A Mechanosensitive K⁺ Channel in Heart Cells

Activation by Arachidonic Acid

DONGHEE KIM

From the Department of Physiology and Biophysics, Chicago Medical School, North Chicago, Illinois 60064

ABSTRACT Mechanosensitive ion channels have been described in many types of cells. These channels are believed to transduce pressure signals into intracellular biochemical and physiological events. In this study, the patch-clamp technique was used to identify and characterize a mechanosensitive ion channel in rat atrial cells. In cell-attached patches, negative pressure in the pipette activated an ion channel in a pressure-dependent manner. The pressure to induce half-maximal activation was 12 ± 3 mmHg at +40 mV, and nearly full activation was observed at ~20 mmHg. The probability of opening was voltage dependent, with greater channel activity at depolarized potentials. The mechanosensitive channel was identical to the K⁺ channel previously shown to be activated by arachidonic acid and other lipophilic compounds, as judged by the outwardly rectifying current-voltage relation, single channel amplitude, mean open time (1.4 ± 0.3 ms), bursty openings, K⁺ selectivity, insensitivity to any known organic inhibitors of ion channels, and pH sensitivity. In symmetrical 140 mM KCl, the slope conductance was 94 ± 11 pS at +60 mV and 64 ± 8 pS at −60 mV. Anions and cations such as Cl⁻, glutamate, Na⁺, Ca²⁺, Li⁺, and Ba²⁺ were not permeant. Extracellular Ba²⁺ (1 mM) blocked the inward K⁺ current completely. GdCl₃ (100 μM) or CaCl₂ (100 μM) did not alter the K⁺ channel activity or amplitude. Lowering of intracellular pH increased the pressure sensitivity of the channel. The K⁺ channel could be activated in the presence of 5 mM intracellular [ATP] or 10 μM glybenclamide in inside-out patches. In the absence of ATP, when the ATP-sensitive K⁺ channel was active, the mechanosensitive channel could further be activated by pressure, suggesting that they were two separate channels. The ATP-sensitive K⁺ channel was not mechanosensitive. Pressure activated the K⁺ channel in the presence of albumin, a fatty acid binding protein, suggesting that pressure and arachidonic acid activate the K⁺ channel via separate pathways.

Address reprint requests to Dr. Donghee Kim, Department of Physiology and Biophysics, Chicago Medical School, 3333 Green Bay Road, North Chicago, IL 60064.
INTRODUCTION

The presence of mechanoreceptors in muscle spindles, pacinian corpuscle, vestibular hair cells, and other specialized cells that transduce changes in pressure or stretch into electrical signals via changes in membrane ion permeability is well known. Since 1984, mechanosensitive ion channels have also been identified in many different nonspecialized cells including erythrocytes (Hamill, 1983), oocytes (Methfessel, Witzemann, Takahashi, Mishina, Numa, and Sakmann, 1986; Moody and Bosma, 1989; Yang and Sachs, 1989), skeletal muscle cells (Guhray and Sachs, 1984), neurons (Erxleben, 1989; Morris and Sigurdson, 1989), endothelial cells (Lansman, Hallam, and Rink, 1987), smooth muscle cells (Kirber, Walsh, and Singer, 1988), heart cells (Sigurdson, Morris, Brezden, and Gardner, 1987), lens epithelial cells (Cooper, Tang, Rae, and Eisenberg, 1986), kidney cells (Ubl, Murer, and Kolb, 1988; Sackin, 1989; Kawahara, 1990), liver cells (Bear, 1990), Escherichia coli (Martinac, Buechner, Delcour, Adler, and Kung, 1987), fungus (Zhou, Stumpf, Hoch, and Kung, 1991), yeast (Gustin, Zhou, Martinac, and Kung, 1988), and plant cells (Falke, Edwards, Pickard, and Misler, 1988). Although the exact physiological role for the mechanosensitive ion channels for each cell type is not clearly understood, it is speculated that these channels may be involved in volume regulation, muscle contraction, and cell differentiation and development (Medina and Bregestovski, 1988).

In heart atrial cells, stretch has been shown to induce release of atrial natriuretic peptide (ANP) in vitro and in vivo (Lang, Tolken, Ganten, Luft, Ruskoaho, and Unger, 1985; Bilder, Schofield, and Blaine, 1986; Page, Upshaw-Early, Goings, and Hanck, 1991). Since Ca\(^{2+}\) is involved in ANP release (Page, Goings, Power, and Upshaw-Early, 1990), an ion channel that is sensitive to stretch and permeant to Ca\(^{2+}\) could play an important role in the regulation of ANP release. So far, two types of mechanosensitive ion channels have been described in the heart: a K\(^+\)-selective channel in molluscan ventricular cells (Sigurdson et al., 1987); and nonselective cation channels in neonatal rat ventricular cells, guinea pig heart cells, and chick embryo heart cells (Craelius, Chen, and El-Sherif, 1988; Bustamante, Ruknudin, and Sachs, 1991; Sigurdson, Ruknudin, and Sachs, 1992). More recently, the ATP-sensitive K\(^+\) channel (\(i_{\text{KATP}}\)) in rat atrial cells has been reported to be sensitive to pressure and hypotonic swelling (Van Wagoner and Russo, 1992).

In this paper we report the discovery of a K\(^+\)-selective ion channel that is activated by pressure and is different from other stretch-activated channels described earlier in cardiac cells. This mechanosensitive ion channel was found in both adult rat atrial and ventricular cells. Characterization of the kinetics of the mechanosensitive K\(^+\) channel indicated that it was identical to the K\(^+\) channel previously shown to be activated by various lipophilic compounds such as arachidonic acid.

MATERIALS AND METHODS

Cell Preparation

Single ventricular and atrial cells of adult rat heart (200–300 g; Sprague-Dawley) were prepared by enzymatic digestion as described previously (Kim and Duff, 1990). Hearts were retrogradely perfused via the aorta in a Langendorff apparatus with 0.05% collagenase (type II; Worthing-
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ton Biochemical Corp., Freehold, NJ) and 0.03% hyaluronidase (Sigma Chemical Co., St. Louis, MO) in Ca$^{2+}$-free bicarbonate-buffered physiological solution for 30 min for ventricular muscle or 45 min for atrial muscle. Tissues were then cut into small pieces and mechanically dissociated into single cells. Cells were washed several times with well-oxygenated buffer solution and kept at $\sim$24°C in oxygenated (95% O$_2$/5% air) atmosphere. All cells used for experiments were rod-shaped, with no visible signs of contracture or blebs. Bicarbonate-buffered solution contained (mM): 118 NaCl, 4.7 KCl, 1.2 MgSO$_4$, 1.2 KH$_2$PO$_4$, 25 NaHCO$_3$, and 10 glucose. All cells were used within 6 h.

Electrophysiology

Gigaseals were formed with Sylgard-coated thin-walled borosilicate pipettes with 2–5 MO resistances, and channel currents were recorded using the method described by Hamill, Marty, Neher, Sakmann, and Sigworth (1981). Channel current recorded with an Axopatch 1D patch-clamp amplifier was sampled at 20 kHz, digitized by a PCM adapter (model VR10; Instutech, Elmont, NY; frequency response, 37 kHz), and stored on video tape using a video tape recorder (JVC). The recorded signal was transferred directly in digital form to an Atari ST computer using the VCATCH program, and continuous single channel currents were analyzed with the TAC program. In some cases, currents were low-pass filtered at 5 kHz using an 8-pole Bessel filter (model 902-LPF; Frequency Devices Inc., Haverhill, MA) before entering the data to the computer. The built-in Gaussian digital filter was initially set at 5 kHz (equivalent to a Bessel filter with this 3-dB bandwidth) and later changed to 2 kHz for analysis of open time duration. At this setting of 2 kHz, the minimum detectable event duration is $\sim$80 $\mu$s (% fc) when the 50% threshold detector is used. After obtaining all open time events, durations <100 $\mu$s were deleted for plotting the open time histogram. The A/D board (ITC16-ST computer) used was a 16-bit ADC converter which has linearity up to 100 kHz. Data were analyzed to obtain duration and amplitude histograms and channel activity (averaged $Np_o$) using the analysis protocol described by Sigworth and Sine (1987). $N$ is the number of channels, and $p_o$ is the probability of channel opening. Logarithmic (abscissa) and square root (ordinate) scales were used to represent dwell time distributions. Single channel dwell times were plotted on a logarithmic time scale using binned maximum likelihood fitting with constant logarithmic bin width (10 bins/decade). All experiments were performed at 24–26°C. All values are represented as mean ± SD.

Solutions and Materials

In experiments using physiological solutions, the bath solution contained 135 mM NaCl, 5 mM KCl, 1 mM CaCl$_2$, 2 mM MgSO$_4$, and 10 mM HEPES (pH 7.2). In experiments using excised patches, the standard bath and pipette solutions contained 140 mM KCl, 5 mM EGTA, 2 mM MgCl$_2$, and 10 mM HEPES (pH 7.2). In certain experiments, bath K$^+$ was replaced with an equimolar concentration of Na$^+$, Li$^+$, Cs$^+$, Ca$^{2+}$, Ba$^{2+}$, or choline to determine cation selectivity. To determine anion selectivity, Cl$^-$ was replaced with glutamate. To change solutions, the pipette tip with the attached membrane patch was brought to the mouth of a polypropylene tubing through which flowed a solution containing the desired solution at a rate of $\sim$1.0 ml/min. Continuous inflow and outflow of solutions to and from the perfusion bath (200 $\mu$l capacity) resulted in complete exchange of solution within 1 min, as determined earlier by changes in electrode resistance. ATP, quinidine, ouabain, sodium orthovanadate, tolbutamide, glybenclamide, apamin, amiloride, tetraethylammonium, 4,4'-diisothioxyano-2,2'-disulfonic acid (DIDS), and tetrodotoxin were purchased from Sigma Chemical Co. Nifedipine was purchased from Calbiochem Corp. (La Jolla, CA). Gadolinium chloride (GdCl$_3$) as purchased from Aldrich Chemical Co. (Milwaukee, WI). Arachidonic acid purchased from Sigma Chemical
Co. was kept at -70°C in a freezer until used. Immediately before each experiment the organic solvent in vials containing the fatty acid was evaporated under pure N₂ at 0°C, and bath solution was added to the vials to a final concentration of 10 μM. The solution was sonicated for 15 s and used immediately (Mead, Alfin, Howton, and Popjak, 1985). 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 the desired levels by operating the syringe.

**RESULTS**

**Activation of an Ion Channel with Pressure**

The presence of single channel currents sensitive to pressure was first examined using isolated membrane patches. Cell-attached patches were formed in a physiological bath solution. Generally, a suction pressure of ~5 mmHg was sufficient to form gigaseals, and upon release of pressure only the opening of inwardly rectifying (IK₁) channels were observed. IK₁ was present only in ~15% of the patches examined. Application of negative pressure in the pipette produced either no response or activation of a channel with bursty openings. Fig. 1 shows a cell-attached patch of an atrial cell in which 3.7 mmHg of negative pressure was applied to the pipette. Release of pressure quickly closed the channels. Channel openings recorded at an expanded time scale show the bursty opening characteristics of these channels. Similar activation of the channel was present in the inside-out patch. Application of positive pressure also activated these channels, but the patch current rapidly became unstable and resulted in breaking of the seal. The pressure-activated channels exhibited outward rectification and K⁺ selectivity. The kinetic properties of this mechanosensitive channel are described below in more detail.

At a given negative pressure (~13 mmHg), channel currents in an inside-out atrial patch were recorded at different potentials, and mean open times and single channel amplitudes were measured for each potential (Fig. 2 A). Mean open times did not change markedly with membrane potential and ranged from 1.2 to 1.6 ms. An open...
time duration histogram is shown for a current recorded at +40 mV (Fig. 2 B). The current–voltage relation showed that the current was outwardly rectifying. In symmetrical 140 mM K⁺, the slope conductance was 94 ± 11 pS at +60 mV and 64 ± 8 pS at −60 mV (Fig. 2 C).

**Ion Selectivity and Effects of Ion Channel Inhibitors**

To study ion selectivity of the pressure-activated ion channel, [K⁺] in the bath of inside-out patches was changed from 140 mM to 14 or 28 mM. The reversal potential shifted from 0 mV to 54 ± 4 or 35 ± 5 mV (n = 3), respectively, close to the expected reversal potentials for a K⁺-selective ion channel. Replacing Cl⁻ with glutamate had no measurable effect on the reversal potential or the current–voltage relation, indicating that the channel was not permeable to Cl⁻. In inside-out patches, no measurable outward current was present when K⁺ in the bath was replaced with an equimolar concentration of Na⁺, Cs⁺, or Ba²⁺. This is illustrated in Fig. 3, in which

![Figure 2](https://example.com/figure2.png)

**Figure 2.** (A) Mechanosensitive channel current at various potentials from an inside-out patch. (B) Open time duration histogram of channel openings at +40 mV (mean open time, 1.4 ms). (C) Current–voltage relation in symmetrical 140 mM K⁺ solution.
the bath solution of an inside-out patch was changed sequentially from K⁺ to Na⁺, Cs⁺, and Ba²⁺. However, inward K⁺ currents were still present. 1 mM Ba²⁺ in the pipette abolished the inward K⁺ current. Replacing K⁺ in the pipette with these cations produced opposite changes; i.e., only the outward current was present. The absence of channel currents with Na⁺, Cs⁺, or Ba²⁺ is unlikely to be due to a shift in the pressure–open probability relationship, since no current was observed even at pressure levels up to 40 mmHg. Thus, the mechanosensitive channel in rat atrial cell was K⁺ selective.

The lanthanide gadolinium (100 µM), which blocks stretch-activated ion channels in oocytes (Yang and Sachs, 1989), skeletal muscle cells (Franco and Lansman, 1990), and fungus (Zhou et al., 1991), did not block the mechanosensitive K⁺ channel in atrial cells even up to 1 mM when added to the bath (Fig. 3). 100 µM Ca²⁺ also failed to alter the open probability of the K⁺ channel at a given pressure level. Although

![Figure 3](https://example.com/figure3.png)

Figure 3. Ion selectivity of the mechanosensitive channel. In an inside-out patch held at +40 mV, bath K⁺ was replaced sequentially with equimolar concentrations of Na⁺, Cs⁺, or Ba²⁺. No outward currents were observed. Gd³⁺ (100 µM) or Ca²⁺ (100 µM) added to 140 mM K⁺ bath solution failed to affect the channel activity or amplitude.

not shown in Fig. 3, the channel activity (0.26 vs. 0.27; averages from three patches) and the mean open times (1.51 vs. 1.46 ms; averages from three patches) before and after application with Ca²⁺ remained the same. The effect of tetraethylammonium (10 mM), apamin (10 nM), nifedipine (10 µM), quinidine (100 µM), tetrodotoxin (10 µM), ouabain (100 µM), vanadate (1 mM), and DIDS (100 µM) were tested. None of these drugs altered channel activity or single channel conductance. Amiloride has been shown to block mechanosensitive cation channel in *Xenopus* oocytes (Lane, McBride, and Hamill, 1991) and mechanoelectrical transduction channel of chick hair cells (Jorgensen and Ohmori, 1988), but had no effect on the cardiac K⁺ channel found here. Glybenclamide and a sulfonylurea, tolbutamide (Trube, Rorsman, and Ohno-Shosaku, 1986), which block the *I*ₖₐₜₙ, also failed to affect the mechanosensitive K⁺ channel. Albumin (10 mg/ml), which was reported to inhibit
stretch-activated ion channel in smooth muscle cells (Ordway, Petrou, Kirber, Walsh, and Singer, 1992), failed to inhibit channel activation by pressure in atrial cells.

**Pressure-dependent Changes in Channel Activity**

The effect of pressure on channel activation was studied in more detail in cell-attached patches. The cell membrane potential was held at approximately +40 mV in these experiments. No channel opening was observed in normal atmospheric pressure (0 mmHg). As shown in Fig. 4, negative pressure in the pipette rapidly caused opening of the K⁺ channel, and return to atmospheric pressure resulted in closure of the channel. In some patches, such as that shown in Fig. 4, the channel opened infrequently during the period between the two pressure applications, but generally tended to close if the pressure was not continued for > 1 min. This could be due to the partially stretched membrane even after the pipette pressure has returned to the atmospheric pressure. Higher negative pressure in the pipette caused greater activation of the channel. Near maximal activation of the channel was usually seen at

![Figure 4](https://example.com/figure4)

**FIGURE 4.** Pressure-dependent activation of the K⁺ channel in a cell-attached patch. Pipette potential was held at -40 mV. Pressure in the pipette was changed stepwise from atmospheric (0) to 30 mmHg in the same patch. Although no channel openings were observed before application of any negative pressure, few channel openings were present during the periods between each pressure pulses in ~50% of the patches. The current tracing shown was filtered at 15 Hz. Bath and pipette solutions contained (mM): 140 K⁺, 2 Mg²⁺, 10 HEPES, and 5 EGTA.

or slightly above 20 mmHg of pressure. The mechanosensitive channel activity is better illustrated in Fig. 5. Before applying negative pressure, no channel opening was observed. A channel started to open in short bursts at ~2–3 mmHg and the burst duration increased with increasing pressure. The open probability of the channel was plotted as a function of negative pressure in the pipette. Data obtained from a patch are plotted in Fig. 6. The open 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 were fitted by linear regression to a modified Boltzmann distribution given by \( P_o = \frac{\exp[(p - p_{1/2})/s]}{[1 + \exp(p - p_{1/2})/s]} \), where \( P_o \) is the opening probability, \( p \) is the negative pressure, \( p_{1/2} \) is the pressure at which \( P_o = 0.5 \), and \( s \) is the slope of the plot of \( \ln [P_o/(1 - P_o)] \) vs. pressure. \( p_{1/2} \) was 12 ± 3 mmHg (n = 3). An e-fold change in activity (ratio of probability of being open to probability of being closed) occurred for every 3.3 mmHg change in pressure. These effects of pressure on the K⁺ channel activity were observed in ~40% of the patches (86 of 220). In a few patches (12%), the threshold
FIGURE 5. Current records in Fig. 4 at an expanded time scale, showing the progressive increase in channel burst duration with increasing negative pressure in the pipette. Only one K⁺ channel was present in this patch.

FIGURE 6. Relation between relative open probability and pressure. Mechanosensitive channel activity was recorded from three cell-attached patches, and the open probability values (filled circles) were calculated for each pressure. The data were fitted to the Boltzmann distribution as described in the text. $P_{1/2} = 12 \pm 3$ mmHg ($n = 3$). The channel activity calculated as $Np_o$ during maximal channel activity obtained at pressures at or greater than 40 mmHg ranged from 0.76 to 0.82. The maximal channel activity calculated for a given patch was taken as an open probability of 1.
negative pressure required to activate the K channel was 15–20 mmHg. However, once the channel was activated, the channel in the same patch could subsequently be reactivated at lower pressures similar to those described above.

**Voltage Dependence of the Mechanosensitive Channel**

Channel open probabilities were measured at potentials ranging from -60 to +80 mV from three cells and plotted as shown in Fig. 7. Channels were generally more active at depolarized potentials (the pressure was -9.6 mmHg in these experiments). At potentials negative to reversal potential (-60 to -20 mV) the channel activity did not change significantly. However, at depolarized potentials the channel activity increased progressively with greater depolarization. Because the membrane patch became very unstable above +80 mV, no clear analyzable records could be obtained. Although we have not analyzed in detail the membrane potential–open probability relationship at different pressures, the channel activity was always greater at +60 mV than at -40 mV at different negative pressures (-5 to -20 mmHg) in all patches examined. The mean values from three experiments shown in Fig. 7 were fitted to the Boltzmann distribution given by

$$P_o = \frac{1}{1 + e^{-\frac{V - V_{1/2}}{k}}}$$

where $V_{1/2}$ (65 mV) is the half-maximal voltage and $k$ is the slope. An e-fold change in open probability occurred per 52 mV for the mechanosensitive K+ channel. This can be compared with the peak gNa, which has a limiting slope of e-fold per 4 mV.

**Activation of the Mechanosensitive K+ Channel by Arachidonic Acid**

The single channel kinetics and the current–voltage relation of the mechanosensitive K+ channel described above indicated that this channel was identical to the K+ channel that was recently shown to be activated by arachidonic acid and other lipophilic compounds (Kim and Duff, 1990; Wallert, Ackerman, Kim, and Clapham, 1991). If the pressure- and arachidonic acid–activated channels are indeed the same channel, the fatty acid–activated K+ channel should also be mechanosensitive. This hypothesis was studied by exposing the cytosolic surface of the inside-out patches to arachidonic acid (10 μM) to activate the K+ channel and then determining whether
the activity of the channel increased in response to negative pressure. In an inside-out patch, 11 mmHg negative pressure was first applied to the pipette to determine the presence of a mechanosensitive channel in the membrane patch. One mechanosensitive K⁺ channel was present in the patch shown in Fig. 8 (current

![Graph showing pressure sensitivity of arachidonic acid-activated K⁺ channels](image)

**Figure 8.** Pressure sensitivity of arachidonic acid-activated K⁺ channels in an inside-out patch (membrane potential, +40 mV) in symmetrical 140 mM K⁺. (A) First, a negative pressure of 11 mmHg was applied to the pipette, which resulted in opening of the K⁺ channel. After return to zero pressure and closing of the channel, arachidonic acid (10 μM) was applied to the cytoplasmic surface of the cell. In this patch, arachidonic acid quickly activated a K⁺ channel identical to the pressure-activated channel. Negative pressure in the pipette further increased the channel activity and this was reversible. (B) Current activated by arachidonic acid shown at an expanded time scale. (C) A relative open probability-pressure relation from another inside-out patch. Data were fitted to a Boltzmann distribution as described in the text. Because the extent of activation of the K⁺ channel by arachidonic acid was different for each patch, the open probability-pressure relations varied markedly from patch to patch. $N_{po}$ for the activation by arachidonic acid ranged from 0.004 to 0.3 in 15 patches at +40 mV. The filter bandwidth in A was 15 Hz.

tracing was obtained at filter bandwidth of 15 Hz). After the release of pressure and closing of the channel, arachidonic acid was slowly applied to the bath. Within a few seconds, opening of a channel indistinguishable from that produced by pressure was observed. Single channel openings at a faster time scale are shown in Fig. 8 B. The
kinetic properties of this channel were identical to those of the pressure-activated channel (for detailed kinetic analysis, see Wallert et al., 1991). When the channel activity reached steady state, negative pressures were again applied to the pipette, which produced a further increase in channel activity. No other types of ion channels were activated by pressure or arachidonic acid. After return to atmospheric pressure, the channel activity decreased quickly to the level observed just before suction. The open probability of the K⁺ channel was plotted as a function of applied pressure in the presence of arachidonic acid from another inside-out patch (Fig. 8C). The data points were fitted to the Boltzmann relation as before. Due to variable activation of the K⁺ channel by arachidonic acid in different patches, the pressure–activity relationship varied significantly. However, the sensitivity of the channel to pressure was similar to control conditions without the fatty acid; an e-fold change in channel activity occurred for every ~3 mmHg pressure with arachidonic acid (n = 3). In every membrane patch studied, arachidonic acid did not activate the channel fully even when the concentration was increased to 50–100 μM. At a given level of pressure and channel activity, arachidonic acid increased the channel activity to varying degrees but never to a maximal level. In contrast, pressure application to ~30 mmHg always resulted in maximal activation of the channel whether the membrane was exposed to arachidonic acid or not. Thus, endogenous changes in the membrane produced by pressure seem to be more effective in channel activation than exogenous application of the fatty acid.

Arachidonic acid and pressure presumably activated the same population of K⁺ channels in this patch, as only one type of K⁺ channel was present. If two different K⁺ channels were involved, one would expect two overlapping channels when the mechanosensitive channel has been fully activated. To further determine whether the arachidonic acid–activated and mechanosensitive K⁺ channels are identical entities, the ability of a channel in a patch of membrane to be activated by arachidonic acid and/or pressure was studied. In inside-out patches in which application of arachidonic acid (20 μM) caused activation of the K⁺ channel (19 of 45 patches; 42%), application of negative pressure after the washout of the fatty acid resulted in activation of the same channel in all 19 patches (100%). In those patches in which arachidonic acid failed to activate the channel, pressure also failed to open the channel. Similarly, in patches in which pressure caused activation of the K⁺ channel (14 of 31 patches; 45%), arachidonic acid also elicited opening of the K⁺ channel in all 14 patches (100%). Thus, these results provide additional evidence that the K⁺ channel activated by arachidonic acid and pressure are identical entities.

In our previous study using cultured neonatal rat atrial cells (Wallert et al., 1991), it was found that compounds other than arachidonic acid such as choline, myristic acid, and palmitic acid could also activate the K⁺ channel. In adult rat atrial cells used in this study, these compounds (10 μM) did not activate the K⁺ channel (0 of 16 patches for choline; 0 of 12 for myristic acid; 0 of 12 for palmitic acid). However, linoleic acid was found to activate the K⁺ channel (6 of 13 patches). When the K⁺ channel was activated by linoleic acid, it was also sensitive to pressure in all six patches. Although it is not clear why a compound activates the mechanosensitive K⁺ channel in neonatal atrial cells and not in adult atrial cells, it may be related to differences in membrane lipid composition.
Is the Mechanosensitive K⁺ Channel a Modified Form of the ATP-sensitive K⁺ Channel?

The K⁺ channel with kinetics similar to those activated by arachidonic acid or pressure as described here has been observed in metabolically inhibited cells whose [ATP]ᵢ is markedly reduced (Deutsch and Weiss, 1991). It has also been observed that when arachidonic acid is added to a membrane patch that has several active iK_ATP, iK_ATP quickly inactivates and the arachidonic acid–activated K⁺ channels open (Kim and Duff, 1990). It is therefore possible that the kinetic property of the iK_ATP protein is somehow modified by fatty acids, pressure, or metabolic inhibition to become ATP insensitive and to show outward rectification even in the presence of intracellular Mg²⁺. This possibility was tested using inside-out patches containing only one ATP-sensitive K⁺ channel. Patches containing only one such channel were obtained by forming cell-attached patches using pipette electrodes with high tip resistances. Upon excision of membrane patch from the cell-attached form, activation of iK_ATP occurred due to the washout of cytosolic ATP. The presence of a pressure-activated K⁺ channel was then examined in such patches. Membrane potential was held either at +60 or −40 mV. As shown in Fig. 9, the current with the smaller amplitude at either voltage is the current through the iK_ATP, which shows inward rectifying characteristics; i.e., larger current at hyperpolarized potentials than at depolarized potentials. A negative pressure in the pipette (−10 mmHg) activated another channel current with a larger amplitude at both potentials and with outwardly rectifying property. The bursty pressure-activated channels opened together with the iK_ATP that was open continuously. Addition of 1 mM ATP to the bath quickly shut down the channel with smaller amplitude leaving only the mechanosensitive K⁺ channel (not shown). Identical results were seen in 12 other patches having only one such iK_ATP, indicating clearly that the two types of K⁺ channels are distinct entities with different gating characteristics. We did not observe any consistent activation or inhibition of the iK_ATP with pressure.

Figure 9. Activation of both ATP- and mechanosensitive K⁺ channels in an inside-out patch in symmetrical 140 K⁺. In a patch containing only one active ATP-sensitive K⁺ channel, negative pressure applied to the pipette (10 mmHg) caused opening of the mechanosensitive K⁺ channel. Channel openings are shown at both +60 and −40 mV. At either potential, the mechanosensitive K⁺ channel that opens in bursts and the ATP-sensitive K⁺ channel that is open continuously can be seen.
Effect of pH on the Mechanosensitive Channel

It was shown previously that when the K⁺ channel is activated by arachidonic acid, the channel activity can be further increased simply by lowering the cytoplasmic pH (Kim and Clapham, 1989; Wallert et al., 1991). If the K⁺ channel activated by pressure or arachidonic acid is the same, the mechanosensitive channel would also be expected to be sensitive to pH. To test whether pH affects the sensitivity of the K⁺ channel to pressure, excised inside-out patches were formed and channel activity in response to negative pipette pressure was determined at a cytosolic pH of 7.2 and 6.6. As shown in Fig. 10A, the same negative pressure applied to an inside-out patch produced a greater channel open probability at pH 6.6 than at pH 7.2, and the effect of pH was fully reversible. In five separate experiments, the act of changing perfusion solutions (of same pH) per se had no effect on the channel activity. The single channel conductance and current–voltage relations were not altered by these changes in pH (data not shown). Pressure-dependent changes in channel activity were further examined at pH 7.2 and 6.6 using an inside-out patch. First, the pressure was changed from atmospheric (0 mmHg) to −30 mmHg in small increments at pH 7.2. After returning to 0 mmHg, the pH of the bath solution was switched to 6.6 and the pressure was changed similarly. The probability of channel opening was analyzed at each pressure and plotted as a function of negative pressure in the pipette (Fig. 10B). The data were fitted to the Boltzmann distribution as described in Fig. 6. An e-fold change in channel activity occurred for every 3.4 mmHg at pH 7.2 and 2.1 mmHg at pH 6.6. It is clear from these results that the K⁺ channel is more sensitive to pressure at acidic pH, analogous to the effect of pH on arachidonic acid–activated K⁺ channel activity. These effects of pH could be due to alteration of net charges of membrane lipids leading to slight changes in lipid architecture, or to a more direct effect on the channel protein.

Activation of Whole-Cell Current by Pressure

To determine whether the mechanosensitive current could be detected at the whole-cell level, positive pressure was applied to the interior of the cell via the pipette and the whole-cell current was recorded. The cell membrane potential was held at +40 mV to record outward current in symmetrical 140 mM K⁺ solution. In 182 cells in the whole-cell configuration, application of positive pressure (10–20 mmHg) nearly always resulted in destabilization of the gigaseal such that the leak current became significant during the experiment. In three cells, we were able to measure a clear reversible increase in K⁺ current in response to positive pressure (15 mmHg). A current recording from one such cell is shown in Fig. 11. We were unable to obtain a pressure–current relationship in any of the three cells due to seal breakage after ~1 min. As reported earlier, addition of 10 μM arachidonic acid to the pipette also resulted in an increase in whole-cell current. Further application of pressure, however, caused immediate loss of gigaseal in every cell tested (>50 cells). Although we could not determine the pressure–current relation at the whole-cell level with and without arachidonic acid, the presence of pressure-induced whole-cell current suggests that single mechanosensitive channels do indeed exist in the cell membrane.
A

pH 7.2
5 mmHg

pH 6.6
5 mmHg

pH 6.6
10 mmHg

B

Normalized Open probability

Pressure (mmHg)

pH 6.6
pH 7.2

FIGURE 10. Increased sensitivity of the K⁺ channel to pressure by intracellular acidosis. (A) An inside-out patch was bathed in solution at pH 7.2 (symmetrical 140 K⁺ solution). Negative pressure applied to the pipette caused activation of the K⁺ channel. pH of the bath solution was then changed from 7.2 to 6.6 at the same and higher pressure. (B) Open probability–pressure relations obtained from a patch at pH 7.2 and 6.6. Data were fitted to the Boltzmann distribution as described in the text. P₁/₂ was 7.2 ± 2.2 mmHg at pH 6.6 and 13.2 ± 3.1 mmHg at pH 7.2 (mean ± SD, n = 3).

DISCUSSION

In this study, a K⁺-selective ion channel that is activated by either negative or positive pressure has been found in adult rat atrial cells. The sensitivity of the K⁺ channel to pressure is similar to that reported for other mechanosensitive channels; i.e., half-maximal activation was achieved at pressures between 10 and 20 mmHg. This channel was observed both in cell-attached and inside-out patches, indicating that cytosolic factors such as Ca²⁺ and nucleotide phosphates were not involved. The mechanosensitive K⁺ channel described here does not appear to be similar to the K⁺...
channel found in molluscan ventricular cells. A low level of spontaneous channel activity was observed in molluscan cells under control conditions (Sigurdson et al., 1987). No such spontaneous opening was seen in rat cardiac cells before pressure application. The current-voltage relation was ohmic for molluscan cell, whereas it was outwardly rectifying in the rat. Lastly, the pressure dependence of the K⁺ channel was different from that in rat heart cells. For example, release of pressure did not result in rapid closure of the channel in molluscan cells (Fig. 4 of Sigurdson et al., 1987).

Interestingly, the kinetics of the mechanosensitive K⁺ channel that we describe here were identical to those of the cardiac K⁺ channels activated by arachidonic acid (Kim and Clapham, 1989; Kim and Duff, 1990; this study) and by other lipophilic compounds (Wallert et al., 1991). Thus, the pressure- and arachidonic acid–sensitive channels showed slightly outwardly rectifying property with similar single channel conductances. Also, the mean open times ranged from 1.2 to 1.6 ms, and opening occurred in bursts for both currents. The observation that the same K⁺ channel can be activated by pressure or by fatty acids raises an interesting possibility that stretch-activated channels may be mediated by the same membrane events produced by addition of fatty acids to the membrane. Could lipophilic compounds such as fatty acids insert into the membrane and somehow alter the existing lipid architecture to cause an effect similar to stretch? Or could the stretch cause release of fatty acids which then directly activate the K⁺ channels? It has been reported that the S channel (K⁺ channel stimulated by serotonin) in *Aplysia* neurons can be activated either by arachidonic acid metabolites generated via the lipoxygenase pathway or by stretch (Piomelli, Volterra, Dale, Siegelbaum, Kandel, Schwartz, and Belardetti, 1987; Vandorpe and Morris, 1992). In toad gastric smooth muscle cells, different classes of fatty acids activated a K⁺ channel that was also sensitive to pressure (Kirber, Ordway, Clapp, Walsh, and Singer, 1992). Unlike the K⁺ channel in heart cells, the pressure-induced K⁺ channel activity in the smooth muscle cells remained active for tens of seconds even after release of pressure. It was found that in smooth muscle cells from rabbit pulmonary artery, the presence of albumin (10 μM) prevented opening of the K⁺ channel by pressure and therefore the authors hypothesized that fatty acids mediated the pressure-induced opening of the channel (Ordway et al.,
In heart cells, we found that albumin (10 mg/ml = 200 μM) did not inhibit activation of the K+ channel and also did not affect the sensitivity of the channel to pressure. The atrial channel activity was closely associated with the degree of pressure in the pipette, and recovery was immediate in the presence or absence of albumin. Therefore, it is unlikely that fatty acids were involved and acted as second messengers in pressure-activated K+ channels in heart cells used in this study. In *E. coli*, amphipathic compounds of hydrophilic and hydrophobic character with positive, negative, or no net charge were found to slowly activate the mechanosensitive channels (Martinac, Adler, and Kung, 1990). These studies support the view that membrane lipids generate the force to modulate the activity of these mechanosensitive ion channels. Although both pressure and fatty acids activate the same K+ channel, neither seems to depend on the other factor. However, there must be a common point in the pathways via which pressure and fatty acids produce their effects in the membrane in order to activate the same channel. The precise membrane events involved in channel activation by tension and fatty acids need further investigation. It would be interesting to determine whether other types of channels that are activated by fatty acids are mechanosensitive and whether these mechanosensitive channels can be activated by certain fatty acids.

Recently it has been reported that *IKATP* in neonatal rat atrial cells could be activated by negative pressure and by hypotonic swelling (Van Wagoner and Russo, 1992). In our studies using adult rat atrial and ventricular cells, *IKATP* was not sensitive to pressure. Although suction caused slight activation of the K+ channel in certain patches, this was not a consistent observation. It is plausible that in certain patches suction may help to accelerate washout of ATP from the membrane inside the pipette and thus cause opening of the K+ channel. Once open, the K+ channel activity was not further increased by negative pressure or decreased by release of pressure.

In guinea pig ventricular cells, metabolic inhibition using rotenone and carbonyl cyanide p-(trifluoromethoxy) phenyl-hydrazone have been reported to cause opening of *IKATP* and an ATP-insensitive K+ channel (Deutsch and Weiss, 1991). This latter channel appears to be similar to the arachidonic acid-activated K+ channel in the adult rat ventricular cells, as judged by channel opening kinetics, loss of inward rectification in the presence of Mg2+, single channel conductance, and insensitivity to ATP (Kim and Duff, 1990; Wallert et al., 1991). We reported earlier that in rat ventricular cells, perfusion of cytoplasmic surface of the membrane with arachidonic acid or other related fatty acids inhibited the *IKATP* and activated the ATP-insensitive K+ channel (now known to be mechanosensitive) in the same patch (Kim and Duff, 1990). Therefore, it is plausible that the two K+ channels are actually the same protein but that the gating properties of the *IKATP* were somehow modified either by pressure or arachidonic acid to give rise to the ATP-insensitive K+ channel. Our results shown in Fig. 8 indicate, however, that they are separate channels. The possibility that other types of K+ channels, such as the resting K+ channel (*IK1*) or delayed rectifier K+ channel, are modified by suction or fatty acids cannot be ruled out at this time. The muscarinic K+ channel is probably not involved since it is absent in ventricular cells where the mechanosensitive K+ channels are also present. In certain cell-attached patches with *IK1* present, both *IK1* and mechanosensitive K+
channels were observed. In a given patch of membrane covered by a pipette with a resistance of ~4 MΩ, usually one or two mechanosensitive channels were present. In similar patches, 4–5 iK\textsubscript{ATP} (ventricular and atrial) and 6–10 muscarinic K\textsuperscript{+} channels (atrial cells with acetylcholine in the pipette) were generally observed. Thus, these findings also suggest that the mechanosensitive K\textsuperscript{+} channel belongs to a separate class of K\textsuperscript{+} channels.

It has been reported that arachidonic acid and other fatty acids activate two different types of K\textsuperscript{+} channels labeled as iK\textsubscript{AA} and iK\textsubscript{PC} (Wallert et al., 1991). Although we have described the pressure sensitivity of the iK\textsubscript{AA} type of current in this paper, iK\textsubscript{PC} type of K\textsuperscript{+} channels were also observed in a few patches with kinetics identical to those reported previously. We also found the iK\textsubscript{PC} type of channel to be sensitive to pressure in a manner similar to iK\textsubscript{AA}. The sensitivities of the two types of channels were similar, with half-maximal open probability found at ~12 mmHg of negative pressure. We have never seen the two types of K\textsuperscript{+} channels in the same patch, suggesting that they may be the same protein with different gating kinetics produced as a result of different membrane lipid arrangement around the channel protein. We have not studied in detail the pressure sensitivity of the channels at the whole-cell level due to our inability to maintain stable gigaseal. However, the biophysical properties of both iK\textsubscript{AA} and iK\textsubscript{PC} have been characterized in detail at the whole-cell and single channel levels by Wallert et al. (1991). Therefore, the single channels sensitive to pressure are unlikely to be an artifact of patch-clamp recording, a situation reported to exist in Lymnaea stagnalis neurons (Morris and Horn, 1991) in which mechanosensitive single channel currents were not observed at the whole-cell level.

The mechanosensitive K\textsuperscript{+} channel described above for atrial cells was also found in freshly dissociated rat ventricular cells (data not shown). The single channel conductance (106 ± 12 pS at +60 mV and 72 ± 10 pS at −60 mV), ion selectivity (K\textsuperscript{+}), pH sensitivity, and pressure sensitivity were essentially identical to those found in atrial cells. The K\textsuperscript{+} channel in ventricular cells was also insensitive to all the drugs that we have tested in atrial cells. We have previously described such a K\textsuperscript{+} channel in rat ventricular cells that was activated by arachidonic acid (Kim and Duff, 1990). Thus, both atrial and ventricular cells possess the same mechanosensitive K\textsuperscript{+} channel that is also activated by certain lipophilic substances such as arachidonic acid. The density of the K\textsuperscript{+} channel was similar in both cell types, as judged by the number of K\textsuperscript{+} channels elicited by pressure using pipettes of similar resistances.

**Physiological Significance**

Normally, the pressure fluctuates between ~0 and 120 mmHg in ventricles and between ~0 and 12 mmHg in atria. How much of these pressure changes is sensed by the cardiac cells and whether the K\textsuperscript{+} channels are activated in vivo are not known. If cardiac cells in vivo have the same pressure sensitivity, the mechanosensitive K\textsuperscript{+} channels in cells that sense the pressure changes would be expected to open and close during each cardiac cycle and participate in the regulation of resting membrane potential and repolarization. As the atrium is only a few cell layers thick, atrial cells are more likely to sense the pressure changes, as do the endocardial endothelial cells that are in direct contact with the blood. Since stretch-activated channels are present...
In endothelial cells (Lansman et al., 1987; Oleson, Clapham, and Davies, 1988), the modulation of heart function by stretch may be mediated more effectively by release of vasoactive factors from endothelial cells.

In pathophysiological conditions such as ischemia or hypoxia, the mechanosensitive K⁺ channel may become more relevant. We have suggested previously that the arachidonic acid-activated K⁺ channel may become active when heart cells become ischemic or severely hypoxic, at which time the free cytosolic arachidonic acid (or other fatty acid) level is elevated (Kim and Duff, 1990). The activation of the K⁺ channel could thus explain, at least in part, the loss of K⁺ observed during ischemia (Kleber, 1984). The reduction of intracellular pH that occurs in ischemia would further increase the probability of channel opening and augment K⁺ efflux during ischemia. How this is related to the pressure sensitivity of the K⁺ channel is not clear. It has been reported that prolonged ischemia is associated with cell swelling (Sparks, Wangler, and DeWitt, 1984). The mechanosensitive K⁺ channels in the membrane of such cells may become active and further contribute to K⁺ loss. Whether such K⁺ loss is beneficial or detrimental for the heart cells is not clear at this time.

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