Pflügers Arch (1989) 414 (Suppl 1): S183–S184

Ca^{2+}-activated K^+ channels contribute to the resting potential of vascular myocytes. Ca^{2+}-sensitivity is increased by intracellular Mg^{2+}-ions

U. Trieschmann and G. Isenberg

Department of Physiology, University of Cologne, D-5000 Köln 41, F.R.G.

Introduction

The Ca^{2+}-activated maxi-K^+ channel has been described for many smooth muscle cells; we show here that it can contribute to the resting membrane potential.

Methods

Myocytes were isolated from either coronary or pial vessels of the pig. They were superfused with a salt solution composed of (mM): NaCl 150, KCl 5.4, $MgCl_2$ 1.2, glucose 20, HEPES/NaOH 10 (pH 7.4, 24°C). For whole-cell recordings the patch electrodes were filled with (mM): KCl 150, $MgCl_2$ 2, EGTA 0.02, HEPES/KOH 10 (pH 7.4).

Results and discussion

In these "physiological conditions", the resting potential (V_m) spontaneously fluctuates between -55 and -70 mV (Fig. 1A). Under voltage-clamp conditions the whole-cell current (-55 mV) shows about 50 ms brief shots of outward current, the amplitudes ranging between 1 and 2 pA. In its ampitude and time course, the current resembles the current through single Ca^{2+}-activated maxi-channels (i_{KCa}), as recorded from cell-free patches with buffered $[Ca^{2+}]_c$ (e.g. Magleby and Palotta 1983). The K^+ channel blocker tetraethylammonium (TEA) reversibly inhibits currents and fluctuations of V_m.

Although suggested by Fig. 1, the hypothesis of i_{KCa} contributing to V_m has not yet been proven by single channel analysis. Usually i_{KCa} has been analysed at positive potentials and micromolar cystolic calcium concentrations $[Ca^{2+}]_c$ (both interventions increase the open probability, P_o), and extrapolation of data to the resting potential only suggests that P_o is "almost zero". Here we are trying to quantify these very low P_o values at negative V_m and submicromolar $[Ca^{2+}]_c$. In addition, we will show that P_o is significantly larger when cytosolic Mg^{2+}-ions are present.

Single channel currents were recorded from inside out patches facing symmetrical 150 mM KCl solutions (10 mM Tris/HEPES, pH 7.4), where the Ca^{2+}-activated maxi-K^+ channel has a single channel conductance of 172 ±6.8 pS (n=6). The solution facing the cytosolic site of the membrane was complemented with 10 mM Ca-EGTA which was adjusted to the desired $[Ca^{2+}]_c$-values. At V_m=-40 mV and 100 nM $[Ca^{2+}]_c$, $N.P_o$ was as low as 0.0007 ± 0.0002 (n=8). Elevation of $[Ca^{2+}]_c$ to 316 nM increased $N.P_o$ to 0.003 ± 0.01 (compare Fig. 2A with C).

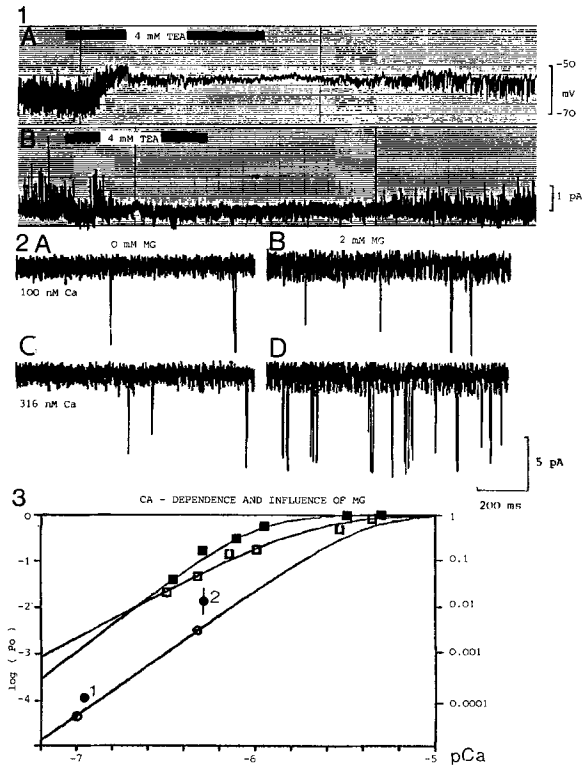

Fig. 1. Effect of 4 mM TEA **A** on the resting membrane potential and **B** on the whole-cell current (holding potential: -55 mV). **Fig. 2.** Inside-out patches, V_h= -40 mV; each trace represents 7 superimposed records of 1000 ms; **A, B** at 100 nM $[Ca^{2+}]_c$: 0 / 2 mM $MgCl_2$, $N.P_o$= 0.0007 / 0.0017; **C, D** at 316 nM $[Ca^{2+}]_c$: 0 / 2 mM $MgCl_2$, $N.P_o$ = 0.003 / 0.006. **Fig. 3.** Influence of $MgCl_2$ on the Ca^{2+}-dependence; squares: V_h= +40 mV, open squares / filled squares: 0 / 4 mM $MgCl_2$, Hill coefficient: 2.1 / 2.9; circles: V_h= -40mV, O: 0 mM $MgCl_2$, $\bullet_{1/2}$: 2 / 4 mM $MgCl_2$.

Addition of 2 mM $MgCl_2$ more than doubled $N.P_o$ (Fig.

2A to B and C to D), for 316 nM $[Ca^{2+}]_c$ and 4 mM $MgCl_2$ by a factor of 4.6 ± 1.7 (mean ± S.D., n=4, Fig 3). This increase was statistically significant (p=0.005). Addition of $MgCl_2$ marginally replaces calcium from Ca-EGTA. The slight increase in $[Ca^{2+}]_c$ is expected to contribute to the above magnesium-effect with a factor of 1.15 only. Thus, we postulate that Mg^{2+}-ions have a direct effect on the K^+ channel.

Magnesium ions have been suggested to increase the calcium-sensitivity of P_o (Golowasch et al. 1986). This possibility was analysed by plotting log P_o versus pCa. Since at -40 mV the channel opens only once every minute, data in Fig. 3 had to be recorded at +40 mV instead. P_o was normalized by maximal P_o recorded at 2 μM $[Ca^{2+}]_c$. With $[Mg^{2+}]_c$ = 4 the curve was steeper than the one without magnesium (Hill-coefficients increased from 2.1 ± 0.3 to 2.9 ± 0.3). In addition, the pCa of half maximal P_o was shifted from pCa 6.0. Thus, we can indeed call this magnesium effect a calcium-sensitizing effect.

Returning to the possible contribution of i_{KCa} to the resting potential, our results suggest a P_o of 0.0001 for V_m=-40 mV and $[Ca^{2+}]_c$=100 nM. The density of maxi-K^+ channels was estimated to about 8/μm^2 or 16000 per cell, therefore about 2 channels should open simultaneously. This number may be smaller if V_m is -55 mV. At -55 mV and with a bath $[K^+]$ of 5.4 mM K^+, i_{KCa} had an amplitude of 1.0 pA. This current generated on the cells' input resistance (3.4 ± 1.5 GΩ) a voltage drop of -5 mV. Our whole-cell recordings demonstrated similar values for the outward currents as well as for the fluctuations in V_m. We conclude, therefore, that the current through Ca^{2+}-activated maxi-K^+ channels does contribute to the resting potential of vascular and smooth muscle cells.

References

Magleby KL, Palotta BS (1983) Calcium dependence of open and shut interval distributions from calcium-activated potassium channels in cultured rat muscle. J Physiol 344:585-604

Golowasch J, Kirkwood A, Miller CH (1986) Allosteric effects of Mg^{2+} on the gating of Ca^{2+}-activated K^+-channels from mammalian skeletal muscle. J exp Biol 124:5-13