Characterization of a newly found stretch-activated \( K_{Ca,ATP} \) channel in cultured chick ventricular myocytes

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Kawakubo, Takashi, Keiji Naruse, Tatsuaki Matsubara, Nigishi Hotta, and Masahiro Sokabe. Characterization of a newly found stretch-activated \( K_{Ca,ATP} \) channel in cultured chick ventricular myocytes. Am. J. Physiol. 276 (Heart Circ. Physiol. 45): H1827–H1838, 1999.—With the use of the patch-clamp technique, five kinds of stretch-activated (SA) ion channels were identified on the basis of their single-channel conductances and ion selectivities in cultured chick ventricular myocytes. Because a high-conductance \( K^+ \) selective channel predominated among these channels, we concentrated on characterizing its properties mostly using excised inside-out patches. With 145 mM KCl solution in the pipette and the bath, the channel had a conductance of 199.8 ± 8.2 pS (n = 22). The ion selectivities among \( K^+ \), Na\(^+\), Ca\(^{2+}\), and Cl\(^-\) as estimated from their permeability ratios were \( P_{Na(P)K} = 0.03 \), \( P_{Ca(P)K} = 0.025 \), and \( P_{Cl(P)K} = 0.026 \). The probability of the channel being open (\( P_o \)) increased with the Ca\(^{2+}\) concentration in the bath ([Ca\(^{2+}\)]\(_b\); dissociation constant \( K_d = 0.51 \mu M \) at +30 mV) and membrane potential (voltage at half-maximal \( P_o = 39.4 \) mV at 0.35 \( [Ca^{2+}]_b \)). The channel was blocked by gadolinium, tetraethylammonium, and charybdotoxin from the extracellular surface and, consequently, was identified as a Ca\(^{2+}\)–activated \( K^+ \) (\( K_{Ca} \)) channel type. The channel was also reversibly activated by ATP applied to the intracellular surface (\( K_d = 0.74 \) mM at 0.10 \( [Ca^{2+}]_b \), at +30 mV). From these data taken together, we concluded that the channel is a new type of \( K_{Ca} \) channel that could be designated as an “SA \( K_{Ca,ATP} \) channel.” To our knowledge, this is the first report of \( K_{Ca} \) channel in heart cells.

patch clamp; stretch-activated channel; calcium-activated potassium channel; adenosine 5'-triphosphate-activated channel

It has been demonstrated that several types of Ca\(^{2+}\)-activated \( K^+ \) (\( K_{Ca} \)) channels are present in various muscular and nonmuscular preparations (16, 25). Among these channels, a large-conductance type (big \( K_{Ca} \) channel) similar to that first reported by Marty (16) is the most popular and extensively studied type. Big \( K_{Ca} \) channels from a variety of tissues share common properties of conductance, ion selectivity, voltage dependency, and Ca\(^{2+}\) dependency (4, 16, 25). Because of their abundance and large conductance, \( K_{Ca} \) channels are supposed to participate in repolarizing the membrane potential after depolarization. Recently, it has been suggested that \( K_{Ca} \) channels in smooth muscle may serve as a negative-feedback pathway by responding to fluctuations in membrane potential and intracellular Ca\(^{2+}\) concentration, and in this way they may serve to regulate the level of vascular tone (5).

Recently, special types of \( K_{Ca} \) channels that are activated by some factors in addition to membrane potential and intracellular Ca\(^{2+}\) concentration have been reported. Robertson et al. (22) showed that a \( K_{Ca} \) channel in pulmonary arterial smooth muscle cells isolated from rats can be activated by Mg\(^{2+}\)–ATP, and therefore they called it a \( K_{Ca,ATP} \) channel. The existence of \( K_{Ca} \) channels modulated by membrane stretch has also been reported in the apical membrane of cultured medullary thick ascending limb cells (28), in the apical membrane of rat and rabbit cortical collecting tubules (21), in G292 osteoblastic-like cells (7), and in embryonic rat neuroepithelial cells (17). Curiously enough, there has been no report of the \( K_{Ca} \) channel in cardiac myocyte despite such a ubiquitous distribution of \( K_{Ca} \) channels in a variety of cell types.

In the past decade, a new type of ion channel called stretch-activated (SA) channel, which is supposed to be activated by membrane tension (26), has emerged. SA channels are also carried by a variety of cell types (18). In cardiac myocytes some types of \( K^+ \) channels have been reported to be stretch activated (23, 29). It is believed that this type of channel would be activated when cells are mechanically deformed. This aspect of SA channels is particularly intriguing in heart cells because these cells are always subjected to periodic stretch and sometimes receive mechanical overload that induces arrhythmias or hypertrophy.

Originally, we started our study to further characterize the SA channels in heart cells using a preparation similar to that used by Ruknudin et al. (23). We were able to identify five different types of SA channels with conductances ranging from 25 to 200 pS. Because the channel with the largest conductance (200 pS) was most frequently observed, virtually in most patches, we decided to characterize this channel and found that it is a big \( K_{Ca} \) channel. In this report we present our detailed analyses of the biophysical and pharmacological properties of the newly found \( K_{Ca} \) channel in cultured chick heart cells. The channel turned out to be modulated by both membrane stretch and intracellular ATP, which is a completely new aspect in regard to the big \( K_{Ca} \) channels. Furthermore, this is the first report of the existence of a big \( K_{Ca} \) channel in heart cells.

MATERIALS AND METHODS

Cell culture. Ventricles were dissected from 10- to 12-day-old White Leghorn embryos under sterile conditions. Cell suspensions were prepared by exposing the ventricles to...
nominally Ca\(^{2+}\)-Mg\(^{2+}\)-free saline containing 2 mg/ml collagenase (Sigma Chemical) for 10 min at 37°C. Cells were grown in Dulbecco’s modified Eagle’s medium (GIBCO BRL) supplemented with heat-inactivated horse serum (10% vol/vol), chick embryo extract (2% vol/vol), penicillin, and streptomycin. The cultures were maintained in an atmosphere of 95% air-5% CO\(_2\) at 37°C and 95% relative humidity. Cells from 3 to 14 days of culture were used for experiments because the patches from younger cells were more fragile, preventing us from making stable recordings.

Single-channel recording and analysis. We used the patch-clamp technique for high-resolution detection of single-channel currents. Patch electrodes were fabricated from 100-μm glass capillaries (Drummond Scientific) in two stages on a vertical electrode puller (pp-83, Narishige) and were heat polished on a microforge (MF-83, Narishige). Tips of freshly pulled pipettes were filled by being placed in a 1-ml sample vial containing a filtered solution for 3–5 min, and then the shanks were backfilled. Pipettes were mounted on a micromanipulator and connected to the probe of a patch-clamp amplifier (model 3900, Dagan Instruments). Mechanical stretch in the patch was made by applying negative pressure in the pipette with a pneumatic transducer tester (DPM-1B, BIO-Tek Instruments) connected to a pipette holder. Most recordings were done with excised inside-out patches, although cell-attached patches were used in a limited case. All experiments were conducted at room temperature (23 ± 2°C). Currents were stored on VHS videotapes through a PCM recorder (VR-10B, Instrutech). The stored raw data were replayed through a four-pole Bessel filter and analyzed using software (PAT vers. 6.02) written by Dr. John Dempster (University of Strathclyde, Glasgow, UK). Channel-opening events were detected by using the 50% amplitude-threshold criterion from segments of records of 10–200 s in length. Single-channel current amplitude was measured from the peak-to-peak distance on the amplitude histogram or directly from chart records. The probability of the channel being open \(P_o\) was calculated from amplitude histograms as the ratio of open peak area to total area. In patches containing \(N\) number of channels with low \(P_o\), in which there were very few overlapped openings, \(P_o\) was determined from the equation:

\[
P_o(\%) = \frac{1 - P_c^{100}}{100}
\]

where \(P_c\) is the probability of the channel not being open in the record. In most kinetic analyses, i.e., determination of mean open and closed times, we used the data from the patches containing a single channel. In inside-out patches, membrane potential was defined as the negative value of the pipette potential. Upward current deflections in Figs. 1–5, 7, and 8 represent cation movement from the cytoplasmic side to the external side of the membrane and vice versa for anions.

Solutions. The standard external solution (in the pipette) facing the extracellular surface of the patch contained 145 mM KCl, 1 mM CaCl\(_2\), 10 mM HEPES, and 10 mM glucose adjusted to pH 7.40 with KOH. The internal solution (in the bath) facing the cytoplasmic surface of the patch was the same as that in the pipette except that the concentration of CaCl\(_2\) was below 1 mM. When the Ca\(^{2+}\) concentration was below 10 µM, it was adjusted with EGTA on the basis of calculations made using the program EqCal (BioSoft) according to the stability constants from Owen (20). Most experiments were done with excised inside-out patches and with these solutions, unless otherwise noted.

RESULTS

SA channels in cultured myocytes. Figure 1 shows typical examples of five different types of SA single-channel currents recorded from separate inside-out patches. As shown, negative pressures in the pipette caused \(P_o\) to increase without apparent adaptation in every case. We first categorized the channels on the basis of their conductances as determined from current-voltage (I-V) relations under two different ionic conditions: 1) 145 mM KCl in the pipette and 145 mM KCl in the bath, or 2) 145 mM NaCl in the pipette and 145 mM KCl in the bath. With 145 mM KCl saline in the pipette and the bath, we identified four types of SA channels with conductances of ~25 pS (Fig. 1A and B), 50 pS (Fig. 1C), 100 pS (Fig. 1D), and 200 pS (Fig. 1E). The
25-pS type was further separated into two subtypes, a K⁺-selective channel (Fig. 1B), with a permeability ratio of Na⁺ to K⁺ (P_{Na}/P_{K}) of 0.10, and a nonselective cation channel (Fig. 1A), with a P_{Na}/P_{K} of 1.0. The 50-, 100-, and 200-pS channels were K⁺ selective (P_{Na}/P_{K} = 0.08, 0.05, and 0.03, respectively). These results are roughly in line with the earlier report of Ruknudin et al. (23). Because the activity of the 200-pS channel predominated in most patches observed, we focused on this type and characterized its biophysical and pharmacological properties. Interestingly, as shown by the following results, this channel was voltage dependent and strongly activated by Ca²⁺ from the cytoplasmic side of the membrane, which suggests that it is a big KCa channel.

Channel density of 200-pS channel. Channel frequencies in 100 patches were 4 channels for nonselective 25-pS type, 22 channels for K⁺-selective 25-pS type, 21 channels for 50-pS type, 39 channels for 100-pS type, and 245 channels for 200-pS type. The frequencies for 25-pS (nonselective), 25-pS (K⁺ selective), 50-pS, and 100-pS channel types were somewhat similar to those reported by Ruknudin et al. (23); however, our frequency for 200-pS channel was much higher than that reported by Ruknudin et al. The reason for this difference may be that they used cell-attached patches with intracellular surfaces exposed to a lower Ca²⁺ concentration than that (0.35–1.0 µM) used for our inside-out patches. The estimated intracellular Ca²⁺ concentration in our preparation was 0.1–0.35 µM (see Fig. 7D). The membrane area of the patch could be estimated to be 2–5 µm², based on the geometric measurements obtained using high-power videomicroscopy (26). Therefore, the channel densities of the 200-pS channel seemed to be in the range of 0.5–1.2 channels/µm².

Ion selectivity. The ion selectivity of the 200-pS channel was estimated from I-V curves for inside-out patches by exposing the cytoplasmic surface of intracellular surfaces exposed to a lower Ca²⁺ concentration than that (0.35–1.0 µM) used for our inside-out patches. The estimated intracellular Ca²⁺ concentration in our preparation was 0.1–0.35 µM (see Fig. 7D). The membrane area of the patch could be estimated to be 2–5 µm², based on the geometric measurements obtained using high-power videomicroscopy (26). Therefore, the channel densities of the 200-pS channel seemed to be in the range of 0.5–1.2 channels/µm².
The permeability ratio $P_{\text{E}}$ of the channel was not changed (control 68.3% vs. Mg$^{2+}$[Na$^{+}$]).

Using the backfill procedure, we tested the effect of $\text{H}^{18}$30 STRETCH-ACTIVATED K$_{\text{Ca,ATP}}$ CHANNEL IN HEART CELLS on the Gd$^{3+}$ channel using the backfill procedure (3), in which 145 mM KCl in the pipette, thus yielding an estimated dissociation constant ($K_d$) of $\sim$0.4 mM. These data are in accordance with the TEA-induced reduction of the single-channel conductance in a variety of big K$_{\text{Ca}}$ channels (hereafter, we refer to K$_{\text{Ca}}$ channels by name).

Calcium and voltage dependencies. The effect of intracellular Ca$^{2+}$ on the channel activity was studied by exposing the cytoplasmic side of the patch to a range of various Ca$^{2+}$ concentrations ([Ca$^{2+}$]). Figure 4A demonstrates Ca$^{2+}$ activation of the 200-pS channel in an inside-out patch with various [Ca$^{2+}$]$_{\text{b}}$ at a membrane potential of $+30 \text{ mV}$ in a symmetrical 145 mM KCl solution. Although $P_0$ was extremely low (0.1%) at 0.1 $\mu\text{M}$ [Ca$^{2+}$]$_{\text{b}}$, $P_0$ increased with the increase in [Ca$^{2+}$]$_{\text{b}}$. Ca$^{2+}$ dependency of the open and closed times was estimated from the dwell-time histograms. The histograms of open-time distribution were well fitted by single-exponential functions, whereas those of closed times were fitted by double-exponential functions (Fig. 4B). Ca$^{2+}$ dependency of $P_0$ and the mean open and closed times are summarized in Fig. 4C, in which the mean long closed time decreased and the mean open time increased with the increase in [Ca$^{2+}$]$_{\text{b}}$, whereas short closed time showed little change. This type of [Ca$^{2+}$]$_{\text{b}}$ dependency, with a decrease in long closed time and an increase in open time with an increase in [Ca$^{2+}$]$_{\text{b}}$, was also seen in other preparations (25).

Fast blocking of the 200-pS channel by externally applied tetraethylammonium (TEA) using the backfill procedure is shown in Fig. 3D. In contrast to the all-or-none type of blockade described above, TEA decreased the mean single-channel current in a dose-dependent manner and increased the open-channel current noise of the 200-pS channel. This type of “flickery block” is characteristic of a blocking process with relatively fast kinetics. The single-channel conductance was 151 pS with 0.1 mM TEA, 126 pS with 0.3 mM TEA, and 62 pS with 0.8 mM TEA backfilled in the pipette, thus yielding an estimated dissociation constant ($K_d$) of $\sim$0.4 mM. These data are in accordance with the TEA-induced reduction of the single-channel conductance in a variety of big K$_{\text{Ca}}$ channels (hereafter, we refer to K$_{\text{Ca}}$ channels by name).

Effects of $\text{K}^+$-channel blockers. In general there are two types of channel blockers, fast and slow blockers. Fast blockers induce a graded decrease of the single-channel currents, whereas slow blockers make “all-or-none” types of gaps in the open-channel currents, which sometimes are hard to discriminate from channel closing. To avoid this difficulty, we used a high Ca$^{2+}$ concentration (1 mM) in the bath to keep the open channel open for most of the time over a wide potential range. First, we tested the effect of charybdotoxin (CTX), a specific blocker for big K$_{\text{Ca}}$ channels (2), on the 200-pS channel using the backfill procedure (3), in which 145 mM KCl solution containing 20 nM CTX was backfilled. Figure 3A shows time-dependent changes in the channel activities at $+30 \text{ mV}$, which reflect gradual diffusion of the drug to the patch membrane. The $P_0$ of the 200-pS channel was significantly decreased 5 min after the start of the backfill and almost completely disappeared after an additional 15 min.

The effect of 20 $\mu$M gadolinium (Gd$^{3+}$), a potent blocker of SA channels, was also examined using the same method. As shown in Fig. 3B, a slow blockade of the 200-pS channel became visible at 5 min, and complete blockade was apparent 15 min after the backfill. Although the kinetics of this Gd$^{3+}$ blockade were not systematically investigated in the present study, its qualitative features seemed to be similar to the Gd$^{3+}$ blockade of the endogenous SA cation channels in Xenopus oocytes (31). Figure 3C shows time courses of $P_0$ from the control, CTX, and Gd$^{3+}$ groups. Using the backfill procedure, we tested the effect of extracellular Mg$^{2+}$ (3 mM) and found that the $P_0$ of the channel was not changed (control 68.3% vs. Mg$^{2+}$ 66.1%, $n = 3$). We also tested the effect of intracellular Mg$^{2+}$ and again observed no significant change in $P_0$ (70.5% vs. 68.1%, $n = 3$).
closed time decreases with voltage, whereas the mean short closed time remains unchanged. A similar tendency was reported in other preparations (6, 7, 25).

Figure 6 shows $P_o$ as a function of membrane potential at various $[\text{Ca}^{2+}]_0$ from 45 separate inside-out patches. $P_o$ increased with depolarization at all $[\text{Ca}^{2+}]_0$ tested, and the data set for each $[\text{Ca}^{2+}]_0$ was reasonably fitted by Eq. 1. The value for $K_1$ was 18.07 ± 0.72 ($n = 5$).

The theoretical activation curves in Fig. 6 give $P_o$ values at membrane potentials of $-20, -10, 0, +10, +20$, and $+30$ mV with various $\text{Ca}^{2+}$ concentrations, from which we could obtain Hill plots. Hill coefficients were $4.34 ± 0.17$ mV ($n = 6$).

From the above results (i.e., high unitary conductance (200 pS) and sensitivity to $\text{Ca}^{2+}$, voltage, TEA, and CTX), we concluded that the 200-pS SA channel was a $\text{Ca}^{2+}$-activated $K^+$ channel ($K_{\text{Ca}}$ channel).

Pressure dependency. We next investigated SA properties of the $K_{\text{Ca}}$ channel. In both inside-out and cell-attached patches in cultured chick cardiac cells, the activity of the $K_{\text{Ca}}$ channel was increased by the application of negative pressure in the patch pipette. Figure 7A shows typical current traces of responses to the pressure in the pipette. Typically, membrane stretch by negative pressure was applied in a stepwise fashion with a 10-mmHg increment, and the response of the channel activity lagged behind the pressure change by only a few seconds. Positive pressure in the pipette also activated the $K_{\text{Ca}}$ channel in a dose-dependent manner. Conditions were same as in A except that pipette was backfilled with 145 mM KCl containing 1 mM TEA.
times broke the seal in 10–15 s, preventing us from obtaining good results.

The effect of membrane stretch on the open-close kinetics was analyzed on the basis of open- and closed-time distributions. Dwell-time histograms of those times at 0 and 50 mmHg of negative pressure are shown in Fig. 7B. For this channel, a double-exponential function was required to fit the closed-time distribution, and a single-exponential function was enough to fit the open-time distribution, suggesting that there are at least two closed states \((C_1\) and \(C_2\)) and one open state \((O_1)\). Such a property is similar to the results reported in other types of SA channels (9, 12). With this particular patch, the mean long closed time decreased and the mean open time increased with membrane stretch (Fig. 7C). The change in open time was much smaller than that in long closed time, suggesting that suction in the pipette increased \(P_o\) primarily by decreasing the mean long closed time of the channel. This is also similar to the findings in the previously mentioned reports (9, 12). Five additional patches showed basically a similar tendency. With the assumption of a simple linear kinetics model \((C_1 \leftrightarrow C_2 \leftrightarrow O_1)\), only the transition from \(C_1\) to \(C_2\) is stretch dependent.

We also examined the effect of membrane stretch on the \(K_{Ca}\) channel in cell-attached patches. Membrane stretch activated the \(K_{Ca}\) channels in a similar manner, although there was a high degree of variability in their responses. Figure 7D summarizes the data from excised inside-out patches with 0.1 \((n = 11)\) or 0.35 µM \((n = 12)\) \([Ca^{2+}]_b\) from excised inside-out patches with no \(Ca^{2+}\) in the pipette or bath \((n = 4)\), and from cell-attached patches \((n = 8)\). From these results, the intracellular \(Ca^{2+}\) concentration in our preparations could be estimated between 0.1 and 0.35 µM, a reasonable range for the resting heart cell. In the apical

Fig. 4. \(Ca^{2+}\) dependency of 200-pS SA channel. A: single-channel current traces of 200-pS channel in an inside-out patch at various \(Ca^{2+}\) concentrations in bath \([Ca^{2+}]_b\). \(P_o\) of channel in an inside-out patch increased as \([Ca^{2+}]_b\) increased. Membrane potential was held at +30 mV. Pipette and bath contained 145 mM KCl. B: dwell-time histograms of open (OT), long closed (ICT), and short closed times (sCT) for patch in A at 0.35 µM \([Ca^{2+}]_b\). OT histogram could be fitted by a single-exponential function, and ICT and sCT histograms could be fitted by a double-exponential function with time constants indicated. C: plot of \(P_o\) vs. \([Ca^{2+}]_b\). Data (●) were taken from patch in A. Dotted line was drawn according to a Boltzmann-type function. \([Ca^{2+}]_b\) dependence of OT, ICT, and sCT are also indicated. In response to a change in \([Ca^{2+}]_b\), ICT decreased from 514.24 ms at 0.1 µM \([Ca^{2+}]_b\) to 1.91 ms at 0.8 µM \([Ca^{2+}]_b\), whereas OT increases from 2.12 to 11.83 ms.
membrane of cultured medullary thick ascending limb cells, the activation of $K_{Ca}$ channels by membrane stretch was reported to be attributed to some indirect processes, such as $Ca^{2+}$ influx mediated by SA cation channels or volume-induced $Ca^{2+}$ releases from cytoplasmic stores (9, 28). However, we observed stretch-dependent channel activation with 0 mM $CaCl_2$ and 4 mM EGTA in the pipette and bath (Fig. 7D). To rule out the possibility of $Ca^{2+}$ crossing from the patch pipette into a nonstirred layer at the cytoplasmic surface of the membrane patch, we performed a set of cell-attached experiments with 0 mM $CaCl_2$ and 4 mM EGTA in the patch and bath solutions. Under these conditions, we still observed an increase of $P_o$ by negative pressure in the pipette (data not shown).

ATP dependency. Because Robertson et al. (22) have shown that $K_{Ca}$ channels in pulmonary arterial smooth muscle cells isolated from rats can be activated by $Mg^{2+}$-ATP, we also tested the effect of ATP on our $K_{Ca}$ channel in inside-out patches. Aliquots of concentrated ATP solution were successively added to the bath to give various final concentrations at the cytoplasmic side ($[ATP]_b$). Figure 8A shows typical current traces demonstrating progressive activation of the $K_{Ca}$ chan-
In the pipette. ADP and AMP activated the KCa,ATP, and negative pressure (20 mmHg) was applied
outside-out patches. The channel could therefore be identified as a stretch-, Ca2+-, and ATP-activated K+ (SA KCa,ATP) channel.

DISCUSSION

In the present study, we have revealed the presence of five distinct types of SA channels in cultured chick ventricular myocytes by using the patch-clamp technique. Among them, a K+ channel with the largest conductance predominated and was identified as a KCa channel on the basis of several characteristics, including high K+ selectivity, a large unitary conductance, voltage and Ca2+ dependencies, and sensitivities to TEA and CTX. The KCa channels in this study were also activated by application of ATP to the intracellular surface of inside-out patches. The channel could therefore be identified as a stretch-, Ca2+-, and ATP-activated K+ (SA KCa,ATP) channel.

Calium and voltage dependencies. The gating of the KCa channels in chick cardiac myocytes was sensitive to changes in [Ca2+]b between 0.1 and 1.0 µM (Fig. 4). A KCa channel with a similar sensitivity to [Ca2+]b (<0.1 µM) was reported in smooth muscle cells (25), whereas channels with higher sensitivity (activation at <0.01 µM) in pituitary cells (30) and lower sensitivity (1–10 µM activation range) in cultured rat muscle cells (4) have been reported. The Hill coefficient of our KCa channel in cardiac myocytes was between 4.0 and 5.0. For other preparations the values have been determined to be 2.87 in cultured rat skeletal muscle (4), 2.82 in rat pulmonary arterial smooth muscle cells (1), and 1.63 in rat brain synaptosomal cells (19). Most of the values from mammalian skeletal muscle have been reported to be between 1.5 and 3.0.

A comparison of the voltage sensitivity of the present KCa channels with those in various preparations may be made by using the inverse slope of the plot of the natural logarithm of the ratio [Pf/(1 – Po)] versus membrane potential. Our channel had an inverse slope of 14.8–18.5 mV for smooth muscle cells of the guinea pig taenia coli (11), and 15–20 mV for canine colonic myocytes (6). The inverse slope of most KCa channels has been determined to be 9 mV in dopal anterior pituitary cells (30), 15–16 mV in cultured rat skeletal muscle (4), 11.3 mV for smooth muscle cells of the guinea pig taenia coli (11), and 15–20 mV for canine colonic myocytes (6). Thus the inverse slope of most KCa channels consistently lies between 10 and 20 mV, suggesting that the voltage-sensing mechanism of KCa channels, including ours, seems to be well conserved. The Ca2+ and voltage dependencies of our KCa channel lie in the range of those previously reported KCa channels.

Activation by membrane stretch. An interesting aspect of the KCa channel in cardiac myocytes described in this study is its mechosensitive property, a direct activation by membrane stretch. Stretch activation of some KCa channels, however, has been ascribed to other mechanisms rather than to stretch itself, e.g., increases of intracellular Ca2+ concentration by Ca2+ influx through a SA cation channel that permits an influx of Ca2+ or volume-induced release of Ca2+ from cytoplasmic stores (21). Although we cannot completely preclude such mechanisms in our channel, we have ob-
served stretch activation of the $K_{Ca}$ channels under conditions designed to eliminate changes in $Ca^{2+}$ concentration in the pipette and bath solutions. In cell-attached patches with 0 mM $Ca^{2+}$ in the patch pipette and the bath, membrane stretch by suction also increased the $P_0$ of the $K_{Ca}$ channel in a manner similar to that in excised patches, suggesting that the stretch activation of our $K_{Ca}$ channel does not require any intracellular messengers. These results strongly suggest that the channel is activated directly by membrane stretch.

Ruknudin et al. (23) have reported that one of five SA channels found in their study had a large conductance (190 pS), a value similar to ours (23). However, our channel is much more permeable to $K^+$ over $Na^+$ than their channel, and their channel had no clear voltage dependence. At present, without other data to compare, we cannot completely conclude whether our channel is different from theirs.

Activation mechanism by ATP. In the present study, we found that the $K_{Ca}$ channel is activated by physiological levels of ATP from the cytoplasmic side of the membrane. It is possible that ATP activation of the $K_{Ca}$ channel involves protein phosphorylation that requires ATP. Activation of $K_{Ca}$ channels by cAMP-dependent phosphorylation has been reported in a variety of cells (8, 14, 24). We tested the involvement of such a cAMP-dependent protein phosphorylation by using di-
butyryl cAMP, a membrane-permeable cAMP analog, which had no significant effect when applied to the inside-out patch (see Table 1). We also tested the effect of cAMP on the channel in the intact cell to check whether there is a cAMP-dependent intracellular mechanism that could modulate the channel activity. However, even in cell-attached patches, extracellular application of dibutyryl cAMP up to 1 mM had no effect on the channel activity. It is unlikely that activation of the $K_{\text{Ca}}$ channel involves cAMP-dependent protein phosphorylation. What is puzzling is that adenosine 5'-O-(3-thiotriphosphate) (ATP$_{\gamma}$S) had no effect on the $K_{\text{Ca}}$ channel (see Table 1). At present we do not know whether the channel discriminates the difference between ATP and ATP$_{\gamma}$S or whether ATP hydrolysis is involved in the ATP activation. However, the latter mechanism seems to be unlikely because Mg$^{2+}$ required for ATP hydrolysis had no effect on the ATP activation.

On the other hand, it is known that muscarinic K$^+$ channels in atrial myocytes of guinea pig hearts are directly activated by GTP and guanosine 5'-O-(3-thiotriphosphate) (GTP$_{\gamma}$S) in the absence of agonists such as acetylcholine (11). We applied GTP$_{\gamma}$S (1 mM) to the cytoplasmic surface of inside-out patches, but little
activation of the \( K_{Ca} \) channels was detected. Thus G protein-mediated signal transduction systems may not play a major role in the activation of our \( K_{Ca} \) channel.

Recently, Lemos and Takeda (15) have reported that application of 10 \( \mu M \) ATP to the external solution containing a normal concentration of \( Ca^{2+} \) caused a large increase in \( K_{Ca} \)-channel activity in cell-attached patches. This is due to the ATP-induced increase in \( Ca^{2+} \) concentration from both \( Ca^{2+} \) release from internal stores and influx from the extracellular solution. In the present study, external solutions that contained ATP did not activate \( K_{Ca} \) channels in cell-attached patches (data not shown), and when ATP was in the bath, there was no difference in \( P_{o} \) with or without \( Ca^{2+} \) in the pipette in excised inside-out patches (data not shown). Therefore, it is not likely that the activation of our \( K_{Ca,ATP} \) channel by ATP involves an ATP-induced increase in intracellular \( Ca^{2+} \) concentration or \([Ca^{2+}]_{o}\) (15). These results strongly suggest that the ATP-activation of our \( K_{Ca} \) channel is mediated by a direct interaction of ATP with the channel.

It is known that, under physiological conditions, the intracellular ATP concentration is a few millimoles per liter. However, we observed in cell-attached patches that the activity of the \( K_{Ca,ATP} \) channels was extremely low, whereas in inside-out patches, 1 mM ATP at the cytoplasmic surface caused a significant activation. It is possible that in cell-attached patches some kind of cytosolic factors may suppress the ATP-induced activation of the \( K_{Ca,ATP} \) channel.

Physiological role of \( K_{Ca,ATP} \) channels. In this study we used only embryonic heart cells; therefore, it may be too early to discuss the physiological role of the \( K_{Ca,ATP} \) channel. Taking this limitation into account, we offer some speculation on the possible functions of this channel in the heart.

Intracellular \( Ca^{2+} \) concentration in heart cells under physiological conditions is roughly 0.1 \( \mu M \) in the diastolic phase and 1.0 \( \mu M \) in the systolic phase. The corresponding membrane potentials are roughly \(-80 \) and \(+30 \) mV. Intracellular ATP concentration in heart cells is suggested to be 4–5 mM in both phases. With a 0.1 \( \mu M \) \([Ca^{2+}]_{o}\), at \(-80 \) mV, \( P_{o} \) of the \( K_{Ca,ATP} \) channel in this study proved to be quite low (Fig. 6), and ATP is not likely to increase \( P_{o} \) at this potential (Fig. 8). On the other hand, with a 1.0 \( \mu M \) \([Ca^{2+}]_{o}\), at +30 mV, corresponding to the systolic phase, \( P_{o} \) increased to the maximal level even with no ATP (Fig. 6). Thus ATP may not affect the \( K_{Ca,ATP} \) channel under normal conditions.

Under pathological conditions, e.g., during ischemia, intracellular \( Ca^{2+} \) concentration is suggested to be 0.8–1.0 \( \mu M \) in the diastolic phase and 1.3–1.5 \( \mu M \) in the systolic phase. Intracellular ATP concentration during ischemia decreases to 25–30% of its normal level, namely, 1.0–1.3 \( \mu M \), in both phases. With a 0.8 \( \mu M \) \([Ca^{2+}]_{o}\), at \(-80 \) mV, \( P_{o} \) of the channel seems to be >30% (Fig. 6). A 1 \( \mu M \) [ATP] does not seem to affect the \( P_{o} \) at this potential (Fig. 8D). The channel may be fully activated in the systolic phase during ischemia, as under normal conditions. Thus, under pathological conditions in both phases, the channel would remain at a highly activated level, contributing to hyperpolarization of the membrane potential and reduction of the excitability. The effect of ATP on the channel under both conditions is not clear, because \([Ca^{2+}]_{o}\), seems to dominate in controlling the voltage dependence of the channel. ATP may contribute to a more subtle regulation of the \( K_{Ca,ATP} \)-channel activity. On the other hand, membrane stretch seems to increase the \( P_{o} \) of the channel (Fig. 7D) and to accelerate the hyperpolarizing process.

Albarwani et al. (1) have suggested that \( K_{Ca,ATP} \) channels in pulmonary arterial smooth muscle cells isolated from the rat are involved in controlling pulmonary vascular tone under hypoxic conditions. After hypoxia, the sensitivity of \( K_{Ca,ATP} \) channels to intracellular \( Ca^{2+} \) concentration, and therefore to voltage, is reduced as a consequence of reduced intracellular ATP concentration. The activity of these channels is therefore reduced, exerting a depolarization, which would potentially favor \( Ca^{2+} \) influx through voltage-gated \( Ca^{2+} \) channels, ultimately increasing pulmonary vascular tone. This may also mean that activation of \( K_{Ca,ATP} \) channels causes dilation of pulmonary arteries. Taguchi et al. (27) have suggested that dilation of cerebral arteries in response to increases in the intracellular concentration of cAMP is mediated by activation of \( K_{Ca} \) channels. Brayden and Nelson (5) also indicated that activation of \( K_{Ca} \) channels by any means could lead to vasodilation of myogenic arteries. Thus one important role of \( K_{Ca} \) channels might be to exert vasodilation. Kume et al. (13) have suggested that \( \beta \)-adrenergic stimulation of \( K_{Ca} \) channels may cause relaxation of tone in airway smooth muscle, namely, bronchodilation by cAMP-dependent and membrane-delimited pathways. Likewise, \( K_{Ca,ATP} \) channels in the heart may be

Table 1. Effect of other chemicals on \( K_{Ca,ATP} \) channel

<table>
<thead>
<tr>
<th>Chemical</th>
<th>n</th>
<th>Control</th>
<th>Addition of chemical</th>
<th>Chemical and suction</th>
</tr>
</thead>
<tbody>
<tr>
<td>ATP</td>
<td>5</td>
<td>0.62 ± 0.18</td>
<td>55.35 ± 11.28</td>
<td>76.73 ± 4.81</td>
</tr>
<tr>
<td>ADP</td>
<td>5</td>
<td>8.28 ± 1.05</td>
<td>20.22 ± 4.75</td>
<td>63.86 ± 11.50</td>
</tr>
<tr>
<td>AMP</td>
<td>5</td>
<td>1.25 ± 0.59</td>
<td>6.56 ± 2.58</td>
<td>23.72 ± 12.72</td>
</tr>
<tr>
<td>ATP*S</td>
<td>6</td>
<td>1.14 ± 0.44</td>
<td>2.89 ± 0.96</td>
<td>14.47 ± 8.01</td>
</tr>
<tr>
<td>MgATP†</td>
<td>3</td>
<td>0.88 ± 0.44</td>
<td>58.31 ± 15.72</td>
<td>77.34 ± 10.51</td>
</tr>
<tr>
<td>CTP</td>
<td>5</td>
<td>0.54 ± 0.36</td>
<td>5.00 ± 1.74</td>
<td>7.95 ± 1.91</td>
</tr>
<tr>
<td>GTP</td>
<td>5</td>
<td>0.22 ± 0.04</td>
<td>0.67 ± 0.29</td>
<td>1.93 ± 1.14</td>
</tr>
<tr>
<td>GTP*E</td>
<td>5</td>
<td>0.07 ± 0.04</td>
<td>0.18 ± 0.08</td>
<td>0.65 ± 0.28</td>
</tr>
<tr>
<td>Adenosine</td>
<td>6</td>
<td>0.34 ± 0.27</td>
<td>1.36 ± 0.94</td>
<td>2.58 ± 1.72</td>
</tr>
<tr>
<td>UTP</td>
<td>5</td>
<td>0.47 ± 0.29</td>
<td>1.44 ± 0.24</td>
<td>3.47 ± 1.47</td>
</tr>
<tr>
<td>Glibenclamide†</td>
<td>5</td>
<td>81.21 ± 5.36</td>
<td>87.31 ± 3.13</td>
<td>9.12 ± 7.31</td>
</tr>
<tr>
<td>Dibutyryl cAMP</td>
<td>5</td>
<td>0.44 ± 0.29</td>
<td>1.43 ± 0.80</td>
<td>3.13 ± 1.23</td>
</tr>
<tr>
<td>Dibutyryl cAMP*</td>
<td>5</td>
<td>0.25 ± 0.09</td>
<td>0.23 ± 0.10</td>
<td>0.67 ± 0.28</td>
</tr>
</tbody>
</table>

Values of open probability (\( P_o \)) in \( Ca^{2+} \)-activated potassium (\( K_{Ca} \)) channels are means ± SE; \( n \) = no. of patches. All experiments were performed in inside-out patches unless otherwise noted. \( Ca^{2+} \) concentration in bath solution (\([Ca^{2+}]_{o}\)) was 0.1 \( \mu M \) unless otherwise noted; \( Ca^{2+} \) concentration in pipette solution was 1 \( mM \) in all experiments. All solutions in pipette and bath contained 145 mM KCl, 10 mM HEPES, and 10 mM glucose. ATP*S, adenosine 5’-O-(3-thiotriphosphate); GTP*E, guanosine 5’-O-(3-thiotriphosphate). *Experiments were performed with cell-attached patches. †Experiments were performed with \([Ca^{2+}]_{o}\) of 1 \( \mu M \).
involved in dilation or relaxation of the heart. However, it remains to be determined whether the $K_{Ca,ATP}$ channel is expressed in matured heart cells and how the $K_{Ca,ATP}$ channel is involved in the heart under physiological and pathological conditions.

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