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Vanadate as an activator of ATP – sensitive potassium channels in mouse skeletal muscle

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Abstract. The inside-out mode of the patch-clamp technique was used to study adenosine-5′-triphosphate (ATP)-sensitive K^+ channels in mammalian skeletal muscle. Vanadate, applied to the cytoplasmic face of excised patches, was a potent activator of ATP-sensitive K^+ channels. Divalent cations (Mg^{2+}, Ca^{2+}) were a prerequisite for the activating process. The maximal effect was achieved using 1 m*M* vanadate dissolved in Ringer, increasing the open-state probability about ninefold. The active 5+ redox form of vanadate which stimulates ATP-sensitive K^+ channels is likely to be decavanadate $V_{10}O_{28}^{6-}$. ATP concentration-response curves have Hill coefficients near three in internal Na^+-rich Ringer and between one and two in internal KCl solutions. Half-maximal channel blockage was observed at ATP concentrations of 4 and 8 μ*M* in Ringer and KCl solutions, respectively. Internal vanadate shifted the curves towards higher ATP concentrations without affecting their slopes. Thus 50% channel blockage occurred at 65 μ*M* ATP in internal Ringer containing 0.5 m*M* vanadate. The results indicate that the affinity and stoichiometry of ATP binding to ATP-sensitive K^+ channels are strongly modulated by internal cations and that the ATP sensitivity is weakened by vanadate.

Key words: Skeletal muscle – Potassium channel – Patch clamp – ATP – Vanadate

Introduction

Ionic channels that are selective for K^+ ions and are blocked by intracellular adenosine triphosphate have been described in membranes of cardiac, skeletal and smooth muscle, of insulin-secreting cells, of central neurones and peripheral nerve and of epithelial cells (for a review see Ashcroft and Ashcroft 1990). In skeletal muscle, these ATP-sensitive K^+ channels can be inhibited by intracellular application of reagents that modify SH-groups, and it has been shown that the functionally important sulphydryl group is likely to be near the ATP-binding site of the channel (Weik and Neumcke 1989 a). A similar arrangement of an essential SH-group in the ATP-binding site has previously been inferred for the Na^+, K^+-ATPase (Patzelt-Wenczler et al. 1975). This analogy between the ATP-sensitive K^+ channel and the Na^+, K^+-ATPase stimulated the experiments described in this paper. We have studied the effects of vanadate, a well-known inhibitor of the Na^+, K^+-ATPase, on ATP-sensitive K^+ channels in mouse skeletal muscle and found a marked channel activation in the presence of internal Mg^{2+} ions, as reported by Dunne et al. (1989) for an insulinoma cell line. Activation of ATP-sensitive K^+ channels by vanadate depends on internal Na^+ and K^+ ions and leads to a reduced ATP-sensitivity of the channels. We interpret the results by suggesting that vanadate and internal cations modify internal sites on, or associated with the ATP-sensitive K^+ channels.

Some of the results have been published as an abstract (Weik and Neumcke 1989 b).

Materials and methods

Flexor digitorum muscles of adult mice were dissociated into single fibres, and the surface of a muscle fibre was patch clamped as described previously (Woll et al. 1989; Weik and Neumcke 1989 a). After forming a seal, the membrane patch was excised and currents through single ion channels were recorded in the inside-out mode of the patch-clamp technique (Hamill et al. 1981) with an L/M-EPC-5 amplifier (List Electronics, Darmstadt, FRG). Currents were measured at room temperature (20–21 °C) for a range of membrane potentials between −40 and −60 mV (taken as potential differences between the intra- and extracellular sides of the patch). The currents

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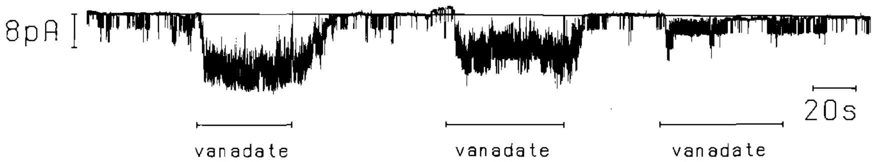

Fig. 1. A long (360 s) period of single-channel currents recorded from ATP-sensitive K^+ channels in internal Ringer and during repeated applications of 1 m*M* vanadate as indicated. Membrane potential −60 mV

were always recorded under steady-state conditions, i.e. one or more seconds after a step to the indicated membrane potential and after complete equilibration with a new internal solution.

Solutions

The pipettes were filled with a K^+-rich external solution of composition (in m*M*): 155 KCl, 3 $MgCl_2$, 0.5 EGTA (ethylene glycol bis (β-aminoethylether)-N,N,N′,N′-tetra-acetic acid), 10 HEPES (4-(2-hydroxyethyl)-1-piperazine-ethanesulphonic acid). The solution was titrated to pH 7.4 with 1 *M* KOH.

Muscle fibres were prepared and stored in mammalian Ringer whose composition is given in Table 1. Ringer was also the first internal solution in contact with the sarcoplasmic side of the excised membrane patches. After exposure of the inner membrane surface to the Na^+-rich Ringer solution containing m*M* concentrations of Ca^{2+} and Mg^{2+} ions, the internal bath solution was replaced with one or more of the K^+-rich solutions A to D (see Table 1).

ATP was added to the internal solutions as Na_2ATP (Boehringer, Mannheim, FRG), and the pH was readjusted to pH 7.4 with NaOH or KOH. Vanadate solutions were freshly prepared before the experiments by dissolving Na_3VO_4 (BDH Chemicals, Poole, UK) in the appropriate internal solutions and by titrating to pH 7.4 with HCl. The vanadate solutions had an orange-yellow colour which indicates that at least part of the vanadate was present as decavanadate ($Na_6V_{10}O_{28}$) in the 5+ redox state (Darr and Fridovich 1985). During heating (50 °C for about 2 h) the vanadate solutions became colourless because decavanadate was then slowly transformed into the 5+ redox forms metavanadate ($Na_4V_4O_{12}$) and orthovanadate (Na_3VO_4; see Darr and Fridovich 1985). The K^+-rich internal solution without divalent cations (solution D) was prepared without EDTA (ethylenediaminetetraacetic acid), because this chelating agent forms complexes with vanadate, whereas there is only little interaction between EGTA and vanadate (Darr and Fridovich 1985).

Table 1. Compositions of internal solutions (m*M*). All solutions were buffered with 10 m*M* HEPES and titrated to pH 7.4 with 1 *M* NaOH (Ringer) or with 1 *M* KOH (A, B, C, D)

	Ringer	A	B	C	D
NaCl	150	–	–	–	–
KCl	5	155	155	155	160
$CaCl_2$	2	2	–	5	–
$MgCl_2$	1	1	5	–	–
EGTA	–	–	1	–	–

Data acquisition and analysis

The currents were digitized (Sony PCM 501/ES) and stored on video tape. For analysis the data were replayed, filtered by a four-pole low-pass Bessel filter with 0.4 kHz corner frequency and digitized at 0.3 ms intervals. Subsequently, uninterrupted current recordings, mostly of 30 s duration, were analyzed numerically and under visual control to determine relative open-probabilities of ATP-sensitive K^+ channels. The half-amplitude criterion (Colquhoun and Sigworth 1983) was used to detect the openings and closings of one or more channels in the patch. All open probabilities p_0 of the channels were normalized with respect to a control p_0 value, and these relative p_0 values are given in % units. In the vanadate experiments maximum channel activation was achieved during the first application of 1 m*M* vanadate dissolved in Ringer (see Fig. 1). Since the open-probabilities of this maximum channel activation did not scatter much between different experiments, the mean p_0 value from four patches was used as control for the effects of vanadate dissolved in different internal solutions (Fig. 3, open column for Ringer).

Hill coefficients n_H for channel blockage by ATP or for channel activation by vanadate were determined as the steepest slopes of the Hill plots $y = \log(p_0/(100 - p_0))$ versus $x = \log[S]$ with [S] denoting the ATP or vanadate concentration.

Results

Channel activation by vanadate

The currents illustrated in Fig. 1 were recorded from a membrane patch containing several ATP-sensitive K^+ channels. At the beginning, up to three channels opened simultaneously in internal Ringer suggesting that there are only three ATP-sensitive K^+ channels in the patch. However, internal application of 1m*M* vanadate strongly increased the channel activity and revealed the presence of more channels. Channel activation by internal vanadate occurred within a few seconds and was fully reversible. If vanadate was applied repeatedly, the effects became progressively smaller with every application as shown in Fig. 1. This decaying action of vanadate is not caused by desensitization or run-down of ATP-sensitive K^+ channels because channel activity in internal Ringer without vanadate persists for up to 30 min (not shown). Compared to the strong effect of vanadate dissolved in Ringer, only minor stimulation could be detected using K^+-rich internal solutions with m*M* concentrations of Ca^{2+} and Mg^{2+} ions (Fig. 2A–C). Without these divalent cations almost no stimulating effect of vanadate

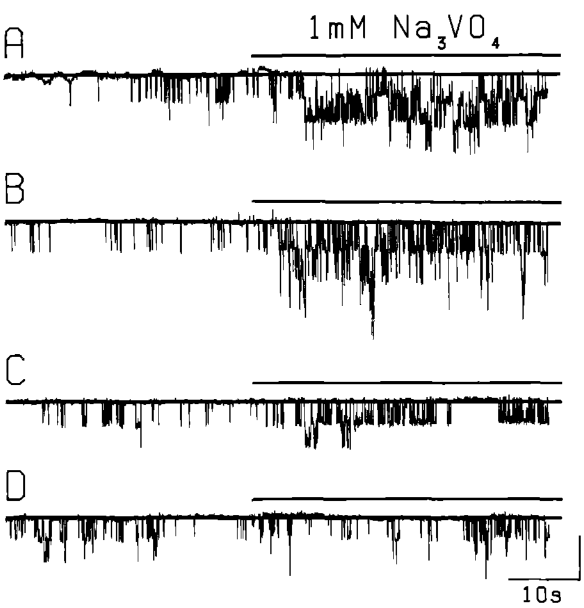

Fig. 2A–D. Effects of internal 1 m*M* vanadate dissolved in different solutions. Internal solutions are **A** solution A (155 m*M* KCl, 2 m*M* $CaCl_2$, 1 m*M* $MgCl_2$), **B** solution B (155 m*M* KCl, 5 m*M* $MgCl_2$), **C** solution C (155 m*M* KCl, 5 m*M* $CaCl_2$), **D** solution D (160 m*M* KCl). The records **A** to **D** are from different experiments and they begin 1 to 2 min after the patch was excised. Membrane potentials are −60 mV (**B**) and −50 mV otherwise

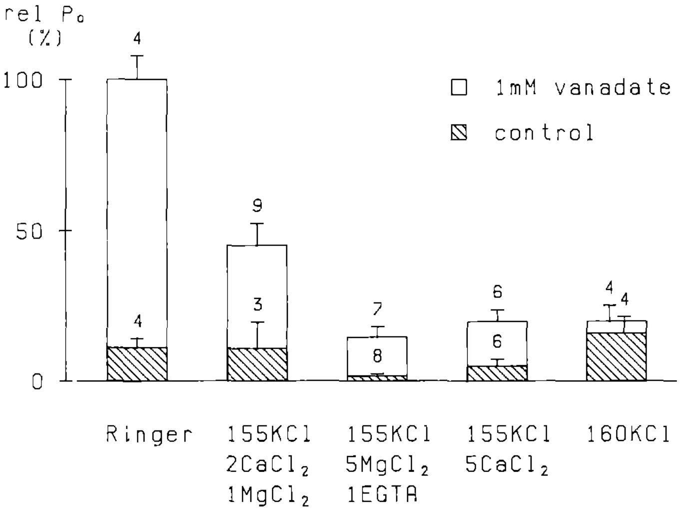

Fig. 3. Relative open-probabilities p_0 of ATP-sensitive K^+ channels in various internal solutions without (*hatched columns*) and with 1 m*M* vanadate (*open columns*). The compositions of the K^+-rich internal solutions A, B, C, D (in m*M*) are listed below the columns. Open-probabilities in Ca^{2+}-containing internal solutions (Ringer, solutions A, C) plus 1 m*M* vanadate were determined from currents recorded during the first minute of maximal channel activation in the respective solutions containing vanadate. All p_0 results were normalized with respect to the mean p_0 value determined in internal Ringer plus 1 m*M* vanadate. *Numbers* and *bars* above the columns denote the number of patches and the SEM values

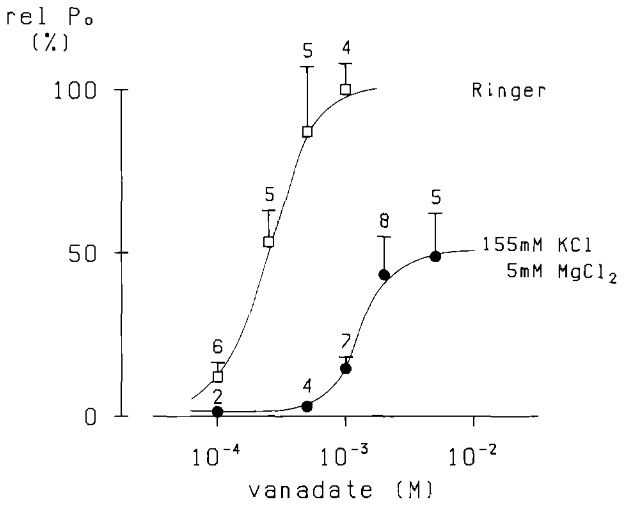

Fig. 4. Concentration-response curves of channel activation by vanadate in Ringer (□) and in solution B (155 m*M* KCl + 5 m*M* $MgCl_2$: ●). The abscissa denotes the nominal Na_3VO_4 concentration dissolved in the solutions. Open-probabilities in Ringer were determined from currents recorded during the first minute of maximal channel stimulation at different concentrations of vanadate. The p_0 results were normalized with respect to the mean p_0 value in Ringer plus 1 m*M* vanadate. *Numbers* and *bars* denote the number of patches and the SEM values. The *curves* through the mean values were drawn by eye. Half maximal channel activation at vanadate concentrations of 0.25 m*M* (Ringer) and 1.5 m*M* (solution B). The Hill coefficients of the concentration-response curves for channel activation are $n_H = 2.6$ (Ringer) and near 4 (solution B)

could be detected (Fig. 2D). All vanadate effects are reversible as shown in Fig. 1 for internal Ringer.

The records illustrated in Fig. 2 are short segments of currents before and during application of 1 m*M* vanadate in various internal solutions. Owing to the stochastical nature of channel gating, quantitative results cannot be extracted from such single traces. Therefore, open-probabilities were determined for several patches before, during and after application of vanadate and normalized as described in Methods. These relative open-probabilities are shown in Fig. 3.

Immediately after application, the strongest channel activation by 1 m*M* vanadate was observed in the Na^+-rich Ringer solution (Fig. 3). Relative to this condition, the relative open-probability p_0 in the K^+-rich solution B assumed a maximum of 49% at 5 m*M* vanadate (Fig. 4). As a function of the nominal vanadate concentration, the Hill coefficients n_H of channel activation by vanadate were 2.6 in Ringer and near 4 in solution B, and thus revealed a cooperative binding process in both solutions.

As described in the Methods, the vanadate solutions prepared before the experiments and used for channel activation had an orange-yellow colour and thus contained decavanadate. Colourless vanadate solutions, in which decavanadate had been transformed into other 5+ redox forms of vanadate, were much less effective in activating ATP-sensitive K^+ channels (not shown). This suggests that it is the decavanadate complex ($V_{10}O_{28}^{6-}$) which is primarily responsible for channel activation.

Channel blockage by ATP

Blockage of ATP-sensitive K^+ channels in skeletal muscle by internal ATP is strongly affected by internal cations and by vanadate. Figure 5 shows ATP concentration-response curves before and during treatment with 0.5 m*M*

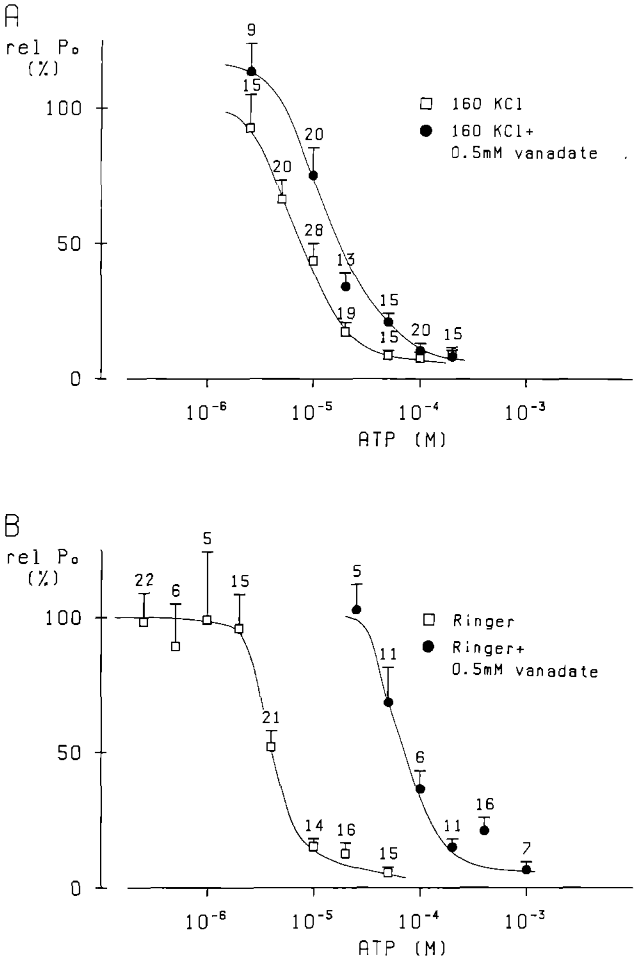

Fig. 5A, B. Concentration-response curves of channel blockage by ATP in various internal solutions. **A**, solution D (160 mM KCl) without (□) or with 0.5 mM vanadate (●). **B**, Ringer without (□) or with 0.5 mM vanadate (●). The p_0 results were normalized with respect to mean p_0 values in ATP-free control solutions before and after ATP application. The control solutions were: solution D (**A**, □ and ●), Ringer (**B**, □) and Ringer plus 0.5 mM vanadate (**B**, ●). *Numbers* and *bars* denote the number of measurements and the SEM values. The *curves* through the mean values were drawn by eye. Half-maximal channel blockage at ATP concentrations of 8 μM (**A**, □), 12 μM (**A**, ●), 4 μM (**B**, □) and 65 μM (**B**, ●). The Hill coefficients of the concentration-response curves for the channel blockage are $n_H = 1.4$ (**A**, □), 1.8 (**A**, ●), near 3 (**B**, □ and ●)

vanadate in internal KCl solution D (upper part A) and in internal Ringer (lower part B). Replacing internal KCl by Ringer solution increases the steepness of the curves for normal and vanadate-activated channels (compare Hill coefficients listed in the legend to Fig. 5). On the other hand, vanadate primarily shifts the midpoint of the curves towards higher ATP concentrations without affecting their slopes. The reduced effectiveness of ATP during treatment with vanadate was hardly detectable for the internal KCl solution D (Fig. 5A), but very pronounced for internal Ringer (Fig. 5B). Thus half of the channels were closed at an ATP concentration of 4 μM in Ringer, but 65 μM ATP was needed for inhibition of 50% of the channels after activation by 0.5 mM vanadate.

Discussion

ATP-binding properties are dependent on internal cations

Using an internal KCl solution without divalent cations (solution D), our data (Fig. 5A) suggest an approximate 1:1 stoichiometry of ATP-binding to ATP-sensitive K^+ channels as described by Spruce, Standen and Stanfield (1987). Substitution of this internal KCl solution by Ringer, which contains the blocking cations Mg^{2+}, Ca^{2+} and Na^+, caused a marked increase in the cooperativity and sensitivity of the ATP-binding (Fig. 5B). This implies that the ATP-receptor is modified in such a way that up to three ATP-molecules are able to bind to the protein at the same time. Furthermore, the midpoint of the concentration-response curves of channel blockage by ATP depends on the internal cations (50% channel blockage at ATP concentrations of 8 μM in KCl solution and of 4 μM in Ringer, see Fig. 5). This reflects a higher blocking potency of ATP in Ringer compared to KCl solution in agreement with our previous findings (Weik and Neumcke 1989a).

Vanadate as an activator of ATP-sensitive K^+ channels

As described for channel inhibition by ATP, channel activation by vanadate also depended on the cations used. Our results show that (i) the divalent cations Mg^{2+} and Ca^{2+} were necessary for channel activation, (ii) Mg^{2+} produced a sustained effect whereas Ca^{2+} induced a decay of the activation process, (iii) these two divalent cations had cooperative actions and (iv) replacing K^+ by Na^+ further enhanced channel activity (see Figs. 1, 2 and 3).

Channel activation by vanadate cannot be explained by the formation of complexes between vanadate and divalent cations, since replacing the monovalent cation K^+ by Na^+ further enhanced channel activation by vanadate (Fig. 3, first and second columns). On the other hand, changes of the open-probability by internal polyphosphates, which are polyvalent anionic agents like vanadate, are smaller and attributable to complex formation with Mg^{2+} ions (unpublished results).

There exists a striking difference between normal and vanadate-treated ATP-sensitive K^+ channels. As shown in Fig. 5B, in the presence of vanadate in Ringer, K^+ channels are about 16 times less sensitive to ATP than under control conditions. Vanadate treatment does not alter the slope of the concentration-response curve, i.e. does not change the cooperativity of the ATP-binding. Therefore, the mechanism, by which vanadate modifies the ATP-sensitive K^+ channels, cannot be explained by changes of the number of binding sites for ATP. Instead, the reduced ATP-sensitivity during vanadate treatment might be due to interactions between ATP and vanadate. This possibility is excluded by the results shown in Fig. 5A. If vanadate is dissolved in a 160 mM KCl solution, the ATP-sensitivity of the channel is little affected. The small changes in the concentration-response curves are mainly due to the small activating effect of vanadate in this solution (Fig. 3, last column).

Other effects of vanadate

The active form of vanadate inhibiting the Na^+, K^+-ATPase is thought to be orthovanadate VO_4^{3-} (Chasteen 1983), whereas the activation of ATP-sensitive K^+ channels in skeletal muscle seems to be primarily induced by decavanadate $V_{10}O_{28}^{6-}$ (see Results). Furthermore, micromolar concentrations of vanadate are sufficient to inhibit the pump (Chasteen 1983), while relative high concentrations (up to millimolar total vanadate) are necessary to stimulate the channel (Fig. 4). These findings suggest different vanadate-binding sites in the Na^+, K^+-ATPase and in the ATP-sensitive K^+ channel of skeletal muscle. ATP-sensitive K^+ channels in other tissues may have different properties, e.g. in insulinoma cell lines this type of channel can be stimulated using only micromolar vanadate, and it was suggested that vanadate activates a G-protein associated with the channel (Dunne et al. 1989). On the other hand, channel activation by vanadate in skeletal muscle does not seem to involve a G-protein, because the effects of vanadate were only seen at millimolar concentrations (Fig. 4).

ATP-sensitive K^+ channels in skeletal muscle are stimulated by decavanadate and blocked by ATP. In contrast, synergistical actions of decavanadate and ATP have been described for phosphofructokinase (Choate and Mansour 1979) and adenylate cyclase (Pai et al. 1977). In the case of adenylate cyclase, Pai et al. (1977) showed that decavanadate binds to the phosphate binding region of the ATP binding site. However, we cannot explain the totally different effects of ATP and decavanadate with a one-receptor hypothesis. As described previously (Weik and Neumcke 1989a), ATP-anions and Mg · ATP both are effective in blocking the channels, whereas decavanadate needs divalent cations to be effective. This would argue against one receptor for both substances.

Conclusions

Activation of ATP-sensitive K^+ channels in skeletal muscle by internal vanadate shows several peculiarities which are not observed in other transport systems. The channel activation requires millimolar concentrations of total vanadate, the active form is likely to be decavanadate; vanadate and ATP exert antagonistic actions and the vanadate- and ATP-binding sites seem to be separated from each other. In addition, channel activation by vanadate and channel blockage by ATP are strongly affected by internal mono- and divalent cations. These complex interrelations between the effects of internal cations and anions are likely to be important factors in regulating ATP-sensitive K^+ channels of skeletal muscle in vivo.

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