J. Physiol. (1985), **363**, *pp.* 441–462
With 11 text-figures
Printed in Great Britain

PROPERTIES OF ADENOSINE-TRIPHOSPHATE-REGULATED POTASSIUM CHANNELS IN GUINEA-PIG VENTRICULAR CELLS

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(*Received 16 October 1984*)

SUMMARY

1. A class of K channels in cardiac muscle is reversibly blocked by intracellular adenosine 5′-triphosphate (ATP). The characteristics of this K channel were studied by recording single-channel currents in ventricular cells isolated enzymatically from guinea-pig heart. The reversal potential of single-channel currents agreed well with the K equilibrium potential. Blockers of other K channels, such as tetraethylammonium and 4-aminopyridine, decreased the mean open time of the channel.

2. The chord conductance increased as the 0·24th power of the K concentration on the outer surface of the membrane, and showed a marked inward-going rectification on strong depolarizations. The degree of rectification was larger with increasing Na concentration on the inner side of the membrane.

3. The kinetics of the channel were almost voltage independent, but depended on the concentration of intracellular ATP. The conductance of the channel was not affected by ATP.

4. When channel kinetics were examined in the presence of ATP, the distribution of open times and closed times was fitted well with a sum of two exponential components. When ATP concentration was increased, the time constants obtained from the open-time histogram decreased and those from the closed-time histogram increased, resulting in a decrease of the open-state probability.

5. The channel was blocked by ATP, adenosine 5′-diphosphate,5′-adenylylimidodiphosphate, guanosine 5′-triphosphate and uridine 5′-triphosphate, but not by adenosine 5′-monophosphate, creatine phosphate, creatine or adenosine. Plots of the open-state probability *versus* the ATP concentration revealed Michaelis–Menten saturation kinetics with strong co-operativity of multiple receptor sites (Hill coefficient 3–4, concentration of half-saturation 0·5 mM). It was concluded that this K channel has three or four receptor sites selective for triphosphate nucleotide on the inner surface of the membrane, and that the channel is blocked through the binding of agonists to the receptors.

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INTRODUCTION

It has been well established that the plateau of the cardiac action potential is shortened when energy metabolism of the heart cell is depressed by depleting oxygen or by applying CN or 2,4-dinitrophenol (Trautwein, Gottstein & Dudel, 1954; DeMello, 1959; MacLeod & Daniel, 1965; McDonald & MacLeod, 1973*a*, *b*). Recent voltage-clamp experiments have revealed an increase of an outward K current underlying the shortening of the action potential (Vleugels, Vereecke & Carmeliet, 1980; Isenberg, Vereecke, Van der Heyden & Carmeliet, 1983). The nature of this K conductance, however, is still not clear. Recently, it was found that poisoning the cell with CN induced an outward current which was depressed by micro-injection of ATP into the cell (Taniguchi, Noma & Irisawa, 1983). As a candidate for the K channel carrying the increased outward current, a class of K channels (ATP-regulated K channels) was found in patch-clamp experiments in guinea-pig ventricular and atrial cells (Trube & Hescheler, 1983, 1984; Noma, 1983). The ATP-regulated K channel was also recorded from the isolated atrioventricular node cell, which can generate spontaneous action potentials. This K channel was not activated by raising the Ca concentration at the inner side of the membrane (Kakei & Noma, 1984). The lack of Ca sensitivity of the K channel is in contrast to Ca-activated K channels (Pallotta, Magleby & Barrett, 1981; Lux, Neher & Marty, 1981; Marty, 1981; Barrett, Magleby & Pallotta, 1982). It is likely that this K channel is usually blocked by intracellular ATP, but becomes active when the intracellular ATP is lowered under the influence of the metabolic inhibitors.

In the present study, we examined the ATP-regulated K channel in single ventricular cells to characterize its conductance and kinetic properties and its interaction with ATP and other related substances.

METHODS

Isolation of the cardiac cells. The technique used to isolate single cardiac cells has been described elsewhere (Kakei & Noma, 1984; Matsuda & Noma, 1984). Briefly, hearts were dissected from guinea-pigs of 300–400 g body weight, mounted on Langendorff-type apparatus and perfused with a Ca-free Tyrode solution containing 0·04 % (w/v) collagenase (Sigma, type 1). After 1 h collagenase treatment the heart was softened, so that gentle agitation of small pieces of tissue in the normal Tyrode solution easily dispersed single cells in the recording chamber. The rod shape of the cell and the clear striations of sarcomeres were important criteria used for selecting viable cells for study. Experiments were performed at 35–36 °C.

Solutions. The composition of Tyrode solution was (in mM): NaCl, 136·1; KCl, 5·4; $CaCl_2$, 1·8; $MgCl_2$, 0·5; $NaHPO_4$, 0·33; glucose, 5·5; and the pH was adjusted to 7·4 with 5 mM-HEPES–NaOH. In the patch-clamp experiment, the control pipette solution contained (in mM): KCl, 150; $CaCl_2$, 2; and HEPES–NaOH, 5 (pH 7·4). Pipette solutions containing different K concentrations were prepared by substituting NaCl for equimolar KCl. In experiments using inside-out patch recordings, the bath solution was switched from the control Tyrode solution to a high-K relaxing solution before applying the suction pipette. The standard high-K relaxing solution contained (in mM): KCl, 140; $MgCl_2$, 0–1·0; KH_2PO_4, 0·5; EGTA, 0·1–1·0; adenosine 5′-triphosphate (Sigma, dipotassium salt, ATP), 2·0; and HEPES–KOH, 5 (pH 7·4). In experiments testing the interaction between the channel activity and ATP-related substances, guanosine 5′-triphosphate (Yamasa, trisodium salt, GTP), uridine 5′-triphosphate (Yamasa, trisodium salt, UTP), or 5′-adenylylimidodiphosphate (P-L Biochemicals, AMPPNP) was added in the high-K relaxing solution containing 0·5 mM-ATP. Tetraethylammonium chloride (Katayama Chemical, TEA), 4-aminopyridine (Sigma, 4-AP),

creatine phosphate (dipotassium salt, Calbiochem-Behring Corp.), creatine (Sigma), adenosine (Kohjin Co. Ltd.), adenosine 5′-monophosphate (Yamasa, AMP), adenosine 5′-diphosphate (Yamasa, ADP), or adenosine 3′,5′-cyclic monophosphate (Yamasa, cyclic AMP) was added to the high-K relaxing solution (0·5 mM-ATP). In some experiments (Fig. 5) 45 mM-KCl in the high-K relaxing solution was replaced with 90 mM-sucrose, 45 mM-NaCl or 45 mM-Tris Cl. Solutions containing different concentrations of Na were prepared by mixing the sucrose-containing solution with a solution containing 45 mM-NaCl.

Patch-clamp technique. The technique for the patch-clamp recordings was essentially the same as described by Hamill, Marty, Neher, Sakmann & Sigworth (1981), except that the technique for preparing inside-out patches was modified as described below. Before applying the Si-coated and heat-polished pipette (5–10 MΩ) to the cell, the bath solution was switched from the normal Tyrode solution to the high-K relaxing solution. After establishing a high-resistance seal near the centre of the cell, a part of the surface membrane on one end of the rod cell was chemically skinned by local application of saponin in order to equilibrate the intracellular medium with the bath solution (open cell-attached patch). This was achieved by positioning a glass pipette (2–3 μm tip diameter) containing 1 % (w/v) saponin just downstream from the cell, so that passive diffusion of saponin disrupted a part of the cell membrane. Opening of the cell membrane was detected by a sudden, slight local swelling of the cell observed under the microscope. A complete disruption of the cell membrane was confirmed by the intracellular organelles diffusing out of the cell if the procedure for disrupting the cell membrane was made in the rounded ventricular cell. To avoid break-down of the gigaseal by a further diffusion of saponin, the saponin pipette was immediately removed. During the procedure, the perfusion of the recording chamber was maintained while the saponin pipette approached upstream. After the disruption of the cell membrane, the activity of the ATP-regulated K channel appeared within 30 s when the high-K relaxing solution contained ATP of a concentration of less than 2 mM. In the open cell-attached patch, the channel activity seemed more intact and survived much longer (as long as 1 h) in comparison with the more conventional, excised, inside-out patch, suggesting that the integrity of the structure (cytoskeleton) just under the cell membrane may be important for maintaining normal channel activity. If the ATP concentration in the bath was decreased to less than 0·2 mM, contracture of the cell developed (rigor bridge) making stable recordings difficult. All data from the patch-clamp experiments described in this paper were obtained from open cell-attached patches. The bath solution was grounded through an agar/Ag–AgCl bridge, and the pipette potential was clamped at various levels. The membrane potential described in this study indicates the transmembrane potential at the inner side of the membrane. The currents and potentials were recorded with a patch-clamp amplifier (EPC-7, List Co. Ltd.) and stored on magnetic tapes (TEAC R-210) for later analysis using a computer (HITAC E-600).

RESULTS

Responses of two different K channels to disruption of the cell membrane

In the high-K relaxing solution and at a K concentration of 150 mM on the outer surface of the patch membrane, activity of the inward-rectifier K channels was recorded in the cell-attached patch at negative potentials (Fig. 1*A*; for the inward rectifier K channel, see Kameyama, Kiyosue & Soejima, 1983; Sakmann & Trube, 1984*a*, *b*). When part of the surface membrane was disrupted by local application of saponin in the presence of 2 mM-ATP in the bath, no obvious change was observed in the activity of the inward-rectifier K channels (Fig. 1*A*, upper trace). At positive potentials no channel current was observed before or after the disruption of the membrane (not shown). However, if the cell membrane was disrupted in the bath solution containing less than 2 mM-ATP, activation of a different class of channel was observed at membrane potentials positive to the K equilibrium potential (about 0 mV; lower trace in *A*). At negative potentials, this new class of channel current was also evident, with an amplitude almost twice as large as that of the inward-rectifier

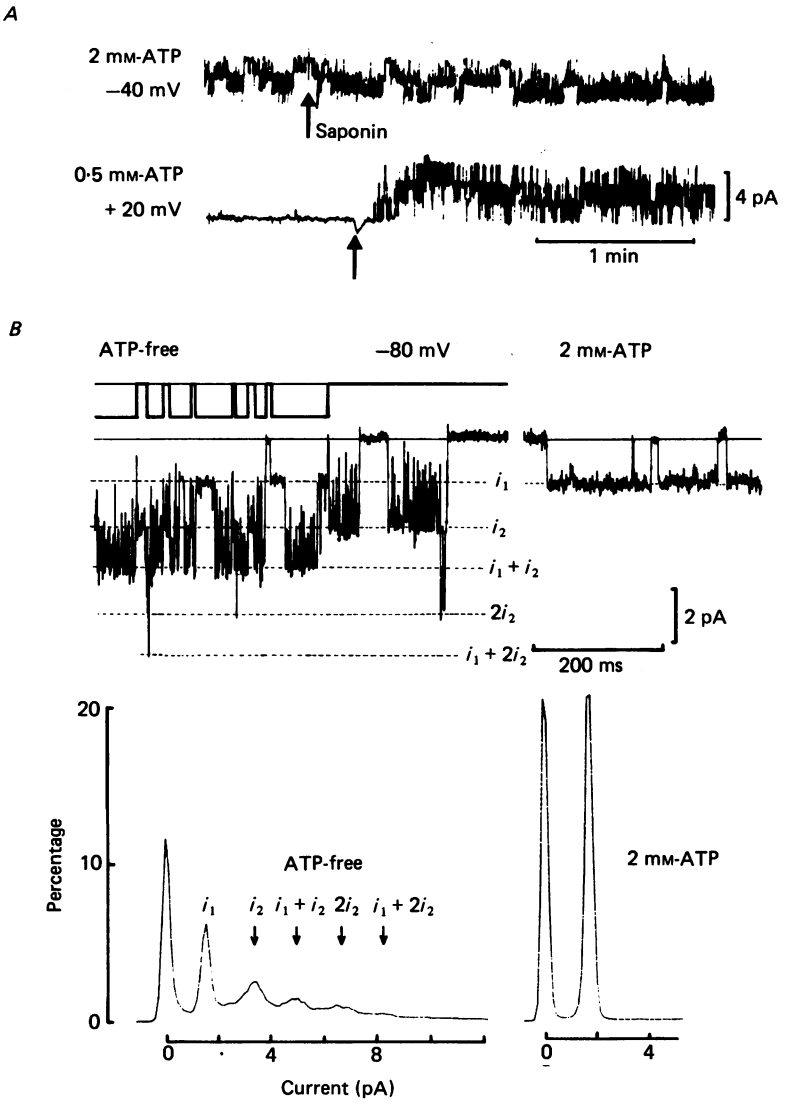

Fig. 1. *A*, effects of disrupting the cell membrane on the inward-rectifier K channel (upper trace) and on the ATP-regulated K channel (lower trace). During the cell-attached patch recording of the inward-rectifier K-channel current at the membrane potential of −40 mV (inward current in the downward direction), part of the cell membrane was disrupted by local application of saponin as indicated by the arrow. The bathing solution was the standard high-K relaxing solution containing 2 mM-ATP. In the lower trace, the high-K relaxing solution contained 0·5 mM-ATP and the membrane potential was +20 mV. 10 s after disrupting the cell membrane, as indicated by the arrow, outward channel currents appeared. The current was displayed through a low-pass filter of 100 Hz on a chart recorder. *B*, superposition of the two different K channels recorded 2 min after starting the perfusion of the ATP-free solution. The line drawing indicates openings of a channel. When the membrane potential was kept at −80 mV with the pipette solution containing 50 mM-K, the current steps were composed of two different unit amplitudes (i_1, i_2). Amplitude histograms are displayed in the lower panels. In the right-hand panels, the ATP concentration on the inner side of the membrane was raised to 2 mM. The current was displayed through a low-pass filter of 1 kHz.

K channel. The new channel had large fluctuations of current during the open state and was found to be an ATP-regulated K channel, because it was blocked by ATP at the inner side of the membrane (Noma, 1983).

In order to discriminate openings of each channel at negative potentials, current records obtained 2 min after decreasing the ATP concentration in the bath to zero were examined, as shown in Fig. 1*B*. The unit amplitude of the inward-rectifier K channel (i_1) and that of the ATP-regulated K channel (i_2) are indicated by the dotted line superimposed on the records or by the arrows in the amplitude histogram in Fig. 1*B*. The other current levels corresponded to the sum of unit amplitudes, indicating overlapping opening of channels. Thus, the open-state probability of the inward-rectifier K channel was calculated by dividing the sum of the integrals of the peaks indicated with i_1, i_1+i_2, and i_1+2i_2 by the total number of sampling points. It was 0·36, which was lower than that obtained during the superfusion of 2 mM-ATP (0·52) in the same membrane patch (right-hand graph in Fig. 1*B*). If the perfusion of ATP-free solution was continued, the open-state probability of the inward-rectifier K channel decreased further, but the appearance of multiple ATP-regulated K channels interfered with the measurement of open-state probability of the inward-rectifier K channel. When the ATP concentration was decreased from 2·0 to 0·25 mM in three other experiments, reversible steady responses were obtained. The open-state probability of the inward-rectifier K channel decreased from $0{\cdot}46 \pm 0{\cdot}06$ to $0{\cdot}09 \pm 0{\cdot}01$. Thus, it was concluded that decreasing the ATP concentration on the inner side of the membrane activates the ATP-regulated K channel, whereas it decreases the open-state probability of the inward-rectifier K channel.

In the experiments shown in Fig. 1*B*, the two classes of channel were recorded in the same patch. Therefore, the relative densities of their distributions may be estimated without knowing the area of the patch membrane. The maximum number of superpositions seen in a particular experiment was taken as the number of channels in the patch. This approximation may be justified because the open-state probability is rather high for the inward-rectifier K channel at an ATP concentration of 2 mM (see also Kameyama *et al.* 1983; Sakmann & Trube, 1984*a*, *b*) and for the ATP-regulated K channel at ATP concentrations below 0·5 mM (larger than 0·5, see Fig. 10). In six experiments, the ratio of the number of inward-rectifier K channels to the number of ATP-regulated K channels ranged from 0·5 to 1·75, with an average of 1·12. This finding may indicate that the densities of both channels are almost equal.

Conductance properties of the ATP-regulated K channel

The reversal potential and the channel conductance were measured by recording the current–voltage relations at different K concentrations. The estimation of the unit amplitude, however, was complicated by the presence of marked fluctuations of the current during the channel openings. Therefore, the fluctuations of channel current were examined before measuring the unit amplitude of the single-channel current. The power density spectrum, calculated from the current records (frame length 20 ms, with an anti-aliasing filter of 5 kHz) during continuous opening of the channel was compared with that measured during closure of the channel. The power density spectrum for the open-channel current was almost flat from 0·2 to 5 kHz, and was about five times larger than the power of the noise during the channel closure. However, there was no pronounced relaxation component (Lorentz spectrum) visible

in the spectrum over the range of frequency from 0·2 to 5 kHz. If the fluctuation of the current level is due to very brief openings and closings of the channel, the corresponding spectral components might be suppressed by the 5 kHz low-pass filter.

The distribution of the current level during the channel-open state, indicated by

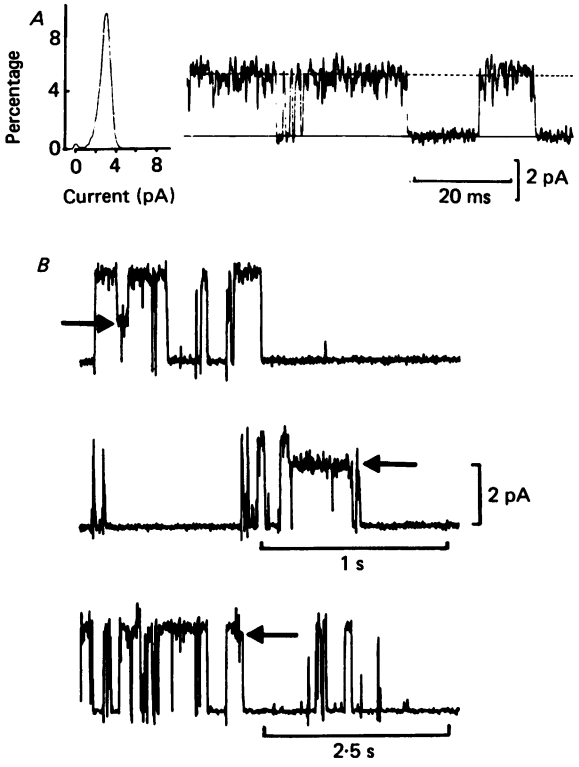

Fig. 2. *A*, current fluctuations of the open ATP-regulated K channel. In the amplitude histogram, the left small peak indicates the current level during the channel closure and the right larger peak the current during the channel opening. The current record at 0 mV was sampled through a low-pass filter of 2·5 kHz. The horizontal continuous line indicates the zero-current level and the dashed line indicates the unit amplitude of the channel. Outward currents are displayed upwards. *B*, sublevel openings (arrows) of the ATP-regulated K channel. The pipette contained control Tyrode solution, and the bathing solution was the high-K relaxing solution containing 0·7 mM-ATP. The pipette potential was 0 mV. The upper two records were obtained from the same open cell-attached patch. The current was displayed through a low-pass filter of 250 Hz.

the second peak of the amplitude histogram, showed a smooth peak as shown in Fig. 2*A*. The location of the peak was not affected by changing the cut-off frequency of the low-pass filter from 0·1 to 5 kHz. As a first approximation, the unit amplitude was measured as the interval between the first peak, which indicates the zero-current level, and the second peak. When the unit amplitude was less than 0·5 pA, the peaks were not clearly separated because of the small signal-to-noise ratio, and the unit amplitude was determined by eye.

The other complication in measuring the unit amplitude arose from the presence of sublevels of the open-channel current. The current level stays for a definite period at several intermediate levels, as shown in Fig. 2*B* (arrows), suggesting the existence of multiple conductance states of the channel. The amplitude of these sublevels varied between 20 and 90 % of the main level. Since the probability of staying at the sublevels was extremely low, the sublevels of the current could not be detected in the amplitude histograms.

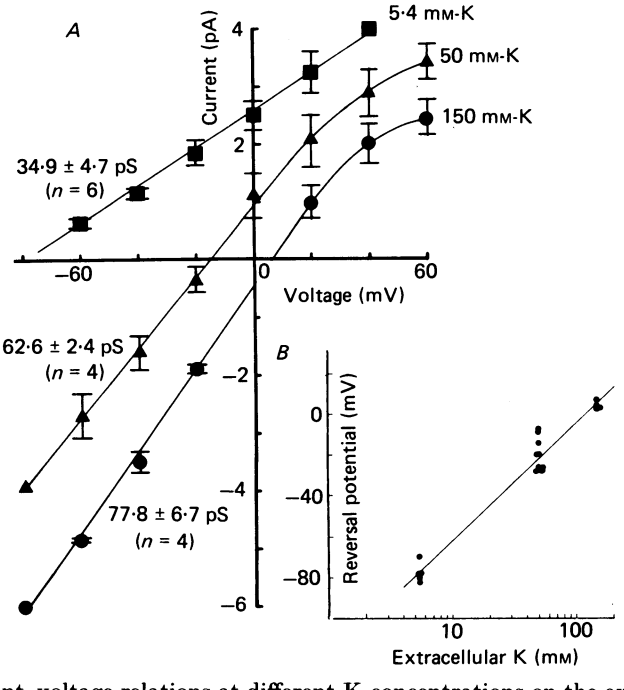

Fig. 3. *A*, current–voltage relations at different K concentrations on the external side of the membrane, recorded by the open cell-attached patch with the high-K relaxing solution in the bath. The means ± S.E. of mean were calculated from four to six experiments, from which a series of voltage jumps were recorded. *B*, the reversal potentials (ordinate) were measured in each experiment and plotted against the K concentration in the pipette solution (abscissa). The line was drawn by the least-squares fitting method.

In Fig. 3*A* the current–voltage relation of the main level of the single-channel current were measured with 5·4, 50, and 150 mM-K in the external solution. Each point gives the mean ± S.E. of mean of four to six experiments. With increasing K concentration, the channel conductance increased and the reversal potential shifted to the right. The reversal potentials were plotted against the logarithm of the K concentration at the outer side of the membrane in Fig. 3*B*. The line obtained by the least-squares fit showed a slope of 58 mV for a 10-fold increase in the K concentration, indicating that the channel was predominantly selective for K ions.

The slope conductances were almost linear except at strong depolarizations. The dependence of the slope conductance on the K concentration at around the reversal potential is examined in Fig. 4. The data are fitted well by a line having a slope of

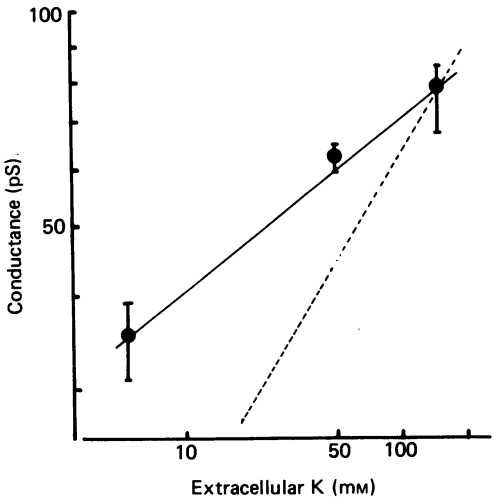

Fig. 4. The relation between the single-channel conductance (γ in pS) and the K concentration (in mM) on the external side of the membrane. The mean values ± S.D. were obtained from six, eight and five experiments at 5·4, 50 and 150 mM-extracellular K. The line was drawn from the equation,

$$\gamma = 23{\cdot}6\ [K]_o^{0{\cdot}24}.$$

The dashed line was calculated from the constant-field equation.

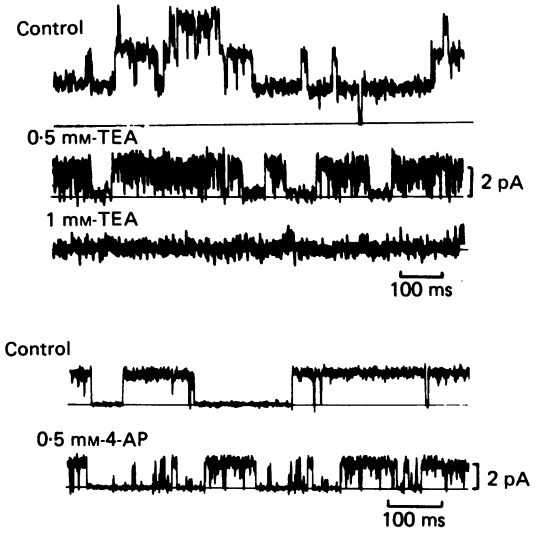

Fig. 5. Effects of TEA and 4-AP on the ATP-regulated K channel. After recording the control current at 0 mV with normal Tyrode solution in the pipette and standard high-K relaxing solution in the bath, TEA or 4-AP (0·5 mM) was added to the bathing solution. The current traces were displayed through a low-pass filter of 1 kHz.

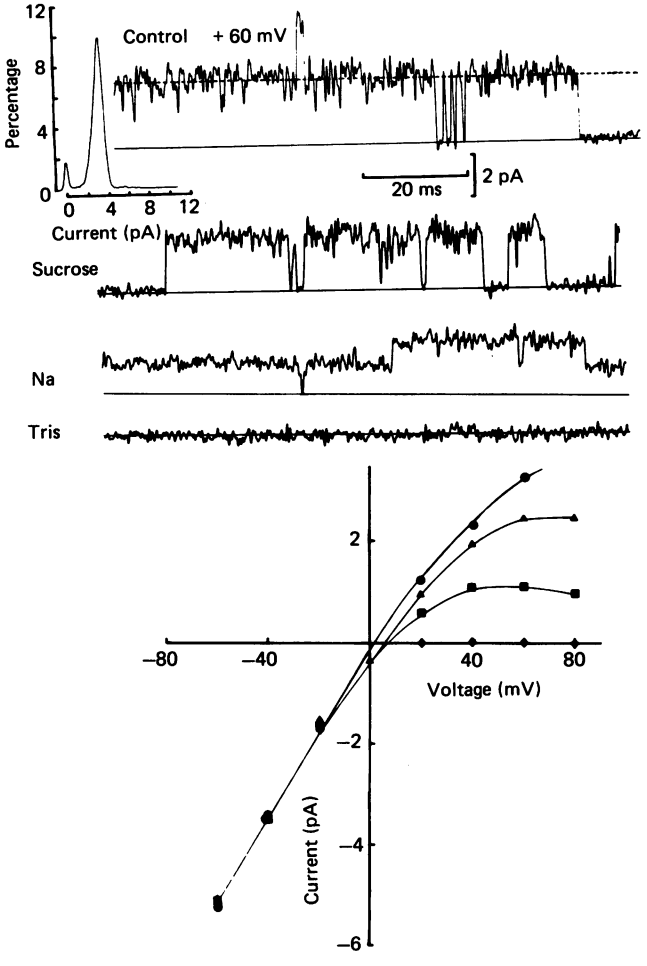

Fig. 6. Effects of Na and Tris ions in the internal solution on the outward current through the ATP-regulated K channel. 45 mM-KCl in the high-K relaxing solution (●) was replaced with 90 mM-sucrose (▲), 45 mM-NaCl (■) or 45 mM-Tris Cl (◆), and the current–voltage relations were measured with each solution in the bath. The current–voltage curves were obtained from the same experiment. The pipette solution contained 150 mM-KCl. The current traces at +60 mV were recorded through a low-pass filter of 1 kHz.

0·24. The dotted line in Fig. 4 indicates the relation obtained from the constant-field equations (Goldman, 1943). It is evident that the slope conductance of the ATP-regulated K channel is less sensitive to extracellular K concentration than expected from the Goldman equations. Assuming that the constant-field equation is valid close to the zero-current potential, the permeability coefficient at the concentration of 150 mM-K was $1{\cdot}4 \times 10^{-13}$ cm^3/s. This value is larger than the value of 10^{-13} cm^3/s for the acetylcholine-sensitive K channel (Sakmann, Noma & Trautwein, 1983) or of 5×10^{-14} cm^3/s for the inward-rectifier K channel in cardiac muscle (Sakmann & Trube, 1984*a*). The inward-going rectification on depolarization was observed at

every K concentration and became evident at membrane potentials positive to +60 mV at 50 and 150 mM-extracellular K.

Effects of the K-channel blockers

The ATP-regulated K channel was blocked by both TEA and 4-AP, which are known as selective K-channel blockers. TEA (Fig. 5, upper panel) or 4-AP (lower panel) at 0·5 mM, when applied to the inner surface of the membrane, induced short interruptions of the single-channel current. The interruptions of the single-channel current became more frequent with increasing drug concentration and at 1 mM no obvious opening was recorded. These effects of both TEA and 4-AP were reversible.

Effects of intracellular Na on the inward rectification of the K channel

The inward rectification of the ATP-regulated K channel seems to be different from that of the inward-rectifier K channel; the outward current of the latter channel is difficult to record, but the former rectifies only at strong depolarizations. Furthermore, the rectification of the ATP-regulated K channel was affected by the ionic composition on the inner side of the membrane (Fig. 6). When examining the effect of intracellular Na on the rectification of the channel, we tried to find a substitute for K which was inert in order to avoid an additional effect of varying intracellular K. However, substitution of K with sucrose or Tris by itself depressed the outward current without affecting the channel conductance for the inward current (Fig. 6). In the presence of Tris ions, outward current was not recorded. Since the effect of sucrose was relatively small, the solution containing 90 mM-sucrose was used as the control and sucrose was substituted by NaCl. When compared with the unit amplitude measured with the 90 mM-sucrose solution at +40 mV, the average amplitude ($n = 5$) of the outward current at 10, 20 and 45 mM-Na were 76, 69 and 53 %, respectively. These findings indicate that Na depressed the outward current through the ATP-regulated K channel in a dose-dependent manner. The saturation of the amplitude of the outward current with larger depolarizations in the presence of intracellular Na indicates that the blocking effect of Na is voltage dependent. The current noise during the open state was not increased by substitution of K with Na (2·5 kHz low-pass filter). This finding is in contrast to the marked increase of open-channel noise of the Ca-activated K channels in chromaffin cells with increasing Na concentration (Marty, 1983). The presence of large anions on both sides of the membrane, such as glutamate, aspartate or HEPES did not affect the channel conductance.

Kinetic properties of the channel: time course of the summated current

The gating kinetics of the ATP-regulated K channel were almost independent of the membrane potential. This was demonstrated by examining the relaxation of the average current during voltage jumps. In Fig. 7, a patch electrode having an outer tip diameter of 4–5 μm was used and multichannel currents (ten to twenty channels) were recorded; thus, a smooth mean current was obtained by averaging ten to thirty records for each clamp step. The pipette was filled with 50 mM-K solution and the membrane potential was jumped from the holding potential of −60 mV to +20 mV (K reversal potential −27 mV). After obtaining the control records, the ATP concentration was increased from 0·5 to 2·0 mM (arrow, Fig. 7*A*). The channel activity

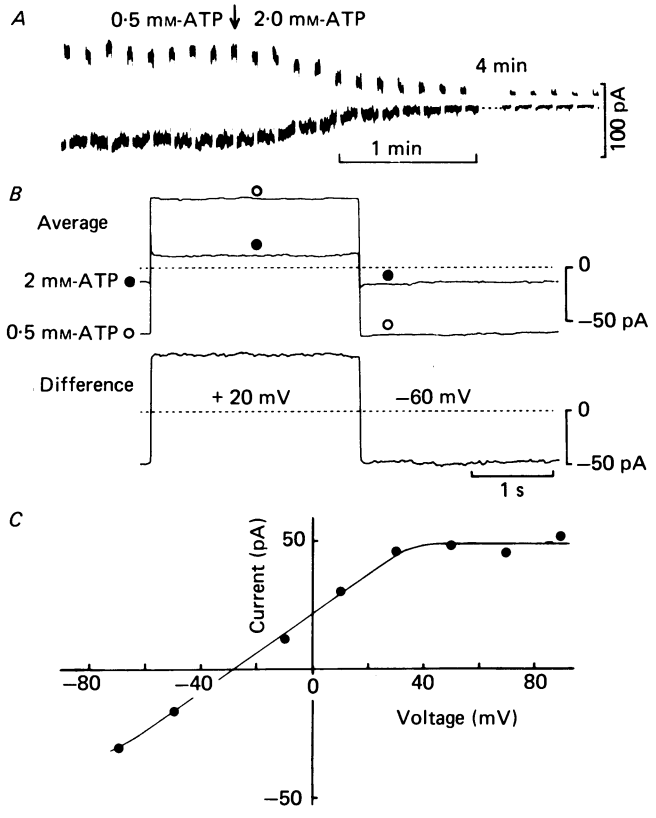

Fig. 7. Time independency of the average currents through ATP-regulated K channels. Multichannel currents were recorded using a gigaseal electrode containing 50 mM-K pipette solution with an open cell-attached patch. The clamp steps were made across the K equilibrium potential of −28 mV from −60 mV to +20 mV. The concentration of ATP in the high-K relaxing solution was increased from 0·5 to 2·0 mM at the arrow. The jumps of the zero channel current level indicate that the input resistance is about 8 GΩ. Average currents were calculated for each ATP concentration as shown in *B*, and the difference in the average current is shown in the lower trace. The current–voltage relation of the average current is shown in *C*.

was almost completely depressed 5 min after the increase in the ATP concentration. Under this condition, close inspection on the oscilloscope at a higher gain revealed openings of the inward-rectifier K channels at −60 mV, but infrequent openings of the ATP-regulated K channel at +20 mV. Assuming a minor change in the activity of the inward-rectifier K channel, the current component produced by the ATP-regulated K channels was measured as the difference between two average traces (● at 2·0 mM-ATP and ○ at 0·5 mM-ATP). No significant time-dependent change was observed in the difference current (Fig. 7*B*, lower trace).

If the gating mechanism is independent of the transmembrane potential, i.e. the open-state probability is constant, the current–voltage relation for the average current should be similar to that for the unitary current. In Fig. 7*C*, the current–voltage relation for the difference current was measured using a similar protocol to that in

Fig. 7*A*, but only four to six records were averaged for each step. The linear relation over the range from −70 to +30 mV and saturation of the current amplitude at potentials more positive than +40 mV are quite similar to the current–voltage relation for the unitary current as shown in Fig. 3*A*. These findings indicate that voltage dependence is insignificant for the gating mechanism of the ATP-regulated K channel.

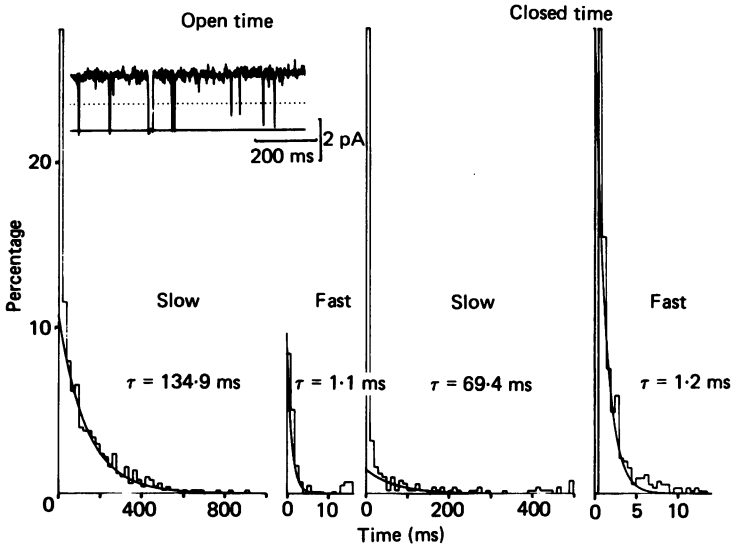

Fig. 8. Distribution of open times and closed times with 0·5 mM-ATP on the inner side of the membrane. The transmembrane potential is +20 mV. The current was sampled at 2 kHz through a low-pass filter of 1 kHz. A threshold level for discrimination of open and closed states was set at half of the main open level, as shown by the dotted line in the inset trace. The histograms were first constructed with a bin of 20 ms for open-time histograms and with a bin of 10 ms for closed-time histograms, and the slow components were fitted with single exponentials. The excess number of events above the theoretical curve were replotted using a 0·5 ms bin for the closed-time histogram and a 1·0 ms bin for the open-time histogram. Time constants (τ) are shown.

Modulation of the kinetics by intracellular ATP

When the concentration of ATP was increased from 0·5 mM, the unit amplitude of the channel current was not affected, but the probability of the channel-open state decreased. This fact indicates that channel kinetics depend on intracellular ATP. In order to evaluate this property, the open-time histogram and the closed-time histogram were calculated at various concentrations of ATP.

The open and closed times of the channel were measured from records showing no overlaps of the unitary current through the entire period of recording, indicating that the membrane patch contained only one ATP-regulated K channel. At potentials negative to the reversal potential, however, the openings of the inward-rectifier K channel interfered with the measurement. Therefore, all data were obtained at a membrane potential of +20 mV, which is positive to the K reversal potential by 47 mV (50 mM-extracellular K/145 mM-intracellular K).

Distributions of both the open and closed times could not be fitted with single-exponential functions, at least two components being necessary. In Fig. 8, the ATP concentration was 0·5 mM and the time constants of the exponential distribution were 1·1 and 134·9 ms for the open-time histogram, and 1·2 and 69·4 ms for the closed-time histogram, respectively.

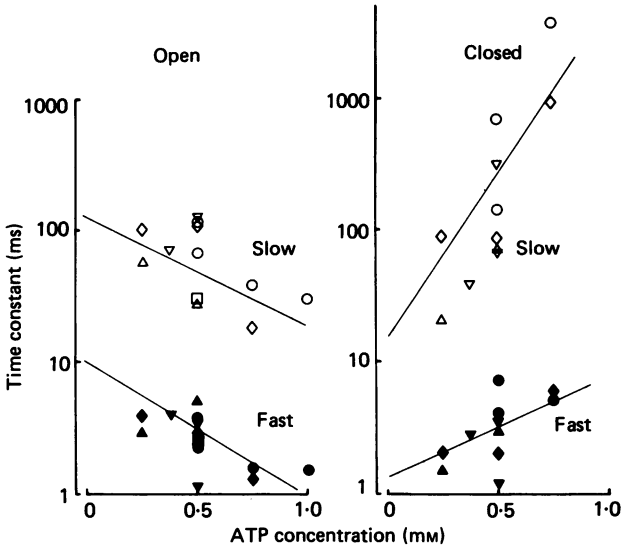

Fig. 9. Time constants for distribution of the open and closed times at various ATP concentrations. Different symbols indicate different experiments, filled symbols for the fast component and open symbols for the slow component. The line was fitted by eye. The same recording conditions were used as in Fig. 8.

The above open-time and closed-time histograms might be modulated by the blocking action of ATP. In order to avoid such an effect of ATP, the open-time and closed-time histograms were measured in the absence of ATP in the bath. However, in most cases the measurements were confounded by a gradual decay of the channel activity and the development of rigor contracture.

Time constants for the distribution of the open and closed times were determined at various ATP concentrations and are plotted in Fig. 9. When the concentration of ATP in the high-K relaxing solution was increased, both the fast and slow time constants for the open-time histograms decreased. On the other hand, the time constants for the distribution of the closed times increased with increasing ATP concentration. At present we have no kinetic model which can explain the modulation of the channel kinetics caused by ATP. The findings on the open-time and closed-time histograms, however, correspond well to a decrease of the open-state probability at higher ATP concentrations.

The dose–response relation for the blocking effect of ATP

In order to clarify the interaction of ATP with the binding site of the K channel, the relation between intracellular ATP and channel activity was measured. In the experiments shown in Fig. 10, the open cell-attached patch was prepared and bath

solutions containing different concentrations of ATP were alternately perfused. The open-state probability (p) is given by the equation;

$$p = I/(Ni), \tag{1}$$

where I is the arithmetic mean of the channel current within the patch membrane, N the total number of channels functioning within the patch and i the unit amplitude

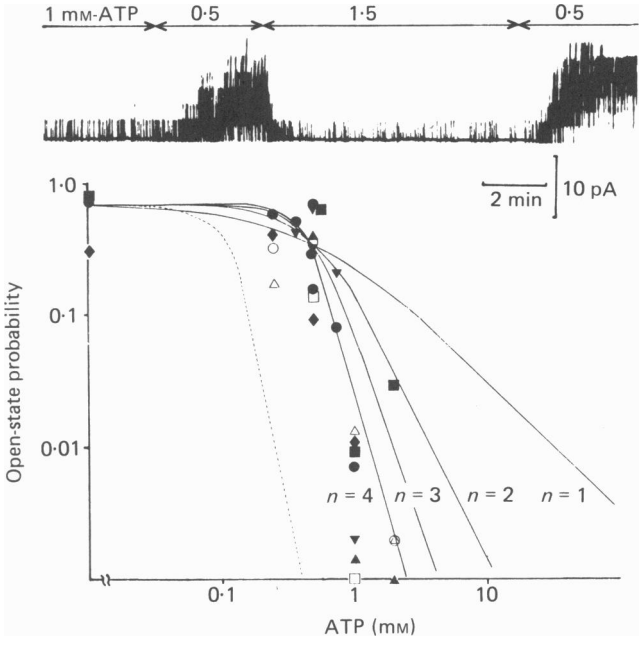

Fig. 10. Dose–response relation for the blocking action of ATP on the K channel. The upper trace is a chart recording of channel activity during perfusion of an open cell-attached patch with different ATP concentrations. The membrane potential was 0 mV and the pipette contained the normal Tyrode solution while the bath was perfused with the high-K relaxing solution. The open probability of the channel was calculated using eqn. (1). Different symbols indicate different experiments. The curves were drawn using eqn. (2) with $p_{max} = 0{\cdot}8$, $K_{\frac{1}{2}} = 0{\cdot}5$ mM and various values of the Hill coefficient (n) as indicated in the graph. The dashed curve was taken from the data obtained from the excised inside-out patch recording (Noma, 1983).

of the single-channel current. The value of N was approximated as the maximum number of overlaps of the openings in the same membrane patch during the perfusion of ATP-free solution.

The open-state probability decreased as the ATP concentration was increased between 0·2 and 2 mM. With ATP-free solution, the open-state probability was about 0·8. In the graph, theoretical dose–response relations are superposed on the experimental data. The dose–response relations were calculated by the empirical equation,

$$p = p_{max}/(1-(A/K_{\frac{1}{2}})^n), \tag{2}$$

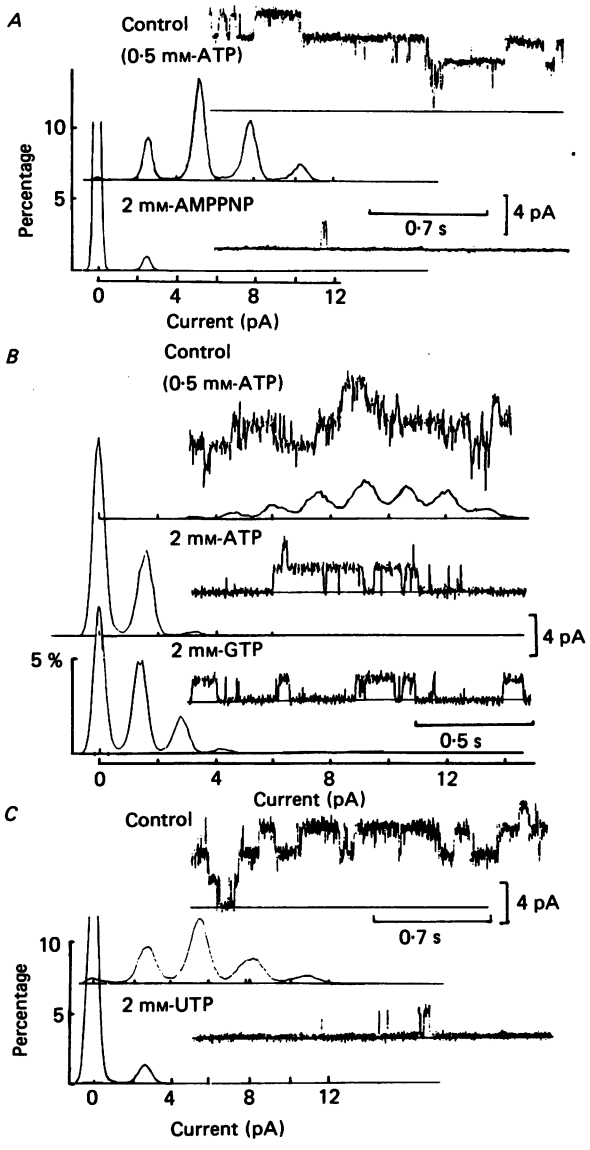

Fig. 11. Effects of 2 mM-AMPPNP (*A*), GTP (*B*) and UTP (*C*) on the ATP-regulated K channel. Control current records were obtained in the presence of 0·5 mM-ATP on the inner side of the membrane in each experiment, and the effects of adding these drugs were examined. In order to evaluate the effect with a long time span, the amplitude histograms (graphs to the left of each record) were measured from records of more than 30 s in duration. The line indicates the zero channel current level. Multichannel current was preferred in order to get average effects. The pipette solution was normal Tyrode solution and the bath solution was the high-K relaxing solution. On application of 2 mM-AMPPNP (*A*), the development of a cell contracture interfered frequently with the recordings. In *B* the effect of GTP is compared with ATP.

where p_{max} is the maximum value of the open-state probability (0·8), A is the concentration of ATP, $K_{\frac{1}{2}}$ is the concentration of half-saturation (0·5 mM), and n is the Hill coefficient. Obviously, the data are not fitted with a Hill coefficient of 1, but required a coefficient of more than 3, indicating co-operativity of multiple ATP-binding sites.

The dose–response relation obtained using the excised inside-out patch recordings (Noma, 1983) is illustrated in the same graph by the dotted curve. The curve has a similar slope as in the open cell-attached patch, but the dose–response relation in the latter is shifted to the right.

Effects of ATP and ATP-related substances on the gating of the channel

As shown in the previous report (Noma, 1983), the blocking potency of ADP was lower by one order of magnitude when compared with ATP, and AMP had no effect below 10 mM. In the metabolic reactions in the cell, ATP functions as a Mg complex. However, the blocking effect of ATP on the channel was observed even in the absence of Mg in the high-K relaxing solution. The block of the channel does not require hydrolysis of ATP, since 2 mM-AMPPNP, in which the terminal bridge O of ATP is replaced with N, and thus cannot be a donor of the high-energy phosphate bond, blocked the ATP-regulated channel as shown in Fig. 11.

In order to examine base specificity in the blocking action, another pyrimidine nucleotide, GTP, and the purine nucleotide, UTP, were applied on the inner surface of the membrane. They also reversibly blocked channel activity (Fig. 11*B* and *C*), indicating weak base specificity. The blocking effect was not observed when adenosine (1 mM), creatine phosphate (2 mM), or creatine (2 mM) was added to the internal solution. These findings may confirm the view that the hydrolysis is not involved in the blocking action of ATP and that the presence of three or two phosphate groups in the molecule is a prerequisite for the blocking action.

DISCUSSION

According to the conduction properties demonstrated in the present study, the ATP-regulated K channel may be classified among highly selective K channels of high conductance called 'maxi K channels' by Latorre & Miller (1983). The K channel can also be placed in a class of chemical-receptor-dependent K channels, such as the end-plate channel in the skeletal muscle (for review, see Sakmann & Adams, 1979), the Ca-activated non-selective cation channel (Colquhoun, Neher, Reuter & Stevens, 1981; Yellen, 1982; for macroscopic current, see Matsuda, 1983), Ca-activated K channels (Pallotta *et al.* 1981), Na-activated K channel (Kameyama, Kakei, Sato, Shibasaki, Matsuda & Irisawa, 1984) and muscarinic acetylcholine-receptor-regulated K channels (Sakmann *et al.* 1983; Soejima & Noma, 1984). In contrast to the potential-dependent channels, such as the inward-rectifier K channel (Kameyama *et al.* 1983; Sakmann & Trube, 1984*b*), the current through the ATP-regulated K channels is time independent and may be responsible for the increase in the outward current during various anoxic conditions (Noma & Shibasaki, 1984).

Distribution of the ATP-regulated K channel in various heart muscles

The K channels sensitive to intracellular ATP have been found in nodal cells (Kakei & Noma, 1984) and also in atrial cells (Noma, 1983, 1985). Two differences in kinetics are noticed between the nodal K channel and the ventricular K channel. First, in the nodal cell a depolarization of the cell-attached patch activated outward single-channel currents. In excised patch recordings, two-thirds of the channels examined showed no time dependency, but the rest of them showed increased activity with depolarization. In the ventricular cell clear voltage dependency could be found neither in the patch recordings nor in the whole-cell current recordings (Noma & Shibasaki, 1985). The other difference is that the open-time histogram was fitted with a single exponential in the nodal cells, while in the ventricular cell fitting required a sum of two exponential components. At present we do not have any clear explanation for these differences.

In spite of the above differences in kinetic measurements, the inward-going rectification as well as the conductances near the reversal potential (31–32 pS at 5·4 mM-K, and 40–42 pS at 20 mM-K) agreed well with those of the K channel in the present study (34·9 ± 4·7 pS at 5·4 mM-K, and about 46 pS at 20 mM-K in Fig. 4). The activity of the ATP-regulated K channel in the ventricular cell was not affected by varying the Ca concentration on the inner side of the membrane by up to 10 μM (Noma, 1983) as in the nodal K channel. It might be suggested that the nodal and ventricular channels belong to the same class of ATP-regulated K channel and that the ATP-regulated K channels are distributed throughout the mammalian cardiac muscle.

Large conductance of the ATP-regulated K channel

The conductance of the ATP-regulated K channel (80 pS) is higher than those of the inward-rectifier K channel (45 pS) and the ACh-sensitive K channel (55 pS), but lower than that of the Na-activated K channel (220 pS) in the presence of 100–150 mM-K on the outer side of the membrane. The conductances of these channels, however, are much higher than the delayed-rectifier K channel in the *Helix* ganglion (2 pS at 110 mM-extracellular K, Reuter & Stevens, 1980) or the inward-rectifier K channels in the skeletal muscle (10 pS at 150 mM-extracellular K, Schwarz, Neumcke & Palade, 1981; Ohmori, Yoshida & Hagiwara, 1981; Fukushima, 1981) and in the tunicate egg (7 pS at 100 mM-extracellular K, Hagiwara & Takahashi, 1974). In spite of the large conductance, the ATP-regulated K channel showed high selectivity for K ions, and responded to the open-channel blocker, TEA, in a similar manner as did the small 'classical' K channels; short interruptions appeared during the channel openings. Furthermore, Na ions, when added to the internal solution depressed the outward conductance of the ATP-regulated K channel. A similar blocking effect of Na has been reported for the delayed-rectifier K current (Bezanilla & Armstrong, 1972). Therefore, it is unlikely that the channel has a large-radius conductivity pore. Latorre & Miller (1983) assumed that the maxi K channel contains a tunnel region along which the applied voltage drops and works as a selective filter. The large conductance is assumed to be due to a relatively shorter tunnel as compared to the low-conductance channel. The presence of substates in the ATP-regulated K

channel, however, may support an alternative model, which has been proposed by Sakmann & Trube (1984*a*) for the inward-rectifier K channel. They observed substates of lower conductance in the inward-rectifier K channel and hypothesized that the main state could be the result of the simultaneous opening of four conducting subunits, each of which has a conductance of about 7 pS (145 mM-extracellular K). It may be speculated that the number of subunits of the ATP-regulated K channel is larger than that of the inward-rectifier K channel. The structure of each subunit may be comparable to that of low-conductance K channels.

Rectification of the ATP-regulated K channel

The chord conductance of the ATP-regulated K channel became smaller as the membrane potential deviated more positive from the reversal potential (see also Trube & Hescheler, 1984). At around the reversal potential, the current–voltage relation was almost linear, especially with a low K concentration on the outer side of the membrane. This is in contrast to the inward-rectifier K channel, whose outward current is difficult to record because of its high degree of rectification. We found in the present study that the degree of rectification of the ATP-regulated K channel depends on the composition of the cations in the internal medium. The dependence of the rectification on intracellular Na may explain the relatively strong rectification of the ATP-regulated K channel observed by Trube & Hescheler (1984); the amplitude of the outward current saturated at 1·5 pA. The 8 mM-Na used in their internal solution might have depressed the outward current more extensively as the membrane potential became more positive.

Marty (1983) similarly reported observations that the outward current through the Ca-activated K channel is depressed by intracellular Na ions. In his case the fluctuations of the current during the channel openings increased in the presence of Na on the inner side of the membrane, suggesting that Na ions to some extent can enter the channel but cannot pass through the selectivity filter. In our case, however, the variance of the current fluctuations did not significantly increase when Na ions were applied. These different findings may be explained by a two-step blocking model as proposed by Schwarz *et al.* (1981) for the blocking effect of external Cs on the inward-rectifying K channel in skeletal muscle. They assumed a fast exchange of an artificial blocking particle, i.e. Cs ion, between the extracellular solution and a fast blocking site, and a slow transition between a Cs ion at the first blocking site and a second reaction centre deeper within the channel. With the usual time resolution of the recordings ($\leqq$ 5 kHz), the first kinetic process is not resolved. Thus, the unitary amplitude was decreased without an increase in the current fluctuations. By analogy, if Na cannot reach the second reaction centre assumed in the ATP-regulated K channel, the unit amplitude of the channel current may be decreased without an increase in the open-channel noise. Such instantaneous blocking and unblocking are also suggested in the effect of intracellular cations on the K channels of squid axon (Bezanilla & Armstrong, 1972).

Chemical regulation of the channel kinetics

The summated current through the ATP-regulated K channel did not show clear relaxation on voltage jumps (time independent). This fact may indicate that the K

channel has open and close kinetics, but the rate constants are voltage independent over the physiological membrane potential range. At negative membrane potentials, the ATP-regulated K channel flickers more frequently between its open state and a short closed state (Trube & Heschler, 1984), suggesting a weak voltage dependence of the gating kinetics at negative potentials. On the other hand, the kinetics are controlled by the intracellular ATP concentration; the closing rate constants increased with increasing ATP concentrations and vice versa for the opening rate constants (Fig. 9). This effect of ATP on the K channel is most probably mediated by binding of the ATP molecule to its receptor site, the occupation of which induces a modulation of the gating mechanism by allosteric influence. The binding reaction of ATP to its receptor site on the channel might also be voltage independent, because no relaxation of the current was observed on voltage jumps (Fig. 7*B*). It is unlikely that the channel is plugged by several ATP molecules, which have negative charges (the Hill coefficient was 3–4). Since phosphorylation is not involved in the blocking reaction by ATP (Fig. 11), the mode of regulation of the channel is different from that of the Ca and other voltage-dependent channels which are phosphorylated via β-receptor activation (Trautwein, Taniguchi & Noma, 1982; Osterrieder, Brum, Hescheler, Trautwein, Hofmann & Flockerzi, 1982; Brum, Flockerzi, Hofmann, Osterrieder & Trautwein, 1983; for review, see Tsien, 1977).

Chemical regulation of channel kinetics is also demonstrated in other channels, most of which are responsible for time-independent currents. In atrial muscle, there exists a class of K channels whose kinetics are weakly voltage dependent, but which are activated by the binding of acetylcholine to the muscarinic receptor (Noma & Trautwein, 1978; DiFrancesco, Noma & Trautwein, 1980; Sakmann *et al.* 1983; Soejima & Noma, 1984). Also in cardiac muscle, a class of K channel is activated when the intracellular Na concentration is raised above 20 mM (Kameyama *et al.* 1984). All of the rate constants varied when intracellular Na varied. The kinetics of the channel were also voltage independent. An increase in the intracellular Ca concentration activates a cation-selective channel, whose kinetics are also voltage independent (Colquhoun *et al.* 1981). With respect to voltage independency, these channels differ from Ca-activated K channels, whose open-state probability increases with depolarizations (Lux *et al.* 1981; Pallotta *et al.* 1981; Barrett *et al.* 1982).

Comparison of the dose–response relation with extra- and intracellular ATP receptors

Receptors for ATP, which are linked to ionic channels (purinergic receptors), have been found in the autonomic nervous system and in a number of other tissues (Burnstock, 1978, 1981). The sequence of agonist potency for the ATP-regulated K channel (ATP > ADP, with AMP and adenosine ineffective) is qualitatively similar to the sequence of agonists for P_2 purinergic receptors (ATP > ADP > AMP > adenosine), but different from that for P_1 purinergic receptors (adenosine > AMP > ADP > ATP). The sequence of agonists for the ATP receptor in the mammalian sensory ganglia (Krishtal, Marchenko & Pidoplichko, 1983) is quite similar to the sequence observed in the present study. However, the concentration of half-saturation in the ATP-regulated K channel is higher by 100 times than that in the ATP receptor in the ganglion cell, and the Hill coefficient of the latter is 1 compared to 3–4 in the

present study. The dissociation constant of the K channel is rather similar to that for the Na–K-ATPase, which is about 0·3 mM when measured with respect to the enzyme activity, and about 10-fold lower than the dissociation constant for binding of ATP to the enzyme in the presence of K (for review, see Schwartz, Lindenmayer & Allen, 1975).

Discussions with respect to the threshold concentration of ATP in its blocking action of the K channel will be described in the accompanying paper (Noma & Shibasaki, 1985), which describes internal dialysis of cells with different ATP concentrations. These results will give a basis for considering the contribution of the K channel to the whole-cell current under various anoxic conditions.

We thank Dr D. Cook (VA Medical Center, Seattle) for valuable comments in preparing the manuscript. We also thank Professor H. Irisawa for continuous helpful discussions during the study. The experiments were carried out with the technical help of Mr M. Ohara and Mr O. Nagata. This work was supported by a Grant-in-Aid for Special Project Research (59123005) from the Japanese Ministry of Education, Science and Culture.

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