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Modulation by Mg^{2+} and ADP of ATP-Sensitive Potassium Channels in Frog Skeletal Muscle

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Summary. The patch-clamp technique was used to examine the action of intracellular magnesium ions and ADP in the absence of ATP on skeletal muscle ATP-sensitive potassium channels (K-ATP channels). Inside-out patches were excised from the membrane of sarcolemmal blebs which arise spontaneously without enzymatic treatment after a frog muscle fiber is split in half.

In the absence of nucleotides, K-ATP channel open probability was not significantly affected by intracellular magnesium even at a concentration (20 mM) which fully blocks cardiac and pancreatic K-ATP channels. On the other hand, Mg^{2+} ions (10–20 mM) decreased both inward and outward unitary currents. The percent reduction in inward currents (about 8%) was independent of voltage while the reduction in outward currents was larger at higher voltages, suggesting that the former effect resulted from cancellation of surface charges and the latter from rapid channel block.

With or without Mg^{2+}, intracellular ADP could either stimulate or inhibit K-ATP channel activity. Low concentrations (1–100 μM) of ADP rapidly and reversibly increased average activity by a factor of 2 to 3. This activation was seen in half of the patches tested and was greater in the presence of mM Mg^{2+}. High concentrations (>100 μM) of ADP inhibited activity with a half-block concentration of 450 μM in 0 Mg^{2+}, i.e., more than an order of magnitude the value for ATP. ADP inhibition, like ATP inhibition, was partially relieved by mM Mg^{2+}, suggesting that the Mg^{2+}-bound ADP forms are less effective than free ADP forms.

During exercise, free ADP levels rise and ATP declines while remaining high. Since ADP inhibition appears to compete with ATP inhibition, the two distinct processes of ADP activation and ADP inhibition could therefore promote opening of K-ATP channels during intense muscle work.

Key Words
K-ATP channels · ATP · *Rana esculenta* · single channel recording · patch clamp

Introduction

Ionic channels which conduct preferentially potassium ions and are inhibited by intracellular ATP are present in many different tissues (Ashcroft & Ashcroft, 1990). The role of these ATP-sensitive K^+ channels (K-ATP) is best understood in pancreatic β-cells. In these cells, K-ATP channels appear to control the resting membrane potential, therefore providing a means of coupling metabolism to cell excitability (Ashcroft & Ashcroft, 1990). This function makes them an essential element in the cascade linking plasma glucose levels to secretion of insulin.

Considerable amounts of data on K-ATP channels have also been gathered in cardiac muscle and, to a lesser extent, in smooth and skeletal muscles. Nevertheless, the role of K-ATP channels in muscle function is not well established, one major obstacle to a full understanding being that it is rather difficult—for obvious mechanical reasons—to use the patch-clamp technique on a working muscle cell.

In skeletal muscle fibers, K-ATP channels which can be present at a remarkably high density (Spruce, Standen & Stanfield, 1985; Vivaudou, Arnoult & Villaz, 1991) appear to be all closed at rest (Spuler, Lehmann-Horn & Grafe, 1989). One could imagine that they could open during exercise when metabolic energy is used up and ATP levels fall. The opening of such a large number of channels would explain the large efflux of K^+ observed in exercising muscle (Sjøgaard, 1990) and would induce fatigue-like symptoms by reducing fiber excitability. Unfortunately, this scenario remains hypothetical as it is not yet clear what physiological conditions will cause K-ATP channels to open and what physiological effects this opening will have.

In particular, it is not known precisely to what extent ATP content declines during sustained exercise: depending on experimental protocol and muscle type, values from 20% (Westerblad et al., 1991) to 60% (Green et al., 1992) have been reported. A fall in bulk ATP might not be the only trigger: other signals associated with muscle activity, such as acid-

ification (Davies, 1990), local variations in free ATP, increase in ADP or increase in free Ca^{2+} could be important.

Indeed, we have shown previously (Vivaudou et al., 1991) that frog skeletal muscle K-ATP channels are much more sensitive to free ATP than to ATP complexed with Mg^{2+} and that ADP could reduce significantly this ATP sensitivity by a mechanism akin to a competition for the same nucleotide binding site.

In this work, we have focused on the individual and combined actions of Mg^{2+} ions and ADP in the absence of ATP. Besides enhancing inward rectification, magnesium ions were found to reduce the linear conductance to inward currents. Channel open probability was not significantly altered by concentrations of Mg^{2+} as high as 20 mM. ADP could both inhibit and activate K-ATP channels. Elevated concentrations of ADP blocked activity in a dose-dependent fashion. As with ATP, blocking efficacy was reduced by mM Mg^{2+} suggesting that the complex MgADP is a weaker inhibitor than free ADP. Concentrations of ADP less than 100 μM augmented channel activity in half of the patches tested. Added magnesium was not necessary for ADP activation, but it could be a cofactor as it enhanced it.

A preliminary report of this work has been published in abstract form (Forestier & Vivaudou, 1992).

Materials and Methods

Preparation

Using the patch-clamp technique, (Hamill et al., 1981) single-channel currents were recorded in inside-out patches excised from the membrane of split-fiber blebs.

Formation of these large sarcolemmal blebs was induced by splitting individual fibers of the iliofibularis thigh muscle of the adult frog, *Rana esculenta,* in a relaxing solution as described previously in detail (Vivaudou et al., 1991). This mechanical procedure avoids the enzymatic digestion used in other adult skeletal muscle preparations (Standen et al., 1984; Woll, Lönnendonker & Neumcke, 1989).

Gigaseals could also be obtained, although with more difficulty, by applying the patch pipette directly to the external surface of fibers which did not produce blebs after being split in half. Blebs and nonblebs patches had identical electrophysiological properties, thus strengthening the hypothesis that blebs are formed from sarcolemmal membranes rather than from internal membranes extruded from the bulk of the fiber.

Experimental Setup

Patch pipettes (3–20 MΩ) were pulled from borosilicate capillaries (Kimax-51 34502) on a horizontal puller (BB-CH Mechanex).

The intracellular face of the patches was perfused by placing the tip of the pipette in the outlet flow of one of 30 polyethylene tubes (300 μm internal diameter) from which the different solutions continuously flowed by gravity (5–15 cm elevation). Solution switching could be done manually in less than 1 sec by moving the battery of tubes with a custom-built remote hydraulic manipulator and was monitored by a position transducer attached to the manipulator.

Ionic currents were measured with a Bio-Logic RK300 amplifier equipped with a 10-GΩ feedback headstage, and filtered at a 0.3–3 kHz cutoff frequency. Currents, together with either voltage or the solution identification signal issued by the position transducer, were stored on digital audio tapes (Bio-Logic DTR-1200 DAT recorder).

Solutions

The patch pipette contained 150 mM K^+, 145 mM Cl^-, 2 mM Mg^{2+}, and 5 mM PIPES. The cytoplasmic side of the patch was bathed with solutions which contained various concentrations of nucleotides and Mg^{2+} with 150 mM K^+, 40 mM Cl^-, 1 mM EGTA, 5 mM PIPES, and the concentration of methanesulfonate$^-$ (about 100 mM) imposed by bulk charge neutrality. Concentrations of free (i.e., not bound to Mg^{2+}) and bound nucleotides were estimated using the software program ALEX (Vivaudou et al., 1991). Values given in the text represent total concentrations, unless expressly specified. In bath solutions containing no added magnesium, contaminant Mg^{2+} was measured by ICP (inductively-coupled plasma) emission spectrometry to be 7 μM plus 0.7 μM per mM ATP plus 1.4 μM per mM ADP.

All solutions were adjusted to pH 7.1 with KOH. Experiments were performed at room temperature (20–24°C). ATP and ADP (potassium salts) were purchased from Sigma. To prevent degradation, stock solutions of 120 mM ATP or ADP and 60 mM PIPES were titrated to pH 6.8 with KOH and stored at −30°C.

Data Analysis

Stored signals were sampled (1–10 kHz) and processed with an IBM-compatible 80486-class microcomputer equipped with a Labmaster acquisition board. For presentation of long stretches of data, additional Gaussian filtering and undersampling was done numerically. Final cutoff and sampling frequencies are indicated in the figure captions as f_c and f_s, respectively. Slow fluctuations of the no-channel-open baseline of the current signal were removed by interactive fitting of the baseline with a spline curve and subtraction of this fit from the signal.

Stimulation, acquisition, analysis, and presentation were performed with custom software and with the help of Bio-Logic BioPatch 3.10 for fitting of amplitude histograms to a sum of Gaussians (Simplex method), and MicroCal Origin 1.28 for fitting of dose-response curves to a sigmoid (Levenberg-Marquardt method).

Unitary currents were determined either directly from the difference between current levels in the raw data or from analysis of amplitude histograms. The number of active channels N in a patch was often large (more than 350 in one case) and was difficult to estimate with accuracy. Channel open probability P_o was therefore not computed. Instead, quantities proportional to P_o, mean current I or NP_o, were used to evaluate channel activity. Mean patch current which is given by $I = NP_oi$, where i = unitary current, was computed by averaging data points over a portion

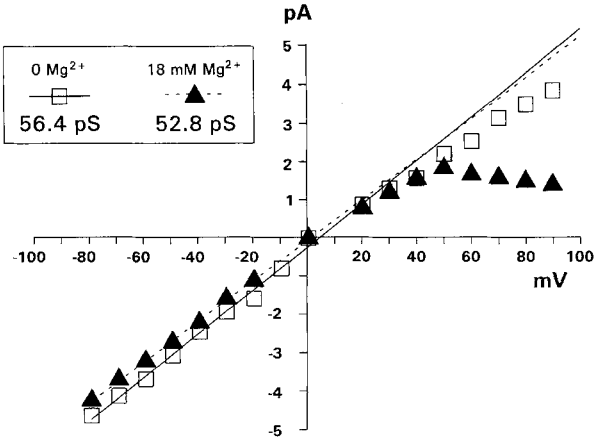

Fig. 1. Mg^{2+} accentuates inward rectification and decreases inward current conductance. *I-V* curves obtained in the same patch with 18 mM intracellular Mg^{2+} and 1 mM ATP (triangles) and with 0 Mg^{2+} and 0.5 mM ATP (squares). Straight lines are linear least-square fits to data points below 0 mV which yield conductances of 56.4 pS (r^2 = 99.1%) and 52.8 pS (r^2 = 99.3%) for 18 mM and 0 Mg^{2+}, respectively. Patch 25M5.

of the signal delimited by interactive cursors. NP_o was computed from Gaussian fits of amplitude histograms using the expression:

$$NP_o = \sum_{k=0}^{N} kP_k$$

where P_k = probability that k channels are simultaneously open.

Results are presented as mean ± SD (n = sample size).

Results

Mg^{2+} Reduces Inward and Outward Unitary Currents

In symmetrical elevated K^+ with variable amounts of Mg^{2+} in the bath, K-ATP channels display a linear conductance below 0 mV of 58.2 ± 6.0 pS (n = 53). This conductance was found to be slightly dependent on Mg^{2+} since it decreased with increasing Mg^{2+} (Fig. 1). In three experiments where *I-V* curves were obtained at different Mg^{2+} concentrations in the same patch, the inward current conductance in 10 to 20 mM Mg^{2+} was 8.2 ± 2.5% less than in 0 Mg^{2+}. At a constant negative potential, this action of Mg^{2+} translated into a dose-dependent reduction in the amplitude of the inward elementary currents (Fig. 2).

This effect of Mg^{2+} on inward currents was rather different than the attenuation by Mg^{2+} of outward currents which was so pronounced at high positive potentials that the slope of the *I–V* curve became negative (Fig. 1).

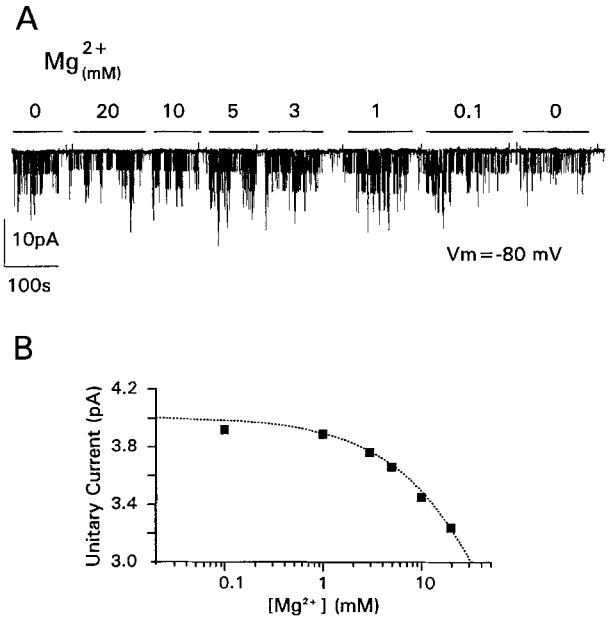

Fig. 2. Dose-dependent reduction by Mg^{2+} of inward elementary currents without effects on open probability. (*A*) Currents recorded at various concentrations of internal magnesium in the absence of ATP. Between applications of test solutions, the patch was perfused with a control solution containing 3 mM ATP and 5 mM Mg^{2+}. f_s = 250 Hz, f_c = 100 Hz. (*B*) Corresponding plot of the elementary current amplitude *vs.* total magnesium concentration. Patch 19M2.

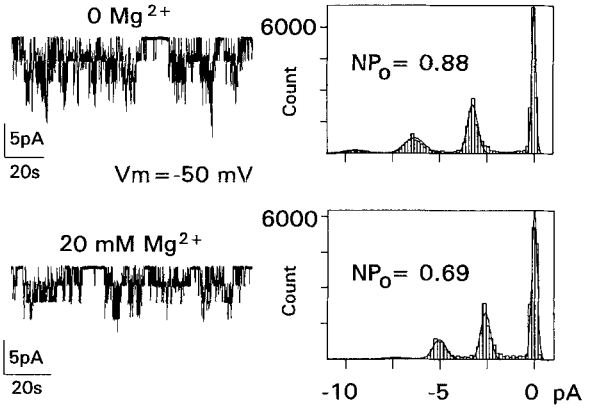

Fig. 3. Channel activity in the absence of Mg^{2+} and in the presence of excess Mg^{2+} (f_s = 200 Hz, f_c = 50 Hz) and corresponding amplitude histograms (80 bins). No ATP. Patch 20M3.

Mg^{2+} Alone Does Not Affect Channel Activity

Mg^{2+} ions are known to produce a dose-dependent reduction in the open probability of K-ATP channels from pancreatic β-cells (Findlay, 1987*a*; Ashcroft & Kakei, 1989) and from cardiac myocytes (Findlay, 1987*b*). As illustrated in Figs. 2 and 3, in skeletal

muscle blebs, internal magnesium had little or no effect upon channel open probability even at a concentration as high as 20 mM, which completely inhibits its K-ATP channels in other tissues. In 10 patches (symmetrical K^+, negative potential), 20 mM Mg^{2+} caused either no change, or a slight increase, or, more often, a slight decrease in the average patch current: after subtraction of the effect of Mg^{2+} on elementary conductance, channel activity in Mg^{2+} was 91 ± 20% of control, i.e., no statistically significant difference could be found overall.

It should be noted that, in a few patches, during the time of an experiment some applications of 5–20 mM Mg^{2+} caused an unmistakable (up to 50%) reduction in the average current while most applications had no effects. One could speculate that there exists conditions (state of the channel or associated enzymes, patch environment, . . . etc.) which we do not control and which favor inhibition by Mg^{2+}.

Inhibition by ADP is Reduced by Mg^{2+}

In frog skeletal muscle, we have found that ADP is a full, reversible inhibitor of K-ATP channels (n = 8) (Fig. 4). The relationship between average current I_{ADP} and nucleotide concentration |ADP| could be modeled by the equation

$$I_{ADP} = I_{Max}/[1 + (|ADP|/K_{1/2})^h]$$

where I_{Max} was the maximum current, $K_{1/2}$ the concentration producing half-maximal inhibition and h the Hill coefficient. An equivalent expression was used for ATP inhibition.

In the absence of Mg^{2+}, $K_{1/2}$ for ADP was 552 ± 248 μM (n = 4). This value should be compared to the $K_{1/2}$ for ATP which was 40.6 ± 35.2 μM in the same conditions (n = 11; no Mg^{2+}). Like ATP sensitivity (Vivaudou et al., 1991), ADP sensitivity differed greatly from one patch to another. It could also vary with time in the same patch, either increasing or decreasing without any evident correlation with the degree of rundown.

Raising magnesium reduced ADP inhibition, increasing the half-maximal inhibitory dose by up to one order of magnitude (Fig. 4). In 5 mM Mg^{2+}, $K_{1/2}$ for total ADP was 3.1 ± 2.5 mM (n = 6), more than five times the value in 0 Mg^{2+}. This suggests that MgADP is less potent than ADP not complexed with Mg^{2+}. In fact, $K_{1/2}$ for free computed ADP was 1.3 ± 1.4 mM (n = 6) in 5 mM Mg^{2+}, closer to the value of 0.55 mM in 0 Mg^{2+}.

In 5 mM Mg^{2+}, Hill coefficients h for inhibition by free ADP and free ATP were 0.94 ± 0.22 (n = 6) and 1.24 ± 0.35 (n = 25), respectively.

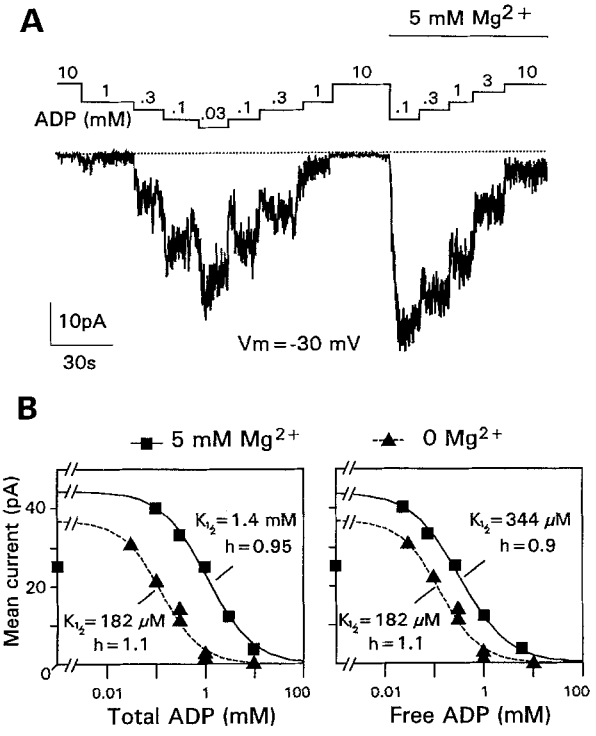

Fig. 4. Mg^{2+} attenuates ADP inhibition (*A*) Currents recorded in a patch containing more than 80 channels at various concentrations of total added ADP without Mg^{2+} and with 5 mM Mg^{2+}. f_s = 50 Hz, f_c = 10 Hz. (*B*) Corresponding dose-response plots of the mean current at each concentration of ADP *vs.* total ADP concentration (left panel) and computed free ADP (right panel), in the absence of Mg^{2+} (triangles) and in 5 mM Mg^{2+} (squares). Value of mean current in 0 ADP is an average from two applications preceding and following the record in panel *A*. Patch 4M2.

As presented below, ADP can also augment K-ATP current and this activation is promoted by Mg^{2+}. In particular, the lesser amplitude of the 0 ADP current in Fig. 4*B* suggests that ADP activation as well as inhibition was taking place. A critical reader could therefore argue that currents are greater in Mg^{2+} not because of relief of inhibition but rather because of increase in activation. This is unlikely because (i) there seems to be little overlap between partially activating and inhibiting ADP concentrations, (ii) the ADP doses used for inhibition were high enough to produce maximum activation, and (iii) Mg^{2+} induced a shift in the ADP dose-response in patches where no ADP activation was observed.

Low Concentrations of ADP Can Augment K-ATP Channel Activity

In our preparation, without ATP, ADP at concentrations ranging from 1 to 100 μM increased activity in more than half of the patches tested (17 out of 29).

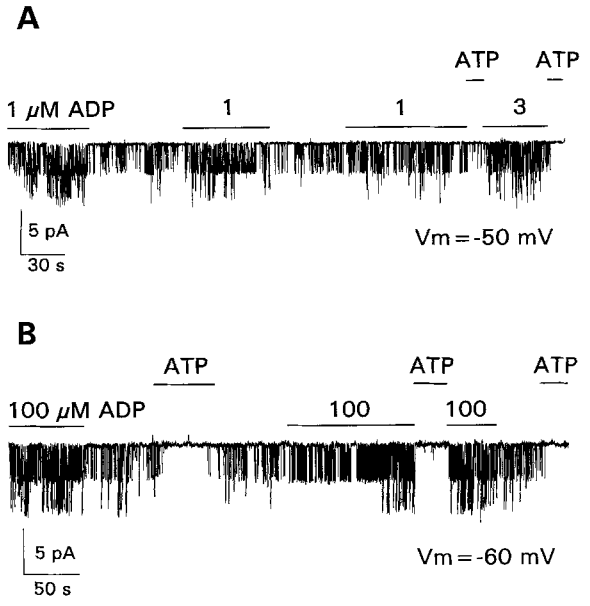

Fig. 5. K-ATP channel activity is potentiated by low concentrations of ADP in presence of Mg^{2+} (5 mM) and absence of ATP. (*A*) Activation by 1 or 3 μM ADP. Patch 14M4.(*B*) Activation by 100 μM ADP. Patch 5M1. Solution marked ATP contained 3 mM ATP and 5 mM Mg^{2+}. Control nucleotide-free solution had 5 mM Mg^{2+}. f_s = 50 Hz, f_c = 30 Hz.

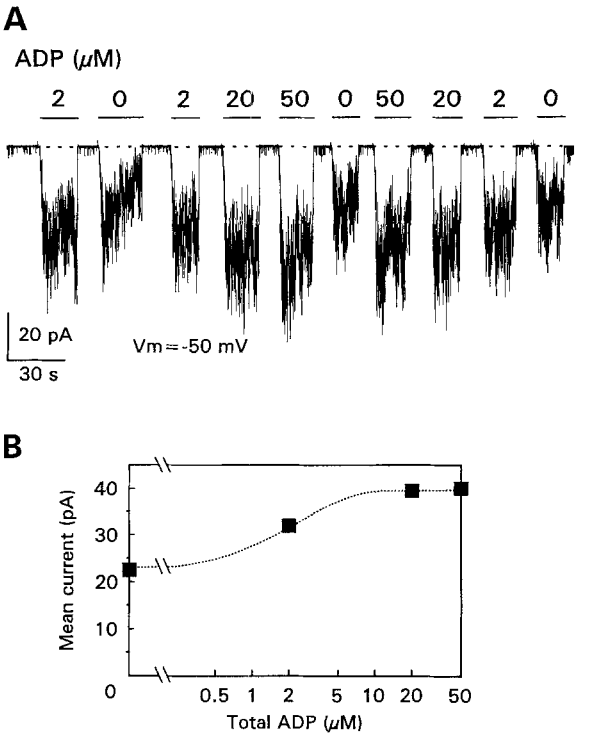

Fig. 6. Dose-dependent activation by ADP. (*A*) Protocol showing the dependency of channel activation upon ADP (5 mM Mg^{2+}, 0 ATP). ADP applications were separated by application of a blocking solution (3 mM ATP, 5 mM Mg^{2+}). f_s = 400 Hz, f_c = 200 Hz. (*B*) Corresponding plot of mean current *vs.* ADP concentration. Patch 25M5.

Figures 5 and 6 demonstrate that activation by ADP was dose-dependent, reversible, and repeatable. Half-maximal dose for activation was approximately 2 μM. Largest activation was achieved at approximately 50 μM ADP. In seven experiments in the presence of 5 mM Mg^{2+}, channel activity was increased by 1 to 100 μM ADP to 275 ± 96% of control. After complete loss of activity due to rundown, ADP was unable to induce any channel reopening.

To determine if ADP activation required Mg^{2+}, we have tested the role of Mg^{2+} in the activation by ADP: with no Mg^{2+} added (i.e., less than 10 μM of contaminant; *see* Materials and Methods), activation was still possible, but always less pronounced than with 5 mM Mg^{2+} (Fig. 7). ADP activation without Mg^{2+} was registered in 6 out of 15 patches. In five experiments, ADP with Mg^{2+} augmented K-ATP current but failed to do so without Mg^{2+}.

Discussion

In skeletal muscle, activity of K-ATP channels which have been partially inhibited by ATP is augmented by either Mg^{2+} or ADP (Vivaudou et al., 1991). Mg^{2+} which has a high affinity for ATP relieves channel inhibition probably because the Mg^{2+}-bound form of ATP is less potent an inhibitor than other soluble forms of ATP. The action of ADP is more direct: our previous observations (Vivaudou et al., 1991) suggested that ADP, a weak inhibitor, acts by competing with ATP for the same binding site.

In pancreatic (Dunne & Petersen, 1986; Kakei et al., 1986; Misler et al., 1986) and cardiac (Findlay, 1988; Lederer & Nichols, 1989) tissues, ADP also decreases the sensitivity to ATP while free ATP is a stronger inhibitor than Mg-ATP only in pancreatic β-cells (Dunne et al., 1987, 1988; Ashcroft & Kakei, 1989).

In order to further understand the metabolic regulation of K-ATP channels and their tissue-specific characteristics, we sought in this work to find out if Mg^{2+} ions and ADP have effects on skeletal muscle K-ATP channels other than the above-mentioned interactions with ATP inhibition.

Intracellular Mg^{2+} Reduces Unitary Currents by Two Mechanisms

We have found that Mg^{2+} not only reduces outward unitary currents, as it does in other tissues (Ashcroft

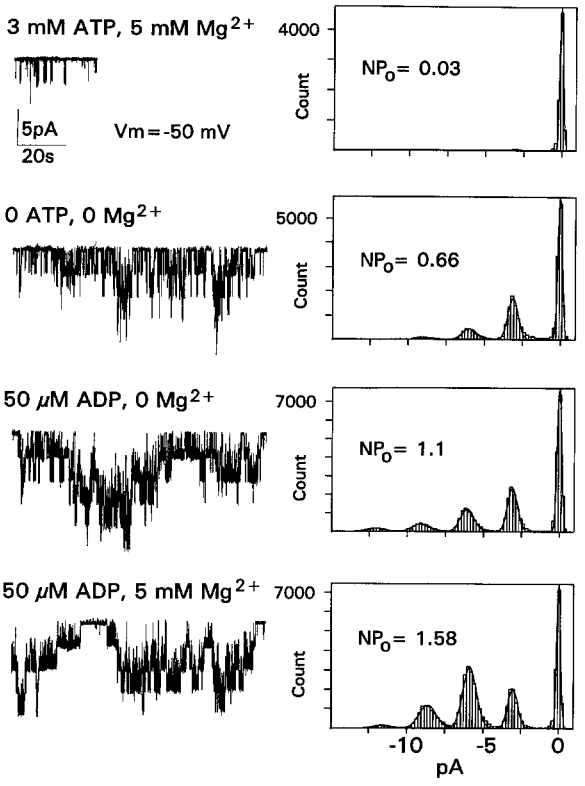

Fig. 7. Effect of internal magnesium on ADP activation. Current traces at left are the successive portions of a continuous record. Amplitude histograms (80 bins) at right were built from these traces and processed to evaluate NP_o (*see* Materials and Methods). Note that the bottom histogram appears to reflect a nonbinomial distribution: Our present data are insufficient to tell if such a response is typical but, if confirmed, this observation could be explained by heterogeneity of the response of the channels to MgADP. f_s = 200 Hz, f_c = 50 Hz. Patch 21M4.

& Ashcroft, 1991), but it also reduces inward current conductance. The former effect could be interpreted as a rapid block of the channel by Mg^{2+} ions entering the pore under the action of outward electrostatic forces (Horie, Irisawa & Noma, 1987) and indeed we have observed a definite increase in open channel noise consistent with this interpretation. The latter effect—attenuation of inward currents which has been also noted by Findlay (1987*b*)—appears quite distinct by its linear voltage dependence and its smaller magnitude. A possible mechanism could be that Mg^{2+} ions partly cancel negative surface charges near the cytoplasmic mouth of the channel by screening or directly binding to those charges, thus reducing the voltage gradient arising from the local surface potential created by those negative charges (MacKinnon, Latorre & Miller, 1989).

Channel Open Probability is not Significantly Altered by Mg^{2+}

Our results show that, in the absence of ATP and ADP, Mg^{2+} ions influence little, or at all, the average activity of K-ATP channels in skeletal muscle. Even at concentrations well above physiological values, Mg^{2+} had no consistent effects on open probability. There is a clear difference on this point between skeletal muscle fibers and other cells such as pancreatic β-cells (Findlay, 1987*a*; Ashcroft & Kakei, 1989) and cardiac cells (Findlay, 1987*b*) where significant reductions in open probability are achieved with as little as 100 μM of Mg^{2+}.

Mg^{2+} Weakens Inhibition by High Doses of ADP

Like ATP, ADP is capable of inhibiting K-ATP channels (Ashcroft & Ashcroft, 1990). Findlay (1988) reported that, in rat ventricular myocytes, the inhibition by ADP was reduced in the presence of internal magnesium while Lederer and Nichols (1989) concluded that, in the same tissue, the complex MgADP did not cause any inhibition of channel activity.

In the absence of ATP, frog skeletal muscle K-ATP channels were blocked dose-dependently by concentrations of ADP higher than about 100 μM in 5 mM Mg^{2+}. Without Mg^{2+}, the concentration for half-maximal inhibition was 450 μM (more than 10 times the value for ATP) and full inhibition could be reached at 10 mM. ADP inhibition was clearly relieved by mM Mg^{2+}, which could mean that MgADP is not as good an inhibitor as free ADP. Similar arguments led to similar conclusions for MgATP and free ATP (Vivaudou et al., 1991). Moreover, for both ADP and ATP, Hill coefficients close to 1 were obtained. These similarities could reflect the existence of a single nucleotide inhibitory site on the channel protein with higher affinity for the more negatively charged free forms of the nucleotides (Ashcroft & Kakei, 1989). Such a common site would also explain the lowering of ATP inhibition by ADP.

Activation by Low ADP with and without Mg^{2+}

The activating effect of low concentrations of ADP in the absence of ATP has been described in cardiac ventricular cells (Findlay, 1988; Tung & Kurachi, 1991), insulin-secreting cells (Dunne & Petersen, 1986) and pancreatic β-cells (Bokvist et al., 1991; Hopkins et al., 1992). In all of these studies, ADP

activation required Mg^{2+}. Furthermore, activation appeared to be linked to the degree of rundown, either increasing as channels inactivated because of rundown (Dunne & Petersen, 1986; Findlay, 1988) or decreasing somewhat with time (Bokvist et al., 1991).

Our results demonstrate that, without ATP, ADP at concentrations too low to cause any significant inhibition could also enhance significantly K-ATP channel activity. In skeletal muscle, activation by ADP could be observed even in the virtual absence of Mg^{2+} (i.e., <10 μM) although it was clearly potentiated by mM Mg^{2+}. Since ADP concentrations were probably too low to inhibit the channels, the potentiation by Mg^{2+} could not be attributed merely to buffering of the inhibitor free ADP as described in the previous section. Instead, one could hypothesize that MgADP is a better activator than free ADP.

This effect was rapid in its onset as well as its termination and its locus is therefore likely to be near or on the channel protein. But the effect was rather inconsistent in its magnitude and probability of occurrence in a given patch (about 50% of the patches did not respond) as reported by others (Dunne & Petersen, 1986; Findlay, 1988). Moreover, no obvious correlation between rundown and ADP stimulation could be detected. Since there was no clear difference in terms of electrophysiological properties between a patch where ADP caused activation and one where it did not, it could be that ADP stimulation depends on membrane-associated processes which may be altered in an excised patch. Thus, activation is likely not to arise from simple binding of ADP to the channel as appears to be the case for ATP or ADP inhibition.

Functional Implications

Our data show that the only significant effect of Mg^{2+} ions in the absence of nucleotides will be to enhance inward rectification. The observed reduction in inward current conductance is probably too small to have a physiological impact and Mg^{2+} did not cause any statistically meaningful decrease in open probability.

In the absence of ATP, ADP elicited either activation or inhibition. Mg^{2+}-bound ADP was less effective at blocking the channel than free ADP while it was the opposite for activation. There appeared to be little overlap between the two processes since, in the presence of 5 mM Mg^{2+}, activation was maximal at about 50 μM ADP (free ADP $\approx$ 15 μM) and noticeable inhibition required more than 200 μM ADP (free ADP $\approx$ 70 μM). During fatiguing exercise, free ADP levels in a working muscle have been estimated to increase from $\approx$30 to $\approx$200 μM (Westerblad et al., 1991). In that range of ADP and in the presence of blocking concentrations of ATP, both ADP activation and inhibition might take place and, since ADP inhibition might relieve ATP inhibition, both could add up to trigger K-ATP channel opening. This mechanism would operate in the right direction if K-ATP channels are indeed involved with fatigue, and in particular with the accompanying K^+efflux (Sjøgaard, 1990). Determining the relative importance of such a mechanism compared to others such as activation by acidosis (Davies, 1990) will probably necessitate a more complete mechanistic view of the K-ATP channel and a more precise knowledge of the temporal and spatial exercise-induced variations in metabolites.

This paper is dedicated to the late Christian Roche, our technician, who greatly helped in designing and building the apparatus we have used. We thank Dr.Michel Villaz for helpful comments on the manuscript and Dr. Daniel Cook for providing a preprint of his latest article. This work was supported by CEA (Commissariat à l'Energie Atomique), AFM (Association Francaise contre les Myopathies), and CNRS (Centre National de la Recherche Scientifique).

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