Biochimica et Biophysica Acta, 1108 (1992) 119–122

BBAMEM 70706

Rapid Report

Dual effects of calcium on ATP-sensitive potassium channels of frog skeletal muscle

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(Received 8 April 1992)

Key words: Skeletal muscle; Potassium ion channel; ATP binding; Calcium ion dependence; (Frog)

The block of ATP-sensitive K^+ channels by ATP were studied in inside-out patches of frog toe muscles. Seal formation within Ca^{2+}-free Ringer induces many open channels which can be irreversibly blocked by subsequent addition of Ca^{2+}. However, such Ca^{2+}-blocked and ATP-blocked channels can be influenced by the addition of ATP and Ca^{2+}: the block by ATP is partially but irreversibly cancelled.

One of the most abundant K^+ channels in excitable tissues is the ATP-sensitive K^+ (K_{ATP}) channel, which is blockable by cytosolic ATP. It has been discovered in a wide variety of tissues [1–6]. K_{ATP} channels seem to have important physiological roles, e.g., their closure induces electrical activity and thereby insulin release in pancreatic β-cells [7] or their opening may increase the K^+ permeability in metabolically exhausted skeletal muscle [8,9]. However, it is not fully understood how K_{ATP} channels can open in intact cells. They are blocked by ATP concentrations in the micromolar range [1,5,10], whereas intracellular concentrations are 3–10 mM [11,12] and change little even during metabolic exhaustion [12]. To explain the opening of K_{ATP} channels additional regulatory mechanisms must be postulated, e.g., the binding of other nucleotides [13]. Recently Davies [14] has shown that a fall in cytosolic pH, which may occur during muscular activity, increases the open-state probability of K_{ATP} channels of frog skeletal muscle. We present evidence here that an increase in cytosolic free Ca^{2+}, in the physiological range, can also open K_{ATP} channels of frog skeletal muscle which are blocked by ATP. This is in so far surprising, because, in the absence of ATP, cytosolic free Ca^{2+} had the opposite effect, as has already been shown for K_{ATP} channels of cardiac muscle [15]. Part of the results has been published [16].

We studied inside-out patches [17] of frog (*Rana esculenta*) interosseal toe muscles dissected enzymatically [18]. The pipette solution contained in mM: KCl 100, $MgCl_2$ 2, EGTA 0.5, $CaCl_2$ 0.2, Hepes 5 (pH 7.4). Seals were obtained with either Ringer (Figs. 1 and 2) or Ca^{2+}-free Ringer (0.5 mM EGTA, Fig. 3) as the bath solution. The cell free patch was then immediately transferred to a separate small chamber with the control bath solution containing in mM: KCl 100, $MgCl_2$ 0.2, EGTA 0.5, Hepes 5 (pH 7.4). Test solutions were applied to the cytosolic side of the patch. Under control conditions at room temperature (20–25°C) we observed only one type of channel, the K_{ATP} channel. It had a reversal potential near 0 mV (−4.3 mV) and the I–V relation was linear from −90 to 20 mV. The calculated slope conductance was 54.8 pS, a value which is close to that reported by Spruce et al. [2]. We usually performed experiments at a holding potential of −50 or −60 mV. With one exception we normally saw 5–10 active channels in the patch and no obvious rundown was observed. Addition of 50 μM Na_2-ATP to the cytosolic bath solution led to a reversible (not shown) block of K_{ATP} channel activity. The calculated concentration [19] of free ATP was 26 μM. Since the calculated free concentration of Mg^{2+} was 173 μM of the 200 μM $MgCl_2$ it is likely that most of the rest of the ATP is Mg-ATP. However, both ATP and Mg-ATP seem to block the K_{ATP} channel of frog skeletal muscle in the same way [14]. This holds at least true for enzymatically treated muscle fibres, which are used

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here, whereas in mechanically preparated fibres Mg^{2+} seems to have an effect on the block of ATP [20]. Currents were recorded with a List EPC 5 patch-clamp amplifier and stored on videotape. For later analysis with a PDP 11 computer they were then filtered at 0.5 kHz (−3 dB) and digitized at 2.5 kHz. Amplitude histograms were obtained from 1 min segments 30 s after every solution change or after the end of the previous segment (open-channel currents are assigned positive values). In almost all patches we studied too many channels were active for a detailed kinetic analysis. As a measure of overall channel activity in the patch during these 1 min segments, the actual current amplitude in each bin (400 μS) was summed up and divided by the number of bins. This value is referred to as the mean current amplitude (i).

Fig. 1 shows results from one of 20 experiments with similar results. In the control bath solution (Fig. 1A) up to 6 K_{ATP} channels were open simultaneously and i = 12.0 pA. After addition of 50 μM ATP (Fig. 1B) i was reduced to 2.1 pA and only up to two channels opened simultaneously. Subsequent application of a solution with 550 μM $CaCl_2$ (Fig. 1D), giving a calculated free Ca^{2+} concentration of 50 μM [19], increased i again to 7.7 pA. Up to 5 channels were now simultaneously active in the patch. The calculated concentration of free ATP changed only negligibly after the addition of Ca^{2+}. Washout of Ca^{2+} for several minutes with the ATP containing bath solution did not reduce the number of active K_{ATP} channels in the patch (Fig. 1F) and i showed no significant change (8.2 pA). The effect of Ca^{2+} on the block by ATP was therefore irreversible under these conditions. It is also obvious from Fig. 1 that there was no time dependent run down in channel activity in the presence of ATP or during the washout period of Ca^{2+}. Also the single channel current amplitude remained unchanged. The kinetic analysis of one experiment with a single active K_{ATP} channel in the patch showed that ATP blocked the channel by prolonging the interburst intervals and reducing the open-state probability (P_{open}), which is in agreement with the results of Spruce et al. [2] and Woll

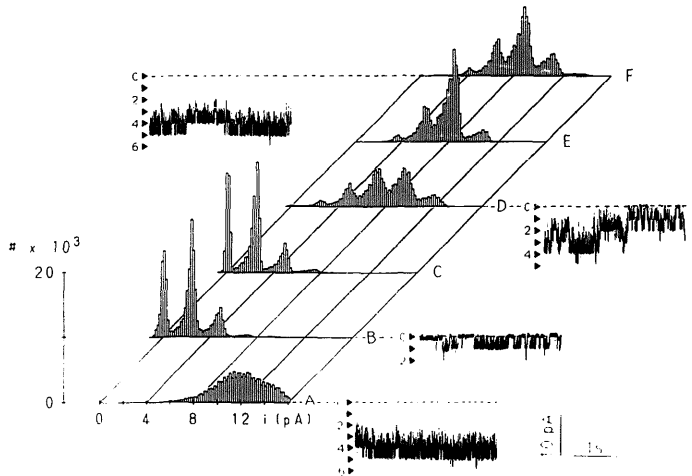

Fig. 1. Effect of ATP and ATP+Ca^{2+} on the activity of K_{ATP} channels from an inside-out patch at a holding potential of −50 mV under symmetrical K^+ concentrations (100 mM). The control bath solution contained Mg^{2+} (0.2 mM) and was Ca^{2+} free (0.5 mM EGTA). The figure shows amplitude histograms (# = number of data points) and corresponding current traces for A: control activity, B and C: after addition of 50 μM ATP to the bath (concentration of free ATP 26 μM, see text Fig. 2), D: after subsequent addition of 550 μM Ca^{2+} (free concentration 50 μM), E and F: after washout of Ca^{2+}. The single-channel current amplitude in the control solution was 2.8 pA and did not change during the experiment. The dashed lines in the current traces indicate the 0 or closed (C) level, the numbers correspond to current levels were 2, 4, or 6 channels were open. The 'z'-axis in the figure indicates the order of solution changes and resembles thereby the 'time'-axis of the experimental protocol.

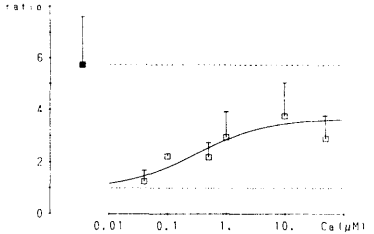

Fig. 2. Concentration-response curve of the effect of Ca^{2+} on the activity of K_{ATP} channels. The i value for channel activity in the presence of 50 μM ATP (free concentration 26 μM) was set as 1 and marked by the lower dashed line. The response is given as the ratio of this value and the corresponding test values. The filled square (and the upper dashed line) is the control value and the open squares show the increase in channel activity with increasing free Ca^{2+} concentrations. All free ion concentrations were calculated according to Trube [19] with stability constants taken from Martell and Smith [27]. Mean values of 4–7 experiments with S.E. for free Ca^{2+} concentrations of (in μM): 0.04, 0.1, 0.5, 1.0, 10, and 50 for a total Ca^{2+} concentration of (in μM): 160, 280, 430, 470, 510, and 550, respectively. The solid curve is a fit of a logistic function to the data: the inflection point amounts to 300 μM Ca^{2+} and the ratio reaches 3.7 for large quantities of Ca^{2+}.

et al. [21]. Increasing the free Ca^{2+} concentration to 0.1 μM caused a further shortening of the interburst intervals which led to an increase in channel activity, although the mean open time was irreversibly reduced from 8.5 to 6.5 ms. The concentration response curve of free Ca^{2+} on the activity of K_{ATP} channels in the presence of 50 μM ATP (Fig. 2) shows that a concentration of 100 nM is already sufficient to increase the activity of K_{ATP} channels. The calculated half-maximal effective concentration of free Ca^{2+} was 300 nM. Intracellular free Ca^{2+} concentrations in frog skeletal muscle at rest seem to be lower than 10 nM [22,23] and increase to 5–8 μM during an action potential [23,24]. This led us to the idea that the rise in intracellular free Ca^{2+} during force development, especially in metabolically exhausted fibers, may indeed open K_{ATP} channels and thereby increase K^+ conductance and stabilize the resting membrane potential. However, it seems that this regulation is not a simple process, since the effect of free Ca^{2+} was irreversible in our experiments. Findlay [15] has shown that Ca^{2+} also has a direct effect on K_{ATP} channels of rat ventricular myocytes. Channel activity was irreversibly blocked by 5 μM free Ca^{2+}. This is in contrast to the findings of Spruce et al. [10], who found no effect of cytosolic free Ca^{2+} on P_{open} of K_{ATP} channels of frog skeletal muscle. However, we found that in the absence of ATP, free Ca^{2+} on the cytosolic side also inhibits K_{ATP} channel activity in frog skeletal muscle. Fig. 3 shows one representative experiment out of five with similar results. Under control conditions i was 17.6 pA (Fig. 3A) and up to 6 K_{ATP} channels were simultaneously active in the patch. Addition of 0.5 μM free Ca^{2+} reduced i to 2.9 pA and only up to three channels open simultaneously (Fig. 3B). This did not significantly change after 1 min of washout of Ca^{2+} (Fig. 3C): i = 3.2 pA. Since this effect of Ca^{2+} was also irreversible, it is likely that cytosolic

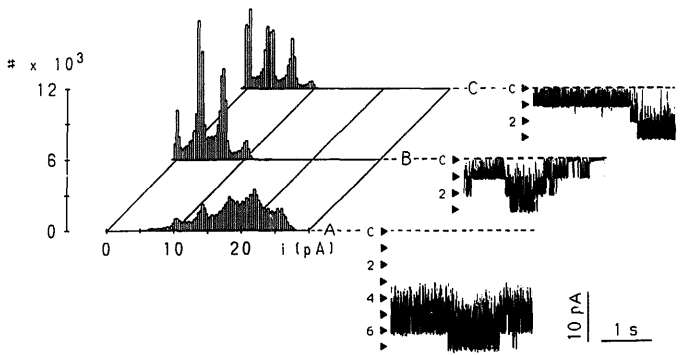

Fig. 3. Effect of free Ca^{2+} (0.5 μM) on the activity of K_{ATP} channels from an inside-out patch at −60 mV. A: Control activity, B: after addition of 0.5 μM free Ca^{2+}, C: after washout of Ca^{2+}. Seal formation was done in Ca^{2+}-free Ringer. All other conditions as in Fig. 1. Single-channel current amplitude under control conditions was 3.5 pA and changed only negligibly during the experiment.

free Ca^{2+} changes the state of K_{ATP} channels, which cannot be reversed in cell free patches.

Findlay [15] showed that the addition of Mg-ATP could induce recovery of channel activity and proposed that an intracellular phosphorylation process might counteract the effect of free Ca^{2+}. However, in our experiments with ATP, Mg-ATP was not able to reverse the effect of Ca^{2+}. Davies et al. [25] discussed that K_{ATP} channels in skeletal muscle may not only play an important role during metabolic exhaustion of the muscle, but also under physiological conditions. They suggest that these channels open to reduce excitability of individual fibres within a motor unit or open to increase the local extracellular K^+ concentration which then in turn will have other functions, e.g., cause vasodilation within the muscle [25]. Davies et al. [25] attribute the openings of skeletal muscle K_{ATP} channels mainly to the fall in intracelluiar pH due to metabolism during exercise. However, under all these conditions, i.e., metabolic exhaustion [8] as well as after every action potential [23], there is a rise in intracellular free Ca^{2+}. Furthermore, it has been shown that internal free Ca^{2+} promotes the activation of a K^+ conductance in exhausted muscle fibres [8] and that this increase in K^+ permeability is predominantly due to an activation of K_{ATP} channels [9]. As shown with our experiments, cytosolic free Ca^{2+} can open K_{ATP} channels blocked by ATP, although this effect was irreversible in the cell free patch. Provided that intracellular factors which reverse the effect of Ca^{2+} exist, Ca^{2+} can be an important and powerful factor in regulating K_{ATP} channel activity.

Dr. T. Plant has given helpful comments on this paper. This research was supported by the Deutsche Forschungsgemeinschaft (Ne 287/3-2).

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