Original Article

Magnesium Removal Impairs the Regulatory Role of Rat Endothelium

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Vascular endothelium has been shown to play an important role in the regulation of vascular tone and, hence, impairment of the endothelium may induce hypertension. Although magnesium (Mg) deficiency could induce hypertension, the role of Mg on the endothelium is unclear. We examined the effects of Mg removal on endothelium-dependent and -independent responses using ring preparations of femoral arteries obtained from spontaneously hypertensive rats (SHR) and Wistar-Kyoto rats (WKY). Norepinephrine (10^{-9} – 10^{-4} M) evoked concentration-dependent contractions in arteries with endothelium. The maximal response was greater in SHR than in WKY. Removal of external Mg augmented the contraction in WKY but not in SHR. As a result, the contraction obtained in arteries with endothelium was identical in the two groups. Removal of the endothelium enhanced the contraction in both strains, with a greater response occurring in WKY than in SHR in Krebs, but not in Mg-free, solution. As a result, in arteries without endothelium, the contractions were identical in WKY and SHR both in Krebs and Mg-free solutions. Acetylcholine (10^{-9} – 10^{-4} M) evoked concentration-dependent relaxation in arteries with, but not in those without, endothelium obtained from WKY and SHR. The relaxation did not differ between the two strains, nor was it altered by Mg removal. Thus, Mg removal impairs inhibitory function of the endothelium against contraction induced by norepinephrine, without affecting endothelium-dependent relaxation in response to acetylcholine, in the rat femoral artery. The effect of Mg removal is not apparent in SHR. The fact that after removal of external Mg the contraction in response to norepinephrine in arteries with endothelium is identical in WKY and SHR suggests that a normotensive artery with Mg deficiency may mimic a hypertensive artery through endothelial impairment. (*Hypertens Res* 2000; 23: 669–675)

Key Words: endothelium, magnesium, norepinephrine, spontaneously hypertensive rats, Wistar-Kyoto rats

Introduction

A possible causal relationship between magnesium and blood pressure has been discussed during the last two decades (1–5). A chronic oral or acute intravenous administration of magnesium (Mg) causes a decrease in blood pressure in many species, including humans (3, 6–9), though some reports have shown no decrease in blood pressure following Mg administration (10, 11). In contrast, an acute hypomagnesemia with normal calcium

level is associated with systemic vasoconstriction and an increase in arterial pressure (2–4, 12–15). An inverse correlation between dietary Mg and blood pressure has also been reported (4, 16). These reports suggest that the serum level of Mg is likely an important factor in regulating blood pressure.

In vitro studies demonstrated that an increase in external Mg evokes relaxation in vascular smooth muscle (6–8), which might result in a decrease of vascular resistance. The relaxation is caused by an inhibition of calcium entry through calcium channels, calcium release from in-

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tracellular stores (1, 17, 18), or calcium availability in vascular smooth muscle (19). On the other hand, cellular examination revealed a markedly low Mg content in subjects with hypertension, which might contribute to an increased vascular tone (20, 21).

Recently, a considerable contribution of the endothelium in the regulation of vascular tone has been demonstrated (22). The endothelium releases relaxing and contracting factors in response to many vasoactive substances. Since the release mechanisms involve calcium entry into the endothelium, Mg may modulate the endothelial function by interfering with the signal transduction of calcium. Indeed, the removal of Mg from extracellular fluid causes a transient relaxation followed by a sustained contraction in arteries with endothelium; in arteries without endothelium, however, the removal of Mg causes vasoconstriction (23). Since endothelial cells exhibit morphological and functional changes in tandem with the progression of hypertension (24, 25), the responses of hypertensive endothelium to changes in extracellular Mg concentration would be of pathophysiological interest. However, only a few reports describe the effect of changes in Mg concentration on endothelial function in a hypertensive model. Thus, this study was designed to investigate the role of Mg in endothelium as it relates to the development of hypertension by evaluating the responses to norepinephrine and acetylcholine in the femoral arteries of spontaneously hypertensive rats (SHR) and Wistar-Kyoto rats (WKY).

Methods

Experimental Set-Up

Male SHR and WKY, 10 to 13 weeks of age (Charles River Japan, Yokohama), were anesthetized with diethyl ether. All procedures were in accordance with our institutional guidelines. The rats were exsanguinated, and the femoral artery was removed and immersed in cold modified Krebs-Ringer bicarbonate solution of the following composition (in mM): NaCl, 118.6; KCl, 4.7; CaCl₂, 2.5; KH₂PO₄, 1.2; MgSO₄, 1.2; NaHCO₃, 25.1; edetate calcium disodium, 0.026; and glucose, 10.1 (Krebs solution). The artery was cleaned of surrounding tissues and cut into rings (outside diameter 0.7 to 0.8 mm, length: 1.0 mm) under a dissection microscope. Care was taken to avoid damage to the intimal surface of the artery. In some arterial rings, the endothelium was removed by gentle rubbing of the intimal surface with stainless wire. The presence or absence of the functional endothelium was confirmed later by the presence or absence of a relaxation in response to 10⁻⁷ M acetylcholine, respectively.

The rings were mounted by means of two L-shaped stainless steel hooks in a water-jacketed bath chamber (5 ml) filled with the Krebs solution (37°C) bubbled with a

mixture of 95% O₂ and 5% CO₂. One of the hooks was fixed to the chamber, and another was attached to a force-displacement transducer (UM-203, Kishimoto, Kyoto) for the measurement of isometric tension development. The rings were passively stretched to generate a resting tension of 300 mg and allowed to equilibrate for 60 min. Then, the rings were maximally contracted by exposing them to a 60 mM KCl solution (which was obtained by equimolar replacement of NaCl by KCl in the Krebs solution) two times at a 45-min interval.

Tension Development

All the experiments were performed in the presence of timolol maleate (3×10^{-7} M) to block β -adrenoceptors. The antagonist was applied 30 min prior to the experiments. Cumulative concentration-response curves for norepinephrine (10^{-9} – 10^{-4} M) were obtained in arteries with and without endothelium. Concentration-response curves for acetylcholine (10^{-9} – 10^{-4} M) were constructed in arteries with endothelium precontracted with the EC₇₅ value of norepinephrine. The responses to norepinephrine and acetylcholine were obtained in the Krebs solution or a Mg-free solution. In experiments where we studied the effects of Mg removal, the Krebs solution was replaced with the Mg-free solution 30 min before the start of the experiments.

Drugs and Solution

The following drugs were used: acetylcholine chloride, L-norepinephrine bitartrate (Sigma Chemical Co., St. Louis, USA), and timolol maleate (Banyu Pharmaceutical, Co., Ltd., Tokyo). A stock solution was prepared in distilled water, separated into aliquots, and stored frozen. These aliquots were diluted with the Mg-free solution just before use. The Mg-free solution was prepared by omitting MgSO₄ from the Krebs solution.

Calculations and Statistical Analysis

Vascular tensions were expressed as a percentage of the contraction developed by the 60 mM KCl solution, and relaxations were expressed as a percent inhibition of the preceding contractions. The concentration inducing half-maximum contraction (EC₅₀) and the concentration producing half-maximal inhibition of a preceding contraction (IC₅₀) were determined graphically in each concentration-response curve and expressed as the pD₂ value ($-\log M$). Data are presented as mean $\pm$ SEM. In each set of experiments, *n* equals the number of animals studied. Unpaired Student's *t*-tests and analysis of variance were used for assessing statistical significance where appropriate. A *p* value less than 0.05 was considered to be significant.

Fig. 1. Concentration-response curves to norepinephrine in femoral arteries with (●) and without (○) endothelium obtained from 10- to 13-week-old Wistar-Kyoto rats in Krebs (left) and Mg-free (right) solutions. Note that the inhibitory effect of the endothelium against the contraction induced by norepinephrine is reduced in Mg-free solution as compared to that in Krebs solution. Mg removal augments the contraction in arteries with endothelium.

Table 1. Maximal Response and pD_2 Value for Norepinephrine in Femoral Arteries with and without Endothelium Obtained from Wistar-Kyoto Rat (WKY) and Spontaneously Hypertensive Rats (SHR)

	Krebs solution	Mg-free solution	<i>p</i> value (Krebs vs. Mg-free)
WKY with endothelium	<i>n</i> =7	<i>n</i> =7	
Maximal contraction (%)	42.5±5.7	71.9±10.6	<0.05
pD_2	5.55±0.03	6.01±0.12	<0.01
WKY without endothelium	<i>n</i> =7	<i>n</i> =9	
Maximal contraction (%)	113.8±2.7 [<0.001]	100.6±2.9 [<0.001]	<0.01
pD_2	6.49±0.06 [<0.001]	7.11±0.08 [<0.001]	<0.001
SHR with endothelium	<i>n</i> =8	<i>n</i> =8	
Maximal contraction (%)	90.6±7.8	78.3±2.5	0.16
pD_2	5.89±0.15	5.97±0.11	0.74
SHR without endothelium	<i>n</i> =8	<i>n</i> =9	
Maximal contraction (%)	116.6±3.0 [<0.01]	98.8±2.2 [<0.001]	<0.001
pD_2	6.59±0.15 [<0.01]	6.96±0.13 [<0.001]	0.90

Values are expressed as mean±SEM. [], *p* value vs. arteries with intact endothelium; *n*, number of animal studied.

Results

Removal of Magnesium in Quiescent Artery

In WKY, Mg removal from the Krebs solution induced small contractions in arteries with ($2.7\pm0.5\%$ of 60 mM KCl-induced contraction) and without endothelium ($3.5\pm0.8\%$; NS). In SHR, the contraction was $2.3\pm0.2\%$ and $3.3\pm0.5\%$ in arteries with and without endothelium, respectively (NS). The contraction was not different be-

tween WKY and SHR.

Norepinephrine

WKY

Norepinephrine (10^{-9} - 10^{-4} M) evoked concentration-dependent contractions in arteries with endothelium in the Krebs solution (Fig. 1). The contraction was augmented after the removal of external Mg. In contrast, Mg removal decreased the maximal response in arteries without endothelium although the pD_2 value was slightly increased

Fig. 2. Concentration-response curves to norepinephrine in femoral arteries with (■) and without (□) endothelium obtained from 10- to 13-week-old spontaneously hypertensive rats (SHR) in Krebs (left) and Mg-free (right) solutions. Note that Mg removal does not affect the inhibitory effect of the endothelium against the contraction induced by norepinephrine in SHR.

Fig. 3. Concentration-response curves to acetylcholine in femoral arteries with endothelium obtained from Wistar-Kyoto rats (WKY; left) and spontaneously hypertensive rats (SHR; right) in Krebs (●, ■) and Mg-free (○, □) solutions. Note that the relaxation induced by acetylcholine is not different between WKY and SHR in the two solutions.

(Fig. 1 and Table 1). In the Krebs solution, removal of the endothelium markedly enhanced the contraction, while the enhancement was somewhat small in the Mg-free solution. The increase in the maximal response after

the endothelial removal was 172% in the Krebs solution and 40% in the Mg-free solution.

SHR

A concentration-dependent contraction in response to norepinephrine was also obtained in SHR (Fig. 2). In the Krebs solution, the contraction obtained in arteries with, but not without, endothelium, was greater in SHR than in WKY ($p < 0.001$). In contrast to WKY, Mg removal did not alter the contraction in arteries with endothelium in SHR (Fig. 2 and Table 1). In the Mg-free solution, the contraction obtained in arteries with endothelium was identical in SHR and WKY (Table 1). In SHR, the removal of the endothelium increased the contraction in both solutions to the same extent (Fig. 2). In arteries without endothelium, the maximal response was smaller in the Mg-free solution than in the Krebs solution, as observed in WKY. In arteries without endothelium, the contraction was not different between WKY and SHR in either solution. These changes by Mg removal were restored by the addition of external Mg in WKY and SHR.

Acetylcholine

In arteries with endothelium, acetylcholine (10^{-9} – 10^{-4} M) evoked concentration-dependent relaxation in the Krebs solution (Fig. 3, left). The relaxation was completely inhibited in the presence of atropine sulfate (10^{-5} M; data not shown). In WKY, neither the maximal relaxation ($90.6 \pm 1.2\%$) nor the pD_2 value (7.66 ± 0.15) was affected by Mg removal ($83.6 \pm 6.4\%$ and 7.70 ± 0.09 , respectively; NS). Similar results were obtained in SHR (maximal relaxation $93.2 \pm 3.3\%$ vs. $91.8 \pm 4.2\%$, NS; pD_2 value 7.64 ± 0.12 vs. 7.66 ± 0.11 , NS, in Krebs and Mg-free solution, respectively). High concentrations of acetylcholine (10^{-6} – 10^{-4} M) tended to contract the artery in the Mg-free solution, but this did not reach statistical significance.

Discussion

The present study demonstrates that Mg removal impairs the inhibitory effect of the endothelium against contraction induced by norepinephrine. This may explain, at least in part, the pressor effect of Mg-deficiency.

In WKY, the contraction induced by norepinephrine was much more pronounced in arteries without endothelium than in those with endothelium, confirming our previous observation (26). This demonstrates that the endothelium negatively modulates vascular tone by releasing endothelium-derived relaxing factors (EDRF) during contractions evoked by norepinephrine. The inhibitory effect of the endothelium must be clinically important, since the effect was observed against contractions induced by the neurotransmitter. The contraction was augmented after the removal of external Mg, indicating that the inhibitory effect of the endothelium is impaired by the Mg removal. The facts seem to be incompatible with the reports that

the release of EDRF, such as nitric oxide and prostacyclin, is dependent on calcium influx into endothelial cells and that a decrease in external Mg increases the calcium influx (27, 28). Indeed, an acute decrease in extracellular Mg results in a transient relaxation followed by a sustained contraction in arteries with intact endothelium (29–31). It is possible that an increase in calcium influx caused by Mg removal may be transient and, therefore, the response by the release of EDRF cannot be detected in the present experimental condition where external Mg was removed 30 min before the addition of norepinephrine. Or the arteries in the quiescent condition might not show any relaxant responses when Mg is removed.

In Krebs solution, the contraction induced by norepinephrine in arteries with endothelium was greater in SHR than in WKY. Since the contraction in arteries without endothelium was identical in WKY and SHR, the result is explained by an impairment of the hypertensive endothelium to inhibit vascular contraction (26). Thus, an impaired endothelial function, but not vascular smooth muscle, is responsible for the increased vascular contraction in SHR. Interestingly, the contraction of WKY arteries with endothelium obtained in Mg-free solution was not different from that of SHR arteries obtained in Krebs solution. That fact clearly demonstrates that Mg removal impairs endothelial function and that functionally normal endothelium could mimic hypertensive endothelium in a Mg-deficient condition. As a result, a normotensive artery exhibits responses similar to those of a hypertensive artery due to the endothelial impairment, suggesting that the release or availability of EDRF is decreased. This may partly explain the *in vivo* observation that hypomagnesemia increases arterial pressure (2–4, 12). In contrast to WKY, the inhibitory effect of the endothelium in SHR was not affected by Mg removal. In SHR, the effect of Mg removal on endothelial function could be minimal, since Mg removal may not further impair the reduced inhibitory effect of the endothelium.

In order to address mechanisms underlying the impairment of endothelial function after Mg removal, we investigated the effects of Mg removal on vascular tone in quiescent arteries and on endothelium-dependent relaxation induced by acetylcholine. Mg removal evoked small contractions in both WKY and SHR, which was not affected by the presence of the endothelium. Basal release of endothelium-derived factors may be minimal, at least in the experimental condition. Similarly, relaxations induced by acetylcholine were identical in Krebs and Mg-free solution in both strains. This result indicates that stimulated (receptor-mediated) release of EDRF by acetylcholine in the femoral artery is not different between the two strains (32), which contrasts with the result obtained with norepinephrine. The response to the release of EDRF could be altered according to the autacoids used. Still, the response to acetylcholine seems to

be incompatible with the finding of previous studies that the release of EDRF is diminished in animal models of hypertension. However, the impaired relaxation by acetylcholine is not responsible for the decreased release of EDRF, but it is responsible for the increased formation of endothelium-derived contracting factors that may play a part in animals older than those examined in the present study (33, 34). The increase in the release of EDRF might be counterbalanced by a decrease in efficacy of acetylcholine due to a conformational change of the receptor in Mg-free solution (2, 35). It might also be possible that calcium influx into the endothelium is maximally activated by the stimulation with acetylcholine. Additionally, acetylcholine stimulates endothelium to release endothelium-dependent hyperpolarizing factor (EDHF) (36). Although no data are available on the relationship between Mg deficiency and EDHF, the release of EDHF by acetylcholine, in addition to EDRF, might have modulated the relaxation of the arteries of WKY and SHR in the presence or absence of Mg and resulted in a similar relaxation by acetylcholine in both strains. The effect of Mg removal seems to be, therefore, minimum.

In Mg-free solution, the maximal response to norepinephrine in arteries without endothelium was attenuated both in WKY and SHR, indicating that contractile response of the smooth muscle to a neurotransmitter via α -adrenoceptors in the hypertensive artery is similar to that in the normotensive artery. Contractile property of vascular smooth muscle is also suspected to be altered by Mg removal, which may affect the response of arteries to norepinephrine. Mg removal may induce changes in calcium utilization and conformational changes of receptors, or potentiate the activities of catecholamines by acting at sites in vascular smooth muscle other than the receptors (2, 35). A reduction of external Mg concentration below physiological level increases maximal responses, whereas, in agreement with this study, total Mg removal seems to decrease maximal responses of vascular smooth muscle to contractile agonists (2, 19, 37, 38). Although it is unclear why the absence of Mg attenuates maximal contraction in arteries without endothelium, the finding might be partly explained by the fact that Mg removal inhibits intracellular calcium release (38). In SHR, an increased free cytosolic calcium or basal calcium influx might have prevented any further increase in calcium influx after Mg removal (39-41).

In conclusion, the contraction induced by norepinephrine in the rat femoral artery with endothelium is enhanced in SHR as compared to WKY due to the reduced inhibitory function of the impaired endothelium. In WKY, removal of the external Mg increases the sensitivity of vascular smooth muscle to norepinephrine and impairs the inhibitory function of the endothelium, while the effect of Mg removal is not apparent in SHR. These results indicate that Mg plays an important role in maintain-

ing the physiological function of endothelium and that a normotensive artery with Mg deficiency may mimic a hypertensive artery through endothelial impairment.

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