS and 9-AC on the stretch-activated Cl^- channel in outside-out patch recordings. (*A*) (*top*): Effect of DIDS (0.1 mM) on the positive pressure-activated Cl^- channel in outside-out patch recordings at 37°C. After application of positive pressure (15.5 mm Hg) via the pipette, channel activity appeared. A subsequent application of DIDS (0.1 mM) to the bath inhibited channel activity. The current was low pass filtered at 2 kHz at +60 mV. (*bottom*): The relative P_o during exposure to DIDS (0.1 mM) was measured in five different cells. P_o was normalized by the control value. The vertical bar through each bar represents the SD. The value during exposure to DIDS was significantly smaller than that in control and after washout of DIDS ($P < 0.01$). In contrast, the value was not significantly different (NS) between the control and after washout of DIDS. (*B*) (*top*): Effect of 9-AC (0.1 mM) on the positive pressure-activated Cl^- channel in outside-out patch recordings at 37°C. After application of positive pressure (15.5 mm Hg) via the pipette, channel activity appeared. A subsequent application of 9-AC (0.1 mM) to the bath inhibited channel activity. The current was low pass filtered at 2 kHz at +60 mV. (*bottom*): The relative P_o during exposure to 9-AC (0.1 mM) was measured in five different cells. P_o was normalized by the control value. The vertical bar through each bar represents the SD. The value during exposure to 9-AC was significantly smaller than that in control and after washout of 9-AC ($P < 0.01$). The value was not significantly different (NS) between the control and after washout of 9-AC.

single channel kinetics, our described stretch-activated Cl^- channel should be compared with these channels in the future.

Characteristics of the Channel

Our described stretch-activated Cl^- channel exhibited slow bursting openings separated by interburst periods. The channel may have the one open-channel state, because single channel open time distributions were best described by a single exponential function. In contrast, the channel may have at least two closed states because of the double exponential fitting of the closed time distributions. The open and closed time constants did not show significant voltage dependence. Although the unitary amplitude of an individual channel and the number of channels in the patch did not change with pressure, P_o was influenced by pressure (*see* Fig. 4). This would contribute to the macroscopic current response to pressure because macroscopic current (I) can be expressed by Eq. 2. In the equation, N and i were almost unchanged by the pressure. Pressure-induced change of the macroscopic current magnitude can be mainly influenced by single channel P_o. In addition, pressure-dependent change of P_o may be caused by the pressure-dependent change of the interburst closed interval (τ_{cs}, *see* Table 2). Because the time constant for open time distribution (τ_o) and the closed time within burst (τ_{cf}) were unchanged with pressure, channel gating kinetics may not be influenced by the pressure.

It has been documented that the stretch-activated

and/or the volume-sensitive ion channels in many cell types are Ca^{2+} dependent (Eckert, 1977; Grinstein, Dupre & Rothstein, 1982). The experimental condition of whole-cell and outside-out patches in the present study can achieve sufficiently low level of $[Ca^{2+}]_i$. Channel activation in the excised inside-out patch condition suggests that cytosolic Ca^{2+} as well as intracellular signaling cascades are not involved in the activation of the channel. The Ca^{2+}-activated Cl^- channel activation occurs at pCa < 8.3 (Zygmunt & Gibbons, 1991).

Physiological and Pathophysiological Implications

Elevation of atrial pressure above normal during atrial and ventricular dysfunction in a pathological state may stimulate the channel. Since the atrium is only a few cell layers thick, atrial cells may be more sensitive to pressure change than ventricular cells. Because intra-atrial pressure elevated to >5 mm Hg is associated with heart failure and other atrial and ventricular dysfunction, such a pathological condition may trigger the channel activation. Cardiac cell swelling can occur during myocardial ischemia and reperfusion (Macknight & Leaf, 1977; Tranum-Jensen et al., 1981). Cell swelling may generate membrane stretch or tension which can cause channel activation. However, the exact role of the channel is yet unknown. Further electrophysiological studies related to an in vivo condition are likely to provide important insights into the physiological and pathophysiological role of the channel.

References

Bahinski, A. Nairn, A.C., Greengard, P., Gadsby, D.C. 1989. Chloride conductance regulated by cyclic AMP-dependent protein kinase in cardiac myocytes. *Nature* **340:**718–721

Bear, C.E. 1990. A nonselective cation channel in rat liver cells is activated by membrane stretch. *Am. J. Physiol.* **253:**C421–C428

Beuckelmann, D.J., Nabauer, M., Erdmann, E. 1993. Alterations of K^+ currents in isolated human ventricular myocytes from patients with terminal heart failure. *Circ. Res.* **73:**379–385

Brezden, B.L., Gardner, D.R., Morris, C.E. 1985. A potassium-selective channel in isolated Lymnaea stagnalis heart muscle cells. *J. Exp. Biol.* **123:**175–189

Cheng, H.-C., Kemp, B.E., Pearson, R.B., Smith, A.J., Misconi, L., Van Patten, S.C., Walsh, D.A. 1986. A potent synthetic peptide inhibitor of the cAMP-dependent protein kinase. *J. Biol. Chem.* **25:**989–992

Christensen, O. 1987. Mediation of cell volume regulation by Ca^{2+} influx through stretch-activated channels. *Nature* **330:**66–68

Colquhoun, D., Hawkes, A.G. 1983. The principles of the stochastic interpretation of ion-channel mechanisms. *In:* Single-Channel Recording. B. Sakmann and E. Neher, editors. pp. 135–175. Plenum Publishing, New York

Colquhoun, D., Sigworth, F.J. 1983. Fitting and statistical analysis of single-channel records. *In:* Single-Channel Recording. B. Sakmann and E. Neher, editors, pp. 191–263. Plenum Publishing, New York

Cooper, K.E., Tang, J.M., Rae, J.L., Eisenberg, R.S. 1986. A cation channel in frog lens epithelia responsive to pressure and calcium. *J. Membrane Biol.* **93:**259–269

Duan, D., Nattel, S. 1994. Properties of single outwardly rectifying Cl^- channels in heart. *Circ. Res.* **75:**789–795

Duan, D., Fermini, B., Nattel, S. 1995. α-Adrenergic control of volume-regulated Cl^- currents in rabbit atrial myocytes. *Circ. Res.* **77:**379–393

Eckert, R. 1977. Bioelectric control of ciliary activity. *Science* **176:**473–481

Ehara, T., Matsuura, H. 1993. Single-channel study of the cyclic AMP-regulated chloride current in guinea-pig ventricular myocytes. *J. Physiol.* **464:**307–320

Grinstein, S., Dupre, A., Rothstein, A. 1982. Volume regulation by human lymphocytes. Role of calcium. *J. Gen. Physiol.* **79:**849–868

Guharay, F., Sachs, F., 1984. Stretch-activated single ion channel currents in tissue culture embryonic chick skeletal muscle. *J. Physiol.* **352:**685–701

Hagiwara, N., Masuda, H., Shoda, M., Irisawa, H. 1992. Stretch-activated anion currents of rabbit cardiac myocytes. *J. Physiol.* **456:**285–302

Hamill, O.P. 1983. Potassium and chloride channels in red blood cells. *In:* Single-Channel Recording. B. Sakmann and E. Neher, editors. pp. 191–263. Plenum Press, New York

Hamill, O.P., Marty, A., Neher, E., Sakmann, B., Sigworth, F.J. 1981. Improved patch-clamp techniques for high-resolution current recording from cells and cell-free membrane patches. *Pfluegers Arch.* **391:**85–100

Harvey, R.D., Hume, J.R. 1989. Autonomic regulation of a chloride current in heart. *Science* **244:**983–985

Heidbuchel, H., Vereecke, J., Carmeliet, E. 1990. Three different potassium channels in human atrium: contribution to the basal potassium conductance. *Circ. Res.* **66:**1277–1286

Isenberg, G., Klockner, U. 1982. Calcium tolerant ventricular myocytes prepared by preincubation in KB medium. *Pfluegers Arch.* **395:**6–18

Kim, D. 1992. A mechanosensitive K^+ channel in heart cells: activation by arachidonic acid. *J. Gen. Physiol.* **100:**1021–1040

Kirber, M.T., Walsh Jr., J.V., Singer, J.J. 1988. Stretch-activated ion channels in smooth muscle: a mechanism for the initiation of stretch-induced contraction. *Pfluegers Arch.* **412:**339–345

Koumi, S-i., Wasserstrom, J.A. 1994. Acetylcholine-sensitive muscarinic K^+ channels in mammalian ventricular myocytes. *Am. J. Physiol.* **266:**H1812–H1821

Koumi, S-i., Backer, C.L., Arentzen, C.E. 1995*a.* Characterization of the inwardly-rectifying K^+ channel in human cardiac myocytes: Alterations in channel behavior in myocytes isolated from patients with idiopathic dilated cardiomyopathy. *Circulation* **92:**164–174

Koumi, S-i., Arentzen, C.E., Backer, C.L., Wasserstrom, J.A. 1994. Alterations in muscarinic K^+ channel response to acetylcholine and to G protein-mediated activation in atrial myocytes isolated from failing human hearts. *Circulation* **90:**2213–2224

Koumi, S-i., Backer, C.L., Arentzen, C.E., Sato, R. 1995*b.* β-Adrenergic modulation of the inwardly-rectifying potassium channel in isolated human ventricular myocytes: Alteration in channel response to β-adrenergic stimulation in failing human hearts. *J. Clin. Invest.* **96:**2870–2881

Lansman, J.B., Hallam, T.J., Rink, T.J. 1987. Single stretch-activated ion channels in vascular endothelium cells as mechanotransducers? *Nature* **325:**8111–8113

Macknight, A.D.C., Leaf, A. 1977. Regulation of cellular volume. *Physiol. Rev.* **57:**510–573

Martinac, B., Buechner, M., Delcour, A.H., Adler, J., Kung, C. 1987.

Pressure sensitive ion channels in *E. coli*. *Proc. Natl. Acad. Sci. USA* **84:**2297–2301

Oz, M.C., Sorota, S. 1995. Forskolin stimulates swelling-induced chloride current, not cardiac cystic fibrosis transmembrane-conductance regulator current, in human cardiac myocytes. *Circ. Res.* **76:**1063–1070

Sackin, H. 1987. Stretch-activated potassium channels in renal proximal tubule. *Am. J. Physiol.* **253:**F1253–F1262

Sakai, R., Hagiwara, N., Kasanuki, H., Hosoda, S. 1995. Chloride conductance in human atrial cells. *J. Mol. Cell. Cardiol.* **27:**2403–2408

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

Sorota, S. 1992. Swelling-induced chloride-sensitive current in canine atrial cells revealed by whole-cell patch-clamp method. *Circ. Res.* **70:**679–687

Tranum-Jensen, J., Janse, M.J., Fiolet, J.W.T., Krieger, W.J.G., D'Alnoncourt, C.N., Durrer, D. 1981. Tissue osmolality, cell swelling, and reperfusion in acute regional myocardial ischemia in the isolated porcine heart. *Circ. Res.* **49:**364–381

Tseng, G-N. 1992. Cell swelling increases membrane conductance of canine cardiac cells: evidence for a volume-sensitive Cl^- channel. *Am. J. Physiol.* **262:**C1056–C1068

Zhang, J., Rasmussen, R.L., Hall, S.K., Lieberman, M. 1993. A chloride current associated with swelling of cultured chick heart cells. *J. Physiol.* **472;**801–820

Zhou, X.-L., Stumpf, M.A., Hoch, H., Kung, C. 1991. A mechanosensitive channel in whole cells and in membrane patches of the fungus. *Uromyces. Science* **253:**1415–1417

Zygmunt, A.C., Gibbons, W.R. 1991. Calcium-activated chloride current in rabbit ventricular myocytes. *Circ. Res.* **68:**424–437