J. Membrane Biol. 154, 45–51 (1996)

The Journal of
Membrane
Biology

© Springer-Verlag New York Inc. 1996

Identification of ATP-sensitive Potassium Channel in Frog Ventricular Myocytes

M. Munemori, K. Yamaoka, I. Seyama

Department of Physiology, School of Medicine, Hiroshima University, Kasumi 1-2-3, Minamiku, Hiroshima 734, Japan

Received: 22 May 1996/Revised: 5 July 1996

Abstracts. ATP-sensitive potassium channels were found in frog ventricular myocytes using the inside-out patch-clamp technique. The channel was selectively permeable to K^+. Single-channel conductance was 32.6 pS at 3.0 mM of $[K^+]_o$ and 132 mM $[K^+]_i$ and 77.3 pS at 114 mM $[K^+]_o$ and 132 mM $[K^+]_i$. ATP did not affect single-channel conductance. The open probability of the channel was decreased by intracellular application of ATP in both the presence and absence of 2 mM $MgCl_2$. The coexistence of Mg^{2+} with ATP shifts the dose-response curve for the open probability of ATP-sensitive K^+ channel against ATP rightward. The shift of the curve indicates that Mg-ATP is less effective than free ATP in inhibiting the channel. An open-time histogram was fitted by a single exponential function with a time constant of 1.63 ± 0.17 msec ($n = 5$) in an ATP-free medium. Mean open time (1.57 ± 0.10 msec; $n = 5$) was not altered but the inter-burst time (closed time between bursts) lengthened in 10 μM ATP.

Key words: ATP-sensitive K^+ channel — *Rana catesbeiana* — Patch clamp — Heart ventricle

Introduction

ATP-sensitive potassium channels (K_{ATP}-channel) have been found to be distributed widely among various cells, such as cardiac cells (Noma, 1983), pancreatic β-cells (Cook & Hales, 1984; Findlay, Dunne & Petersen, 1985), skeletal muscle cells (Spruce, Standen & Stanfield, 1985), smooth muscle cells (Standen et al., 1989) and cultured neurons from rat cerebral or cerebellar cortices (Ashford et al., 1988). In cardiac cells, it has been suggested that activation of the K_{ATP}-channel shortens the plateau under anoxic conditions (Noma, 1983; Noma & Shibasaki, 1985), thereby protecting the cardiac cells from death by decreasing the entry of Ca^{2+} from the extracellular side and/or reducing the consumption of ATP. However, its physiological role is still under debate because concentration of ATP effective for the channel closure may be too low to support cell survival. A useful way of elucidating the physiological role is to use a comparative physiological approach. Because the amphibian heart does not have a coronary circulation system and is in a poikilothermic state, it is worthwhile looking for a K_{ATP}-channel in the frog heart and, if it exists, to compare its characteristics with those of its mammalian counterparts. Pilsudski, Rougier & Tourneur (1990) have suggested the presence of K_{ATP}-channels in frog atrial and ventricular cells by showing a large increase in the outward membrane current during depolarization in the presence of cromakalim, a specific K_{ATP}-channel opener.

We have found K_{ATP}-channels in the frog heart, which retain characteristics similar to those of mammalian hearts.

Materials and Methods

Cell Preparation

The method for isolating single ventricular cells from the frog (*Rana catesbeiana*) was essentially the same as that in a previous experiment (Seyama & Yamaoka, 1988). Briefly, a heart was dissected and rinsed in normal Ringer solution with 100 units/ml of heparin. The rinsed heart was mounted on a Langendorff apparatus and retrogradely perfused via the aorta with a Ca^{2+}-free solution containing collagenase (0.1 mg/ml; Yakult, Tokyo, Japan) and trypsin (0.06 mg/ml; type I, Sigma Chemical, St. Louis, MO) for 20 min at 32°C. The isolated ventricle was then cut into pieces and dispersed by pipette agitation. The single cells were kept in a solution containing a low Ca^{2+} concentration (200 μM) for 30 min and then centrifuged for 1 min at 93 × *g*

Correspondence to: I. Seyama

and stored in Leibovitz's L-15 medium for experimental use (GIBCO, Grand Island, NY).

Solutions

The normal Ringer solution contained (in mM): 113.5 NaCl, 5.4 KCl, 1.8 $CaCl_2$, and 5.0 *N*-2hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid (HEPES) (pH 7.2). The Ca^{2+}-free solution contained 93.5 NaCl, 5.4 KCl, 5.0 $MgSO_4$, 20 glucose, 20 Taurine, and 10 HEPES (pH 7.2).

The intracellular bath solutions contained 110 KCl, various concentrations of K_2 ATP, 10 ethylene glycol-(b-aminoethyl ether)-*N*,*N*,*N'*,*N'*-tetraacetic acid (EGTA), 10 HEPES, and the pH was adjusted to 7.0 by adding approximately 22 KOH. In several experiments, an appropriate amount of $MgCl_2$ was added. Intracellular pCa ($-\log[Ca^{2+}]$) was fixed at 8.0 by adding $CaCl_2$ in the EGTA-containing bath solution using Schoenmakers' program (1992).

The extracellular pipette solutions contained 110 KCl, 1 $CaCl_2$, and 10 HEPES with 4 KOH added to adjust the pH (7.2). To determine ion selectivity of the K_{ATP} channel, various $[K^+]_o$ solutions were used (3, 10 or 30 KCl and 107, 100 or 80 NaCl, 1 $CaCl_2$, and 10 HEPES) with the pH adjusted by NaOH. The bath solution could be exchanged within 10 sec from one to another. All experiments were performed at room temperature (24–26°C).

Electrophysiological Recording and Analysis

The experiments were carried out in the inside-out configuration using the conventional patch-clamp technique (Hamill et al., 1981). The resistance of the patch pipettes filled with the pipette solution was 5–10 MΩ. The records obtained by patch-clamp amplifiers (EPC-5; List-electronic, Darmstadt/Eberstadt, Germany or Axopatch 200A; Axon Instruments, Foster City, CA) were stored using a DAT tape-recorder (DTC-1000 ES; SONY, Tokyo, Japan). Data were filtered through the 1 kHz low-pass filter of an EPC-5 amplifier (4-pole Bessel filter). The data recorded by DAT were sampled at 2 kHz for further analysis using pCLAMP 5.5 (Axon Instruments, Foster City, CA). In later experiments, when highly resolved recordings were required for dwell-time analysis, we used Axopatch 200A and data were filtered through the 5 kHz low-pass filter of an Axopatch 200A amplifier (4-pole Bessel filter with a cutoff frequency of 5 kHz) and sampled at 50 kHz. Data for the life time of bursts and the inter-burst time (closed time between bursts) were obtained from the records filtered at a 0.1 kHz cutoff frequency in five cells. The statistical significance between groups was determined by the Mann-Whitney test, $P < 0.05$ being considered statistically significant.

After isolating the inside-out patch, the K_{ATP}-channels were subject to a marked rundown within the first several minutes in approximately 30% of cells. To obtain valid results, data collection was started 5 min after establishment of the inside-out patch configuration. Activity of the K_{ATP}-channel was expressed by NP_o, which is defined as the ratio of mean patch current to unit amplitude during the observed time. NP_o in various ATP solutions was normalized by referring to that in the ATP-free solution, which was applied before and after recording the currents in various ATP solutions. The arithmetic mean of NP_o in the ATP-free solutions before and after applying various ATP solutions was used as a reference to minimize errors introduced by the rundown. In the presence of Mg^{2+}, data were referred to those in ATP-free solution containing 2 mM Mg^{2+}. Each test solution was applied for 1 min, and records were analyzed for the midway 40 sec, excluding the data of the initial and last 10 sec. Because the membrane was held at −40 mV, K_{ATP} channels were not affected by Mg^{2+} rectification (Horie, Irisawa & Noma, 1987).

Results

Identification of ATP-sensitive K^+ Channel and its *I-V* Relation

When a K^+-rich pipette was attached to the dissociated frog ventricular myocytes, activity of the inward rectifier K^+ channels was usually recorded (Munemori et al., 1996). On excising the inside-out patch from the cell into high K^+-bath solution (132 mM K^+), conspicuous activity different from the inward rectifier K^+ channel current was recorded in the ATP-free medium (*see* records in Fig. 1*A*). The channel open events appeared in bursts. Flickering during bursts tended to disappear as the membrane became depolarized.

Amplitude histograms were constructed from the original records with the bin width of 0.1 pA (Fig. 1*B*). The histogram showed two peaks, which were fitted with Gaussian distributions. The amplitude of the single-channel current was determined as the difference between the two peaks of the Gaussian functions. Single-channel conductance was estimated from the amplitude histograms to be 71.4 ± 3.1 pS ($n = 9$) in the ATP-free solution and 69.9 ± 3.8 pS ($n = 9$) in 10 μM ATP. No significant change in the channel conductance was observed with the application of ATP ($P > 0.5$).

Recordings of the single-channel current were also made with 3, 10, 30 mM $[K^+]_o$ in the patch pipette while the bath solution contained 132 mM $[K^+]_i$. Channel conductance obtained from the slopes of straight lines fitted for data from 3, 10, 30 and 114 mM $[K^+]_o$ in the *I-V* relation were 32.6, 43.0, 46.9 and 77.3 pS, respectively (Fig. 1*C*). The reversal potential was simply determined from the membrane potential at which the straight line for the *I-V* curve crossed zero current level. The reversal potentials in 30 and 114 mM $[K^+]_o$ were −39.0 and 1.7 mV, respectively. However, when we switched the pipette solution from 114 mM $[K^+]_o$ to 3 or 10 mM $[K^+]_o$, current records at membrane potentials below −60 mV suddenly became too noisy to determine their direction or amplitude. Thus, only one reliable datum, for −80 mV in 10 mM $[K^+]_o$, was available. The reversal potentials of −79.2 and −59.0 mV in 3 and 10 mM $[K^+]_o$ were obtained by fitting a straight line to data from −40 to +40 mV and then extrapolating it to zero current level. These reversal potentials are plotted against $[K^+]_o$ in Fig. 2*D*. The line indicates values predicted by the Nernst equation, which roughly agree with the experimental data.

All characteristics described above for the channel events are similar to those of the K_{ATP} channels described previously (Noma, 1983; Spruce, Standen & Stanfield, 1985; Findlay, 1988; Lederer & Nichols, 1989).

Dose-response Curve

The dose-response curves for ATP in the presence and absence of 2 mM Mg^{2+} were constructed by measuring

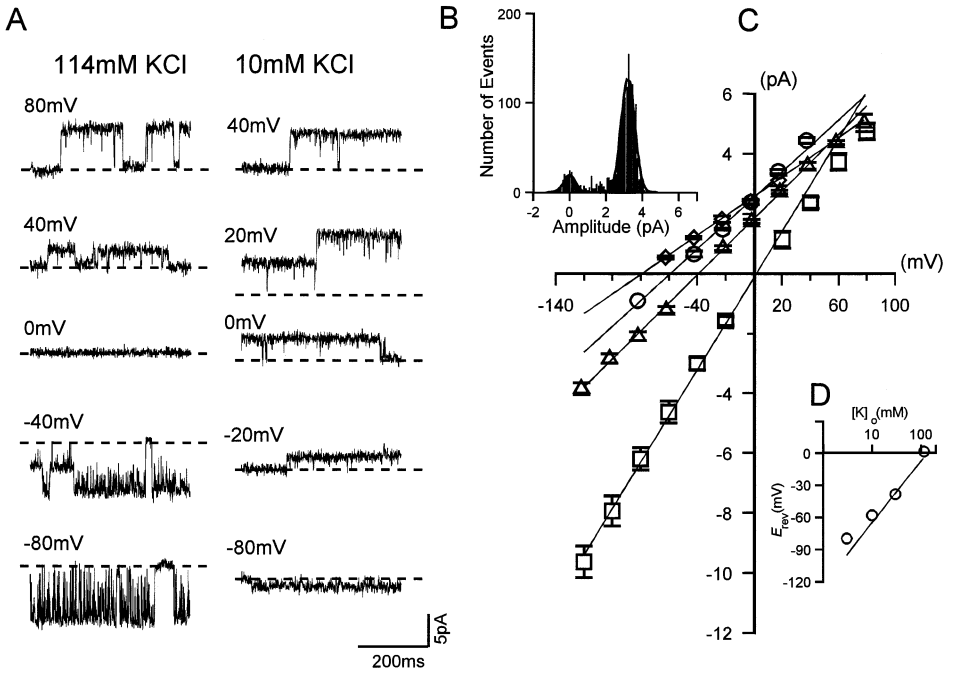

Fig. 1. Relation between single-channel current and membrane potential in 3, 10, 30 and 114 mM K^+ pipette solutions. (*A*) Original current records. In the left hand column are data for 114 mM $[K^+]_o$, and in the right hand column those for 10 mM $[K^+]_o$. Numerals in the left hand corner of each record indicate the membrane potential. Broken lines indicate the closed-channel level. Scale bars are applicable to all records. (*B*) Amplitude histogram. Unitary conductance was determined to be 3.2 pA by Gaussian curve fitting. (*C*) The straight line is the best fit by the least-squares method of a line to data in the range of −120 to +20 mV for 114 (open squares) and 30 mM $[K^+]_o$ (open triangles) and −40 to +40 mV for 10 (open circles) and 3 mM $[K^+]_o$ (open diamonds). Numbers of observations are 9 for 3 mM $[K^+]_o$, 4 for 10 mM $[K^+]_o$, 9 for 30 mM $[K^+]_o$ and 6 for 114 mM $[K^+]_o$. The vertical bar means SE of the mean. (*D*) Relation between reversal potential (E_{rev}) and $[K^+]_o$. The straight line was drawn by using the Nernst equation. Experimentally determined junction potentials in various $[K^+]_o$ were corrected.

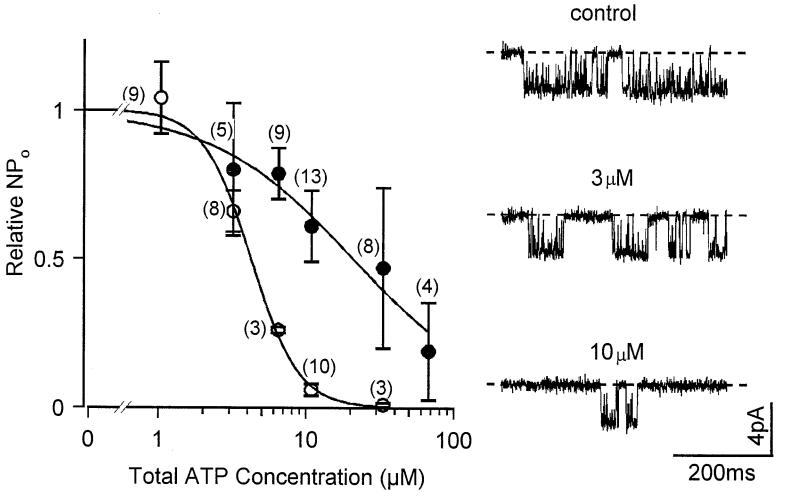

Fig. 2. Dose-response curve for K_{ATP}-channel activity in response to change in $[ATP]_i$. The line is drawn by fitting Hill's equation, $1/[1 + (ATP/ID_{50})^n]$ (where ATP = intracellular ATP concentration, ID_{50} = concentration of half-maximal inhibition, n = Hill coefficient) to the data. The vertical bar means SE of the mean. Numbers of observations are indicated in parentheses. The abscissa indicates total ATP concentration. Closed and open circles are data with and without 2 mM $MgCl_2$ in the bath solution. ID_{50} and n in the presence and absence of Mg^{2+} was 18.9 μM, 0.92 and 3.94 μM, 2.74, respectively. Records obtained in different concentrations of ATP (ATP-free, 3 and 10 μM ATP) in the absence of Mg^{2+} are shown in the right column.

NP_o (Fig. 2). The solid lines were drawn according to the equation $1/[1 + ([ATP]/ID_{50})^n]$, where [ATP] is ATP concentration, ID_{50} the concentration of half-maximal inhibition and n the Hill coefficient. Addition of 2 mM Mg^{2+} shifted an ID_{50} of 3.94 μM in the absence of Mg^{2+} to 18.9 μM accompanying a shift of the Hill coefficient from 2.74 to 0.92. The results indicate that inhibitory action of Mg^{2+}-bound ATP is weaker than that of free ATP.

Dwell-Time Analysis

The dwell time of channel events was analyzed for recordings filtered at 5 kHz and 0.1 kHz. The former re-

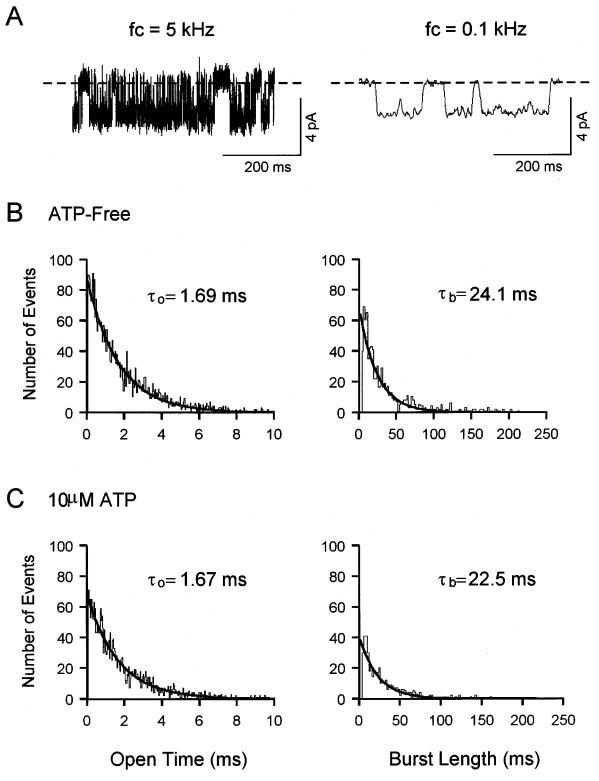

Fig. 3. Histograms of open time and life time of bursts of K_{ATP}-channels. (*A*) Representative records filtered with low pass cutoff frequencies (f_c) of 5 kHz and 0.1 kHz. The dashed line indicates zero current level. Histograms of open time and life time of the bursts are shown in *B* for ATP-free medium and *C* for 10 μM ATP. The bin width is set to 40 μs for open time and 2 msec for the lifetime of the bursts. The potential in the pipette was held at +40 mV in the solutions of 114 mM $[K^+]_o$ and 132 mM $[K^+]_i$ throughout the experiment. τ_o and τ_b in ATP-free are estimated to be 1.69 and 24.1 msec and those in 10 μM ATP to be 1.67 and 22.5 msec, respectively.

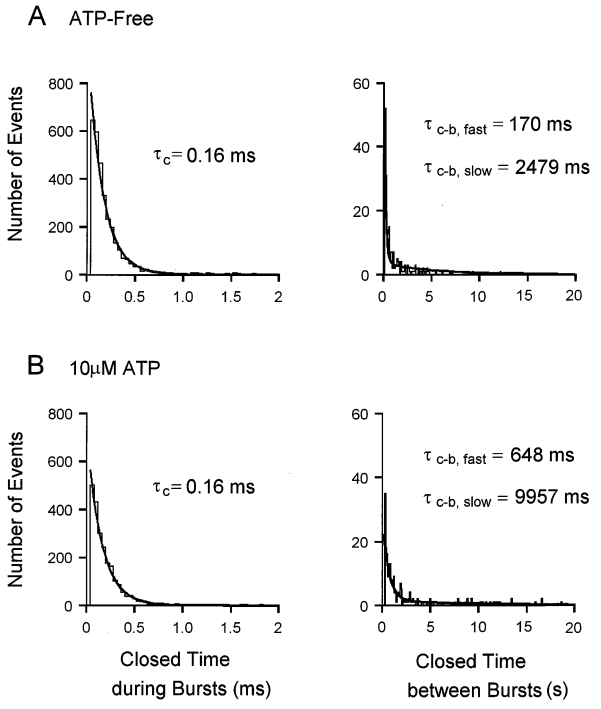

Fig. 4. Histograms of closed time during bursts and inter-burst time of K_{ATP}-channel. Histograms of closed time during the bursts and inter-burst time are shown for ATP-free medium in *A*, and for 10 μM ATP in *B*. The bin width is set to 40 μsec for closed time during the bursts and 100 msec for inter-burst time. τ_c in ATP-free medium is estimated to be 0.16 and that in 10 μM ATP to be 0.16 as well. The two time constants for inter-burst time ($\tau_{c\text{-}b,\ fast}$ and $\tau_{c\text{-}b,\ slow}$) were calculated to be 170 and 2479 ms in the absence of ATP and 648 and 9957 msec in the presence of 10 μM ATP.

cordings were used to define the open and closed times mostly during the burst. In the latter case, the flickering events during the burst was largely removed as shown in Fig. 3*A*, and the current recordings were used for convenience to define the life time of bursts and the inter-burst duration. A single exponential curve was fitted to the open-time (Fig. 3) and closed-time distributions (Fig. 4). The mean open time was determined to be 1.63 ± 0.17 msec ($n = 5$) in an ATP-free medium and 1.57 ± 0.10 msec ($n = 5$) in 10 μM ATP. The mean closed time was estimated to be 0.16 ± 0.01 msec ($n = 5$) in ATP-free medium and 0.15 ± 0.01 msec ($n = 5$) in 10 μM ATP. Neither of mean open time nor the closed time was significantly affected by 10 μM ATP ($P > 0.05$).

The mean life time of bursts was estimated to be 24.1 msec in the ATP-free solution and 22.5 msec in 10 μM ATP. Averages of arithmetic means of the life times of bursts were 37.7 ± 24.1 msec ($n = 5$) in ATP-free medium and 20.5 ± 7.1 msec ($n = 5$) in 10 μM ATP. Histogram of inter-burst times is shown in Fig. 4*B*, which was constructed by combining data obtained in five cells. This is because the number of inter-burst events was too small in a given experiment, since their mean lifetime was in the range of seconds. The histogram of inter-burst times was fitted by two exponential functions, having time constants of 170 and 2479 msec in ATP-free medium and 648 and 9957 msec in 10 μM ATP. Averages of arithmetic means for inter-burst times were 402 ± 198 msec ($n = 5$) when ATP-free and 2723 ± 2108 ($n = 5$) in 10 μM ATP. The difference was statistically significant ($P < 0.05$). These results show that the only parameter affected by ATP was the inter-burst time.

Another noteworthy point is that the open time tended to increase as the membrane potential was depolarized (Fig. 5). A dependency of open time on membrane potential was also reported in K_{ATP}-channel in rat ventricular cells (Zilberter et al., 1988).

Effect of Glibenclamide on Activity of K_{ATP}-Channels

Because glibenclamide is known to be a selective blocker of K_{ATP}-channels (Sturgess et al., 1985; Fosset et

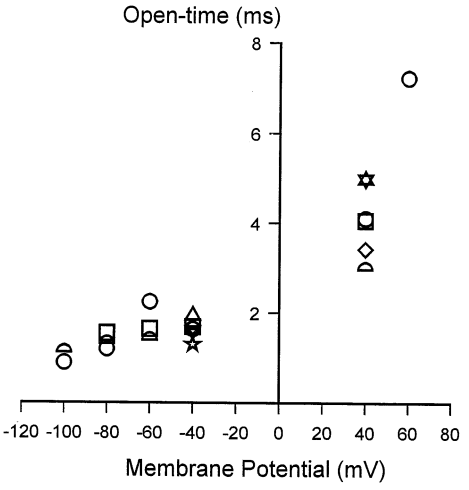

Fig. 5. Voltage dependency of open time. Mean open time of measurements at various membrane potentials are plotted. Each symbol indicates a series of data obtained from the same cell. These data were taken in the solutions of 114 mM $[K^+]_o$ and 132 mM $[K^+]_i$ without ATP.

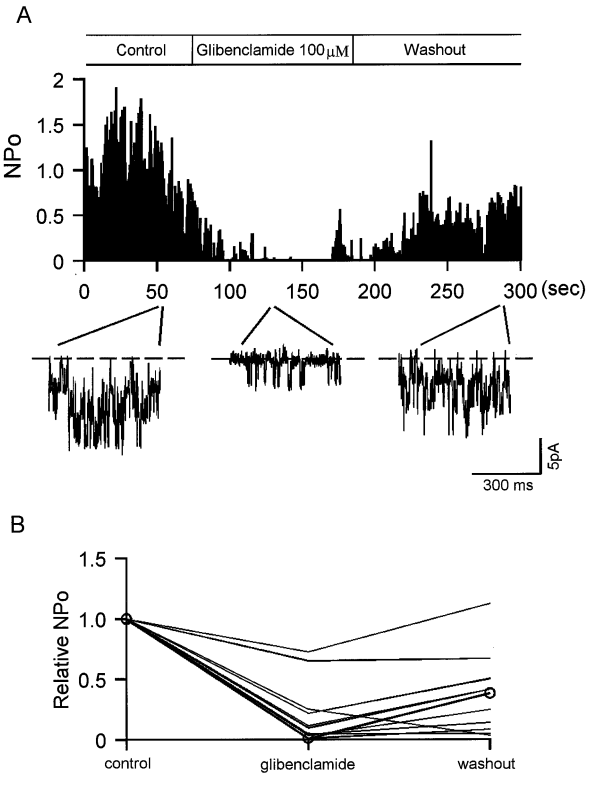

Fig. 6. Suppression of K_{ATP}-channel by 100 μM glibenclamide. (*A*) Change in NP_o during application of 100 μM glibenclamide after the formation of the inside-out patch. Channel activity was reversibly inhibited by application of 100 μM glibenclamide. Representative records are shown in each state during control, application and washout of glibenclamide. The potential in the pipette was held at +40 mV in the solutions of 114 mM $[K^+]_o$ and 132 mM $[K^+]_i$ without ATP. (*B*) Summary of the effect of 100 μM glibenclamide on 11 cells. Averaged NP_o before application of glibenclamide is referred to control in each cell and expressed as unity. Fractions of NP_o to its control are shown during the application and washout of glibenclamide in each cell.

al., 1988; Pilsudski, Rougier & Tourneur, 1990), we tested its effect on K_{ATP}-channels in frog ventricular myocytes. Representative data in which the activity of K_{ATP}-channel is expressed as NP_o are shown in Fig. 6*A*.

The responses of K_{ATP}-channels to glibenclamide are shown in Fig. 6*B* as relative NP_o from a series of eleven experiments. Internal application of 100 μM glibenclamide reduced the channel activity to 20.1 ± 8.0% (n = 11) of control ($P < 0.05$), except in two experiments. Partial recovery of NP_o was observed by washing off glibenclamide that was most probably due to incomplete washout of glibenclamide and/or the process of rundown. These results indicate that glibenclamide effectively suppresses K_{ATP}-channels of frog ventricular myocytes. Sensitivity of K_{ATP}-channels to this reagent is qualitatively similar to that reported in other literature (Venkatesh, Lamp & Weiss, 1991; Findlay, 1993; Ripoll, Lederer & Nichols, 1993).

Discussion

In this study, we have shown the existence of ATP-sensitive K^+ channels in frog cardiac myocytes. Properties of the channels can be summarized as follows. (i) Single channel conductance in 114 mM $[K^+]_o$ with 132 mM $[K^+]_i$ medium is estimated to be 77.3 pS and this channel is highly selective to K^+. (ii) Channel open events appear in bursts and the kinetics of channel gating is dependent on membrane potential. (iii) ATP bound with Mg^{2+} is less effective than free ATP in inhibiting the channel. (iv) The open time histogram is well fitted with a single exponential curve, with time constants of 1.63 msec in ATP-free medium and of 1.57 msec in 10 μM ATP. Change in ATP concentration affects neither the amplitude of the single-channel current nor the mean open time. Neither the closed time during bursts nor the lifetime of the bursts was affected by ATP. However, application of ATP lengthened the inter-burst time. (v) Glibenclamide suppresses this channel as it does other K_{ATP}-channels.

We failed to observe a change in mean open time as a result of applying 10 μM ATP. The result is not consistent with data reported in skeletal muscle K_{ATP}-channels (Spurce, Standen & Stanfield, 1987; Woll, Lönnendonker & Neumcke, 1989) and mammalian cardiac muscles (Kakei, Noma & Shibasaki, 1985; Nichols, Lederer & Cannel, 1991), in which mean open time is shortened by ATP. Thus, it is unlikely that ATP binds exclusively to the channel in the open state in frog heart. Qin, Takano & Noma (1989) and Fan, Nakayama & Hiraoka (1990) obtained results similar to ours in that

ATP did not change mean open time. These studies focused on kinetics within bursts, revealing consistent results that ATP did not change mean open time and closed time. In the report by Fan, Nakayama & Hiraoka (1990), however, a reduction in the lifetime of bursts was also pointed out. We did not find any change in the lifetime of bursts but only in the inter-burst time on application of 10 μM ATP. We thus tend to believe that ATP binds to the channels in the closed states forming the inter-burst time and the channels bound by ATP remain stalled in closed states longer than those free from ATP.

In frog ventricular myocytes, K_{ATP}-channel appear to be much less sensitive to ATP in a medium containing Mg^{2+} than in a Mg^{2+}-free medium, as shown in Fig. 2. The result is similar to data from β-cells (Findlay, 1988; Ashcroft & Kakei, 1989) and skeletal muscle (Vivaudou, Arnoult & Villaz, 1991). The role of Mg^{2+} in the inhibitory action of ATP to K_{ATP}-channels, however, is a subject of controversy. In pancreatic cells, Aschcroft and Kakei (1989) obtained a coincidence of the dose-response curve for computed free ATP in a medium containing Mg^{2+} with that in a Mg^{2+}-free medium, so they argued that only free ATP is effective in suppressing the channel, but not Mg-ATP. In skeletal muscle blebs, on the other hand, Vivaudou et al. (1991) adopted a procedure similar to that of Aschcroft and Kakei (1989) but failed to obtain coincidence of the two dose-response curves. They thus argued that Mg-ATP can block the channel but less potently than free ATP. In frog heart myocytes, the results obtained qualitatively agree with the latter as shown in Fig. 2, so we believe that Mg^{2+}-ATP is less potent in inhibiting the channel than free ATP. Our only difference from their results is the reduction of Hill's coefficient from 2.74 in the ATP medium to 0.92 in Mg-ATP medium, probably attributable to a conformational change in the K_{ATP}-channel complex in the absence of Mg^{2+}. Nevertheless, some reports assert that Mg^{2+} functions as an inert or an active molecule in changing the sensitivity of K_{ATP}-channel to ATP. Lederer and Nichols (1989) showed that, regardless of the presence and absence of Mg^{2+}, the dose-response curves against free ATP coincide with each other. Findlay (1988) used almost the same protocol as we did and reached a conclusion, different from ours, that the K_{ATP}-channel of rat ventricular myocytes does not discriminate free ATP from Mg-ATP, with the dose-response curve against total ATP in the solution containing Mg^{2+} shifting slightly further leftward than with the application of ATP alone.

We wish to express our sincere thanks to Dr. M. Yakehiro for his valuable advice. One of the authors (M.M.) would also like to thank Professor G. Kajiyama, First Department of Internal Medicine, Hiroshima University, for providing him with the opportunity to do this research. This work was supported by the Uehara Memorial Foundation.

References

Ashcroft, F.M., Kakei, M. 1989. ATP-sensitive K^+ channels in rat pancreatic β-cells: modulation by ATP and Mg^{2+} ions. *J. Physiol.* **416:**349–367

Ashford, M.L.J., Sturgess, N.C., Trout, N.J., Gardner, N.J., Hales, C.N. 1988. Adenosine-5′-triphosphate-sensitive ion channels in neonatal rat cultured central neurons. *Pfluegers Arch.* **412:**297–304

Cook, D.L., Hales, C.N. 1984. Intracellular ATP directly blocks K^+ channels in pancreatic β-cells. *Nature* **311:**271–273

Fan, Z., Nakayama, K., Hiraoka, M. 1990. Pinacidil activates the ATP-sensitive K^+ channel in inside-out and cell-attached patch membranes of guinea-pig ventricular myocytes. *Pfluegers Arch.* **415:**387–394

Findlay, I. 1988. ATP^{4-} and ATP · Mg inhibit the ATP-sensitive K^+ channel of rat ventricular myocytes. *Pfluegers Arch.* **412:**37–41

Findlay, I. 1993. Sulphonylurea drugs no longer inhibit ATP-sensitive K^+ channels during metabolic stress in cardiac muscle. *J. Pharmacol. Exp. Ther.* **266:**456–467

Findlay, I., Dunne, M.J., Petersen, O.H. 1985. ATP-sensitive inward rectifier and voltage- and calcium-activated K^+ channels in cultured pancreatic islet cells. *J. Membrane Biol.* **88:**165–172

Fosset, M., DeWeille, J.R., Green, R.D., Schmid-Antomarchi, H., Lazdunski, M. 1988. Antidiabetic sulfonylureas control action potential properties in heart cells via high affinity receptors that are linked to ATP-dependent K^+ channels. *J. Biol. Chem.* **263:**7933–7936

Hamill, O.P., Marty, A., Neher, E., Sakmann, B., Sigworth, F.J. 1981. Improved patch-clamp techniques for high-resolution current recording from cells and cell-free membrane patches. *Pfluegers Arch.* **391:**85–100

Horie, M., Irisawa, H., Noma, A. 1987. Voltage-dependent magnesium block of adenosine-triphosphate-sensitive potassium channel in guinea-pig ventricular cells. *J. Physiol.* **387:**251–272

Kakei, M., Noma, A., Shibasaki, T. 1985. Properties of adenosine-triphosphate-regulated potassium channels in guinea-pig ventricular cells. *J. Physiol.* **363:**441–462

Lederer, W.J., Nichols, C.G. 1989. Nucleotide modulation of the activity of rat heart ATP-sensitive K^+ channels in isolated membrane patches. *J. Physiol.* **419:**193–211

Munemori, M., Yamaoka, K., Yusuf, I., Sumii, K., Otsuka, H., Yamasaki, K. 1996. Effect of N-ferulolyl tyramine (an analogue of tyramine) on inwardly rectifying potassium channel in frog ventricular myocytes. *Hiroshima J. Med. Sci.* **45:**31–35

Nichols, C.G., Lederer, W.J., Cannel, M.B. 1991. ATP dependence of K_{ATP} channel kinetics in isolated membrane patches from rat ventricle. *Biophys. J.* **60:**1164–1177

Noma, A. 1983. ATP-regulated K^+ channels in cardiac muscle. *Nature* **305:**147–148

Noma, A., Shibasaki, T. 1985. Membrane current through adenosine-triphosphate-regulated potassium channels in guinea-pig ventricular cells. *J. Physiol.* **363:**463–480

Pilsudski, R., Rougier, O., Tourneur, Y. 1990. Action of cromakalim on potassium membrane conductance in isolated heart myocytes of frog. *Br. J. Pharmacol.* **100:**581–587

Qin, D.Y., Takano, M., Noma, A. 1989. Kinetics of ATP-sensitive K^+ channel revealed with oil-gate concentration jump method. *Am. J. Physiol.* **257:**H1624–1633

Ripoll, C., Lederer, W.J., Nichols, C.G. 1993. On the mechanism of inhibition of K_{ATP} channels by glibenclamide in rat ventricular myocytes. *J. Cardiovasc. Electrophysiol.* **4:**38–47

Schoenmakers, T.J., Visser, G.J., Flik, G., Theuvenet, A.P. 1992. CHELATOR: an improved method for computing metal ion concentrations in physiological solutions. *Biotechniques* **12:**870–874, 876–879

Seyama, I., Yamaoka, K. 1988. A study of the electrical characteristics of sodium currents in single ventricular cells of the frog. *J. Physiol.* **401:**257–275

Spruce, A.E., Standen, N.B., Stanfield, P.R. 1985. Voltage-dependent ATP-sensitive potassium channels of skeletal muscle membrane. *Nature* **316:**736–738

Spruce, A. E., Standen, N. B., Stanfield, P.R. 1987. Studies of the unitary properties of adenosine-5′-triphosphate-regulated potassium channels of frog skeletal muscle. *J. Physiol.* **382:**213–236

Standen, N.B., Quayle, J.M., Davies, N.W., Brayden, J.E., Huang, Y., Nelson, M.T. 1989. Hyperpolarizing vasodilators activate ATP-sensitive K^+ channels in arterial smooth muscle. *Science* **245:**177–180

Sturgess, N.C., Ashford, M.L., Cook, D.L., Hales, C.N. 1985. The sulphonylurea receptor may be an ATP-sensitive potassium channel. *Lancet* **31:**474–475

Venkatesh, N., Lamp, S.T., Weiss, J.N. 1991. Sulfonylureas, ATP-sensitive K^+ channels, and cellular K^+ loss during hypoxia, ischemia, and metabolic inhibition in mammalian ventricle. *Circ. Res.* **69:**623–637

Vivaudou, M.B., Arnoult, C., Villaz, M. 1991. Skeletal muscle ATP-sensitive K^+ channels recorded from sarcolemmal blebs of split fibers: ATP inhibition is reduced by magnesium and ADP. *J. Membrane Biol.* **122:**165–175

Woll, K.H., Lönnendonker, U., Neumcke, B. 1989. ATP-sensitive potassium channels in adult mouse skeletal muscle: Different modes of blockage by internal cations, ATP and tolbutamide. *Pfluegers Arch.* **414:**622–628

Zilberter, Y., Burnashev, N., Papin, A., Portnov, V., Khodorov, B. 1988. Gating kinetics of ATP-sensitive single potassium channels in myocardial cells depends on electromotive force. *Pfluegers Arch.* **411:**584–589