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K_{ATP} channels of mouse skeletal muscle: mechanism of channel blockage by AMP-PNP

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Abstract. Single ATP-sensitive potassium channels (K_{ATP} channels) were studied in inside-out membrane patches excised from mouse skeletal muscle. Channel blockage by the non-hydrolysable ATP analogue AMP-PNP was investigated in the absence or presence of 1 mM $MgCl_2$ with K^+-rich solutions bathing the internal membrane surface. Currents through single K_{ATP} channels were recorded at −40 and +40 mV. AMP-PNP (5 to 500 μM; Li salt) reduced the open-probability p_o of K_{ATP} channels and decreased the single-channel currents at high nucleotide concentrations by approximately 10%. Half maximal reduction of p_o at −40 mV was observed at nucleotide concentrations of 29 μM in the absence and of 39 μM in the presence of Mg^{2+}. The steepness of the AMP-PNP concentration-response curves was strongly affected by Mg^{2+}, the Hill coefficients of the curves were 0.6 in the absence and 1.6 in the presence of 1 mM $MgCl_2$. The efficacies of channel blockage by AMP-PNP at −40 and +40 mV were not significantly different. The results indicate that a K_{ATP} channel can bind more divalent Mg^{2+}-complexes of AMP-PNP than trivalent protonated forms of the nucleotide and that channel blockage is hardly affected by the membrane electric field. To estimate the contribution of lithium ions to the observed results, we studied the effects of LiCl (0.8 to 10 mM) in the Mg^{2+}-free solution on the single channel current i. At a Li^+ concentration of 10 mM, i was hardly affected at −40 mV but reduced by a factor of 0.75 at +40 mV. The results are interpreted by a fast, voltage-dependent blockage of K_{ATP} channels by internal Li^+ ions.

Key words: Skeletal muscle – Potassium channel – Channel blockage – ATP – AMP-PNP

Introduction

ATP-sensitive potassium channels (K_{ATP} channels) are inhibited by intracellular ATP and other nucleotides (for a review see Ashcroft and Ashcroft 1990). However, in the presence of intracellular Mg^{2+} ions the nucleotides may also promote channel opening or induce channel reactivation after spontaneous slow decline (run-down) of channel activity. For K_{ATP} channels of cardiac and skeletal muscle such activatory effects of nucleotides have been described for various nucleoside di- and triphosphates (e.g. Findlay 1988a; Tung and Kurachi 1991; Allard and Lazdunski 1992; Benz and Kohlhardt 1992; Forestier and Vivaudou 1993). In contrast, the adenosine triphosphate analogue AMP-PNP inhibits K_{ATP} channels of ventricular myocytes and of pancreatic β-cells both in the absence and presence of Mg^{2+} ions (Findlay 1988b; Ashcroft and Kakei 1989; Schwanstecher et al. 1992). Thus, this non-hydrolyzable ATP analogue is suitable as an agent to investigate the blockage of K_{ATP} channels by intracellular nucleotides without superimposed stimulatory nucleotide effects.

This paper reports the results of such an analysis. Our experiments were performed on skeletal muscle fibres which contain a large number of K_{ATP} channels in the sarcolemma (Spruce et al. 1985). Specifically, we have studied the dependence of channel blockage by AMP-PNP on the charge and the nature of the nucleotide complex, the influence of the membrane potential and the time scale of channel blockage by the nucleotide. Furthermore, we estimated the contribution of internally applied lithium ions to the observed results.

Some of the results have been published in abstract form (Hehl and Neumcke 1993a) and are part of the PhD thesis of S. Hehl.

Materials and methods

Flexor digitorum brevis muscles of adult mice were dissociated into single fibres by treatment with collagenase

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(SIGMA Type I, SIGMA Deisenhofen or Boehringer Collagenase B, Boehringer, Mannheim, Germany, 8 mg/3 ml Ringer) at 37 °C for 90 min. After seal formation, the membrane patch was excised and currents through single K_{ATP} channels recorded in the inside-out configuration of the patch-clamp technique (Hamill et al. 1981) with an L/M-EPC-7 amplifier (List Electronics, Darmstadt, Germany). Currents were measured at room temperature (20–23 °C) and were always recorded under steady-state conditions, i.e. some seconds after a step to the indicated membrane potentials and approximately 2 min after solution exchange. The currents were either recorded on video tape (Woll et al. 1989) or digitized at 0.1 ms intervals and stored on computer files (Hehl et al. 1994).

Solutions

The pipettes were filled with a K^+-rich external solution of composition (mM): 155 KCl, 3 $MgCl_2$, 0.5 EGTA (ethylene glycol bis(β-aminoethyl ether)-N,N'-tetraacetic acid), 10 HEPES (4-(2-hydroxyethyl)-1-piperazine ethanesulphonic acid). The solution was titrated to pH 7.4 with 1 N KOH. The muscle fibres were prepared and stored in mammalian Ringer containing (mM): 150 NaCl, 5 KCl, 2 $CaCl_2$, 1 $MgCl_2$, 10 HEPES, titrated to pH 7.4 with 1 N NaOH. Seals were formed and membrane patches excised in Ringer containing 2 mM $CaCl_2$ which leads to a slow Ca^{2+}-dependent inactivation of K_{ATP} channels (Hehl et al. 1994). After appearance of single-channel activity, the pipette was moved from the main solution pool to a small chamber in which Ringer was exchanged for a K^+-rich solution composed of (mM): 160 KCl, 1 EGTA, 10 HEPES with or without 1 $MgCl_2$. Lithium chloride (SERVA Feinbiochemica GmbH, Heidelberg, Germany) and AMP-PNP · Li_4 (Boehringer, Mannheim, Germany) were dissolved in the K^+-rich internal solutions, and the pH value readjusted to 7.4 with 1 N KOH.

Data analysis

Membrane potentials are defined as potential differences between the intra- and extracellular sides of the membrane patch. Membrane currents recorded on video tape were evaluated as described previously (Bodewei et al. 1992). From data stored in computer files point amplitude histograms were constructed from all sampled points of 30 s periods of current recordings (see Fig. 2). The histograms were fitted by Gaussian functions, and the open-probability p_0 of a K_{ATP} channel was calculated as

$$p_0 = \frac{A_1/A_0}{N + A_1/A_0}, \quad (1)$$

where A_0, A_1 are the areas under the Gaussian curves for closed channels and for one open channel and N the number of active channels in the patch (Hehl et al. 1994). Since the channel number cannot be estimated from short periods of current recordings (Colquhoun and Hawkes 1983), N was taken as the maximal number of simultaneously open channels observed during the experiment (Horn 1991). The current i through one open channel was obtained as the difference between the peak positions of the Gaussian curves A_0 and A_1. The effects of AMP-PNP on the parameters p_0 and i were expressed as relative open-probability (p_0 in the presence of the nucleotide normalized with respect to the mean p_0 values before and after nucleotide application) and as the ratio of single channel currents i in the presence and absence of AMP-PNP.

Relative open-probabilities (rel p_0) are given as percentages. Hill coefficients h for channel blockage by AMP-PNP were obtained by fitting the relation

$$\text{rel } p_0 = 100\left[\frac{1}{1+(c/K_D)^h}\right] \quad (2)$$

to rel p_0-values with c denoting the AMP-PNP concentration and K_D the concentration producing 50% channel blockage.

Results of several experiments are given as means ±SEM. We used the two-sided t-test to decide whether the ratio between the relative-open probabilities at +40 mV and −40 mV (Fig. 4) and the ratio of the single-channel currents in the presence and absence of AMP-PNP (Fig. 5) differs from unity at the 5% significance level.

The concentrations of various AMP-PNP complexes in the K^+-rich internal solutions (Table 1) were calculated with the program REACT kindly provided by Dr. G. L. Smith, Institute of Physiology, University of Glasgow, UK. The equilibrium dissociation constants for the H^+, Ca^{2+}, Mg^{2+} complexes of AMP-PNP were taken from Pettit and Siddiqui (1976). The pK value of the K · AMP-PNP complex could not be found in the literature. It was assumed to be 1.2, since the analogous ATP value is 0.903 (Botts et al. 1965), and because all metal complexes of AMP-PNP are generally a little more stable than those of ATP (Yount et al. 1971). In our experiments, the nucleotide AMP-PNP was applied as lithium-salt AMP-PNP · Li_4 in concentrations up to 500 μM. Under these conditions the contribution of the Li · AMP-PNP complex can be neglected (the pK value of the Li · ATP complex is 1.69, see Botts et al. 1965).

Table 1. Concentrations of AMP-PNP complexes for 10 μM AMP-PNP in K^+-rich internal solutions with 1 mM $MgCl_2$ or without added $MgCl_2$. All nucleotide concentrations are given in μM. It is assumed that the nominally Mg^{2+}-free solution contains traces of $MgCl_2$ at a concentration of 1 μM

AMP-PNP complex	K^+-rich solution with 1 mM $MgCl_2$	K^+-rich solution without $MgCl_2$
AMP-PNP^{4-}	0.08	0.58
AMP-PNP · H^{3-}	1.04	7.89
AMP-PNP · K^{3-}	0.20	1.47
AMP-PNP · Mg^{2-}	8.68	0.06

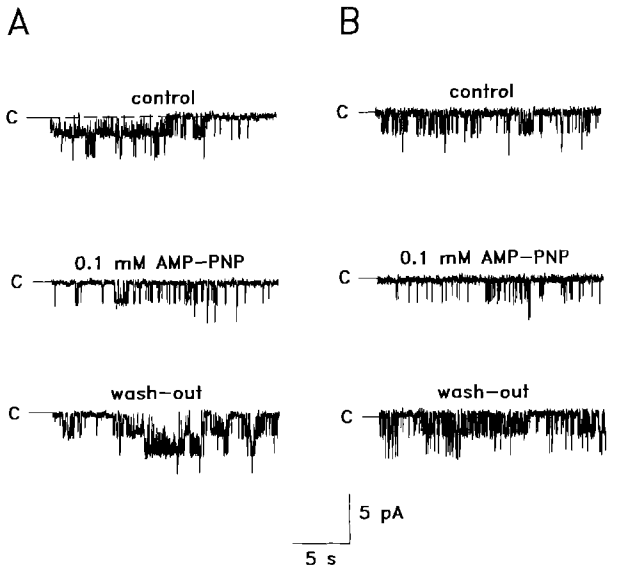


Fig. 1. Inward currents through K_{ATP} channels at −40 mV before (control), during (100 μM AMP-PNP) and after (wash-out) application of the nucleotide in the presence **A** or absence **B** of 1 mM $MgCl_2$ in the K^+-rich internal solution. C denotes the closed channel state. The relative open-probabilities p_0 in the presence of 100 μM AMP-PNP normalized with respect to the mean p_0-value before and after nucleotide application are 6% **A** and 13% **B**. Records in **A** and **B** are from two different patches

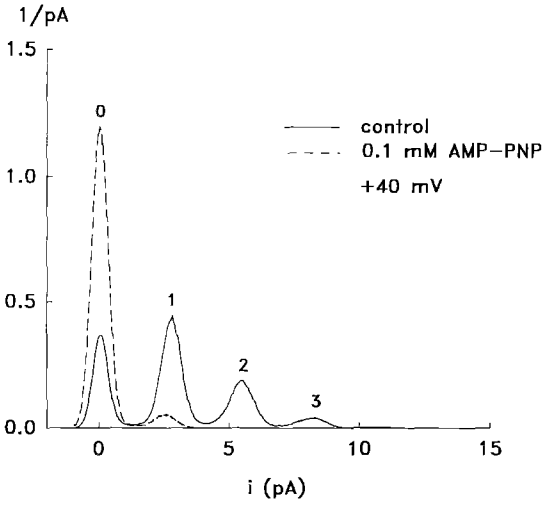


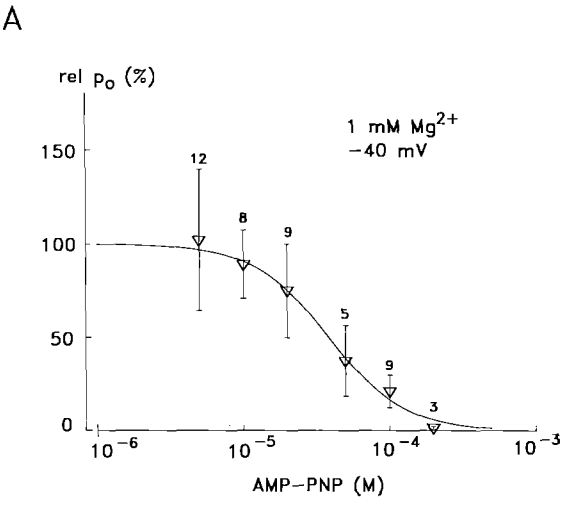
Fig. 2. Amplitude histograms of current recordings at +40 mV from K_{ATP} channels in the Mg^{2+}-free, K^+-rich internal solution (*solid line*) and after addition of 100 μM AMP-PNP (*dashed line*). The areas of the amplitude distributions are normalized to unity, hence the ordinates of the histograms are in units of 1/pA. The open-probabilities p_0 of K_{ATP} channels as calculated from the areas of the distributions are $p_0 = 27.3\%$ (control) and $p_0 = 1.5\%$ (100 μM AMP-PNP)

Results

Effects of AMP-PNP on the relative open-probability of K_{ATP} channels

Figure 1 illustrates the effects of 100 μM AMP-PNP on inward currents through K_{ATP} channels in the presence (left-hand part A) or absence (right-hand part B) of $MgCl_2$ in the K^+-rich internal solution. In this and in all other experiments blockage of K_{ATP} channels by AMP-PNP occurred within a few seconds and was almost fully reversible. The relative open-probabilities of the channels in the presence of AMP-PNP (100 μM) were 6% for the records shown in Fig. 1 A and 13% for those of Fig. 1 B. This indicates a stronger blockage of K_{ATP} channels by AMP-PNP in the presence of Mg^{2+} ions at a nucleotide concentration of 100 μM. This was confirmed in experiments on more membrane patches (Fig. 3).

AMP-PNP also inhibits outward currents through K_{ATP} channels as demonstrated with the amplitude histograms of Fig. 2. The histograms were constructed from current recordings at +40 mV in the Mg^{2+}-free, K^+-rich



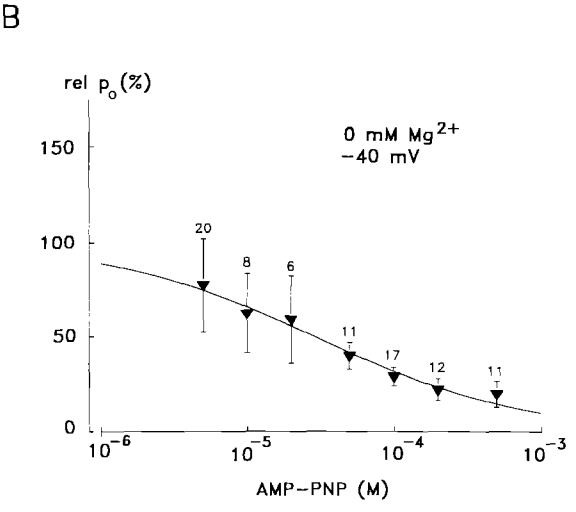


Fig. 3. Concentration-response curves of channel blockage by AMP-PNP at −40 mV in K^+-rich internal solutions in the presence **A** or absence **B** of Mg^{2+} ions. The open-probabilities p_0 were normalized with respect to the mean p_0-values before and after application of AMP-PNP determined in the same patch and at the same membrane potential. Numbers and bars denote the number of experiments and the SEM values. The curves through the symbols represent fits of the measured relative p_0-values by Eq. (2), the values of the parameters are **A** $K_D = 39$ μM, $h = 1.6$ and **B** $K_D = 29$ μM, $h = 0.6$

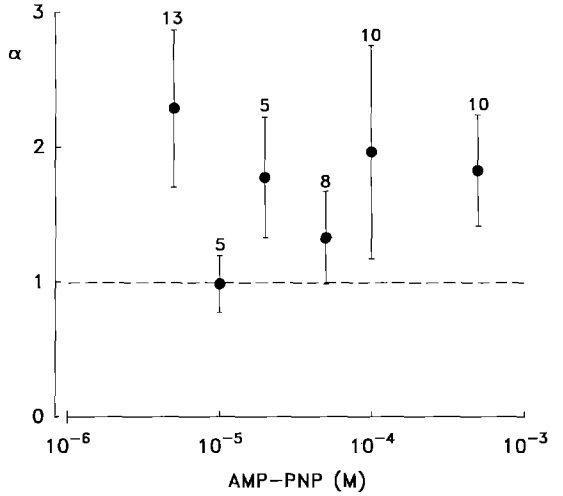


Fig. 4. Voltage-dependence of channel blockage by AMP-PNP in the Mg^{2+}-free, K^+-rich internal solution. α is the ratio of the relative open-probabilities rel p_0 at +40 mV to rel p_0 at −40 mV as determined from measurements in the same patch. Symbols, numbers and bars denote mean values, number of experiments and SEM values respectively

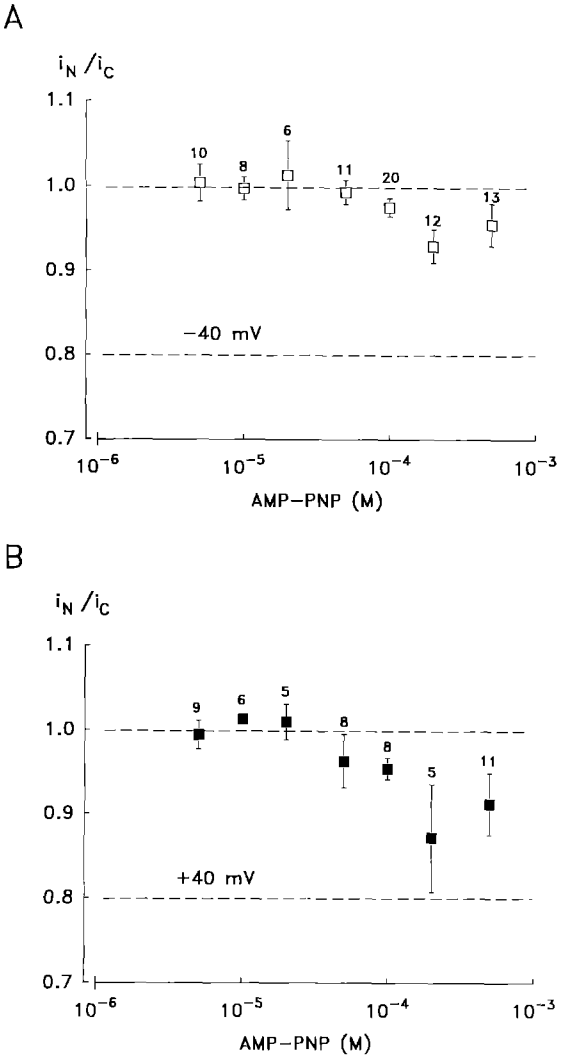


Fig. 5. Effects of AMP-PNP in the Mg^{2+}-free, K^+-rich internal solution on single channel currents at −40 mV **A** and +40 mV **B**. The ordinates represent the ratio of channel currents in the presence of AMP-PNP (i_N) to the current before nucleotide application (i_C) as determined from measurements in the same patch. Symbols, numbers and bars denote mean values, number of experiments and SEM values respectively

internal solution and exhibit peaks from up to three open channels under control conditions but only a peak for one open channel in the presence of AMP-PNP (100 μM). Hence, the nucleotide also blocks K_{ATP} channels very effectively at positive potentials (compare also the values of the open-probabilities of K_{ATP} channels given in the legend to Fig. 2).

A summary of relative open-probabilities at −40 mV and at various concentrations of AMP-PNP is shown in Fig. 3. The figure demonstrates that AMP-PNP blocks K_{ATP} channels at all concentrations investigated in the presence (Fig. 3A) as well as in the absence (Fig. 3B) of Mg^{2+} ions in the K^+-rich internal solution. Half-maximal channel blockage was reached near 35 μM in both cases, but the slopes of the concentration-response curves are very different. Compared to the Mg^{2+}-containing solution, the curve in the absence of Mg^{2+} ions is less steep, and the relative open probabilities do not reach zero even at a high concentration of 500 μM AMP-PNP. The Hill coefficients of the curves are 1.6 in the presence (Fig. 3A) and 0.6 in the absence of Mg^{2+} ions (Fig. 3B).

Voltage-dependence of channel blockage by AMP-PNP

A possible dependence of channel blockage by AMP-PNP on the membrane potential was studied by comparing relative open-probabilities (rel p_0) at +40 and −40 mV. Figure 4 shows ratios $\alpha = \text{rel } p_0(+40\text{ mV})/\text{rel } p_0(-40\text{ mV})$ at various concentrations of AMP-PNP in the Mg^{2+}-free, K^+-rich internal solution. With the exception of the α value at 10 μM, the ratios are larger than unity. Hence, there is a tendency towards a stronger blockage of K_{ATP} channels by AMP-PNP complexes at negative membrane potentials. However, the standard error of the ratios α is considerable, and the results do not differ from unity at the two-sided 5% significance level.

Ratios α equal to or above unity were also obtained with AMP-PNP in the K^+-rich internal solution containing 1 mM $MgCl_2$: The α values were 2.15 ± 0.67 ($n = 11$) at an AMP-PNP concentration of 5 μM, 1.00 ± 0.21 ($n = 8$) at 10 μM, 3.47 ± 2.32 ($n = 10$) at 20 μM and 6.46 ± 3.12 ($n = 8$) at 100 μM. Again, the α values do not differ from unity at the two-sided 5% significance level.

Effects of AMP-PNP on the single-channel current

A decline of the open-probability of K_{ATP} channels in the presence of AMP-PNP (Fig. 3) implies an "intermediate" time scale of channel blockage by the nucleotide (Hille 1992). In addition, AMP-PNP could act as a "fast" blocker of K_{ATP} channels with kinetics which are too fast to record. This possibility was studied for the Mg^{2+}-free internal solution only, because Mg^{2+} ions are fast chan-

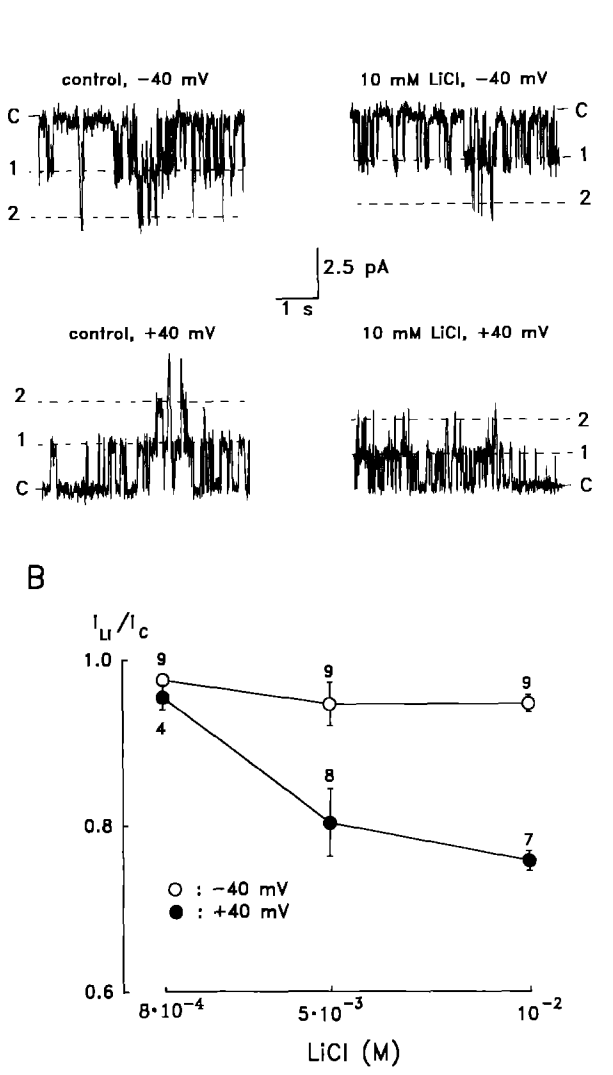


Fig. 6. A Segments of current recordings from K_{ATP} channels before (control) and after addition of 10 mM LiCl to the Mg^{2+}-free, K^+-rich internal solution at −40 mV (*upper records*) and +40 mV (*lower records*). C, 1 and 2 denote the closed channel state and the current levels of 1 and 2 open channels. The single channel currents are −2.8 pA (control, −40 mV); −2.6 pA (10 mM LiCl, −40 mV); 2.4 pA (control, +40 mV) and 1.8 pA (10 mM LiCl, +40 mV). All records are from one patch. **B** Effects of LiCl in the Mg^{2+}-free, K^+-rich internal solution on the single channel current *i* at −40 mV (*open circles*) and +40 mV (*filled circles*). Ratios of the single channel currents (i_{Li}/i_C) were calculated by dividing the currents (i_{Li}) in the presence of LiCl (0.8, 5 or 10 mM) by the initial control values (i_C) of the same patch and at the same potential. Symbols, numbers and bars denote mean values, number of experiments and SEM values respectively

nel blockers themselves at positive membrane potentials (Woll et al. 1989), and this would hamper the detection of fast AMP-PNP effects.

A characteristic property of "fast" channel blockers is the lowering of the single-channel current as seen in a displacement of peaks in amplitude histograms. Thus the positions of the peaks 1 in the histograms of Fig. 2 are at 2.77 pA under control conditions and at 2.51 pA during application of 100 μM AMP-PNP. Figure 5 shows ratios of single-channel currents *i* before and during treatment with AMP-PNP at two membrane potentials $V =$ −40 mV (Fig. 5A) and +40 mV (Fig. 5B). At nucleotide concentrations between 50 and 500 μM the mean values of the current ratios are smaller than unity at both potentials. This excludes the possibility that the observed alterations of the single-channel currents were caused by a mere voltage shift of the $i(V)$ curves. The deviations of the current ratios from unity are significant at the two-sided 5% level for 100 μM AMP-PNP at +40 and −40 mV, for 200 μM AMP-PNP at −40 mV and for 500 μM AMP-PNP at +40 mV.

Voltage-dependence of channel blockage by Li^+-ions

In our experiments the nucleotide AMP-PNP was applied as the lithium-salt AMP-PNP · Li_4. To differentiate between the effects of the nucleotide and those of the Li^+ ion, we studied the effects of LiCl at concentrations of 0.8, 5 and 10 mM in the Mg^{2+}-free, K^+-rich internal solution. Figure 6A shows segments of current recordings at −40 and +40 mV before and during addition of 10 mM LiCl to the internal solution. At −40 mV the single-channel current *i* was not significantly affected by the presence of Li^+-ions (reduction of *i* to 91.8%), whereas the same LiCl concentration at +40 mV produced a marked decrease in the single-channel current to 74.6%. Figure 6B summarizes the results from more experiments and illustrates that the effects of internal Li^+ ions are both concentration- and voltage-dependent.

Discussion

Blockage of K_{ATP} channels by divalent and trivalent AMP-PNP complexes

The AMP-PNP concentration-response curves have different slopes and Hill coefficients for K^+-rich internal solutions with or without $MgCl_2$ (Fig. 3). This difference is probably due to the presence of different nucleotide complexes in the two internal solutions. As demonstrated in Table 1, AMP-PNP is mainly present as the divalent Mg^{2-} complex in the Mg^{2+}-containing solution and as the trivalent protonated H^{3-} form in the Mg^{2+}-free solution. The higher negative charge of AMP-PNP in the Mg^{2+}-free solution hardly affected the nucleotide concentration producing half-maximum channel blockage, but it reduced the steepness of the concentration-response curve (Fig. 3). Thus, the decrease of the open-probability of the channels at the high AMP-PNP concentrations of 100 and 200 μM is stronger in the *presence* of Mg^{2+}, in agreement with the results on rat pancreatic β-cells (Ashcroft and Kakei 1989; Schwanstecher et al. 1992) and on rat ventricular myocytes (Findlay 1988b). In contrast, blockage of K_{ATP} channels in skeletal muscle at the low AMP-PNP concentrations of 10 and 20 μM is stronger in the *absence* of Mg^{2+} (Fig. 3).

The weak concentration-dependence of channel blockage by AMP-PNP in the absence of internal Mg^{2+} ions (Fig. 3B) was described by a Hill coefficient of 0.6. Such a flat concentration-response curve could arise from nega-

tive cooperativity between K_{ATP} channels for blockage by the trivalent AMP-PNP · H^{3-} anions (Hehl and Neumcke 1993 b). On the other hand, channel blockage by the divalent AMP-PNP · Mg^{2-} complexes has a Hill coefficient of 1.6 (Fig. 3 A) and could occur without cooperativity between channels.

Another explanation of the different slopes of the AMP-PNP concentration-response curves in the presence and absence of Mg^{2+} ions would be that the negative charge of the nucleotide affects the stoichiometry of channel block by internal nucleotides. In general, a higher Hill coefficient of the concentration-response curve implies channel models with more sequential nucleotide-binding sites (Nichols et al. 1991). Our results therefore suggest that a K_{ATP} channel can accommodate more AMP-PNP · Mg^{2-} complexes than AMP-PNP · H^{3-} anions which could arise from a weaker electrostatic repulsion between the divalent complexes. These arguments apply to K_{ATP} channels of skeletal muscle and may not be valid for other tissues. For example, AMP-PNP concentration-response curves of K_{ATP} channels in mouse pancreatic β-cells have similar slopes in the absence and presence of internal Mg^{2+} (see Fig. 3 of Schwanstecher et al. 1992) and thus reveal no obvious dependence of the stoichiometry on the charge of the nucleotide complex.

The steepness of the concentration-response curves for blockage of K_{ATP} channels by nucleotides is not only determined by the charge of the nucleotide complex but also by other factors. Thus blockage of K_{ATP} channels in skeletal muscle by ATP instead of AMP-PNP in a K^+-rich, Mg^{2+}-free solution occurs with a higher Hill coefficient of 1.4, and even steeper concentration-response curves were found for ATP in a Na^+-rich internal solution (see Fig. 5 of Neumcke and Weik 1991). Hence, channel blockage by nucleotides is also dependent on the structure of the nucleotide complex and on the ion composition of the internal solution.

Voltage-dependence of blockage of K_{ATP} channels

A voltage-dependent blockage of K_{ATP} channels in skeletal muscle has been described for various inorganic and organic cations in solutions bathing the internal surface of the membrane, e.g. for Na^+, Ca^{2+}, Mg^{2+} (Woll et al. 1989), for tetraethylammonium (Davies et al. 1989) and for 4-aminopyridine (Davies et al. 1991). As expected from the positive charge of these agents, their blocking effects become more pronounced at positive membrane potentials. As shown in this investigation, Li^+ belongs to this class of voltage-dependent channel blockers (Fig. 6).

An aim of the present study was to test for a voltage-dependence of blockage of K_{ATP} channels by internal nucleotides. If nucleotide binding to K_{ATP} channels were influenced by the membrane electric field, the binding of the negatively charged AMP-PNP should be stronger at more negative potentials, and this voltage dependence should be more pronounced for the trivalent anion AMP-PNP · H^{3-} compared to the divalent AMP-PNP · Mg^{2-} complex. Neither prediction could be verified in our experiments. The results shown in Fig. 4 reveal a weak, but not significant voltage-dependence of the relative open-probability in the presence of AMP-PNP. Moreover, the ratios α of the relative open-probabilities at +40 and −40 mV shown in Fig. 4 and given in Results for Mg^{2+}-free and Mg^{2+}-containing internal solutions respectively are not smaller in the presence of divalent cations. Thus, the mean α values at 100 μM AMP-PNP were 1.97 in the absence and 6.46 in the presence of internal Mg^{2+} ions. These results are contrary to the expectation that blockage by the prevailing AMP-PNP · H^{3-} anions of the Mg^{2+}-free solution should have a stronger voltage dependence than that by AMP-PNP · Mg^{2-} complexes of the solution with 1 mM $MgCl_2$. Hence, nucleotide binding to K_{ATP} channels is hardly affected by the membrane electric field and seems to occur at superficial cytoplasmic sites of the channel protein.

Fast blockage of K_{ATP} channels

The inorganic and organic cations listed above lower the single-channel current of K_{ATP} channels at positive voltages. This may be explained by a flickery block of K_{ATP} channels with fast kinetics which are filtered by our recording system. Such a fast blockage has been described also for ATP · Mg^{2-} complexes inhibiting an ATP-sensitive ion channel in rat central neurons (Ashford et al. 1988). In our study we have explored the possibility of a similar fast blockage of K_{ATP} channels in skeletal muscle by negatively-charged nucleotides. Our results indeed indicate a lowering of the single-channel currents at AMP-PNP concentrations above 50 μM (Fig. 5). However, the effects are only of the order of 10%, only four of the current ratios i_N/i_C differ significantly from unity, and the results are affected by the presence of Li^+ ions in the solutions containing AMP-PNP · Li_4. For example, at a voltage of −40 mV the single-channel current is lowered by a factor of 0.930 ± 0.020 ($n = 12$) in the presence of 200 μM AMP · Li_4 and by a factor of 0.976 ± 0.005 ($n = 9$) after addition of 0.8 mM LiCl (Fig. 6 B). Similar problems in differentiating between the effects of the negatively charged AMP-PNP complex and the positively charged Li^+ ion on single-channel currents arise at the other AMP-PNP concentrations studied and in the analysis of measurements performed at +40 mV. Hence, no conclusive evidence for a fast blockage of K_{ATP} channels by nucleotides could be obtained in this investigation.

In conclusion, the main effect of the non-hydrolysable nucleotide AMP-PNP on K_{ATP} channels in skeletal muscle is a reduction of the open-probability of the channels. Channel blockage by AMP-PNP depends on the negative charge of the nucleotide complex, is hardly affected by the membrane potential and occurs at cytoplasmatic parts of the channel molecule which are distinct from the binding sites of voltage-dependent channel blockers.

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