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Stretch-activated K^+ Channels in Rat Atrial Myocytes

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Mechanical stimuli to the cardiac myocytes initiate many biochemical and physiological events. Stretch-activated cation channels have been suggested to mediate these events. In this study, cell-attached and inside-out excised-patch clamp methods were used to identify stretch-activated cation channels in adult rat atrial myocytes. Channel openings were increased in cell-attached configuration when negative pressure was applied to the pipette, and also in inside-out excised patches by negative pressure. The channel was not permeable to Cl^-, Na^+ and Cs^+, but selectively permeable to K^+, and the degree of activation was dependent on the magnitude of negative pressure (full activation at ~ −50 mmHg). In symmetrical 140 mM KCl, the slope conductance was 51.2±3 pS between the potentials of −80 and 0 mV and 55±6 pS between 0 and +80 mV (n=5). Glibenclamide (100 μM) or ATP (2 mM) failed to block the channel openings, indicating that it is not ATP-sensitive K^+ channel. Arachidonic acid (30 μM), which has been shown to activate a K^+ channel cooperatively with membrane stretch, did not affect the channel activity. $GdCl_3$ (100 μM) also did not alter the activity. These results demonstrate that the mechanical stretch in rat atrial myocytes activates a novel K^+-selective cation channel, which is not associated with other K^+ channels such as ATP-sensitive and arachidonic acid-activated K^+ channel.

Key Words: Stretch-activated K^+ channels, Mechanical stretch, Atrial myocytes

INTRODUCTION

Mechanical stimulation causes many intracellular biochemical and physiological events in the heart. Stretching of isolated cardiac tissues was reported to depolarize the cell membrane during diastole, alter the action potential, and induce premature ventricular beat and arrhythmias (Lab, 1978; Boland & Troquet, 1980; Dean & Lab, 1989; Franz et al, 1989; Hansen et al, 1990; Franz et al, 1992; Hansen, 1993). Stretching of cardiac myocytes causes programmed cell death, alteration of contractility and structural deformation (Cheng et al, 1995). Stretching of atrium has been reported to induce secretion of atrial natriuretic peptide both *in vitro* and *in vivo* (Lang et al, 1985; Bilder et al, 1986; Page et al, 1991; Bruneau & de Bold, 1994; Jiao & Baertschi, 1995). *In vitro* stretching of cardiac tissue including atrium and ventricle has been reported to induce hypertrophy by activation of gene transcription and protein synthesis (Izumo et al, 1988; Haneda et al, 1989; Boer et al, 1994; Miki et al, 1994). There are evidences that at least alteration of heart rate, arrhythmias and depolarization during diastole results from the activation of stretch-activated ion channels (Bustamante et al, 1991; Hagiwara et al, 1992; Sasaki et al, 1992; Craelius, 1993; Hoyer et al, 1994). It is therefore suggested that stretch-activated channels (SACs) may act as a mechano-transducer, which converts a mechanical stimulus into an electrical signal.

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There are SACs showing different ionic selectivity in the heart. After discovery of SACs in chick skeletal muscle (Guharay & Sachs, 1984), SACs with non-selectivity to cations have been observed in the heart (Kim, 1993; Ruknudin et al, 1993; Hu & Sachs, 1996; Zhang et al, 2000). They have some common characteristics such as similar ionic selectivity and the absence of voltage- and time-dependency. SACs with K^+-selectivity have also been detected in human, rat and mouse heart (Kim, 1992; Terrenoire et al, 2001), and appear to be of the TREK-1 family of 2P domain channels (Patel et al, 2001; Terrenoire et al, 2001). In addition, K_{ATP}-channel (Van Wagoner, 1993) and K_{ACh}-channel (Pleumsamran & Kim, 1995) have also been known to have stretch sensitivity. These SACs with K^+ selectivity are thought to hyperpolarize, shorten action potentials, decrease energy expenditure, and weaken excitability of cardiac myocytes by lowering Ca^{2+} influx. As for another type of SACs, Cl^--selective SACs with a single channel conductance of 8.6 pS were also shown in human atrial myocytes (Sato & Koumi, 1998).

In order to elucidate the role of specific SACs in the mediation of biochemical and physiological events associated with the mechanical stimulus to the rat atrial myocytes, I found and characterized a group of SACs with K^+-selectivity. Negative pressure was applied to the membrane patch in cell-attached or inside-out configuration to activate SACs. The presently described SACs are K^+-selective, but differ significantly from previously reported stretch-activated K^+ channels, such as K_{ATP}-channel,

ABBREVIATIONS: SACs, stretch-activated channels; DIDS, 4,4'-Di-isothiocyanatostilbene-2,2'-disulphonic acid.

K_{ACh}-channel and arachidonic acid-activated K^+ channel, which have different pharmacological and biophysical properties.

METHODS

Cell preparation

Young adult Sprague-Dawley rats (200~250 g) were anesthetized using 100 mg/kg sodium pentobarbital. Hearts were isolated and perfused quickly with normal Tyrode solutions via the aorta onto a Langendorff apparatus. Temperature of perfusates was set at 37°C. After a few min, the perfusate was changed to nominally Ca^{2+}-free Tyrode solution (50 ml), and then to the same solution containing 0.12 mg/ml collagenase (Yakult, Japan) and 50 μM $CaCl_2$. After 14 min perfusion, the hearts were removed from the Langendorff apparatus and placed in a high-K^+, low-Cl^- storage medium. The atria and their appendages were dissected into small pieces and mechanically dispersed with a fire polished Pasteur pipette in the same solution. Isolated myocytes were stored in the high-K^+, low-Cl^- storage medium at 4°C until use. Dissociated atrial myocytes were mostly rod shaped and only a few were rounded-up under light microscopy. All experiments were performed on rod shaped and well defined myocytes within 24 h of isolation.

Solutions

The high-K^+, low-Cl^- storage medium contained (in mM) 50 L-glutamate, 50 KCl, 20 taurine, 20 KH_2PO_4, 3 $MgCl_2$, 10 glucose, 10 HEPES (N-[2-hydroxyethyl]piperazine-N'-[2-ethanesulfonic acid]), 0.5 EGTA (ethyleneglycol-bis (β-aminoethyl ether)-*N,N,N/,N/*-tetraacetic acid), and pH was adjusted to 7.3 by KOH. For the experiment with cell-attached configuration, a normal Tyrode external solution and a high Cs^+ pipette solution were used. The normal Tyrode solution contained (in mM): 143 NaCl, 5.4 KCl, 0.5 $MgCl_2$, 1.8 $CaCl_2$, 5.5 glucose, 5 HEPES, gassed with 100% O_2 at 37°C. Isotonic bath or pipette K^+ solutions contained 140 KCl, 2 $MgCl_2$, 5 EGTA and 10 HEPES. Isotonic Na^+ or Cs^+ solutions were prepared by substituting K^+ with Na^+ or Cs^+. In all ionic replacement studies, liquid junction potentials at the agar-bridge bath electrode were determined separately, and all voltages were corrected for offsets that were <5 mV. Arachidonic acid was obtained from Sigma Chemical, made as $>$ 1,000-fold stock solutions in dimethyl sulfoxide (DMSO), and stored under N_2 at -80°C. Arachidonic acid was dissolved to the final working concentration just before use. ATP (0.1~5 mM) and glibenclamide (0.1 mM) were added to either the extracellular or intracellular solutions according to the experimental protocols described in the text. Unless otherwise noted, all chemicals were from Sigma (St. Louis, MO, USA).

Electrophysiological recordings

Conventional cell-attached and inside-out configurations were used to record single channel activities. Axopatch-200 amplifier (Axon Instrument, Cal., U.S.A.) was used to clamp the membrane voltage of patches. Recording pipettes were fabricated and fire polished from 1.5 mm o.d. glass (Clark Electromedical, UK) to produce microelectrodes with resistance of 4~5 MΩ. Tips of microelectrodes were coated with Sylgard. The giga-seal was formed by applying a negative pressure of -10 mmHg and the seal resistance was usually over 20 GΩ. The current signals were filtered at 1~10 kHz, and stored on a video tape via a PCM adapter (VR-10B, Instrutech, Elmont, N.Y.). For the analysis of membrane currents, the data were transferred to a computer (IBM-PC, pentium-133) with pCLAMP v 6.0.5. software (Axon Instruments, Burlingame, CA, USA) through an analogue-to-digital converter interface (Digidata-1200, Axon Instruments, Burlingame, CA, USA). For most experiments, the temperature was set at room temperature (20~24°C). All averaged and normalized data are presented as means±S.E.M.

Application of mechanical stretch

A mechanical stretch of a membrane patch was obtained by applying a negative pressure (~50 mmHg) to the patch pipette. The pressure was calibrated using a water manometer and generated by a syringe and applied to the patch pipette. Pressure levels measured as millimeters of H_2O were converted to millimeters of mercury. The negative pressure at the membrane patch is the pressure measured from the manometer minus the pressure due to a small amount of solution in the pipette tip (~0.1 mmHg). This small amount of pressure was not included in my calculations. The possible presence of other small forces in the pipette produced by capillary action was not considered.

Data analysis and quantification of channel activity

The open probability (P_o) was calculated from the mean total current (*I*) divided by the single-channel current amplitude (*i*) and the maximum number of channels observed in a patch (*N*), such that $P_o=I/iN$. The *i* was determined from the amplitude histogram of the current record, and the *N* was determined from a visual inspection of the record after activation of the channel by a maximum pressure (~80 mmHg). For determining mean open and closed times, an open closed transition was considered valid if it remained in the state for at least two sample periods (250 μs). The threshold of opening transition was set at one-half of the unitary current (Colquhoun & Sigworth, 1983). Event-duration histograms for both the open time and fast closed time were constructed by binning at the sampling rate.

RESULTS

Single channel recording of stretch-activated channels in the cell-attached configuration

Fig. 1 shows a record of unitary currents in the cell-attached configuration. Spontaneous channel activity was observed, possibly due to resting tension in the patch (Sokabe et al, 1993). Application of negative pressure (-50 mmHg) increased the channel activity and release of the pressure readily decreased it to the control level. The response was reproducible at any time during the experiment. Channel activity was not increased or decreased during the pressure step.

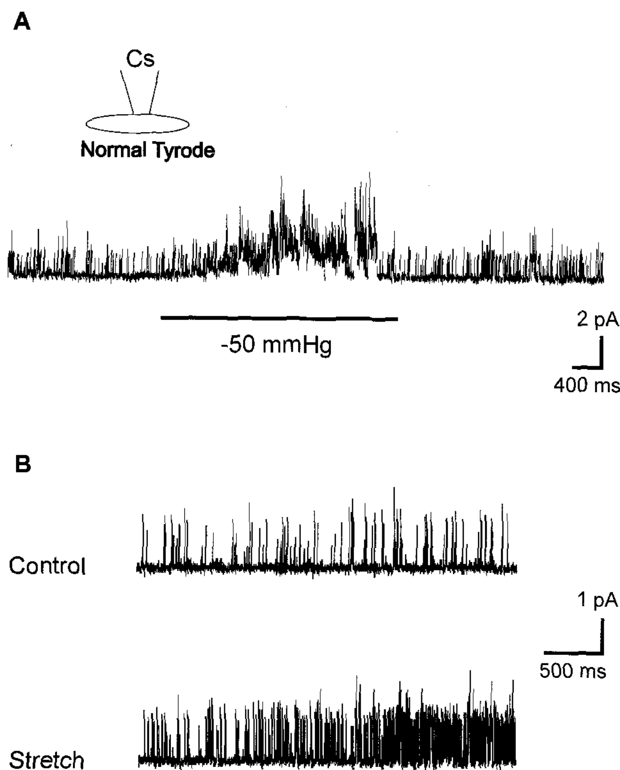


Fig. 1. Stretch-increased channel activity in a cell-attached patch. A, Channel activity was increased by a mechanical stretch on a cell-attached patch with Cs^+ pipette and normal Tyrode bath. The mechanical stretch was achieved by applying a negative pressure of -50 mmHg to the patch pipette. Channel activity was reversibly increased by a mechanical stretch. The membrane potential of cell-attached patch was $+19$ mV. B, Expanded traces showing increased channel activity by mechanical stretch without change in channel amplitude. Traces were obtained from other patch containing single stretch-activated channel.

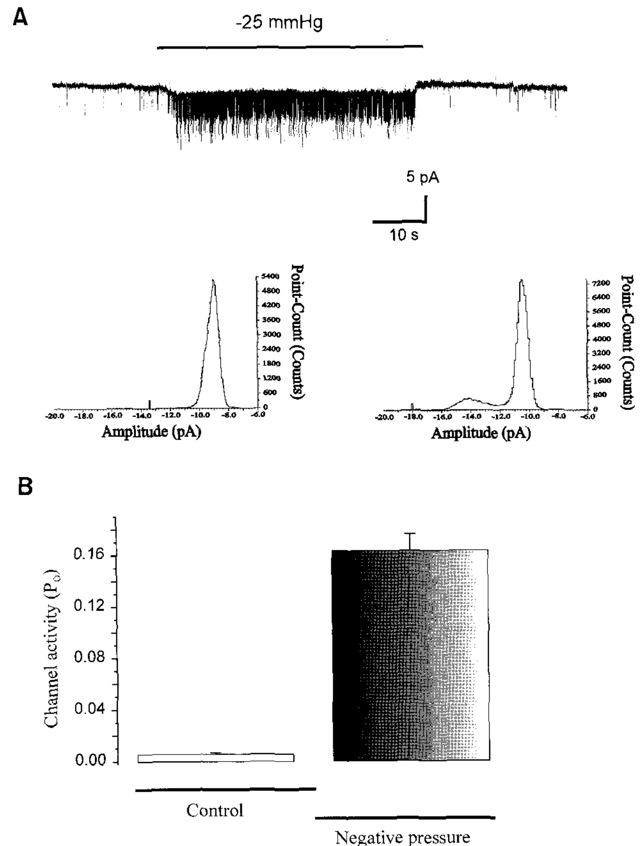


Fig. 2. Stretch-increased channel activity in the inside-out excised patch configuration. A, Representative recording of channel opening activated by a negative pressure under symmetrical K^+. Low panels represent all point histograms before and after stretch. Application of negative pressure to the patch pipette raised the open probability of unitary current in a reversible manner. B, Comparison of channel activity between control and under negative pressure.

Single channel recording of stretch-activated channels in the inside-out configuration

The effect of negative pressure (-25 mmHg) on the unitary currents under symmetrical K^+ was examined in the inside-out excised patch configuration (Fig. 2). Before the negative pressure, only few spontaneous openings (probability of being open <0.01) of unitary currents with large amplitude (-4.2 pA at a membrane potential of -80 mV) were observed. After the negative pressure, there was a dramatic increase in the channel activity (probability of being open$\cong$0.17) in 11 patches out of 64 patches tested. As long as pressure was applied to the patch, channel openings did not show a decrease in amplitude or frequency. Channel openings disappeared as soon as pressure was released. Application of positive pressure rapidly made patch clamp recordings unstable and resulted in the breaking of the seal.

At a given negative pressure (-25 mmHg), currents were recorded at step potentials, and then the amplitude of unitary currents was measured for each test potential (Fig. 3A). The unitary current-voltage (I-V) relationship was linear in symmetrical 140 mM KCl. The mean slope conductance by the linear regression was 51.2 ± 3 pS between the potentials of -80 and 0 mV, and 55 ± 6 pS between 0 and $+80$ mV (Fig. 3B; n=5). Mean open- and mean closed-time were also obtained at each test potential. At -80 mV, they were calculated to be 1.4 ± 0.2 msec, ~0.7 msec, respectively. They did not change markedly with membrane potential. In case of mean open-time, it ranged from 1.2 to 1.6 msec.

Ionic selectivity of stretch-activated channels

To study the ion selectivity of stretch-activated channels, 140 mM KCl in the bath were changed gradually to equimolar NaCl or CsCl in the inside-out patch configuration (Fig. 4). Gradual changing of bath solution abolished outward currents at $+40$ mV (Fig. 4A), while inward currents at -40 mV unaffected (data not shown). When KCl in the pipette and bath was replaced with symmetric Na^+ or Cs^+, channel opening with the same characteristics described above was not observed with application of negative pressure (data not shown). It means that the pressure-activated channel is highly selective for K^+, compared with Na^+ or Cs^+. Since the Cl^- equilibrium potential (0 mV) calculated from the experimental condition

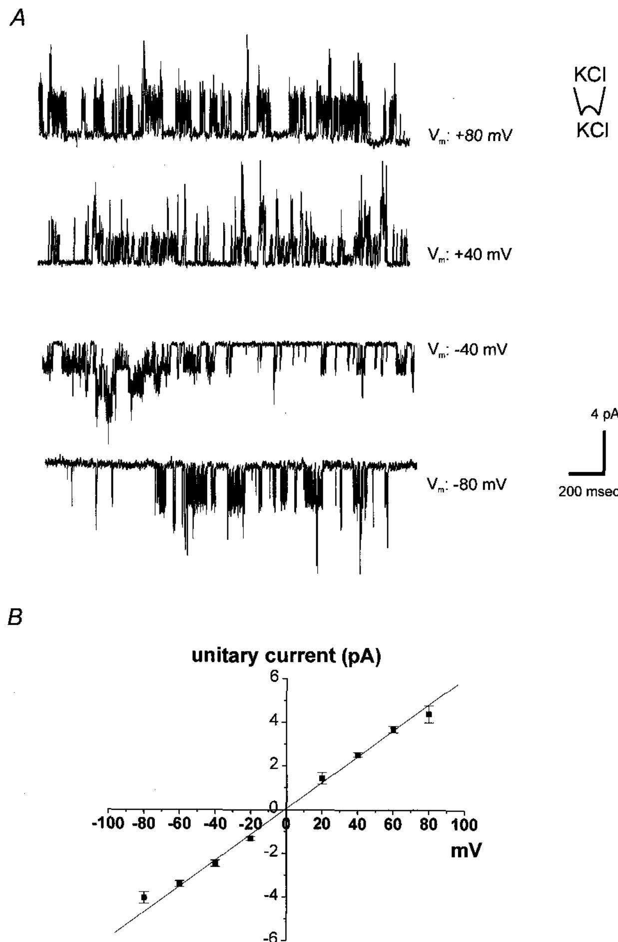


Fig. 3. Voltage dependency of stretch- activated channels. A, Stretch-activated K^+ currents obtained at various membrane potentials in inside-out membrane patch. Channel openings apparently appeared in burst. B, Unitary current-voltage relationship. Slope conductance was 51.2 pS between −80 and 0 mV and 55 pS between 0 and +80 mV. Least-squares fit (r=0.9) was superimposed to give a straight line. Current-voltage relationship was nearly linear.

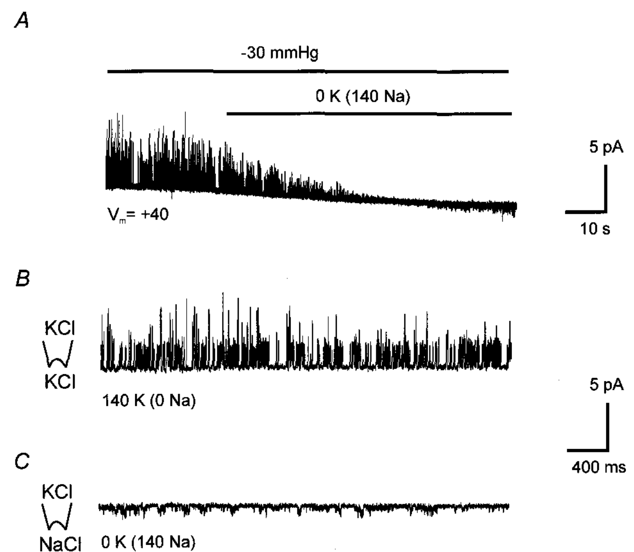


Fig. 4. Ionic selectivity of stretch-activated channel recorded under symmetrical K^+ condition. A, The effect of changing bath solution. As the concentration of K^+ in the bath solution decreased from 140 mM (0 mM Na^+) to 0 mM (140 mM Na^+), amplitude of unitary currents activated by negative pressure gradually decreased, indicating that it is selective to K^+. B, Expanded traces comparing unitary currents in 140 mM KCl with equimolar NaCl.

was far from the reversal potential (>> 40 mV) of the pressure-activated unitary currents under the condition of 140 mM NaCl in the bath and 140 mM KCl in the pipette, it is concluded that the channel is not highly selective for Cl^-.

Pressure dependence of stretch-activated K^+ channels

The effect of pressure on channel activation was studied in more detail. As shown in Fig. 5A, pressure dependent increase of channel opening was obvious. Expanded traces in Fig. 5B demonstrate that the pressure step raised only the open probability without affecting single channel conductance. The open probability of the channel at different pressure was each obtained from all point histogram of current amplitude at a membrane potential of −80 mV (Fig. 5C). Data points were fitted by a linear regression to the modified Boltzmann distribution given by $P_o=\{\exp[(p-p_{1/2})/s]\}/\{1+\exp[(p-p_{1/2})/s]\}$, 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 $[P_o/(1-P_o)]$ versus pressure (Kim, 1992). $P_{1/2}$ was −28±3 mmHg. Maximal activation of the channel was usually seen near −50 mmHg of pressure. Fig. 6 shows the distribution of open and closed times of the channel at different pressure level. The open and closed time histograms were analyzed from the current records filtered at cut-off frequency of 1 kHz. At a negative pressure of −10 mmHg, open time distribution was best fitted by a single exponential function with a mean open life time of 1.39±0.21 msec (n=5). Closed time distribution was also fitted by a single exponential function with a mean closed time distribution of 0.74±0.1 msec (n=5). Increasing pressure from −10 mmHg to −30 mmHg affected both mean open and closed life times significantly ($p<0.05$). Mean open life time increased from 1.39 msec to 2.03 msec, whereas mean closed life time decreased from 0.74 msec to 0.44 msec. It indicates that stretch increases the open probability of these channels by increasing the duration of open time and decreasing the duration of closed time.

Effects of glibenclamide and ATP on stretch-activated K^+ channels

The K^+ channel with kinetics similar to those activated by pressure as described in the present study has been observed in metabolically inhibited cells whose $[ATP]_i$ is markedly reduced (Deutsch et al, 1991). To exclude the possibility of the activation of ATP-sensitive K^+ channel (K_{ATP}) by negative pressure, the effect of glibenclamide or $[ATP]_i$ on the cytoplasmic surface of inside-out excised-patch was tested. As shown in Fig. 7, 0.1 mM glibenclamide or 2 mM ATP applied to the bath had no effect on the

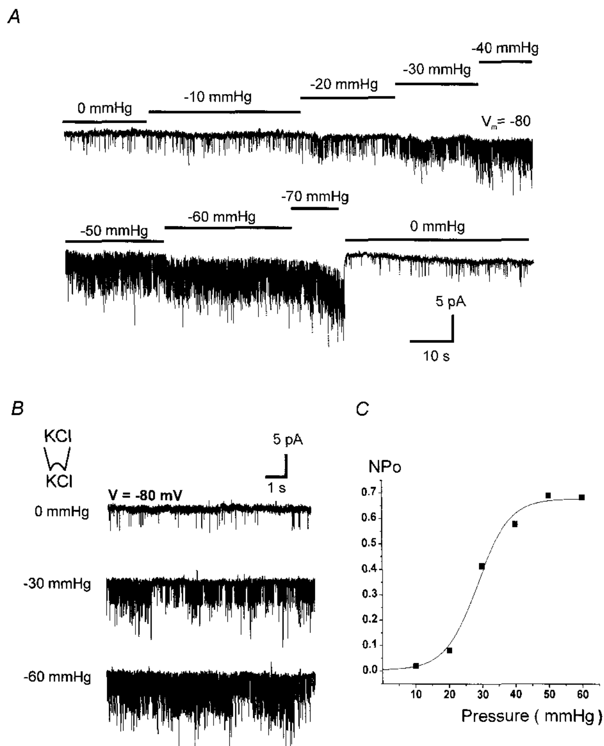


Fig. 5. Tracings showing that channel activity varies with the applied pressure in a dose-related fashion. A, Membrane currents at a step increase of negative pressure from 0 to −70 mmHg. Pressure dependent increase of membrane current is obvious and reversible. B, Expanded traces showing the increase of channel activity without change in single channel conductance. C, Relation between the open probability and pressure. The data were fitted to the Boltzmann distribution as described in the text. $P_{1/2}$ = −28 mmHg.

amplitude and probability of being open of the pressure-activated K^+ channel. Furthermore, simultaneous opening of glibenclamide-sensitive inwardly rectifying K_{ATP} channel and pressure-activated K^+ channel was observed (data not shown), excluding the possibility that the pressure-activated channel might be a K_{ATP} channel whose opening was increased by a negative pressure. The open probability and amplitude of unitary currents of K_{ATP} channel were not affected by application of negative pressure (~−80 mmHg).

Effects of arachidonic acid on stretch-activated K^+ channels

The K^+ channel with similar characteristics has been observed in arachidonic acid applied cells, where arachidonic acid and negative pressure increase the open probability of unitary current cooperatively (Kim, 1992; Kim et al, 1995). The relatively large unitary conductance (64.4 pS at −80 mV) and outward rectifying I-V relationship of the arachidonic acid activated K^+ channels favored the idea that the K^+ channels in the present study are different from them. To confirm the possibility, the effect of arachidonic acid on the open probability of stretch-activated K^+ channels was tested. As shown in Fig. 8, application of arachidonic acid (30 μM) to the cytoplasmic surface of the

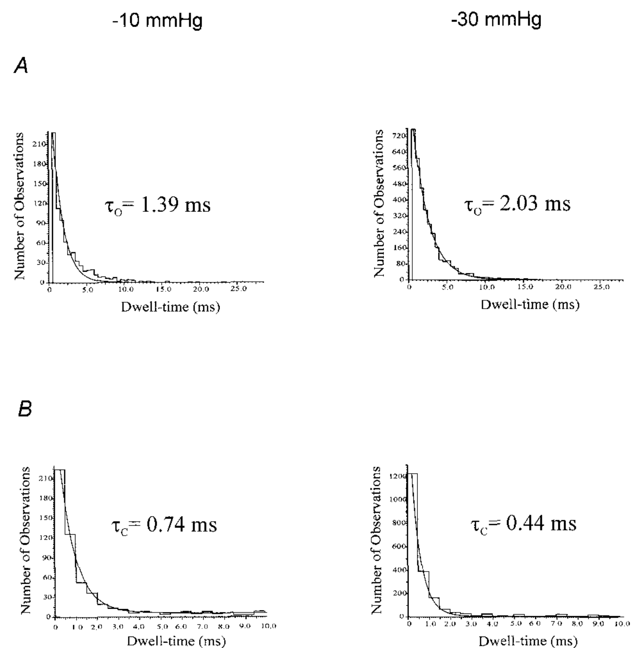


Fig. 6. The effect of pressure on the histograms of the open- (A) and closed- (B) time distribution of stretch-activated K^+ channels. The left and right panel denote the histogram under the pressure of −10 mmHg and −30 mmHg, respectively. Application of pressure increased the open time duration, whereas it decreased the closed time duration.

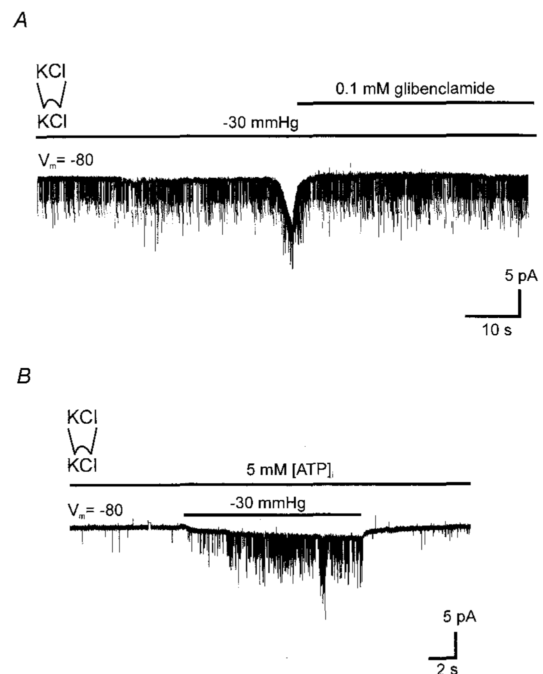


Fig. 7. The effect of glibenclamide and ATP on stretch-activated K^+ channels. A, Glibenclamide (0.1 mM) applied to the cytosolic surface of the patch failed to affect the open probability or amplitude of the unitary currents activated by negative pressure. B, The effect of stretch under continuous perfusion of ATP on the unitary currents. Stretch-activated unitary currents were still activated. ATP was applied to the cytosolic surface of the patch.

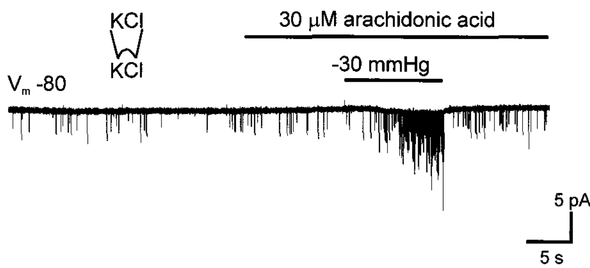
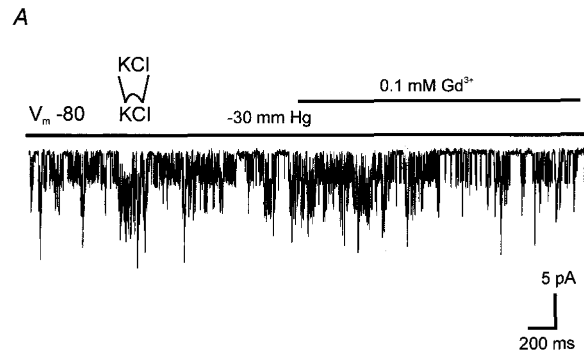


Fig. 8. The effect of arachidonic acid on stretch-activated K^+ channels. Application of arachidonic acid (30 μM) alone to the cytoplasmic surface of the inside-out patch failed to affect the open probability or amplitude of the unitary currents, while negative pressure still activated them.

inside-out patch failed to affect the open probability or amplitude of unitary current in both the resting and pressure-applied membrane patch.

Effects of other channel blockers

The lanthanide gadolinium (0.1 mM), which blocks stretch-activated ion channels in oocytes (Yang & Sachs, 1989), skeletal muscle cells (Franco & Lansman, 1990) and fungus (Zhou et al, 1991), did not block the stretch-activated K^+ channels when added to the bath (Fig. 9). Ba^{2+} (0.1 mM) also failed to alter the open probability of the stretch-activated K^+ channels at a given pressure level (data not shown, n = 4). The effects of tetraethyl ammonium (10 mM) and DIDS (0.1 mM) were also tested, and none of these drugs altered the open probability or amplitude of the unitary currents.



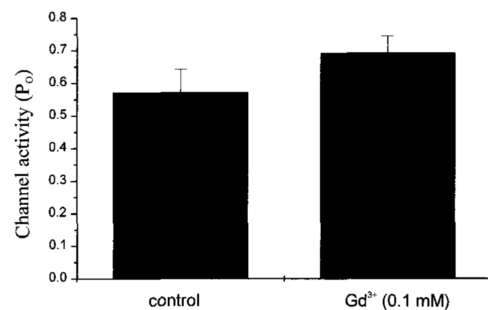


Fig. 9. The effect of gadolinium on stretch-activated K^+ channels. Application of gadolinium chloride (0.1 mM) to the cytoplasmic surface of the inside-out patch failed to affect the open probability or amplitude of the unitary currents activated by negative pressure.

DISCUSSION

I demonstrated the presence of a K^+ selective channel, which was activated by application of negative pressure on isolated membrane patch in rat atrial myocytes. The sensitivity of the K^+ channel to pressure is similar to that reported for other stretch-activated channels (Kim, 1992); i.e., half-maximal activation was achieved at a pressure of -28 mmHg. This channel was observed in both cell-attached and inside-out patches, indicating that cytosolic factors such as nucleotide phosphates and Ca^{2+} were not involved.

K_{ATP} channels in neonatal rat atrial cells were reported to be activated by application of negative pressure and by hypotonic swelling (Van Wagoner, 1993). Their I-V relation is inward rectifying and they are blocked by glibenclamide (Babenko et al, 1998; Baron et al, 1999). I-V relation in the present study, however, was linear and glibenclamide (0.1 mM) had no effect on the open probability of unitary currents. Furthermore, K_{ATP} channels with the same characteristics were also recorded in my study. They were not sensitive to pressure. Although it appeared that the negative pressure slightly increased the open probability of K_{ATP} channel in certain patches, it was not responsive to further increase or release of pressure. It might be explained by the hypothesis that the negative pressure could affect the open probability of K_{ATP} channels by decreasing the accessibility of $[ATP]_i$ to the K^+ channel (Kim, 1992).

Interestingly, the kinetics of the stretch-activated K^+ channels described herein is very similar to that of the cardiac K^+ channels, which are activated by arachidonic acid (Kim, 1992; Kim et al, 1995). The mean open time ranged from 1.2 to 1.6 msec and my result was 1.4 msec. However, the single channel conductance and I-V relationship presently described are significantly different from the arachidonic acid-activated K^+ channels. I-V relationship in my results was linear rather than outward rectifying and showed smaller single channel conductance in both outward and inward directions (51.2 vs. 64 pS between -80 and 0 mV; 55 vs. 94 pS between 0 and $+80$ mV). Furthermore, arachidonic acid alone was not effective in activating stretch-activated K^+ channels, whereas mechanical stretch to the same patch could immediately activate them. Ba^{2+}, which has been known to block arachidonic acid-activated K^+ channels (Kim, 1992; Kim et al, 1995), did not block the K^+ channel activated by negative pressure (data not shown). In addition, I observed stretch-activated K^+ channel currents whose I-V relationship and single channel conductance were the same as those of arachidonic acid-activated K^+ channel currents. In those cases, the increase in the open probability by application of negative pressure was not obvious in every patch, and the decrease in the open probability upon release of pressure was not instantaneous especially in the range of positive membrane potential. It is thought that at lease two types of stretch-activated K^+ channels exist in adult rat atrial mycytes, and their relative contribution should be studied in more detail.

It was also reported that cardiac muscarinic K^+ channel activity responds to a mechanical stretch in inside-out excised patches (Pleumsamran & Kim, 1995). In the absence of acetylcholine, muscarinic K^+ channels show no channel activity, and negative pressure fails to activate them. In the presence of acetylcholine, however, muscarinic K^+ channel activity appears and mechanical stretch augments it (Pleumsamran & Kim, 1995). Muscarinic K^+ channel activity with the same characteristics was also observed in my previous study in the presence of acetylcholine or GTPγS, but not in their absence (Cho et al, 2001). Stretch-activated K^+ currents in the present study were recorded in the absence of acetylcholine or GTPγS. And furthermore, the mean slope conductance of unitary inward currents carried by muscarinic K^+ channels in my previous study (Cho et al, 2001) was 42.4 ± 0.7 pS (n=4), whereas that of stretch-activated K^+ currents in the present study was 51.2 ± 3 pS (n=5). Therefore, it is evident that the unitary currents activated by application of negative pressure are not muscarinic K^+ currents.

Normally, the pressure ranges between 0 and 12 mmHg in atria. The net effect of stretch above the normal pressure ranges on the regulation of resting membrane potential and normal cardiac cycle has been known to be dependent on the mode of stretch. Hypotonic stress, which induces a cell volume increase, has been known to induce the activation of K^+- and Cl^-- selective channel and subsequent volume loss (Sasaki et al, 1992; Sorota, 1992; Tseng, 1992; Van Wagoner, 1993; Zhang et al, 1993; Fransen et al, 1995). It has been suggested that the increased K^+ permeability by the elevation of intra-atrial pressure or hypotonic stress will hyperpolarize the resting membrane potential of cardiac myocytes and produce slowing of the heart rate (Kim, 1992; Pleumsamran & Kim, 1995). On the other hand, direct stretch or mechanical stimulus on atrial myocytes has been known to depolarize the resting membrane potential or initiate stretch-induced arrhythmias in cardiac tissues by activation of non-selective cation channel (Stacy et al, 1992). My results suggest that a membrane expansion by negative pressure also activates K^+-selective channels. Assuming that hypotonic stress induces membrane expansion, the stretch-activated K^+ channel recorded in my experiment seems to represent the increase of K^+ permeability in hypotonic stress. Whether the stretch-activated K^+-channel is also activated by longitudinal stretch was not examined in single channel level, because of technical difficulty. Indirect evidence from the change of reversal potential by longitudinal stretch in whole-cell recording suggests the selective activation of non-selective cation channel in the condition of stretch along the long axis of atrial cell (Zhang et al, 2000). Assuming that a longitudinal stretch can simulate the increased intra-atrial pressure, membrane depolarization is expected in that condition. It is not clear whether hypotonic stress depolarizes or hyperpolarizes the membrane, because membrane expansion activates both non-selective (Zhang et al, 2000) and K^+-selective stretch-activated channels. There remain future studies on how membrane senses the mode of stretch and determines the selective activation of ion channels.

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