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Internal Ca^{2+} ions inactivate and modify ATP-sensitive potassium channels in adult mouse skeletal muscle

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Abstract

The effects of internal Ca^{2+} ions on single ATP-sensitive potassium channels (K_{ATP} channels) were studied in inside-out membrane patches excised from mouse skeletal muscle. Channel activity was high when patches were excised in a Ca^{2+}-free, Na^+-rich solution and declined irreversibly within seconds in the presence of internal Ca^{2+} (0.1 and 2 mM). After Ca^{2+}-dependent inactivation of the channels, the ATP concentration-response curve was steeper and 50% channel blockage occurred at a lower ATP concentration than before inactivation. ATP (50 μM) in a K^+-rich solution bathing the intracellular membrane surface reduced the open-probability of K_{ATP} channels to 18% before and to 10% after exposure of the patch to internal Ca^{2+} (0.1 mM). The block of K_{ATP} channels by ATP (50 μM) was also enhanced by internal Ca^{2+} at a concentration of 13 μM. It is concluded that internal Ca^{2+} ions can both inactivate K_{ATP} channels and modify the active channels. K_{ATP} channels modified by Ca^{2+} are blocked more strongly by ATP than unmodified channels.

Key words: Patch clamp; Potassium channel; Adenosine triphosphate; Skeletal muscle; Calcium dependent inactivation; (Mouse)

1. Introduction

The surface membrane of skeletal muscle fibres contains a large number of ATP-sensitive potassium channels (K_{ATP} channels) that are blocked by intracellular ATP and are selective for K^+ ions [1]. For patch-clamp experiments of single K_{ATP} channels in intact skeletal muscle fibres, the muscle surface must be cleaned by collagenase and the isolated muscle fibres stored in Ringer's solution [2,3]. After this treatment, seals can be formed and inside-out patches excised in this solution. Our usual procedure was then to record currents through single K_{ATP} channels with Ringer and subsequently with other solutions bathing the intracellular membrane surface [4].

We now report that a much higher number of active K_{ATP} channels are observed if, prior to patch excision, Ringer in the bath is exchanged for a Ca^{2+}-free, Na^+-rich solution. Addition of $CaCl_2$ then leads to a slow Ca^{2+}-dependent inactivation of K_{ATP} channels as described for ventricular myocytes [5]. The subject of this paper is a comparison of the affinities and stoichiometries of ATP-binding to K_{ATP} channels before and after Ca^{2+}-dependent inactivation to investigate whether internal Ca^{2+} ions also modify the properties of the active channels. We also study whether Ca^{2+} ions can relieve the ATP block of K_{ATP} channels as reported for frog skeletal muscle [3].

2. Materials and methods

Single muscle fibres from Flexor digitorum brevis muscles of female adult mice were prepared as described previously [2]. The muscles were dissociated into single fibres by treatment with collagenase (Sigma Type I, Sigma, Deisenhofen, Germany or Boehringer Collagenase B, Boehringer, Mannheim, Germany) for 1.5 h at a concentration of 8 mg/3 ml Ringer and at a temperature of 37°C. Currents through single K_{ATP} channels were recorded in the inside-out configuration of the patch-clamp technique [6] using an L/M-EPC-7 amplifier (List, Darmstadt, Germany). The membrane currents were recorded on a pen-recorder (linearcorder Mark VII, Graphtec, March, Germany), see Fig.

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1, and stored for subsequent analysis. Currents were stored either on video tape or on an IBM compatible computer (386 DX, 33 MHz, 2 × 120 MB hard disc, 18 ms access time). In the latter case, the currents were filtered by a four-pole low-pass Bessel filter with 1 kHz corner frequency and digitized at 0.1 ms intervals by a LabMaster Interface (DMA/TM100 OEM, Beltech, Eindhoven, The Netherlands). The program SUPER-PATCH (Dr. T. Böhm, Halle, Germany) was used for setting the voltage pulses and for on-line recording of the membrane currents. All experiments were performed at room temperature (20–23°C).

2.1. Solutions

The pipettes were filled with a K^+-rich solution which bathed the extracellular membrane surface (hereafter called external solution) which was composed of (mM): 155 KCl, 3 $MgCl_2$, 0.5 EGTA, 10 Hepes (pH 7.4).

The compositions of the Na^+-rich and K^+-rich solutions which bathed the intracellular membrane surface (hereafter called internal solutions) are listed in Table 1. The Na^+-rich solution A (mammalian Ringer) was also used during the preparation of the muscles and for storage of isolated muscle fibres. The K^+-rich solutions D1, D2 contained EDTA (ethylenediaminetetraacetic acid), and the solutions G1, G2 were buffered with EGTA. The compositions of the solutions were chosen to minimize the concentrations of free Ca^{2+} and Mg^{2+} ions (solution D1), to lower the Ca^{2+} concentration (solution G1) or to establish Ca^{2+} concentrations near 100 μM (solution D2) or 10 μM (solution G2). Seals were formed and membrane patches excised either directly in Ringer or after replacing the bath solution by the Ca^{2+}-free, Na^+-rich solution B. After excision of the membrane patch, the pipette was moved from the main solution pool to a small chamber in which the

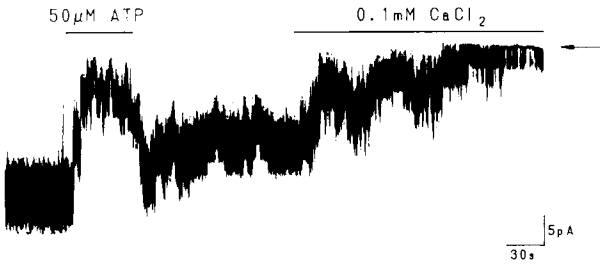

Fig. 1. Currents through K_{ATP} channels at −50 mV in Na^+-rich internal solutions. The current level during closure of all channels is marked by an arrow, and inward currents are plotted downwards. The recordings begin at the time of patch excision in an internal Na^+-rich solution without Ca^{2+} (solution B). 50 s after excision 50 μM ATP in solution B was applied for 58 s. After wash-out of the nucleotide the internal solution B was exchanged for the Na^+-rich solution C containing 0.1 mM $CaCl_2$. The currents were recorded with a pen-recorder and filtered at 100 Hz.

Table 1
Compositions of Na^+-rich and K^+-rich internal solutions (mM)

	A	B	C	
(a) Na^+-rich solutions				
NaCl	150	160	160	
KCl	5	–	–	
$CaCl_2$	2	–	0.1	
$MgCl_2$	1	1	1	
EGTA	–	0.5	–	
	D1	**D2**	**G1**	**G2**
(b) K^+-rich solutions				
KCl	160	160	160	160
$CaCl_2$	–	0.6	–	0.51
$MgCl_2$	–	–	1	1
EDTA	0.5	0.5	–	–
EGTA	–	–	0.5	0.5

All solutions were buffered with 10 mM Hepes and titrated to pH 7.4 with 1 M NaOH (Na^+-rich solutions) or with 1 M KOH (K^+-rich solutions). The Na^+-rich solution A was used as mammalian Ringer.

Na^+-rich solution could be exchanged for a K^+-rich solution.

ATP was added to the internal solutions as $Na_2 \cdot$ ATP (Boehringer, Mannheim, Germany), and the pH value of all ATP containing solutions was readjusted to 7.4 with NaOH (solutions A, B) or KOH (solutions D1, D2, G1, G2).

2.2. Analysis

Membrane potentials are defined as potential differences between the intra- and extracellular sides of the membrane patch. Membrane currents, stored on video tape during the experiment, were processed as described in detail previously [2,7]. Data stored in computer files were evaluated using the program ASCD (Dr. G. Droogmans, Laboratorium voor Fysiologie, KU Leuven, Belgium). Amplitude histograms were constructed from 30 s periods of current recordings and fitted by Gaussian functions. From the areas A_0, $A_1 \ldots A_N$ under the Gaussian curves for closed channels and for $1 \ldots N$ open channels the open-probability p_o of a K_{ATP} channel was calculated as

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

or

$$p_o = \frac{1}{N} \cdot \sum_{i=1}^{N} i \cdot A_i \quad (2)$$

where N is the number of active channels in the membrane patch. N was taken as the maximal number of simultaneously open channels observed during the experiment [8]. If channel activity is low and the amplitude histograms show clear peaks for closed channels and for one open channel (Fig. 5), p_o can be easily

determined from Eqn. 1. At high channel activities when all channels are rarely closed simultaneously (Fig. 3), p_o must be calculated from Eqn. 2. Eqns. 1 and 2 follow from a binomial distribution for the superposition of openings from independent channels. The independence between K_{ATP} channels was tested in control experiments in which the activity of several channels was recorded in the internal solution G1 in the absence or presence of 50 μM ATP. The areas under the Gaussian curves for 0, 1, 2... N open channels were found to follow approximately a binomial distribution.

If discrete current steps from the opening and closing of single channels could not be resolved (e.g., see the beginning of the recordings in Fig. 1), the sums of the membrane currents during 15 s periods of steady-state conditions were calculated from currents stored on video tape and taken as a measure of the open-probability. Since this method did not directly yield p_o values and because the activity of K_{ATP} channels varies greatly among different membrane patches, the open-probabilities were always normalized, for details see the legends to Figs. 2, 4 and 6. The resulting relative p_o values are expressed as percentages. Results from several experiments are given as mean values ± S.E. Hill coefficients n for channel blockage by ATP (Fig. 2) were obtained as the steepest slope of the Hill plot.

The concentrations of free Ca^{2+} and Mg^{2+} ions and of various ATP complexes in the internal solutions used (Table 2) were calculated with the program RE-

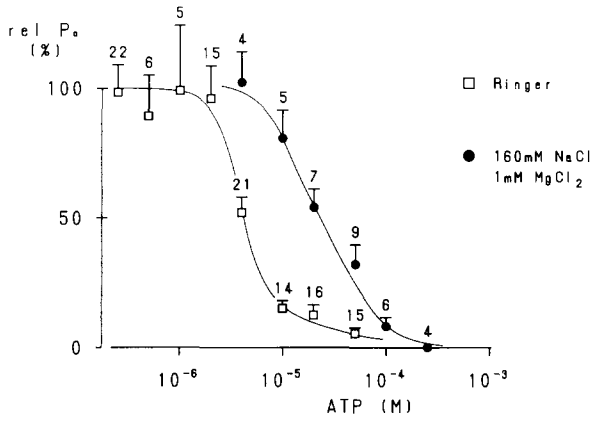

Fig. 2. Concentration-response curves of channel blockage by ATP in Na^+-rich internal solutions. The relative open-probabilities (rel p_o) refer to the respective ATP-free solution. The values (●) are obtained from membrane patches excised in an internal solution without Ca^{2+} (solution B) and from measurements in this solution. Membrane potential −50 mV. The values (□) are from measurements in solution A (mammalian Ringer) containing 2 mM $CaCl_2$ and were taken from Ref. 10, Fig. 5B. Membrane potential −40 to −60 mV. Symbols, numbers and bars denote mean values, number of measurements and S.E. values. The curves through the mean values were drawn by eye. Half-maximal channel blockage at ATP concentrations of 22 μM (●) and 4 μM (□). The Hill coefficients of the concentration-response curves are $n = 1.6$ (●) and near 3 (□).

Table 2
Concentrations of free Ca^{2+}, Mg^{2+} ions and of various ATP complexes for 50 μM Na_2ATP in solutions A, B, D1, G1, G2

Solution	A	B	D1	G1	G2
Ca^{2+}	1978	–	–	–	12.9
Mg^{2+}	976	926	–	925	956
ATP^{4-}	1.16	2.18	17.92	2.22	2.14
$ATP{\cdot}H^{3-}$	0.55	1.03	8.45	1.05	1.02
$ATP{\cdot}K^{3-}$	0.05	–	23.61	2.92	2.82
$ATP{\cdot}Na^{3-}$	1.86	3.72	0.02	≈ 0	≈ 0
$ATP{\cdot}Ca^{2-}$	22.15	–	–	–	0.27
$ATP{\cdot}Mg^{2-}$	24.23	43.07	–	43.81	43.75

All concentrations are given in μM.

ACT kindly provided by Dr. G.L. Smith, Institute of Physiology, University of Glasgow, UK. The equilibrium dissociation constants for the proton and metal complexes of ATP were taken from Fabiato and Fabiato [9].

3. Results

3.1. Ca^{2+} effects in Na^+-rich solutions

A large number of active K_{ATP} channels were observed when membrane patches of mouse skeletal muscle were excised into Ca^{2+}-free internal solutions. Fig. 1 shows an example for a patch excised in the nominally Ca^{2+}-free, Na^+-rich internal solution B. Addition of 50 μM ATP to this solution reduced the mean inward current; the effect of the nucleotide was rapid and the response limited by the speed of the solution

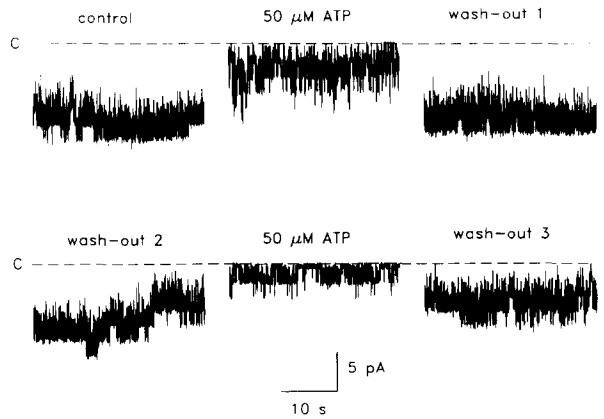

Fig. 3. Currents from K_{ATP} channels at −40 mV in the Ca^{2+}-free, K^+-rich solution D1 before (upper row) and after (lower row) treatment with internal Ca^{2+} ions. The current levels during closure of all channels are marked by the letter C. The patches were excised in the Ca^{2+}-free, Na^+-rich solution B and transferred to the K^+-rich solution D1 (control). Subsequently, 50 μM ATP was added to solution D1 and washed out (wash-out 1). Thereafter, solution D2 containing 600 μM $CaCl_2$ was applied for approx. 2 min and washed out (wash-out 2). 50 μM ATP was then added for a second time and washed out (wash-out 3).

exchange. In contrast, 0.1 mM $CaCl_2$ in the Na^+-rich internal solution C produced a slow inactivation of K_{ATP} channels within 3 min, and currents from the opening and closing of single channels were finally detectable at the end of the recording period. A subsequent exchange of the internal solution C for the Ca^{2+}-free solution B did not restore the initial high activity of K_{ATP} channels (not shown). Thus, the Ca^{2+}-dependent inactivation of the channels is only partially reversible.

In separate experiments, membrane patches were excised in solution B and transferred into mammalian Ringer (solution A) containing a higher $CaCl_2$ concentration of 2 mM (not shown). The degree of the Ca^{2+}-dependent inactivation was then stronger than that observed with 0.1 mM $CaCl_2$, but it showed considerable variation between different membrane patches. Thus, the ratio 90 ± 39 (mean $\pm$ S.E., $n = 5$) of the open-probabilities of the channels in solutions B and A was affected by a large standard error.

The blockage of K_{ATP} channels by internal ATP showed remarkable differences before and during treatment with internal Ca^{2+} ions: The addition of 50 μM ATP to the Ca^{2+}-free, Na^+-rich solution B reduced the mean current through the channels to approx. 25% (Fig. 1), whereas at the same nucleotide concentration the open-probability of the channels in internal mammalian Ringer containing 2 mM $CaCl_2$ was lowered to 5% [10]. Fig. 2 shows complete concentration-response curves for blockage of K_{ATP} channels by ATP in the absence and presence of internal Ca^{2+} ions. Compared to Ca^{2+}-free conditions (solution B), the curve for Ringer (solution A) is shifted towards lower ATP concentrations and is steeper. One possible explanation for the differences in the ATP concentration – response curves could be the presence of ATP · Ca^{2-} complexes in solution A which are absent in solution B (see Table 2). In addition, the differences could originate from a modification of ATP binding to K_{ATP} channels by internal Ca^{2+} ions. The following experiments, performed in K^+-rich internal solutions, were designed to test for the presence of a Ca^{2+}-induced modification of K_{ATP} channels.

3.2. Ca^{2+} effects in K^+-rich solutions

Fig. 3 shows current recordings from an experiment in which channel blockage by 50 μM ATP in the Ca^{2+}-free, K^+-rich internal solution D1 containing 0.5 mM EDTA was investigated before (recordings in upper row) and after (recordings in lower row) exposure of the patch to internal Ca^{2+} ions. Patches were treated with internal Ca^{2+} ions between the periods 'wash-out 1' and 'wash-out 2' by exposing the patch to the internal solution D2 containing 600 μM $CaCl_2$ and 0.5 mM EDTA (free Ca^{2+} concentration 100 μM) for approx. 2 min. After wash-out of $CaCl_2$, the open probability of K_{ATP} channels was lower and their ATP affinity higher than before treatment with internal Ca^{2+} ions. Quantitative results from this and from 7 additional experiments are shown in Fig. 4. Owing to Ca^{2+}-dependent inactivation of K_{ATP} channels, the open-probability of the K_{ATP} channels during the 'wash-out 2' phase was $40.6 \pm 17.4\%$ ($n = 8$) relative to the initial

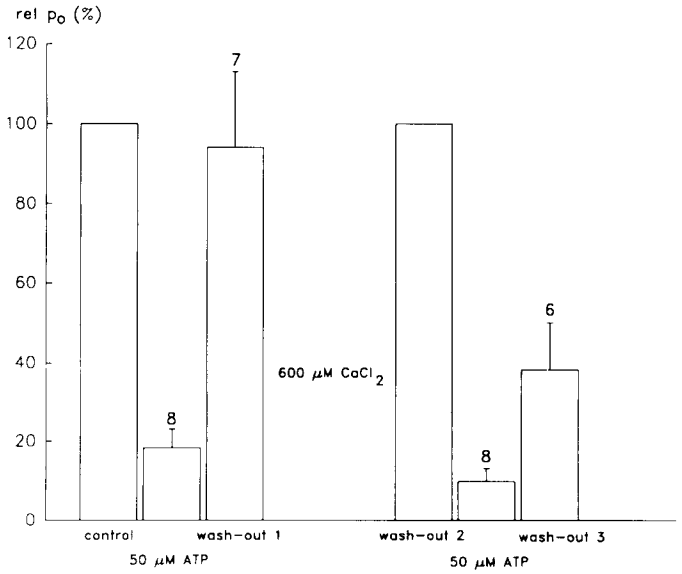

Fig. 4. Relative open-probabilities (rel p_o) of K_{ATP} channels in internal solution D1 before and after the patch was exposed to internal Ca^{2+} ions. The figure gives a summary of 8 experiments performed with the protocol described in the legend to Fig. 3. During 'wash-out 1' the open probabilities could be determined in 7 experiments and during 'wash-out 3' in 6 experiments. The probabilities are normalized with respect to measurements before the first and second application of ATP respectively. Bars above the columns indicate the S.E. values.

control value of the respective experiment. The rel p_o values of the channels which remained active after wash-out of Ca^{2+} ions were again taken as 100% (wash-out 2). This representation reveals that ATP (50 μM) had different effects before and after treatment with Ca^{2+} ions: The nucleotide decreased the open-probability of the channels to $18.2 \pm 4.8\%$ ($n = 8$) before and to $9.8 \pm 3.3\%$ ($n = 8$) after exposure to Ca^{2+} ions. The two values are significantly different (paired t-test at the 5% significance level). Hence, ATP blocks K_{ATP} channels more strongly after an exposure to internal Ca^{2+} ions.

In frog skeletal muscle, free Ca^{2+} ions can relieve the block of K_{ATP} channels by ATP [3]. Figs. 5 and 6 show the results of comparable experiments on mouse skeletal muscle (performed with the 0.5 mM EGTA containing solutions G1 and G2). Fig. 5 illustrates

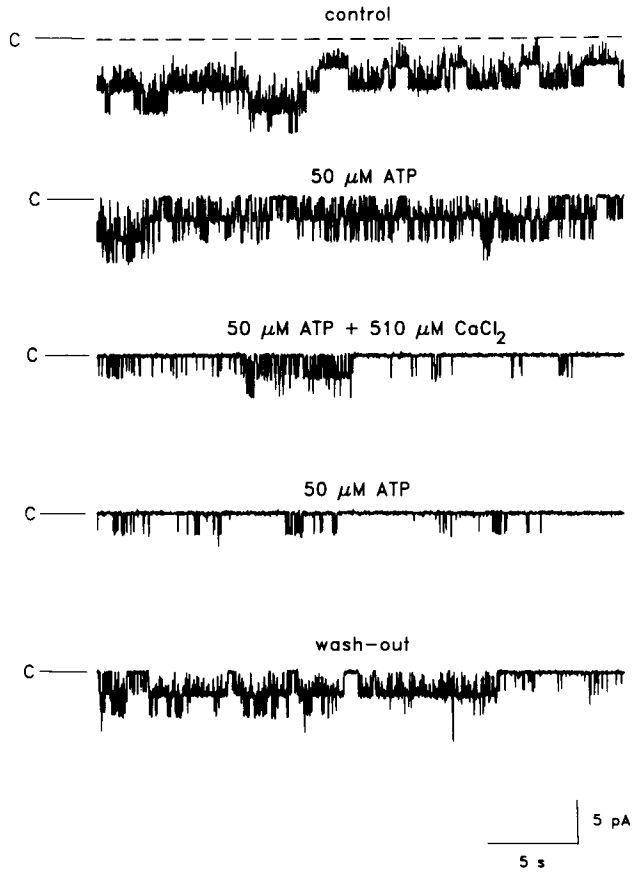

Fig. 5. Currents from K_{ATP} channels at -40 mV in internal solutions G1, G2 in the absence and presence of ATP and $CaCl_2$. The current levels during closure of all channels are marked by the letter C. The patches were excised in the Ca^{2+}-free, Na^+-rich solution B and transferred to the K^+-rich solution G1 (control). The subsequent internal solutions were solution G1 with 50 μM ATP (50 μM ATP), solution G2 with 50 μM ATP (50 μM ATP + 510 μM $CaCl_2$), solution G1 with 50 μM ATP (50 μM ATP) and solution G1 (wash-out).

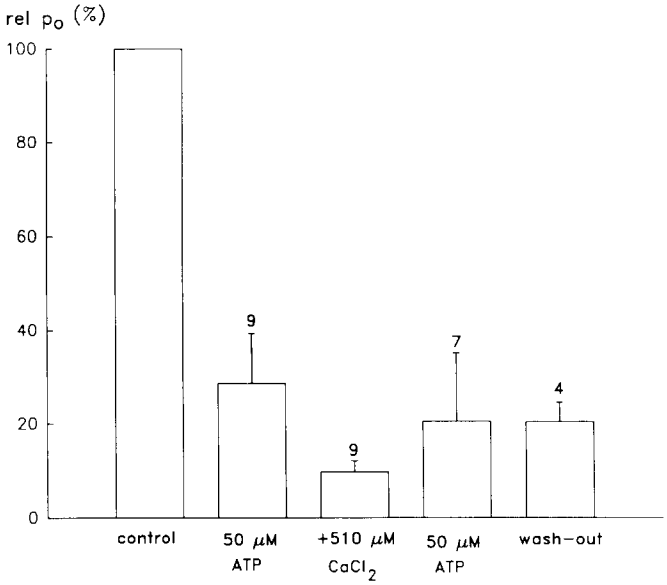

Fig. 6. Relative open-probabilities (rel p_o) of K_{ATP} channels in internal solutions G1, G2 in the absence and presence of ATP and $CaCl_2$. The probabilities are normalized with respect to measurements in solution G1 before application of ATP (control). The figure gives a summary of all experiments performed with the protocol described in the legend to Fig. 5. Bars and numbers above the columns indicate S.E. values and number of experiments.

sections of current recordings from a patch excised in the Ca^{2+}-free, Na^+-rich solution B and transferred to the Ca^{2+}-free, K^+-rich solution G1 containing 0.5 mM EGTA (control). Subsequently, the patch was exposed to 50 μM ATP, to 50 μM ATP in the presence of 510 μM $CaCl_2$ (50 μM ATP in solution G2, free Ca^{2+} concentration 12.9 μM) and again to 50 μM ATP without $CaCl_2$ and to solution G1 without ATP and $CaCl_2$ (wash-out). In contrast to the findings on frog muscle, the addition of $CaCl_2$ to an ATP-containing solution did not increase the activity of K_{ATP} channels but decreased their open probability. This result was confirmed in a total of 9 patches (Fig. 6). Additional experiments were performed with the same protocol as employed by Krippeit-Drews and Lönnendonker [3]: patches were excised in solution A (mammalian Ringer) containing 2 mM $CaCl_2$ and immediately transferred to the Ca^{2+}-free, K^+-rich solution G1. Referred to the initial control value in solution G1, the open probabilities were $12.5 \pm 3.9\%$ in the presence of 50 μM ATP, $3.6 \pm 1.4\%$ with 50 μM + 510 μM $CaCl_2$, $9.1 \pm 5.1\%$ after wash-out of $CaCl_2$ and $47.9 \pm 12.3\%$ after wash-out of $CaCl_2$ and ATP (4 experiments, not shown). Hence, internal Ca^{2+} ions do not relieve the ATP block of K_{ATP} channels in patches which were excised either in Ca^{2+}-free or in Ca^{2+}-containing, Na^+-rich solutions.

4. Discussion

4.1. Internal Ca^{2+} ions inactivate K_{ATP} channels

Compared to the rapid block of K_{ATP} channels by ATP, Ca^{2+}-dependent inactivation of the channels proceeds on a slower time scale of the order of several seconds (Fig. 1). Similar results were reported for K_{ATP} channels in rat ventricular myocytes [5]. Inactivation of K_{ATP} channels in mouse skeletal muscle occurs in Na^+-rich (Fig. 1) and in K^+-rich (Fig. 3) internal solutions and is only partially reversible on washing with Ca^{2+}-free solutions (Fig. 5). A similar decline of the open-probability of K_{ATP} channels induced by internal Ca^{2+} ions was found in patch clamp experiments on frog [3] and mouse [11] skeletal muscle fibres. In contrast, no effects of micromolar concentrations of free internal Ca^{2+} ions were reported for K_{ATP} channels in sarcolemmal vesicles of frog skeletal muscle [1] and for K_{ATP} channels isolated from the transverse tubules of rabbit skeletal muscle and incorporated into lipid bilayers [12]. The origin of these discrepancies is unclear but could be related to the different preparations and procedures used to study currents through single K_{ATP} channels.

Inactivation of K_{ATP} channels by internal Ca^{2+} ions has no effect on the single-channel current (Fig. 5). Thus, channel inactivation reduces the open-probability of all channels in the patch, i.e., the product $N \cdot p_o$ of the number N of active channels and the open-probability p_o of a single channel. The effects of Ca^{2+} ions on the parameters N and p_o could not be evaluated separately, because we cannot discriminate between a decrease of N or p_o in our multi-channel experiments.

For rat ventricular myocytes, Findlay [5] suggested that the run-down of channel activity and the irreversible loss of channel activity which was provoked by internal Ca^{2+} might reflect aspects of the same process. In contrast, the process of Ca^{2+}-dependent inactivation of K_{ATP} channels in skeletal muscle must be distinguished from the spontaneous run-down of channel activity which occurs even in Ca^{2+}-free solutions but on a slower time scale (Ref. 11, see also current recordings in Fig. 1 after wash-out of ATP). In the insulin-secreting cell line CRI-G1, the run-down of K_{ATP} channels depends on the presence of internal Mg^{2+} or Mn^{2+} ions and cannot be prevented by experimental protocols favouring channel phosphorylation [13]. Hence, Ca^{2+}-dependent inactivation and run-down of K_{ATP} channels in skeletal muscle and in insulin-secreting cells seem to proceed by different mechanisms, but details of both processes are still unknown.

4.2. Internal Ca^{2+} ions modify K_{ATP} channels

Application of internal Ca^{2+} ions alters the ATP concentration-response curve of the channels which remain active after Ca^{2+}-dependent inactivation (Fig. 2). Compared to the curve obtained in the Ca^{2+}-free solution B, the curve for the Ca^{2+}-containing solution A is steeper, and half of the channels are blocked at a lower ATP concentration. As an interpretation of these differences we suggest that internal Ca^{2+} ions not only inactivate K_{ATP} channels but also modify active channels by increasing the affinity and stoichiometry of nucleotide binding.

Direct evidence for channel modification by Ca^{2+} was obtained in experiments in which the effects of ATP in a Ca^{2+}-free, K^+-rich solution were compared before and after treatment with internal Ca^{2+} ions (Fig. 3). The results of these experiments show that modification of K_{ATP} channels by internal Ca^{2+} ions increases channel blockage by ATP; the relative open-probabilities in the presence of 50 μM ATP were 18% before and 10% after treatment with Ca^{2+} ions (Fig. 4). At the same nucleotide concentration, the rel p_o values in the Na^+-rich solution B without Ca^{2+} and in solution A with 2 mM $CaCl_2$ were 32% and 5%, respectively (Fig. 2). The differences between the two sets of experiments could be explained by the presence of different ATP complexes in the internal solutions used (Table 2), by the different $CaCl_2$ concentrations in solutions A and D2 and by the higher efficacy of ATP in Na^+-rich compared to K^+-rich internal solutions after exposure of the patch to internal Ca^{2+} ions [4]. In general, the two sets of experiments show that K_{ATP} channels modified by Ca^{2+} ions are blocked more strongly by ATP than unmodified channels in Na^+-rich as well as in K^+-rich internal solutions. This direct effect of internal Ca^{2+} ions on K_{ATP} channels in excised membrane patches of skeletal muscle must be distinguished from an indirect intrinsic action of Ca^{2+} in intact ventricular myocytes by which the ATP sensitivity of K_{ATP} channels is decreased [14].

4.3. Internal Ca^{2+} ions do not relieve the ATP block of K_{ATP} channels in mammalian muscle

In our experiments, blockage of K_{ATP} channels by ATP was enhanced during (Fig. 2) or after (Fig. 4) addition of Ca^{2+} ions to the internal solution. In contrast, cytosolic free Ca^{2+} ions were found to relieve the ATP block of K_{ATP} channels in frog skeletal muscle [3]. We have performed comparable experiments on mouse skeletal muscle fibres and have studied the effects of ATP and $CaCl_2$ in K^+-rich solutions with 0.5 mM EGTA as in the experiments of Krippeit-Drews and Lönnendonker [3]. As illustrated in Figs. 5 and 6, there is no relief of channel blockage but instead a decrease of the open-probability of K_{ATP} channels after adding $CaCl_2$ to an ATP-containing solution. Hence, internal Ca^{2+} ions cannot open K_{ATP} channels blocked by ATP in mouse skeletal muscle in contrast to

the results reported for the frog. This species difference suggests that K_{ATP} channels have different properties or regulatory mechanisms in mammalian and amphibian skeletal muscle.

Inactivation and modification of K_{ATP} channels in excised membrane patches of mouse skeletal muscle by internal Ca^{2+} ions will reduce the number of active K_{ATP} channels and increase the fraction of channels blocked by ATP. Both processes would imply that opening of K_{ATP} channels in skeletal muscle will be less likely after a rise of the myoplasmic Ca^{2+} concentration. However, as reported for ventricular myocytes [14], superimposed indirect intrinsic effects of Ca^{2+} ions in intact muscle fibres could weaken the ATP-sensitivity of K_{ATP} channels. The effects would promote the opening of K_{ATP} channels in metabolically exhausted muscle fibres [15] owing to an increased Ca^{2+} influx into the fibre.

5. Acknowledgements

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