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Membrane Stretch Augments the Cardiac Muscarinic K^+ Channel Activity

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Abstract. Arachidonic acid has been shown to activate K^+-selective, mechanosensitive ion channels in cardiac, neuronal and smooth muscle cells. Since the cardiac G protein (G_K)-gated, muscarinic K^+ (K_{ACh}) channel can also be activated by arachidonic acid, we investigated whether the K_{ACh} channel was also sensitive to membrane stretch. In the absence of acetylcholine (ACh), K_{ACh} channels were not active, and negative pressure failed to activate these channels. With ACh (10 μM) in the pipette, applying negative pressure (0 to −80 mm Hg) to the membrane caused a reversible, pressure-dependent increase in channel activity in cell-attached and inside-out patches (100 μM GTP in bath). Membrane stretch did not alter the sensitivity of the K_{ACh} channel to GTP. When G_K was maximally activated with 100 μM GTPγS in inside-out patches, the K_{ACh} channel activity could be further increased by negative pressure. Trypsin (0.5 mg/ml) applied to the membrane caused activation of the K_{ACh} channel in the absence of ACh and GTP; K_{ACh} channel activity was further increased by stretch. These results indicate that the atrial muscarinic K^+ channels are modulated by stretch independently of receptor/G protein, probably via a direct effect on the channel protein/lipid bilayer.

Key words: Mechanosensitivity — Arachidonic acid — Muscarinic K channel — Pressure — G protein

Introduction

Acetylcholine binds to muscarinic receptors in atrial cells and activates an inwardly rectifying K^+ channel (K_{ACh} channel) via a GTP binding protein referred to as G_K [1]. Other receptor agonists such as phenylephrine [14], and platelet-activating factor [18] have been reported to activate the K_{ACh} channel as a result of stimulation of phospholipase A_2 and formation of arachidonic acid and its metabolites [18, 24]. Activation of the atrial K_{ACh} channel by direct application of arachidonic acid (or its metabolites) has also been demonstrated [9, 13]. The mechanism of activation of the K_{ACh} channel by arachidonic acid or its metabolites is not clearly known.

In addition to activation of the K_{ACh} channel in atrial cells, arachidonic acid has been shown to activate K^+-selective ion channels in cardiac muscle cells [7], smooth muscle cells [11] and neuronal cells [10, 20]. In cardiac and smooth muscle cells, the K^+ channel could also be activated by other types of free fatty acids such as myristic or linoleic acids which are not substrates for arachidonic acid-metabolizing enzymes (i.e., lipoxygenases). This suggests that the K^+ channel is directly modulated by the fatty acid itself [19]. Interestingly, the K^+ channels modulated by arachidonic acid were also found to be sensitive to membrane stretch [7, 11, 30]. In these electrophysiological studies, application of negative pressure to the membrane patch via the pipette caused an increase in K^+ channel activity in a reversible, pressure-dependent manner. These findings suggest that certain K^+ channels modulated by arachidonic acid (and perhaps other fatty acids) could also be sensitive to membrane stretch.

As the K_{ACh} channel is able to be activated by arachidonic acid, we tested whether the K_{ACh} channel was also sensitive to membrane stretch. We used cell-attached and inside-out patches from neonatal and adult rat atrial cells to study whether the membrane stretch produced by negative pressure applied via the patch pipette would augment the open probability of the K_{ACh} channel under different experimental conditions. Since the channel is normally activated through the receptor-coupled G protein pathway, we examined the possible alteration in G protein-K_{ACh} channel interaction, and the involvement of the G protein in the stretch-induced changes in K_{ACh} channel function.

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Materials and Methods

Cell Preparation

Hearts from 1–2-day-old newborn rats (Sprague-Dawley) were dissociated with collagenase and trypsin [6]. Rats were rapidly decapitated, and right and left atrial tissues from whole hearts were excised and placed in Ca^{2+}-free Hank's medium (Sigma). The tissues were then cut into small pieces (<1 mm^3) with a sharp blade, and placed in Hank's balanced salt medium containing 0.015% collagenase type II and 0.09% trypsin (Worthington). Tissues were incubated at 37°C and agitated for 7 min. Suspended cells were then removed and added to an equal volume of 50% fetal calf serum to inhibit the activity of the enzymes. Remaining tissues were incubated in a fresh enzyme solution and allowed to dissociate for another 7 min. This procedure was repeated 5 times. Dissociated cells were collected, centrifuged, and placed in the growth medium consisting of culture medium (Dulbecco's Modified Eagle's Medium; Sigma), 10% fetal calf serum, and 0.1% penicillin-streptomycin. Cells were plated on glass coverslips and placed in a 37°C incubator gassed with 5% CO_2/95% air for 18–24 hr before being used. Single atrial cells of adult rats (200–300 g; Sprague-Dawley) were prepared by enzymatic digestion as described previously. After anesthesia with ether, hearts were removed and retrogradely perfused via the aorta in a Langendorff apparatus with 0.05% collagenase (type II, Worthington) and 0.03% hyaluronidase (Sigma) in Ca^{2+}-free bicarbonate-buffered physiological solution for 45 min. Atrial 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 ~24°C in oxygenated (95% O_2/5% air) atmosphere. All cells used in the experiments were of elongated shape with no visible signs of contracture or blebs. Bicarbonate-buffered solution contained (mM) NaCl 118, KCl 4.7, $MgSO_4$ 1.2, KH_2PO_4 1.2, $NaHCO_3$ 25, pyruvate 10, and glucose 10. All cells were used within 6 hr.

Electrophysiology

Gigaseals were formed using Sylgard-coated thin-walled borosilicate pipettes (Kimax) with 2–5 megaohm resistances [4]. Channel currents were recorded with an Axopatch 1D patch clamp amplifier, digitized with a PCM adapter (VR10, Instrutech, Elmont, NY), and stored on videotape using a videotape recorder (JVC). The recorded signal was transferred directly in digital form to an Atari ST computer using the VCATCH program. Continuous single-channel currents were then analyzed with the TAC program. The built-in Gaussian digital filter was set at 2 kHz (equivalent to a Bessel filter with this −3dB bandwidth). At this setting of 2 kHz, the minimum detectable event duration is ~80 μsec (1/6fc) when the 50% threshold detector is used. After obtaining all open time events, durations less than 100 μsec were deleted for plotting the open time histogram. Data were analyzed to obtain duration histogram, amplitude histogram and channel activity (averaged NP_o) using the analysis protocol described by Sigworth & Sine [25]. N is the number of channels, and P_o is the probability of a channel being open. Current tracings shown in figures were filtered at 100 Hz.

Solutions and Materials

The pipette and bath solutions contained (in mM): 140 KCl, 2 $MgCl_2$, 10 HEPES and 5 EGTA (pH 7.2). To change solutions perfusing the cytosolic surface of the inside-out patches, the pipette with the attached membrane was brought to the mouth of the polypropylene tubing through which flowed the desired solution at a rate of ~1 ml/min. To study the effect of pressure on channel activity, the negative pressure in the pipette was produced using a tubing with one end connected to the patch pipette and the other end to a mercury manometer. Rapid changes in pipette pressure could be achieved to the desired levels by operating a calibrated syringe attached to the tubing between the pipette and the manometer. Acetylcholine, GTP, GTPγS, and ATP were purchased from Boehringer Mannheim Chemicals (Indianapolis, IN). Trypsin (Type II) was purchased from Sigma Chemical Co. (St. Louis, MO). All experiments were performed at 24–26°C. All values are represented as mean ± SE.

Results

Effect of Stretch on K_{ACh} Channel Activity

Cell-attached patches were used first to study the effect of membrane stretch on the K_{ACh} channel activity. ACh (10 μM) was added to the pipette solution to activate the K_{ACh} channels. With 140 mM KCl in the bath solution (reversal potential, ~0 mV), cell membrane potential was held at −70 mV to record inward currents. Patches containing only the K_{ACh} channels were chosen. The resting (I_{K1}) and ATP-sensitive K channels were present in less than half of the patches, and their presence could be easily discerned from their markedly different single-channel conductances (25 and 80 pS). The channel activity in the cell-attached state was allowed to reach a relatively steady-state level (~30 sec) at the atmospheric pressure in the pipette (0 mm Hg). Negative pressure was then applied to the membrane via the pipette (−60 mm Hg) and monitored using a mercury manometer. As shown in Fig. 1, negative pressure produced a marked increase in K_{ACh} channel activity. The single-channel conductances before and during pressure application were 35 ± 1 and 36 ± 1 pS, respectively (n = 3), indicating that we were studying the same K_{ACh} channels (*see below*). The channel activity (NP_o), averaged every second, was plotted as a function of time as illustrated in Fig. 1*B*, clearly showing the pressure-induced increase in channel activity during pressure application.

The effect of a range of negative pressures on channel activity was determined in cell-attached patches. Percent changes in channel activity were plotted as a function of negative pressure in Fig. 2. The K_{ACh} channel activity in cell-attached patches at the atmospheric pressure (0 mm Hg) was 0.17 ± 0.01 (n = 7). At −20 mm Hg, no significant change in channel activity was present ($P > 0.05$). Above −20 mm Hg, increases in negative pressure caused a significant ($P < 0.05$) and progressive augmentation of K_{ACh} channel activity. We were unable to obtain the results at levels beyond −80 mm Hg as every patch either broke or was leaky such that channel analysis could not be done.

During the course of these studies, we observed two other stretch-activated channels that have been described previously: a K^+-selective and a nonselective cation channels [7, 8]. Due to their distinctly different kinetics from those of the K_{ACh} channel, we were able to clearly

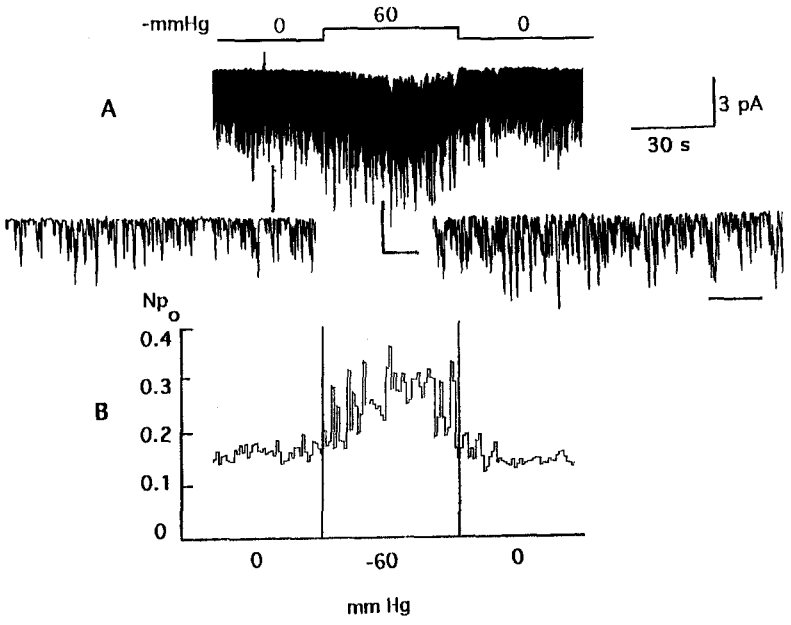

Fig. 1. A current tracing from a cell-attached patch showing a reversible increase in K_{ACh} channel activity by membrane stretch. Cell-attached patch was formed with 10 μM ACh in the pipette. The cell membrane potential was held at −70 mV to record inward currents. (*A*) At steady-state, negative pressure (−60 mm Hg) was applied to the patch via pipette and held for ~1 min. Tracings at expanded time scale are also shown. Bar on the right indicate 200 msec. (*B*) Channel activity shown in the top tracing was averaged every second and plotted as a function of time.

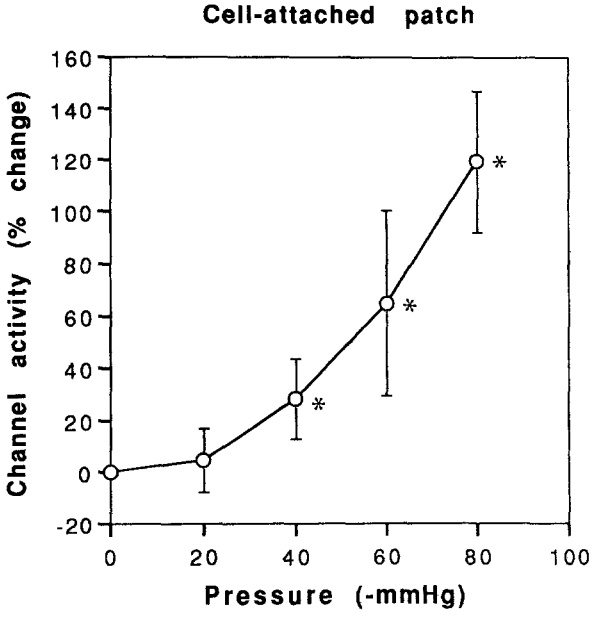

Fig. 2. A graph showing the relationship between negative pressure applied to the membrane and percent increase in K_{ACh} channel activity (Np_o) above control in cell-attached patches. Control value is the channel activity at 0 mm Hg. The experiment was done at the cell membrane potential of −70 mV. Data were obtained from ~30 sec of current tracing. Each point was the mean of 5–7 values, and the vertical lines indicate standard error. Asterisk (*) indicates significant difference from the control value observed at 0 mm Hg ($P < 0.05$).

distinguish each channel type (*see* Discussion). For the sake of clarity, we have not used patches containing these channels which appeared in ~30% of the patches.

To determine whether we could also observe augmentation of K_{ACh} channel activity by negative pressure in inside-out patches, K_{ACh} channels were activated with 10 μM ACh in the pipette and 100 μM GTP applied to the cytoplasmic side of the membrane. Figure 3 shows the pressure-dependent increase in K_{ACh} channel activity where the negative pressure applied to the membrane was increased in stepwise manner from 0 to −100 mm Hg in an inside-out patch. From the channel openings shown in expanded time scale (Fig. 3*B*), it is clear that stretch caused an increase in K_{ACh} channel activity in a reversible manner. Amplitude histograms showed that all openings were multiples of the single-channel amplitude. Thus, the amplitudes were 2.2 ± 0.1 pA at 0 mm Hg, and 2.3 ± 0.1 pA, 4.6 ± 0.2 pA and 7.0 ± 0.3 pA at −100 mm Hg (n = 4 each; Fig. 3*C*).

The percent changes in channel activity as a function of negative pressure from measurements in inside-out patches were plotted in Fig. 4. In these inside-out patches, the K_{ACh} channel activity at the atmospheric pressure (0 mm Hg) was 0.23 ± 0.02 (n = 7). Unlike cell-attached patches, some inside-out patches were able to withstand the −100 mm Hg pressure without breaking, thereby permitting analysis of channel activity at this pressure. Due to a large variation in channel activity at high pressure, channel activity at −100 mm Hg was not significantly different from that at −80 mm Hg ($P > 0.05$). The stimulatory effect of membrane stretch on the K_{ACh} channel was present as long as the pressure was maintained (~3 min). In control patches before pressure application, the mean open times in cell-attached and inside-out patches were 0.9 ± 0.1 and 1.0 ± 0.1 msec (n = 6), respectively. The mean open time tended to increase slightly during pressure applications. At −80 mm Hg, the mean open time of K_{ACh} channel openings in inside-out patches was 1.2 ± 0.1 msec (n = 5), a value significantly greater than that at 0 mm Hg ($P < 0.05$). The effect of pressure (0 to −80 mm Hg) on the mean

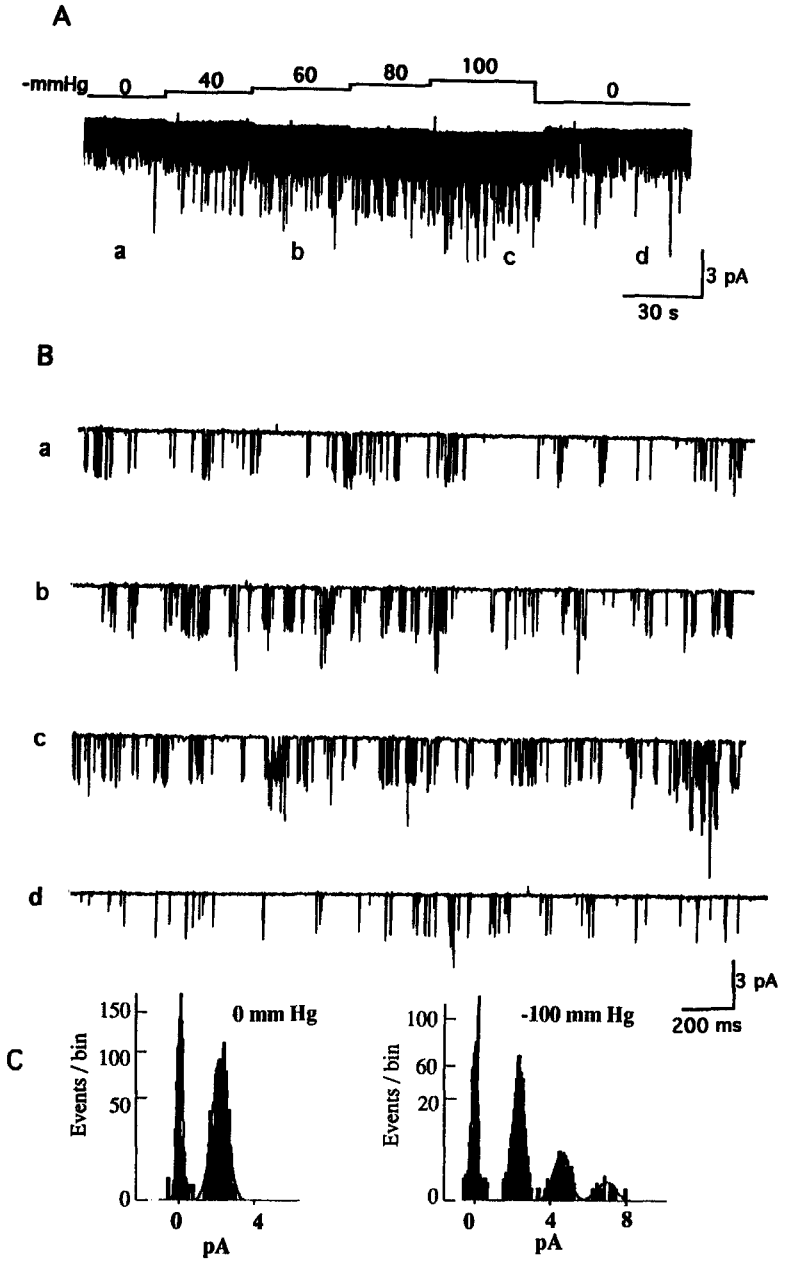

Fig. 3. A current tracing showing the effect of stepwise increases in pressure on K_{ACh} channel activity. (*A*) Inside-out patches were formed with 10 μM ACh in the pipette and 100 μM GTP in the bath. The cell membrane potential was clamped at −80 mV. When the level of activity at zero pressure was at steady state, negative pressure was applied to the patch membrane for ~30 sec. The negative pressure was decreased in 20 mm Hg increments to −100 mm Hg and then returned to zero level. (*B*) Channel openings at expanded time scale are shown for pressure levels at zero, −60, −100 and at zero again. (*C*) Amplitude histograms obtained from channel openings observed at 0 and −100 mm Hg. *See* text for details.

open time was ~20% or less both in cell-attached and inside-out patches. Therefore, the stretch-induced increase in channel activity was due primarily to an increase in the frequency of channel opening. These results obtained from cell-attached and inside-out patches demonstrated that the K_{ACh} channel activity could be increased by stretch.

The increase in K_{ACh} channel activity by stretch is unlikely to be due to an increased number of channels in the patch membrane produced by negative pressure. The total number of any channels should remain constant in excised, inside-out patches, although the total membrane patch area may be enlarged [26]. Also, in many experiments, the maximum observed number of channels in a given patch of membrane before, during and after the application of negative pressure remained the same. For example, in Fig. 3, simultaneous opening of three channels (marked by solid circles) can be seen during each of the pressure steps. Stretch simply increased the number of multiple openings (two and three channels) but did not produce higher levels of opening.

Effects of Stretch on the ATP-modified K_{ACh} Channel

In earlier studies, the open time duration of K_{ACh} channels was shown to be prolonged by treatment of the

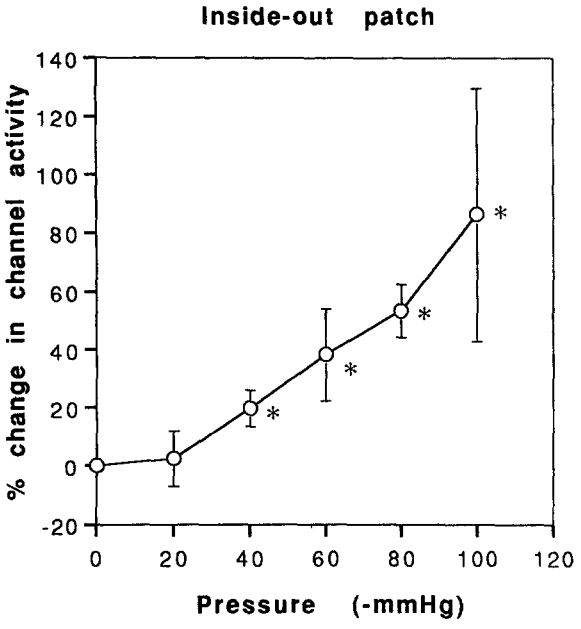

Fig. 4. A graph showing the relationship between negative pressure applied to the membrane and the percent increase in K_{ACh} channel activity above control (zero pressure) in inside-out patches. The cell membrane potential was −70 mV. Data were obtained from ~30 sec of current tracings. Each point is the mean of 5–7 values, and the vertical lines indicate standard error. Asterisk (*) indicates significant difference from the control value observed at 0 mm Hg ($P < 0.05$).

cytoplasmic side of the membrane with ATP [6]. To study whether the K_{ACh} channel in a different kinetic conformation (i.e., with longer open time duration) was also sensitive to membrane stretch, inside-out patches were perfused with 100 μM GTP and 1 mM ATP for ~2 min. Channel openings at steady state in control conditions (0 mm Hg) with ATP in the bath solution are shown in Fig. 5*A*. Channel activity at this state was 0.32 ± 0.12 (n = 4). When the pressure in the pipette was decreased to −80 mm Hg, the channel activity increased markedly ($NP_o = 0.65 \pm 0.23$). Changing of the pressure back to 0 mm Hg caused the channel activity to return to the original control values ($Np_o = 0.29 \pm 0.15$). Amplitude histograms obtained from such channel recordings showed that currents were multiples of the single channel amplitude (2.2 ± 0.1 pA), and therefore that only the K_{ACh} channels were present. Thus, regardless of the kinetic state of the channel, these results showed that membrane stretch caused an increase in open probability of the K_{ACh} channel.

Effect of Stretch on the K_{ACh} Channel Sensitivity to GTP

The increase in K_{ACh} channel activity caused by stretch could be the result of an increased sensitivity of the K_{ACh} channel to GTP. To test this hypothesis, the concentration-effect relationships for GTP-induced activation of the K_{ACh} channel were obtained at 0 and −60 mm Hg of pressure applied to inside-out patches. Figure 6 shows a typical tracing obtained from such an experiment. With 10 μM ACh in the pipette, GTP was applied to the cytoplasmic side of the membrane starting at 0.1 μM and its concentration increased stepwide to 20 μM. Channel activity (NP_o) observed at 20 μM was taken as 1.0, and the relative channel activities were plotted as a function of cytosolic [GTP]. Data points were fitted to the Hill equation:

$$f = 1/\{1 + (K_{1/2}/[\text{GTP}])^n\}$$

where f is the relative NP_o, $K_{1/2}$ is the concentration that produces half maximal activation, [GTP] is the concentration of GTP, and n is the Hill coefficient. At 0 mm Hg pressure, $K_{1/2}$ was 0.90 μM and n was 4.2 ($n = 5$). When similar experiments were done while applying a negative pressure of 60 mm Hg to inside-out patches, $K_{1/2}$ was 0.96 μM and n was 4.1 (Fig. 6*B*). Therefore, the increased K_{ACh} channel activity produced by stretch at a given concentration of GTP was not due to an altered sensitivity of the G_K/K_{ACh} channel to GTP.

Effect of Stretch on the K_{ACh} Channel Activated by GTPγS

To further examine the possibility that stretch causes an increase in K_{ACh} channel activity as a result of an increased level of activated G_K, we used a concentration of GTPγS (100 μM) that produced a maximal generation of activated G_K in the presence of ACh. As shown in Fig. 7*A*, applying GTPγS to the cytoplasmic side of the membrane resulted in an activation of the K_{ACh} channel within 30 sec, and the activity remained relatively at steady state. Negative pressure (−60 mm Hg) was applied for ~30 sec at three different times as shown in Fig. 7*A*. During each pressure application, the K_{ACh} channel activity increased significantly ($P < 0.05$) compared to those present before and after the application. The results from six patches are summarized in Fig. 7*B*. These results indicated that the stretch-induced increase in the open probability of the K_{ACh} involved mechanisms other than G_K.

Effect of Stretch on the K_{ACh} Channel Activated by Trypsin

It has been reported that the K_{ACh} channel could be activated by applying trypsin to the cytoplasmic side of the membrane [12] presumably via removal of an inhibitory domain of the channel protein. We therefore studied

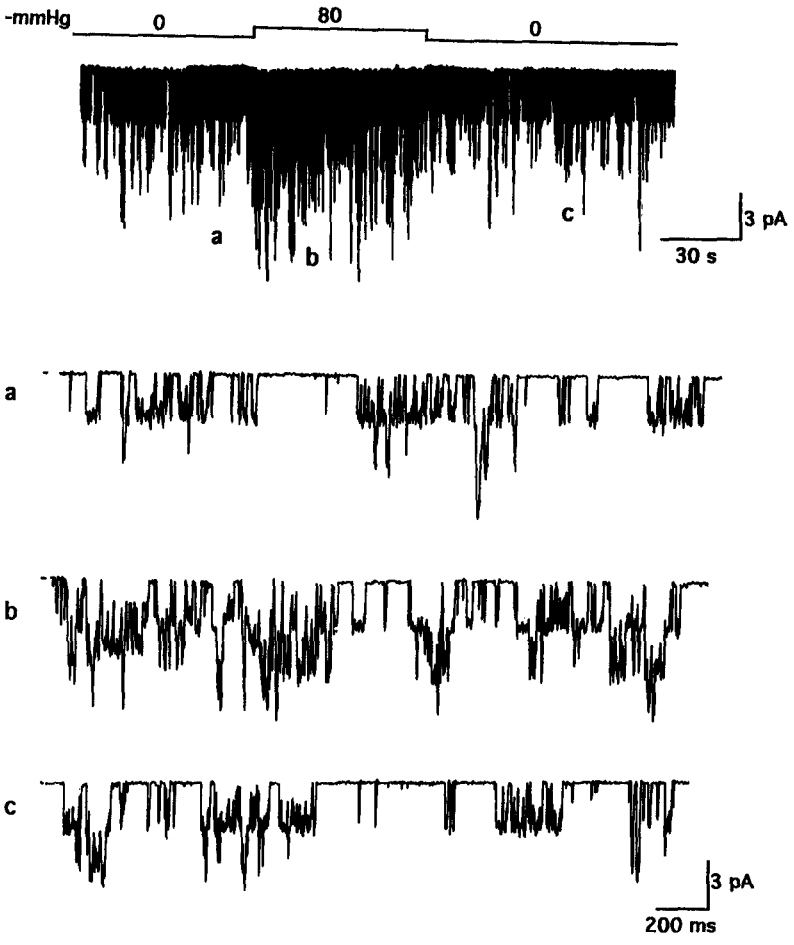

Fig. 5. A current tracing showing the effect of membrane stretch on the channel opening in an inside-out patch treated with 1 mM ATP. Inside-out patches were formed with 10 μM ACh in the pipette, and 100 μM GTP and 1 mM ATP in the bath. The cell membrane potential was held at −70 mV. In the presence of ATP, the mean open time of channels was 5.2 ± 1.1 msec (n = 4), a value several fold greater than that observed in the absence of ATP (0.9 ± 1.0 msec). Negative pressure (−80 mm Hg) applied to the membrane for ~1 min produced a marked increase in channel activity, as seen from expanded current tracings observed before, during and after the membrane stretch.

whether the K_{ACh} channel activated in such a manner independently of G protein pathway was also sensitive to stretch. In inside-out patches, application of 0.5 mg/ml trypsin to the cytoplasmic side of the membrane slowly caused activation of the K_{ACh} channel. The channel current activated by trypsin was inwardly rectifying, and had a single-channel conductance of 36 ± 1 pS (n = 4) in 140 mM CKI at both sides of the membrane. The mean open time was 1.0 ± 0.1 msec (n = 4). These kinetic properties were identical to those activated via G_K. Channel activity reached steady state usually after ~3 min of trypsin treatment and remained so for at least 10 min. Application of negative pressure to the pipette (−60 mm Hg) produced a reversible increase in K_{ACh} channel activity (71 ± 22%, n = 5 patches; Fig. 8*B*). When the pressure was applied at different times during the trypsin treatment, a significant increase in channel activity was observed only during the pressure application, almost identical to that observed with GTPγS. Thus, these results support the view that membrane stretch could cause an increase in K_{ACh} channel activity perhaps via a direct effect on the K_{ACh} channel without an involvement of G_K.

Effect of Stretch on the K_{ACh} Channel Activity in Adult Atrial Cells

Since channel modulation in cultured cells might be different from that found in freshly isolated cells, we examined whether the muscarinic K^+ channels of atrial cells freshly dissociated from adult atria also possessed similar mechanosensitivity. Figure 9 shows the effect of membrane stretch in atrial cells prepared from adult rat heart. Inside-out patches were formed with 10 μM ACh and 100 μM GTP in the bath to activate the K_{ACh} channels. Negative pressure (−80 mm Hg) applied to the membrane patch elicited an increase in channel activity (NP_o) in all six patches tested. The average increase in K_{ACh} channel activity at −80 mm Hg was 72 ± 31% (n = 6) above the control level, a value not significantly different from that observed in cultured neonatal rat atrial cells (54 ± 9%; $P > 0.05$). Similarly, the increase in K_{ACh} channel activity was also observed in response to negative pressure (−80 mm Hg) in five of five inside-out atrial patches prepared from adult guinea pig heart. In guinea pig atrial cells, the average increase in K_{ACh} channel activity was 82 ± 28% above control, a value not

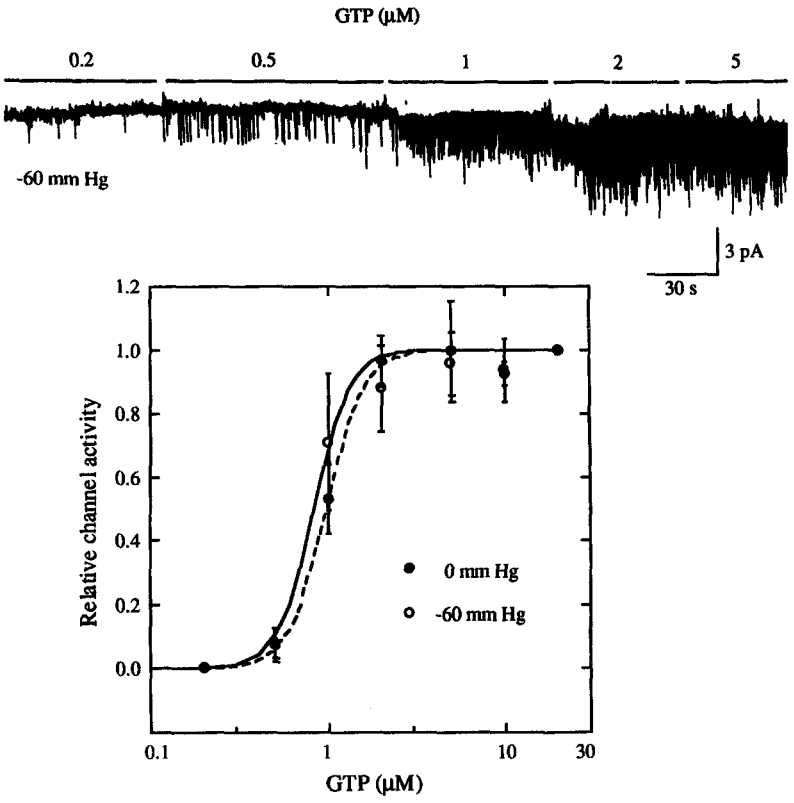

Fig. 6. Effect of stretch on the sensitivity of the K_{ACh} channel to intracellular GTP. An inside-out patch with ACh in the pipette was perfused intracellularly with increasing concentrations of GTP (0.1–20 μM). Channel activity (Np_o) at each concentration of GTP was determined and plotted as function of [GTP]. Channel activity determined at 20 μM was taken as 1.0. Solid circles are values obtained from patches under atmospheric pressure (0 mm Hg; n = 5). Open circles are values obtained from patches maintained under negative pressure (–60 mm Hg; n = 5). Data were fitted to Hill equation as described in the text. $K_{1/2}$ values were 0.90 and 0.96 μM, and n (Hill coefficient) values were 4.2 and 4.1 at 0 and –60 mm Hg pressure, respectively.

significantly different from that observed in adult rat atrial cells ($P > 0.05$). These results showed that the cardiac muscarinic K^+ channels, whether they are from neonatal or adult atria, were sensitive to membrane stretch.

Lack of Effect of Stretch on the K_{ACh} Channel in the Absence of GTP

In the absence of ACh in the pipette and GTP in the bath solution in inside-out patches, no K_{ACh} channel activity was observed except an occasional opening once every 5–10 sec. This is expected since GTP binding protein function is required for channel opening. Application of negative pressure to the patch (–60 to – 80 mm Hg) failed to activate the K_{ACh} channel in every patch tested ($n = 14$). With 10 μM ACh in the pipette and no GTP in the bath, K_{ACh} channel activity was also absent, and application of pressure (–80 mm Hg) did not activate the channel (n = 12). These results indicate that the K_{ACh} channel can not be opened by stretch when it is in the inactive, closed conformation.

Discussion

The present study was initiated by the earlier observation that arachidonic acid-activated K^+ channels in cardiac muscle cells, smooth muscle cells and neuronal cells were sensitive to stretch [7, 10, 11, 20] and that arachidonic acid and some of its metabolites could activate or modulate the K_{ACh} channel [9, 13, 24]. Although not shown in this paper, we were also able to observe activation of the K_{ACh} channel by applying 20 μM arachidonic acid to the bath solution in cell-attached patches in the absence of ACh. Therefore, it was of interest to examine whether the K_{ACh} channel could also be sensitive to membrane stretch. In many studies, whether an ion channel is activated or modulated by membrane stretch has generally been studied by applying negative or positive pressure to the membrane patch via the pipette [4, 25, 29]. Using similar methods, we found that the atrial muscarinic-gated K^+ channel was sensitive to membrane stretch. Unlike other true stretch-activated ion channels in which a normally closed channel was opened by applied pressure, the K_{ACh} channel first needed to be opened by G_K (or trypsin) in order for its activity to be further increased by stretch. In this sense, the K_{ACh} channel is a stretch-modulated ion channel. This mechanosensitivity adds further complexity to the existing regulatory mechanism of the K_{ACh} channel in which molecules such as G_K, GTP, ATP, arachidonic acid and its metabolites all influence the K_{ACh} channel function.

In an earlier study using video microscopy, it was reported that negative pressure applied to the patch increased the size of the patch membrane, presumably due to influx of new lipids [26]. Therefore the increase in K_{ACh} channel activity observed in response to suction

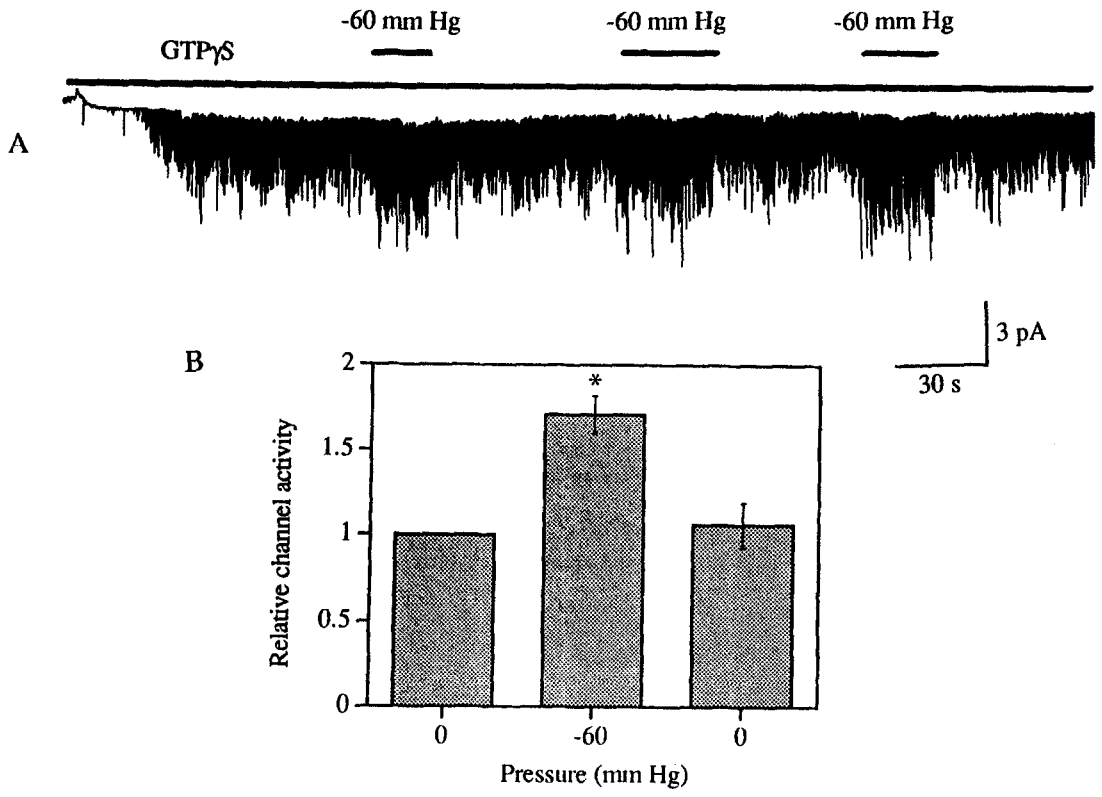

Fig. 7. Effect of stretch on the K_{ACh} channel activated by GTPγS. (*A*) An inside-out patch was formed without ACh in the pipette. GTPγS (100 μM) was applied to the bath. At relative steady state, negative pressure (~60 mm Hg) was applied for ~30 sec three separate times as shown. (*B*) Channel activities before, during and after the pressure application were determined from six patches and plotted. Channel activity before pressure application was taken as 1.0. Asterisk (*) indicates significant difference from the control value ($P < 0.05$).

may simply be the result of recruitment of new K_{ACh} channel into the patch membrane. As we have not measured the membrane area, we could not rule out the contribution of such changes in patch membrane area to the net increase in K_{ACh} channel activity. However, three observations indicate that the primary mechanism of the increase in K_{ACh} channel activity is due to increased frequency of opening of existing channels. (i) The maximum number of channels open before, during and after a pressure application were the same in many (although not all) patches. For example, in Figs. 3, 5 and 9, the maximum number of channels observed did not change during the course of the experiments. If the increase in K_{ACh} channel activity was due to influx of new proteins, one would expect to see higher levels of channel opening given sufficient recording time periods. To further rule out the possible changes in the number of channels, we attempted to study the effect of stretch on the whole-cell current. Unfortunately, the seal resistance deteriorated rapidly in the whole-cell configuration during application of pressure in every cell studied even at 20 mm Hg (>50 cells). Therefore, we were unable to obtain evidence for the pressure-sensitivity of the K_{ACh} channel at the whole-cell level. (ii) Membrane stretch did not alter the number of K_I or K_{ATP} channels in every patch that contained these channels. In our previous studies with the mechanosensitive K^+ channel [7], the number of observed channels in each patch did not increase with pressure even at pressures as high as −100 mm Hg. Only the frequency of opening was increased. (iii) Negative pressure increased the K_{ACh} channel activity in excised, inside-out patches in which the total number of channels would not be expected to be increased. Therefore, the increase in K_{ACh} channel activity by stretch was most likely via increased frequency of opening of existing channels.

The sensitivity of the K_{ACh} channel to stretch was lower than that of the recently identified stretch-activated K^+ channel (K_{FA} channel; ref 7) and nonselective cation channels [8] under similar experimental conditions. The pressures at which half maximal activation of the channel occurred were ~15 mm Hg for the K_{FA} channel and ~1.5 mm Hg for the nonselective cation channel. We were unable to determine the half maximal pressure for the K_{ACh} channel as the activity continued to increase with changing pressure until the patch broke. Since K_{ACh} channel activity was not significantly affected by −20 mm Hg, it is clear that the K_{ACh} channel was much less sensitive to stretch than the other two stretch-activated channels. However, the pressure sensitivity

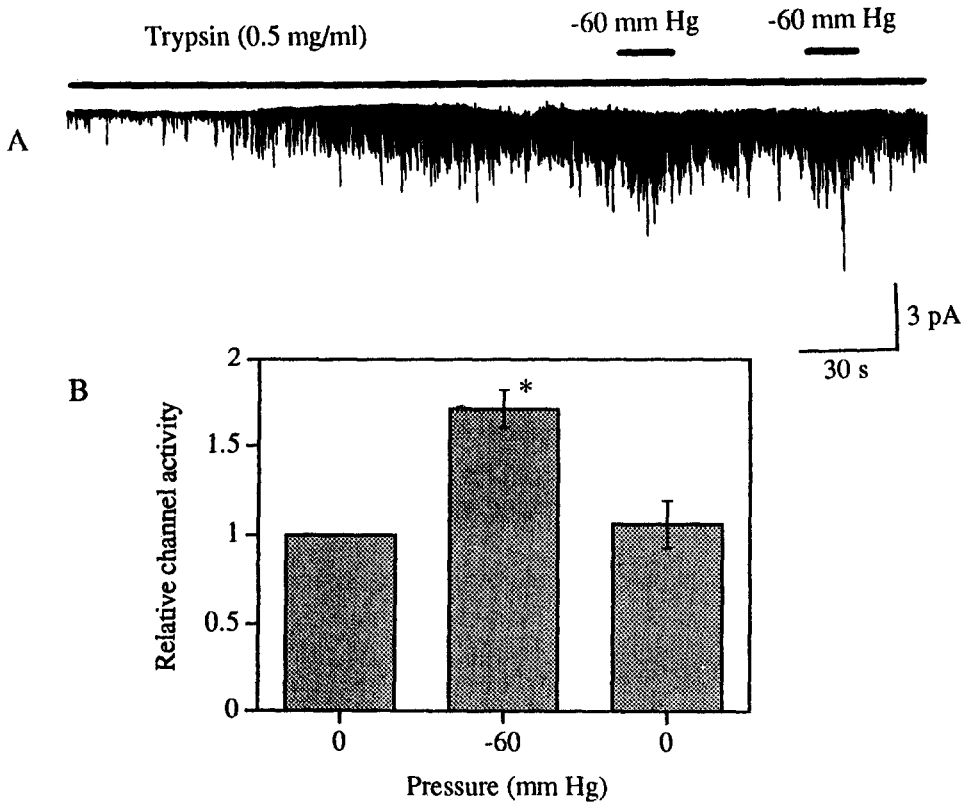

Fig. 8. Effect of stretch on the K_{ACh} channel activated by trypsin. (*A*) An inside-out patch was formed without ACh in the pipette. Trypsin (0.5 mg/ml) was applied to the bath. When a steady state level of activity was present, negative pressure (–60 mm Hg) was applied for ~30 sec two separate times as shown. (*B*) Channel activities before, during and after the pressure application were determined from five patches and plotted. Channel activity before pressure application was taken as 1.0. Asterisk (*) indicates significant difference from the control value ($P < 0.05$).

and characteristics of the K_{ACh} channel is comparable to that of the Aplysia S-channel which is activated by FMRFamide via a specific receptor, membrane stretch or arachidonic acid metabolites [30]. Like the K_{ACh} channel, the S-channel continued to increase in activity with pressure levels up to –100 mm Hg, and exhibited a slight increase in open time duration. It is possible that the K_{ACh} channel and the Aplysia S-channel share some common mechanisms in the stretch-induced augmentation of channel open probability. Whether other types of ion channels such as Ca^{2+} [5] and Cl^- [23] channels which have been shown to be modulated by arachidonic acid are also stretch sensitive has not been examined but would be interesting to know.

How membrane stretch activates or modulates ion channel activity is not known at present, although it has been speculated that cytoskeletal elements and the lipid bilayer may be involved [27]. The study of the mechanism by which stretch augments K_{ACh} channel activity is further complicated by the involvement of ligand-receptor-G protein-ion channel cascade. Changes in coupling efficiency at any level of this cascade will influence the K_{ACh} channel activity. The K_{ACh} channel is known to be directly activated by one of the subunits of G protein (G_K). In several studies, direct application of purified subunits of either Gα or Gβγ was reported to cause activation of the K_{ACh} channel [15, 31]. Therefore, if membrane stretch caused a change in the amount of G_K available to the channel by stimulating the muscarinic receptor-G protein coupling, by promoting GTP-GDP exchange on the G protein, or by reducing the rate of GTP hydrolysis, one would expect an increase in K_{ACh} channel activity. Our data suggest that these are unlikely mechanisms as stretch was able to increase the K_{ACh} channel activity even after the channel was activated by a high concentration of GTPγS, a nonhydrolyzable analogue of GTP, which would have maximally stimulated the G protein.

It was possible that stretch promoted the interaction between G_K and the relevant channel domain to increase K_{ACh} channel activity simply by modifying the lipid architecture surrounding the channel protein or by further exposing the G_K-binding domain to G_K. The results obtained using trypsin to activate the muscarinic K^+ channel independently of G_K show that stretch can increase the channel activity even in the absence of GTP and therefore G_K. Therefore, it seems likely that stretch increases the channel activity by acting directly on the

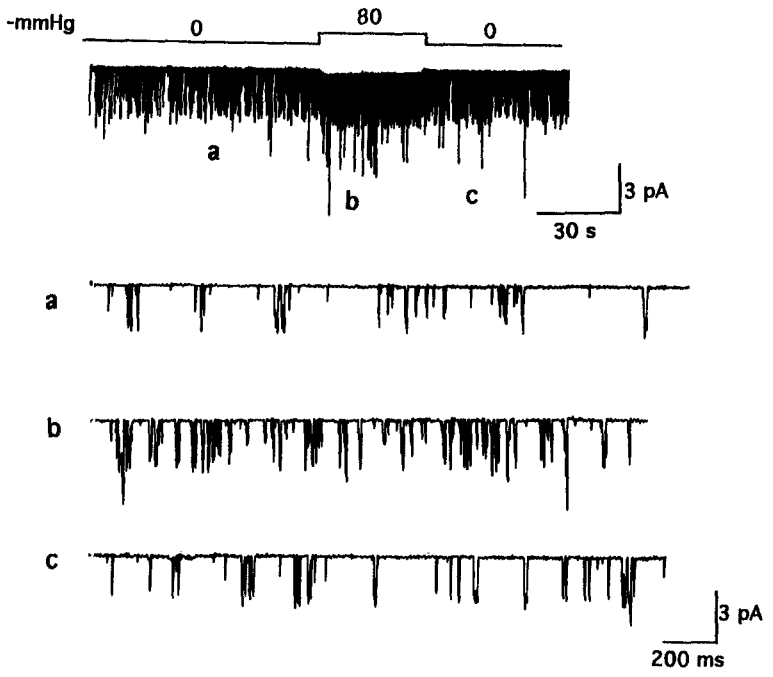

Fig. 9. A current tracing showing the effect of membrane stretch on the K_{ACh} channel in an adult rat atrial cell. An inside-out patch was formed with 10 μM ACh in the pipette and 100 μM GTP in the bath to activate the K_{ACh} channel. The cell membrane potential was held at −80 mV. Application of negative pressure produced a marked increase in channel activity in a reversible manner. Expanded current tracings show channel openings before, during and after the membrane stretch. In this patch, 2.2-fold increase in channel activity was produced by applying −80 mm Hg pressure to the membrane patch.

open K_{ACh} channel in the lipid bilayer. Recent studies have shown that cytoplasmic carboxyl-terminal regions of the cloned K_{ACh} channel (GIRK1) is important in channel gating [28] and binding of Gβγ [29]. Therefore, it is possible that stretch somehow affects this cytoplasmic region of the protein to modify channel function.

Mechanosensitive ion channels are found in many types of mammalian cells [17], plant cells [2], and in microorganisms such as bacteria and yeast [3, 16]. They are believed to be important in the regulation of cell volume, and in certain cases cell differentiation and proliferation. Whether the atrial muscarinic K^+ channels are involved in atrial cell volume regulation is not clear. However, atrial K_{ACh} channel plays an critical role in the modulation of cardiac rate and rhythm by changing the membrane K^+ permeability. At a given level of vagal activity, one would expect that stretch of the atrial wall would lead to augmentation of the K_{ACh} channel activity and thereby produce further slowing of the heart rate. As the sensitivity of the K_{ACh} channel to pressure is low, the physiological role of the mechanosensitivity of the K_{ACh} channel under normal physiological conditions where the atrial pressure fluctuates between zero and ~15 mm Hg is questionable. Under pathophysiological conditions in which the atrial pressure could rise above 20 mm Hg, K_{ACh} channels may become important in modulating cardiac function.

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