Modulatory effect of extracellular Mg^{2+} ions on K^+ and Ca^{2+} currents of capillary endothelial cells from rat brain

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Abstract **Using whole-cell patch-clamp recording, we demonstrate that exposure of single rat brain capillary endothelial cells to different extracellular Mg^{2+} concentrations (0.3, 4.8 and 9.6 mM) affects the conductance of K^+ and Ca^{2+} currents elicited under control conditions (1.2 mM). Extracellular Mg^{2+} concentrations ($[Mg^{2+}]_o$) of 4.8 and 9.6 mM reversibly depress outward K^+ currents by about 30 ± 12% ($n = 10$) and 34 ± 13% ($n = 10$), at all activating potentials, respectively. Using identical concentrations reversibly depressed the Ca^{2+} current by about 40 ± 16% ($n = 8$) and 46 ± 18% ($n = 6$), respectively. Using a low Mg^{2+} concentration of 0.3 mM, the K^+ current activation was unexpectedly and mildly increased by about 15 ± 5% ($n = 5$), and the inward Ca^{2+} current was attenuated. When studying this effect of low $[Mg^{2+}]_o$ on 'pure' Ca^{2+} currents, free of outward currents, we found that this inward current was depressed by about 38 ± 16% ($n = 8$), and its threshold for activation, in the current-voltage relationship, was shifted to more negative potentials. It is concluded that high $[Mg^{2+}]_o$ hinders the entry of Ca^{2+} through low-voltage activated Ca^{2+} channels and thereby attenuates a Ca^{2+}-regulated K^+ conductance. At a low $[Mg^{2+}]_o$ (0.3 mM), Mg^{2+} shifts the steady-state inactivation of the voltage-activated Ca^{2+} channel to more negative potentials by about 8 mV ($n = 6$), probably due to a negative screening effect, i.e. a reduction of positive charges on the cell membrane. This may contribute to an apparent increase in K^+ conductance by an, as yet, unknown mechanism.**

Key words: Ionic current; Voltage clamp; Mg^{2+} effect; Endothelial cell; Capillary; Brain

1. Introduction

The capillary endothelium of the brain is involved, essentially, in the preservation of the blood-brain barrier and in the regulation of brain microcirculation [1–3]. As a biological membrane, that lines the inner walls of blood vessels, the endothelium is exposed to many environmental alterations of the blood components. It is known that magnesium ions (Mg^{2+}) play important roles in the control of vascular reactivity by modulating myogenic tone and the contractile response of vascular smooth muscle cells to various physiological and pharmacological stimuli [4]. This modulatory effect of Mg^{2+} is thought to result from an interaction with calcium flux across the cell membrane, and its release from internal stores [5–7]. Recent studies on endothelial cells, concerning this property, demonstrate that changes in the $[Mg^{2+}]_o$ can modify the state of the cytosolic free calcium [5].

In this context, the aim of the present study was to investigate whether, in fact, the modulatory effect of $[Mg^{2+}]_o$ could be related to some modulatory effect on the excitability of the cell membrane. We investigated, therefore, in voltage-clamp experiments the effect of different $[Mg^{2+}]_o$ on transmembrane currents of a cell line (E49) cloned from rat brain capillary endothelial cells (BCEC) [8], by using the patch-clamp technique in the whole-cell mode [9]. These capillary cells, as demonstrated previously, contain different K^+ channels, but express, predominantly, a low-threshold voltage-gated Ca^{2+} channel (T type), but no Na^+ channels [10,11].

2. Materials and methods

2.1. Cell culture

Endothelial cells (ECs) were isolated from brain capillaries of 2 week-old rats according to the method of Bowman et al. [12] and cloned by Pietruschka and co-workers [8] to a cell line E49. They were further identified as differentiated endothelial cells through their specific fluorescence induced by the uptake of an acetylated-low density lipoprotein (Dil-Ac-LDL] as described by Voyta et al. [13].

2.2. Recording conditions

Voltage-clamp experiments, using the whole-cell patch-clamp technique [9], were performed on BCECs attached to a glass coverslip and superfused in a polycarbonate chamber with a bath solution consisting of (in mM): NaCl, 135; KCl, 5.6; $CaCl_2$, 2.5; $MgCl_2$, 1.2; glucose, 5.5; HEPES, 10; adjusted to pH 7.4 with NaOH. Patch electrodes were prepared from borosilicate glass (Science Products, Germany) and had a resistance of about 3 MΩ when filled with an internal solution consisting of (in mM): K^+-aspartate, 80; KCl, 50; $MgCl_2$, 1.2; EGTA, 10; HEPES, 10; pH 7.28 with KOH. All solutions were adjusted with sucrose to an osmotic pressure of 300 mOsml. To obtain 'pure' inward Ca^{2+} currents, Na^+ and K^+ ions of the external and pipette solutions were replaced by *N*-methyl-D-glucamine (NMDG) and external Ca^{2+} was increased to 10 mM. BCECs were clamped at holding potentials of −60 or −80 mV to record K^+ or Ca^{2+} currents, respectively. Currents were recorded during 150 ms test pulses to various membrane potentials to determine the current-voltage (*I-V*) relationship. To obtain the Ca^{2+} current steady-state inactivation curve, a test pulse of 50 ms to −20 mV (maximal current activation) was applied from different conditioning prepulses of 150 ms duration (different holding potentials: −90 to −30 mV). The curve was fitted to the Boltzmann equation:

$$I/I_{max} = 1/\{1 + \exp[-(CP - V_{0.5})/k]\}$$

where CP is the amplitude of a conditioning prepulse, $V_{0.5}$ equals the half-maximal inactivation voltage and k is the inactivation slope. An EPC-7 patch-clamp amplifier (Heka Elect., Germany), in combination with a personal computer (HP 486/66M, Germany), controlled generation of voltage-clamp pulse protocols and on-line acquisition, as well as off-line data analyses (CED 1401 voltage-clamp software; Cambridge Elect. Device, UK). All experiments were performed at a temperature of 34°C. Different $[Mg^{2+}]_o$ were applied to the bath through a switching device connected to separate reservoirs by means of miniature valves (Lee Co., Germany), which permitted fast solution changes.

Where appropriate, means ± S.E.M. were calculated and examined for statistical significance by means of Student's *t*-test. Values of $P < 0.05$ were considered to be statistically significant.

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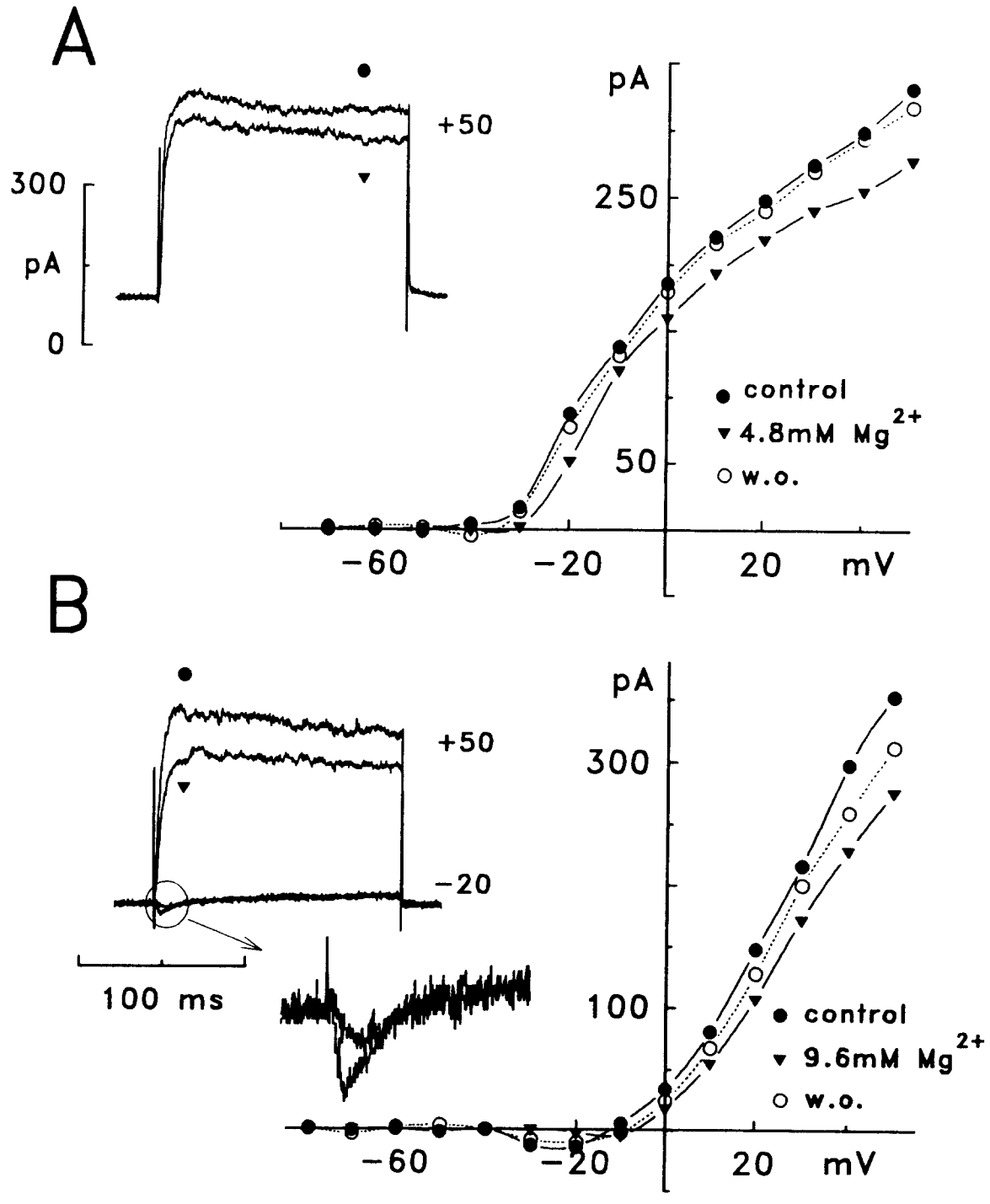

Fig. 1. Effect of two different high $[Mg^{2+}]_o$ (4.8 and 9.6 mM) on outward K^+ and inward Ca^{2+} currents. (A) *I-V* curve of the K^+ current from one cell of $n = 10$, elicited from a Hp of −60 mV when exposed to control conditions (1.2 mM Mg^{2+}, filled circles), high Mg^{2+} (4.8 mM, filled triangles), and after recovery (wash out, open circles). The inset, left panel, shows the original current traces elicited by a test pulse to +50 mV, under control (filled circle) and high Mg^{2+} conditions (filled triangle). (B) *I-V* curve of large K^+ and small Ca^{2+} currents elicited from a Hp of −80 mV, under control conditions (filled circles), and plotted from the current amplitude at the time indicated by the filled symbols versus the membrane potential. The figure illustrates the inhibitory effect of 9.6 mM Mg^{2+} on the K^+ (filled triangle) and Ca^{2+} current (inset: original traces indicated by the arrow).

3. Results and discussion

Fig. 1 illustrates the effect of changing the $[Mg^{2+}]_o$ from 1.2 to 4.8 or to 9.6 mM, on outward and inward currents of BCECs. Fig. 1A shows the *I-V* curve of K^+ currents elicited from a holding potential (Hp) of −60 mV, of one representative experiment, from $n = 10$. It can be seen that the current amplitude was depressed reversibly by 4.8 mM Mg^{2+} at all activating potentials. In this case, as shown in the inset of the figure, with original trace currents elicited at a potential of +50 mV, the inhibition amounted to 17% (30 ± 12%, $n = 10$). As illustrated in Fig. 1B, when $[Mg^{2+}]_o$ was changed from 1.2

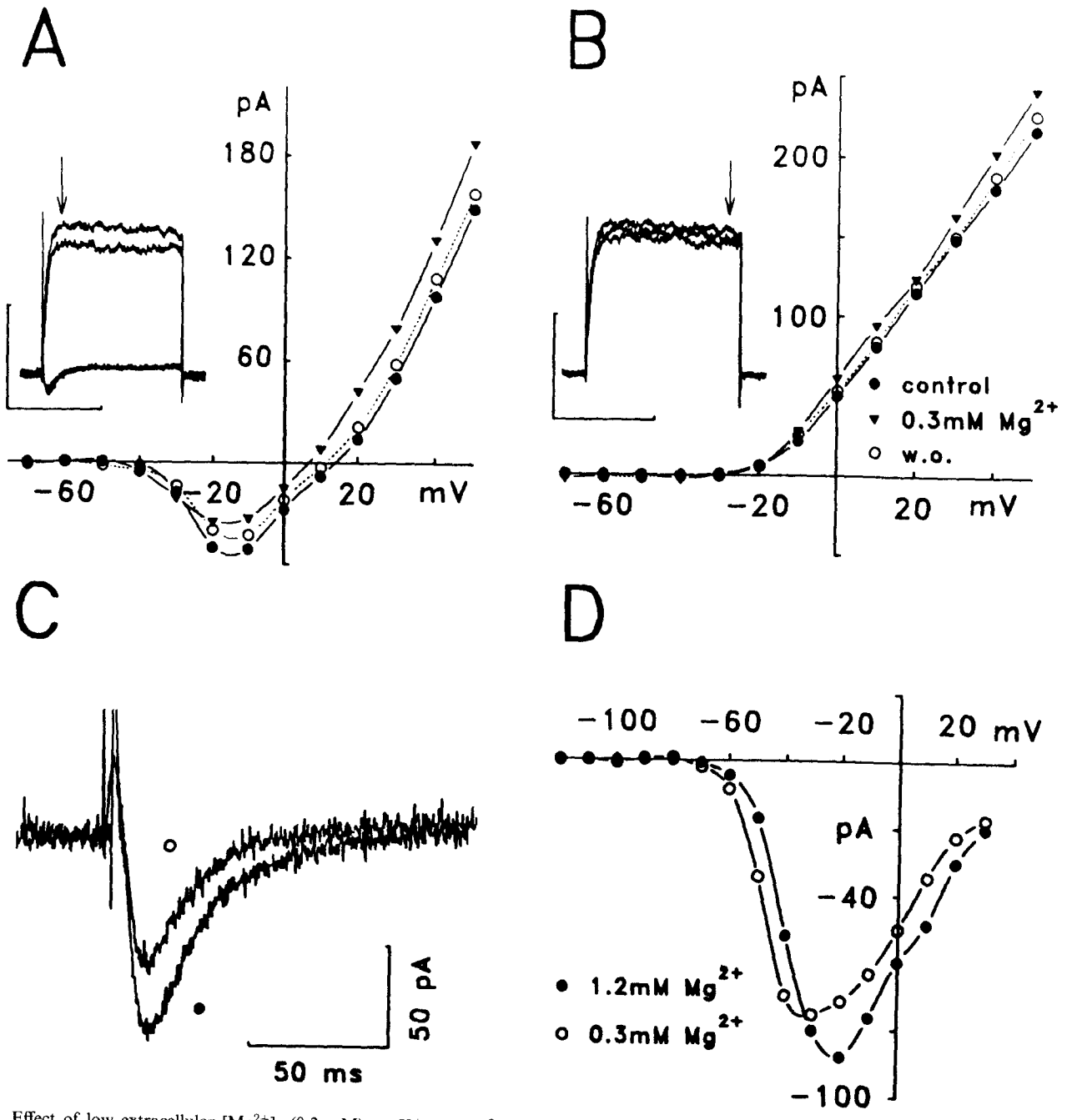

Fig. 2. Effect of low extracellular $[Mg^{2+}]_o$ (0.3 mM) on K^+ and Ca^{2+} currents. (A) *I-V* curve of K^+ and Ca^{2+} currents elicited from a Hp of −80 mV to show the mild activation induced by low Mg^{2+} on the K^+ current measured at the time indicated by the arrow ('instantaneous current'), as well as the inactivation of the Ca^{2+} current. Inset: original current traces of K^+ (outward) and Ca^{2+} current. (B) *I-V* curve of the K^+ current plotted from the current amplitude at the time indicated by the arrow ('steady-state current'). Inset: original traces of K^+ currents elicited during control, low Mg^{2+} and wash out conditions. (C) Progress of a 'pure' Ca^{2+} current elicited from a Hp of −80 mV, during control conditions (filled circle) and after low $[Mg^{2+}]_o$ (open cirle) in one cell of $n=8$. (D) *I-V* curve plotted from the maximal Ca^{2+} current amplitude shown in (C), versus the membrane potential, to illustrate a mild shift to the left produced by low $[Mg^{2+}]_o$. The calibration bars in the inset of (A,B) correspond to 200 pA (vertical) and 100 ms (horizontal).

to 9.6 mM, the K^+ current, elicited this time at a Hp of −80 mV, was depressed in the same form, by approx. 20% ($34 \pm 13\%$, $n=10$). The inhibitory effect on K^+ currents, produced by 4.8 and 9.6 mM $[Mg^{2+}]_o$, was statistically significant as compared to control values ($P<0.05$) but not between them. Since inward Ca^{2+} currents could be elicited from this Hp, the inhibitory effect of 9.6 mM Mg^{2+} on this current, of about 60% ($46 \pm 18\%$, $n=6$), can be observed in the inset. This blocking effect of high Mg^{2+} was also found on pure Ca^{2+} currents elicited with no contaminating K^+ currents (not shown). The blocking property of high Mg^{2+} on low voltage-activated Ca^{2+} currents (T-type) is consistent with similar findings reported for sensory neurones [14]. Since Mg^{2+} is a mild Ca^{2+} channel blocker [15,16], in addition, its inhibitory effect on the K^+ current corroborates the presence of Ca^{2+}-activated K^+ channels in BCECs, as previously demonstrated with charybdotoxin [10]. On the other hand, our new data support the inhibitory and protective properties of Mg^{2+} on

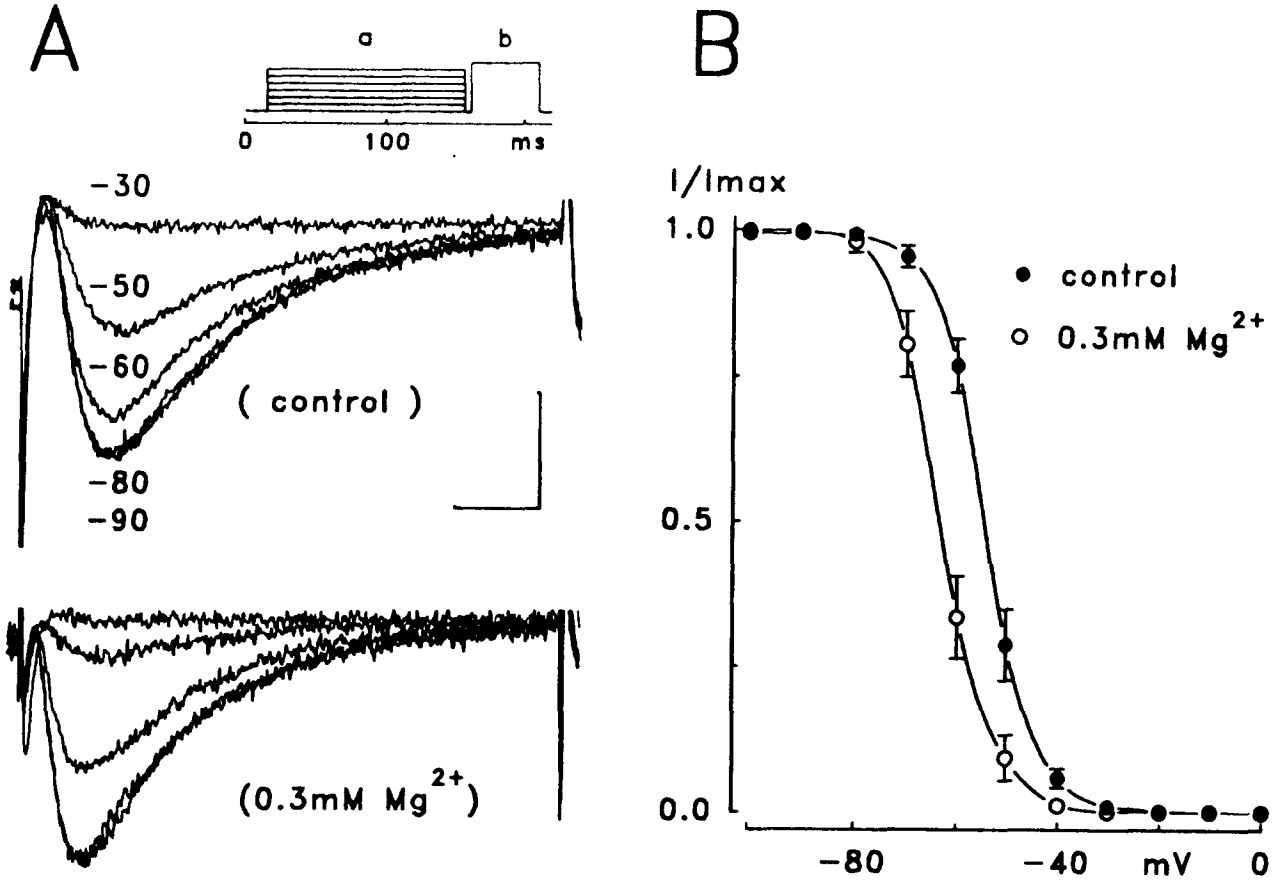

Fig. 3. 'Pure' Ca^{2+} currents and their steady-state inactivation curve. (A) Illustrates not contaminated ('pure') Ca^{2+} currents elicited by a test pulse **b** that starts from different conditioning holding potentials (prepulse **a**) of a pulse protocol illustrated in the inset. Note the original current traces under control conditions (1.2 mM Mg^{2+}, upper panel) and under low Mg^{2+} (0.3 mM, lower panel). The calibration bars correspond to 20 pA (vertical) and 10 ms (horizontal). (B) Ca^{2+} currents elicited by the test pulse **b** from the same cell exposed first to a solution with control extracellular Mg^{2+} (1.2 mM) and low Mg^{2+} concentration (0.3 mM). Note the conditioning holding potentials located near the Ca^{2+} current (control) that were elicited by a single test pulse to −20 mV.

blood vessel tone [6]. When BCECs were exposed to a low Mg^{2+} concentration of 0.3 mM, both the K^+ and the Ca^{2+} current, elicited together from a Hp 0f −80 mV, unexpectedly were slightly increased and depressed, respectively, as illustrated in Fig. 2A. However, this activating effect on the K^+ current was observed, principally, in the 'instantaneous' component of the current, as shown in the *I-V* curve of Fig. 2A, whereas the 'steady-state' component, measured after 140 ms from onset, did not exhibit a significant change (Fig. 2B). It would appear that through a change of normal (1.2 mM) to low $[Mg^{2+}]_o$ (0.3 mM) the LVA Ca^{2+} current was more affected, which, as illustrated in Fig. 2C with an original trace of a pure Ca^{2+} current, was apparently attenuated. This effect is further illustrated in Fig. 2D, which shows the *I-V* curve plotted from the peak amplitude of this inward current versus the membrane potential, that clearly demonstrated that under low Mg^{2+}, this curve was shifted slightly to the left (n=8, $P < 0.05$).

Since this unexpected reduction of Ca^{2+} currents induced by low $[Mg^{2+}]_o$ is in contradistinction to its inhibitory effect at high concentrations (Fig. 1B), we suspected some kind of a negative screening effect rather than a 'true' current inhibition. Therefore, we further analyzed the steady-state inactivation of the Ca^{2+} current under low $[Mg^{2+}]_o$ conditions (0.3 mM). Fig. 3 demonstrates the sequence of these experiments. Fig. 3A illustrates superimposed Ca^{2+} currents elicited by the test pulse **b** from the pulse protocol (inset, upper panel), under control (upper panel, $n = 9$) and low $[Mg^{2+}]_o$ conditions (lower panel, $n = 9$). Cells were first stimulated with a prepulse (**a**) of 150 ms duration from various holding potentials (range −90 to −10 mV) followed by a single test pulse (**b**) of 50 ms to −20 mV (maximal current activation). Fig. 3B illustrates this action of low $[Mg^{2+}]_o$ on the steady-state inactivation curve more clearly. The curve was plotted by normalizing each Ca^{2+} current, as shown in Fig. 3A, to the maximal inward current measured during the test pulse versus the conditioning voltage of the prepulse. Both curves (control and low Mg^{2+}) fitted to the Boltzmann equation (see Section 2) illustrate that low $[Mg^{2+}]_o$ shifted the half-maximal inactivation voltage from -54.5 ± 1.8 mV (control) to -62.6 ± 1.7 mV (low Mg^{2+}), by about 8.07 ± 2.9 mV to a more hyperpolarizing potential ($n = 6$, $P < 0.01$).

These results support the contention that extracellular Mg^{2+} affects both the inactivation and activation of the Ca^{2+} channel and, therefore, in this way, the contractility of blood vessels via direct effects on vascular muscle and indirect effect on ECs, per se. Taking into account all our results, it could be suggested that elevation of extracellular Mg^{2+} would be expected to depolarize BCECs by depressing K^+ channels (Fig. 1), assuming that the membrane potential of BCECs is

controlled primarily by this ion [10]. In contrast, decreasing its concentration could bring the cell toward hyperpolarization according to the Ca^{2+} inactivation (Fig. 3). Lowering $[Mg^{2+}]_o$, as shown herein, appears to increase slightly the instantaneous K^+ current by an, as yet, unknown mechanism related to Ca^{2+} channel inactivation. However, since the current amplitude itself does not seem to change, this increase might not be implicated in the contractility of blood vessels.

As the concentration of extracellular Mg^{2+} has been shown recently to influence endothelial-derived relaxant factors (EDRF) [17,18], and nitric oxide (NO) generation [19], it is distinctly possible that the electrophysiological actions shown herein may be pivotal in elaboration of EDRF and NO. It may be of more than passing interest to note here: (1) that low $[Mg^{2+}]_o$ promotes EDRF-dependent vasodilation of intact canine coronary arteries [18,19]; and (2) the $[Mg^{2+}]_o$ used here, (0.3 mM), has been found recently, through the use of Mg^{2+} ion-selective electrodes, to be present in serum in some patients with ischemic heart disease and stroke [20].

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