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EFFECTS OF MAGNESIUM ON CONTRACTILE RESPONSES INDUCED BY ELECTRICAL TRANSMURAL STIMULATION AND NORADRENALINE IN RABBIT THORACIC AORTA

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1 In rabbit isolated thoracic aortae, effects of magnesium ions on the contraction and ^{3}H-efflux in response to electrical transmural stimulation and on the contractile responses induced by noradrenaline and KCl were investigated.

2 Addition of magnesium (1.2, 3.6, 12.0 and 24.0 mM) to the bathing solution inhibited the electrically induced contractions in a dose-related manner; the inhibition was complete with a concentration of 24.0 mM.

3 The increase in ^{3}H-efflux induced by electrical transmural stimulation was inhibited by the addition of magnesium to the superfusing fluid, but a complete block was not obtained even in high concentrations of magnesium.

4 Magnesium ions (1.2, 3.6, 12.0 and 24.0 mM) inhibited the contractile responses induced by low concentrations of noradrenaline (2×10^{-8} M) and KCl (2×10^{-2} M). However, the responses induced by higher concentrations of noradrenaline (5×10^{-7} and 10^{-5} M) and KCl (3 and 4×10^{-2} M) were enhanced by low concentrations of magnesium.

5 Magnesium ions affect both presynaptic and effector sites in rabbit thoracic aortae but in a different manner; magnesium manifests only an inhibitory effect on noradrenaline release from the adrenergic nerves, but dual effects on reactivity of vascular smooth muscle, depending on concentrations of magnesium and stimulants; it is suggested that the change in vascular reactivity is more important than the reduction in transmitter release when magnesium inhibits the response to nerve stimulation.

Introduction

It is well known that magnesium ions are essential in the stimulus-secretion coupling mechanisms, affecting the release of transmitter from motor nerves (Eccles, 1961; Wernig, 1972), chromaffin cells in adrenal medulla (Douglas & Rubin, 1963), splenic nerve of cat (Kirpekar & Misu, 1967), and adrenergic nerve of artery (George & Leach, 1975). Furthermore, using isolated vascular smooth muscles, Altura & Altura (1971), Turlapaty & Carrier (1973) and Jurevics & Carrier (1973) reported that magnesium ions are involved in the regulation of the permeability of the cell membrane to various ions and that they modulate vascular reactivity.

In the present experiments, responses of rabbit thoracic aorta to electrical stimulation and noradrenaline were examined in order to clarify the pre- and postjunctional effects of magnesium.

Methods

Rabbits (about 2.5 kg) of either sex were killed by a blow on the head, and carotid arteries were cut. The chest was opened and the thoracic aorta removed. A spiral strip of tissue, 0.4 cm wide, 1.5 cm long was mounted in a 20 ml organ bath filled with modified Krebs solution of the following composition (mM): NaCl 120.7, KCl 5.9, $MgCl_2$ varied, $CaCl_2$ 2.5, $NaHCO_3$ 15.5, NaH_2PO_4 1.2 and glucose 11.5 in distilled, deionized water. The solution was maintained at 37°C, constantly bubbled with a gas mixture of 95% O_2 and 5% CO_2 and had a pH of 7.3 to 7.4. The strip was attached to a force displacement transducer and isometric contractions were recorded on a biophysiograph, from an initial tension of 1.5 grams. Whenever the solution was changed to one with a different magnesium concentration, a 15 min period elapsed before transmural stimulation was

applied, or before noradrenaline or KCl was added cumulatively to the bath.

For electrical transmural stimulation, the strip was suspended between parallel platinum plate electrodes in the solution and stimulated at 10 min intervals with rectangular pulses of 0.3 ms duration at 30 Hz for 30 s at various voltages.

For radioisotope experiments, aortic strips about 1.5 cm long were equilibrated in the modified Krebs solution for 1 h and then transferred to solution containing (−)-[7-^{3}H]-noradrenaline (specific activity 6.6 Ci/mmol, Amersham/Searl Corp.) at 0.1 μM for 1 hour. The incubating solution contained ascorbic acid 0.1 mg/ml and sodium edetate 1.5 μg/ml to prevent auto-oxidation of noradrenaline. To investigate ^{3}H-efflux from the aorta by electrical transmural stimulation, a superfusion technique was used (Kurahashi & Fujiwara, 1976). The pre-incubated strip was suspended in air between parallel platinum wire electrodes and stimulated every 15 min with rectangular pulses (80 V, 0.3 ms duration, 30 Hz) for 30 s, whilst being superfused with the modified Krebs solution at a flow rate of 4 ml/minute. Three aliquots of superfusate were collected beforehand and five during or immediately after stimulation periods, each fraction being collected for 1 minute. Of the 4 ml in each sample, 1 ml was used to determine radioactivity.

The superfusate sample was added to 10 ml Bray's solution of the following composition; 2,5-diphenyloxazol PPO 4 g, 1,4-bis 2 [(4-methyl-5-phenyloxazolyl)] benzene dimethyl POPOP 200 mg, naphthalene 60 g, methanol 100 ml, ethylene glycol 20 ml and dioxane 880 ml. The radioactivity was measured in a Packard Tri-Carb liquid scintillation spectrometer and expressed as ct/min per fraction collected in 1 minute.

Drugs used were (−)-noradrenaline bitartrate (Sigma), potassium chloride (Nakarai) and magnesium chloride (Nakarai).

Results

Effect of magnesium on the contractions induced by electrical transmural stimulation in bathed tissues

The contractions of aortic strips to electrical transmural stimulation were completely blocked by addition to the bath of tetrodotoxin (5×10^{-7} M) or phentolamine (10^{-6} M) for 15 minutes. The size of the response was voltage-dependent, 20, 40 and 80 V causing increases in tension of 0.41 ± 0.05 g ($n = 4$), 0.52 ± 0.04 g ($n = 4$) and 0.65 ± 0.04 g ($n = 8$), respectively. These responses were inhibited progressively by addition of magnesium (1.2, 3.6, 12.0 and 24.0 mM), as shown in Figure 1, although no changes were seen in the resting tension. The contraction induced by low intensity stimulation (20 V) was more susceptible to magnesium than that to 40 or 80 volts. These inhibitory effects of magnesium occurred about 10 min after the solution had been changed and recovered to the original response about 20 min after its removal.

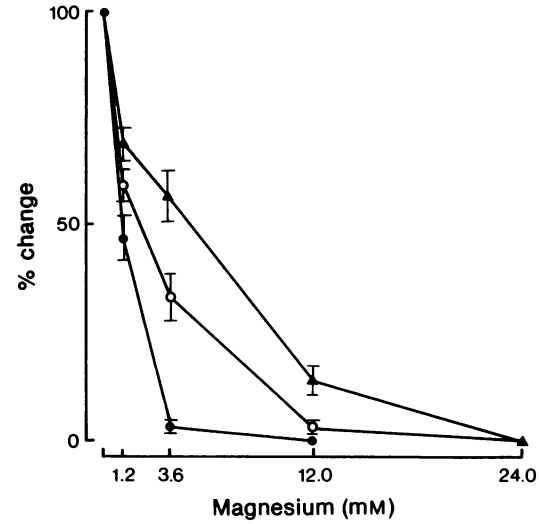

Figure 1 Effects of addition of magnesium to magnesium-free solution on contractile responses induced by electrical transmural stimulation (duration, 0.3 ms; frequency, 30 Hz; strength (●) 20 V, (○) 40 V, (▲) 80 V; for 30 s) in rabbit isolated thoracic aorta. The 100% indicates control responses in magnesium-free medium induced by electrical transmural stimulation at various voltages. Dose-response curves were obtained from 4 (20 V, 40 V) or 8 (80 V) preparations and calculated as a mean % inhibition for each point. Vertical lines show s.e. mean.

Effect of magnesium ions on the ^{3}H-efflux caused by electrical transmural stimulation in superfused tissues

Spontaneous efflux of ^{3}H from the aorta preloaded with [^{3}H]-noradrenaline declined quickly in the first 10 min, then gradually reached a plateau within 30 minutes. After this period, each tissue responded to transmural stimulation with an increase both in tension and in ^{3}H-efflux which was voltage-dependent. The overflow of ^{3}H was greatest in the superfusate fraction collected during stimulation and gradually declined to the basal level within the next 5 minutes. In each experiment at least 6 stimulations were applied at 15 min intervals and no significant decay of ^{3}H-efflux in response to stimulation was noted over this period. The efflux was blocked by superfusion with bretylium (10^{-5} M) for 10 minutes.

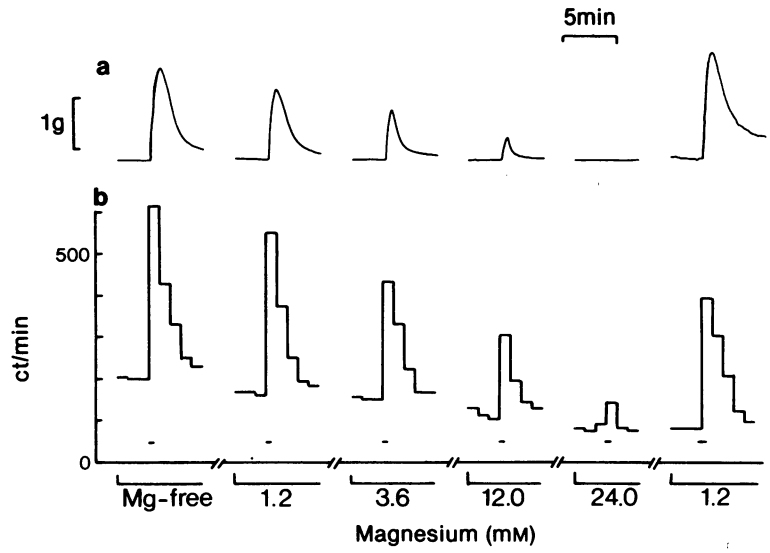

Figure 2 Representative recordings of the effects of adding magnesium to magnesium-free solution on mechanical responses (a) and ^{3}H-efflux (b) induced by electrical transmural stimulation (duration, 0.3 ms; frequency, 30 Hz; supramaximum voltage; for 30 s) in superfused rabbit thoracic aorta. Tissues were preincubated with [^{3}H]-noradrenaline (10^{-7} M) for 1 hour.

Neither the resting tension nor the basal level of ^{3}H-efflux were affected by increasing the magnesium concentration up to 24 mM. However, the contractions induced by stimulation at 80 V were progressively reduced as the magnesium concentration was increased, being completely abolished when the concentration reached 24.0 mM. The concomitant increases in ^{3}H-efflux were also progressively reduced although a complete block was not obtained even at the highest concentration of magnesium (Figure 2). It should be noted that the increase in tension to transmural stimulation at 80 V (30 Hz for 30 s, pulse duration 0.3 ms) was 1.47 ± 0.13 g, $n = 8$, being larger than that caused by 2×10^{-8} M noradrenaline (0.89 ± 0.01 g, $n = 19$) but smaller than that to 5×10^{-7} M (2.31 ± 0.21 g, $n = 23$).

Effect of magnesium ions on contractile responses induced by noradrenaline and KCl

The addition of noradrenaline (10^{-9} to 10^{-5} M) or KCl (1 to 4×10^{-2} M) to the magnesium-free medium caused concentration-dependent contractions. The EC_{50} values of noradrenaline were unchanged in the presence of 1.2 or 3.6 mM magnesium but were increased with 12.0 or 24.0 mM, whilst the EC_{50} values of KCl were increased in the presence of 3.6, 12.0 and 24.0 mM magnesium. In contrast, the maximum contractile responses to noradrenaline and KCl were enhanced by addition of magnesium although the extent of enhancement was not related to the concentration. The increase in EC_{50} values produced by addition of 24.0 mM of magnesium was approximately 22 times for noradrenaline and 3 times for KCl, and the enhancement of maximum contraction was 1.3 and 1.45 fold for noradrenaline and KCl, respectively (Figure 3).

The contractile responses to three different concentrations of noradrenaline and KCl were examined in the presence of various concentrations of magnesium. The contraction induced by a low concentration of noradrenaline (2×10^{-8} M) was inhibited concentration-dependently by the addition of magnesium but the contraction to the high concentration of noradrenaline (10^{-5} M) was augmented by all magnesium concentrations used. The response to the middle concentration of noradrenaline (5×10^{-7} M) was augmented by low magnesium concentrations (1.2, 3.6 and 12.0 mM) but inhibited by magnesium 24.0 mM (Figure 4a). In the case of KCl the contractile responses to the lowest concentration (2×10^{-2} M) were inhibited concentration-dependently by addition of magnesium, but the responses induced by higher concentrations (3 and 4×10^{-2} M) of KCl were enhanced by lower concentrations of magnesium and inhibited by the higher concentrations (Figure 4b).

Discussion

The experiments described here show that in the rabbit isolated thoracic aorta, magnesium ions affect

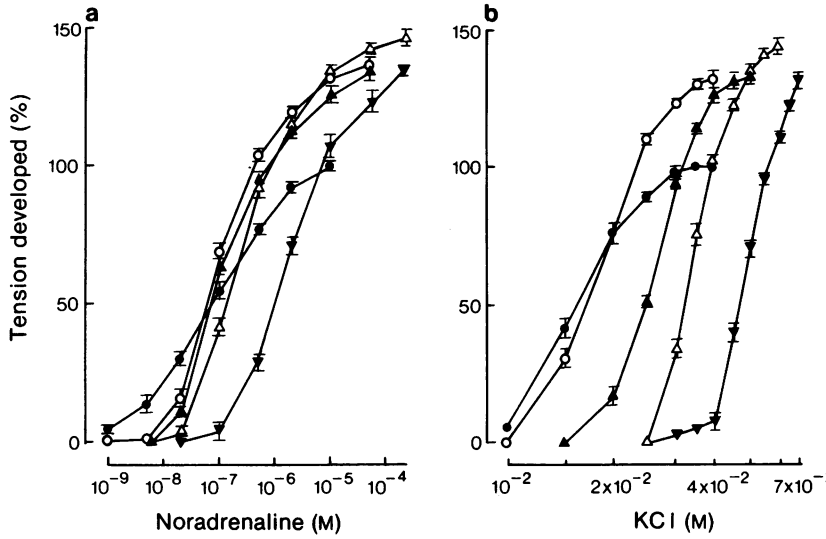

Figure 3 Effects of adding magnesium to magnesium-free solution on concentration-response curves to noradrenaline (a) and KCl (b) in rabbit thoracic aorta. Points were calculated as a % of the contraction induced by noradrenaline (10^{-5}M) or KCl (4×10^{-2}M) in magnesium-free medium (=100%). Each concentration-response curve ((●) magnesium-free; (○) magnesium 1.2 mM; (▲) magnesium 3.6 mM; (△) magnesium 12.0 mM; (▼) magnesium 24.0 mM) was obtained from 4 to 23 preparations and calculated as a mean % contraction for each point. Vertical lines show s.e. mean.

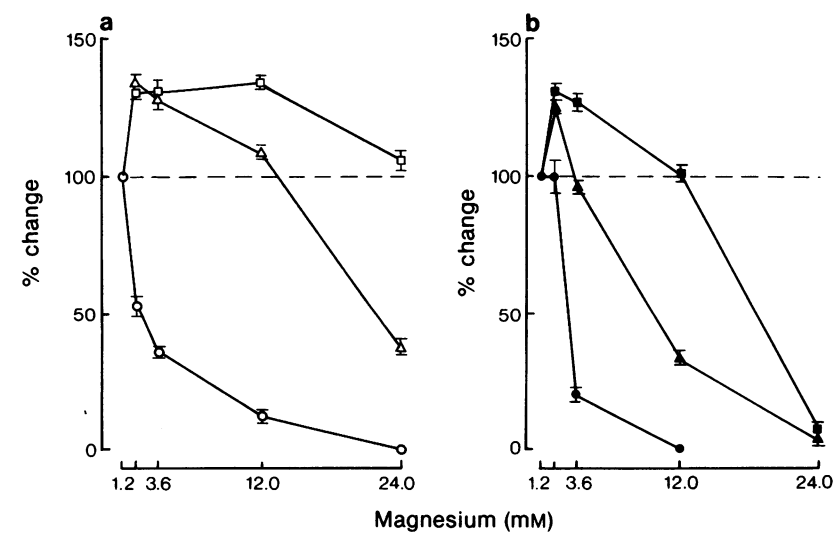

Figure 4 Effects of adding magnesium to magnesium-free solution on contractile responses induced by (a) noradrenaline (○) 2×10^{-8}M; (△) 5×10^{-7}M; (□) 10^{-5}M and (b) KCl (●) 2×10^{-2}M; (▲) 3×10^{-2}M; (■) 4×10^{-2}M in rabbit thoracic aorta. Points were calculated as % of the control responses in magnesium-free solution induced by various concentrations of noradrenaline and KCl. Concentration-response curves were obtained from 4 to 23 preparations and calculated as a mean % inhibition for each point. Vertical lines show s.e. mean.

both presynaptic and effector sites but in a different manner; magnesium inhibits noradrenaline release from the adrenergic nerves, but has dual effects on the reactivity of vascular smooth muscles, depending on the concentrations of magnesium and of the stimulants used.

It is well known that contractions induced by electrical transmural stimulation of vascular preparations are due to noradrenaline release from the adrenergic nerve terminals (Su & Bevan, 1970; Kurahashi & Fujiwara, 1976). Calculations from the present results show that in the absence of magnesium, the response to electrical stimulation at 80 V is equivalent in size to that produced by an exogenous noradrenaline concentration of about 6×10^{-8} M. It is clear that the responses to 6×10^{-8} M noradrenaline were progressively inhibited as the magnesium concentration was increased and that inhibition was complete with 24 mM magnesium. Thus it might be anticipated that responses to electrical stimulation would be abolished completely in the presence of 24 mM magnesium, regardless of whether there was a reduction in the amount of noradrenaline released by the nerves. To this extent the postsynaptic effect of magnesium can be considered as more important than the presynaptic effect in explaining the inhibition of responses evoked by nerve stimulation. The effect of magnesium on the adrenergic nerve terminals was only inhibitory and this is probably due to stabilization of the membrane. Several investigators (Kuriyama, 1964, on hypogastric nerve-vas deferens of guinea-pig; Kirpekar & Misu, 1967, on splenic nerve of cat; Wernig, 1972, on crayfish neuromuscular junction; Muller & Finkelstein, 1974, on frog neuromuscular junction) have shown that increasing the magnesium concentration in bathing or perfusion media inhibits transmitter release from adrenergic or cholinergic nerve terminals.

The presence of magnesium in the bathing solution caused a concentration-dependent decrease in the vascular sensitivity to noradrenaline and KCl, being exhibited either as the inhibition of contractions induced by low concentrations of noradrenaline (2×10^{-8} M) and KCl (2×10^{-2} M) or as the increase in EC_{50} values. This effect is possibly brought about by magnesium regulating the permeability of the muscle cell membrane to calcium and influencing the capacity of calcium binding to vascular smooth muscles, which then causes membrane stabilization, as previously suggested for vascular and other tissues (Altura & Altura, 1971; Turlapaty & Carrier, 1973; Jurevics & Carrier, 1973).

In contrast, the increase in the maximum contractile responses to noradrenaline and KCl suggests that magnesium also activates, beyond the vascular membrane, the contractile machinery. Indeed, it has recently been shown by Fitzpatrick, Landon, Debbas & Hurwitz (1972) that the uptake of calcium into microsomal and mitochondrial fractions of vascular smooth muscle is dependent on the magnesium salt of adenosine triphosphate. If these calcium ions sequestered into microsomal and mitochondrial fractions are available when vascular smooth muscles are sufficiently activated by certain stimulants even in the presence of magnesium, the vascular smooth muscle could respond with much stronger contractions than would occur in magnesium-free solution. Such a mechanism might explain why the contractile responses to high concentrations of noradrenaline (10^{-5} M) and KCl (4×10^{-2} M) were enhanced in the presence of magnesium in concentrations up to 12.0 mM. However, with 24.0 mM magnesium the contractile response to noradrenaline (10^{-5} M) was augmented whereas that to KCl (4×10^{-2} M) was greatly inhibited. This difference between the response to noradrenaline and KCl is probably due to different mechanism of contraction of the vascular smooth muscle by these agents. It has been reported by Bohr (1963), Hinke (1965), Van Breemen, Farinas, Berba & MacNaughton (1972), and Steinsland, Furchgott & Kirpekar (1973), that noradrenaline brings about vascular contractions by releasing intracellularly bound calcium, and that KCl does so by influx of calcium from extracellular spaces.

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