Mechanosensitive Gating of Atrial ATP-Sensitive Potassium Channels

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Cell-attached and inside-out excised-patch recording techniques were used to search for mechanosensitive ion channels in neonatal and adult rat atrial myocytes. A channel activated by negative pressure applied to the patch, with a single-channel conductance of 52 pS in symmetric potassium solutions, was frequently observed. This channel has been identified as the atrial ATP-sensitive potassium (K\textsubscript{ATP}) channel on the basis of its potassium selectivity, as well as its inhibition by ATP or tolbutamide in the inside-out excised patch. Mechanosensitive modulation of the K\textsubscript{ATP} channel has not previously been reported. In the presence of 1 mM ATP, 10–50 μM pinacidil (a specific K\textsubscript{ATP} channel agonist) does not significantly increase basal K\textsubscript{ATP} channel activity; however, these concentrations of pinacidil potentiated the mechanosensitive modulation of the K\textsubscript{ATP} channel. A hypotonic swelling protocol (a mechanical stimulus) was used in an effort to determine whether mechanosensitive modulation of this channel can generate significant whole-cell currents. Under perforated-patch whole-cell recording conditions, superfusion of atrial myocytes with a 240 mosm/kg solution (control solution, 290 mosm/kg) stimulated whole-cell currents with a magnitude similar to those activated by 10 μM pinacidil. These results demonstrate that the gating of the atrial K\textsubscript{ATP} channel is mechanosensitive and suggest that mechanosensitive modulation may be an additional and significant mechanism, modulating channel activity under both physiological and pathological conditions. (Circulation Research 1993;72:973–983)

**KEY WORDS** • stretch-activated channels • glibenclamide • tolbutamide • potassium channel openers • pinacidil

The cardiac atria function as the volume sensors of the cardiovascular system. Distension of the atria leads both to the secretion of atrial natriuretic peptide and to activation of the baroreceptors. Abrupt distension of the heart is proarrhythmic and can decrease the refractory period; chronic distension causes cardiac hypertrophy. The transduction mechanisms underlying these processes are not understood. Mechanosensitive ion channels have been postulated to be involved in modulating the volume status, resting potential, calcium permeability, and overall excitability of the tissues in which they are found. In view of the physiological role of the atrium, it seemed to be a tissue likely to benefit from the presence of mechanosensitive ion channels. The present study was initiated to determine whether cation-permeable mechanosensitive channels were present in the atria and then, if present, to begin to identify their functional role. Since this study was initiated, there has been a report identifying non-selective cation channels with single-channel techniques in neonatal rat atrial myocytes, as well as reports of whole-cell mechanosensitive chloride currents in canine atrial myocytes.

During my work I discovered that rat atrial myocytes possess at least one ion channel, the ATP-sensitive potassium (K\textsubscript{ATP}) channel, whose activity can be mechanically modulated but which differs significantly from the mechanosensitive channels previously reported in other cardiac tissues. The K\textsubscript{ATP} channel was first discovered in the heart and has since been found to be present at high density in cardiac muscle, pancreas, smooth muscle, skeletal muscle, and brain. The regulation and distribution of these channels in the cardiovascular system have been studied extensively and reviewed recently. The role of these channels in cardiac physiology has been the subject of some speculation because of an apparent dissociation between the concentration of ATP required to block the channel in the excised patch (~100 μM) and the typically high concentration of ATP in a cardiac myocyte (4–5 mM) in all but the most pathological of circumstances. It has been convincingly demonstrated, however, that activation of even a small fraction of the total number of K\textsubscript{ATP} channels (whether via ATP depletion, hormonal effects, or other modulatory mechanisms) can significantly modulate the cellular resting potential and action potential duration. Thus, it is important to evaluate the contribution of mechanosensitive stimuli as an additional mechanism modulating the activity of the atrial K\textsubscript{ATP} channel.

**Materials and Methods**

**Neonatal Atrial Myocyte Preparation**

Neonatal atrial myocytes were isolated from American Wistar rat pups (1–3 days old) using a method...
based on that of Engelmann et al.12 After decapitation into liquid nitrogen, the hearts were rapidly excised and trimmed to remove excess fascia, and the atria were separated from the ventricles. All of the atria from a litter (10–15 animals) were pooled. The tissue was minced and digested with type II collagenase (Worthington Biochemical Corp., Freehold, N.J.) for 10-minute intervals. Supernatant from the digest was collected, and the dissociated cells were filtered and centrifuged. The supernatant was discarded, and the pellet was resuspended. These dissociated cells were then transferred into a centrifuge tube containing 10 ml chilled Joklik-minimum essential medium (GIBCO Laboratories, Grand Island, N.Y.) and 125 μl fetal calf serum (GIBCO). The cells were kept on ice between collections. These steps were repeated until the pellet began decreasing in yield (four to five times). After the final collection, the cell count was determined with a hemocytometer, and the cells were resuspended in serum-free PC-1 culture (Ventrex Laboratories, Portland, Me.) at a density of 10⁶/ml. Cells were plated at 0.25 × 10⁶ cells per dish in 35-mm (Falcon 3001) culture dishes. They were incubated in PC-1 media at 37°C and 5% CO₂ for 36 hours; then the media was replaced with a serum-free mixture containing two parts DME/F-12 (a mixture of Dulbecco's modified Eagle's medium with F-12 Ham medium, 1:1, Sigma Chemical Co., St. Louis, Mo.) and one part PC-1. An antibiotic-antimycotic mixture (GIBCO) was used in all media at recommended concentrations to suppress bacterial, fungal, and yeast growth in culture. Neonatal atrial myocytes were usually studied within 4–7 days of isolation and were identified both from their beating spontaneously in culture and from their spindle-shaped morphology. The cell density was low enough that individual cells remained isolated during the period of study. Only clearly isolated individual atrial cells were used in the electrophysiological studies.

**Adult Atrial Myocyte Preparation**

American Wistar or Sprague-Dawley rats (250–300 g) were preinjected intraperitoneally with heparin (2,000 units/kg) and then anesthetized with a lethal dose of pentobarbital (100 mg/kg). When the rats were fully anesthetized, the heart was excised and placed in a beaker of cold buffer. The aorta was trimmed at the branch and cannulated. Blood was flushed from the heart with 1.5 ml cold buffer; then the cannula was attached to a dual-channel Langendorff perfusion apparatus at a buffer flow rate of 10 ml/min. The heart was perfused with 50 ml calcium-containing buffer and then flushed with 70 ml calcium-free solution in a nonrecirculating mode. Additional perfusate (80 ml) was allowed to recirculate, and a collagenase/bovine serum albumin (BSA) stock (Worthington type II/Sigma fraction V; final concentrations, 1 mg/ml each) was added to the media. After 20 minutes of collagenase perfusion, calcium was added back to the media over three 5-minute intervals until total free calcium was 1 mM. Flow was incrementally increased up to 16 ml/min. When the back pressure on the heart began to decrease, the heart was removed from the perfusion apparatus. The atria were isolated and minced into a beaker of collagenase-containing media, placed in a shaking water bath at 37°C, and aerated with humidified 95% O₂–5% CO₂. The tissue was triturated periodically to disperse the cells. After shaking 10–15 minutes, the cells were filtered through a gauze mesh and centrifuged for 1 minute at low speed (400 rpm), and the supernatant was discarded. Cells were rinsed once with 0.5% BSA collagenase-free media, then resuspended in 4% BSA media. After the cells settled for 2 minutes, the supernatant was again discarded. Cells were then resuspended in an incubation buffer at room temperature until use (within 24 hours). The yield of rod-shaped atrial cells from this procedure was in the range of 60–80%.

**Single-Channel Recording**

Conventional single-channel recording techniques13 were used in either the cell-attached or inside-out excised-patch configuration. Single-channel currents were recorded with an Axopatch 1C patch clamp amplifier. Current tracings were filtered at 1 kHz, and both current and pressure tracings were digitized into an IBM-compatible computer at 2 kHz per channel using LabMaster DMA hardware and Axotape software (Axon Instruments, Foster City, Calif.). Single-channel patch pipettes were made from Corning 7052 glass, pulled to 2–10 MΩ resistance, and coated with Sylgard (Dow Corning, Midland, Mich.). Cell-attached and excised-patch experiments were performed at room temperature (22–24°C). Only patches with at least a 10-GΩ initial seal resistance were used for these studies. All figures (including cell-attached and excised-patch studies) are plotted using whole-cell voltage conventions (e.g., an upward current reflects the movement of cations from the inside of the cell/patch to the outside). Single-channel amplitudes were measured from current histograms (obtained with the Fetchan single-channel analysis module of pCLAMP) as the difference between the mean of the closed current peak and the peak of interest. In some cases, single-channel amplitudes were compared by fitting amplitude histograms as sums of gaussian distributions using a least-squares algorithm (in the PSTAT module of PCLAMP, for less than three open states) or fitted by eye, also as a sum of gaussian distributions, with MATHCAD 3.1 (Mathsoft). Changes in open probability were determined in patches exhibiting one or two open levels with a threshold detection program (self-written, Borland C++) that evaluated the fraction of time the current was greater than user-specified threshold value and binned that fraction into episodes delimited by changes in the pressure tracing that was simultaneously recorded.

Changes in pressure were applied to the membrane patch using a syringe attached to the sidearm port of the pipette holder and measured with a pressure transducer (Spectramed DTX) attached to a Gould RS-3200 amplifier. Negative pressure is plotted as a downward deflection in the pressure tracing.

The standard pipette solution for cell-attached and excised-patch studies contained (mM) potassium acetate 140, KCl 5, MgCl₂ 2, and HEPES 5, pH 7.4 (with NaOH). The bath solution contained (mM) potassium acetate 130, MgCl₂ 2, CaCl₂ 0.02, glucose 10, and HEPES 10, pH 7.4 (with NaOH). The osmolality of both solutions was 290–295 mosm/kg. Solutions were held in 60-ml syringes mounted above the stage; solutions flowed via gravity at 5–10 ml/min through a
six-position switch into the bath (35-mm Falcon tissue culture dishes). The chamber volume was 1–2 ml, and complete solution changes required 30–60 seconds. All single-channel experiments were performed at room temperature (20–24°C) except for those described in Figure 7.

Whole-Cell Current Measurements

Whole-cell currents were recorded from adult atrial myocytes using the nystatin-perforated patch-clamp technique.14,15 Patch pipettes were pulled from Corning 8161 glass and coated with Sylgard. Pipettes (2–4 MΩ) were dipped in nystatin-free whole-cell pipette solution and then filled with nystatin-containing whole-cell pipette solution consisting of (mM) KCl 140, Tris 10, K2-EGTA 5, and MgCl2; 2, pH 7.4 (with HEPES). Nystatin stock solutions were prepared fresh daily (6 mg in 100 μl dimethyl sulfoxide), diluted, and sonicated in the whole-cell buffer just before use. After gigaseal formation, changes in the access resistance were monitored with repetitive −10-mV test pulses. Experiments were initiated when the series resistance was stable and under 20 MΩ. Series resistance was monitored at 5-minute intervals during an experiment; cells with high series resistance values (>20 MΩ) were discarded. The holding potential during all protocols was −50 mV. The control (isotonic) bath solution was (mM) NaCl 115, glucose 10, HEPES 10, KCl 5, MgCl2, 2, CaCl2, 1, CoCl2, 1, and mannitol 50, pH 7.4 (with NaOH), with an osmolality of 290 mosm/kg. The hypotonic solution was identical in ionic strength to the control solution, except that it lacked the mannitol and had an osmolality of 240 mosm/kg. Whole-cell experiments were performed at 30°C using a microscope stage heater (20/20 Technology, Whitehouse Station, N.J.), and bathing solutions were prewarmed in jacketed water baths. Bath solutions were bubbled with 100% O2.

Materials

Pinacidil was donated by Lilly Research Laboratories, Indianapolis, Ind. ATP, glibenclamide, and nystatin were obtained from Sigma. Pipette glass was obtained from Garner Glass Co., Claremont, Calif.

Results

Mechanosensitive channel activity has been frequently observed in cell-attached patches of atrial myocytes isolated from either neonatal or adult rat atria. Since the observations were qualitatively similar in both preparations, all data presented in this report were obtained from acutely isolated adult atrial myocytes, with the exception of Figure 5. Figures 1A and 1B are typical tracings showing the responses of a cell-attached patch to the application of negative pressure in the pipette. Channel activity is sometimes present under resting conditions (as in Figure 1A) but increases significantly with the application of negative pressure. Stretch-activated channel activity has been observed in 25–30% of the cell-attached patches (n>500) under conditions in which potassium was both the major permeant cation in the patch pipette and the major cation in the bath (to null the resting potential). Since a nonspecific cation channel has been reported in other cardiac myocyte preparations,16 the selectivity of the mechanosensitive channels in neonatal atrial myocytes was examined. Changing the pipette potassium concentration from 140 to 14 mM (Figure 1A versus 1B) decreased the amplitude of the currents and shifted the current–voltage relation. Addition of 100 μM Gd3+ (which blocks nonspecific cation channels17) to the patch pipette did not block the predominant type of mechanosensitive channel activity observed in the cell-attached configuration (n=6). When the pipette solution contained isotonic Ba2+ (to facilitate detection of a divalent ion-permeable channel), no mechanosensitive currents were detected. With isotonic salts of Na+, K+, or Cs+, the stretch-activated currents were observed only in the outward direction. Figure 1C demonstrates that mechanosensitive modulation of these channels can occur over a wide voltage range. It also shows that, although the onset of the increase in activity is closely linked to the mechanical stimulus, a residual increase in channel activity sometimes persists after the removal of the stimulus. This effect is more common at extremely negative potentials.

The averaged current–voltage relation for this channel is shown in Figure 2. The single-channel conductance of this channel in the cell-attached configuration was 52±1.5 pS (mean±SEM, n=8) with 140 mM potassium acetate in the pipette (full solution compositions are listed in “Materials and Methods”). With varying concentrations of potassium in the pipette (14–140 mM), the reversal potential followed the Nernst potential for potassium (inset, Figure 2), demonstrating that the channel is highly selective for potassium ions. Because of its high density in cell-attached patches of adult atrial myocytes, subsequent work has focused on this potassium channel.

The effect of varying the pressure applied to a cell-attached patch is illustrated in Figure 3A. The fraction of time the channel was open (determined with a threshold crossing routine) and the opening rate are plotted in Figure 3B. It is apparent that both the number of openings and the open probability reversibly increase as a function of the pressure applied to the patch pipette. This type of response is typical of the pressure–activity relation observed in cell-attached patches from atrial myocytes. As is evident here, the relation between pressure applied to the patch and the probability of channel opening is typically nonlinear. The “threshold” for an increase in channel activity is typically between −5 and −10 mm Hg.

Mechanosensitive ion channels have often been assumed to comprise a unique group of channels. However, given the fact that at least six distinct potassium conductances have already been described in the cardiac myocyte,18 the properties of the channel observed were compared with other cardiac channels previously characterized. The single-channel conductance was significantly higher than the conductance for either the delayed rectifier (27 pS) or muscarinic (42 pS) K+ channels.19 The conductance and rectification properties of the atrial mechanosensitive potassium channel are nearly identical to the value reported for the KATP channel in rat ventricle20 (48–52 pS) and somewhat lower than the values reported in human atria (73 pS)19 or in guinea pig ventricle (80 pS).21 A class of potassium channels modulated by fatty acids has recently been described.22 In vascular smooth muscle cells, both fatty acids and membrane stretch have been shown to directly
activate the same large-conductance potassium channel. Two channels in this general class, one activated by arachidonic acid and the other by phosphatidylcholine, have recently been reported in neonatal atrial myocytes. The conductance of the phosphatidylcholine-activated channel (60 pS at +40 mV) is similar to

**FIGURE 1.** Tracings showing that deformation of the atrial cell membrane causes a reversible increase in channel activity. In these cell-attached patches from acutely isolated adult atrial myocytes, negative pressure pulses were applied to the patch pipette with a syringe. In both panels A and B, the cellular resting potential was nulled with a bath containing 130 mM potassium acetate. In panel A, the patch pipette contained 140 mM potassium acetate (full solution compositions are listed in "Materials and Methods"), and the potential across the patch was +40 mV. Upward current deflection reflects potassium efflux from the cell. Under these depolarizing conditions, variable degrees of spontaneous ATP-sensitive potassium channel activity are observed; in this patch, one channel was spontaneously active most of the time, and a second was activated by the application of negative pressure. In panel B, the patch pipette contained 14 mM potassium acetate, and the patch potential was 0 mV. If the channel was not selective between cations, no current would be expected at this potential. Panel C shows voltage dependence of the atrial mechanosensitive potassium channel. These tracings were sequentially obtained from a cell-attached patch of an adult atrial myocyte. The test potential is indicated on the left, and the period of mechanosensitive stimulation is indicated by the bar over each tracing. The mean negative pressure applied during the bar was 10±0.5 mm Hg. As above, the bath and pipette solutions contained potassium acetate. The single-channel conductance in this patch was 50.5 pS (single-channel amplitudes were calculated from current histograms, as described in "Materials and Methods").

**FIGURE 2.** Graph showing single-channel current-voltage relation for the predominant mechanosensitive ion channel in adult atrial myocytes, recorded in the cell-attached patch configuration. The curves plotted are the mean values at each concentration. The mean slope conductance with 140 mM potassium acetate in the pipette was 52±1.5 pS (mean±SEM, n=8) between the potentials of −100 and −20 mV. The inset plots the reversal potential as a function of the pipette potassium concentration. Each data point represents the mean±SEM for the potassium concentration listed. The number of experiments for each concentration of pipette potassium was as follows: 14 mM, n=6; 28 mM, n=3; 70 mM, n=8; and 140 mM, n=8. The curve plotted is the predicted relation for a (Nernstian) potassium-selective channel, assuming that the cytosolic potassium concentration is the same as the bath concentration (130 mM). No correction for ionic activity (versus concentration) was used in generating the curve.
that of the mechanosensitive channel observed in the present study.

The inside-out excised-patch technique was used to clarify the identity of the most frequently encountered mechanosensitive K⁺ channel. Immediately on excision, activity of the K_{ATP} channel became evident. When 1 mM ATP was added to the bathing solution, most of the spontaneous channel activity in the patch was suppressed; application of negative pressure to an excised patch under these conditions causes several of the channels that were closed by ATP to reopen during the negative pressure. The number of channels that respond varies with the amount of pressure applied to the patch. Pressures of −5 to −25 mm Hg were typically applied in these studies to avoid membrane breakdown and excessive mechanical artifacts on the current tracing. To obtain a conventional whole-cell recording configuration from a cell-attached patch, rupturing pressures typically range from −100 to −200 mm Hg.

To confirm that the mechanosensitive currents were derived from the same channel exhibiting spontaneous activity, the top panel of Figure 4 illustrates a current recording in which 10-second data segments were trace-evoked (+) or spontaneously active (−); the amplitude histogram for these segments is shown in the bottom panel of Figure 4. This recording was made 4 minutes after excision, a time when most of the original channel activity had declined, although the channels could again be stimulated to open by the application of negative pressure. “Rundown” is a hallmark of K_{ATP} channel activity. Under the experimental conditions used here, spontaneous activity usually decayed to low levels within 2 minutes in the absence of ATP in the bath solution, although the channel could be mechanically stimulated to open for at least 10–15 minutes after that time. Acetate has been used as the dominant anion in these experiments to avoid measuring chloride currents. It is suspected that the anion in the bathing media may have an influence on the rundown of the channel, although this has not yet been thoroughly investigated.

Although only one to three channels remained simultaneously open in the absence of negative pressure, it is clear from the current tracing that there were at least five channels in the patch and that application of suction substantially increased the probability of multiple simultaneous openings. When the current histograms from both periods are fit as sums of gaussian curves restricted to multiples of a unit open-channel amplitude, it is apparent that the channel activated by negative pressure is the same one that was spontaneously open (Figure 4, bottom panel).

In similar excised-patch experiments, further confirmation of the identity of the channel was obtained by noting that spontaneous channel activity could be consistently and completely inhibited by perfusion of the patch with physiological levels of ATP (5 mM). This concentration of ATP also blocks most of the mechanosensitive channel activity. Because of the high density of K_{ATP} channels in adult atrial myocytes, it is difficult to determine the relation between [ATP] and the mechanosensitivity of the channel. Because the density of K_{ATP} channels is significantly lower in neonatal myocytes, the dose–response relation between channel open probability and [ATP] has been explored in neonatal atrial cells in which the maximum number of simultaneous channel openings observed was never greater than two. Raw current recordings of a neonatal single-channel patch that survived an entire [ATP] versus pressure dose–response series are shown in Figure 5A. The channel open probability from this experiment at each [ATP] is plotted in Figure 5B. As in the adult myocyte studies, the pressures applied were not greater than −30 mm Hg, to avoid rupturing the patches during the experiment. In eight neonatal myocytes studied over the range of 0.1–5 mM ATP, 1 mM ATP caused approximately a 50% decrease in the maximum open probability obtained at −20 to −30 mm Hg.
The sulfonamide antidiabetic drugs (e.g., tolbutamide and glibenclamide) have specific binding sites on rat heart sarcolemmal membranes; these sites are either on or associated with the K\textsubscript{ATP} channel.\textsuperscript{25} Figure 6 shows that 100 \mu M tolbutamide can block both the spontaneous and stretch-evoked activation of the K\textsubscript{ATP} channel. Glibenclamide has similar effects at concentrations of approximately 1 \mu M, although its block of both the spontaneous activity and of the mechanosensitive activity occurs significantly more slowly.

On the basis of the single-channel conductance, ionic selectivity, and sensitivity to inhibition by both ATP and the sulfonamide drugs, I conclude that one of the dominant mechanosensitive ion channels in atrial myocytes is the K\textsubscript{ATP} channel. These single-channel experiments demonstrate that the K\textsubscript{ATP} channel is mechanosensitive in both the cell-attached and inside-out patch configurations. Further confirmation of this identification has been obtained in experiments in which the effects of pinacidil, a relatively selective K\textsubscript{ATP} channel agonist, have been investigated. The effectiveness of pinacidil is dependent on the cytosolic [ATP], with the drug more effective at lower [ATP].\textsuperscript{26} Figure 7 illustrates an adult cell patch bathed in 1 mM ATP, in which most of the basal channel activity was suppressed, but mechanosensitive stimuli could still evoke a measurable increase in channel activity. Addition of pinacidil at concentrations insufficient to modulate the channel open probability on its own potentiated the mechanosensitive responses of the patch, more than doubling the open probability at a pressure of approximately −20 mm Hg. This type of response was observed in four of seven patches.

Morris and Horn\textsuperscript{37} reported an inability to demonstrate macroscopic currents, which were expected because of previous single-channel studies in neuronal growth cones. They suggested that the mechanosensitivity of ion channel gating might be an artifact of the single-channel recording technique. In an attempt to address this possibility and determine whether or not whole-cell K\textsubscript{ATP} channel currents could be mechanically induced, the perforated-patch technique\textsuperscript{14,15} (which maintains the integrity of cytosolic regulatory components) was used to record whole-cell currents from adult atrial myocytes perfused with either isotonic or hypotonic solutions of constant ionic composition. This technique is adequate for recording and controlling the voltage-dependent whole-cell currents in atrial myocytes, as assessed by control experiments (data not shown). In an attempt to begin to examine the effects of mechanosensitive stimulation on whole-cell currents, repetitive voltage-ramp currents (−100 to +50 mV over 10 seconds, repeated at 30-second intervals) were recorded from myocytes that were bathed in either isotonic (290 mosm/kg) or hypotonic (240 mosm/kg) solutions of the same ionic composition, both containing glucose and bubbled with oxygen to ensure the metabolic viability of the cell. Sustained superfusion of the myocyte with a hypotonic solution causes an increase in both the holding and ramp-evoked currents at potentials above the potassium reversal potential (Figure 8A).

To confirm that hypotonic swelling–induced current was due to activation of the K\textsubscript{ATP} channel, the difference

![Figure 4](http://circres.ahajournals.org/)

**Figure 4.** Tracings (top panel) and histogram (bottom panel) showing that mechanosensitive K⁺ channel activity is preserved in excised patches. While spontaneous activity appears, application of negative pressure increases the number of channels simultaneously open. **Panel A:** This 128-second current and patch pressure tracing is from an adult atrial myocyte, beginning 4 minutes after excision, after the spontaneous channel activity had disappeared. Potassium acetate pipette and bath solutions were used, as in Figure 1. The patch potential was −40 mV. Neighboring 10-second current segments (either stretch-evoked [+] or spontaneously activated [−]) were chosen for the amplitude histograms. **Panel B:** All-point current histogram for the stretch-evoked (+) and spontaneous (−) current segments above. The bin width was 0.2 pA. Pressure and current tracings were each sampled at 2 kHz; thus, each current segment corresponds to a total sample of 20,000 points. The histogram values for the spontaneous activity are plotted as filled squares; the values obtained from the period of negative pressure are plotted with open squares. Up to five distinct open-channel amplitude peaks can be resolved in the stretch-evoked (pressure, −20 mm Hg) amplitude histogram, whereas only two open-channel peaks are observed in the spontaneously active current histogram. The histograms were fitted as sums of gaussians (with MATHCAD 3.1), restricted to multiples of a unitary amplitude of −2.25 pA. The spontaneous activity is fit with a solid line; the stretch activity is fit with a dashed line.
currents (hypotonic minus control) generated by hypotonic stimulation were compared with the difference currents recorded when the myocyte was superfused with pinacidil (Figure 8B). The current–voltage curves stimulated by either pinacidil or hypotonic swelling had the same shape, and both could be blocked by the addition of 1 μM glibenclamide to the bath solution (Figure 8C). Results like these were observed in nine cells at 30°C using the perforated-patch technique, although at higher temperatures (36–37°C) the cell frequently burst and died (four of five cells) when glibenclamide was applied after hypotonic stimulation. These results suggest that activation of the KATP channel may be an additional compensatory mechanism in the volume regulation process.

Discussion

The single-channel recording studies demonstrate that the gating of the atrial KATP channel can be modulated by mechanical stimuli. Given the range of factors that can modulate the activity of the KATP channel, the involvement of the channel in normal cardiac physiology is still the subject of some debate. In addition to the [ATP] in the cell, these factors include the [ADP], the [ATP]/[ADP] ratio, pH, G protein interactions, and protein kinase C activity. The present results demonstrate that deformation of the cell membrane is an additional modulator of KATP channel activity. Whether the deformation directly changes the conformation of the channel or acts indirectly through activation of another modulatory system (e.g., protein kinase C or a phospholipase) has not yet been established. The fact that not all increases in channel activity immediately return to baseline on release of the pressure stimulus suggests that the latter indirect pathway may be involved. Whatever the molecular mechanism, these results demonstrate that mechanosensitive modulation of KATP channel activity complements the other known pathways and may under some circumstances dominate the regulation of channel activity. It is interesting to note that, although it has not been systematically explored, mechanosensitive modulation of single KATP channel activity has also been observed in pancreatic beta cells (S. Misler, personal communication), suggesting that this phenomenon may not be unique to atrial myocytes. Further studies in other cell types will be needed to determine how ubiquitous this phenomenon is.

Structure–function studies have not yet determined whether the sulfonylurea-binding protein and the channel protein are the same or whether they are closely associated independent subunits. In the present study, the sulfonylurea drugs glibenclamide and tolbutamide were able to decrease or eliminate the mechanosensitive stimulation of KATP channel activity. Thus, it appears that the functional relation between the sulfonylurea-binding protein and the channel remains intact. The mechanism by which pinacidil interacts with the KATP channel is still unknown. A better understanding of the regulation of this channel will evolve once its structure is better understood. At the most naive level, mechanosensitive stimulation favors the transition from the...
with which mechanosensitive modulation is detected; the phenomenon is most frequently observed when using thin-walled hard glasses. In view of the observation of Morris and Horn, who suggested that the mechanosensitive modulation may be an artifact of the single-channel recording configuration, it was important to ascertain whether the single-channel currents observed had a whole-cell current correlate. The "threshold" pressure for channel activation in these studies was much lower (from -5 to -10 mm Hg) than the threshold for activation of the stretch-activated potassium channel in *Lymnaea* neurons (typically ranging from -30 to -50 mm Hg), suggesting that the ion channels in the atrial myocyte are more sensitive to deformation, but comparison of pressures applied in different experimental systems may not be valid. Indeed, correlating the pressure applied to the patch pipette in a single-channel recording configuration with any estimate of membrane tension is impossible without visualizing the patch. This was not possible in the present experiments.

The hypotonic swelling experiments demonstrate that there is a consistent, rapid activation of whole-cell difference currents that can be attributed to K$_{ATP}$ channel activation. This is evidence that swelling, per se, is an adequate stimulus to activate whole-cell K$_{ATP}$ channel currents. Although the experimental conditions were designed to supply adequate metabolic substrates (10 mM glucose, bubbling the bath with 100% O$_2$), the perforated-patch technique does not clamp cytosolic [ATP], an important variable in K$_{ATP}$ channel regulation. The alternative whole-cell clamp configuration is not a more attractive experimental alternative, because of the inherent dialysis of all of the other modulators of the K$_{ATP}$ channel.

Thus, the perforated-patch studies demonstrate that a mechanical stimulus (cell swelling) can generate whole-cell currents by activation of the same channels that can be activated in a mechanosensitive fashion using single-channel techniques. These results suggest that mechanosensitive stimulation is physiologically important as a means of activating whole-cell K$_{ATP}$ channel current in atrial myocytes. More detailed studies are in progress to quantify the relation between the degree of cell swelling and the activation of K$_{ATP}$ channel current.

The hypotonic swelling experiments performed suggest that a significant fraction of the K$_{ATP}$ channels in a myocyte are activated by the swelling process. The whole-cell current (I) is the sum of the activity of all of the individual ion channels in the cell: $I = \gamma \cdot N \cdot P_o$, where $\gamma$ is the single-channel current, $N$ is the total number of channels, and $P_o$ is the single-channel open probability. If one assumes a uniform channel density of one channel per square micrometer, a 60-pF cell (typical for an adult atrial myocyte) would possess approximately 6,000 K$_{ATP}$ channels. Under the experimental conditions used for the swelling experiments (low extracellular potassium), the single-channel conductance of the K$_{ATP}$ channel is reduced, probably to the range of 30-40 pS, which would result in $\gamma$ of approximately 1 pA (at -50 mV). Assuming this conductance, a holding current of 300 pA would correspond to a product ($N \cdot P_o$) of 300. In the worst case ($P_o = 1$), this would reflect the simultaneous total activation of approximately 300 channels, or 5% of the total K$_{ATP}$ channels.
FIGURE 7. Tracings (panel A) and graph (panel B) showing that pinacidal (PIN) potentiates the mechanosensitive modulation of the ATP-sensitive potassium (K<sub>ATP</sub>) channel. The series of tracings in panel A were sequentially obtained from the same inside-out adult atrial myocyte patch at a potential of +60 mV. Most of the spontaneous channel activity was suppressed by bathing the patch in 1 mM ATP, which was added at the beginning of the first tracing and then was present throughout the experiment. The temperature in the bath was maintained at 30°C with a thermal stage controller. The tracings on the right are excerpted from the tracings on the left during the final pressure step, showing that the currents in all cases were derived from the same channel type. The excerpted first tracing on the right (pre-ATP) was obtained in the first 10 seconds of the top sweep at the left, before the added ATP had reached a steady-state block of the channel. Addition of ATP greatly decreases the mean open time of the channel. Mean open probability values (or fractional open times) were derived as previously described (e.g., see Figure 2). The values obtained during each pressure step are plotted in panel B. The pressures and open probabilities plotted are the means for each period. The maximum open probability more than doubled at both concentrations of PIN and was readily reversed on washout.

Since P<sub>o</sub> for any individual channel is unlikely to approach unity with a stimulus as uniformly distributed as hypotonic swelling, a whole-cell current of this magnitude is likely to reflect the activation of an even greater fraction of the channels in the myocyte. A reversible dilution of cytosolic ATP by swelling would be an alternative explanation for the increased current. This seems unlikely, since, with an effective half-maximal inhibitory concentration (K<sub>i</sub>) for channel inhibition by ATP in the range of 100 μM, doubling of cell volume (a worst-case scenario) would likely decrease the [ATP] from 5 to 2.5 mM, with a constant ratio for [ATP]/[ADP]. This would not account for activation of 5% of the available channels. Further studies, using alternative means of deforming the myocyte, will be required to clarify the types of stimuli that are capable of generating whole-cell mechanosensitive currents resulting from the activation of either the K<sub>ATP</sub> channel or any other mechanosensitive channel.

In view of the observed developmental differences in K<sub>ATP</sub> channel density between neonatal and adult atrial myocytes, as well as reported changes in [ATP] sensitivity of the K<sub>ATP</sub> channel in hypertrophied myocytes, it is tempting to speculate that channel density may be related to cell diameter, with more channels present at higher membrane tensions; however, further studies are required to evaluate this and other questions about the physiological and pathophysiological significance of mechanosensitive activation of the cardiac K<sub>ATP</sub> channel.

Regardless of the stimulus (depletion of [ATP], channel agonist, or deformation of the cell membrane), K<sub>ATP</sub> channel activation will hyperpolarize the myocyte, shorten the action potential, decrease calcium influx, and thus reduce myocyte excitability and energy consumption. These effects are likely to account for the protective role of K<sub>ATP</sub> channel activation in states of ischemia or hypoxia. Several studies have demonstrated that, if the heart is subjected to brief periods of ischemia, the muscle tissue is protected from subsequent, more severe insults. A body of literature is accumulating that suggests that it is activation of the K<sub>ATP</sub> channel during the initial brief ischemic period that subsequently reduces the energy demand and mediates the protective effect. The experimental ischemia (caused by occlusion of an artery) causes a transient but profound dilation of the heart. An intriguing recent report suggests that dilation of the heart alone, in the absence of ischemia, is enough to reduce the infarct area by 50%. The results presented above suggest that mechanosensitive activation of the K<sub>ATP</sub> channels during the dilation may mediate this effect.

Abrupt dilation of the intact human atria with an early stimulus during pacing increases atrial pressure and decreases both the effective and absolute refractory period. This observation is most consistent with the mechanosensitive activation of a repolarizing current, such as K<sub>ATP</sub>, rather than a nonselective cation channel current, which would be expected to exert a further...
depolarizing influence, lengthening both the action potential duration and the refractory period.

Although the present study has focused on the mechanosensitive modulation of $K_{ATP}$ channel activity, it is important to note that in the course of this work I have detected several other mechanosensitive ion channels, and their characterization will require further study. In addition, a nonselective cation channel, with a conductance of 21 pS, has recently been identified in neonatal atrial myocytes. No mention was made with respect to the distribution of this channel in adult myocytes. Another recent report has very clearly documented the presence of mechanosensitive single-channel currents and the contribution of that nonspecific cation conductance to whole-cell mechanosensitive currents in vascular smooth muscle cells. In addition, chloride currents, which are of key importance in myocyte volume regulation, have been demonstrated to be stimulated by cell swelling in canine atrial myocytes, thus suggesting that mechanosensitive chloride channels may exist as well. The experiments described above, using acetate as the dominant anion, would not have detected these currents.

**FIGURE 8.** Tracings showing that swelling and pinacidil stimulate similar whole-cell voltage-ramp currents in an adult atrial myocyte. The perforated-patch technique was used to monitor the whole-cell currents. The cell was initially bathed in an isotonic (control) media at 30°C and maintained with a thermal stage controller. Solutions were gassed with 100% O$_2$. The initial cell capacitance was 52 pF, and the series resistance 8 MΩ. Series resistance and capacitance were both evaluated at 5-minute intervals and did not change significantly over the course of the experiment. All tracings are from the same cell. Panel A: Ramp currents were recorded for 10 seconds at 30-second intervals during the course of the experiment, sampling the current at 200 Hz. The potential was stepped from the holding potential of $-50$ to $-100$ mV for 1 second and then ramped up to $+50$ mV over 10 seconds, using pCLAMP 5.51 software and LabMaster hardware (Axon Instruments, Foster City, Calif.). Raw tracings in the presence of control (290 mosm/kg) or hypotonic saline (240 mosm/kg) are presented and show the effect of hypertonic treatment on the current elicited by the ramp protocol. Panel B: Difference currents obtained by subtracting the control ramp current from either the hypotonic solution or the pinacidil (10 μM)-evoked ramp currents are shown. The difference currents elicited by either stimuli exhibit identical current–voltage shapes, as well as similar reversal potentials and peak amplitudes. Panel C: Steady-state $(-50$ mV) $ATP$-sensitive potassium ($K_{ATP}$) channel activity was monitored by tracking the holding current in the perforated patch mode (top tracing). Hypo, hypotonic saline; Glib, glibenclamide. This potential is more positive than the potassium reversal potential (−84 mV), whereas the current at −100 mV (lower tracing, measured at the initial negative deflection) is below the reversal potential. In the absence of a $K_{ATP}$ channel agonist or hypertonic stimulation, both the holding current and ramp-evoked currents frequently remained stable in the perforated-patch configuration for more than 1 hour.

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