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M. Saito · M. Ohmura · A. Kondo · K. Miyake

Effect of magnesium on the function of the rabbit corpus cavernosum

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Abstract Contraction and relaxation of the smooth muscle, including the corpus cavernosum, are mediated by changes in the intracellular concentration of calcium. Since magnesium modulates the movement of calcium, it can modify the function of the erectile tissue. We designed this study to investigate the effects of magnesium in doses ranging from 5 to 30 mM on the function of the rabbit corpus cavernosum in vitro. The resting tension of tissue strips was significantly reduced by exposure to a solution high in magnesium (5–30 mM). The contractile response to field stimulation under resting conditions, and the contraction to phenylephrine, were significantly decreased by magnesium (5–30 mM). There were no differences in the contractile strength of the corpus cavernosum to KCl. Although the relaxation induced by field stimulation under preincubation with 200 μM phenylephrine was abolished in the presence of 30 mM magnesium, there were no differences at a concentration of 5 mM or of 10 mM magnesium. The relaxation induced by sodium nitroprusside under precontraction with 200 μM phenylephrine was further increased by magnesium dose dependently. A high concentration of magnesium (30 mM) enhanced both bethanechol-induced and ATP-induced relaxations under precontraction with phenylephrine. Our study demonstrated that magnesium reduced the receptor-mediated contraction of the rabbit corpus cavernosum and enhanced the relaxation of this tissue induced by sodium nitroprusside, bethanechol, and ATP.

Key words Rabbit corpus cavernosum · Impotence · Magnesium · Calcium · Smooth muscle

M. Saito (✉) · M. Ohmura · A. Kondo · K. Miyake
Department of Urology, Nagoya University School of Medicine, Tsurumae 65, Showa, Nagoya 466, Japan
Fax: (+81) 52 741 3040

Introduction

Erectile function is mediated by the interaction of several forms of neurotransmission, including the local production and release of nitric oxide [5, 8, 15, 17, 20]. Contraction leading to the detumescence of the corpus cavernosum is mediated primarily by α-adrenergic stimulation. An erection is initiated and sustained by a relaxation of the corpus cavernosum, primarily through the action of nitric oxide. Nitric oxide (endothelial-derived relaxant factor) can be released directly from nerve terminals on smooth muscle, and via cholinergic activation of the endothelial cells [5, 8, 17, 20]. Smooth muscle contraction and relaxation are mediated by changes in the intracellular concentration of free calcium [3, 4, 7, 11, 16]. The function of the penile erectile tissue is also mediated by calcium [14].

Magnesium is known to modify smooth muscle contractility [10]. Since this agent is reported to be a calcium channel inhibitor [2, 9, 10], it can be used as an adjuvant in treating hypertension [21]. In addition, alterations in the extracellular concentration of magnesium could modify the formation and/or the release of nitric oxide, with a resultant decrease in tonus of the arterial smooth muscle [18]. These findings suggest that magnesium may modify the penile erectile function.

We designed this study to investigate the effect of magnesium on the response of the rabbit corpus cavernosum to various kinds of stimulation.

Materials and methods

Eleven male Japanese white rabbits obtained from Chubu Kagaku, Aichi, Japan (mean weight 3 kg), were used. All rabbits were sedated with an intramuscular injection of ketamine (25 mg/kg) and xylazine (6 mg/kg). Anesthesia was maintained by intravenous injection of sodium pentobarbital (25 mg/kg). The penis was removed at the level of attachment of the corporal body to the ischium. The grossly dissected organ preparation was then placed in Krebs' solution at room temperature. Subsequently, most of the overlying skeletal muscle was carefully removed to avoid damaging the underlying

tunica albuginea. Once it was fully exposed, a slit was made in the proximal end of the tunica and extended distally. The corpus cavernosum was sharply dissected free from the tunica bilaterally. Two samples of corporal tissue from each rabbit (total 22 tissue samples) were used for the experiments.

Longitudinal sections of the rabbit corpus cavernosum having an unstretched length of about 10 mm were placed in an organ bath containing 10 ml Krebs' solution (NaCl 119 mM, KCl 4.7 mM, $MgSO_4$ 1.2 mM, KH_2PO_4 1.2 mM, $CaCl_2$ 2.5 mM, $NaHCO_3$ 25 mM and glucose 11 mM) at 37 °C. The tissue was allowed to equilibrate for 60 min in a mixture of 95% oxygen and 5% carbon dioxide at a resting tension of 2 g. One end of each strip was connected to a force displacement transducer (Sanei model 45196), and changes in muscle tension were measured and recorded on a Rectigraph-8k (Sanei).

Field stimulations utilized platinum electrodes that were set on both sides of the muscle strips in the organ baths. Transmural nerve stimulation was performed with a DPS-160 field stimulator (Dia-Medical System) that delivered biphasic square wave pulses of 50 V, 0.5 ms in duration. The interval between stimulations was 2 min. After an equilibration at 2 g resting tension, field stimulation was applied at various frequencies from 2 to 60 Hz. Under resting conditions without precontraction, the contractile response to field stimulation was evaluated.

The frequency response study to field stimulation was repeated under precontraction with 200 μM phenylephrine, an α-adrenergic agonist. After the contraction induced by 200 μM phenylephrine had plateaued, the relaxation induced by field stimulation was observed.

The dose-response effects of phenylephrine (0.14–200 μM) and the contraction in the KCl-Krebs' solution (KCl, 60 mM) were examined at a resting tension of 2 g. The KCl-Krebs' solution was prepared by replacing NaCl with an equimolar amount of KCl. We observed the relaxation of the corpus cavernosum induced by one dose of ATP (adenosine 5'-triphosphate; 2 mM), bethanechol (600 μM), and sodium nitroprusside (100 μM) under precontractile conditions with 200 μM phenylephrine.

After completing the first study using normal Krebs' solution, we changed the incubation medium to one containing 5 mM $MgCl_2$. This medium was prepared by replacing sodium with an equimolar amount of magnesium. A second study was performed after 30 min incubation in this solution. This procedure was repeated with $MgCl_2$, 10 mM and 30 mM. A high KCl solution also contained $MgCl_2$ (5, 10, 30 mM), which was replaced by an equimolar amount of NaCl.

Preliminary studies demonstrated that, under standard conditions, the tissue generated similar results for four sequential studies produced in normal Krebs' solution at 30-min intervals. The effect of magnesium on resting basal tension of the tissue strips was evaluated by comparing the tension of these strips after equilibration for 60 min in normal Krebs' solution versus that at the end of 30 min incubation in a solution high in magnesium.

ATP, phenylephrine, bethanechol, and sodium nitroprusside were all purchased from Sigma. The contractile responses to field stimulation, bethanechol, and KCl were expressed as grams tension/100 mg tissue. The relaxations induced by field stimulation, and by the administration of ATP, bethanechol, and sodium nitroprusside, were expressed as the percentage relaxation of total tonic tension, calculated as the maximum decrease in tissue tension (grams) during drug application and field stimulation divided by the total tonic tension of the tissue strips under precontraction with 200 μM phenylephrine.

Statistical comparisons were made using the unpaired Student's *t*-test. A probability level of $P < 0.05$ was accepted as being statistically significant.

Results

The resting tissue tension of the corpus cavernosum was significantly and dose dependently reduced by

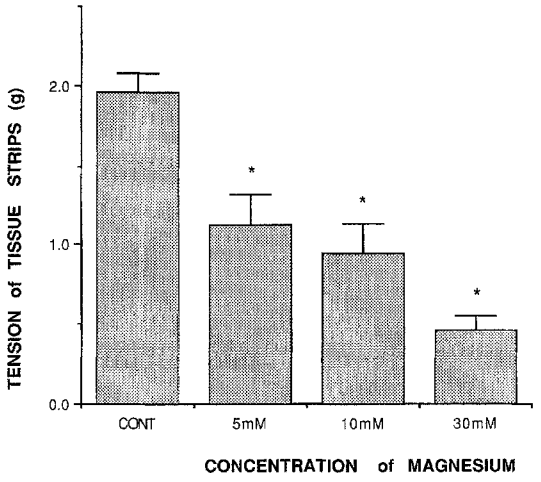

Fig. 1 Effect of magnesium on basal tension of the corpus cavernosum. *Each point* is the mean ± SEM of nine duplicate observations. *CONT* control values observed in Krebs' solution. * significant difference from the tension in normal Krebs' solution. $P < 0.05$

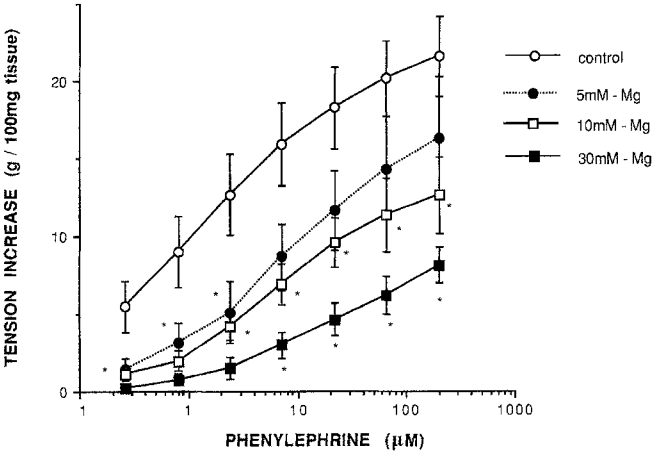

Fig. 2 Effect of magnesium on the dose-response curve to phenylephrine. *Each point* is the mean ± SEM of five individual observations. * significant difference from the response in normal Krebs' solution. $P < 0.05$

$MgCl_2$, 5–30 mM (Fig. 1). The contraction of the corporal tissue produced by phenylephrine was significantly decreased dose dependently by a solution that contained a high concentration of magnesium (Fig. 2). Under 2 g resting tension without precontraction, the contraction induced by field stimulation was decreased significantly as the concentration of magnesium was increased (Fig. 3). Although field-stimulated relaxation under precontraction with 200 μM phenylephrine was abolished in a solution containing 30 mM magnesium, no differences were observed at magnesium concentrations of 5 mM or 10 mM (Fig. 4). Under precontraction with 200 μM phenylephrine, the relaxation induced by sodium nitroprusside was further increased by magnesium dose dependently (Fig. 5). The relaxations induced by bethanechol and ATP were enhanced by exposure to 30 mM magnesium (Figs. 6, 7).

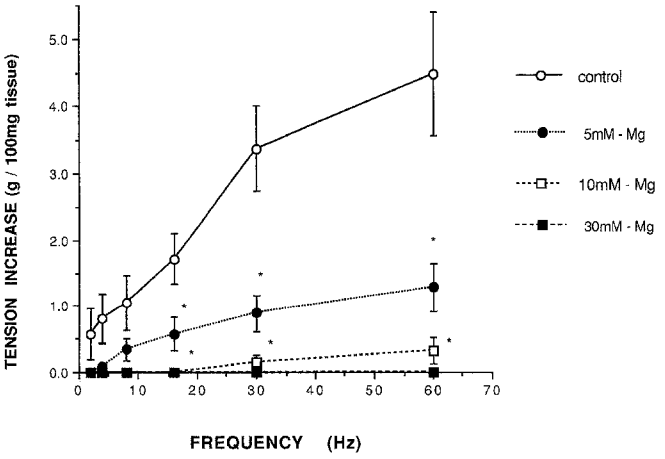

Fig. 3 Effect of magnesium on the contraction to field stimulation under 2 g resting tension without precontraction. *Each point* is the mean ± SEM of five individual observations. * significant difference from the response in normal Krebs' solution. $P < 0.05$

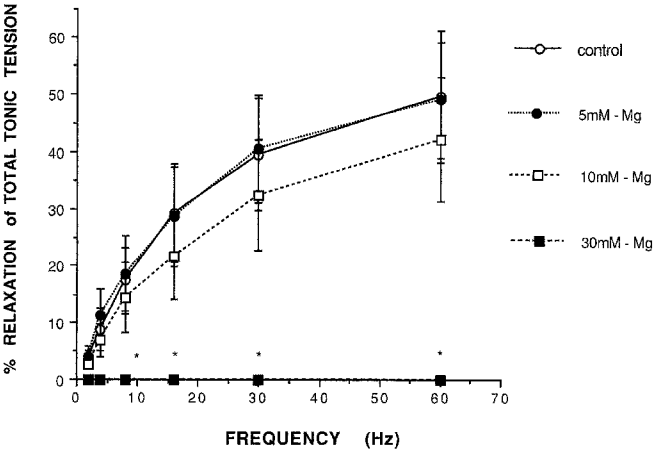

Fig. 4 Effect of magnesium on the relaxation induced by field stimulation under precontraction with 200 μM phenylephrine. *Each point* is the mean ± SEM of five individual observations. * significant difference from the response in normal Krebs' solution. $P < 0.05$

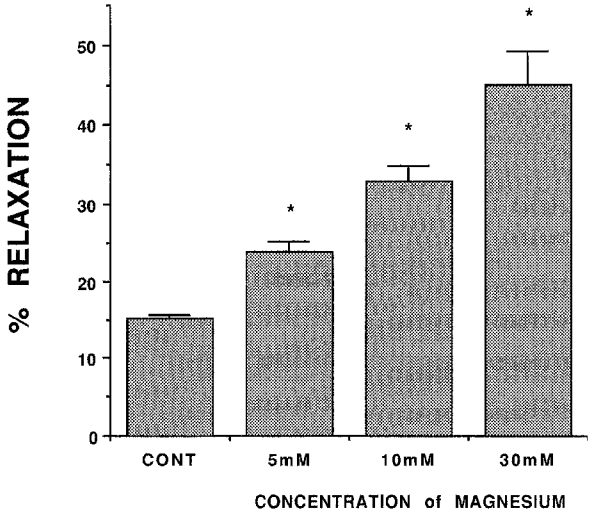

Fig. 5 Effect of magnesium on the relaxation induced by sodium nitroprusside under precontraction with 200 μM phenylephrine. *Each point* is the mean ± SEM of six individual observations. *CONT* control values observed in Krebs' solution. * significant difference from the response in normal Krebs' solution. $P < 0.05$

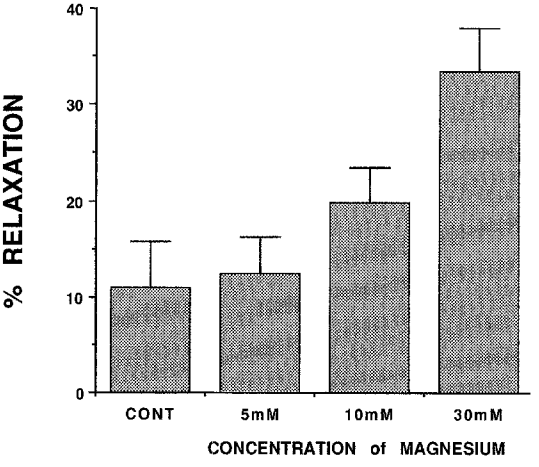

Fig. 6 Effect of magnesium on the relaxation induced by bethanechol under precontraction with 200 μM phenylephrine. *Each point* is the mean ± SEM of five individual observations. *CONT* control values observed in Krebs' solution. * significant difference from the response in normal Krebs' solution. $P < 0.05$

There was no change in the response of the corpus cavernosum to KCl in the presence of any concentration of $MgCl_2$ (Fig. 8).

Discussion and conclusion

The corpus cavernosum is contracted by α-adrenergic neurotransmission, which leads to detumescence. An erection is maintained by the continuous relaxation of the corpus cavernosum induced by a decrease in α-neurotransmission, the activation of muscarinic cholinergic receptors, and the activation of non-adrenergic and non-cholinergic neurotransmitters (nitric oxide and ATP) [5, 8, 15, 17, 20]. Cholinergic stimulation initiates the local release of the endothelial-derived relaxation factor, which is nitric oxide, and which mediates direct relaxation of the corpus cavernosum [17].

The response of smooth muscle, including the corpus cavernosum, to various forms of stimulation depends mainly on changes in the concentration of intracellular free calcium [3, 4, 7, 11, 14]. The study using Fura-2 calcium fluorescence demonstrated that the contractions of the corpus cavernosum in response to both α-adrenergic stimulation and field stimulation were mediated by an increase in the intracellular concentration of free calcium. However, calcium movement was inconsistent during relaxation [14].

Magnesium exerts its effect on smooth muscle as a nonspecific calcium channel inhibitor [2, 9, 10]. In

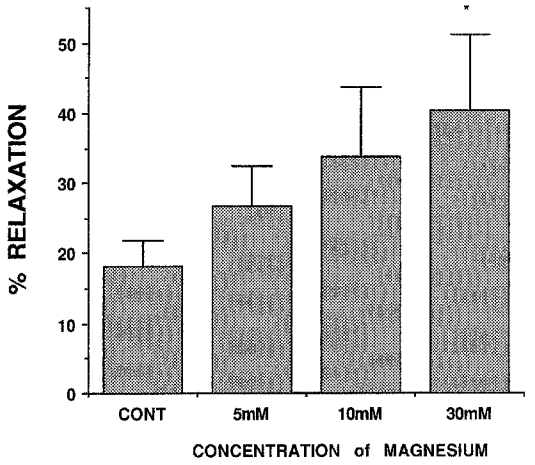

Fig. 7 Effect of magnesium on the relaxation induced by ATP under precontraction with 200 μM phenylephrine. *Each point* is the mean ± SEM of seven individual observations. *CONT* control values observed in Krebs' solution. * significant difference from the response in normal Krebs' solution. $P < 0.05$

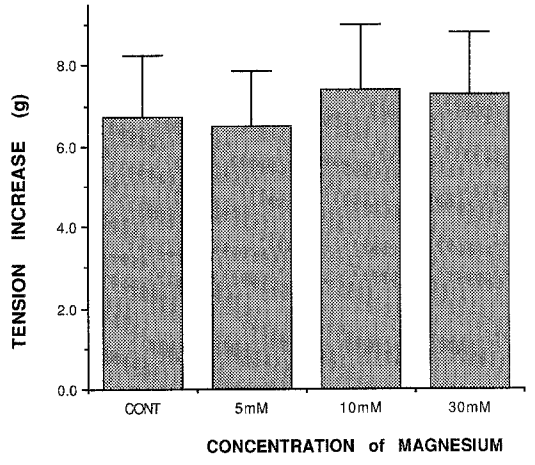

Fig. 8 Effect of magnesium on the contraction induced by a solution high in KCl. *CONT* control values observed in Krebs' solution. *Each point* is the mean ± SEM of six individual observations

addition, this agent reduces the release of intracellular calcium from the sarcoplasmic reticulum [10]. Magnesium is an essential cofactor for activation of various enzyme systems [10]. There is evidence for involvement of the endothelium in the vasodilatation induced by magnesium [1, 19].

This study demonstrated that magnesium reduced the basal tonus of erectile tissue, consistent with results of studies conducted in vascular smooth muscle [12, 22]. These studies suggest that magnesium reduces the resting calcium permeability via the smooth muscle cell membrane.

Magnesium nonselectively inhibits both the voltage-dependent calcium channels and the receptor-operated calcium channels, whereas calcium channel blockers inhibit the voltage-dependent calcium channels relatively selectively [10]. Magnesium significantly inhibited the contractions of corpus cavernosum in response to phenylephrine and to field stimulation without precontraction. However, the contractile response to KCl was unaffected by an increase in extracellular concentration of magnesium (5–30 mM). In our previous study, the magnesium significantly reduced the response of the rat detrusor to KCl [13]. The sensitivity of the arteries to magnesium showed a considerable difference based on animal species as well as the part from which the artery was taken [10]. The sensitivity of the corpus cavernosum to magnesium in the response to KCl may be extremely low.

The contraction and relaxation to field stimulation were completely abolished in the presence of 30 mM magnesium. However, a direct stimulation of the receptor gave a significant response. These observations suggested that 30 mM magnesium may disturb the nerve-mediated response. The relaxations induced by sodium nitroprusside, bethanechol, and ATP were enhanced by an increase in the extracellular concentration of magnesium. Bethanechol-induced relaxation was endothelium dependent (via an effect on the release of nitric oxide) [17]. Since changes in the extracellular concentration of magnesium can modify the formation and release of the endothelial-dependent relaxing factor known to be nitric oxide [18], magnesium can augment the relaxation induced by bethanechol enhancing the formation and/or the release of nitric oxide. Sodium nitroprusside and nitric oxide are each thought to produce smooth muscle relaxation by activating soluble guanylate cyclase, and by increasing the cyclic GMP [6, 10, 16]. The effect of sodium nitroprusside on vascular smooth muscle was also attenuated by a solution that was free of magnesium [10]. These studies suggest that magnesium may enhance the activation of intracellular guanylate cyclase.

ATP in the corporal tissue is thought to induce a response via the purinergic receptors [20]. ATP-induced relaxation increases the intracellular calcium concentration [14]. Since magnesium is a nonselective calcium channel inhibitor that decreases intracellular calcium concentration [10], the ATP-induced relaxation of the corpus cavernosum should be inhibited by magnesium. However, the present study showed that the ATP-induced relaxation was enhanced by magnesium. In the presence of bethanechol, which relaxes the corporal tissue, ATP contracted the corpus cavernosum with an increase in the intracellular concentration of calcium [8, 14]. The controversy remains as to the mechanisms involved in the ATP-mediated corporal relaxation and contraction.

In conclusion, magnesium decreased tension and decreased the contractile strength of the corpus cavernosum to α-adrenergic stimulator and to nerve-mediated stimulation. The relaxations induced by sodium nitroprusside, bethanechol, and ATP were enhanced.

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