Augmentation by external Mg ions of β -adrenoceptor-mediated actions in the longitudinal muscle of rat uterus

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1 The longitudinal muscle isolated from the uterus of oestrogen-treated rats was not spontaneously active in Locke solution, and electrical stimulation evoked phasic contraction. Isoprenaline (3×10^{-11} – 10^{-8} M) and dibutyryl cyclic AMP (db cyclic AMP, 0.1–0.8 mM) depressed the phasic contraction; the depression was enhanced in the presence of 0.6 mM Mg.

2 The contracture generated by 40 mM K was partially relaxed by isoprenaline (10^{-11} – 10^{-8} M) and db cyclic AMP (0.1–0.8 mM). Mg (0.6 mM) enhanced the isoprenaline-induced relaxation, but not that induced by db cyclic AMP.

3 The membrane potential of the muscle was -61 mV, and electrical stimulation induced an action potential which consisted of spike and plateau components. Application of isoprenaline and db cyclic AMP mainly reduced the duration of the plateau potential. The effect was potentiated by 0.6 mM Mg.

4 The membrane was hyperpolarized, accompanied by a decrease in membrane resistance, when 10^{-8} M isoprenaline or 0.8 mM db cyclic AMP was applied. The effects of isoprenaline were prominently augmented in the presence of 1.2 mM Mg, while those of db cyclic AMP were slightly potentiated.

5 Forskolin (0.1 μ M) or papaverine (10 μ M) inhibited the phasic contraction and the K-contracture. The effect on the phasic contraction was potentiated by 0.6 mM Mg, while that on the K-contracture was not affected.

6 Forskolin shortened the action potential at 0.3 μ M, and hyperpolarized the membrane with a decrease in membrane resistance at 3.0 μ M. The membrane effects were augmented by 0.6 and 1.2 mM Mg, respectively.

7 It was hypothesized that external Mg ions could affect at least two processes involved in actions at β -adrenoceptors on rat myometrium; receptor-agonist interaction and cyclic AMP-mediated inhibition of membrane excitability.

Introduction

Mg ions are an essential constituent of serum, but less attention has been paid to their physiological significance than to other cations (e.g., Na, K and Ca ions). In vascular smooth muscle, some processes in the plasma membrane are thought to be modulated by external Mg ions: the excitability of the muscle membrane can be depressed by the stabilizing action of Mg ions (Bohr, 1964); Mg inhibits the entrance of Ca ions from the extracellular space following membrane excitation (Viveros & Somjen, 1968). Hormonal effects on smooth muscle are also influenced by external Mg ions: the effects of vasopressin and oxytocin on vascular, intestinal and myometrial smooth

muscle are potentiated by Mg ions (Somlyo *et al.*, 1966; Woo & Somlyo, 1967; Schild, 1969). These actions of Mg are thought to be exerted at the receptor level. It was found that inhibitory effects of isoprenaline (β -adrenoceptor agonist) on uterine circular muscle of the oestrogen-treated rat were potentiated by adding Mg ions to the external solution, but the underlying mechanism remained obscure (Osa & Ogasawara, 1979).

The purpose of the present work was to clarify the process influenced by external Mg ions on the β -adrenoceptor action in rat myometrium. Experiments were based on the following scheme for the

mechanism of β -action which is generally accepted (see Bülbring & Tomita, 1987, for a recent review): the activation of β -adrenoceptors (binding of agonist to receptor) stimulates adenylate cyclase, and adenosine 3':5'-cyclic monophosphate (cyclic AMP) production is increased; intracellular cyclic AMP activates protein kinase which, in turn, phosphorylates functional proteins on the plasma membrane and cell organelles. As a result, the concentration of intracellular free Ca ions is decreased, affecting the electrical and mechanical activities of smooth muscle.

We have shown in the present paper that the presence of Mg ions in the external solution augments the effects of isoprenaline, forskolin and dibutyryl cyclic AMP on the electrical activity of the plasma membrane in the uterine longitudinal muscle. On the other hand, the effect on K-contracture is potentiated by Mg only in the case of isoprenaline. It is suggested that there are at least two processes in the β -adrenoceptor action affected by extracellular Mg ions.

Methods

Wistar-strain female rats (200–300 g) were ovariectomized. After more than 10 days the rats were given 50 μ g oestradiol-17 β benzoate dissolved in sesame oil subcutaneously, and killed 3 days later. The uteri were excised and cut open along the mesometrium. A strip of longitudinal muscle was dissected 1 mm in width and 5 mm in length. The muscle strip was tied at both ends with silk threads and suspended vertically in a 2 ml chamber; one end was fastened at the bottom of the chamber and the other end was attached to a force transducer. Bathing solution (36°C) continuously flowed over the strip at a rate of 8 ml min⁻¹. The muscle strip was stimulated by current pulses when necessary. For measurement of electrical activity, the muscle strip was mounted in a partition chamber (1 ml in volume) as described by Abe & Tomita (1968), and superfused at a rate of 5 ml min⁻¹. Electrical activity of the muscle cell was recorded intracellularly with glass microelectrodes filled with 3 M KCl (30–50 M Ω in resistance). For simultaneous records of electrical and mechanical activity, one end of the muscle strip was tied with a thread and connected to a force transducer. Signals were amplified and recorded by an ink-writing pen recorder. Membrane responses were in some cases stored on a data recorder and reproduced at appropriate sweep speed and magnitude.

The Locke solution had the following composition (mm): NaCl 130.6, KCl 5.9, NaHCO₃ 8.0, CaCl₂ 2.5 and glucose 11.5, aerated with 95% O₂ plus 5% CO₂ (pH 7.3). The preparations were incubated in the sol-

ution at 36°C for 1–2 h before the experiment began. High-K (40 mM) solution was made by replacing NaCl with equimolar KCl. The solution containing Mg ions was prepared by adding an appropriate quantity of 1 M MgCl₂ to the Locke or high-K solution. Drugs used were isoprenaline hydrochloride (Nikken), dibutyryl cyclic AMP monosodium (db cyclic AMP, Sigma), papaverine hydrochloride (Nakarai), and forskolin (Sigma). Forskolin was dissolved in 95% ethanol, and was stable for at least 3 months (Seamon *et al.*, 1981). It was diluted in perfusing solution to give the desired concentration. The final concentration of ethanol in the solution was less than 0.03%.

Statistical analysis for the significance of differences between means was by Student's *t* test.

Results

Isoprenaline and dibutyryl cyclic AMP

The longitudinal muscle strips excised from oestrogen-treated rat uterus were not normally spontaneously active after 1–2 h incubation in Locke solution, and field current stimulation evoked phasic contraction. Isoprenaline (10⁻¹⁰ M) inhibited the phasic contraction in amplitude and duration (Figure 1a(i)). After washing out isoprenaline, the effects of 10⁻¹⁰ M isoprenaline were again examined in the presence of 0.6 mM Mg. Phasic contraction was more strongly inhibited by isoprenaline in the presence of Mg (Figure 1a(ii)). Dibutyryl cyclic AMP (0.2 mM) also inhibited the phasic contraction (Figure 1b(i)), but the onset of the drug action appeared 3–5 min after the application of db cyclic AMP. The delay is probably due to the time required for db cyclic AMP to penetrate into the muscle cell. The inhibitory effect of db cyclic AMP was again augmented in solutions containing 0.6 mM Mg ions (Figure 1b(ii)).

The membrane and contractile effects of isoprenaline and db cyclic AMP were examined in depolarized muscle (Figure 2). Phasic contractions corresponded well with evoked action potentials in solutions containing 0.6 mM Mg (Figure 2a). When the muscle was exposed to high-K solution, the membrane was depolarized to -25 mV and a contracture was produced. The effects of isoprenaline (3 $\times$ 10⁻⁹ M) and db cyclic AMP (0.4 mM) were examined in the high K solution; neither drug affected the membrane potential, but the contracture was partly relaxed (Figure 2b,c). Therefore, effects of isoprenaline and db cyclic AMP via change in membrane potential can be ignored in the depolarized muscle.

Effects of isoprenaline and db cyclic AMP were

Figure 1 Effects of isoprenaline (a) and dibutyryl cyclic AMP (db cyclic AMP) (b) on the phasic contractions of uterine longitudinal muscle in the absence (i) and presence (ii) of Mg ions. Isoprenaline (10^{-10} M) and db cyclic AMP (0.2 mM) were applied during the periods indicated by solid and broken lines, respectively. Mg (0.6 mM) was added to the bathing solution 1–2 h before the 2nd application of the drugs. Dots indicate electrical stimulation (1 s in duration, supramaximal intensity). Records (a) and (b) were obtained from different preparations.

investigated in the depolarized preparation in the absence and presence of Mg ions (Figure 3). The contractures induced by 40 mM K were partly relaxed by 10^{-10} M isoprenaline and 0.2 mM db cyclic AMP under Mg-free conditions (Figure 3a). In the presence of 0.6 mM Mg, the relaxation of K-

Figure 2 Simultaneous records of electrical (upper trace) and mechanical (lower trace) activities, and effects of isoprenaline and dibutyryl cyclic AMP (db cyclic AMP) in high-K solution. The bathing solution contained 0.6 mM Mg. External K concentration was raised to 40 mM at the arrow (a). Isoprenaline (3×10^{-9} M) and db cyclic AMP (0.4 mM) were applied between the arrows (b and c, respectively). Broken lines show the initial level of resting tension. Dots indicate electrical stimulation (500 ms, supramaximal intensity). All records are from the same preparation.

Figure 3 Effects of isoprenaline (left) and dibutyryl cyclic AMP (db cyclic AMP) (right) on the K-contracture in the absence (a, c) and presence (b) of Mg ions. External K concentration was raised to 40 mM at the upward arrow, and returned to 5.9 mM at the downward one. Isoprenaline (10^{-10} M) and db cyclic AMP (0.2 mM) were applied during the periods indicated by solid and broken lines, respectively. Mg (0.6 mM) was added to the bathing solution 70 min before the second application of isoprenaline (b), and removed 25 min before the third application (c). All records are from the same preparation.

contracture by isoprenaline increased but that by db cyclic AMP was unchanged (Figure 3b).

The inhibitory effects of 10^{-10} M isoprenaline and 0.2 mM db cyclic AMP on the mechanical activities are summarized in Table 1. It was proved that the effect of isoprenaline and db cyclic AMP on the phasic contraction and the effect of isoprenaline on the K-contracture were potentiated by Mg, while the effect of db cyclic AMP on the K-contracture was not affected by Mg.

The effects of isoprenaline and db cyclic AMP on mechanical activity were evaluated at various concentrations both in the absence and presence of 0.6 mM Mg (Figure 4). In the case of phasic contractions (Figure 4a(i)(ii)), the reduction of the amplitude caused by either drug was larger at each concentration in the Mg-containing solution than in the Mg-free solution, except at relatively low concentrations in one preparation (isoprenaline, triangle). The depression of K-contracture by isoprenaline was also potentiated at each concentration by Mg, and the dose-response curves tended to be shifted to the left in the presence of Mg (Figure 4b(i)). In contrast, the dose-response curves for db cyclic AMP were little influenced by Mg (Figure 4b(ii)).

Intracellular recording of membrane activity was carried out, and the effects of isoprenaline and db cyclic AMP were examined in the absence and presence of external Mg ions. The resting membrane potential (mean $\pm$ s.d.) was -61.8 ± 3.2 mV ($n = 37$,

Table 1 Contractile effects of isoprenaline and dibutyryl cyclic AMP (db cyclic AMP) in the absence and presence of Mg

	n	Phasic contraction (% of control)		n	K-contraction (% of control)	
		0 mM Mg	0.6 mM Mg		0 mM Mg	0.6 mM Mg
Isoprenaline 10^{-10} M	5	80.9 $\pm$ 11.7	58.6 $\pm$ 20.5*	6	83.5 $\pm$ 5.4	76.8 $\pm$ 5.9*
db Cyclic AMP 0.2 mM	5	65.5 $\pm$ 16.8	40.4 $\pm$ 19.4*	4	80.1 $\pm$ 6.5	81.8 $\pm$ 8.9

Values are means $\pm$ s.d. *n*, number of preparations. Effects of drugs were first examined in the absence of Mg, and then in the presence of 0.6 mM Mg after 1–2 h exposure to the Mg-containing solution.

The minimum amplitude of contraction during the exposure to drugs (12 min) is expressed as a percentage of the amplitude of contraction immediately before the drug application.

* Significantly different from the value at 0 mM Mg ($P < 0.05$).

Figure 4 Dose-response relationships for the effects of isoprenaline (i) and db cyclic AMP (ii), on the phasic contraction (a) and the K-contraction (b) in the absence (open symbol, broken line) and presence (filled symbol, solid line) of 0.6 mM Mg. In each graph, the same type of symbol represents the same preparation. The drugs were applied cumulatively. In the case of K-contraction, external K concentration was raised to 40 mM 10 min before the application of drugs. The experiments in the presence of Mg were carried out after the preparations were incubated with the Mg-containing solution for 1–1.5 h. Ordinates: the minimum amplitude of contraction during the exposure to drugs is expressed as percentage of the amplitude of contraction immediately before the drug application. Abscissae: concentrations of drugs.

Figure 5 Effects of isoprenaline on the electrical activity in the absence (a) and presence (c) of Mg ions. Isoprenaline (3×10^{-9} M) was applied at the downward arrow and washed out at the upward one. In record (b), Mg (0.6 mM) was added to the bathing solution. Record (c)(i) was taken 30 min after the Mg application. Dots indicate electrical stimulation (500 ms, supramaximal intensity). All records were obtained from the same cell.

no Mg), -62.5 ± 3.8 mV ($n = 22$, 0.6 mM Mg) and -61.6 ± 3.5 mV ($n = 20$, 1.2 mM Mg).

Electrical stimulation evoked an action potential which consisted of an initial spike and a plateau potential under Mg-free conditions; repetitive spike discharge was observed on the plateau (Figure 5a). The main effect of 3×10^{-9} M isoprenaline was to shorten the duration of the action potential. In the presence of 0.6 mM Mg (Figure 5b), the duration of the action potential discharge was slightly reduced, and 3×10^{-9} M isoprenaline progressively shortened the action potential, until finally only a few spikes were generated by current stimulation (Figure 5c).

Figure 6 shows the effects of db cyclic AMP on the membrane activity in the absence and presence of Mg. The action potential did not change in its configuration during the first 4–5 min after the application of 0.4 mM db cyclic AMP under Mg-free con-

ditions (Figure 6a(i)), and thereafter decreased mainly in duration (Figure 6a(ii)). The lag time corresponds with the delay observed in the mechanical response (Figure 1b). In the presence of 0.6 mM Mg, the action potential was greatly shortened by 0.4 mM db cyclic AMP and finally became a single spike (Figure 6b(i)(ii)).

The effects of isoprenaline on the membrane potential and membrane resistance were compared in the absence and presence of external Mg ions (Figure 7). In the cell illustrated, application of 10^{-8} M isoprenaline hyperpolarized the membrane by 8 mV (Figure 7a(i)), and reduced the amplitude of the electrotonic potential by 7.2% (Figure 7a(iii) vs 7a(ii)) in Mg-free solution. Membrane potential and electrotonic potential were little altered by the application of 1.2 mM Mg (physiological Mg concentration), but isoprenaline (10^{-8} M) hyperpolar-

Figure 6 Effects of dibutyryl cyclic AMP (db cyclic AMP) on the electrical activity in the absence (a) and presence (b) of Mg ions. db Cyclic AMP (0.4 mM) was applied at the downward arrow. Mg (0.6 mM) was added to the bathing solution 1.5 h before the 2nd application of db cyclic AMP. Upper and lower traces show electrical activity and stimulating current monitor, respectively. Outward current pulses (500 ms, supramaximal intensity) were applied at intervals of 60 s. Records (a) and (b) were obtained from the same preparation, but from different cells.

Figure 7 Effects of isoprenaline on the membrane potential and membrane resistance in the absence (a) and presence (b) of Mg ions. Isoprenaline (10^{-8} M) was applied between the arrows (a(i), b(i)). Mg (1.2 mM) was added to the bathing solution 55 min before the second application of isoprenaline. Upper and lower traces show membrane potential and stimulating current monitor, respectively. Inward current pulses (1 s) at constant intensity were applied at intervals of 10 s. Magnified electrotonic potentials with a faster sweep mode are reproduced under (a(i)) and (b(i)); (a(ii)), (b(ii)) and (a(iii)), (b(iii)) are the electrotonic potential 10–20 s before and 140–160 s after the application of isoprenaline, respectively. All records were obtained from the same cell.

ized the membrane by 12 mV (Figure 7b(i)), and reduced the amplitude of the electrotonic potential by 16.0% (Figure 7b(iii) vs 7b(ii)). In other cells, membrane hyperpolarization by 10^{-8} M isoprenaline was as follows (mean $\pm$ s.d.): no Mg, 8.5 ± 2.9 mV ($n = 6$); 1.2 mM Mg, 12.3 ± 2.3 mV ($n = 6$); the means are significantly different ($P < 0.025$). Therefore membrane hyperpolarization produced by isoprenaline was potentiated by Mg ions.

In similar experiments with db cyclic AMP, the membrane hyperpolarized gradually and the electrotonic potential decreased slightly, when 0.8 mM db cyclic AMP was applied (not illustrated). The hyperpolarization by 0.8 mM db cyclic AMP was: no Mg, 3.2 ± 1.6 mV ($n = 5$); 1.2 mM Mg, 5.4 ± 1.7 mV ($n = 5$); the means are significantly different ($P < 0.05$). The effect of db cyclic AMP on the membrane potential seems to be less influenced by Mg ions than the effect of isoprenaline.

Forskolin and papaverine

Forskolin increases intracellular cyclic AMP concentration in uterine smooth muscle by directly stimulating adenylate cyclase activity (Marshall & Fain, 1984). Figure 8 shows the influence of external Mg ions on the mechanical effects of forskolin. Forskolin (0.1 μ M) had only a slight inhibitory effect on the phasic contraction in Mg-free solution (Figure 8a(i)), but the effect was much stronger in the presence of 0.6 mM Mg (Figure 8a(ii)). Spontaneous contractions

took place when Mg was removed in this preparation (Figure 8a(iii)). The spontaneous activity was abolished by forskolin, and electrical stimulation evoked phasic contractions of large amplitude. On the other hand, the forskolin (0.1 μ M)-induced inhibition of K-contraction was nearly the same in the absence (Figure 8b(i)) and presence (Figure 8b(ii)) of Mg.

Papaverine inhibits phosphodiesterase activity in rat myometrium (Urano *et al.*, 1974), and cyclic AMP accumulates in the muscle cell. Similar results to those for forskolin were obtained with papaverine for the influence of Mg on the mechanical responses (not illustrated).

Table 2 is a summary of the mechanical effects of forskolin and papaverine in the absence and presence of Mg ions. For both drugs, inhibition of the phasic contraction was significantly augmented by Mg, while inhibition of the K-contraction was not significantly influenced by Mg.

External Mg ions also augmented inhibition of the action potential by forskolin (Figure 9). In Mg-free solution, application of 0.3 μ M forskolin shortened the action potential (Figure 9a). Moreover, it depressed the amplitude of the plateau potential, which perhaps resulted in an increase in amplitude of the spikes superimposed on the plateau. These effects were greater in solutions containing 0.6 mM Mg, and finally the action potential was generated only during the period when the current pulse was applied (Figure 9b).

Figure 8 Effects of forskolin on the phasic contraction (a) and K-contraction (b) in the absence and presence of Mg ions. In the records (b), external K concentration was raised to 40 mM at the upward arrow, and returned to normal at the downward one. Forskolin (0.1 μ M) was applied during the periods indicated by solid lines. Mg (0.6 mM) was added to the bathing solution 2 h before the second application of forskolin (a(ii), b(ii)), and removed 1 h before the third application (a(iii)). The broken line (a(iii)) shows the initial level of resting tension. Dots indicate electrical stimulation (1 s, supramaximal intensity). Records (a) and (b) were obtained from different preparations.

Table 2 Contractile effects of forskolin and papaverine in the absence and presence of Mg

	n	Phasic contraction (% of control)		n	K-contracture (% of control)	
		0 mM Mg	0.6 mM Mg		0 mM Mg	0.6 mM Mg
Forskolin 0.1 μ M	7	82.0 $\pm$ 22.3	34.6 $\pm$ 25.9*	4	70.1 $\pm$ 7.1	72.4 $\pm$ 7.3
Papaverine 10 μ M	6	72.7 $\pm$ 18.4	39.3 $\pm$ 22.1*	4	52.9 $\pm$ 6.5	51.0 $\pm$ 8.5

Values are means $\pm$ s.d. *n*, number of preparations. Effects of drugs were first examined in the absence of Mg, and then in the presence of 0.6 mM Mg after 1–2 h exposure to the Mg-containing solution.

The minimum amplitude of contraction during the exposure to drugs (12 min) is expressed as a percentage of the amplitude of contraction immediately before the drug application.

* Significantly different from the value at 0 mM Mg ($P < 0.025$).

The effects of a higher concentration (3 μ M) of forskolin on the membrane potential and membrane resistance were investigated in the absence and presence of 1.2 mM Mg. Figure 10 is a representative record. The membrane was hyperpolarized by 3 mV (Figure 10a(i),(ii)) and the amplitude of the electrotonic potential decreased by 6.5% (Figure 10a(iv) vs 10a(iii)), when forskolin was applied under Mg-free conditions. In Mg-containing solution, forskolin hyperpolarized the membrane by 10 mV (Figure 10b(i),(ii)) and reduced the amplitude of the electrotonic potential by 13.4% (Figure 10b(iv) vs 10b(iii)).

Discussion

The present results indicate that the effects of isoprenaline, forskolin and db cyclic AMP on the membrane responses (inhibition of action potential, hyperpolarization and reduction of electrotonic potential) were potentiated in the presence of external Mg ions whereas only the inhibition of the K-contracture caused by isoprenaline was markedly potentiated by Mg. In addition, external Mg potentiated the inhibition of the phasic contraction by isoprenaline, forskolin and db cyclic AMP, which

Figure 9 Effects of forskolin on the electrical activity in the absence (a) and presence (b) of Mg ions. Forskolin (0.3 μ M) was applied at the downward arrow and washed out at the upward one. Mg (0.6 mM) was added to the bathing solution 1 h before the 2nd application of forskolin. Dots indicate electrical stimulation (500 ms, supra-maximal intensity). All records are from the same preparation.

Figure 10 Effects of forskolin on the membrane potential and membrane resistance in the absence (a) and presence (b) of Mg ions. Forskolin ($3 \mu\text{M}$) was applied at the downward arrow, and removed at the upward one. Mg (1.2 mM) was added to the bathing solution 80 min before the 2nd application of forskolin. Upper and lower traces show membrane potential and stimulating current monitor, respectively. Inward and outward current pulses (1 s) at constant intensity were applied alternately at intervals of 5 s. Magnified electrotonic potentials with a faster sweep mode are reproduced below (a(ii)) and (b(ii)); (a(iii)), (b(iii)) and (a(iv)), (b(iv)) are the electrotonic potential 10–20 s before and 205–225 s after the application of forskolin, respectively. All records are from the same preparation.

occurs as a result of the action potential. The fact that external Mg ions enhanced the effect of db cyclic AMP indicates that the process depending on external Mg ions exists at a relatively 'late' phase of the β -adrenoceptor response. On the other hand, involvement of Mg in the process prior to cyclic AMP-mediated action ('early' phase) is also possible, which seems to be revealed by the results on K-contracture.

It has been postulated in rat myometrium that membrane hyperpolarization in response to β -adrenoceptor activation is due to an increase in K conductance (Kroeger & Marshall, 1973), which is supported by a decrease in membrane resistance in the present work (Figures 7, 10). In the β -action, cyclic AMP may phosphorylate the K channels either directly or via cyclic AMP-dependent protein kinase as reported in *Helix* neurone (Ewald *et al.*, 1985). Mg is considered to augment the activation of K channels by cyclic AMP through the process. In vascular smooth muscle, Somlyo *et al.* (1972) found that no significant membrane hyperpolarization was

observed on the application of 1 mM db cyclic AMP with 4 mM theophylline after 90 min exposure to Mg-free solution, and further application of Mg, still in the presence of db cyclic AMP and theophylline, hyperpolarized the membrane by about 10 mV. They suggested that the hyperpolarization was caused by the activation of Mg-ATPase mediating cation extrusion rather than through opening of K channels.

Action potential duration in uterine longitudinal muscle of oestrogen-treated rats was shortened by lowering external K and Ca concentration, and was prolonged by the application of TEA and tetracaine (Maruta & Osa, 1987). It was concluded that an increase in K conductance was responsible for the repolarization of the action potential. Since the shortening of the action potential by isoprenaline, forskolin and db cyclic AMP was potentiated in the presence of Mg, it is speculated that cyclic AMP facilitates the activation of delayed outward K current, and the action is potentiated by Mg ions. It is not yet clear whether the K channel concerned

with the repolarization of action potential is identical with the K channel responsible for the membrane hyperpolarization.

In depolarized smooth muscle, it is thought that Ca influx through voltage-dependent Ca channels produces the contracture directly or via Ca-induced Ca release (Bolton, 1979). Since the K-contracture was partially relaxed without any change in membrane potential by isoprenaline or db cyclic AMP (Figure 2), the relaxation would be ascribed to intracellular events. It is known that cyclic AMP (or cyclic AMP-dependent protein kinase), as a result of β -adrenoceptor activation, accelerates the uptake of cytoplasmic free Ca ions into storage sites, and directly interferes with the contractile machinery (Bülbring & Tomita, 1987). Hence, the fact that relaxation by forskolin, db cyclic AMP and papaverine of the K-contracture was not altered by external Mg may indicate that cyclic AMP production by adenylate cyclase and the cyclic AMP-mediated effects as described above are not sensitive to 'extracellular' Mg. On the other hand, enhancement by Mg of the isoprenaline-induced relaxation of K-contracture would be ascribed to an increase in affinity of the β -adrenoceptor for isoprenaline, because the concentration-response curves of isoprenaline shifted to the left in the presence of Mg ions (Figure 4b(i)). Augmentation of receptor-agonist interaction by external Mg ions has also been reported in other systems (oxytocin and vasopressin, Somlyo *et al.*, 1966; Woo & Somlyo, 1967; Schild, 1969; adenosine, Murphy & Snyder, 1983; endothelium-derived relaxant factor, Altura & Altura, 1987).

The site of action of Mg for the interaction of isoprenaline and the β -receptor probably exists on the outer surface of the plasma membrane. On the other hand, it is difficult in the cyclic AMP-mediated membrane response to determine whether the site of

action of Mg is inside or outside the membrane. According to the data obtained by atomic absorption spectrophotometry and nuclear magnetic resonance spectroscopy, the intracellular content of Mg ions was little influenced when external Mg concentration was altered (uterus, Moawad & Daniel, 1971; arterial wall, Palatý, 1971; taenia coli, Nakayama *et al.*, 1987). It is therefore probable that the site where Mg ions participate in the cyclic AMP-mediated membrane response is located on the outer surface of membrane. However, it also seems possible that local Mg existing on and near the inner surface of the membrane is labile and this Mg is responsible for the cyclic AMP-mediated membrane response. This idea is reasonable since cyclic AMP acts on the plasma membrane from the inside. The cyclic AMP-dependent protein kinase purified from bovine brain is reported to have an absolute requirement for a divalent metal such as Mg, Mn and Co when stimulated by cyclic AMP (Miyamoto *et al.*, 1969). In Mg-free external solution, K channels may be hard to activate by the cyclic AMP-dependent protein kinase which exists in the peripheral region of the cytoplasm.

In conclusion, it is proposed that extracellular Mg potentiates the β -adrenoceptor-mediated action in rat myometrium at least by two processes: one is the acceleration of receptor-agonist interaction at the outside of the membrane; the other is presumably the potentiation of cyclic AMP-mediated activation of the K-channel exerted on the inside of a membrane. In view of the effects on K-contractures, a cyclic AMP-mediated Ca uptake by intracellular organelles affected by the extracellular Mg can be excluded.

We thank Dr K.E. Creed for her valuable comments and correcting the English on the manuscript.

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(Received August 16, 1988

Revised October 31, 1988

Accepted November 9, 1988)