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ATP-Sensitive Potassium Channels in Adult Mouse Skeletal Muscle: Characterization of the ATP-Binding Site

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Summary. Single K^+-selective channels were studied in excised inside-out membrane patches from dissociated mouse toe muscle fibers. Channels of 74 pS conductance in symmetrical 160 mM KCl solutions were blocked reversibly by 10 μM internal ATP and thus identified as ATP-sensitive K^+ channels. The channels were also blocked reversibly by mM concentrations of internal adenosine, adenine and thymine, but not by cytosine and uracil. The efficacy of the reversible channel blockers was higher when they were present in internal NaCl instead of KCl solutions. An irreversible inhibition of ATP-sensitive K^+ channels was observed after application of several sulphydryl-modifying substances in the internal solution: 0.5 mM chloramine-T, 50 mM hydrogen peroxide or 2 mM *N*-ethylmaleimide (NEM). Large-conductance Ca-activated K^+ channels were not affected by these reagents. The presence of 1 mM internal ATP prevents the irreversible inhibition of ATP-sensitive K^+ channels by NEM. The results suggest that internal Na^+ ions increase the affinity of the ATP-sensitive K^+ channel to ATP and to other reversible channel blockers and that a functionally important SH-group is located at or near the ATP-binding site.

Key Words skeletal muscle · patch clamp · K^+ channel · adenosine triphosphate · *N*-ethylmaleimide · sulphydryl group

Introduction

ATP-sensitive K^+ channels are a class of ionic channels in biological membranes which are selective for K^+ ions and blocked by intracellular adenosine triphosphate. They were described for the first time by Noma (1983) in the membrane of heart muscle cells. Subsequently, they were also detected in B-cells of pancreatic islets (Cook & Hales, 1984) and in other insulin-secreting cells (Dunne et al., 1986) as well as in sarcolemmal vesicles of frog (Spruce, Standen & Stanfield, 1985) and mammalian (Burton, Dörstelmann & Hutter, 1988) skeletal muscle and in cortical neurons of rat brain (Ashford et al., 1988). We have found that similar channels exist in the surface membrane of adult mouse skeletal muscle and describe some properties of these channels in this paper. In particular, we have studied the binding of the nucleotide ATP, the nucleoside adenosine and some purine and pyrimidine bases to internal channel sites and the modification of the binding reactions by the ionic composition of the internal solution. The ATP-binding site was also explored in experiments with various reversible blockers and irreversible channel inhibitors likely to bind at or near these internal channel sites. Some of these new inhibitors have been found to affect only ATP-sensitive but not Ca-activated K^+ channels and, therefore, are suitable agents for a selective blockage of one type of K^+ channel. Furthermore, experiments will be described which suggest that the irreversible channel inhibitors studied act by modifying a sulphydryl group near the ATP-binding site and that this group plays an important role in the normal function of ATP-sensitive K^+ channels in skeletal muscle.

Some of the results have been published as an abstract (Weik & Neumcke, 1988).

Materials and Methods

Preparation

Muscles were cut off from the hind feet of adult female mice, killed by decapitation, and dissociated into single fibers by treatment with collagenase (Sigma Type I, 3 mg/ml Ringer's solution) for 1–2 hr at about 35°C. The procedure was similar to that described previously for frog interosseal (Allen, Akaike & Albuquerque, 1984; Woll et al., 1987) and mouse flexor digitorum brevis muscles (Brehm & Kullberg, 1987). Muscle fibers were stored refrigerated in Ringer's solution in disposable 35-mm tissue culture dishes (Falcon, Becton Dickinson, Heidelberg) and could be used for about 24 hr for electrophysiological measurements. All experiments were performed at room temperature (19–24°C).

Recording Methods

Patch pipettes were pulled in two stages and fire-polished. When filled with pipette solution (*see* Solutions), pipettes had a resistance of about 10 MΩ. After pressing the pipette gently against the cell membrane, seals were formed by applying negative pressure for a short time. Single-channel currents were recorded from excised membrane patches of the inside-out configuration (Hamill et al., 1981) using an L/M-EPC-5 amplifier (List, Darmstadt). Currents were measured at various membrane potentials (taken as potential differences between the intra- and extracellular sides of the patch) and stored in digitally coded form on video-tape.

At the beginning of an experiment the bath solution on the sarcoplasmic side of the excised patches was Ringer. The pipette with the excised patch was then transferred from the main Ringer pool to a small chamber via a solution bridge. Subsequently, the fluid level was lowered until both compartments were separated from each other. Thus a solution change in the small chamber did not affect the remaining muscle fibers in the Ringer pool.

Solutions

The pipettes were filled with two different external solutions composed of (in mM): 155 KCl, 2 $CaCl_2$, 1 $MgCl_2$, 5 HEPES, pH 7.4 (solution 1) or 155 KCl, 3 $MgCl_2$, 0.5 EGTA, 10 HEPES, pH 7.4 (solution 2). The same results were obtained with both solutions.

Channel blockers and protein-modifying substances were dissolved in the following internal solutions (in mM): (i) Ringer (150 NaCl, 5 KCl, 2 $CaCl_2$, 1 $MgCl_2$, 5 HEPES, pH 7.4), (ii) solution 1, or (iii) 160 KCl, 5 or 10 HEPES, pH 7.4 (solution 3). Some experiments were performed with solution 3 + 5 mM EDTA. The titration of the K^+-rich solutions 1, 2, 3 to pH 7.4 was done by addition of 1 N KOH and the pH adjustment of Ringer with 1 N NaOH. ATP was added as Na_2 ATP (Boehringer, Mannheim), adenosine, adenine, cytosine, uracil and thymine as free bases (all from Sigma, Deisenhofen). Guanine was not tested since this base is almost insoluble in water. Other compounds studied were chloramine-T (Fluka, Neu-Ulm), hydrogen peroxide, *N*-ethylmaleimide (NEM), trinitrobenzenesulphonic acid (TNBS) and pyridoxal-5′-phosphate (all from Sigma). Internal solutions containing the substances listed above were freshly prepared before application, and the pH was readjusted to pH 7.4 with KOH or NaOH.

Analysis

Membrane currents, recorded on video-tape, were played back and filtered with a four-pole low-pass Bessel filter at cutoff frequencies as indicated in the figure legends. Digitization of the currents and further analysis of the data were performed with a microcomputer (DEC LSI 11/23). The programs allowed the visual inspection of selected segments of current traces (as shown in Figs. 1, 2, 5 and 6) as well as the calculation of the open-state probability and of amplitude histograms from long periods of uninterrupted current recordings. The half-amplitude criterion was used to detect channel openings and closings (Colquhoun & Sigworth, 1983). As a measure of the open-state probability, we used the sum of open times of all channels in the patch divided by the total time period of analyzed records. The p_o values computed by this procedure may be greater than 1 if the patch contains several active channels. Therefore, the calculated p_o values were normalized to their control values in the same patch and then denoted as relative p_o. In the experiments with reversible channel blockers, amplitude histograms and p_o values were calculated from 30-sec periods of current recordings. After a change of the internal solution the calculation was started when the membrane currents had reached a new steady state. The efficacy of various reversible blockers of ATP-sensitive K^+ channels is described by the decline of p_o in the presence of the agents with respect to the mean of the p_o values before and after blocker application. Errors from the spontaneous decline of p_o during the experiment were then largely eliminated.

Amplitude histograms of 100 bins were fitted by the sum of Gaussian functions, and the mean of the closed-channel distribution was taken as origin of the abscissa. Thus the peak positions of the open-channel distributions directly give the respective channel currents.

Results

Effects of ATP, Adenosine and Purine, Pyrimidine Bases

The left-hand parts of Fig. 1 show original records from ATP-sensitive K^+ channels at −50 mV before, during and after addition of 10 μM ATP to the internal solution. The control currents in Fig. 1*A* originate from the openings (plotted downward) and closings (plotted upward) of at least three ATP-sensitive K^+ channels in the patch, the current through a single channel is −3.2 pA. The channel currents can also be obtained from the location of the corresponding peaks in the amplitude histogram (arrows in Fig. 1*B*). As shown by the currents in Fig. 1*C*, 10 μM ATP in the internal solution decreases the open probability of the ATP-sensitive K^+ channels. This can also be deduced from the reduced open-channel distributions in the amplitude histogram of Fig. 1*D*. However, the current through an open channel is not affected by ATP.

Upon wash-out of ATP from the internal solution the probability of channel opening increases again and even exceeds the initial control value (*compare* p_o values in the legend to Fig. 1). However, this was not observed in all experiments owing to the variability of the open probability in time-limited periods of current recordings and to the slow decline of channel activity during long experiments. For the experiment shown in Fig. 1 the relative p_o in solution 3 plus 10 μM ATP is 0.47 (*compare* figure legend); the mean value from this and 27 more records is represented by the open (third) column in Fig. 4.

The experiment illustrated in Fig. 1 was per-

formed with approximately symmetrical KCl concentrations in the external and internal solutions. Thus, the reversal potential of the channel currents is near 0 mV, and the channel conductance γ can be estimated from the channel current $i = -3.2$ pA at the membrane potential $E = -50$ mV through $\gamma = i/E = 64$ pS. Single-channel conductances determined more accurately from the slope of $i(E)$ curves in several patches are in the range between 62 and 83 pS with a mean value of 74 pS. The same slopes of current-voltage curves at negative membrane potentials were found with the K^+-rich internal solutions 1 and 3 or with internal Ringer (*not shown*). However, different open probabilities and different blocking actions of ATP were observed in the three internal solutions in the potential range between −40 and −70 mV. With respect to solution 1 (155 mM KCl, 2 mM $CaCl_2$, 1 mM $MgCl_2$) as internal solution, p_o in Ringer is 0.48 ± 0.10 (mean ± SEM, $n = 13$), and with respect to solution 3 (160 mM KCl), p_o in Ringer is 0.35 ± 0.06 ($n = 4$). The efficacy of channel block by ATP was also dependent on the ionic composition of the internal solution. The results from several patches are compiled in Fig. 4 which shows that 10 μM internal ATP reduces the relative p_o value of ATP-sensitive K^+ channels at negative membrane potentials to 0.436 ± 0.065 (mean ± SEM, $n = 28$) in solution 3, to 0.553 ± 0.128 ($n = 14$) in solution 1 and to 0.151 ± 0.03 ($n = 14$) in internal Ringer. In additional experiments with solution 3 + 5 mM EDTA the relative p_o declined to 0.271 ± 0.039 ($n = 21$) during application of 10 μM ATP.

ATP-sensitive K^+ channels cannot be blocked only by a few μM of internal ATP but, at higher concentrations, also by adenosine and by adenine. Figure 2 compares the actions of 5 mM adenine, cytosine and uracil in internal Ringer on the activity of ATP-sensitive K^+ channels. It can be seen that only adenine was able to reduce the open probability, whereas cytosine and uracil did not block the channels. The absence of effects of 5 mM cytosine or uracil on ATP-sensitive K^+ channels was confirmed in other experiments in which the bases were dissolved either in solution 1 or in Ringer as internal solutions. ATP-sensitive K^+ channels could not be blocked only by adenine but also by thymine. The efficacy of this pyrimidine base was comparable to that of the purine base adenine. This is illustrated in Fig. 3 with plots of relative p_o values before, during and after internal application of the two bases.

The block of ATP-sensitive K^+ channels by internal adenosine, adenine and thymine depends on the ionic composition of the internal solution as also observed for ATP (Fig. 4): The reduction of p_o by all blockers is stronger in Ringer than in the K^+-rich solutions 1 and 3. Possible explanations of this result will be given in the Discussion.

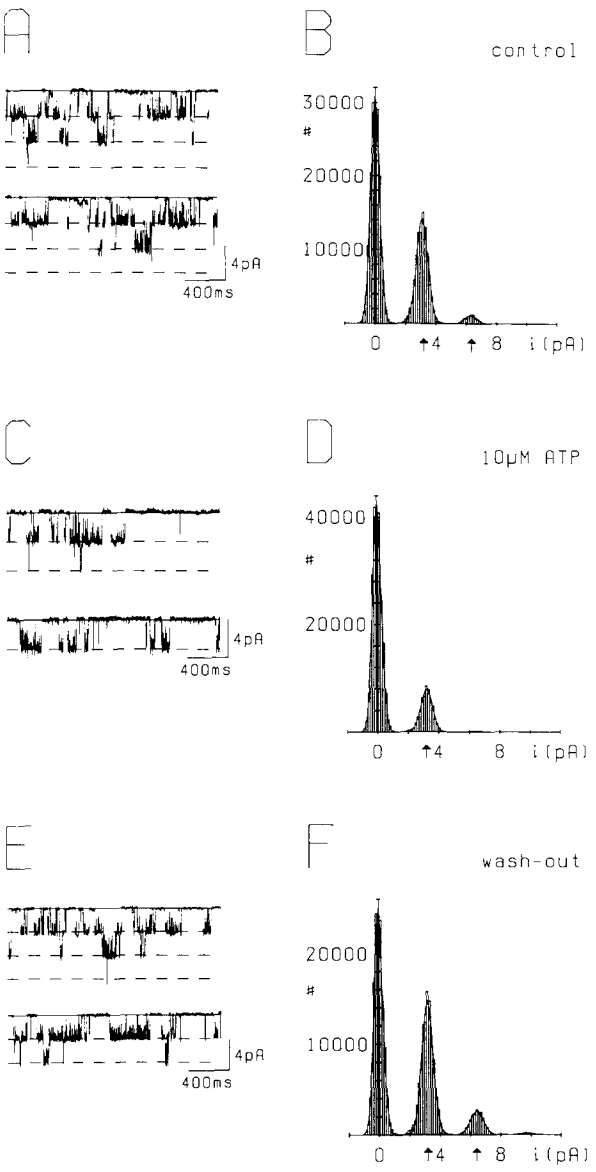

Fig. 1. Currents and amplitude histograms from ATP-sensitive K^+ channels under control conditions (*A*,*B*), in the presence of 10 μM internal ATP (*C*,*D*) and 0.5 min after washout of ATP (*E*,*F*). The full lines in *A*, *C* and *E* denote the closed channel state; interrupted lines indicate the current levels of one, two and three open channels. The amplitude histograms were calculated from 30-sec periods of current recordings and show the number of data points (#) as function of absolute values (*i*) of the channel current. Mean currents from one and two open channels are marked by arrows. p_o values = 0.49 (control), 0.27 (10 μM ATP), 0.67 (washout). Relative decline of p_o in 10 μM ATP: $2 \times 0.27/(0.49 + 0.67) = 0.47$. Membrane potential, −50 mV. Internal solution: solution 3 without or with 10 μM Na_2 ATP. Low-pass filter frequency, 1 kHz; sampling rate, 0.1 msec for calculation of amplitude histograms. Patch 364, temperature 19°C

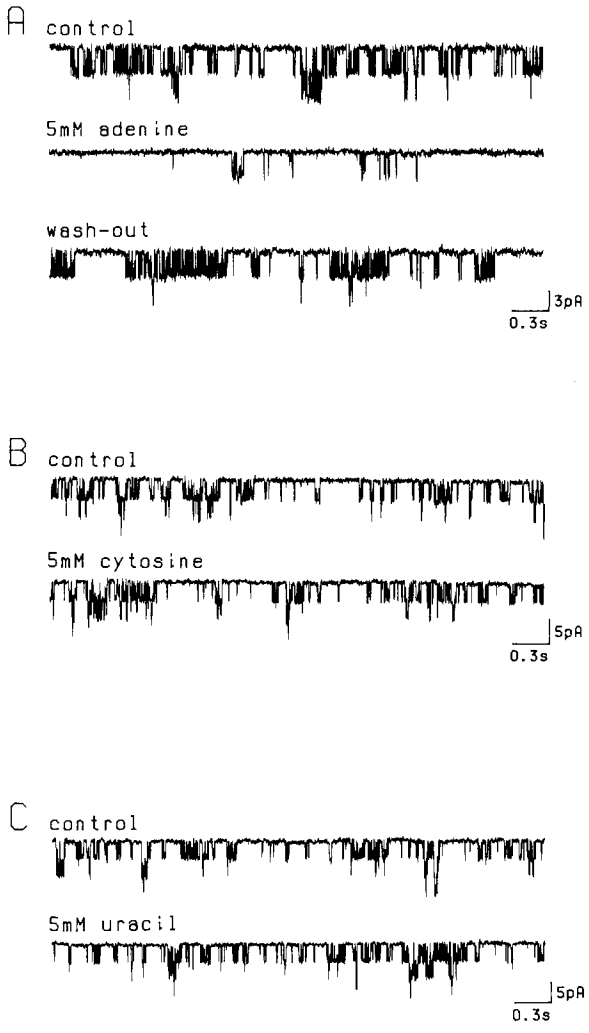

Fig. 2. Effects of 5 mM adenine, cytosine and uracil in internal Ringer's solution. (*A*) Currents at −60 mV before (control), 1 min after application of adenine and 2 min after washout of the base. Patch 203. (*B*) Currents at −60 mV before (control) and 2 min after application of cytosine. Patch 160. (*C*) Currents at −70 mV before (control) and 5 min after application of uracil. Patch 85. Note that adenine, but not cytosine and uracil blocks the channels. Temperatures, 21–24°C. Low-pass filter frequency, 1 kHz

Effects of Protein-Modifying Reagents

Figure 5*A* shows original currents at −70 mV from a membrane patch which contained a Ca-activated and at least two ATP-sensitive K^+ channels. The channel currents of −17.1 pA for the Ca-activated channel and of −4.7 pA for one ATP-sensitive channel can be read off directly from the current records in Fig. 5*A* or taken from the peak positions of the corresponding amplitude histogram in Fig. 5*B*. Figure 5*C* and *D* illustrate that 0.5 mM chloramine-T in the internal solution selectively inhibits the ATP-sensitive K^+ channels, while the Ca-activated K^+ channel is not affected by this oxidizing agent. The actions of chloramine-T on ATP-sensitive K^+ channels are persistent (note missing peaks from ATP-sensitive channels in the amplitude histogram of Fig. 5*D*) and irreversible. A similar selective and irreversible inhibition of ATP-sensitive K^+ channels was observed after the addition of 50 mM hydrogen peroxide to internal solutions (*not shown*).

To decide which chemical groups on the ATP-sensitive K^+ channel react with chloramine-T and with H_2O_2, experiments with other protein-modifying agents were performed. Figure 6 illustrates the effects of *N*-ethylmaleimide (NEM), a substance that reacts with sulphydryl groups of cysteine residues in proteins (Lundblad, 1984). As with chloramine-T and H_2O_2, NEM inhibited ATP-sensitive K^+ channels irreversibly, whereas Ca-activated K^+ channels were unaffected by the reagent.

We have also tested the effects of pyridoxal-5′-phosphate and trinitrobenzenesulphonic acid (TNBS), which are known to modify both ε- and α-amino groups of proteins (Lundblad, 1984). ATP-sensitive K^+ channels were not modified in 160 mM KCl internal solutions containing 2 mM pyridoxal-5′-phosphate or 1 mM TNBS (*not shown*).

Interactions between Channel Blockers and Inhibitors

As shown above, ATP-sensitive K^+ channels can be blocked reversibly by internal ATP and inhibited irreversibly by chloramine-T, H_2O_2 and NEM. The following experiments were performed to obtain information on the location of the ATP-binding site relative to the channel residues which are modified by the inhibitors. An example is illustrated in Fig. 7. The upper part of the figure shows currents from at least three ATP-sensitive K^+ channels which were blocked rapidly after addition of 1 mM internal ATP. A subsequent treatment with 2 mM NEM no longer produced an irreversible channel inhibition, because channel activity reappeared after NEM was first washed out from the internal solution followed by ATP. The open column in the lower part of the figure shows the relative p_o value after washout of NEM and ATP as determined with this protocol in the experiment illustrated and in five additional patches. The mean relative p_o value of 0.16 after treatment with ATP and NEM indicates an incomplete recovery of channel activity. This is partly due to the high concentration of ATP used in the experiments because in control measurements without NEM complete reversibility was not observed 10 min after washout of 1 mM internal ATP

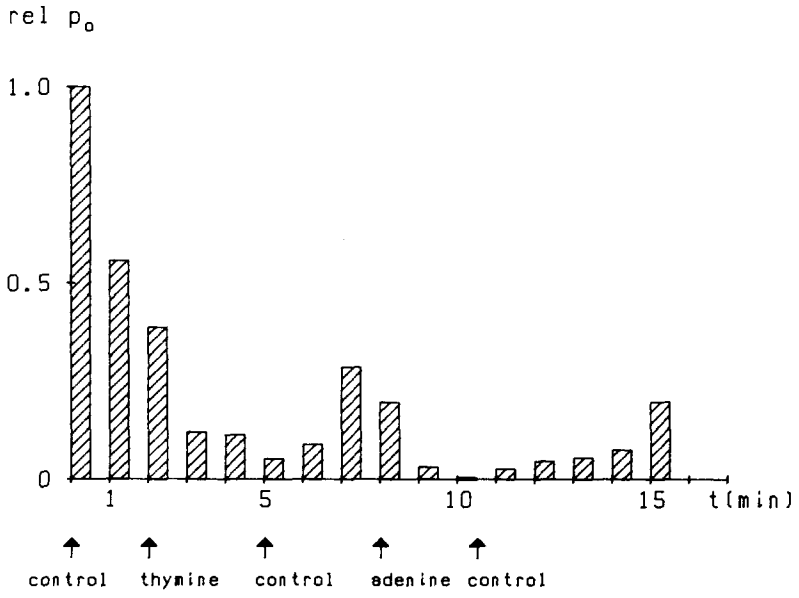

Fig. 3. Relative p_o values of ATP-sensitive K^+ channels in internal Ringer's solution (control) and in the presence and during washout of 5 mM thymine or adenine in Ringer. The p_o values were calculated from currents recorded at −40 mV and were normalized with respect to the initial p_o value shortly after forming the seal. The arrows mark the beginning of the solution change, which was complete after about 1 min. Note that 5 mM thymine and adenine partly and reversibly decrease the open probability. In this experiment with slow solution change and with two different blockers the application times of the blockers and the washout periods were not sufficient for the establishment of steady-state conditions. Patch 151, temperature 21°C

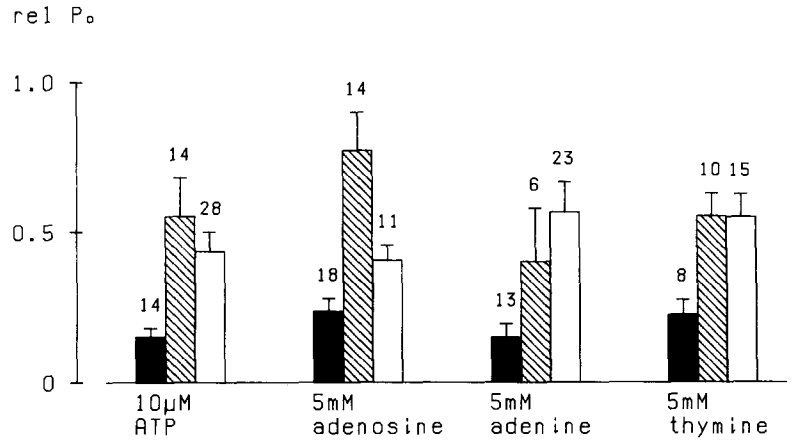

Fig. 4. Relative p_o values in the presence of various internal blockers of ATP-sensitive K^+ channels. The p_o values were determined for blockers dissolved in Ringer (filled columns), solution 1: 155 mM KCl, 2 mM $CaCl_2$, 1 mM $Mg Cl_2$ (hatched columns) or solution 3: 160 mM KCl (open columns). All p_o values were normalized with respect to their control values in the same internal solutions without blockers. Bars and numbers above the columns indicate the SE values of the means and the number of measurements on different patches

(*not shown*). Another reason for the reduced final open probability after treatment with ATP and NEM could be that the channels are not permanently closed by the reversible channel blocker ATP even at the high concentration of 1 mM. Hence, the irreversible inhibitor NEM may reach the channel during the unblocked intervals.

If 2 mM NEM is applied without ATP, ATP-sensitive K^+ channels were inhibited in the presence of this substance (Fig. 6), and the relative p_o value after washout was significantly lower than the value determined in the ATP, NEM experiments (filled column in the lower part of Fig. 7). In two of these experiments NEM did not reach and inhibit all channels during the 2.5-min application period. This explains the finite mean value of 0.01 for the relative p_o after washout of NEM.

Discussion

Channel Block by ATP, Adenosine, Adenine and Thymine

Intracellular ATP and the nonhydrolysable analogue AMP-PNP have approximately equal potencies in blocking ATP-sensitive K^+ channels in skeletal muscle, whereas all other modifications in the ATP molecule seem to reduce its effectiveness (Spruce, Standen & Stanfield, 1987). Similarly, various pyridine nucleotides are less powerful than ATP in inhibiting ATP-sensitive K^+ channels in insulin-secreting cells (Dunne, Findlay & Petersen, 1988). These findings suggest that the base-, the ribose- and the triphosphate-residues of the ATP molecule all participate in the binding of the nucleo-

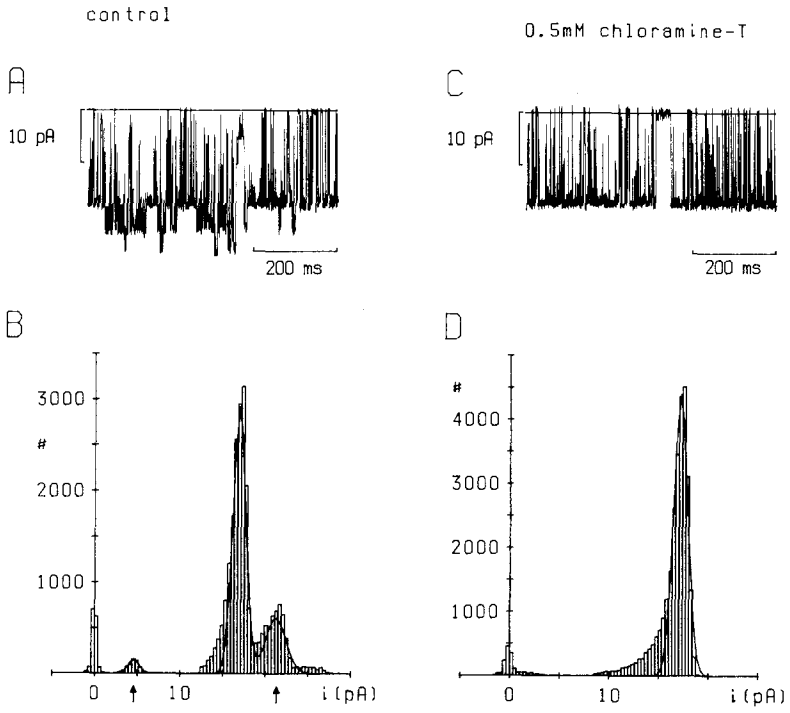

Fig. 5. Effects of chloramine-T in internal solution 1 on Ca-activated and ATP-sensitive K^+ channels. Control currents (*A*) were recorded at −70 mV before application of chloramine-T and arise from the activity of one Ca-activated K^+ channel (single-channel current, −17.1 pA) and of several ATP-sensitive K^+ channels (single-channel current, −4.7 pA). The amplitude histogram (*B*) was calculated from a 10-sec period of control currents; peaks of ATP-sensitive K^+ channels (alone or superposed on the opening of the Ca-activated K^+ channel) are marked by arrows. (*C*) Currents at −70 mV approximately 1 min after application of 0.5 mM chloramine-T. Note blockage of ATP-sensitive K^+ channels and unaffected currents and gating of the Ca-activated K^+ channel. (*D*) Amplitude histogram calculated from a 10-sec period of currents in 0.5 mM chloramine-T; peaks of ATP-sensitive K^+ channels are no longer visible. Low-pass filter frequency, 0.4 kHz; sampling rate, 0.3 msec for calculation of amplitude histograms. Patch 165, temperature 24°C

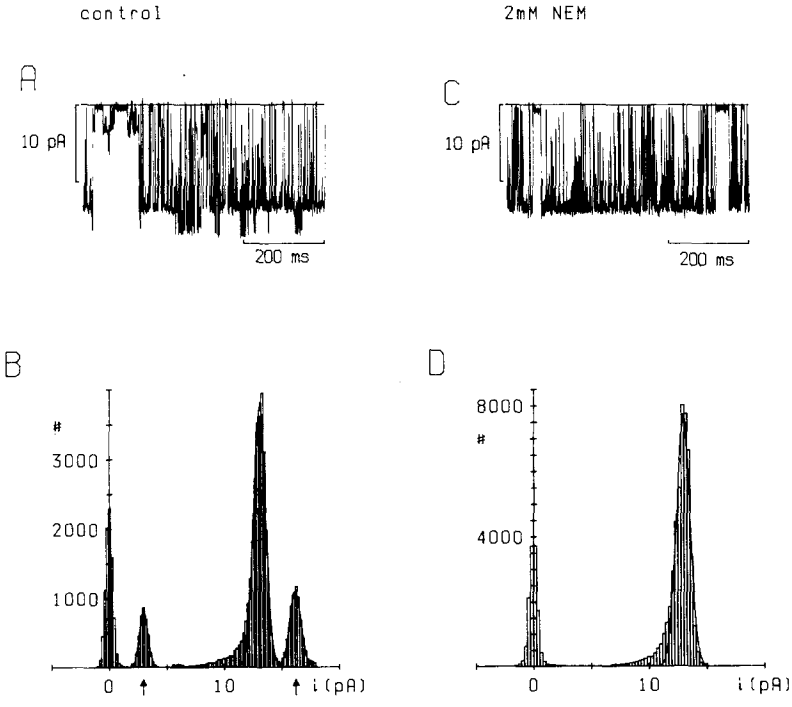

Fig. 6. Effects of NEM in internal Ringer solution on Ca-activated and ATP-sensitive K^+ channels. Control currents (*A*) were recorded at −50 mV before application of NEM and arise from the activity of one Ca-activated K^+ channel (single-channel current, −13.0 pA) and of an ATP-sensitive K^+ channel (single-channel current, −3.0 pA). The amplitude histogram (*B*) was calculated from a 7-sec period of control currents; peaks of the ATP-sensitive K^+ channel (alone or superposed on the opening of the Ca-activated K^+ channel) are marked by arrows. (*C*) Currents at −50 mV approximately 10 min after application of 2 mM NEM. Note blockage of ATP-sensitive K^+ channel and unaffected currents and gating of the Ca-activated K^+ channel. (*D*) Amplitude histogram calculated from a 7-sec period of currents in 2 mM NEM; peaks of the ATP-sensitive K^+ channel are no longer visible. Low-pass filter frequency, 1 kHz; sampling rate, 0.1 msec for calculation of amplitude histograms. Patch 76, temperature 21°C

tide to the channel. This is confirmed in the present study which showed that adenosine and even the free base adenine block ATP-sensitive K^+ channels, but are much less effective than ATP (Fig. 4). The low blocking potencies of the nucleoside and the bases may explain why 1 mM adenosine dissolved in 140 mM KCl solution was not found to affect ATP-sensitive K^+ channels in guinea-pig ventricular cells (Kakei, Noma & Shibasaki, 1985).

As illustrated in Figs. 2–4, internal adenine and thymine block ATP-sensitive K^+ channels, whereas the bases cytosine and uracil at the same concentra-

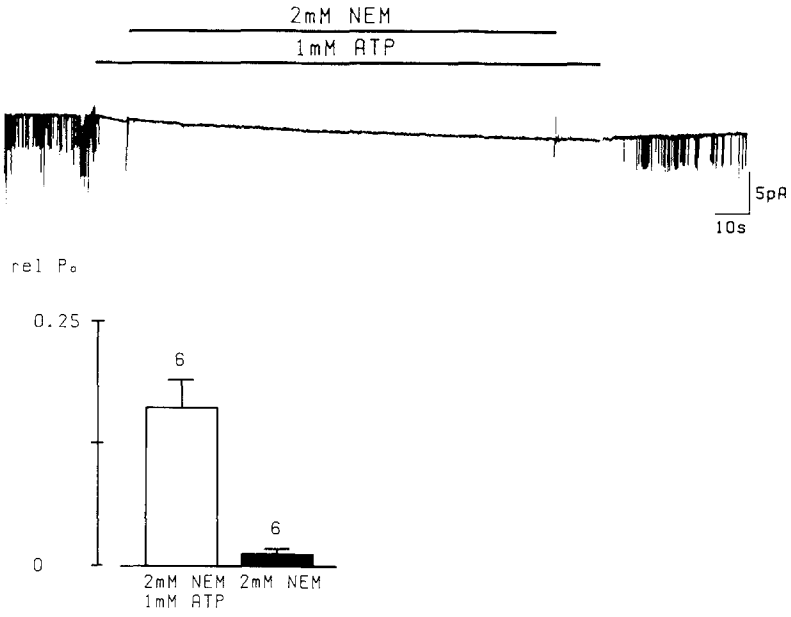

Fig. 7. ATP protects against the action of NEM on ATP-sensitive K^+ channels. The upper part shows currents at −60 mV before, in the presence of 1 mM ATP and 2 mM NEM in internal solution 3 and after washout of the substances. The bars denote the application times of the two agents. The current spikes at the beginning and at the end of NEM treatment are not channel currents but artifacts from the solution change. Note the reappearance of channel activity after washout of NEM and ATP. Low-pass filter frequency, 0.3 kHz. Patch 198, temperature 20°C. The lower part shows p_o values of six patches relative to their initial control values after washout of 2 mM NEM and 1 mM ATP (open column) and 2.5 min after application of 2 mM NEM without ATP (filled column). The bars above the columns indicate the SE values of the means

tions are not effective. An interpretation of this result would be that adenine and thymine bind to the ATP receptor of the channel via the same two hydrogen bonds that connect these complementary bases in the DNA double helix. However, two hydrogen bonds are also formed between adenine of DNA and uracil in a RNA strand so that uracil should be equally effective as thymine in contrast to our results. The observed higher blocking potency of thymine compared to uracil is probably related to the methyl group at position C_5. Hence, this group seems to have a significant role in the binding of thymine to ATP-sensitive K^+ channels.

Efficacy of Reversible Blockers in Different Internal Solutions

The efficacy of the reversible channel blockers ATP, adenosine, adenine and thymine depends on the ionic composition of the internal solution (Fig. 4). In general, the blockers exert their highest potencies in Ringer, whereas the decline of the open probability is less pronounced in KCl solutions. When 10 μM Na_2ATP is dissolved in solution 1 (155 mM KCl, 2 mM $CaCl_2$, 1 mM $MgCl_2$) or in solution 3 (160 mM KCl without EDTA, thus containing traces of Ca^{2+} and Mg^{2+} ions), the nucleotide molecules are almost entirely present as ATP·Mg complexes. These complexes block ATP-sensitive K^+ channels in skeletal muscle as indicated by the hatched and open columns in Fig. 4. An even smaller relative p_o value of 0.271 was obtained for 10 μM ATP in solution 3 with added 5 mM EDTA to minimize the Mg^{2+} concentration (*see* Results). This indicates that ATP anions are more powerful than ATP·Mg complexes in blocking ATP-sensitive K^+ channels in skeletal muscle. The extreme case of ATP anions being the only effective channel blocker was described for insulin-secreting cells (Dunne, Illot & Petersen, 1987; Ashcroft & Kakei, 1987), whereas in rat ventricular myocytes ATP·Mg complexes block ATP-sensitive K^+ channels more strongly than ATP anions (Findlay, 1988). According to our results, the efficacy of the blockers ATP, adenine and thymine is approximately equal in internal KCl solutions without EDTA (containing Ca^{2+}, Mg^{2+} traces) and with mM concentrations of Ca^{2+} and Mg^{2+} ions (open and hatched columns in Fig. 4). On the other hand, addition of mM concentrations of Ca^{2+}, Mg^{2+} seems to weaken the blockage by adenosine. We have no explanation for this difference.

Figure 4 shows that the investigated reversible internal channel blockers are more powerful in Ringer compared to KCl solutions, e.g. 10 μM ATP reduces the relative p_o values to 0.151 and 0.436 in Ringer and 160 mM KCl, respectively. The difference between the two solutions becomes even more pronounced when the p_o values are not normalized to the respective ATP-free solution but to 160 mM KCl. Owing to the presence of Na^+ ions in Ringer, ATP-sensitive K^+ channels are blocked on a fast time scale, especially at positive membrane potentials (Horie, Irisawa & Noma, 1987; Quayle & Stanfield, 1989). In addition, internal Na^+ ions block the channels with slower kinetics at negative membrane potentials at which our experiments were performed (K.H. Woll, U. Lönnendonker and B. Neumcke, *unpublished results*). Furthermore, a

change of the internal K^+ concentration may alter the gating kinetics of ATP-sensitive K^+ channels (Zilberter et al., 1988). All these effects could contribute to the reduced relative p_o of 0.35 in Ringer compared to 160 mM KCl (*see* Results). Hence, relative to this K^+-rich and blocker-free internal solution, the p_o values are 0.436 in 160 mM KCl + 10 μM ATP and $0.151 \times 0.35 = 0.053$ in Ringer + 10 μM ATP. This low open probability in internal solutions containing Na^+ and ATP cannot be explained by the actions of the two channel blockers. Thus the predicted p_o in Ringer + 10 μM ATP relative to 160 mM KCl would be $0.436 \times 0.35 = 0.153$ if both blockers bind to two independent sites. An even higher relative p_o of 0.241 is calculated for competitive binding of Na^+ and ATP for a common blocking site. Instead, internal Na^+ ions seem to have a dual effect on ATP-sensitive K^+ channels: The ions block the channel pore, and they increase the affinity of the channel receptor for ATP. A similar enhancement of ATP binding by internal Na^+ ions has been described for the Na^+, K^+-ATPase in guinea-pig kidney cortex (Hegyvary & Post, 1971) and in ox brain (Skou 1974*a*). Figure 4 illustrates that this modifying effect of internal Na^+ on ATP-sensitive K^+ channels is not restricted to ATP but is also observed for other reversible channel blockers.

A Functionally Important SH-Group on the Channel

ATP-sensitive K^+ channels are inhibited irreversibly by chloramine-T, H_2O_2 and NEM in the internal solution. A common property of all of these agents is the modification of sulphydryl residues in proteins, with NEM exhibiting the greatest specificity for this group (Lundblad, 1984). Despite a preferred specificity for sulphydryl group reactions, NEM may also undergo less favored side reactions with amino groups or with histidine residues (Smyth, Blumenfeld & Konigsberg, 1964; Rack et al., 1984). Of these, amino groups seem not to be involved in the irreversible channel inhibition because treatment with the chemicals pyridoxal-5′-phosphate and trinitrobenzenesulphonic acid, which are known to modify both ε- and α-amino groups of proteins (Flügge & Heldt, 1977; Salem, Lauter & Trams, 1981; Lundblad, 1984), did not affect the open probability of ATP-sensitive K^+ channels. It is also not very likely that NEM molecules in the internal solution penetrate the membrane and inhibit the channel by modifying an external site. This hypothesis is not supported by the observed interactions between NEM and the membrane-impermeant ATP (Fig. 7). Thus the most plausible interpretation of our experiments is that NEM acts on the inside of ATP-sensitive K^+ channels and causes channel inhibition by reacting with a sulphydryl group of a cysteine residue.

Chloramine-T, hydrogen peroxide and NEM inhibit only ATP-sensitive K^+ channels, whereas the single-channel current and the gating properties of the large-conductance Ca-activated K^+ channel in skeletal muscle seem not to be affected by these agents (Figs. 5 and 6). Hence, these reagents may be useful as specific blockers of one type of K^+-selective ion channels.

The SH-Group May Be Near the ATP-Binding Site

The blockage of ATP-sensitive K^+ channels by intracellular ATP shows remarkable differences between various tissues (Ashcroft, 1988): In amphibian skeletal muscle inhibition occurs with approximate 1:1 binding of ATP to a channel (Spruce et al., 1987), whereas Hill coefficients larger than 1 have been reported for ATP-sensitive K^+ channels in cardiac muscle (Kakei et al., 1985) and in some B-cells (Ohno-Shosaku, Zünkler & Trube, 1987; Ribalet & Ciani, 1987). These differences suggest the presence of one ATP-binding site per channel for skeletal muscle but more sites for the other preparations cited. The experiments illustrated in Fig. 7 indicate that the ATP-binding site of the ATP-sensitive K^+ channel in skeletal muscle may be located near the sulphydryl group which is modified by NEM. Thus bound ATP prevents the access of this irreversible channel inhibitor to an essential SH-group. Another interpretation would be that ATP-binding causes a conformational change of the channel molecule after which a distant SH-group can no longer be reached by internal NEM. Furthermore, there may be several functionally important SH-groups on the ATP-sensitive K^+ channel as found for the Na^+, K^+-ATPase (for a review *see* Schuurmans Stekhoven & Benting, 1981). In the transport ATPase one SH-group seems to be located in the ATP-binding site (Patzelt-Wenczler et al., 1975) and others outside. A similar arrangement of essential SH-groups in ATP-sensitive K^+ channels could account for the loss of channel activity during NEM- and ATP treatment (Fig. 7), because only the SH-group of the ATP-binding site would be protected by ATP, whereas additional groups could be reached by NEM. Similarly, ATP can only protect to a certain extent against the inhibitory effect of NEM on Na^+, K^+-ATPase (Skou, 1974*a*). Other striking parallels between the ATP-sensitive K^+ channel in skeletal muscle and the

Na^+, K^+-ATPase are the higher ATP affinity in internal Na^+ instead of K^+ solutions (*see above*) and the abilities of ATP anions and ATP·Mg complexes to bind to the ATP-sensitive K^+ channel and to the Na^+, K^+-ATPase (Skou, 1974*b*). Some common structures in both transport systems are also suggested by the observation that purified Na^+, K^+-ATPase incorporated in lipid bilayers forms ion channels in the absence of ATP with a minimum conductance of about 40 pS (Kumazawa, Tsujimoto & Fukushima, 1986).

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