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ORIGINAL ARTICLE

EXTRACELLULAR MG^{2+} LEVEL AFFECTS THE MAJOR MECHANISM OF ENDOTHELIUM-DEPENDENT RELAXATION IN RESISTANCE ARTERIES

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Abstract

Mg^{2+} affects vascular smooth muscle contraction by direct and endothelium-dependent pathways. The major mechanism of endothelium-dependent relaxation (EDR) in resistance arteries is independent of nitric oxide synthase and cyclooxygenase and mediated by Ca^{2+}-activated K^+ channels (sK and iK). We tested the effect of modified extracellular Mg^{2+} (Mg_e) concentration upon this mechanism, in isolated small mesenteric arteries from rat. EDR was induced by carbachol 10^{-8} - 10^{-4} M, in rings contracted by phenylephrine 10^{-5} M, in the presence of N(G)-Nitro-L-arginine methyl ester (L-NAME) 10^{-4} M and indomethacin 10^{-5} M, with [Mg_e] kept at 2, 1.2, or 0.8 mM. This EDR is enhanced by high [Mg_e] and reduced by low [Mg_e], vs. the one in regular [Mg_e] of 1.2 mM ($n = 6$; $p < 0.01$). Mg_e similarly modulates relaxation induced by SKA-31 (sK/iK activator). These new Mg_e actions do not involve known Mg^{2+} effects on sK/iK and may be relevant in Mg therapy.

Rezumat

Mg^{2+} afectează contracţia muşchiului neted vascular pe căi directe şi dependente de endoteliu. Mecanismul major de relaxare endoteliu-dependentă (RED) în artere de rezistenţă este independent de nitric oxid sintază şi ciclooxigenază, mediat de canale de K^+ activate de Ca^{2+} (sK şi iK). A fost testat efectul modificării concentraţiei de Mg^{2+}extracelular (Mg_e) asupra acestui mecanism, în artere mezenterice mici izolate de la şobolan. RED a fost indusă de carbacol 10^{-8} - 10^{-4} M, în inele contractate de fenilefrină 10^{-5} M, în prezenţă de L-NAME 10^{-4} M şi indometacin 10^{-5} M, cu [Mg_e] menţinută la 2, 1,2 sau 0,8 mM. Această RED este mărită de [Mg_e] crescută şi redusă de [Mg_e] scăzută, vs. cea în [Mg_e] obişnuită de 1,2 mM ($n = 6$; $p <$ 0,01). Mg_e modulează similar relaxarea indusă de SKA-31 (activator sK/iK). Noile acţiuni ale Mg_e nu implică efecte cunoscute ale Mg^{2+} pe sK/iK şi pot fi relevante în terapia Mg.

Keywords: Mg^{2+}, endothelium-dependent relaxation, resistance arteries, EDH

Introduction

Besides blood flow distribution, small arteries and arterioles are key factors of total peripheral resistance, thus of systemic arterial blood pressure (ABP). Many studies assessed the Mg importance for ABP modulation [15]. Extracellular Mg^{2+} (Mg_e) is a vasodilator when [Mg_e] is increased enough from normal values. Mg_e-induced vasodilation involves: direct relaxation of vascular smooth muscle (VSM) andendothelium-dependent relaxation (EDR) of VSM mediated by nitric oxide (NO) [24]. NO-mediated EDR is blunted in absence of Mg_e [1]. But the major EDR mechanism in resistance arteries is independent of NO-synthase (NOS) and cyclo-oxygenase (COX) and mediated by endothelium-dependent hyperpolarization (EDH) [9, 22]. EDH-mediated EDR relies on two types of endothelial Ca^{2+}-activated K^+ channels (K_{Ca}) [4-6, 17]: apamin-sensitive of small conductance (sK) (also called sK_{Ca}, SK3, $K_{Ca}2.3$; gene KCNN3) and charybdo-toxin-sensitive of intermediate conductance (iK) (also called iK_{Ca}, SK4, $K_{Ca}3.1$, IK1; gene KCNN4). EDH-mediated EDR is: counter-regulator of sympathetic vasoconstriction [13]; important in flow autoregulation, myogenic response, and vasomotion [9]; compensatory in endothelial dysfunction, but also involved in its pathogenesis [8, 10-12].

Mg_e effects upon EDH-mediated EDR are unknown; only one study [23], but remotely related addressed this issue, as follows. High [K^+] in cardioplegic solutions inhibits EDH-mediated EDR and this inhibition can be relieved by high Mg concentrations [23], but the effects of Mg_e itself on EDH-mediated EDR were not studied. Two other studies may be close to the subject, but Mg effects on EDH-mediated EDR were not tested [2, 3]. They studied the effect of chronic Mg upon l-NAME-induced hypertension and also tested EDR of isolated arteries [2, 3], but in one study the effect of $[Mg]_e$ in the physiological saline solution (PSS) was not examined [3], while in the other only the effect of high $[Mg]_e$ in the PSS (4.8 mM) was tested on EDR but not on EDH-mediated EDR [2]. In the context of sustained interest in EDH-

mediated EDR [9, 16, 19, 22], the present study was initiated.

Materials and Methods

Male adult Wistar rats (~200 g) were euthanized. Jejunal loops were placed in PSS, containing (mM): NaCl 119; KCl 4.7; $CaCl_2$ 2.5; $MgSO_4$ 1.2; $NaHCO_3$ 25; KH_2PO_4 1.18; glucose 5.5. In PSS at ~5ºC, 1 mm rings from first order branches of mesenteric artery were dissected and mounted in wire myographs. Rings were then oxygenated (95% O_2 + 5% CO_2) and kept at 37ºC (pH 7.2 - 7.4). After 30 minutes equilibration under 1 g passive tension, rings were checked twice for complete EDR by 10^{-5} M carbamoyl-choline (CAR) upon contraction by 10^{-5} M phenyl-ephrine (PHE). Then PSS was either kept with normal [Mg_e], or switched to higher or lower [Mg_e]; 2 mM or 0.8 mM [$MgSO_4$] in PSS, modified by the respective isoosmotic replacements between $MgSO_4$ and NaCl; full randomisation and 30 minutes re-equilibration. In the presence of 10^{-4} M N(G)-Nitro-L-arginine methyl ester (L-NAME) and 10^{-5} M indomethacin (IND), rings were again contracted by PHE 10^{-5} M and randomly tested for EDH-mediated EDR, as induced by cumulative concentrations (10^{-8} M to 10^{-5} M) of either CAR or Naphtho[1,2-d]thiazol-2-ylamine (SKA-31) (specific activator of sK and iK, including tests on resistance arteries [14]). Each effect, for each dose tested, was allowed 5 minutes of stable *plateau* before the next test. Inhibition of active tension at each concentration of the relaxing agent is expressed as % of complete relaxation from contraction by PHE 10^{-5} M; data as mean ± SD (n = 6; two-way ANOVA and t-test; significance for $p < 0.05$). Only one of the six different experimental protocols were applied to each ring. Time-match paired rings confirmed the 30 minutes stability of PHE-induced contraction ($p < 0.05$ for each time-match). This study was approved by the Research Ethics Commission of the host institution. All procedures comply with guide-lines from: Act on Animal Experimentation and Animal Health and Welfare from Romania; Universities Federation for Animal Welfare; Directive 2010/63/EU of the European Parliament and of the Council of 22 September 2010.

Results and Discussion

In the context of massive research on EDR [22], different published studies also tested the interplay of Mg and NO-mediated EDR [1, 24]. In the present study we show that [Mg_e] affects EDH-mediated EDR, the major EDR pathway in resistance arteries. As detailed in the introduction, [Mg_e] effects on EDH-mediated EDR have not been previously tested, not even in the three studies a bit more closely related to the issue [2, 3, 23]. We observed EDH-mediated EDR tends to increase in higher [Mg_e] and decrease in lower [Mg_e], which could be explained by Mg^{2+} actions involving sK/iK, key players in EDH-mediated EDR. In order to test this hypothesis, we used SKA-31 as specific sK/iK activator [14]. The initial observation and hypothesis were confirmed (Figures 1 and 2), but after preliminary tests.

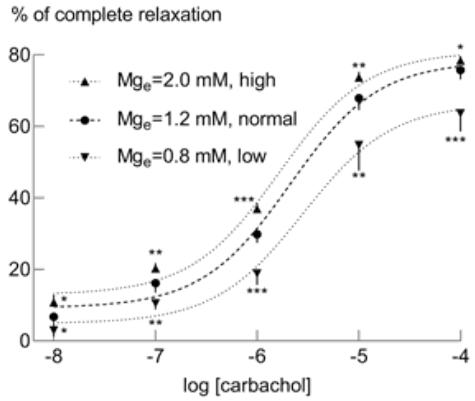

Figure 1.
NO-synthase- and cyclooxygenase-independent endothelium-dependent relaxation of isolated resistance arteries by acetylcholine analogue, affected by extracellular Mg level (Mg_e)
(* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$)

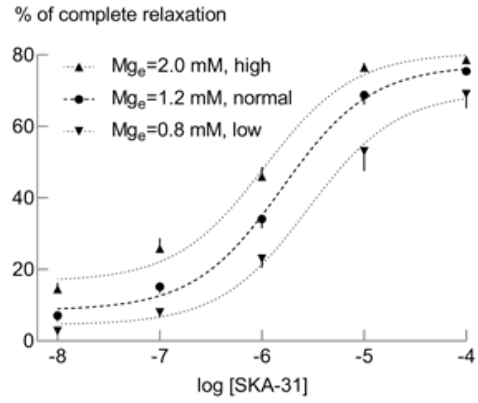

Figure 2.
NO-synthase- and cyclooxygenase-independent endothelium-dependent relaxation of isolated resistance arteries by sK/iK activator, affected by extracellular Mg (Mg_e)
($p < 0.01$ *vs*. normal Mg_e at any [SKA-31])

First we decided that the tested [Mg_e] to be 2 mM and 0.8 mM, i.e. the least modified [Mg_e] expected to affect EDH-mediated EDR without inducing VSM tone changes by themselves. Isometric tension was similar ($p > 0.05$; paired t-test) upon [Mg_e] switch alone, from 1.2 mM to 0.8 mM (n = 6) or to 2 mM (n = 6), in rings at rest (n = 6) or contracted by PHE 10^{-5} M in the presence of L-NAME 10^{-5} M and IND

10^{-5} M (n = 6). Conditions (Figures 1 and 2) differ only by [Mg_e]; controls show [Mg_e] effects on EDH-mediated EDR are not biased by [Mg_e] effects on resting/activated vascular tone.

We cannot detail if [Mg_e] effects (Figures 1 and 2) involve changed intracellular [Mg^{2+}] ([Mg_i]). But we note the only two studies on the effect of Mg_i on sK/iK; patch-clamp shows Mg_i inhibits sK/iK [18, 21]. This cannot explain the [Mg_e] effects we discovered. Mg_e effects on iK/sK and Mg_i effects on EDH-mediated EDR seem unknown. So, sK/iK stimulation by Mg^{2+} has not been described. Our results cannot be explained by sK/iK inhibition by Mg^{2+} (directly [16, 21] or indirectly by reducing Ca^{2+} influx), because such actions would lead to effects opposite to our data. This also applies to iK in endothelial projections, functioning in a feedback loop with L-type Ca^{2+} channels (Ca_L) in VSM [9, 17]. But Mg_e inhibits Ca_L in VSM [20, 25], the major Ca^{2+} influx for VSM contraction. VSM has iberiotoxin-sensitive K_{Ca} with large conductance (BK) (also called BK_{Ca}, $K_{Ca}1.1$, MaxiK, slo1). Mg_e stimulates BK in VSM [7]. Mg_e promotes VSM hyperpolarization and relaxation, by Ca_L inhibition and BK stimulation. Such Mg_e actions in VSM, during EDH-mediated EDR, may explain how the latter is reduced in lower [Mg_e] and enhanced in higher [Mg_e].

The [Mg_e] effects on CAR-induced EDH-mediated EDR (Figure 1) are mimicked by those on relaxation induced by direct sK/iK activation (Figure 2); [Mg_e] affects the sK/iK common path of EDH-mediated EDR, beyond the variable mechanistic details [19, 22]. We underline we tested small [Mg_e] changes, for which preliminary studies confirmed the lack of VSM relaxation/contraction by Mg_e itself. Finally, endothelium-dependent contracting factors (EDCF) [22] may be involved in [Mg_e] effects tested here, except COX-dependent EDCF, since COX was blocked by IND.

Our results represent a change of view, adding [Mg_e] effects on EDH-dependent EDR to known Mg benefits: direct and NO-mediated VSM relaxation, antioxidant, anti-inflammatory. This novel path may be relevant in hypertension with Mg deficit and for benefits of Mg supplementation [15, 26]. Our data support: the benefit of normal and increased [Mg_e], acting *via* normal and enhanced EDH-mediated EDR respectively and the detriment of decreased [Mg_e], acting *via* diminished EDH-mediated EDR. Major advances could arise hereafter, with impact on the future of Mg therapy.

Conclusions

EDH-mediated EDR, the major EDR mechanism in resistance arteries, is inhibited by decreased [Mg_e] and enhanced by increased [Mg_e]. These [Mg_e] effects do not involve any known interaction of Mg^{2+} with endothelial sK/iK, but could be mediated by established Mg_e effects on VSM, i.e. Ca_L inhibition and BK stimulation. [Mg_e] effects on EDH-dependent EDR might be important in Mg pharmacotherapy.

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