Modulatory Role of Magnesium on the Contractile Response of Rat Aorta to Several Agonists in Normal and Calcium-free Medium

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Abstract—Acute withdrawal of external Mg^{2+} increased basal tone of rat isolated aorta incubated in the presence of Ca^{2+}. Above normal levels of Mg^{2+} (1–4 mM) inhibited basal tone while much higher levels of the divalent cation (64–256 mM) evoked contractile responses regardless of the presence of Ca^{2+}. Contractile responses to noradrenaline (1 μM) and KCl (80 mM) were inhibited by addition of cumulative concentrations of Mg^{2+}. Acetylcholine-induced contractions in the presence of physiological concentrations of Mg^{2+} (1 mM) decreased gradually to the basal tone, but a sustained contraction was observed in the absence of this ion. In Ca^{2+}-free medium, acetylcholine-induced phasic responses indicate the existence of an acetylcholine-sensitive Ca^{2+} store. KCl induced contraction only in Krebs solution, although a small residual contraction could be observed in Ca^{2+}-free medium in some experiments. Mg^{2+}-depletion in the extracellular medium increased contractile responses induced by acetylcholine and KCl in Ca^{2+}-free medium. These results suggest that extracellular Mg^{2+} modulates basal tone, Ca^{2+} channels and responsiveness to various agents in the absence of Ca^{2+}.

In experimental studies in-vitro, Mg^{2+} deficiency has been shown to constrict coronary arteries in the dog. Extracellular Mg^{2+} acts as a Ca^{2+} antagonist at the level of the vascular smooth muscle (Altura 1982; Altura et al 1987) or uterine smooth muscle (D'Ocon et al 1987a, b). In addition, general studies have shown the existence of a regulatory system that can be directly activated by pharmacological levels of Mg^{2+} (Ikebe et al 1984; Moreland & Moreland 1991). Moreover, it is also well established that many biochemical systems subserving contractility are sensitive to free Mg^{2+} concentration (Pato & Adelstein 1980; Moreland & Ford 1981, 1982; Ikebe et al 1984), many enzymes are Mg^{2+}-dependent (e.g. Ca^{2+}-activated, Mg^{2+}-dependent ATPase) and there are specific binding sites for Mg^{2+} on contractile proteins (Singh 1987; Murakawa et al 1988).

Recent studies have demonstrated the influence of Mg^{2+} in the extracellular medium on the contractile responses induced by many agonists, including noradrenaline, acetylcholine and 5-hydroxytryptamine in different smooth muscles (Altura & Altura 1985a, b; Murakawa et al 1988). It has also been reported that this divalent cation influences agonist-induced contractile responses in Ca^{2+}-free medium. Thus, in rat uterus, acetylcholine- and oxytocin-evoked contractions are modified by the presence of Mg^{2+} in the incubation medium (D'Ocon et al 1987a, b) and in rat aortic strips, responses to noradrenaline and caffeine (Noguera & D'Ocon 1992) are also altered.

The purpose of this study was to examine the effects of Mg^{2+} on different processes.

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Materials and Methods

Experimental procedure

Helically-cut strips of the thoracic aorta of male Wistar rats, 200–220 g, were prepared and mounted as described by Furchgott & Zawadzki (1980). In some experiments thoracic aortic strips were bisected and both halves were used to perform parallel experiments. Each preparation was suspended in a 10 mL organ bath containing Krebs-bicarbonate solution (Krebs), maintained at 37°C and gassed with 95% O_2–5% CO_2.

An initial load of 1 g was applied to each preparation and maintained throughout a 75–90 min equilibration period. Tension was recorded isometrically on a Philips recorder PM (8222) (Eindhoven, The Netherlands) coupled to a Hewlett Packard amplifier (8805D) (CA, USA) via force-displacement transducers (Gould Statham UC2) (CA, USA).

Endothelium-denuded aortic strips were prepared by rubbing the entire intimal surface. The absence of relaxant response after acetylcholine (10^{-4} M) addition to preparations previously contracted with noradrenaline (1 μM) indicated the absence of a functional endothelium (Furchgott & Zawadzki 1980).

Drugs and solutions

L-Noradrenaline L-tartrate and acetylcholine were obtained from Merck, Darmstadt, Germany. Other reagents were of analytical grade.

All drugs were dissolved in distilled water. The composition of the Krebs solution (mM) was: NaCl 118, KCl 4·75, $CaCl_2$ 1·8, $MgCl_2$ 1·2, KH_2PO_4 1·2, $NaHCO_3$ 25 and glucose 11. Ca^{2+}-free and Mg^{2+}- and Ca^{2+}-free solutions had the same composition except that $CaCl_2$, or $CaCl_2$ and $MgCl_2$ were, respectively, omitted and EDTA (0·1 mM) was added.

Depolarizing solution, 80 mM, was prepared by equimolar substitution of KCl by NaCl in Krebs solution.

Analysis of results

Contractions in Krebs solution are expressed in mg of developed tension and, when elicited in Ca^{2+}-free medium, as a percentage of the values obtained in the presence of Ca^{2+}.

Examination of the effects of alterations in extracellular Mg^{2+} on base-line tone was carried out by altering the external Mg^{2+} concentration in a range of 0–256 mM. Modifications in basal tone in a normal or Ca^{2+}-free Krebs solution are expressed in mg.

Dose–response curves of relaxation to $MgCl_2$ were obtained by addition of cumulative concentrations of the cation to tissues in which sustained contractions were induced by 1 μM noradrenaline or by exposure to an 80 mM KCl-containing solution. Relaxations were expressed as a percentage of the maximum tension obtained by agonist addition. E_{max} represents the maximal relaxation obtained after addition of the highest concentration of $MgCl_2$. A regression of response against $-\log$ C of $MgCl_2$ was performed by the least-squares method for each preparation. The concentration needed to produce 50% inhibition (IC50) was obtained from the linear regression plot of all points between 20 and 80% of the maximal response.

Results are presented as a mean ± s.e.m. for n determinations obtained from different animals. Statistical significance was evaluated by Student's t-test for paired and unpaired data. Differences were considered significant when $P < 0.05$.

Results

Influence of extracellular Mg^{2+} concentration on base-line tone of aortic strips in Ca^{2+}-containing and Ca^{2+}-free solution
Fig. 1a shows the effect of withdrawal of Mg^{2+} from the incubating medium and the effect of additions of cumulative concentrations of this cation on the resting tone of rat aortic strips. In Ca^{2+}-free medium the results were different (Fig. 1b).

Concentration–response curves of relaxation of Mg^{2+} on the contractile responses induced by different agonists
Sustained contractile responses of rat aorta were elicited by depolarizing Krebs solution (KCl 80 mM) in the presence or absence of Mg^{2+}, with a magnitude of 206.0 ± 37.6 (n = 5) and 194.9 ± 36.3 mg (n = 5), respectively. Addition of cumulative concentrations of Mg^{2+} (1–32 mM) to the contractile plateau elicited by KCl in Mg^{2+}-free medium induced concentration-dependent relaxation. E_{max} and IC50 values are summarized in Table 1.

The characteristic acetylcholine-induced contractile response in Krebs solution (133.7 ± 18.5 mg, n = 4) was a contraction that developed and decreased gradually. However, when elicited in Mg^{2+}-free medium, a sustained contractile response with a magnitude of 127.5 ± 12.5 mg (n = 4) was observed. Addition of Mg^{2+} (1 mM) on the contractile plateau decreased the contraction to resting tone value and we could not obtain a concentration–response curve of relaxation by addition of cumulative concentrations of Mg^{2+}.
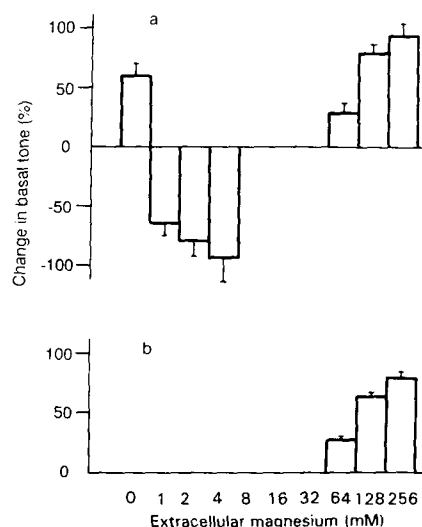

FIG. 1. Modification of basal tone in rat thoracic aorta by altering extracellular magnesium levels in the presence (a) or the absence (b) of extracellular calcium. The numbers below the columns indicate the concentration of magnesium (mM). Vertical bars indicate s.e.m., n = 6–10.

In normal and Mg^{2+}-free Krebs solution, noradrenaline (1 μM) induced sustained contractile responses (212.7 ± 11.0, n = 4, and 206.9 ± 55.9 mg, n = 4, respectively). Contractile responses in Mg^{2+}-free solution were inhibited in a concentration-dependent manner by addition of cumulative doses (1–32 mM) $MgCl_2$. IC50 and E_{max} values are summarized in Table 1.

Influence of Mg^{2+} on acetylcholine-induced contractions in Ca^{2+}-free medium
Addition of acetylcholine (1 mM) to the organ bath containing Krebs solution evoked a transient contractile response of 135.0 ± 24.3 mg (n = 6), which developed and decreased gradually. The tissue was then washed and incubated for 5 min in Ca^{2+}-free medium and the agonist, acetylcholine, was applied (ACh_1), inducing a phasic contraction which gradually returned to the basal level (Table 2). Further additions of acetylcholine failed to induce any contraction, indicating complete depletion of the intracellular Ca^{2+} stores sensitive to acetylcholine. The aortic strip was then incubated for 20 min in the presence of Ca^{2+} (1.8 mM) to ascertain whether the Ca^{2+} depots sensitive to acetylcholine are completely refilled. After the refilling period, the aorta was loaded for 5 min in a Ca^{2+}-free medium and acetylcholine (ACh_2) was added, inducing a phasic contraction similar to that obtained by the first addition of this agonist in the same medium (ACh_1;

Table 1. Parameters of concentration–response curves for the relaxation induced by addition of cumulative concentrations of magnesium on noradrenaline-induced contraction and KCl-depolarized rat aorta.

	Noradrenaline	KCl
E_{max} (%)	96.6 ± 7.2	94.7 ± 11.4
IC50 (mM)	3.9 ± 0.5	$10.0 \pm 2.0^*$

Values are means ± s.e.m., number of experiments = 4. $^*P < 0.01$ compared with the corresponding value for noradrenaline.

Table 2. Influence of magnesium on contractile responses to acetylcholine in Ca^{2+}-free medium.

| | n | Mg^{2+} (mM) | Response | | |
			Krebs (mg)	ACh_1 (%)	ACh_2 (%)
Control	6	1·2	135·0±24·3	27·0±4·3	23·1±3·1
Experimental	4	0	118·8±34·4	45·8±3·4**	40·3±7·9*

All values represent mean ± s.e.m. and are expressed as a percentage of acetylcholine-induced contraction in Krebs solution, n=number of experiments. *P<0·05; **P<0·001 compared with control. ACh_1: addition of the agonist after 5 min incubation in Ca^{2+}-free medium. ACh_2: addition of the agonist after 20 min resting period in Krebs solution and 5 min in Ca^{2+}-free medium.

Table 2). This indicates a recovery of the acetylcholine-sensitive internal Ca^{2+} stores.

The results obtained in other series of experiments are shown in Table 2. After a preincubation time of 5 min in the absence of Ca^{2+} and Mg^{2+}, addition of acetylcholine (ACh_1) to the aorta yielded a significantly increased contractile response ($P<0·001$) with respect to that induced in the absence of Ca^{2+} but in the presence of Mg^{2+} (Table 2). Afterwards, the tissue was incubated for 20 min in Ca^{2+}-containing Mg^{2+}-free solution to refill the intracellular Ca^{2+} stores and then loaded for 5 min in Ca^{2+}- and Mg^{2+}-free solution. A new addition of acetylcholine (ACh_2) induced a contraction similar to that obtained by the first addition of the agonist in this medium (Table 2) which indicates complete refilling of the intracellular Ca^{2+} depots sensitive to acetylcholine.

A similar experimental procedure was used in a new series of experiments but acetylcholine was added after 30 min of preincubation in Ca^{2+}-free medium with (n=4) or without (n=4) Mg^{2+}. In neither case were contractile responses to acetylcholine observed, indicating that the acetylcholine-sensitive intracellular Ca^{2+}-pools disappear after prolonged loading in Ca^{2+}-free medium containing 0·1 mM EDTA.

Influence of Mg^{2+} on depolarizing-induced contractions in Ca^{2+}-free solution

Addition of KCl 80 mM to the organ bath incubated in Krebs solution evoked a sustained contractile response (225·2±18·5 mg, n=18) which rapidly returned to the resting tone after washing with Krebs solution. After 5 min of incubation in the absence of Ca^{2+}, the depolarizing solution induced a small phasic contractile response in 9 of the 18 experiments carried out (15·6±2·7%, n=9; relative to the value obtained in the presence of Ca^{2+}). After washing with Ca^{2+}-free solution a new application of KCl did not evoke further contractions (n=18). In other experiments, after addition of KCl in Krebs solution (184·2±21·6 mg, n=5), the strips were incubated for 30 min in Ca^{2+}-free medium and application of depolarizing solution did not elicit any contractile response (n=5).

In a new series of experiments addition of KCl 80 mM in Krebs solution yielded a sustained contractile response of 248·4±27·5 mg (n=18). After 5 min of incubation time in Ca^{2+}- and Mg^{2+}-free medium, addition of KCl to the aortic strip evoked a phasic contraction in all the experiments (33·7±5·0%, n=18; relative to the value obtained in Ca^{2+}-

containing solution), which was significantly higher than that obtained when Mg^{2+} was present in the incubation medium ($P<0·01$). In other experiments, after addition of KCl in Krebs solution (164·8±18·2 mg, n=5), the strips were incubated for 30 min in Ca^{2+}- and Mg^{2+}-free medium and no contractile response was obtained (n=5).

Discussion

The results of this study indicate that acute withdrawal of Mg^{2+} (0 mM) produces increases in the tone of rat aorta incubated in the presence of Ca^{2+}. The fact that no contraction is induced by Mg^{2+}-depletion when alterations in extracellular Mg^{2+} are carried out in the absence of Ca^{2+}, indicates that the increase in tension is due to the entry of extracellular Ca^{2+} as a result of changes in membrane permeability (Kimura et al 1989). These results agree with those of many previous studies (Turlapaty & Altura 1980; Altura 1982; Altura & Altura 1985a, b).

Concentrations of Mg^{2+} between 8 and 32 mM did not modify the tension in the absence or presence of Ca^{2+}, but higher levels (64–256 mM) promoted concentration-dependent increases in tension. Data obtained by atomic absorption spectrophotometry, nuclear magnetic resonance spectrometry and ^{28}Mg determination indicate that the intracellular content of Mg^{2+} can be only slightly influenced by small changes in extracellular levels of this cation (Shetty & Weiss 1988). However, Palaty (1974) and Shetty & Weiss (1989) reported that in the absence of external Ca^{2+} there is an intracellular Mg^{2+}-dependent fraction that could be altered by elevated concentrations of Mg^{2+}. In addition, many studies have demonstrated that at high concentrations, Mg^{2+} can induce contractions in skinned arteries by activating a regulatory system independent of the Ca^{2+}-calmodulin-dependent phosphorylation of smooth muscle myosin light chain kinase; thus, Mg^{2+}-induced contractions were independent of the presence of Ca^{2+} in the incubation medium (Ikebe et al 1984; Moreland & Moreland 1991). Accordingly, contractions elicited in our experiments by addition of Mg^{2+} (32–256 mM) can be explained by a direct action of this divalent ion on the contractile machinery as a consequence of an elevated increase of the cation in the cytosol.

In the present study, Mg^{2+} inhibited noradrenaline- and KCl-induced contractions and relaxed smooth muscle, indicating inhibition of Ca^{2+} entry activated by agonist addition or depolarization of the cell membrane. Moreover, Mg^{2+} was more effective in inhibiting noradrenaline-elicited contractile responses than KCl-induced responses. Acetylcholine induced sustained contractions in Mg^{2+}-free Krebs solution. Restoration of this cation to physiological levels (1 mM) completely relaxed the contractile response, but the precise mechanism by which Mg^{2+} reduces the tonic contraction induced by acetylcholine is presently unknown.

The fact that when Ca^{2+} is removed a contractile response to acetylcholine can be elicited, suggests a release of Ca^{2+} through a mechanism related to pharmacomechanical coupling, activation of phospholipase C and production of inositol trisphosphate (Ganitkevich & Isenberg 1990). The intracellular Ca^{2+} store sensitive to acetylcholine might be compatible with one of limited capacity because after

washing, further addition of the agonist evoked no contraction, and this indicates complete depletion of the Ca^{2+}-storage sites. This acetylcholine-sensitive intracellular Ca^{2+} store is slowly and spontaneously lost during the loading time in the absence of Ca^{2+}, as after 30 min of incubation in Ca^{2+}-free medium no contraction could be elicited. The characteristics of the contractile mechanism in response to the application of acetylcholine in Ca^{2+}-free medium are similar to those of noradrenaline (Noguera & D'Ocon 1992). Moreover, 20 min of loading time in Ca^{2+}-containing solution is sufficient to recover the contractile response to acetylcholine in Ca^{2+}-free medium.

Our results confirm earlier studies which demonstrated that contractile responses in the absence of Ca^{2+} in rat thoracic aorta can be altered by removing Mg^{2+} from the organ bath (Noguera & D'Ocon 1992). Contractile responses to acetylcholine in Ca^{2+}-free medium are increased by depleting Mg^{2+} in the incubation medium. Recovery of the response to the agonist is complete after a refilling period in the presence of Ca^{2+} but in the absence of Mg^{2+}, which means that refilling of the internal acetylcholine-sensitive Ca^{2+} stores is independent of extracellular Mg^{2+}.

Although it is commonly recognized that depolarization of cell membrane opens Ca^{2+} channels dependent on voltage (Bolton 1979), other mechanisms, such as the release of noradrenaline, which by stimulating α-adrenoceptors leads to a release of intracellular Ca^{2+} (Garcia-Pascual et al 1991), cannot be excluded. The fact that, at least in part of the experiments and after 5 min incubation in the absence of Ca^{2+}, a small contraction could be recorded indicates the existence of a weakly bound Ca^{2+} depot, sensitive to KCl, which is slowly and spontaneously lost during the incubation in the absence of Ca^{2+}. Thus, addition of KCl after 30 min of loading time in Ca^{2+}-free medium elicited no response. This hypothetical Ca^{2+}-pool sensitive to depolarization was previously observed by Högestätt & Andersson (1984) and Somlyo et al (1985) using fluorimetric techniques.

When depolarization of cell membrane is induced in the absence of Ca^{2+} and Mg^{2+}, the contractile activity of KCl is augmented, indicating the presence of a Ca^{2+} pool slightly bound to cell membrane that is spontaneously depleted, but in the absence of Mg^{2+} this loss is retarded. We can see again the role of Mg^{2+} in a process that leads to the depletion of an intracellular Ca^{2+} store.

We can conclude that basal tone is sensitive to alterations in extracellular Mg^{2+}, Ca^{2+} channels are modulated and controlled by Mg^{2+}, and that responsiveness to different agonists in the absence of Ca^{2+} is increased by Mg^{2+} depletion. With these conclusions, the present study extends previous ones and supports the hypothesis that Mg^{2+} deficiency or excess may play a role in the pathogenesis of some coronary spasms in man.

References

Altura, B. M. (1982) Magnesium and regulation of contractility of vascular smooth muscle. Adv. Microcirc. 11: 77–113

Altura, B. M., Altura, B. T. (1985a) New perspectives on the role of magnesium in the pathophysiology of the cardiovascular system. I. Clinical aspects. Magnesium 4: 226–244

Altura, B. M., Altura, B. T. (1985b) New perspectives on the role of magnesium in the pathophysiology of the cardiovascular system. II. Experimental aspects. Magnesium 4: 245–271

Altura, B. M., Altura, B. T., Carella, A., Gebrewold, A., Murakawa, T., Nishio, A. (1987) Mg–Ca interaction in contractility of vascular smooth muscle: Mg versus organic calcium-channel blockers on myogenic tone and agonist-induced responsiveness of blood vessels. Can. J. Physiol. Pharmacol. 65: 729–745

Bolton, T. B. (1979) Mechanisms of actions of transmitters and other substances on smooth muscle. Physiol. Rev. 59: 608–718

D'Ocon, M. P., Anselmi, E., Villar, A. (1987a) Effects of magnesium chloride on the contractile response of uterus to several agonists in Ca-free solution. J. Pharm. Pharmacol. 39: 444–448

D'Ocon, M. P., Anselmi, E., Villar, A. (1987b) Role of stored Ca in contractions of rat uterus induced by acetylcholine: effects of preincubation with Mg-free solution. Arch. Int. Pharmacodyn. 286: 162–170

Furchgott, R. F., Zawadzki, J. V. (1980) The obligatory role of endothelial cells in the relaxation of arterial smooth muscle by acetylcholine. Nature 288: 373–376

Ganitkevich, V., Isenberg, G. (1990) Isolated guinea-pig coronary smooth muscle cells. Acetylcholine induces hyperpolarization due to sarcoplasmic reticulum calcium release activating potassium channels. Circ. Res. 67: 525–528

Garcia-Pascual, A., Costa, G., Isla, M., Jimenez, E., Garcia, S. A. (1991) Potassium-induced contraction in the lamb proximal urethra: involvement of norepinephrine and different calcium entry pathways. J. Pharmacol. Exp. Ther. 256: 127–134

Högestätt, E. D., Andersson, K. E. (1984) Mechanisms behind the biphasic contractile response to potassium depolarization in isolated rat cerebral arteries. J. Pharm. Exp. Ther. 228: 187–195

Ikebe, M., Barsotti, R. J., Hinkins, S., Hatshorne, D. J. (1984) Effects of magnesium chloride on smooth muscle actomyosin adenosine-5'-trisphosphatase activity, myosin conformation, and tension development in glycerinated smooth muscle fibers. Biochemistry. 23: 5062–5068

Kimura, T., Yasue, H., Sakaino, N., Rokutanda, M., Jougasaki, M., Araki, H. (1989) Effects of magnesium on tone of isolated human coronary arteries. Circulation 79: 1118–1124

Moreland, R. S., Ford, G. D. (1981) The influence of magnesium on calcium-activated vascular smooth muscle actomyosin ATPase activity. Arch. Biochem. Biophys. 208: 325–333

Moreland, R. S., Ford, G. D. (1982) The influence of magnesium on the phosphorylation and dephosphorylation of myosin by an actomyosin preparation from vascular smooth muscle. Biochem. Biophys. Res. Commun. 106: 652–659

Moreland, R. S., Moreland, S. (1991) Characterization of Mg-induced contractions in detergent-skinned swine carotid media. Am. J. Physiol. 260 (Cell Physiol. 29): C1224–C1232

Murakawa, T., Altura, B. T., Carella, A., Altura, B. M. (1988) Importance of magnesium and potassium concentration on basal tone and 5-HT-induced contractions in canine isolated coronary artery. Br. J. Pharmacol. 94: 325–334

Noguera, M. A., D'Ocon, M. P. (1992) Different and common intracellular calcium-stores mobilized by noradrenaline and caffeine in vascular smooth muscle. Naunyn Schmiedebergs Arch. Pharmacol. 345: 333–341

Palaty, V. J. (1974) Regulation of the cell magnesium in vascular smooth muscle. Physiology 242: 555–569

Pato, M. D., Adelstein, R. S. (1980) Dephosphorylation of the 2000-dalton light chain of myosin by different phosphatases from smooth muscle. J. Biol. Chem. 255: 6535–6538

Shetty, S. S., Weiss, G. B. (1988) Alterations in ^{28}Mg distribution and movements in rabbit aortic smooth muscle. J. Pharmacol. Exp. Ther. 245: 112–119

Shetty, S. S., Weiss, G. B. (1989) Effects of pH and anion substitution on magnesium accumulation in rabbit aortic smooth muscle. Blood Vessels 26: 65–76

Singh, R. B. (1987) Is verapamil a better magnesium agonist rather than a calcium antagonist? A hypothesis? Medical Hypotheses 24: 1–9

Somlyo, A. P., Wasserman, A. J., Kitazawa, T., Bond, M., Shuman, H., Somlyo, A. V. (1985) Calcium and sodium distribution and movements in smooth muscle. Experientia 41: 981–988

Turlapaty, P. D. M. V., Altura, B. M. (1980) Magnesium deficiency produces spasms of coronary arteries: relationship to etiology of sudden death ischemic heart disease. Science 208: 198–200