Effect of Magnesium on Calcium Responses to Vasopressin in Vascular Smooth Muscle Cells of Spontaneously Hypertensive Rats

RHIAN M. TOUYZ, PASCAL LAURANT and ERNESTO L. SCHIFFRIN

MRC Multidisciplinary Research Group on Hypertension (R.M.T., E.L.S.), Clinical Research Institute of Montreal, University of Montreal, Montreal (Quebec) Canada, Laboratoire Physiologie (P.L.), Pharmacologie et Nutrition Préventive Expérimentale, UFR Médecine et Pharmacie, Université de Franche-Comté, Besançon, France

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ABSTRACT

This study investigated the modulatory effect of magnesium (Mg\(^{2+}\)) on basal and agonist-stimulated intracellular free calcium (Ca\(^{2+}\)) concentration ([Ca\(^{2+}\)]\(_i\)) in vascular smooth muscle cells from spontaneously hypertensive rats (SHR). Effects of increasing extracellular Mg\(^{2+}\) concentration ([Mg\(^{2+}\)]\(_e\)) on vasopressin (AVP)-induced [Ca\(^{2+}\)]\(_i\) responses were determined in primary cultured unpassaged vascular smooth muscle cells from mesenteric and aortic vessels (representing resistance and conduit arteries, respectively) of Wistar Kyoto rats (WKY) and SHR. [Ca\(^{2+}\)]\(_i\) was measured by fura-2 methodology. Underlying mechanisms for Mg\(^{2+}\) actions were determined in Ca\(^{2+}\)-free buffer and in the presence of diltiazem (10\(^{-6}\) M), an L-type Ca\(^{2+}\) channel blocker. Basal and AVP-stimulated [Ca\(^{2+}\)]\(_i\) responses were significantly increased (p < 0.05) in SHR (pD\(_2\) = 8.3 ± 0.1, E\(_{max}\) = 532 ± 14 nM for SHR; pD\(_2\) = 8.0 ± 0.04, E\(_{max}\) = 480 ± 15 nM for WKY). [Mg\(^{2+}\)]\(_e\) dose-dependently reduced basal and agonist-induced [Ca\(^{2+}\)]\(_i\) responses. High [Mg\(^{2+}\)]\(_e\) (4.8 mM) attenuated [Ca\(^{2+}\)]\(_i\) responses to AVP in WKY (E\(_{max}\) = 328 ± 30 nM and SHR (E\(_{max}\) = 265 ± 27 nM) and normalized AVP-elicted hyper-responsiveness in SHR (pD\(_2\) = 30 nM and high [Mg\(^{2+}\)]\(_e\), 8.1 ± 0.3 for SHR, 7.8 ± 0.6 for WKY). Extracellular Ca\(^{2+}\) withdrawal and diltiazem abolished the attenuating effects of high [Mg\(^{2+}\)]\(_e\) in WKY but not in SHR. These findings demonstrate that Mg\(^{2+}\) dose-dependently reduces [Ca\(^{2+}\)]\(_i\) and that high [Mg\(^{2+}\)]\(_e\) attenuates AVP-stimulated [Ca\(^{2+}\)]\(_i\) responses and normalizes sensitivity to AVP in SHR. In WKY, Mg\(^{2+}\) actions are dependent primarily on Ca\(^{2+}\) influx through L-type Ca\(^{2+}\) channels, whereas in SHR, the modulatory effects of [Mg\(^{2+}\)]\(_e\) are mediated both by Ca\(^{2+}\) influx through Ca\(^{2+}\) channels and by intracellular Ca\(^{2+}\) release.

Magnesium, the second most abundant intracellular cation, plays an important physiologic role in regulating vascular tone and contraction and a potentially pathophysiologic role in hypertension. Aberrations in Mg\(^{2+}\) metabolism have been demonstrated in genetic and experimentally induced hypertension, as well as in patients with essential and malignant hypertension (Berthelot et al., 1987; Paolisso and Barbagallo, 1997; Touyz and Milne, 1995). Intracellular and serum Mg\(^{2+}\) concentrations are reduced in SHR and DOCA-salt SHR (Ng et al.; 1992, Touyz et al., 1991; Wells and Agrawal, 1991), dietary or acute administration of Mg\(^{2+}\) reduces blood pressure (Berthelot and Esposito, 1983; Makynen et al., 1995) and the reactivity of vessels from SHR and DOCA-salt hypertensive rats to varying [Mg\(^{2+}\)]\(_e\) is altered (Altu and Altura, 1983; Altura and Altura, 1985; Laurant and Berthelot, 1994). We recently demonstrated that vascular tone and [Ca\(^{2+}\)]\(_i\), responses induced by AVP, a potent vasoconstrictor that has been implicated in the pathogenesis of hypertension, are greater in SHR than in normotensive WKY arteries (Touyz and Schiffrin, 1996a; Yang et al., 1996). In addition, when pressurized mesenteric arteries of SHR were exposed to high [Mg\(^{2+}\)]\(_e\), the AVP-stimulated vasoconstriction and sensitivity to AVP were reduced (Laurant et al., 1997).

The exact cellular mechanisms underlying the modulatory actions of Mg\(^{2+}\) on vascular function are unclear, but the inter-relationships between Mg\(^{2+}\) and Ca\(^{2+}\) may be important. Mg\(^{2+}\) counteracts the actions of Ca\(^{2+}\) and has been suggested to be nature’s physiologic blocker because it exhibits a pharmacologic profile similar to that of synthetic Ca\(^{2+}\) channel antagonists (Alboreh et al.; 1992, Iseri and French, 1984). Mg\(^{2+}\) influences Ca\(^{2+}\) entry, binding, translocation and intracellular mobilization (Altura et al., 1982; Kawai et
al., 1996; Resnick, 1992) in VSMC, so changes in [Mg$^{2+}$]$_i$ lead to changes in [Ca$^{2+}$]$_i$. Increased [Ca$^{2+}$]$_i$ is associated with the activation of protein kinase C and myosin light-chain kinase, which results in vascular smooth muscle contraction (Rembold and Murphy, 1988). In SHR, basal and agonist-stimulated [Ca$^{2+}$]$_i$, responses are increased, and [Mg$^{2+}$]$_i$, and [Ca$^{2+}$]$_i$, are reduced (Adachi et al., 1994; Saito et al., 1995; Sharma and Bhalla, 1989; Touyz et al., 1994). We questioned whether changes in [Mg$^{2+}$]$_i$ contribute to agonist-induced VSMC [Ca$^{2+}$]$_i$, hyper-responsiveness in hypertension. Although a number of studies in normal conditions have demonstrated that increased [Mg$^{2+}$]$_i$, is associated with decreased [Ca$^{2+}$]$_i$, and that agonist-stimulated [Ca$^{2+}$]$_i$, responses are potentiated by reduced [Mg$^{2+}$]$_i$, (Hwang et al., 1992; Zhu et al., 1995; Zhang et al., 1992), little is known about the inter-relationships among Mg$^{2+}$, Ca$^{2+}$ and vascular smooth muscle responses in hypertension.

The purpose of this study was to determine the modulatory effect of Mg$^{2+}$ on basal [Ca$^{2+}$]$_i$, in VSMC from SHR and to assess whether modifications in response to Mg$^{2+}$ influence AVP-stimulated [Ca$^{2+}$]$_i$, hyper-responsiveness in hypertension. We also investigated mechanisms that might underlie the [Ca$^{2+}$]$_i$ actions of Mg$^{2+}$ by assessing the contributory roles of Ca$^{2+}$ influx, intracellular Ca$^{2+}$ release and [Mg$^{2+}$]$_i$. Most previous studies were performed using cultured passaged VSMC. However, passaging of cells results in morphological, biochemical and functional phenotypic changes such that passaged cells are very different from the primary cells from which they were derived. In the present study, we examined VSMC from mesenteric arteries, which contribute to peripheral resistance, and from aortic vessels, which are conduit arteries, and used only primary culture unpassaged cells, which exhibit a contractile phenotype and have undergone little phenotypic change relative to the original smooth muscle cells in blood vessels. SHR were studied at 17 weeks of age, at which stage hypertension is established, and were compared with normotensive control WKY of comparable age.

**Materials and Methods**

**Materials.** The following drugs and chemicals were used in this study: AVP (Peninsula Laboratories Inc, Belmont, CA), fura 2-ace-toxymethyl ester (fura-2AM), mag fura 2AM and pluronic F-127 (Molecular Probes Inc., Eugene, OR), dimethyl sulfoxide (Anachemia Canada Inc., Montreal, Quebec, Canada), dilitiazem (Sigma Chemical Co., St. Louis, MO), DMEM (Gibco Canada, Mississauga, Ontario, Canada) and Hams F-12 medium (Flow Laboratories Inc., McLean, VA). All other chemicals were from Fisher Scientific Co. (Fair Lawn, NJ) and BDH Inc. (Darmstadt, Germany).

**Rats.** The study was approved by the Animal Ethics Committee of the IRCM and was carried out according to the recommendations of the Canadian Council for Animal Care. Male WKY (n = 14) and SHR (n = 20) (Taconic Farms Inc., Germantown, New York) were used. The rats were housed under standardized conditions (12-h light-dark cycle, at constant temperature (22°C) and relative humidity (60%)) in the animal unit at the IRCM.

Systolic blood pressure was recorded in prewarmed (external temperature 37°C) conscious rats by the tail-cuff method, using a photoelectric pulse sensor (model PCPB) and a polygraph (model 7; Grass Instruments Co., Quincy, MA), a few days before experimentation.

**Cell culture.** The rats were killed by decapitation. VSMC derived from thoracic aorta and mesenteric arteries were isolated, phenotypically characterized and propagated, as described in detail previously (Schiffrin et al., 1986; Touyz et al., 1994). Briefly, arteries were cleaned of adventitious and connective tissue, smooth muscle cells were dissociated by digestion, the tissue was filtered and the cell suspension was centrifuged and resuspended in DMEM containing heat-inactivated calf serum, 1-gluconate, HEPES, penicillin and streptomycin. VSMC were grown on round glass coverslips (25 mm in diameter) in plastic 6-well dishes and maintained at 37°C in a humidified incubator in an atmosphere of 95% air, 5% carbon dioxide. Cells were studied at confluency. Before experimentation, confluent cultures of VSMC were rendered quiescent by serum deprivation and maintained in a serum-free medium for 36 h.

**Measurement of [Ca$^{2+}$]$_i$, [Ca$^{2+}$]$_i$, was measured with the ratio- metric fluorescent dye fura-2AM according to previously described methods (Moore et al., 1990; Touyz et al., 1994). On the day of the study, the culture medium was replaced 30 min before loading with warmed (37°C) modified Hanks’ buffered saline containing (in mM) 137 NaCl, 4.2 NaHCO$_3$, 3 NaHPO$_4$, 5.4 KCl, 0.4 KH$_2$PO$_4$, 1.3 CaCl$_2$, 0.5 MgCl$_2$, 0.8 MgSO$_4$, 10 glucose and 5 HEPES (pH = 7.4). The cells, attached to the glass coverslips were washed three times with 2 ml of modified Hanks’ buffer. The washed cells were loaded with fura-2AM (4 μM) that was dissolved in dimethyl sulfoxide containing 0.02% pluronic F-127 and incubated for 30 min at 37°C in a humidified incubator (5% CO$_2$, 95% air). Under these loading conditions, the ratio metric (343/380) fluorescence cell images were homogeneous, which indicates that there was no significant intracellular compartmentalization of fura-2. The loaded cells were then washed three times with Hanks’ buffer and used after a 5-min stabilization period. All washing procedures and experiments were performed at room temperature to minimize compartmentalization and cell extrusion of the dye. Four glass rings (4–5 mm in diameter) were placed on the coverslip containing cells, and a seal was formed between the ring and the coverslip using vacuum grease (Dow Corning, Midland, Michigan). Each ring was filled with 50 μl of warmed Hanks’ buffer.

This method allowed for four separate experiments per coverslip. [Ca$^{2+}$]$_i$, was measured in single cells in cell clusters by fluorescent digital imaging. The advantages of this system are that multiple cells can be examined simultaneously and that the cells under investigation can be imaged throughout the experiment. Cells were investigated using an Axiovert 135 inverted microscope (40× oil immersion objective) and Attofluor Digital Fluorescence System (Zeiss, Germany), using alternating excitation wavelengths of 343 nm and 380 nm. Video images of fluorescence at 520-nm emission were obtained using an intensified CCD camera system (Zeiss) with the output digitized to a resolution of 512×480 pixels. Images of fluorescence ratios were then obtained by dividing, pixel by pixel, the 343-nm image by the 380-nm image after background subtraction. [Ca$^{2+}$]$_i$, was calculated by in situ calibration techniques using the formula of Grynkiewicz et al. (1985):
scribed above for [Ca$$^{2+}$$], measurements, were loaded with mag-fura-2 AM (5 µM) and incubated for 30 min at 37°C in a humidified incubator (95% air, 5% CO$_2$). The loaded cells were then washed three times with warmed (37°C) buffer and incubated for a further 15 min to ensure complete de-esterification. Cells were finally washed once with fresh buffer before measurement of [Mg$$^{2+}$$], [Ca$$^{2+}$$], was determined using an emission wavelength of 520 nm and alternating excitatory wavelengths of 343 nm and 380 nm. The Attofluor system was calibrated by viewing mag-fura-2, tetrapotassium salt solutions containing zero and saturating magnesium concentrations and then including these data in the ratio calculations for construction of a standard curve relating magnesium concentration to the 343/380 ratio. The curve was derived from the Grynkiewicz formula (Grynkiewicz et al., 1985) as above for [Ca$$^{2+}$$], where R is the ratio of fluorescence at 343 and 380 nm, $R_{max}$ and $R_{min}$ are the ratios for mag-fura free acid at 343 and 380 nm in the presence of saturating magnesium and zero magnesium, respectively, and β is the ratio of fluorescence of mag-fura-2 at 380 nm in zero and saturating magnesium. $K_d$ is the dissociation constant of mag-fura-2 for Mg$$^{2+}$$ and is taken as 1.5 mM (Raju et al., 1989).

**Experimental protocols.** To determine whether changes in [Mg$$^{2+}$$], influence AVP-stimulated [Ca$$^{2+}$$], responses, we measured [Ca$$^{2+}$$], effects of AVP (10$^{-9}$M) in cells from WKY that had been pre-exposed for 10 minutes to increasing concentrations of [Mg$$^{2+}$$]. To assess further the actions of [Ca$$^{2+}$$], on agonist-stimulated responses, three [Mg$$^{2+}$$]o concentrations (low, 0.15 mM; normal, 1.3 mM; high, 4.8 mM) were selected, and full AVP dose-response curves were obtained in cells from WKY and SHR. Cells were preincubated in the various [Mg$$^{2+}$$]o solutions for 10 min before AVP (10$^{-12}$ to 10$^{-5}$ M) stimulation. Cells were used for single experiments, and repetitive determinations were not performed.

To investigate whether [Mg$$^{2+}$$], effects are mediated via changes in Ca$$^{2+}$$ influx, we performed experiments using 10$^{-9}$ M AVP in Mg$$^{2+}$$-free Hanks. A concentration of 10$^{-6}$ M AVP was used, because this dose corresponds to $EC_{50}$ response that gives 30% of the maximal response). Furthermore, 10$^{-5}$ M AVP is a high pharmacological concentration and should induce responses that are probably the maximal ones occurring in vivo. The role of Ca$$^{2+}$$ channels was also assessed by repeating the experiments in varying [Mg$$^{2+}$$], in the presence of diltiazem, an L-type Ca$$^{2+}$$ channel antagonist. Diltiazem (10$^{-6}$ M) was added to the cells at the same time that cells were exposed to the various Mg$$^{2+}$$ buffers. Diltiazem was used at a concentration of 10$^{-6}$ M to ensure complete Ca$$^{2+}$$ channel blockade.

**Statistical analysis.** Each experiment was repeated at least three times using different cell preparations. Data obtained from fluorescent digital imaging studies, where multiple cells (10–20 cells) were examined in each experimental field, were calculated as the mean [Ca$$^{2+}$$], per experiment and then as the mean of multiple experiments. Results are presented as mean ± S.E.M. and are compared by Student’s t test or by analysis of variance where appropriate. Tukey-Kramer’s correction was used to compensate for multiple testing procedures. The AVP concentration (M) eliciting 50% of the maximal response ($EC_{50}$) was determined from concentration-response curves, which were fitted by nonlinear regression. Sensitivity to AVP was expressed as $pD_{2}$, which is the log [$EC_{50}$] (in M). Maximal responses to AVP were expressed as $E_{max}$ (in nM). $p < .05$ was considered significant.

**Results**

**Blood pressure and body weight.** The mean systolic blood pressure was significantly higher ($p < .001$) in the SHR group (192 ± 3.0 mm Hg) than in the WKY group (113 ± 1.4 mm Hg). SHR weighed significantly less ($p < .001$) than their normotensive counterparts (320 ± 2.7 g vs 477 ± 7.6 g, SHR vs. WKY).

**Effects of extracellular Mg$$^{2+}$$ on agonist-stimulated [Ca$$^{2+}$$]i in VSMC from WKY and SHR.** Extracellular Mg$$^{2+}$$ inhibited basal and AVP (10$^{-9}$ M)-stimulated [Ca$$^{2+}$$], responses in a dose-dependent manner in mesenteric VSMC from WKY (fig. 1). The threshold for significant inhibitory effects of extracellular Mg$$^{2+}$$ on AVP-induced responses began at physiologic Mg$$^{2+}$$ concentrations of 1 to 2 mM (fig. 1). Low [Mg$$^{2+}$$]o (0.15 mM) increased basal [Ca$$^{2+}$$], by 26 ± 3% and AVP-stimulated [Ca$$^{2+}$$], responses by 16 ± 6% relative to [Ca$$^{2+}$$], in physiologic buffer (fig. 1, lower panel). High [Mg$$^{2+}$$]o (4.8 mM) reduced basal and AVP-stimulated [Ca$$^{2+}$$], transients by 18 ± 4% and 34 ± 8%, respectively, relative to [Ca$$^{2+}$$], responses in normal buffer (fig. 1, lower panel).

In normal Mg$$^{2+}$$ buffer, basal [Ca$$^{2+}$$], in aortic and mesenteric cells was significantly elevated ($p < .05$) in SHR (107 ± 2 nM; aortic; 120 ± 3 nM, mesenteric) compared with WKY (91 ± 5 nM; aortic; 96 ± 2 nM, mesenteric) (fig. 2). Preincubation of cells with low [Mg$$^{2+}$$]o (0.15 mM) significantly increased ($p < .05$) basal [Ca$$^{2+}$$] in mesenteric cells from both WKY (123 ± 5 mM) and SHR (142 ± 9 mM) and in aortic cells from WKY (111 ± 3 mM) compared with basal [Ca$$^{2+}$$] in normal buffer (fig. 2). When cells were preincubated in Mg$$^{2+}$$-rich buffer (4.8 mM), basal [Ca$$^{2+}$$] was reduced in mesenteric (90 ± 3 mM) and aortic cells (87 ± 5 mM) from SHR (fig. 2).

To investigate in greater detail the dependence of AVP elicited [Ca$$^{2+}$$], responses on [Mg$$^{2+}$$], we obtained full AVP dose-response curves in the presence of low (0.15 mM), normal (1.3 mM) and high (4.8 mM) Mg$$^{2+}$$ concentrations. To allow for differences in basal [Ca$$^{2+}$$], between SHR and WKY, we calculated [Ca$$^{2+}$$], responses as the net [Ca$$^{2+}$$], change induced by AVP (the difference between AVP-stimulated [Ca$$^{2+}$$], and basal [Ca$$^{2+}$$]). AVP increased [Ca$$^{2+}$$] in a concentration-dependent manner (fig. 3 and 4). In buffer containing normal Mg$$^{2+}$$, responses to AVP were significantly greater in mesenteric and aortic VSMC from SHR than from WKY (figs. 3 and 4). The $pD_{2}$ values and $E_{max}$ for both cell types were significantly greater in SHR than WKY (tables 1 and 2).

AVP-stimulated responses in low [Mg$$^{2+}$$] were significantly greater in VSMC from SHR than from WKY (figs. 3 and 4) and 4). Compared with normal [Mg$$^{2+}$$], extracellular Mg$$^{2+}$$ withdrawal did not alter AVP sensitivity or $E_{max}$ in WKY or SHR (tables 1 and 2). In low [Mg$$^{2+}$$], $E_{max}$ in SHR was greater than that in WKY (table 2). In Mg$$^{2+}$$-enriched buffer, AVP-stimulated [Ca$$^{2+}$$], responses in SHR were similar to those in WKY (figs. 3 and 4). Compared with responses in buffer containing physiologic Mg$$^{2+}$$ concentrations, high-Mg$$^{2+}$$ buffer (4.8 mM) significantly attenuated ($p < .05$) AVP responses in both cell types in WKY and SHR (tables 1 and 2). High [Mg$$^{2+}$$] significantly decreased ($p < .05$) sensitivity to AVP in mesenteric and aortic VSMC from SHR (table 1), $pD_{2}$ values in SHR being similar to those in WKY (table 1).

**Effects of Ca$$^{2+}$$-free buffer and Ca$$^{2+}$$ channel antagonists on Mg$$^{2+}$$-induced actions.** To determine whether the attenuating actions of high [Mg$$^{2+}$$], are dependent on extracellular Ca$$^{2+}$$, we measured AVP-elicted responses in Ca$$^{2+}$$-free medium and in cells that had been pre-exposed to diltiazem. These studies were performed in mesenteric VSMC. Ca$$^{2+}$$-free medium abolished the attenuating effects of high [Mg$$^{2+}$$] in WKY but not in SHR (fig. 5). Similar
results were obtained in the presence of diltiazem (fig. 6). Thus, in WKY, Mg\(^{2+}\) actions were antagonized when the buffer was depleted of Ca\(^{2+}\) as well as when L-type Ca\(^{2+}\) channels were blocked, which suggests that in normal rats, the attenuating effects of Mg\(^{2+}\) are due mainly to inhibition of Ca\(^{2+}\) influx, mediated specifically through L-type channels. In SHR, Ca\(^{2+}\)-free buffer and Ca\(^{2+}\) channel blockade reduced, but did not abolish, Mg\(^{2+}\) actions, which suggests that in this genetic model of hypertension, the Mg\(^{2+}\)-lowering effects of Mg\(^{2+}\) involve both Ca\(^{2+}\) influx and intracellular Ca\(^{2+}\) mobilization.

**[Mg\(^{2+}\)]\(_i\) in VSMC from WKY and SHR.** To determine whether [Mg\(^{2+}\)]\(_i\) differed in VSMC from WKY and SHR, which could be a mechanism for the observed differential effects found in the two rat strains, we measured [Mg\(^{2+}\)]\(_i\) in resting, unstimulated cells. In VSMC from SHR, basal [Mg\(^{2+}\)]\(_i\) was 0.48 ± 0.03 mM (n = 8), which was significantly lower (p < .01) than [Mg\(^{2+}\)]\(_i\) in VSMC from WKY (0.66 ± 0.01 mM, (n = 7).

**Discussion**

This study demonstrates 1) that AVP-stimulated [Ca\(^{2+}\)]\(_i\) responses are attenuated by [Mg\(^{2+}\)]\(_e\) in a dose-dependent manner, 2) that AVP-induced [Ca\(^{2+}\)]\(_i\) responses are potentiated in SHR in low and normal [Mg\(^{2+}\)]\(_e\) and 3) that high [Mg\(^{2+}\)]\(_e\) normalizes AVP-induced [Ca\(^{2+}\)]\(_i\) responses and sensitivity to AVP in SHR. Furthermore, we have demonstrated that a primary underlying mechanism through which extracellular Mg\(^{2+}\) influences [Ca\(^{2+}\)]\(_i\) is regulation of Ca\(^{2+}\) influx, specifically through L-type Ca\(^{2+}\) channels. In SHR, high [Mg\(^{2+}\)]\(_e\) attenuates [Ca\(^{2+}\)]\(_i\) responses by altering Ca\(^{2+}\) influx as well as by influencing intracellular Ca\(^{2+}\) mobilization.

Extracellular Mg\(^{2+}\) concentration influences the tone and reactivity of veins and arteries, and even small changes in [Mg\(^{2+}\)]\(_e\) exert significant effects on vascular smooth muscle contractility (Altura and Altura, 1980; Altura et al., 1993; Faragó et al., 1991; Gold et al., 1990). Lowering [Mg\(^{2+}\)]\(_e\)
induces rapid contractile responses, potentiation of vasoconstrictor-elicited contraction and attenuation of vasodilation. In vivo and in vitro studies have demonstrated that elevation of \([\text{Mg}^{11}]_{e}\) inhibits spontaneous tone of arteries, dose-dependently dilates vessels and attenuates agonist-stimulated contraction (Altura and Altura, 1985; Noguera and D’Ocon, 1993). In hypertension, these \([\text{Mg}^{11}]_{e}\)-related vascular actions appear to be altered. In vitro studies have shown that the effects of \([\text{Mg}^{11}]_{e}\) are less pronounced in isolated aorta from SHR than from WKY (Altura and Altura, 1983). We recently reported that changes in \([\text{Mg}^{11}]_{e}\) differentially affect vascular tone and reactivity in pressurized mesenteric arteries from SHR and WKY and that high \([\text{Mg}^{11}]_{e}\) significantly decreased AVP-stimulated contractile responses in SHR arteries (Laurant et al., 1997).

To gain clearer insight into the cellular mechanisms that underlie these changes in hypertension, we investigated the effects of varying \([\text{Mg}^{11}]_{e}\) on AVP-stimulated [Ca\(^{2+}\)]\(_i\) responses in VSMC from SHR and control WKY. The novel findings of the present study are related to the modulatory effects of \([\text{Mg}^{11}]_{e}\) on [Ca\(^{2+}\)]\(_i\) responses in SHR. In physiologic \([\text{Mg}^{11}]_{e}\), basal and AVP-stimulated [Ca\(^{2+}\)]\(_i\) responses and sensitivity to AVP were increased in SHR compared with WKY. These results are in agreement with our previous findings, where we reported that AVP-stimulated [Ca\(^{2+}\)]\(_i\) and contractile responses were enhanced in isolated mesenteric arteries and cultured mesenteric VSMC of SHR (Touyz et al., 1996a; Yang et al., 1996).

In the present study, we have demonstrated that AVP-elicited [Ca\(^{2+}\)]\(_i\) responses are also increased in VSMC from aorta of SHR. Low \([\text{Mg}^{11}]_{e}\) increased basal [Ca\(^{2+}\)]\(_i\) and augmented AVP-stimulated responses in SHR compared with WKY. These results indicate that the [Ca\(^{2+}\)] regulatory actions of Mg\(^{2+}\) are altered in hypertension, the effects of Mg\(^{2+}\) withdrawal being greater in SHR than in WKY. In various experimental and genetic models of hypertension, serum Mg\(^{2+}\) concentrations are decreased (Altura et al., 1984; Berthetlot et al., 1987; Seelig, 1989; Touyz et al., 1987). Clinically important hypomagnesemia (serum Mg\(^{2+}\) concentration < 0.6 mM) is associated with malabsorption syndromes, chronic alcoholism, hypoparathyroidism, hyperaldosteronism and diuretic use (Alfrey, 1992). Significant intracellular Mg\(^{2+}\) depletion has been reported in hypertension (essential and malignant forms), insulin resistance, di-

**Fig. 2.** Bar graphs demonstrate basal [Ca\(^{2+}\)]\(_i\) in mesenteric and aortic VSMC from WKY and SHR exposed to buffer containing normal (1.3 mM), low (0.15 mM) or high (4.8 mM) \([\text{Mg}^{11}]_{e}\).*

**Fig. 3.** AVP-induced [Ca\(^{2+}\)]\(_i\) responses in mesenteric vascular smooth muscle cell from WKY and SHR in buffer containing normal (1.3 mM), low (0.15 mM) or high (4.8 mM) \([\text{Mg}^{11}]_{e}\). Each data point is the mean ± S.E.M. of 4 to 8 experiments. *p < .05; **p < .01 vs. WKY counterpart. Comparing curves in low, normal and high \([\text{Mg}^{11}]_{e}\), we found that AVP-induced responses in WKY and SHR were significantly attenuated (p < .01) in high \([\text{Mg}^{11}]_{e}\) compared with normal and low \([\text{Mg}^{11}]_{e}\).
or high (4.8 mM) producing 50% of the maximal response. *p

Furthermore, chronic hypomagnesemia leads to reduced

TABLE 1

AVP pD2 values for intracellular free Ca++ responses in VSMC from mesenteric and aortic vessels of SHR and WKY in buffer containing low (0.15 mM), normal (1.3 mM) or high (4.8 mM) [Mg++]

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<th>WKY</th>
<th>SHR</th>
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<tr>
<td></td>
<td>Mesenteric</td>
<td>Aortic</td>
</tr>
<tr>
<td>Low [Mg++]</td>
<td>8.04 ± 0.29</td>
<td>8.83 ± 0.54</td>
</tr>
<tr>
<td>Normal [Mg++]</td>
<td>8.03 ± 0.04</td>
<td>7.73 ± 0.22</td>
</tr>
<tr>
<td>High [Mg++]</td>
<td>7.82 ± 0.08†</td>
<td>7.85 ± 0.30</td>
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Data were derived from concentration-response curves (figs. 3 and 4). Values are expressed as means ± S.E.M. of 3 to 6 experiments. *p < .05 vs. WKY counterpart. †p < .05 vs. normal [Mg++] counterpart.

TABLE 2

[Ca++] responses (E_max) to vasopressin in mesenteric and aortic VSMC from SHR and WKY in buffer containing low (0.15 mM), normal (1.3 mM) or high (4.8 mM) [Mg++]

<table>
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<th>WKY</th>
<th>SHR</th>
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<tbody>
<tr>
<td></td>
<td>Mesenteric</td>
<td>Aortic</td>
</tr>
<tr>
<td>Low [Mg++]</td>
<td>434 ± 32</td>
<td>413 ± 34</td>
</tr>
<tr>
<td>Normal [Mg++]</td>
<td>480 ± 15</td>
<td>328 ± 43</td>
</tr>
<tr>
<td>High [Mg++]</td>
<td>328 ± 30†</td>
<td>292 ± 30†</td>
</tr>
</tbody>
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E_max values are expressed as nM. †p < .05; ††p < .01 vs. normal [Mg++] counterpart; *p < .05 vs. WKY counterpart.

abates mellitus and left ventricular hypertrophy (Seelig, 1989; Resnick, 1993; Paolisso and Barbagallo, 1997). Persistent exposure to low levels of Mg++ may be associated with reduced Mg++ blockade of Ca++ channels, increased transmembrane Ca++ transport and resultant increased [Ca++]i. Furthermore, chronic hypomagnesemia leads to reduced [Mg++]i. As demonstrated in the present study, [Mg++]i was significantly lower in SHR than in WKY, and as we reported previously, changes in [Mg++]i are directly related to changes in [Mg++]i (Touyz and Schiffrin, 1993). Because [Mg++]i influences [Ca++]i, decreased [Mg++]i may also contribute to the altered modulatory actions of Mg++ in SHR. High [Mg++]i reduced basal [Ca++]i, attenuated agonist-elicited responses and decreased sensitivity to AVP in VSMC from both SHR and WKY. In addition, high [Mg++]i normalized [Ca++]i, hyper-responsiveness in SHR, possibly by potentiating Ca++ channel blockade. These changes in vascular smooth muscle function may lead to reduced vascular tone and contraction and may ultimately result in decreased peripheral resistance. Mg++ supplementation may decrease blood pressure in some forms of hypertension through these cellular mechanisms.

There is general agreement regarding the Ca++-antagonistic properties of Mg++. Mechanisms proposed for the decrease in [Ca++]i induced by increased extracellular Mg++ include disruption of agonist-receptor interactions, alterations of membrane permeability and Ca++ channel blockade (D’Angelo et al., 1992; Hwang et al., 1992; Iseri and French, 1984; Resnick, 1992; Zhu et al., 1995). Changes in [Mg++]i may be reciprocally associated with changes in [Mg++]i, which may in turn influence [Ca++]i (Altura et al., 1982; Noguera and D’Onofrio, 1993). In the present study, we assessed whether Mg++-inhibitory effects are mediated by influencing Ca++ influx and Ca++ release and whether these
components are altered in hypertension. Ca\(^{2+}\)-free buffer, as well as Ca\(^{2+}\) channel blockade by diltiazem, abolished the antagonizing effects of Mg\(^{2+}\) in WKY but not in SHR. These data suggest that in WKY, Mg\(^{2+}\) modulates [Ca\(^{2+}\)], mainly by influencing Ca\(^{2+}\) influx, whereas in SHR, Mg\(^{2+}\) actions may be mediated via both Ca\(^{2+}\) influx and intracellular Ca\(^{2+}\) mobilization. In hypertension, VSMC may be sensitive to changes in [Mg\(^{2+}\)]\(_i\), which could lead to changes in [Mg\(^{2+}\)]\(_i\). Previous studies have indicated that intracellular Mg\(^{2+}\) blocks Ca\(^{2+}\) influx, inhibits Ca\(^{2+}\) release from intracellular stores, potentiates Ca\(^{2+}\) uptake into sarcoplasmic reticulum and reduces [Ca\(^{2+}\)]. (Altura et al., 1982; White and Hartzell, 1988; Yoshimura et al., 1996). [Mg\(^{2+}\)]\(_i\) may therefore directly influence [Ca\(^{2+}\)]. We demonstrated here that in SHR, [Mg\(^{2+}\)]\(_i\) was significantly reduced, which may further contribute to the increased Ca\(^{2+}\) release observed in VSMC from SHR.

In conclusion, the present study demonstrates that Mg\(^{2+}\) dose-dependently decreases AVP-stimulated [Ca\(^{2+}\)], that the effects of extracellular Mg\(^{2+}\) depletion are potentiated in SHR compared with WKY and that [Mg\(^{2+}\)]\(_i\) elevation normalizes AVP-stimulated Ca\(^{2+}\) responses in VSMC from SHR. In WKY, the Ca\(^{2+}\)-attenuating effects of high [Mg\(^{2+}\)]\(_i\), are mediated primarily by inhibiting Ca\(^{2+}\) influx through l-type Ca\(^{2+}\) channels, whereas in SHR, Mg\(^{2+}\) actions appear to involve Ca\(^{2+}\) influx through Ca\(^{2+}\) channels as well as intracellular Ca\(^{2+}\) release. These modulatory effects of Mg\(^{2+}\) on VSMC [Ca\(^{2+}\)]\(_i\), responses in SHR may contribute to altered signaling transduction pathways in hypertension.

References
Seelig M (1989) Cardiovascular consequences of magnesium deficiency and loss: Fig. 5. Effects on [Ca\(^{2+}\)]\(_i\) of 10\(^{-8}\)M AVP in buffer with normal (1.3 mM) or high (4.8 mM) [Mg\(^{2+}\)] concentrations with and without Ca\(^{2+}\) (\(p < .01\) vs. counterpart in Ca\(^{2+}\)-containing buffer; \(p < .05\), \(p < .01\).

Fig. 6. Effects on [Ca\(^{2+}\)]\(_i\) of 10\(^{-8}\)M AVP in buffer with normal (1.3 mM) or high (4.8 mM) [Mg\(^{2+}\)], containing 10\(^{-6}\)M diltiazem. *\(p < .05\), ‡\(p < .01\).
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Send reprint requests to: Rhian M. Touyz, M.D., Ph.D., Clinical Research Institute of Montreal, 110 Pine Avenue West, Montreal (Quebec) Canada, H2W 1R7.