A role for a glibenclamide-sensitive, relatively ATP-insensitive K⁺ current in regulating membrane potential and current in rat aorta

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Abstract

Objective: ATP-sensitive K⁺ channels have been classified based on their inhibition by cytoplasmic ATP. Recent evidence in vascular smooth muscle has suggested that these channels show weak sensitivity to intracellular ATP. However, it is not known whether these channels regulate the resting K conductance in vascular smooth muscles. Therefore, the aim of the present investigation was to characterize this current in rat aorta myocytes and to examine whether it contributes to setting the membrane potential. Methods: The conventional and nystatin-permeabilised whole cell patch clamp techniques were used to characterize the effect of glibenclamide on membrane potential and K current in enzymatically dispersed rat aorta myocytes. Results: The mean resting potential measured in current clamp mode using the permeabilized patch approach was −54±5 mV (n=8). Glibenclamide (10 μM) caused a reversible 24-mV depolarization in these cells. In symmetrical K⁺ (135 mM) solution an inward glibenclamide-sensitive (10 μM) current (−4.1±0.7 pA/pF; n=5), hereafter termed Isub, was observed at a membrane potential of −80 mV when cells held at −60 mV were ramped from 80 to −80 mV. In the absence of any nucleotide in the pipette solution, Isub measured by the conventional whole-cell method was −23.69±4.65 pA/pF (n=9). With 1 and 3 mM ATP in the pipette, the average current density was −25±6.3 pA/pF (n=8), and −9.4±2.7 pA/pF (n=9), respectively. In the absence of ATP, 1 mM GDP significantly (P<0.01) increased Isub (−44.8±8.4 pA/pF; n=13). Inclusion of 1 mM ATP in the GDP-containing pipette solution had no significant effect on the current amplitude (−56.4±10.7 pA/pF; n=7). Isub fell to −11.0±2.9 pA/pF (n=10) if 1 mM GDP and 3 mM ATP were present. In symmetrical K⁺, the Isub observed in the presence of 1 mM ATP in the pipette was increased by more than two-fold in the presence of 10 μM levocromakalim. In PSS containing 5 mM K⁺, a significant glibenclamide-sensitive current was observed at −45 mV membrane potential when cells dialyzed with 1 mM ATP were ramped between −80 to 30 mV. Conclusion: These results demonstrate that Isub channels in rat aorta myocytes differ from classical KATP channels, being relatively insensitive to intracellular ATP. Isub therefore appears to have an important role in contributing to the maintenance of the resting potential in rat aortic smooth muscle. © 1999 Elsevier Science B.V. All rights reserved.

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1. Introduction

K⁺ channels are critical in maintaining the normal resting membrane potential in a variety of excitable cells. Although ATP-sensitive K⁺ (KATP) channels are thought to play little role in maintaining the membrane potential under basal conditions, there have been several reports that the KATP channel blocker glibenclamide caused membrane depolarization, either of intact blood vessels [1] or of cells dialyzed with solutions containing millimolar concentrations of ATP [2]. Although these depolarizations were small, this observation is puzzling because the KATP channel is believed to be closed at normal concentrations of intracellular ATP. More recently, however, Zhang and

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Bolton [3] reported the existence of a type of glibenclamide-sensitive $K^+$ channel (termed the MK channel) which was quite insensitive to ATP in inside-out patch experiments in rat portal vein myocytes. It is not known if this channel is present in other blood vessels and to what extent it might contribute to the resting $K^+$ conductance.

In this study, we have utilized the conventional and nystatin-permeabilized whole cell patch clamp techniques in order to explore the role of glibenclamide-sensitive $K^+$ channels in setting the resting membrane potential in the rat aorta, a widely used vascular preparation.

2. Methods

Adult male Wistar rats (250–300 g) were killed by cervical dislocation in accordance with UK Home Office regulations and with the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1996). The thoracic aorta was removed into ice cold physiological saline solution (PSS), cleaned of fat and connective tissue, cut into small pieces and incubated at 37°C in low $Ca^{2+}$ (15 μM) PSS for 15 min. Tissue was then transferred to the same solution containing 0.23 mg/ml of elastase type I (Sigma) for 20 min, followed by incubation in low $Ca^{2+}$ PSS containing collagenase type I (1 mg/ml), collagenase type XI (1 mg/ml), papain (0.5 mg/ml) and dithiolthreitol (1.1 mM) for a further 20 min. Tissues were washed in enzyme-free low $Ca^{2+}$ PSS, triturated to disperse cells, and then stored in low $Ca^{2+}$ PSS at 4°C for use within 5–6 h. Whole-cell membrane currents were recorded using either nystatin-perforated mode [4] or standard patch clamp techniques as described elsewhere [5]. Membrane currents were typically recorded during voltage ‘ramps’, in which the pipette potential was increased linearly from −80 to +30 or +80 mM over a period of 1 s; ramps were imposed at 0.1 Hz. The membrane potential was determined using the perforated patch technique in the current clamp mode. PSS contained (mM) NaCl, 130; KCl, 5.0; MgCl$_2$, 1.2; CaCl$_2$, 1.5; HEPES, 10; glucose,10. The pH was adjusted to 7.4 with NaOH. Low- $Ca^{2+}$ PSS contained 15 μM $Ca^{2+}$. $Ca^{2+}$-free, high-$K^+$ (135 mM) solution was made by replacing NaCl with an equimolar concentration of KCl and removing $Ca^{2+}$ from the PSS. The pipette solution for perforated patch recording contained (mM): KCl, 110; MgCl$_2$, 2.5; HEPES, 10, EGTA, 10; nystatin 250 μg/ml and the pH was adjusted to 7.2 by KOH. For whole-cell conventional recording of ion currents, ATP (magnesium salt, 0.1, 1.0 or 3.0 mM) or GDP (sodium salt, 1.0 mM) alone or in combination were included in the pipette solution. Values in the text are shown as means±S.E.M (n=sample size). The statistical significance of differences between values was assessed using Student’s unpaired t-test, with $P<0.05$ taken to indicate significance.

3. Results

Fig. 1A illustrates that 10 μM glibenclamide caused a large depolarization of the resting membrane potential ($E_m$) when this was measured in current clamp mode using the perforated patch approach. Following wash, the recovery of $E_m$ was delayed but complete. Fig. 1B shows the mean values of $E_m$ measured in aorta myocytes under control conditions and then after the effect of 10 μM glibenclamide had stabilized. $E_m$ was −54±5 mV (n=8) in PSS, and decreased significantly ($P<0.01$) to −30±7 mV in glibenclamide. Following removal of glibenclamide, $E_m$ recovered to −59±6 mV. These results provide direct evidence for the contribution of $I_{gib}$ to the resting $E_m$ in rat aorta.

Currents were recorded using the nystatin technique in cells held at −60 mV. The voltage was ramped from −80 to 80 mV as shown in Fig. 2A. We measured $I_{gib}$ at a potential of −80 mV in order to minimize the interference
from other K\(^+\) currents. As shown in Fig. 2, there was a small inward current at \(-80\) mV in PSS containing 5 mM K\(^+\). When the superfusing medium was changed to Ca\(^{2+}\)-free high K\(^-\) (135 mM+2 mM TEA) PSS in order to shift the K\(^-\) \(E_{rev}\) to 0 mV and suppress any Ca\(^{2+}\)-activated K\(^-\) current, an inward current of about \(-50\) pA could be observed. Glibenclamide (10 \(\mu\)M) profoundly inhibited this current, which then recovered completely following wash, after a delay similar to that observed for the membrane potential. Fig. 2B illustrates the time-course of changes in the whole-cell current at \(-80\) mV during this procedure. The numbers correspond to the traces shown in Fig. 2A. The mean amplitude of \(I_{glig}\) was \(-42.1 \pm 10.4\) pA \((n=5)\).

Since these data suggested that \(I_{glig}\) channels in rat aorta were open in the resting state at physiological concentrations of intracellular nucleotides, we further examined their regulation by ATP and the nucleotide diphosphate (NDP) GDP, as the actions of this NDP have previously been widely characterized in vascular myocytes [3,6].

One important characteristic of \(I_{glig}\), as previously described [7], is its sensitivity to block by intracellular ATP. However, the current tracings shown in Fig. 3A demonstrate that a large \(I_{glig}\) was also present when the cells were dialyzed with a solution containing 1 mM ATP (with no other nucleotide). The time-course of the whole-cell current recorded at \(-80\) mV is illustrated in Fig. 3B. Glibenclamide 1 \(\mu\)M was as potent as 10 \(\mu\)M in blocking the \(I_{glig}\) (data not shown), although we routinely used the higher concentration because it caused a more rapid effect.

Fig. 3C shows the current–voltage relationship of the whole-cell current recorded with 1 mM GDP (no ATP) in the pipette. The current tracings show that the large inward current which developed when \([K]_{bath}\) was raised to 135 mM K\(^+\) was almost abolished by 10 \(\mu\)M glibenclamide, thereby confirming that the current was mediated by glibenclamide-sensitive K\(^-\)-channels. Fig. 3D depicts the reversible effect of glibenclamide on the whole-cell current at \(-80\) mV. As described above, recovery from glibenclamide block was delayed.

Fig. 4 illustrates the effects on \(I_{glig}\) of varying the concentration of ATP in the presence and absence of GDP. Current amplitude is expressed as current density; cells had a mean cell capacitance of 13.0 \(\pm\) 0.3 PF \((n=229)\). Current amplitude was measured about 2 min after the \([K]_{bath}\) was raised to 135 mM (approximately 5 min after establishment of whole cell recording), because in some cases progressive rundown subsequently occurred (see below).

In the absence of any nucleotide in the pipette, the mean current density of \(I_{glig}\) was \(-23.7 \pm 4.7\) pA/pF \((n=9)\) and the current showed run-down. Inclusion of either 0.1 or 1 mM ATP in the pipette had no significant effect on the current amplitude. \(I_{glig}\) was, however, significantly reduced \((P<0.05)\) when pipette [ATP] was increased to 3 mM.

With 1 mM GDP only in the pipette, \(I_{glig}\) was significantly \((P<0.01)\) greater than in the absence of nucleotide. The current recorded in the presence of 1 mM ATP and 1 mM GDP was not significantly different from that with 1 mM GDP, alone, but was larger than the current present with 1 mM ATP alone \((P<0.05)\). The current amplitude in the presence of 1 mM GDP and 3 mM ATP was similar to that observed when the pipette solution contained only 3 mM ATP.

\(I_{glig}\) showed complete \((99 \pm 5\%\); \(n=4)\) recovery from block by 10 \(\mu\)M glibenclamide when 1 mM ATP was present in the pipette solution. On the other hand, with no ATP in the pipette, recovery from glibenclamide was consistently poor \((12 \pm 9\%\); \(n=5)\). Recovery from glibenclamide amounted to 64 \(\pm\) 5\% \((n=4)\) and 84 \(\pm\) 5\% \((n=5)\) when 1 mM GDP and 1 mM GDP+1 mM ATP, respectively, were present in the pipette solution. This variability presumably represented differences in underlying current.
rundown which had occurred during exposure to glibenclamide and the prolonged recovery period, since separate experiments where glibenclamide was only applied after many minutes showed that a progressive slow rundown occurred with no ATP present, but not in the presence of 1 mM ATP (not shown).

Fig. 5(A,B) demonstrates that $I_{glib}$ was also observed in the presence of 1 mM ATP in the pipette in Ca$^{2+}$-free PSS containing a normal concentration of K$^+$ (5 mM). The current measured at $-45$ mV (in order to minimize contamination by delayed rectifier and Ca$^{2+}$-activated K$^+$ currents) was $20.0 \pm 2.6$ and $-2.3 \pm 1.6$ pA ($n=6$) in the absence and subsequent presence of 1 $\mu$M glibenclamide. The inhibition by glibenclamide was almost completely reversible.

Zhang and Bolton [3] demonstrated that while the MK-type K$_{ATP}$ current was stimulated by levromakalim, the LK-type K$_{ATP}$ channels were insensitive to stimulation by levromakalim. In our study we observed that 10 $\mu$M levromakalim increased the $I_{glib}$ by more than two fold when the whole-cell current was measured with 1 mM ATP in the pipette and the cells were ramped between $-80$ and $80$ mV in symmetrical K$^+$ solution (Fig. 5C,D).

A small delayed rectifier K$^+$ current ($I_{kv}$) was observed, and activated with an apparent threshold of $-30$ mV when cells held at $-60$ mV were stepped to test potentials between $-70$ to $50$ mV (300 ms at 0.1 Hz) in PSS containing 2 mM TEA. The pipette contained 5 mM ATP and the other constituents were the same as described earlier for the pipette solution for the conventional whole-cell recording. This current was insensitive to 10 $\mu$M glibenclamide at all potentials. For example, at $+50$ mV $I_{kv}$ was $31 \pm 7$ and $31 \pm 8$ pA in the absence and subsequent presence of glibenclamide, respectively ($n=4$).

4. Discussion

It is generally believed that ATP-sensitive K$^+$ channels are normally closed because of the high concentration of intracellular ATP. Nevertheless, in both cultured [8] and freshly dispersed porcine coronary artery cells [9], there was evidence of some K$_{ATP}$ channel opening under basal conditions. On the other hand, in cells isolated from rabbit mesenteric artery [10], rabbit portal vein [6] and rat mesenteric artery [11], there was no evidence of any resting current measured in perforated patch mode. These divergent observations suggest the existence of multiple types of glibenclamide-sensitive channels which have
differential sensitivities to intracellular ATP, and which are unequally expressed in different blood vessels.

We report here a K⁺ current in rat aorta myocytes that was sensitive to glibenclamide but was quite insensitive to intracellular ATP. The activation of this current at physiological levels of intracellular nucleotides was evident from a significant resting current (sensitive to 10 μM glibenclamide), measured using the nystatin technique. The observation of a significant Iglib in non-dialyzed rat aorta cells indicates that this K⁺ current may play an important role in maintaining resting K⁺ conductance. This was further substantiated by the finding that glibenclamide caused a reversible 24 mV membrane depolarization from the resting level of about −55 mV. This observation in single cells is consistent with the membrane depolarizing action of glibenclamide in intact rat aorta [12]. A high concentration of glibenclamide (e.g. >100 μM) has been reported to inhibit delayed rectifier K⁺ currents in rabbit portal vein [16]. However, the lack of effect of 10 μM glibenclamide on the Iₖᵥ in rat aorta myocytes suggests that the membrane depolarizing action of this drug is due to selective inhibition of Iglib.

To determine whether the Iglib channels in rat aorta differed from classical K<sub>ATP</sub> channels with respect to sensitivity to intracellular ATP, we used conventional whole-cell recording with various pipette concentrations of ATP. The amplitude of Iglib was similar when pipette [ATP] was raised from 0 to 1.0 mM, although the current then ran down progressively in the absence of nucleotide. When 3 mM ATP was used in the pipette, there was an approximately 60% diminution of Iglib. In comparison, Xu and Lee [13] observed that the IC₅₀ of ATP on the glibenclamide-sensitive current was 350 μM in canine coronary artery smooth muscle cells.

Iglib in rat aorta was stimulated by 1 mM GDP, which is consistent with the ability of this nucleotide to cause K<sub>ATP</sub> channel activation in other vascular smooth muscle cells [6,11,14]. This stimulation was similar in both the absence of ATP, and in the presence of 1 mM ATP, but did not occur in the presence of 3 mM ATP.

Recently, Zhang and Bolton [3] described two types of ATP-sensitive K⁺ channels in rat portal vein myocytes, one blocked by micromolar concentrations of ATP (LK-type) and the other displaying sensitivity to ATP only above a concentration of 1 mM (MK-type). Our results indicate that Iglib in rat aorta has MK-like properties. Apparently, LK-type K⁺ current was absent in these cells because Iglib displayed no sensitivity to ATP below 1 mM. Further, the stimulation by levcromakalim of Iglib in rat aorta is consistent with the similar property of the MK-type channel. Zhang and Bolton [3] drew parallels between the single MK channel current and the whole cell K<sub>NDP</sub> current their laboratory had previously identified. However, the current in rat aorta differs in two respects from K<sub>NDP</sub>. Firstly, Iglib was of similar amplitude in the presence and absence of 1 mM ATP, while Beech et al. [6] showed that the activation of K<sub>NDP</sub> channels by 1 mM GDP was inhibited by 60% in the presence of 1 mM ATP. Secondly, these authors did not report the occurrence of the K<sub>NDP</sub> current under basal conditions.

A glibenclamide-sensitive but ATP-insensitive K⁺ channel has recently been demonstrated in HEK293T cells cotransfected with SUR2B and Kir6.1 subunits [15]. As reviewed by these authors, Kir6.1 is ubiquitously expressed in rat tissues, including smooth muscles, while SUR2B is expressed in vascular smooth muscle cells. This channel appears therefore to resemble the MK channel, and has properties similar to those of Iglib in the rat aorta.

In conclusion, this work makes two novel and complementary observations. The first is that the membrane potential in rat aorta depends strongly on glibenclamide-sensitive K⁺ channels. The second observation, which is predicted by the first, is that Iglib in these cells is relatively insensitive to ATP, which at <1 mM is effective in blocking K<sub>ATP</sub> channels in a variety of other tissues [7]. The steep inhibition of Iglib between ATP concentrations of 1 and 3 mM, which is especially prominent when GDP is present, suggests that in blood vessels where these channels are present, they might play a role, not only in maintaining the resting membrane potential under physiological conditions, but also of controlling vascular tone during metabolic stress associated with relatively small changes in intracellular nucleotides.
Fig. 5. (A,B) Conventional whole-cell recording of $I_{\text{fia}}$ in Ca$^{2+}$-free 5 mM K$^+$ PSS with 1 mM ATP in the pipette in a myocyte ramped from $-80$ to 30 mV for 1 s at every 10 s from a holding potential of $-60$ mV. (A) Effect of 1 $\mu$M glibenclamide on the control current and its recovery following wash out of glibenclamide. (B) Illustrates the glibenclamide-sensitive current which was derived from subtraction of the glibenclamide-resistant current from the control current depicted in Fig. 4A. Note the linearity of the $I_{\text{fia}}$ between $-80$ to 30 mV. (C,D) Effect of 10 $\mu$M levcromakalim on $I_{\text{fia}}$ in a myocyte bathed in 135 mM K$^+$ containing 2 mM TEA. The cell was ramped from $-80$ to 80 mV for 1 s at every 10 s from a holding potential of $-60$ mV. (C) Depicts the current traces in 5 mM K$^+$ PSS followed by Ca$^{2+}$-free 135 mM K$^+$ PSS containing 2 mM TEA in the presence and absence of 10 $\mu$M levcromakalim. Note the enhancement of the basal $I_{\text{fia}}$ by levcromakalim and its subsequent block by 10 $\mu$M glibenclamide. (D) Shows the time-course of development of $I_{\text{fia}}$ and its potentiation by levcromakalim and subsequent block following treatment with 10 $\mu$M glibenclamide.

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References


