Journal of Physiology (1993), **463**, *pp.* 747–766
With 10 *figures*
Printed in Great Britain

ATP-REGULATED K^+ CHANNELS ARE MODULATED BY INTRACELLULAR H^+ IN GUINEA-PIG VENTRICULAR CELLS

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(*Received 12 December 1991*)

SUMMARY

1. The ATP-regulated potassium channel (K^+_{ATP}) was investigated with respect to modulation by intracellular pH (pH_i) by using the inside-out membrane patch clamp technique in ventricular cells isolated from the heart of the guinea-pig. Channels which had been closed by internal ATP (0·3–3 mM) were dose-dependently activated by decreasing the pH_i over the range of pH 7·6–6·0. However, the channel was conversely inhibited when the pH_i was further decreased below 6·0. Inwardly rectifying K^+ channels were also decreased in activity when pH_i fell from 7·2 to 6·0.

2. The channel activation was also observed with constant concentration of free Ca^{2+} (1 nM) and Mg^{2+} (1 mM) in the bathing solution, suggesting that a change in divalent cation concentration is not involved in channel modulation by pH_i.

3. When the dose–response relations of the channel activity for ATP concentrations at different pH_i were examined, the channel activity obtained at 1 μM ATP was increased by decreasing pH from 7·2 to 6·4. The half-maximal inhibition for ATP concentration at pH 7·2 and 6·4 was 20 and 40 μM, respectively, and the Hill coefficient was 2·5 in both curves.

4. In the absence of ATP, internal H^+ was able to reactivate run-down channels but it had less effect on the channel as long as the activity was maintained at a higher level. The increase in the channel activity by H^+ was facilitated with a proceeding of the run-down. However, after the channel was completely inactivated by a long exposure of the membrane patch to ATP-free solution, a reduction of pH could not activate the channel.

5. The decrease of pH from 7·2 to 6·4 reduced single channel conductance from 89·0 to 77·7 pS in the absence of Mg^{2+}, whereas it reduced the conductance only at the negative membrane potentials in the presence of 2 mM Mg^{2+}.

6. Mean open and closed times within the burst-like openings of the channel remained unaffected during the change in pH_i.

7. We conclude that the cardiac K^+_{ATP} channel is modulated by a change in the intracellular pH. The channel modulation consisted of the increase in the channel

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activity and a decrease in the permeability. The former effect was due to the decrease in the sensitivity of the channel to ATP and the reactivation of the channel which is during the process of run-down in activity.

INTRODUCTION

ATP-regulated K^+ channels have been identified in various tissues (Noma, 1983; Cook & Hales, 1984; Spruce, Standen & Stanfield, 1985; Standen, Quayle, Davies, Brayden, Huang & Nelson, 1989; Ashford, Boden & Treherne, 1990). The common characteristic of these channels is their closure by intracellular ATP. Thus the physiological implications of the channels have been primarily discussed in relation to cellular energy metabolism (Trube & Hescheler, 1984; Kakei, Noma & Shibasaki, 1985; Noma & Shibasaki, 1985; Ashcroft for review, 1988). In cardiac myocytes, Noma & Shibasaki (1985) demonstrated that activation of the channel underlies the outward current evoked during metabolic inhibition of the heart cell and results in shortening of action potential duration. It seems the K^+_{ATP} channels may regulate contractility and excitability of cardiac muscle during metabolic inhibition (Fosset, De Weille, Green, Schmid-Antomarchi & Lazdunski, 1988; Wilde, Escande, Schumacher, Thuringer, Mestre, Fiolet & Janse, 1990; Weiss, Venkatesh & Lamp, 1992).

Lederer & Nichols (1989) and Davies (1990) recently reported modulations of the channel by intracellular H^+. The open probability of the K^+_{ATP} channel is increased with lowering pH_i. However, intracellular ATP concentration in normal and even in metabolically poisoned heart may be much greater than the concentration of ATP required for half-maximal inhibition of the channels (Noma, 1983; Kakei & Noma, 1984; Allen, Morris, Orchard & Pirolo, 1985; Elliott, Smith & Allen, 1989). Therefore K^+_{ATP} channel modulation by factors other than ATP alone, i.e. a change in the pH_i, may be more important physiologically, because a fall in the pH_i occurs at an early stage in the anoxic heart (Allen *et al.* 1985).

In the present study, we focused on the effects of internal H^+ on the K^+_{ATP} channel to investigate further ATP concentration dependence of channel activation and permeability properties influenced by H^+ in guinea-pig ventricular myocytes. Internal acidification and its physiological relevance regarding channel activation will be discussed. Some of these results have been reported (Koyano, Kakei, Yoshinaga, Matsuoka & Tanaka, 1990).

METHODS

Preparations

Single ventricular myocytes were isolated from the hearts of guinea-pigs weighing 200–300 g as reported by Taniguchi, Kokubun, Noma & Irisawa (1981). Each animal was anaesthetized with sodium pentobarbitone (25 mg kg^{-1} I.P.) and then artificially ventilated. The chest was opened, the aorta cannulated and the coronary artery then perfused with Tyrode solution. The heart was removed, suspended on Langendorff-type apparatus and perfused with Tyrode solution. The perfusate was switched to nominally Ca^{2+}-free Tyrode solution and a digesting solution which contained 0·01% collagenase (Yakult, Japan; see the paper by Yazawa, Kaibara, Ohara & Kameyama, 1990) and was then perfused for 10 min, followed by perfusion with 100 ml of Kraftbrühe (KB) solution (Isenberg & Klöckner, 1982) to wash out the collagenase. The left

ventricle was removed and cut into small pieces (5 mm) in KB solution and dispersed mechanically into single cells. The dispersed myocytes were stored in KB solution at 4 °C for at least 1 h before use.

Single ventricular myocytes were then carried into the recording chamber (500 μl in capacity) filled with the standard internal solution, and experiments were started after the myocytes had settled at the bottom of the chamber.

Single channel recordings

Standard patch clamp techniques (Hamill, Marty, Neher, Sakmann & Sigworth, 1981) were used to record single K^+_{ATP} channel currents from inside-out membrane patches. Patch pipettes were pulled from hard glass tubing (Narishige Scientific Inst. Lab., Japan), coated with silicon resin to reduce their electrical capacitance and fire-polished just before use. The pipettes had resistances between 5 and 10 MΩ when they were filled with the pipette solution (140 mM KCl). After establishing a gigaohm seal on a cell exposed to a standard internal solution containing 0·3 mM ATP and with a pH of 7·2, the patch membrane was excised. To apply a different test solution to the intracellular side of the membrane patch, the patch pipette was inserted into the mouth of one of a series of tubes (1·5 mm in diameter) from which test solutions were flowing independently at a rate of 2 ml min^{-1} (Yellen, 1982). K^+_{ATP} channel current was recorded by an amplifier (List, EPC7, Germany) and stored with a PCM digital data recorder (TEAC RD-1117Z). Replayed data were then low-pass filtered (24 dB $octave^{-1}$, E-3201A, NF, Japan) at a cut-off frequency indicated in the figure legends and digitized by a 12-bit AD converter to be analysed with a computer (Hewlett Packard, HP-9816, Tokyo, Japan).

To calculate the mean K^+_{ATP} current (I) we constructed the amplitude histogram with a current level of 0·04 pA per bin on abscissa during superfusion with each test solution having a different pH for at least 2 min. The mean current was obtained by integrating open channel distributions on the histogram and by averaging against time. The mean amplitude of the single channel event was determined by the difference between the current level of closed channel distribution and that of the first-step channel events. The probability of the channel being open (open probability) was calculated from the equation described below only when the total number of channels in the membrane patch was determined in ATP-free solution. Following the convention for similar experiments, we referred the channel activity to NP and calculated from the following equation: $I = NPi$, where N is the number of channels functioning in the patch membrane, P is the open probability and i is the unitary current of the K^+_{ATP} channel.

The concentration-jump method (Kakei & Ashcroft, 1987) was used to construct the dose–response relations (Fig. 5) between the channel activity and ATP concentrations at different pH. The patch membrane was held at −60 mV and the membrane patch was exposed to a series of test solutions. The tested sequence is an exposure to the solution at pH 7·2, followed by pH 6·4 and then by the pH 7·2 again at the required ATP concentration. These test solutions were alternated with the exposure to the solution containing 1 mM ATP and pH 7·2 for 1 min to keep the channel activity stable throughout the experiment. The application of the test solution consisted of 20–30 s exposure. The NP at each pH was calculated and normalized to the maximal NP, which usually occurred at pH 6·4 and 1 μM ATP, and then plotted as a function of ATP concentration. In the dose–response relations between the channel activity and ATP, each point was fitted with the following Hill equation;

$$\text{relative activity} = y_{max}/\{1+([L]/K_{\frac{1}{2}})^n\},$$

where y_{max} is the relative channel activity at the maximum, $K_{\frac{1}{2}}$ is the ligand concentration ([L], ATP) for the half-maximal effect on the channel activity and n is the Hill coefficient.

Solutions

The composition of the pipette solution was (mM): KCl, 140; $CaCl_2$, 2; Hepes, 5; pH 7·4 adjusted with NaOH. The Tyrode solution contained (mM): NaCl, 136·9; KCl, 5·4; $CaCl_2$, 1·8; $MgCl_2$, 0·5; NaH_2PO_4, 0·33 and glucose, 5·5; the pH was adjusted to 7·4 with 5 mM Hepes-NaOH. The standard internal solution contained (mM): KCl, 134·5; KH_2PO_4, 0·5; $MgCl_2$, 2; EGTA, 1; Hepes, 5; pH 7·2 with KOH; final K^+ concentration 140 mM. The KB solution contained (mM): KCl, 25; taurine, 10; glutamic acid, 70; oxalic acid, 10; Hepes, 10; KH_2PO_4, 10; EGTA, 0·5 and glucose, 11; the pH was adjusted to 7·4 with KOH. To test various pH_i on K^+_{ATP}-channel activity, the pH of each solution was adjusted with Hepes-KOH. In some experiments, 5 mM Mes (2-[*N*-morpholino]ethane-

sulphonic acid) buffer was used instead of Hepes buffer for solutions having a pH less than 7·2. In order to examine effects of pH_i at constant free Ca^{2+} and free Mg^{2+} concentrations, Ca^{2+}, Mg^{2+}-EGTA buffer was used and titrated to 1 nM Ca^{2+} and 1 mM Mg^{2+} at pH 7·2 and 6·4. The composition of both solutions was (mM): ATP, 0·3; KCl, 108; $CaCl_2$, 0·085; $MgCl_2$, 2·46; KH_2PO_4, 0·5; Hepes, 10; EGTA, 11; pH 7·2 with KOH, and ATP, 0·3; KCl, 113; $CaCl_2$, 0·0025; $MgCl_2$, 1·4; KH_2PO_4, 0·5; Hepes, 10; EGTA, 11; pH 6·4 with KOH. In these solutions final K^+ concentration was adjusted to 140 mM. Adenosine-5′-triphosphate (disodium salt) and adenosine-5′-diphosphate (potassium salt) was purchased from Boehringer and added to bath solutions as required. All the experiments were performed at 37 °C.

RESULTS

Effects of H^+ on the activity of K^+_{ATP} and inwardly rectifying K^+ channels

The activity of most K^+_{ATP} channels tended to decrease rapidly with time when the patch membrane was excised from an intact cell into ATP-free solution ('run-down'; Trube & Hescheler, 1984; Kakei & Noma, 1984; Findlay, 1987). We therefore used a bathing solution containing 0·3 mM ATP to minimize the run-down of K^+_{ATP} channels as has been suggested for pancreatic B-cells (Ohno-Shosaku, Zunckler & Trube, 1987; Ashcroft & Kakei, 1989) and for cardiac cells (Findlay, 1987). The effect of intracellular pH over the pH range 7·2–6·0 on the channel activity in the presence of 0·3 mM and 1 mM ATP is shown in Fig. 1. We first recorded K^+_{ATP} currents in the presence of 0·3 mM ATP at pH 7·2 as a control. After recognizing a steady-state activity of the channel, the intracellular solution was changed to test solutions of different pH (usually pH 7·2, 6·8, 6·4 and 6·0), and finally the current in control solution was recorded again to check for 'run-down' of the K^+_{ATP} channel activity. When significant run-down of the activity was recognized, the data were discarded.

In Fig. 1*A* two types of channel openings are represented; one is a K^+_{ATP} channel with a large conductance and the other the inwardly rectifying K^+ channel (Sakmann & Trube, 1984) with a small conductance and longer burst-like openings. The large conductance channels were identified as K^+_{ATP} channels by a sensitiveness to ATP at the cytoplasmic side of the membrane (Figs 1*C* and 2). The mean amplitudes of these two types of K^+ channels were $5·3 \pm 0·2$ pA ($n = 6$) and $2·2 \pm 0·2$ pA ($n = 4$) at a given membrane potential (−60 mV), indicating that slope conductances of single channel current were 89·5 and 36·7 pS, respectively.

The activity of the K^+_{ATP} channel increased with a reduction of internal pH. The amplitude histogram corresponding to this experiment constructed at pH 7·2 and 6·0 is shown in Fig. 1*B*. The second peak indicates the openings of the inwardly rectifying K^+ channel, and the third and forth peaks (symbolized with circles) indicate those of the K^+_{ATP} channel overlapping with and without the inwardly rectifying K^+ channels, respectively. When the pH was changed from 7·2 to 6·0, more activity was observed for the K^+_{ATP} channel. The open probability at pH 7·2 and 6·0 was 0·0074 and 0·08 respectively, when we assumed that there were two K^+_{ATP} channels in the patch. In contrast, the inwardly rectifying K^+ channel showed decreased channel activity with reduced pH (open probability of 0·89 at pH 7·2 and 0·51 at pH 6·0) when the number of channels is one. The activity of the inwardly rectifying K^+ channel remained unaffected above pH 6·4. It should be noted that the amplitude of the K^+_{ATP} channel was smaller at pH 6·0 than that at pH 7·2 (it decreased from 5·47 to 4·76 pA). Thus, internal acidification seems to influence both the activity and the

single channel conductance of the K^+_{ATP} channel. The effect on the conductance of the K^+_{ATP} channel is analysed further in Fig. 9.

We observed a similar effect of intracellular acidification on the K^+_{ATP} channel activity in the presence of 1 mM ATP (Fig. 1*C*). The control channel activity

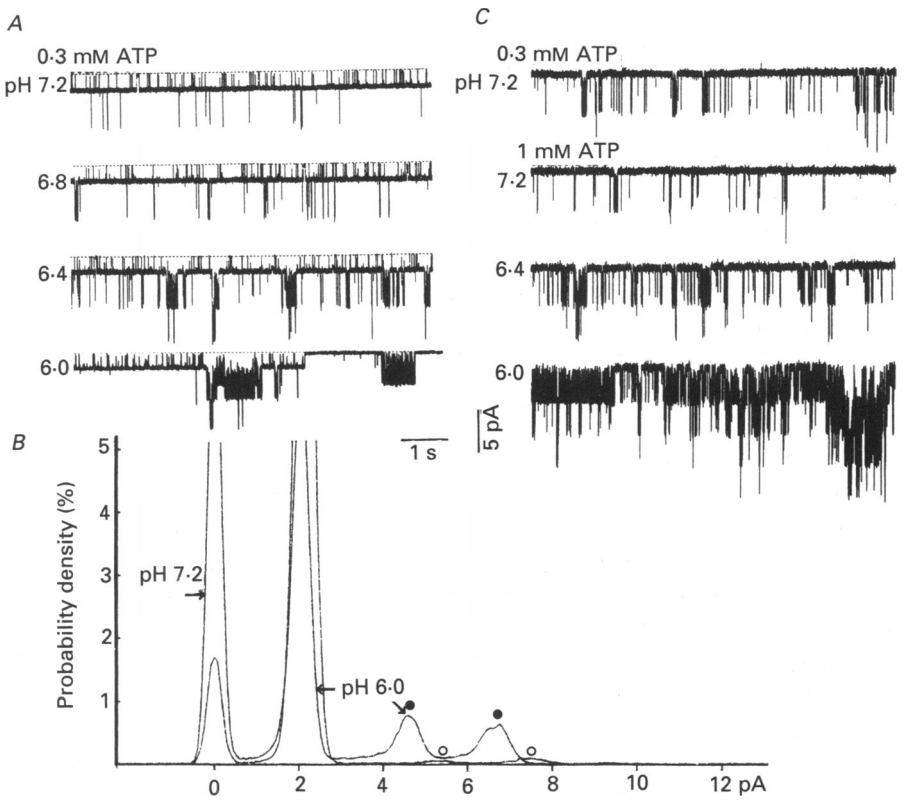

Fig. 1. Effects of intracellular H^+ on both the K^+_{ATP} channel and inwardly rectifying K^+ channel. *A*, single channel recordings obtained from inside-out membrane patches exposed to the bathing solution containing 0·3 mM ATP with the pH indicated beside the current trace. The patch membrane potential was −60 mV. Two types of channel openings can be seen: those of the K^+_{ATP} channel and the inwardly rectifying K^+ channel. After obtaining an inside-out membrane patch in 0·3 mM ATP, pH 7·2 solution, the pH was changed sequentially to 6·8, 6·4 and 6·0 in the presence of 0·3 mM ATP. *B*, the amplitude histogram constructed from recordings longer than 2 min under the experimental conditions indicated in *A* at pH of 7·2 and 6·0. The ordinate expresses the percentage of the total sampled points and the abscissa the bin size in units of 0·04 pA. Each ● near the third and forth peaks of the amplitude histogram indicates the openings of the K^+_{ATP} channel at pH 6·0, and ○ those at pH 7·2. *C*, the effect of pH on the K^+_{ATP} channel in the presence of 1 mM ATP. Channel activity recorded at 0·3 mM ATP and pH 7·2 was substantially inhibited by subsequent introduction of 1 mM ATP (top and second trace). Subsequent acidification relieved the channel inhibition by ATP. The data were filtered and sampled at 2 and 10 kHz, respectively. The dashed lines in *A* indicate the patch current level when the channel was closed.

recorded at 0·3 mM ATP with pH 7·2 (top trace) was significantly inhibited by 1 mM ATP but subsequent reduction of pH_i produced a significant K^+_{ATP} activation. These effects of H^+ were reversible when the pH changed back to 7·2.

In Fig. 2 we examined the pH effect in the presence of 3 mM ATP with and without 2 mM Mg^{2+}. Shown on the top trace of *A*, K^+_{ATP} channel activity with the open

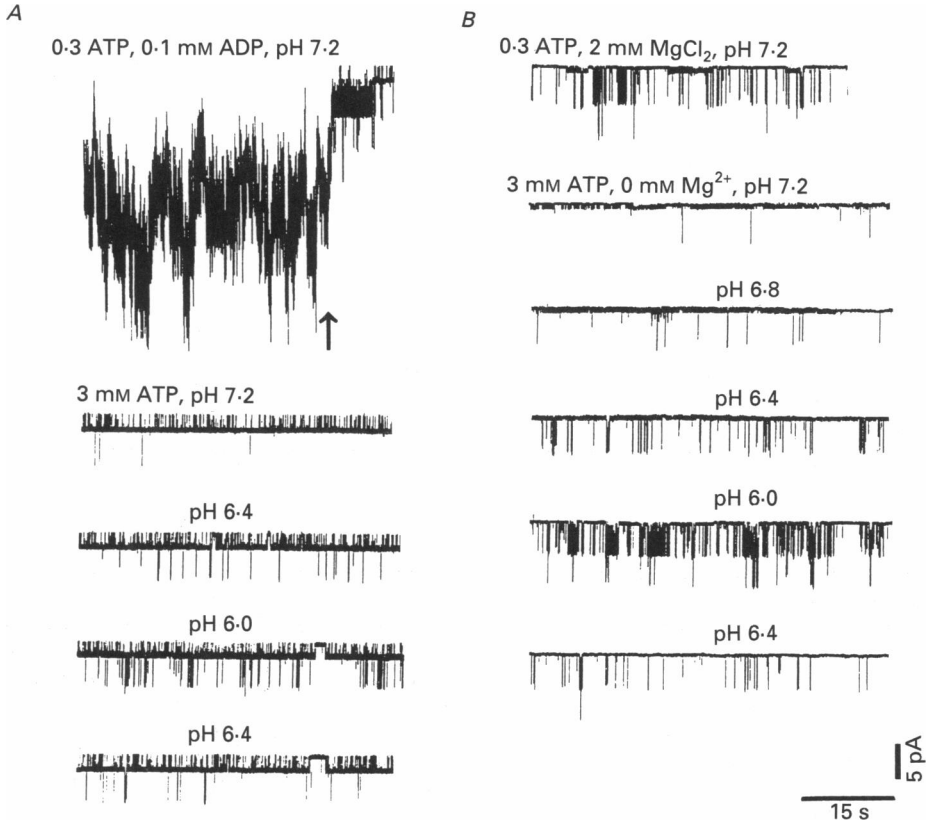

Fig. 2. The effect of H^+ on single K^+_{ATP} channel currents in the presence of 3 mM ATP. The chart recording of K^+_{ATP} channel current was filtered at 1 kHz. *A*, in the presence of 0·3 mM ATP and 0·1 mM ADP, at least eight K^+_{ATP} channels were present in this membrane patch. ATP (3 mM) without ADP was applied at the arrow, then the pH was changed sequentially as indicated above each fractional current trace. *B*, the effect of acid pH on the channel in the absence of Mg^{2+}. Mg^{2+} ions were omitted from the standard internal solution and 1 mM EGTA was replaced by EDTA. The solution exchange was performed sequentially from the top to bottom in the figure. In these experiments, 5 mM Mes-KOH was used instead of Hepes buffer. The patch membrane potential was held at −60 mV.

probability of 0·52 was seen in the solution containing 0·3 mM ATP, 0·1 mM ADP and 2 mM Mg^{2+} which was significantly greater than that in the solution of 0·3 mM ATP alone. This increased activity was due to the decreased sensitivity of the channel to ATP in the presence of ADP as has already been demonstrated (Kakei, Kelly, Ashcroft & Ashcroft, 1986; Findlay, 1987). Subsequent introduction of 3 mM ATP without ADP at the arrow resulted in the rapid inhibition of the K^+_{ATP} channel and

only occasional K^+_{ATP} channel events, overlapping with those of the inwardly rectifying K^+ channel, were seen (second trace). Although many K^+_{ATP} channels were present in this membrane patch, the channel activity was very low when 3 mM ATP was superfused. We, therefore, needed to sample the data for more than 2 min in order

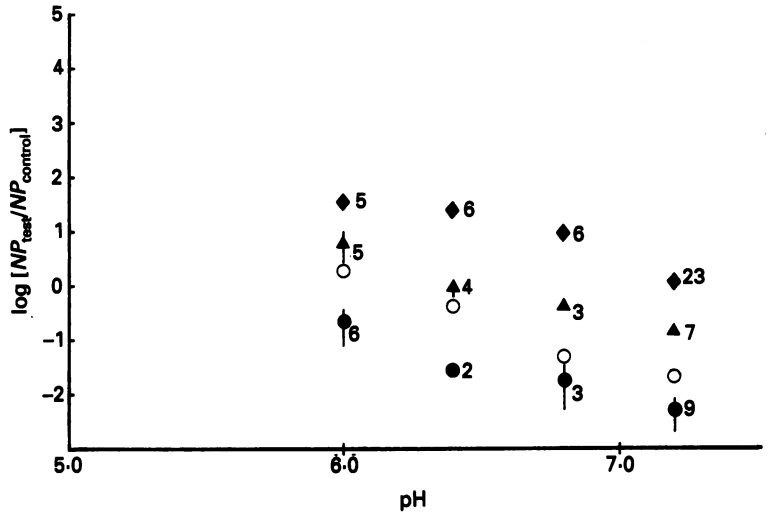

Fig. 3. Dose-dependent activation of the K^+_{ATP} channel by internal H^+. Each point was obtained by making an amplitude histogram and calculating the mean current and unitary amplitude of K^+_{ATP} channel during the application of the test pH solution. The mean current recorded in intracellular solution containing 0·3 mM ATP at pH 7·2 was taken as the control level. The channel activity calculated during the test solution (i.e. at a different pH, NP_{test}) was then expressed as a fraction of $NP_{control}$. The logarithm of relative *NP* against that of control was plotted as a function of pH_i. Means ± S.E.M. were expressed as vertical bars when they were larger than symbols. The number beside each symbol indicates number of experiments carried out. ●, results using 3 mM ATP, 2 mM Mg^{2+}; ○, using 3 mM ATP, 0 mM Mg^{2+}; ▲, using 1 mM ATP, 2 mM Mg^{2+}; and ◆, using 0·3 mM ATP, 2 mM Mg.

to calculate the open probability, because of the longer interburst intervals. The open probability of the K^+_{ATP} channel at pH 7·2, 6·4 and 6·0 was 0·0004, 0·005 and 0·019, respectively (the number of channels was 8). It is clear that protons have an activating effect on the channel even in the presence of 3 mM ATP and that this effect is reversible. A similar effect could be detected also in the absence of Mg^{2+}, as illustrated in part *B* of the figure. The relative increase in the channel activity (*NP*) at low pH as compared to that at pH 7·2 was 3·2 ± 0·6, 13·1 ± 2·8 and 79 ± 8·6 at pH 6·8, 6·4 and 6·0, respectively, in five separate membrane patches.

The relationship between channel activity and pH_i at different ATP concentrations is summarized in Fig. 3. The dose-dependent increase in channel activity with decreasing pH is obvious. The observation that the channel could be activated by H^+ even at ATP concentrations greater than 1 mM contrasts to that reported by Lederer & Nichols (1989), who showed little activation effect of H^+ at such ATP

concentration. In the presence of 3 mM ATP, the channel activity was greater at 0 mM Mg^{2+} than at 2 mM Mg^{2+}. MgATP was more potent for the channel inhibition than ATP^{4-} as has been reported by Findlay (1988*a*).

K^+_{ATP} channel inhibition in extremely low pH solutions

We examined whether the pH effect on channel activity was detectable when the pH was reduced below 6·0. We used a 5 mM Mes buffer rather than Hepes which is

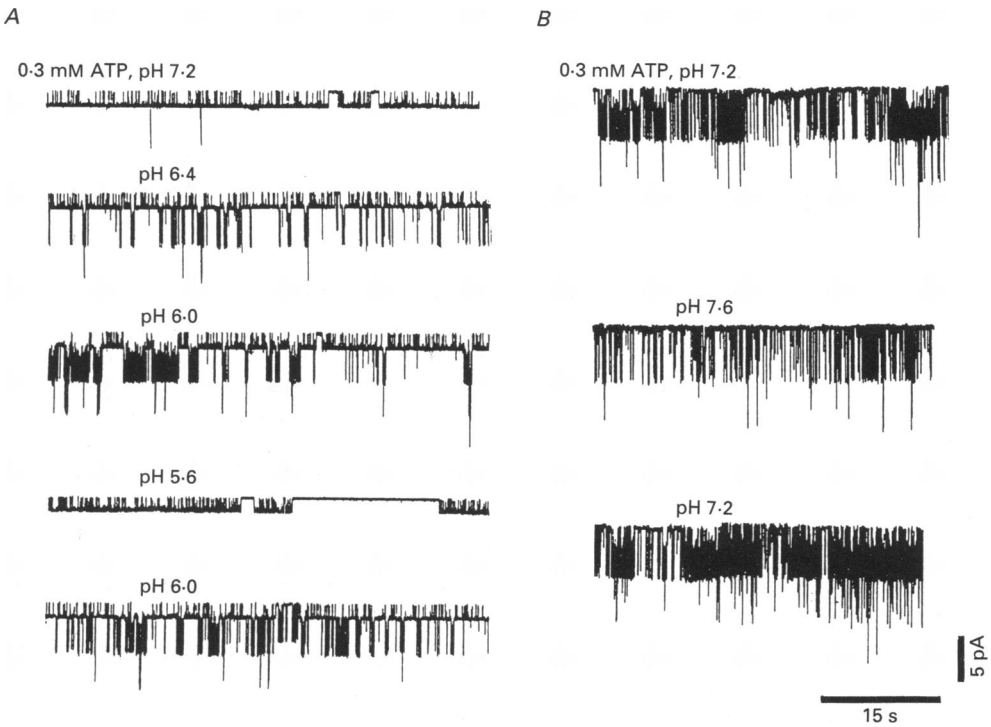

Fig. 4. Channel inhibition both in strong acid pH_i range and in alkaline pH_i. *A*, chart record of the fractional K^+_{ATP} channel current filtered at 1 kHz at various pH_i. Strong acidification to pH 5·6 decreased the channel activity reversibly. 5 mM Mes buffer was used. The small conductance channel openings were those of the inwardly rectifying K^+ channel. *B*, alkaline pH to 7·6 produced less activity of the channel. We used 5 mM Hepes buffer. The membrane potential was held at −60 mV.

expected to be more appropriate for buffering pH at low pH. Figure 4*A* shows that the channel was similarly activated by acidification between pH 6·4 and 6·0 but was reversibly inhibited at pH 5·6. We observed a complete inhibitory effect by pH_i below 6·0 on the channel in eight out of eleven separate patches. The three other patches showed no significant effect as compared to the activity at pH 6·0. It is, therefore, suggested that internal H^+ may have a dual effect on channel gating, activation and inhibition. We recognized the same effects on the channel activity of protons in the Hepes-buffered solution. We also observed that the channel was less active at pH 7·6 than at pH 7·2 (Fig. 4*B*). The relative channel activity at pH 7·6 was

30·0 ± 6·0 % ($n = 4$) of that at pH 7·2. Although the dual effects of protons on the channel activity interfered with a measurement of the half-maximal effect (the negative log of the dissociation constant (pK_a)) for the pH in Fig. 3, we found that the slope factor at 0·3, 1 and 3 mM ATP was 1·3, 1·3 and 1·6, respectively, when judged by a least squares method.

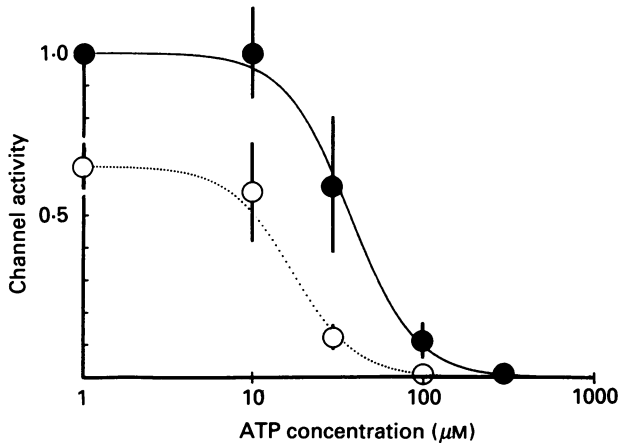

Fig. 5. The effect of pH_i on the dose-dependent inhibition of the channel by ATP. The K^+_{ATP} channel current was recorded at each ATP concentration with two pH values (pH 7·2 (○) and 6·4 (●)) and normalized to that at pH 6·4 and 1 μM ATP. Each curve was drawn according to the Hill equation by the least squares fit ($n = 5$).

Effects of H^+ on ATP-inhibition curve of the channel

Lederer & Nichols (1989) demonstrated that a reduction of pH_i from 7·25 to 6·25 shifted the sensitivity of the K^+_{ATP} channel to ATP from $K_{\frac{1}{2}}$ of 25 to 50 μM. We examined pH_i effects on the dose–response relations of the channel inhibition for ATP (Fig. 5). The data were normalized as unity to that revealing maximal channel activity (*NP*) which was usually obtained at pH 6·4 and 1 μM ATP. Both curves could be well fitted by the Hill equation with a Hill coefficient of 2·5 and with $K_{\frac{1}{2}}$ of 20 μM (pH 7·2) and 40 μM (pH 6·4). The shift of the channel-inhibition curve by the reduction of pH was much less than that reported for the skeletal muscle cell (Davies, Standen & Stanfield, 1992), but it was close to that reported for the rat cardiac myocytes (Lederer & Nichols, 1989). They reported that the effect of low pH_i was accompanied by a change in the Hill coefficient for the ATP-inhibition curve. In the present study, the channel activation by lowering pH_i could be observed even at 1 μM ATP. Because the ATP-inhibited gate of the channel should be maximally opened at such a low ATP concentration, we cannot explain the pH effect solely by a change in the sensitivity of the channel to ATP.

Effects of H^+ on K^+_{ATP} channels in ATP-free solution

The channel could be activated by internal H^+ in the absence of ATP as shown in Fig. 6. The solution exchange protocol is indicated above the chart recordings of K^+_{ATP} channel current (*A* and *B*). When the ATP concentration in the solution was

changed from 0·3 to 0 mM ATP, a transient increase of the channel openings was seen (see also inset). Subsequent rapid decrease in the channel activity in ATP-free solution indicates that the channel ran down in this solution and revealed a new steady-state activity. Under this condition, a reduction of pH to 6·8 produced a

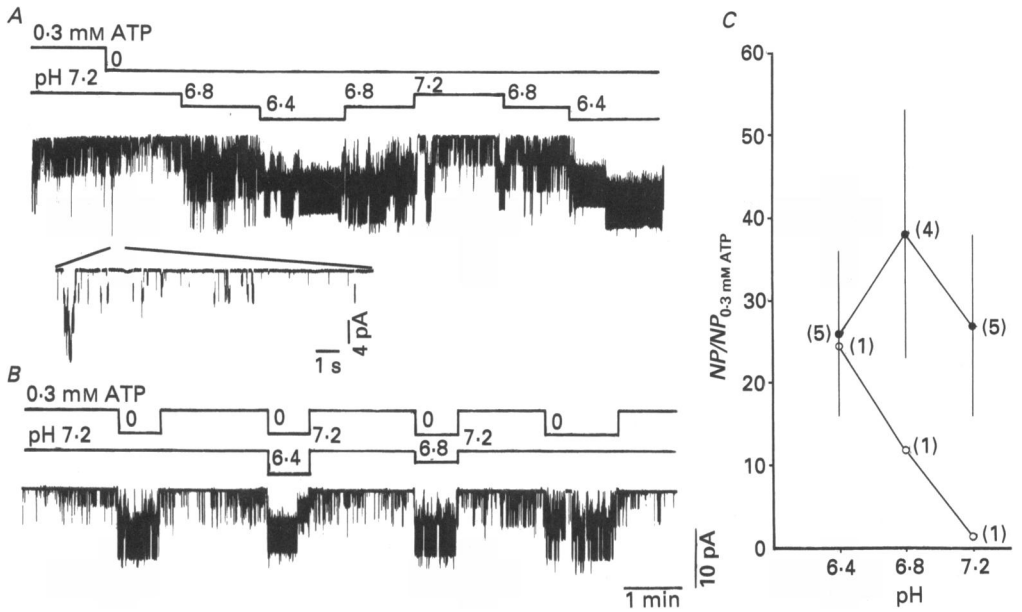

Fig. 6. The effect of pH on the K^+_{ATP} channel in the absence of ATP. *A* and *B*, single K^+_{ATP} channel currents recorded from the chart recorder, the solution-exchanging protocol for ATP and pH_i is shown above current traces. The time-expanded current trace (filtered 2 kHz and sampled 10 kHz) displayed as an inset to *A* shows a rapid increase and subsequent decrease in the channel activity with time after the ATP concentration in the bathing solution was changed from 0·3 to 0 mM, resulting in a steady-state activity similar to that at 0·3 mM ATP. However, the level of the channel activity at 0 mM ATP is kept stable by alternating application of 0·3 mM MgATP in *B*. *C*, dose dependence of the channel activation for H^+ in both protocols. The open circles are from the protocol displayed in *A* and the filled circles from that represented in *B*. The *NP* at pH 7·2 and 0·3 mM ATP was chosen as control to compare the relative effect of H^+ on the channel activity. Patch membrane potential was held at −60 mV. The number beside each symbol indicates the number of replicate experiments.

remarkable increase in channel activity and much more activity was observed at pH 6·4. An increase in the channel activity was 24-fold compared to that of control at pH 7·2 (Fig. 6*C*, open circles).

When the run-down was prevented, however, by alternately exposing the patch to the test solution and then to a recovery solution containing 0·3 mM ATP at pH 7·2 (Ohno-Shosaku *et al.* 1987; Findlay, 1988*a*; Ashcroft & Kakei, 1989; Lederer & Nichols, 1989), internal acidification in the ATP-free solution produced little effect on channel activity (Fig. 6*C*). A similar finding has been reported for skeletal muscle (Davies, 1990) and for rat ventricular myocytes (Lederer & Nichols, 1989). Although

it is undisputed that the fully active channel cannot increase the open probability further unless the rapid open–shut kinetics is changed by protons, the increase in the channel activity by a reduction of pH_i in the ATP-free solution seemed to be related to the extent to which the channel is in a run-down state.

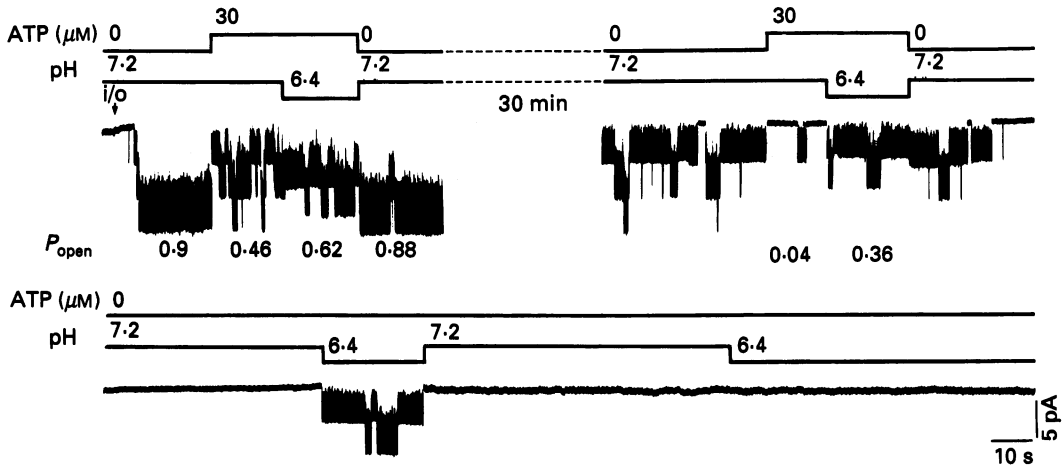

Fig. 7. The effect of pH_i on the K^+_{ATP} channel during the superfusion of ATP-free solution. At the arrow the patch membrane was excised to form the inside-out patch mode (i/o). Open probability (P_{open}) was calculated as described in Methods and was indicated below the current trace. During the interruption of the current trace for 30 min, the channel activity decreased with time. Both upper and lower current traces are continuous. The patch membrane potential was held at −60 mV and the current was filtered at 500 Hz.

We explored the pH effect during a process of channel run-down in Fig. 7. In one experiment shown in the figure, the channel revealed the maximal open probability of 0·9 (the number of channels was three) immediately after excising the patch membrane on the cell into the ATP-free internal solution. During an exposure of the patch membrane to 30 μM ATP with pH 7·2 and 6·4, the channel did not run down because a complete reversibility of the open probability at subsequent 0 mM ATP with pH 7·2 could be seen. In this situation the channel was activated by lower pH, suggesting that the protons reduced the ATP sensitivity. Exposure to ATP-free solution for 30 min resulted in a gradual decrease in channel activity (run-down). An application of 30 μM ATP after the activity reached a steady state produced an inhibition of the channel, so that the channel still retained the ATP sensitivity. However, subsequent reduction of the pH to 6·4 evoked more activation of the channel (9-fold increase in P_{open}) than that observed at the beginning of this experiment (1·3-fold increase). The activation caused by lowering pH was observed even when the channel openings disappeared (the former half of the lower trace in Fig. 7), but it was no longer recognized after ATP-free solution was further superfused. The channel seemed to be reactivated by H^+ during a run-down process until the channel was irreversibly inactivated. We propose that the pH_i effect on the

channel gating consists of lowering the sensitivity of the channel to ATP and the reactivation of the channel during the run-down process.

Is channel activation due to changes in Ca^{2+} or Mg^{2+} concentration?

In the standard internal solution we used, the reduction of pH increases free Ca^{2+} concentration from $1{\cdot}3 \times 10^{-10}$ M at pH 7·2 to $4{\cdot}3 \times 10^{-9}$ M at pH 6·4. It has been

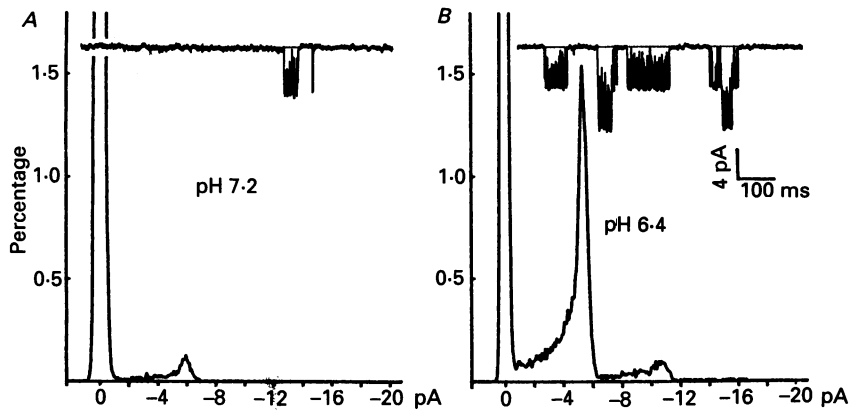

Fig. 8. Effects of pH_i decrease on K^+_{ATP} channels at constant concentrations of free Ca^{2+} and Mg^{2+}. Free Ca^{2+} and free Mg^{2+} concentrations were buffered to 1 nM Ca^{2+} and 1 mM Mg^{2+} at pH 7·2 and 6·4. The composition of the solutions is described in the Methods. Patch membrane potential was held at −60 mV. The data were filtered at 1 kHz.

reported that K^+_{ATP} channels are inhibited by internal divalent cations such as Ca^{2+} and Mg^{2+} at micromolar levels (Kakei & Noma, 1984; Findlay, 1987; Horie, Irisawa & Noma, 1987). Internal Ca^{2+} blocked the K^+_{ATP} channel when the Ca^{2+} concentration was greater than 0·01 μM (Findlay, 1987). However, a change in nanomolar levels (< 10 nM) of Ca^{2+} concentration may conversely modulate the channel activity. Accordingly a question may arise as to whether free Ca^{2+} concentration changes when pH_i in the solutions is lowered, and whether, as a consequence, the activity of the channel increases. In our study this did not happen. As shown in Fig. 8 we observed consistent modulatory effects of pH_i on the channels, when free Ca^{2+} and Mg^{2+} concentrations were buffered to 1 nM for Ca^{2+} and to 1 mM for Mg^{2+}, calculated using association constants provided by Martell & Smith (1974). In this experiment, the open probability was 0·0049 at pH 7·2 and 0·065 at pH 6·4. Similar results were obtained in six other experiments. These results indicate that the pH_i effect is independent of changes in Mg^{2+} and Ca^{2+} concentrations in the internal solution.

Permeability of K^+_{ATP} channels and pH_i

The effect of H^+ on the gating of the channel was accompanied by a reduction in the single channel unitary current, which decreased by 13% when pH_i was reduced from 7·2 to 6·0 (Fig. 1*B*). Single channel unitary currents at a patch membrane potential of −80 and +60 mV and at pH 7·2 and 6·4 are shown in the presence and

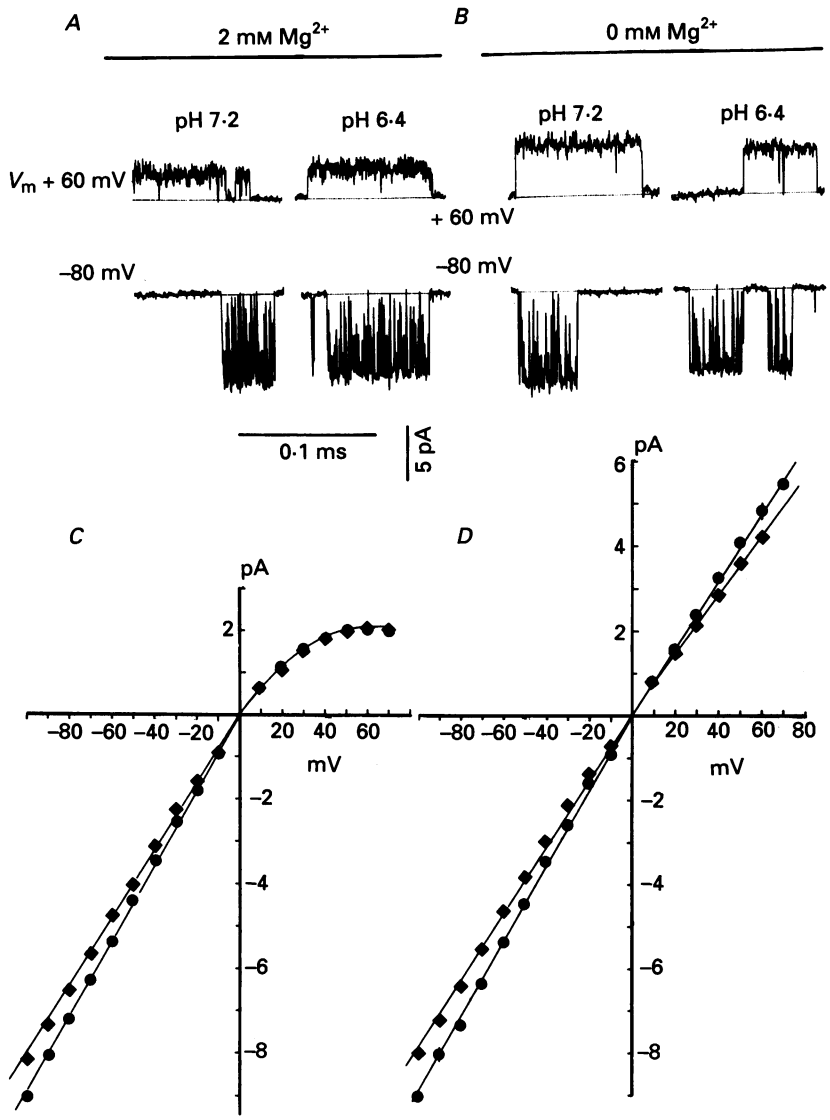

Fig. 9. Inhibition of single channel conductance by internal H^+. Single K^+_{ATP} channel currents at a patch membrane potential of +60 and −80 mV and the current–voltage relations in the presence (*A* and *C*) and absence (*B* and *D*) of 2 mM Mg^{2+} in the intracellular solution. The thin lines in the current traces indicate the closed levels of the channel. In *B* and *D*, Mg^{2+} was omitted and EGTA was replaced by equimolar EDTA. The lines in the current–voltage relations were drawn by eye. The mean current amplitudes from six different inside-out patches were averaged and plotted as means ± S.E.M. which is indicated by the vertical bar and illustrated when it is larger than the symbols. ●, data from pH 7·2; ◆, data from pH 6·4.

absence of Mg^{2+} in Fig. 9*A* and *B*. In the presence of 2 mM Mg^{2+}, the current amplitude decreased significantly when pH_i was changed from 7·2 to 6·4 at −80 mV, but there was no difference at +60 mV. The lack of a decrease in the outward current

amplitudes may be due to blocking of the open channel by Mg^{2+} (Findlay, 1987; Horie *et al.* 1987). We re-examined the effect of H^+ on the K^+_{ATP} channel permeability in the absence of Mg^{2+}. By removing Mg^{2+} from the internal solution, the decrease in the outward current amplitude (inward rectification) was abolished (left panel in

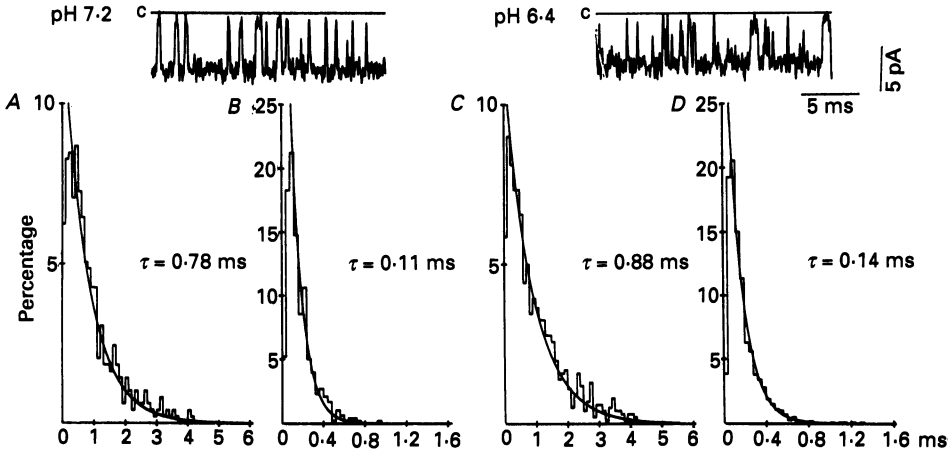

Fig. 10. Effect of H^+ on open time and closed time histograms. Each histogram was constructed with a bin size of 0·10 ms for the open time and 0·04 ms for the closed time. In the closed time histograms, only closed events within the burst-like openings were analysed. Open time histograms at pH 7·2 (*A* and *C*) and closed time histograms at pH 6·4 (*B* and *D*) were constructed from single K^+_{ATP} channel recordings and expressed as a percentage of the total number of events at each bin size. The histograms were fitted with a single exponential function and the time constant was obtained using a least squares method. The critical level used to distinguish between an open or closed event was placed at half the value of the single channel current amplitude. Original current traces are illustrated above the histograms, where c and lines indicate the closed level of the channel. The ATP concentration and the patch membrane potential were 1 mM and −60 mV, respectively. The total number of events collected for each histogram was 1024. The current was filtered at 10 kHz and sampled at 50 kHz.

B and closed circles in *D*). When pH_i was lowered, we observed almost the same amount of reduction in the single channel amplitude at positive and negative membrane potentials, as illustrated in *D*. The inhibition of the current amplitude by internal acidification was not associated with an increase in the open channel noise in contrast to the blocking produced by Mg^{2+} (upper traces in *A* and *B*). It therefore seems that the inhibitory effect of H^+ on the channel current amplitude is different in its mechanism from that produced by internal Mg^{2+}.

The single channel slope conductance when measured at negative potentials was $79{\cdot}9 \pm 0{\cdot}62$ pS (pH 6·4, $n = 5$) and $89{\cdot}53 \pm 0{\cdot}51$ pS (pH 7·2, $n = 5$). These conductances were similar to those obtained in the absence of Mg^{2+}; $77{\cdot}7 \pm 1{\cdot}1$ pS (pH 6·4, $n = 5$) and $89{\cdot}01 \pm 0{\cdot}92$ (pH 7·2, $n = 5$). The difference in conductances between pH 7·2 and 6·4 was statistically significant (Student's *t* test; $P < 0{\cdot}001$). A similar effect was reported for the K^+_{ATP} channel of the frog skeletal muscle cells (Davies, 1990).

Kinetic properties and pH_i

Intracellular H^+ increased the number of openings of the channel (Fig. 1), but did not influence the time constants of open and closed time distributions within a burst of openings (Fig. 10). Mean open times and mean closed times within a burst of openings were $0{\cdot}77 \pm 0{\cdot}03$ ms and $0{\cdot}14 \pm 0{\cdot}02$ at pH 7·2 and $0{\cdot}77 \pm 0{\cdot}06$ and $0{\cdot}14 \pm 0{\cdot}01$ at pH 6·4 in the presence of 1 mM ATP ($n = 3$), and $1{\cdot}01 \pm 0{\cdot}05$ and $0{\cdot}14 \pm 0{\cdot}02$ at pH 7·2 and $1{\cdot}04 \pm 0{\cdot}06$ and $0{\cdot}14 \pm 0{\cdot}01$ at pH 6·4 in the presence of 0·3 mM ATP ($n = 4$). Thus it seems that internal H^+ does not influence the rapid, open and closed events within the burst. Rather, a decrease in pH_i may increase the number of openings within the burst and may decrease the mean lifetime of interburst closed events as shown in Fig. 1*A* and *C*, resulting in an increase in the open probability of the channel.

DISCUSSION

K^+_{ATP} channel modulation by H^+

In the present paper we have shown that the K^+_{ATP} channel is modulated by intracellular H^+. A reduction of pH_i resulted in an increase in the channel activity. This effect consisted of a shift of the $K_{\frac{1}{2}}$ of ATP-dependent channel inhibition from 20 to 40 μM without a change in the steepness and the increase in the open probability of the run-down channel. However, the channel was inhibited by further acidification below 6·0. Lowering the pH also decreased the single channel conductance.

Modulation of K^+_{ATP} channels by internal H^+ has also been found recently in pancreatic B-cells (Misler, Gillis & Tabcharani, 1989), frog skeletal muscle cells (Davies, 1990) and rat ventricular myocytes (Lederer & Nichols, 1989). Internal H^+ increased the channel open probability in skeletal muscle cells and ventricular myocytes, but decreased it in B-cells. The results presented in this paper confirm previous reports from muscle cells (Lederer & Nichols, 1989; Davies, 1990). In the skeletal muscle cells, Davies *et al.* (1992) reported that the K^+_{ATP} channel was activated by protons only in the presence of ATP. They found that the $K_{\frac{1}{2}}$ of the channel inhibition for ATP was changed from 17 μM at pH 7·2 to 260 μM at pH 6·3. Thus, the shift of the $K_{\frac{1}{2}}$ was much smaller in the cardiac myocyte than in the skeletal muscle cell.

Lederer & Nichols (1989) reported that the pH_i effect was associated with an increase in the Hill coefficient from 2 at pH 7·25 to 3 at pH 6·25. In the experiments in Fig. 5, however, we observed little effect of the pH reduction on the steepness of the dose–response relations of the channel activity for ATP. The ineffectiveness on the slope was also observed for skeletal muscle cell (Davies *et al.* 1992).

Mechanism of the effect on the gating of the channel by H^+

Internal acidification caused an increase in the open probability of the K^+_{ATP} channel. The following three reasons suggest that channel activation does not result from changes in metabolite concentrations such as MgATP, ATP^{4-}, Mg^{2+} and Ca^{2+}, the concentrations of which may be changed on decreasing pH and influence channel activity (Findlay, 1987; Findlay, 1988*a*; Lederer & Nichols, 1989). (1) The pH effect

was observed when the Mg^{2+} and Ca^{2+} concentrations were held constant in the internal solution. (2) When we calculated various species of ATP in the internal solution containing 1 mM ATP and 2 mM Mg^{2+} at both pH 7·2 and 6·4 using the binding constant given by Martell & Smith (1974), we found that the concentrations of MgATP and ATP^{4-} are 0·87 mM and 0·036 mM, respectively, at pH 7·2 and both are decreased to 0·82 mM and 0·018 mM at pH 6·4. The concentration of $HATP^{3-}$ should be increased from 8·2 μM at pH 7·2 to 32 μM at pH 6·4. MgATP is a more potent inhibitor than ATP^{4-} for the K^+_{ATP} channel of the cardiac cell and the $K_{\frac{1}{2}}$ was increased from 18 to 30 μM by removing Mg^{2+} in the internal solution (Findlay, 1988*a*). In the K^+_{ATP} channel of pancreatic B-cells, it is suggested that $HATP^{3-}$ has an inhibitory effect on the channel (Ashcroft & Kakei, 1989). If we assume that $HATP^{3-}$ is as effective as ATP^{4-} on the channel inhibition, a decrease in the ratio of (MgATP):[$(ATP^{4-})+(HATP^{3-})$] results in an increase in the $K_{\frac{1}{2}}$ towards the higher ATP concentration in the presence of Mg^{2+}, but this increased $K_{\frac{1}{2}}$ should not exceed the $K_{\frac{1}{2}}$ obtained in the absence of Mg^{2+}. Thus we suggest that the increase (a double increase) of the $K_{\frac{1}{2}}$ for ATP-dependent inhibition produced by a reduction of pH_i can not be fully explained by the small changes in each concentration of MgATP and of the sum of both ATP^{4-} and $HATP^{3-}$. (3) Even if the channel is equally sensitive to both MgATP and ATP^{4-} as has been reported by Lederer & Nichols (1989) or by Davies *et al.* (1992), we cannot explain the pH effect for the same reasons as that mentioned above. Thus, we suggest that protons increase the open probability of the channel by lowering the ATP sensitivity. In the skeletal muscle, Davies *et al.* (1992) proposed the model that protons compete with ATP for the site where ATP binds to close the channel.

Likewise, the fact that protons can activate the channel during the run-down process in ATP-free solution (Figs 6 and 7) suggests that the protons increase the channel activity by a different mechanism from that described in the previous paragraph. We suggest that the activation of the K^+_{ATP} channel by protons is a consequence of both a change in the ATP sensitivity and the reactivation of the channel as long as the channel is to some extent run down. It has been reported that ADP has similar dual effects on the K^+_{ATP} channel in both insulin-secreting cells and cardiac muscle: a change in the ATP sensitivity of the channel and reactivation of run-down channel (Dunne & Petersen, 1986; Kakei *et al.* 1986; Findlay, 1988*b*).

Many other types of K^+ channel seem to be inhibited by internal H^+, as for example the Ca^{2+}-activated K^+ channels from pancreatic B-cells (Cook, Ikeuchi & Fujimoto, 1984) and from rabbit tracheal smooth muscle cells (Kume, Takagi, Satake, Tokuno & Tomita, 1990). In opossum kidney cells, inwardly rectifying K^+ channels were activated by a more alkaline pH (Ohno-Shosaku, Kubota, Yamaguchi & Fujimoto, 1990): the potassium current of cultured Schwann cells was also inhibited by external H^+ (Hoppe, Lux, Schachner & Kettenmann, 1989). All these channels were influenced by changes within the physiological pH_i range, whereas in the cardiac K^+_{ATP} channel, it was blocked by a strong acidic pH_i below 6·0. It seems that the channel inhibition by H^+ may be a common property among potassium channels.

Inhibition of the permeability for potassium ions by H^+

Hydrogen ions influenced not only the open probability of the channel but also the single channel conductance. The reduction in the single channel conductance was 16% of the control amplitude per pH unit in our results and 19% per pH unit in skeletal muscle (Davies, 1990). Reduction of the conductance was observed at all the potentials in the absence of Mg^{2+} despite the acidification only on the inner surface of the membrane. A decrease in the unitary current amplitude was demonstrated also for the acetylcholine receptor (AChR) channel by Imoto, Busch, Sakmann, Mishina, Konno, Nakai, Bujo, Mori, Fukuda & Numa (1988). In *Torpedo* AChR channels expressed in *Xenopus* oocytes, they found that anionic rings formed by the negatively charged amino acid residues (glutamate and/or aspartate residues) on the transmembrane segment is a determinant of the channel conductance, and that ionic permeability may be reduced by changing the number of charges of these residues. Assuming that the K^+_{ATP} channel has a similar structural characteristic, protonation of the K^+_{ATP} channel may neutralize some of the negatively charged amino acid residues at the cytoplasmic side of the transmembrane segment of the channel, resulting in the reduction of unitary amplitudes. A reduction in the single channel conductance by decreasing external pH was also reported for ACh-activated channels from BC3H-1 cells (Pappone & Barchfeld, 1990).

Physiological relevance

Activation of the K^+_{ATP} channel has been thought to be a major component of the increased potassium conductance, which appears under anoxic conditions or in response to the application of metabolic poisons (Noma & Shibasaki, 1985; Fosset *et al.* 1988; Weiss *et al.* 1992). A decrease in pH_i, ATP/ADP, ATP/AMP and phosphocreatine levels seems to occur with a fall in the ATP concentration, when myocyte metabolism is reduced by ligation of the coronary artery (Opie, 1976). Measurement of the cytoplasmic ATP concentration in the anoxic heart by nuclear magnetic resonance showed that the decrease in ATP concentration was preceded by a decline of pH_i and phosphocreatine levels (Allen *et al.* 1985) and showed little decrease in the ATP levels even when action potential durations had already shortened (Elliott *et al.* 1989). Phosphocreatine was without effect on the K^+_{ATP} channel (Kakei *et al.* 1985; Nichols & Lederer, 1990). Could the fall in pH_i produced by ischaemia be responsible for the increase in potassium conductance by activating the K^+_{ATP} channel? It has been reported that internal pH is decreased to around pH 6·8 in response to a disturbance of energy metabolism in heart cells (Allen *et al.* 1985; Vanheel, Leybaert, De Hemptinne & Leusen, 1989). We attempted to estimate mean outward current through K^+_{ATP} channels during internal acidification. From inside-out membrane patch experiments in the presence of 3 mM ATP, mean currents obtained with pHs of 7·2, 6·8, 6·4 and 6·0 were $0·0051 \pm 0·0027$ ($n = 9$), $0·0193 \pm 0·0114$ ($n = 3$), $0·0125 \pm 0·0057$ ($n = 4$) and $0·2140 \pm 0·1260$ pA ($n = 5$), respectively. We assumed a specific membrane capacitance of 1 μF cm^{-2}, a cell capacitance of 50 pF (which is a rather low value), and a patch membrane area of 2 μm^2 which was obtained from the equation (see Sakmann & Neher, 1983) of:

$$a = 12·6(1/R + 0·018),$$

where a is a patch membrane area (μm^2) and R is the pipette resistance, which is between 5 and 10 MΩ in our experiments. The single K^+_{ATP} channel conductance is 35 pS at 5·4 mM K^+ in the external solution (Kakei *et al.* 1986), so that the calculated mean current per cell at pH values of 7·2, 6·8, 6·4 and 6·0 and at a membrane potential of 0 mV was $5·9 \pm 3·1$, $23·5 \pm 13·9$, $15·9 \pm 7·3$ and 290 ± 170 pA, respectively. Faivre & Findlay (1990) demonstrated that an increase of only 50 pA whole-cell background current at 0 mV may be enough to decrease the action potential duration to 50 % of control using the K^+_{ATP} channel opener SR 44866. In a computer-simulated measurement, a similar amount of current increase through the K^+_{ATP} channel could cause a decline of the action potential duration (Nichols & Lederer, 1990). We therefore suggest that a reduction in pH_i below pH 6·8 may be responsible for the increase in the outward current.

It has been suggested that the K^+_{ATP} channel is phosphorylated in MgATP solution and as a consequence the channel activity is maintained (Findlay, 1987; Ohno-Shosaku *et al.* 1987; Takano, Qin & Noma, 1990). As shown in Figs 6 and 7, the run-down channel was more sensitive to change in pH than a not run-down channel. We speculate that the dephosphorylated state during metabolically disturbed conditions to some extent facilitates the activation of the channel by the reduction of pH_i.

However, our estimation of the increase in the K^+_{ATP} channel current level during pH reduction was based on some unknown parameters. The cytosolic ATP concentration in the intact cell may be at a millimolar level, possibly greater than 3 mM, and it may not be changed so much during the early stages of anoxia (Elliott *et al.* 1989). It has been known that not only a change in ATP concentration but also the ATP/ADP ratio influences K^+_{ATP} channel activity (Findlay, 1988*b*; Lederer & Nichols, 1989; Weiss *et al.* 1992). In addition, pH_i reduction during hypoxia is relatively small when CO_2–bicarbonate buffer was used instead of Hepes buffer (Vanheel *et al.* 1989). At present, we do not conclude that intracellular acidosis can fully explain the underlying mechanism of the increase in the potassium conductance in anoxia. It is expected that a more complex modulation of the channel activity by ADP, ATP and internal H^+ determines the appearance of the outward current through the K^+_{ATP} channel.

We thank Dr F. M. Ashcroft (University Laboratory of Physiology, Oxford) for valuable comments in preparing the manuscript. This work was supported by a grant from the Ministry of Education, Science and Culture of Japan.

REFERENCES

ALLEN, D. G., MORRIS, P. B., ORCHARD, C. H. & PIROLO, J. S. (1985). A nuclear magnetic resonance study of metabolism in the ferret heart during hypoxia and inhibition of glycolysis. *Journal of Physiology* **361**, 185–204.

ASHCROFT, F. M. (1988). Adenosine-5′-triphosphate-sensitive potassium channels. *Annual Reviews of Neuroscience* **11**, 97–118.

ASHCROFT, F. M. & KAKEI, M. (1989). ATP-sensitive K^+ channels in rat pancreatic B-cells: modulation by ATP and Mg^{2+} ions. *Journal of Physiology* **416**, 349–367.

ASHFORD, M. L. J., BODEN, P. R. & TREHERNE, J. M. (1990). Glucose-induced excitation of hypothalamic neurons is mediated by ATP-sensitive K^+ channels. *Pflügers Archiv* **415**, 479–483.

COOK, D. L. & HALES, C. N. (1984). Intracellular ATP directly blocks K-channels in pancreatic B-cells. *Nature* **311**, 271–273.

COOK, D. L., IKEUCHI, M. & FUJIMOTO, W. (1984). Lowering of pH_i inhibits Ca^{2+}-activated K^+ channels in pancreatic B-cells. *Nature* **311**, 269–271.

DAVIES, N. W. (1990). Modulation of ATP-sensitive K^+ channels in skeletal muscle by intracellular protons. *Nature* **343**, 375–377.

DAVIES, N. W., STANDEN, N. B. & STANFIELD, P. R. (1992). The effect of intracellular pH on ATP-dependent potassium channels of frog skeletal muscle. *Journal of Physiology* **445**, 549–568.

DUNNE, M. J. & PETERSEN, O. H. (1986). Intracellular ADP activates K^+ channels that are inhibited by ATP in an insulin-secreting cell line. *FEBS Letters* **208**, 59–62.

ELLIOTT, A. C., SMITH, G. L. & ALLEN, D. G. (1989). Simultaneous measurement of action potential duration and intracellular ATP in isolated ferret hearts exposed to cyanide. *Circulation Research* **64**, 583–591.

FAIVRE, J. & FINDLAY, I. (1990). Action potential duration and activation of ATP-sensitive potassium current in isolated guinea-pig ventricular myocytes. *Biochimica et Biophysica Acta* **1029**, 167–172.

FINDLAY, I. (1987). ATP-sensitive K^+ channels in rat ventricular myocytes are blocked and inactivated by internal divalent cations. *Pflügers Archiv* **410**, 313–340.

FINDLAY, I. (1988*a*). ATP^{4-} and ATP Mg inhibit the ATP-sensitive K^+ channel of rat ventricular myocytes. *Pflügers Archiv* **412**, 37–41.

FINDLAY, I. (1988*b*). Effects of ADP upon the ATP-sensitive K^+ channel in rat ventricular myocytes. *Journal of Membrane Biology* **101**, 83–92.

FOSSET, M., DE WEILLE, J. R., GREEN, R. D., SCHMID-ANTOMARCHI, H. & LAZDUNSKI, M. (1988). Antidiabetic solfonylureas control action potential properties in heart cells via high affinity receptors that are linked to ATP-dependent K^+ channels. *Journal of Biological Chemistry* **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. *Pflügers Archiv* **391**, 85–100.

HOPPE, D., LUX, H. D., SCHACHNER, M. & KETTENMANN, H. (1989). Activation of K^+ currents in cultured Schwann cells is controlled by extracellular pH. *Pflügers Archiv* **415**, 22–28.

HORIE, M., IRISAWA, H. & NOMA, A. (1987). Voltage-dependent magnesium block of adenosine-triphosphate-sensitive potassium channel in guinea-pig ventricular cells. *Journal of Physiology* **387**, 251–272.

IMOTO, K., BUSCH, C., SAKMANN, B., MISHINA, M., KONNO, T., NAKEI, J., BUJO, H., MORI, Y., FUKUDA, K. & NUMA, S. (1988). Rings of negatively charged amino acids determine the acetylcholine receptor channel conductance. *Nature* **335**, 645–648.

ISENBERG, G. & KLÖCKNER, R. (1982). Calcium-tolerant ventricular myocytes prepared by preincubation in a 'KB medium'. *Pflügers Archiv* **395**, 6–18.

KAKEI, M. & ASHCROFT, F. M. (1987). A microflow superfusion system for use with excised membrane patches. *Pflügers Archiv* **409**, 337–341.

KAKEI, M., KELLY, R. P., ASHCROFT, S. J. H. & ASHCROFT, F. M. (1986). The ATP-sensitivity of K^+ channel in rat pancreatic B-cells is modulated by ADP. *FEBS Letters* **208**, 63–66.

KAKEI, M. & NOMA, A. (1984). Adenosine-5′-triphosphate-sensitive potassium channel in the atrioventricular node cell of the rabbit heart. *Journal of Physiology* **352**, 265–284.

KAKEI, M., NOMA, A. & SHIBASAKI, T. (1985). Properties of adenosine-triphosphate-regulated potassium channels in guinea-pig ventricular cells. *Journal of Physiology* **363**, 441–462.

KOYANO, T., KAKEI, M., YOSHINAGA, M., MATSUOKA, T. & TANAKA, H. (1990). Internal H^+ opens ATP-regulated K^+ channels in guinea-pig ventricular cells. *Circulation* **82**, suppl. III, 520.

KUME, H., TAKAGI, K., SATAKE, T., TOKUNO, H. & TOMITA, T. (1990). Effects of intracellular pH on calcium-activated potassium channels in rabbit tracheal smooth muscle. *Journal of Physiology* **424**, 445–457.

LEDERER, W. J. & NICHOLS, C. G. (1989). Nucleotide modulation of the activity of rat heart ATP-sensitive K^+ channels in isolated membrane patches. *Journal of Physiology* **419**, 193–211.

MARTELL, A. E. & SMITH, R. M. (1974). *Critical Stability Constants*, vol. 1, *Amino Acids*, vol. 2, *Amines*. Plenum Press, New York.

MISLER, S., GILLIS, K. & TABCHARANI, J. (1989). Modulation of gating of a metabolically regulated, ATP-dependent K^+ channel by intracellular pH in B cells of the pancreatic islet. *Journal of Membrane Biology* **109**, 135–143.

NICHOLS, C. G. & LEDERER, W. J. (1990). The regulation of ATP-sensitive K^+ channel activity in intact and permeabilized rat ventricular myocytes. *Journal of Physiology* **423**, 91–110.

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. *Journal of Physiology* **363**, 463–480.

OHNO-SHOSAKU, T., KUBOTA, T., YAMAGUCHI, J. & FUJIMOTO, M. (1990). Regulation of inwardly rectifying K^+ channels by intracellular pH in opossum kidney cells. *Pflügers Archiv* **416**, 138–143.

OHNO-SHOSAKU, T., ZUNCKLER, B. J. & TRUBE, G. (1987). Dual effects of ATP on K^+ currents in mouse pancreatic B-cells. *Pflügers Archiv* **408**, 133–138.

OPIE, L. H. (1976). Effects of regional ischemia on metabolism of glucose and fatty acids. *Circulation Research* **38** (suppl. 1) I52–68.

PAPPONE, P. A. & BARCHFELD, G. L. (1990). Modifications of single acetylcholine-activated channels in BC3H-1 cells. *Journal of General Physiology* **96**, 1–22.

SAKMANN, B. & NEHER, E. (1983). Geometric parameters of pipettes and membrane patches. In *Single-Channel Recording*, ed. SAKMANN, B. & NEHER, E., pp. 37–51. Plenum Press, New York.

SAKMANN, B. & TRUBE, G. (1984). Conductance properties of single inwardly rectifying potassium channels in ventricular cells from guinea-pig heart. *Journal of Physiology* **247**, 641–657.

SPRUCE, A. E., STANDEN, N. B. & STANFIELD, P. R. (1985). Voltage-dependent, ATP-sensitive potassium channels of skeletal muscle membrane. *Nature* **316**, 736–738.

STANDEN, N. B., QUAYLE, S. J., 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. J., COOK, D. L. & HALES, C. N. (1985). The sulphonylurea receptor may be an ATP-sensitive potassium channel. *Lancet* **8453**, 474–475.

TAKANO, M., QIN, D. & NOMA, A. (1990). ATP-dependent decay and recovery of K^+ channels in guinea-pig cardiac myocytes. *American Journal of Physiology* **258**, H45–50.

TANIGUCHI, J., KOKUBUN, S., NOMA, A. & IRISAWA, H. (1981). Spontaneously active cells isolated from the sino-atrial and atrio-ventricular nodes of the rabbit heart. *Japanese Journal of Physiology* **31**, 547–558.

TRUBE, G. & HESCHELER, J. (1984). Inward-rectifying channels in isolated patches of the heart cell membrane: ATP-dependence and comparison with cell-attached patches. *Pflügers Archiv* **401**, 178–184.

VANHEEL, B., LEYBAERT, L., DE HEMPTINNE, A. & LEUSEN, I. (1989). Simulated ischemia and intracellular pH in isolated ventricular muscle. *American Journal of Physiology* **257**, C365–376.

WEISS, J. N., VENKATESH, N. & LAMP, S. T. (1992). ATP-sensitive K^+ channels and cellular K^+ loss in hypoxic and ischaemic mammalian ventricle. *Journal of Physiology* **447**, 649–673.

WILDE, A. A. M., ESCANDE, D., SCHUMACHER, C. A., THURINGER, D., MESTRE, M., FIOLET, J. W. T. & JANSE, M. J. (1990). Potassium accumulation in the globally ischemic mammalian heart. A role for the ATP-sensitive potassium channel. *Circulation Research* **67** (4), 835–843.

YAZAWA, K., KAIBARA, M., OHARA, M. & KAMEYAMA, M. (1990). An improved method for isolating cardiac myocytes useful for patch-clamp studies. *Japanese Journal of Physiology* **40**, 157–163.

YELLEN, G. (1982). Single Ca^{2+}-activated nonselective cation channel in neuroblastoma. *Nature* **296**, 357–359.