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Effects of $[Ca^{2+}]_i$ and temperature on minK channels expressed in *Xenopus* oocytes

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Slowly activating, voltage-dependent minK channels cloned from rat kidney were expressed in *Xenopus* oocytes. Increase in the bath temperature from 22 to 32°C resulted in a dramatic acceleration of minK channel activation. The extraordinarily high Q_{10} of minK channel activation was voltage-dependent, being higher at more negative potentials (Q_{10} at −20 mV: 7.02; at 20 mV: 4.0). While activation of minK channels was highly voltage-dependent at 22°C, voltage had only little effect on minK channel activation at 32°C. Increase in $[Ca^{2+}]_i$, which has recently been shown to increase the maximal conductance g_{max} at room temperature, did not affect g_{max} at 32°C. However, increase of $[Ca^{2+}]_i$ caused acceleration of minK channel activation at both temperatures. The interaction of $[Ca^{2+}]_i$ and temperature on g_{max} and activation rate of minK channels described here is very similar to recent findings on Ca- and temperature-effects on the slowly activating potassium conductance I_{Ks} in guinea pig cardiac myocytes.

Slow potassium channel; MinK channel; *Xenopus* oocyte; Q_{10}; Temperature

1. INTRODUCTION

Recent advances in molecular biology have allowed the cloning of several families of voltage-gated potassium channels, which can be according to their molecular structure distinguished in two general classes. The first class of mammalian K^+ channels is homologous to the *Drosophila* genes *Shaker*, *Shab*, *Shal* and *Shaw* [1]. Members of the second K^+ channel type have been termed minK channels [2]. This type of channel has been originally cloned from rat kidney and was subsequently found in heart and uterus tissue of different species [3–6]. MinK channels can be distinguished by their small size and unique structure of the channel subunits (130 amino acids, only one transmembrane domain, N-terminal extracellularly and C-terminal intracellularly located). Expressed in *Xenopus* oocytes minK channels have been shown to be regulated by changes in $[Ca^{2+}]_i$, protein kinase C and changes in the cytoskeleton, but the quarternary protein structure of minK channels is unclear [6–9]. Preliminary data suggest that assembly of minK channel subunits might play a role in the slow channel activation [10,11]. The slow activation of minK channels displays a close relation to the slowly activating conductance I_{Ks} recorded in guinea pig cardiac myocytes [4,12]. Recently, a high temperature and Ca-dependence of I_{Ks} activation was observed [13]. In this study effects of $[Ca^{2+}]_i$ and temperature on minK channels were investigated to obtain more information about mechanisms involved in minK channel activation and its putative relation to I_{Ks} recorded in guinea pig cardiac myocytes.

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2. MATERIALS AND METHODS

The methods of cloning and subcloning the minK channels and RBK1 have been previously described [7,14]. The oocytes were each injected with 0.5 ng capped RNA transcribed in vitro as previously described and two electrode voltage clamp recordings were performed 9–10 days after injection [14]. The external solution contained NaCl 96 mM, KCl 2 mM, $CaCl_2$ 1.8 mM, $MgCl_2$ 1 mM and HEPES 5 mM (pH 7.4, 220 mOsm). MinK channel currents were evoked by depolarizing the oocyte from a holding potential of −80 mV for 20 s to potentials from −40 to 40 mV in steps of 20 mV. In some batches of non-injected oocytes long depolarizing steps to 40 mV evoked a slowly activating Na^+-inward current [15]. In these batches minK channels were only recorded at voltages up to 20 mV. The ionophor A23187 (Sigma) was added from a 10 mM stock solution (in DMSO) to the control solution to give a final concentration of 1 μM. At this concentration no Cl^--currents were evoked, but the effects on minK channels were maximal [7].

Values given for the rate of activation and the maximal current were derived from fitting the current to a double exponential function. Numerical values are reported as mean ± S.E.M.; the number of observations (n) was always 5.

3. RESULTS

3.1. *Effects of temperature change on minK channels*

Depolarization of *Xenopus* oocytes, which had been previously injected with mRNA encoding the rat minK channel, evoked a slowly activating potassium current as previously described [7]. It is very difficult to describe the activation kinetics of minK channels since they do not reach a steady state current during the routinely

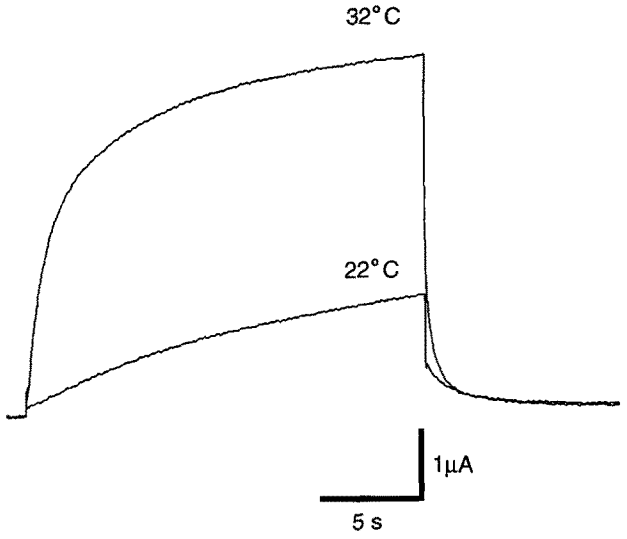

Fig. 1. Effects of temperature change from 22°C to 32°C on minK channel activation and current amplitude. The oocyte was clamped for 20 s at a potential of 0 mV; holding potential was −80 mV.

used 20 s step. To get a reasonable estimate of the activation kinetics of minK channels a sum of two exponentials plus a constant was fitted to the currents. The current amplitude for the fast component was about 80% of the total current. The time constant for activation of a very slow component was with more than 30 s (at 20 mV) far longer than the depolarizing voltage steps, so that they were not considered for further analysis. The time constant for the fast and dominant component (τ_1) was at 22°C and −20 mV 21.8 ± 4.5 s and at 20 mV 9.3 ± 1.3 s. The estimated voltage needed for half-maximal activation of minK channels was −28.1 ± 0.1 mV and the calculated maximal conductance (g_{max}) was 13.7 + 0.0 μS/oocyte. Increasing the bath temperature to 32°C dramatically accelerated the activation and increased g_{max} of minK channels (Fig. 1). At −20 mV τ_1 was reduced to 3.0 ± 1.0 s and at 20 mV τ_1 was reduced to 2.3 ± 1.1 s, which corresponds to a Q_{10} of 7.3 at −20 mV and a Q_{10} of 3.1 at 20 mV. At 32°C there were no significant differences in the rate of activation for the voltages tested (Fig. 2A). g_{max} was increased from 13.7 ± 0.0 μS/oocyte at 22°C to 30.1 ± 0.9 μS/oocyte at 32°C (Fig. 2B). Effects of temperature increase on minK activation and g_{max} were rapidly reversible by decreasing the bath temperature back to 22°C.

3.2. *Effects of an increase in $[Ca^{2+}]_i$ on minK channel activation and g_{max}*

Superfusion of *Xenopus* oocytes expressing minK channels with the ionophore A23187 (1 μM) resulted at 22°C in an increase in g_{max} and an acceleration of activation, similar to what has been previously described [6,7]. At 20 mV τ_1 was reduced from 9.8 ± 0.4 s to 6.5 ± 0.7 s after A23187 (Fig. 3A). In addition, A23187 increased g_{max} from 11.4 ± 0.3 μS to 14.9 ± 0.3 μS without changing $V_{1/2}$ significantly (Fig. 3B). When applied at 32°C A23187 did not result in an increase of g_{max} and the effects on the activation kinetics of minK channels were suppressed (Fig. 4).

4. DISCUSSION

A modest temperature dependency with a Q_{10} between 1.5 and 2.5 has been reported for rapid activating, voltage-gated Na^+- and K^+-currents in neuronal as well as in muscle tissues [16,17]. Considering the well-defined complex molecular structure of these classical voltage-gated ion channels such minor effects of temperature changes are not surprising, since for channel activation rather small conformational changes of the channel protein are sufficient. On the other hand for the very slowly activating, voltage-gated potassium conductance I_{Ks} recorded in guinea pig cardiac myocytes an increase in temperature resulted in a dramatic acceleration of activation with a Q_{10} of 4–6 [13]. MinK channels expressed in *Xenopus* oocytes show a similar slow activation as I_{Ks} in guinea pig cardiac myocytes. In addition, the RNA encoding minK channels has been found in heart tissue of several species including guinea pig ([4,6] unpublished observation). A similar regulation of

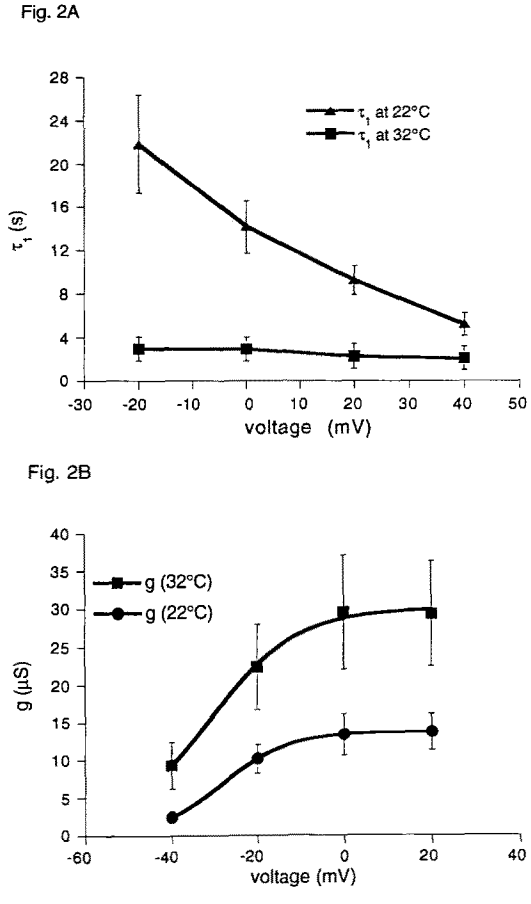

Fig. 2. Effects of temperature change on the voltage-dependence of activation rate (A) and maximal conductance (B) of minK channels.

minK channels and I_{Ks} by second messengers has also been described [6,7,8,18]. Most recently, an identical sensitivity to novel class III antiarrhythmic compounds has been observed for I_{Ks} in guinea pig cardiac myocytes and minK channels expressed in *Xenopus* oocytes [19]. The uncommon high temperature sensitivity of minK channels described in this study further supports a relation of their molecular structure to I_{Ks} in guinea pig cardiac myocytes. A further very intriguing finding of this study is the different voltage-sensitivity of minK activation at different temperatures. The result that the rate of activation looses its voltage-dependence at high temperature indicates a very unique mechanism of minK channel activation.

The role of $[Ca^{2+}]_i$ in minK channel activation appears very complex. An increase in $[Ca^{2+}]_i$ clearly accelerates activation and increases g_{max} significantly at low temperatures, while at higher temperatures the role of $[Ca^{2+}]_i$ was negligible. Busch and Maylie [13] reported for I_{Ks} in guinea pig cardiac myocytes somewhat related findings, where high buffering of $[Ca^{2+}]_i$ could prevent β-receptor mediated positive regulation only at 22°C but not at 32°C. In the same study it was also shown that the Q_{10} for I_{Ks} activation was affected by $[Ca^{2+}]_i$, where low $[Ca^{2+}]_i$ resulted in a higher temperature sensitivity. Although these experiments are only indirectly comparable with the findings described here, they indicate a similar role of $[Ca^{2+}]_i$ for regulation of I_{Ks} as well as for minK channels. There are two obvious interpretations for this observation: either one step in channel activation has a different Ca^{2+}-dependence at different temperatures or there are different mechanisms involved in activation. However, the actual mechanism of minK channel activation remains unclear. Changes in the cytoskeleton due to changes in $[Ca^{2+}]_i$ have been shown to regulate minK activation [9]. The interaction between minK channels and the cytoskeleton might therefore be a good target for investigations which could give a better insight in minK channel activation.

Fig. 3A

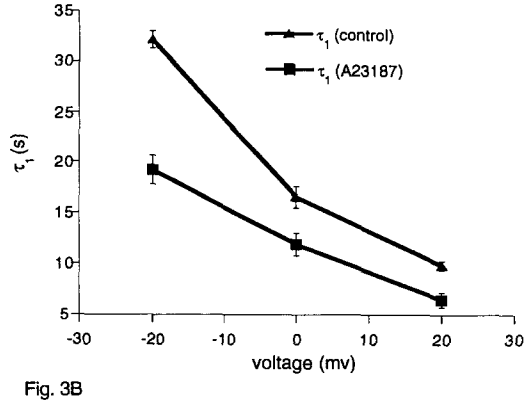

Fig. 3B

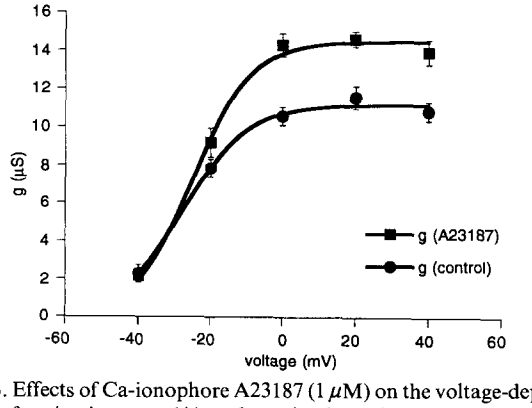

Fig. 3. Effects of Ca-ionophore A23187 (1 μM) on the voltage-dependence of activation rate (A) and maximal conductance (B) of minK channels at 22°C.

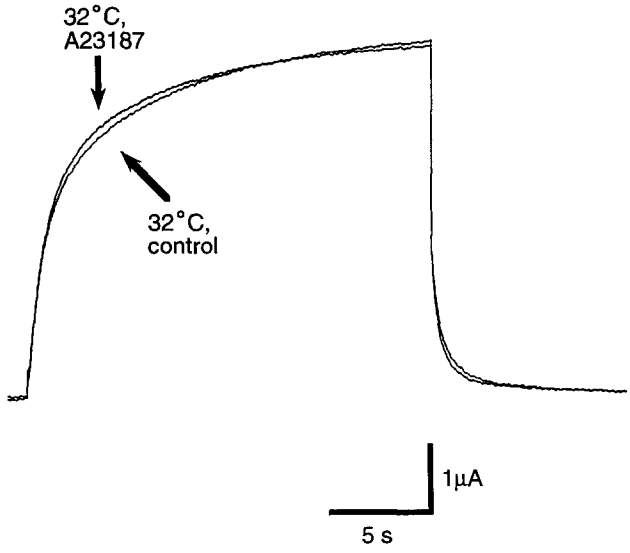

Fig. 4. Lack of a significant effect of A23187 (1 μM) at 32°C. Currents through minK channels were evoked by depolarizing the oocyte for 20 s to 0 mV from a holding potential of −80 mV.

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