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THE EFFECT OF MAGNESIUM ON THE RESPONSE OF SMOOTH MUSCLE TO 5-HYDROXYTRYPTAMINE

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1 The responses to 5-hydroxytryptamine (5-HT) of rabbit isolated mesenteric artery and vein and longitudinal smooth muscle of guinea-pig ileum were examined in Krebs solution containing 0, 1.2 or 2.4 mM Mg^{2+}.

2 When the concentration of Mg^{2+} was raised the spontaneous contractile activity of rabbit mesenteric vein was depressed. The responses to 5-HT in rabbit mesenteric artery and vein and guinea-pig ileum were greater in the absence of Mg^{2+}. The initial fast component of 5-HT-induced contractions in rabbit mesenteric vein was reduced more consistently than the subsequent slow component by increasing the Mg^{2+} concentration.

3 Exposure of mesenteric vein to Ca-free solution containing ethyleneglycoltetra-acetic acid (EGTA) promptly abolished 5-HT contraction in normal-Mg but not in low-Mg Krebs solution.

4 In mesenteric veins, no difference was observed in either the 'lanthanum-resistant' uptake of ^{45}Ca or total tissue Ca, measured by atomic absorption spectrophotometry, after 60 min exposure to either low-Mg or normal-Mg Krebs solution. On the other hand, after 5 min exposure, the 'lanthanum-resistant' uptake of ^{45}Ca was greater in the absence of Mg^{2+} than in the presence of higher Mg^{2+} concentrations.

5 It is suggested that Mg^{2+} depressed the 5-HT response at least partly by reducing the availability of Ca^{2+} from a rapidly equilibrating intracellular pool.

Introduction

Over the last decade a considerable amount of data has been accumulated concerning the effect of magnesium on drug action, particularly in vascular smooth muscle. In the presence of 1.2 mM Mg^{2+} in the bathing solution, the sensitivity and maximum response of canine femoral artery, rabbit mammary strips, and human umbilical vein to vasopressin were found to be greater than in a Mg-free solution (Somlyo, Woo & Somlyo, 1966). This was confirmed by Altura (1975) in rat aorta, who further demonstrated the same effect for several vasopressin analogues. Similarly, the responses of rabbit aorta to adrenaline (Altura & Altura, 1971), and rat aorta to prostaglandins A_1, B_1 and $F_{2\alpha}$ (Altura, Altura & Waldemar, 1976) were also potentiated in the presence of Mg^{2+}. By contrast, the sensitivity and maximum response to acetylcholine, KCl and angiotensin were significantly greater in the absence of Mg^{2+} (Altura & Altura, 1971).

The mechanism by which Mg^{2+} alters the response to drugs is not well defined. Mg^{2+} may interact at several sites in smooth muscle to produce the effects observed. These sites might include receptors, plasma membrane, intracellular Ca^{2+} pools, sarcoplasmic reticulum, contractile proteins and certain enzymes.

The normal serum Mg level in man is reported to be in the range of 0.76 to 1.00 mM (Jackson & Meier, 1968) or 0.7 to 0.9 mM (Massry, 1977). Serum levels below this range may occur in diabetes mellitus (Jackson & Meier, 1968), chronic alcoholism and after treatment with certain diuretics (Massry, 1977). Elevation of serum Mg above the normal range may occur in uraemia or after ingestion of antacids (Jackson & Meier, 1968). Although of obvious practical importance, it is not known whether the altered serum Mg levels observed in these conditions could affect drug response.

Only a few and non-decisive experiments have been reported on the interaction of Mg^{2+} with 5-hydroxytryptamine (5-HT) (Altura & Altura, 1971). The present study has a dual purpose: to investigate the effect of Mg^{2+} on the response of vascular and non-vascular smooth muscle to 5-HT and to examine the mechanism whereby this interaction could occur.

Methods

Male New Zealand white rabbits (2.2 to 3.5 kg) were killed by a blow on the neck. The superior mesenteric artery and vein were removed, placed in oxygenated Krebs solution and carefully cleared of surrounding fat and connective tissue. A helical strip was prepared from the artery, whereas, the vein was divided into three longitudinal strips. One end of each strip was attached to a metal bubbler and the other end to a Grass FT-03 force-displacement transducer. Isometric tension was recorded on a Grass Model 7 Polygraph.

The artery was sequentially exposed to three Mg levels in the following order: normal, low, and high. Each strip of the vein was exposed to only one of these Mg levels. Normal-Mg Krebs solution had the following composition (mM): NaCl 118, KCl 4.7, NaH_2PO_4 1.0, $CaCl_2$ 2.5, $NaHCO_3$ 25.0, dextrose 11.1, and $MgCl_2$ 1.2. High-Mg Krebs solution contained 2.4 mM $MgCl_2$, while low-Mg Krebs solution contained no $MgCl_2$. Osmolarity of the three different solutions was kept constant by the addition or deletion of 1.8 mM NaCl. Each strip was then equilibrated for 60 min in the respective Krebs solution at 37°C under 1g tension and continuously bubbled with a 95% O_2 and 5% CO_2 gas mixture. During the equilibration period, the bathing solution was exchanged with fresh Krebs solution several times to prevent the accummulation of metabolites.

In experiments with non-vascular smooth muscle, male albino guinea-pigs (510–760 g) were killed by cervical dislocation and the ileum, free of mesentery, was removed. Three adjacent segments from the middle of the ileum were each stripped of approximately 3 cm of longitudinal smooth muscle according to the method of Rang (1964). Each longitudinal smooth muscle strip was then equilibrated in either low, normal or high Mg Krebs solution for 60 min under 0.5 g tension at 37°C continuously bubbled with 95% O_2 and 5% CO_2 gas mixture.

Following the initial 60 min equilibration, the response to 5-HT was recorded in each smooth muscle preparation. The drug was dissolved in distilled water and added to the tissue bath in concentrations ranging from 0.01 to 300 μM in volumes not exceeding 0.5 ml. Each dose of 5-HT was washed out after the maximum tension for that dose had been attained. The next 5-HT concentration was given after the baseline tension had been maintained for 5 minutes.

In another set of experiments, rabbit mesenteric veins were divided into two longitudinal segments. Each strip was exposed to normal-Mg or low-Mg Krebs solution, as previously described, and contractions produced by 3×10^{-6}M 5-HT were recorded. Both vascular strips were then placed in normal-Mg Krebs solution for 60 min after which the same strip as before was exposed to Mg-free solution. Five min later, the tissues were transferred to Ca-free solutions containing 10^{-6}M ethyleneglycoltetra-acetic acid (EGTA) which is known to bind Ca^{2+} selectively but not Mg^{2+} (Sillen & Martell, 1971). The response to 3×10^{-6}M 5-HT was then repeatedly recorded in both vascular strips for a 60 min period.

^{45}Ca experiments

The uptake of ^{45}Ca was measured in longitudinal strips of rabbit mesenteric vein by the lanthanum method (Van Breeman, Farinas, Casteels, Gerba, Wuytak & Deth, 1973). This method, based on the greater affinity of La^{3+} than Ca^{2+} for membrane Ca^{2+} binding sites has been used to measure intracellular uptake of ^{45}Ca.

In one group of experiments, three venous segments were equilibrated for 60 min under 1 g tension at 37°C in normal-Mg Krebs solution continuously bubbled with a 95% O_2 and 5% CO_2 gas mixture. Following the equilibration period, the vessels were exposed for 5 min to 10 ml of either low-Mg, normal-Mg, or high-Mg Krebs solution containing 2 μCi of ^{45}Ca (Amersham Searle Corp.). In a second group, the mesenteric vein was divided into four longitudinal strips, each equilibrated for 60 min in normal-Mg Krebs solution. Two strips were then placed for 5 min in low-Mg and two in normal-Mg Krebs solution, each containing 2 μCi of ^{45}Ca with or without 10^{-5}M 5-HT. In a third group of experiments, two strips were equilibrated for 60 min in low-Mg solution while the other two were equilibrated in normal-Mg Krebs solution. They were then exposed to 2 μCi ^{45}Ca with or without 5-HT for 5 min as outlined above. Following ^{45}Ca uptake, every strip was transferred to a La^{3+} solution of the following composition (mM): NaCl 118, (or 1.8 mM, more or less depending on $MgCl_2$ concentration), KCl 4.7, dextrose 11.1, $CaCl_2$ 2.5, $LaCl_3$ 10, Tris maleate 5.0 and $MgCl_2$ O, 1.2, or 2.4. After 3 min, the strips were incubated for 45 min in a Ca-free La^{3+} solution, i.e. as above but without $CaCl_2$ added. Thereafter, the tissues were rinsed 4 times in chilled Krebs solution and digested overnight in 1 ml of Nuclear Chicago Solubilizer (Amersham Searle Corp.) at 55°C. The digest was acidified with 2 N HCl and 15 ml of scintillation fluid was added (Aquasol, New England Nuclear). Radioactivity was measured in a Nuclear Chicago Mark II liquid scintillation counter and results expressed as d min^{-1} mg^{-1} wet tissue weight.

Determination of total tissue Ca content

Total tissue Ca content of rabbit mesenteric veins was determined by atomic absorption spectrophotometry in the laboratory of Dr I. C. Radde. Each vein was

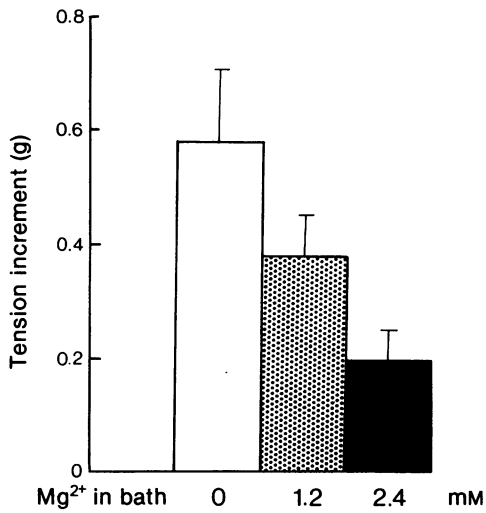

Figure 1 Effect of magnesium on spontaneous contractions of mesenteric vein in eight rabbits. Means with bars indicating standard errors are shown. Contractions were significantly greater in 0 mM Mg^{2+} ($P < 0.01$) and 1.2 mM Mg^{2+} ($P < 0.02$) than in 2.4 mM Mg^{2+}.

divided into four longitudinal strips. The strips were then equilibrated in normal-Mg Krebs solution at 37°C for 60 min under 0.5 g tension and continuously bubbled with a 95% O_2 and 5% CO_2 gas mixture. Two strips were then incubated in either low-Mg or normal-Mg Krebs solution for 30 min, while the remaining two were incubated in either low-Mg or normal-Mg Krebs solution for 60 minutes. After the incubation period, the tissues were stored at −20°C until a pool of 6 strips for each experimental condition had been accumulated. Total tissue Ca content was then determined according to the method of MacIntyre (1961) using a Pye-Unicam SP1900 flame spectrophotometer.

Statistical evaluation

All results are expressed as mean ± s.e. mean and compared for significance by the *t*-test for paired samples. Concentration-response curves were also compared for significance by analysis of covariance with log concentration as the covariable. The parallelism of the slopes as well as the vertical separation of the curves was assessed.

Drugs

5-Hydroxytryptamine (serotonin-creatinine sulphate, BDH Chemicals Ltd.) was used in these experiments. EGTA was obtained from Sigma Chemical Corp.

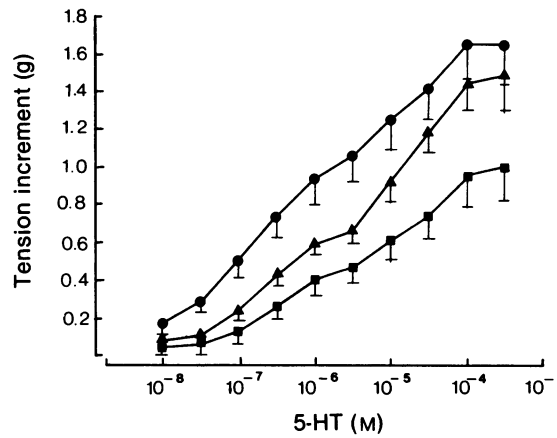

Figure 2 5-Hydroxytryptamine (5-HT) concentration-response curves for 8 rabbit mesenteric veins exposed to Krebs solution containing three different Mg^{2+} levels. Means with bars indicating standard errors are shown. (●) Low-Mg Krebs solution; (▲) normal-Mg Krebs solution; (■) high-Mg Krebs solution.

Results

Effect of Mg^{2+} on spontaneous contractile activity

During the equilibrium period, spontaneous contractions by strips of rabbit mesenteric vein were observed. Initially, the contractions were erratic, but after 20 to 30 min a well-defined pattern became apparent. Measurement of rate and magnitude of the spontaneous contractions revealed that the tension developed by strips was significantly greater both in low-Mg ($P < 0.01$) and normal-Mg ($P < 0.02$) Krebs solution than in high-Mg Krebs solution (Figure 1). The rate of the spontaneous contractions was depressed by Mg^{2+} from 3.25 ± 0.44 contractions per min in low-Mg Krebs solution to 2.73 ± 0.67 contractions per min in high-Mg Krebs solution. This difference was not significant. Helical strips of mesenteric artery did not exhibit any spontaneous activity. Longitudinal smooth muscle strips from guinea-pig ileum also developed spontaneous contractions during the equilibrium period. These contractions were quite irregular and could not be properly evaluated.

Effect of Mg^{2+} on responses to 5-hydroxytryptamine

Figure 2 illustrates that in the absence of Mg^{2+} the response of rabbit mesenteric vein to every concentration of 5-HT tested was greater than either in normal-Mg or high-Mg Krebs solution. Concentration-response curves obtained at the three Mg levels, com-

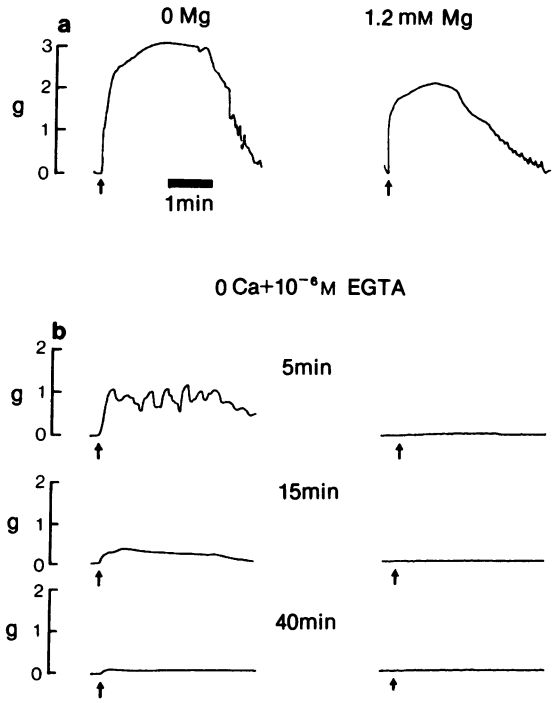

Figure 3 Tracings obtained from two strips of mesenteric vein in low-Mg and normal-Mg Krebs solutions before (a) and after (b) exposure to Ca-free solution containing EGTA. ↑ indicates addition of 3×10^{-6} M 5-hydroxtryptamine to the tissue bath. Time elapsed after O Ca plus EGTA exposure is shown.

pared by analysis of covariance, were significantly different, $P < 0.01$.

At 5-HT concentrations greater than 3×10^{-8}M, the vascular strips characteristically responded to the drug with a contraction consisting of a fast component followed by a slow component. A respresentative example of original records can be seen in Figure 3a. The fast component of 5-HT induced contraction was 2.5 g in low-Mg Krebs solution and 1.6 g in normal-Mg Krebs solution while the slow component was 0.6 g and 0.5 g respectively. Analysis of these components revealed that when the 5-HT total response was significantly reduced by Mg^{2+}, the fast component of the contraction was also significantly lowered (Table 1), while the slow component was inconsistently affected.

The response of rabbit mesenteric artery to 5-HT was also increased in Mg-free Krebs solution; however, the differences were statistically significant ($P < 0.05$) only at 10^{-6} and 10^{-5}M 5-HT.

The response of longitudinal smooth muscle from guinea-pig ileum to 5-HT in Krebs solution of varying Mg^{2+} content is shown in Figure 4. In the absence of Mg^{2+}, the response was consistently greater than that in the presence of 1.2 or 2.4 mM Mg^{2+}. When compared by analysis of covariance, the concentration-response curves obtained in low-Mg, normal-Mg and high-Mg Krebs solution were significantly different ($P < 0.001$). The rightward shift of the concentration-response curves and the reduction of maximum response by higher Mg^{2+} levels suggest a non-competitive antagonism between 5-HT and Mg^{2+}.

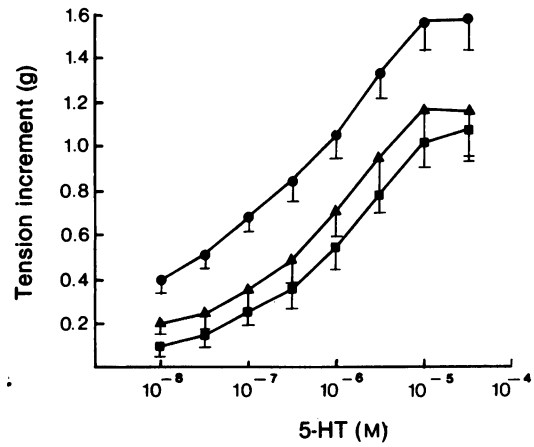

Figure 4 5-Hydroxytryptamine (5-HT) concentration-response curves of guinea-pig ileum longitudinal smooth muscle exposed to Krebs solution containing three different Mg levels. Means and standard error of 8 experiments are shown. (●) Low-Mg Krebs solution; (▲) normal-Mg Krebs solution; (■) high-Mg Krebs solution.

Effect of Mg^{2+} on 'lanthanum-resistant' uptake of ^{45}Ca

Uptake of ^{45}Ca was not significantly different when strips of rabbit mesenteric vein were exposed to low-Mg or normal-Mg Krebs solution for 60 min, the values obtained being 26.6 ± 1.1 and 33.1 ± 7.0 d min^{-1} mg^{-1} respectively. On the other hand, when the strips were exposed for only 5 min to a given Krebs solution after equilibration in normal-Mg solution, the uptake of ^{45}Ca was greater in low-Mg Krebs solution than in either normal-Mg or high-Mg Krebs solution (Figure 5). The addition of 10^{-5}M 5-HT to the bathing solution did not significantly alter the 'lanthanum-resistant' uptake of ^{45}Ca by the vascular strips. This was true both for the veins equilibrated in low-Mg and normal-Mg Krebs solution either for 5 min or 60 minutes.

Table 1 Fast components of 5-hydroxytryptamine (5-HT) contraction of rabbit mesenteric vein

Mg^{2+} in bathing solution (mM)	5-HT (M)							
	10^{-7}	3×10^{-7}	10^{-6}	3×10^{-6}	10^{-5}	3×10^{-5}	10^{-4}	3×10^{-5}
0	0.35 ± 0.07	0.53 ± 0.09	0.75 ± 0.11	0.85 ± 0.13	1.03 ± 0.13	1.14 ± 0.14	1.40 ± 0.16	1.36 ± 0.17
1.2	0.19 ± 0.03 **	0.30 ± 0.03 ***	0.43 ± 0.05 ***	0.50 ± 0.06 **	0.70 ± 0.08 *	0.88 ± 0.09	1.06 ± 0.11	1.10 ± 0.14
2.4	0.11 ± 0.03 ***	0.19 ± 0.05 ***	0.30 ± 0.07 **	0.36 ± 0.06 ***	0.41 ± 0.07 ***	0.58 ± 0.09 **	0.70 ± 0.11 ***	0.76 ± 0.13 **

Values expressed as tension increment in grams, means ± s.e. for 8 experiments.
* $P < 0.05$; ** $P < 0.02$; *** $P < 0.01$ refer to levels of statistical significance determined by t-test for paired samples when values obtained in 0 Mg^{2+} Krebs solution were compared with those in 1.2 mM Mg^{2+} or 2.4 mM Mg^{2+} Krebs solution.

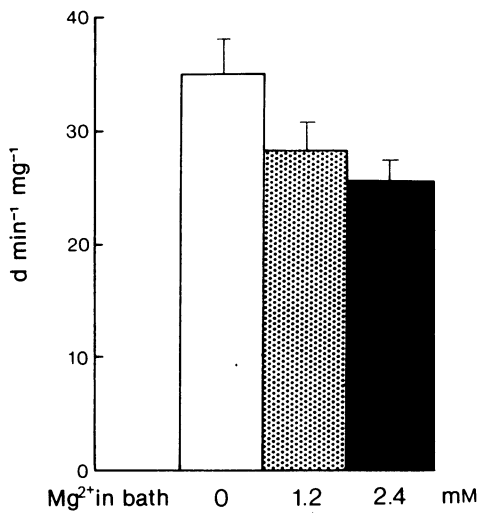

Figure 5 'Lanthanum-resistant' uptake of ^{45}Ca by 8 rabbit mesenteric veins following 5 min exposure to Krebs solution containing 0, 1.2 and 2.4 mM Mg^{2+}. Means and standard error are shown as d min^{-1} mg^{-1} wet weight. Uptake was significantly greater ($P < 0.05$) in vessels incubated in low-Mg Krebs solution than in normal-Mg Krebs solution.

Effect of EGTA on response to 5-hydroxytryptamine

These experiments were performed on the mesenteric veins of four rabbits in order to examine the dependence of Mg^{2+} effect on Ca^{2+} influx during 5-HT-induced contractions. The replacement of normal-Mg Krebs solution with a Ca-free solution containing EGTA 10^{-6}M abolished the 5-HT-induced contraction (Figure 4). In contrast, in the strip of the same vein exposed to low-Mg Krebs solution, the lack of Ca^{2+} had much less of an effect (Figure 4) and 5-HT still produced a contraction, albeit diminished in amplitude for more than 40 min; a similar pattern of activity was observed in the other three vessels.

Effect of Mg^{2+} on total tissue Ca content

In pooled samples, each sample consisting of 6 strips of rabbit mesenteric vein, total tissue Ca content after 30 and 60 min exposure to low-Mg Krebs solution was 7.20 and 9.25 mM Ca/kg wet weight of tissue respectively. Values obtained after 30 and 60 min exposure to normal-Mg Krebs solution were 8.25 and 8.85 mM Ca/kg wet weight of tissue respectively.

Discussion

In these experiments increases in concentration of Mg^{2+} in the bathing solution have been shown to depress the responses of rabbit isolated mesenteric artery and vein and guinea-pig ileum to 5-HT. These results are in agreement with the trend observed in rabbit aortic strips (Altura & Altura, 1971).

Mg^{2+} has been shown to affect differentially the responses of smooth muscle to several agonists. The alterations observed may be due to the interaction of Mg^{2+} at several sites such as receptors, plasma membrane, intracellular Ca^{2+} pools, contractile proteins and enzymes such as ATPase and adenylate cyclase. For example, the greater response to vasopressin by various tissues in the presence of 1.2 mM Mg^{2+}, compared to that in Mg-free solution, has been attributed to an increased affinity of the hormone for its receptor (Somlyo *et al.*, 1966). However, Altura (1975) reported that at several Mg^{2+} levels, concentration-response curves for vasopressin were not displaced in a parallel fashion and concluded that besides the receptor, Mg^{2+} acts elsewhere to alter the response to vasopressin. Our experiments demonstrate that if Mg^{2+} affected the response to 5-HT by altering 5-HT affinity for its receptor, it would do so in an opposite direction to that for vasopressin. The rightward shift of the concentration-response curves and reduced maximum response observed with increasing Mg^{2+} concentrations in both vascular and intestinal smooth muscle preparations indicate a noncompetitive antagonism of Mg^{2+} with the drug. These data suggest, but do not prove, that Mg^{2+} acts at some site or sites other than the receptor, to depress the response to 5-HT. Further experiments employing 5-HT antagonists or binding assays of 5-HT in the presence of varying Mg^{2+} concentrations should be performed to gain a better insight into the possible effect of Mg^{2+} on the 5-HT receptor.

Alteration of the response of smooth muscle to drugs by Mg^{2+} may be the result of interaction with the contractile proteins or enzymes. Exposure to Mg-free physiological solutions for 1 h resulted in the loss of about 25% of total tissue Mg from rat uterus (Moawad & Daniel, 1971) and 29% from rat tail artery (Palaty, 1971). Altura & Altura (1971) reported the loss of about 40% of total tissue Mg from rabbit aortic strips under similar conditions and concluded that sufficient Mg^{2+} remained in the cells for the function of enzymes. Interference with enzymes and contractile proteins seems unlikely as an explanation for the effect of Mg^{2+} on smooth muscle contractions.

Several investigators have suggested that Mg^{2+} may affect drug responses by altering the amount of Ca^{2+} available for contraction. One possibility is that Mg^{2+} and Ca^{2+} compete for divalent cation binding sites in the cell and that displacement of Ca^{2+} by Mg^{2+} would make more Ca^{2+} available for contraction (Turlapaty & Carrier, 1973; Altura & Altura, 1974). This could explain why Mg^{2+} increases the maximum response of smooth muscle to adrenaline,

Ba^{2+} (Altura & Altura, 1971) and noradrenaline (Jurevics & Carrier, 1973) but would certaintly not explain the increased maximum response to acetylcholine, angiotensin (Altura & Altura, 1971) and 5-HT in the absence of Mg^{2+}. Another possibility is that Mg^{2+} may reduce Ca^{2+} flux into the smooth muscle cell. This is suggested by the increased magnitude of spontaneous contractions of rabbit mesenteric vein and rat portal vein (Sigurdsson & Uvelius, 1975) and rat aorta (Altura & Altura, 1974) in the absence of Mg^{2+}. The increase in spontaneous contractile activity is dependent on extracellular Ca^{2+} concentration as demonstrated by Altura & Altura (1976) who showed an enhancement of the effect of Mg-free Krebs solution by EDTA and abolition by EGTA. The effect of Mg^{2+} on Ca^{2+} influx may occur through a generalized change of membrane permeability or through direct interaction with Ca^{2+} transport across the membrane. Altura & Altura (1971) found that in strips of rabbit aorta exposed to Mg-free Krebs solution, Na, K and water content remained unchanged while Ca content was significantly greater than in strips exposed to normal-Mg Krebs solution. Exposure of rat ventricular septa to physiological solution containing 5 mM Mg^{2+} depressed ^{45}Ca uptake but had no effect on ^{42}K and ^{24}Na exchange (Shine & Douglas, 1974). These results are against Mg^{2+} affecting drug response and spontaneous contractile activity through a generalized reduction of membrane permeability.

Our experiments are compatible with the hypothesis that Mg^{2+} affects the availability of Ca^{2+} from an intracellular pool involved in 5-HT contraction. The 'lanthanum-resistant' uptake of ^{45}Ca after only 5 min exposure to Krebs solution of varying Mg^{2+} content was greatest in the absence of Mg^{2+} and declined with higher Mg^{2+} concentrations, In contrast, the 'lanthanum-resistant' uptake of ^{45}Ca was not altered after 60 min exposure to low-Mg solution, a finding similar to that of Carrier, Hester, Jurevics & Tenner (1976) in rabbit aortic strips. In addition, we found that total tissue Ca content measured by atomic absorption spectrophotometry was only slightly altered under similar conditions although its significance could not be assessed. Futhermore, 5-HT did not increase the uptake of 'lanthanum-resistant' ^{45}Ca in our experiments. This may be the result of the insensitivity of the method for demonstrating very slight changes in Ca^{2+} influx into vascular smooth muscle. On the other hand it may be interpreted in the light of an intracellular Ca^{2+} pool being responsible for the potentiation of 5-HT contractions by low-Mg Krebs solution.

The EGTA experiments seem to lend further support to this hypothesis. In a normal-Mg, Ca-free solution containing the Ca^{2+}-chelating agent EGTA, 5-HT contraction was promptly abolished whereas in the strip exposed to low-Mg solution, the fast component of 5-HT contraction, although diminished, did not disappear for over 40 minutes. Such a different effect of Ca-free EGTA solution could occur if exposure of the tissue to low-Mg Krebs solution altered Ca-Mg exchange in a rapidly equilibrated intracellular pool, thereby allowing more intracellular Ca^{2+} to be available for 5-HT contraction.

In conclusion, Mg^{2+} has been demonstrated to depress significantly the magnitude of both spontaneous and 5-HT-induced contractions of vascular and non-vascular smooth muscle. The mechanism partly responsible for this effect of Mg^{2+} is thought to be the reduced availability of Ca^{2+} from a rapidly equilibrating intracellular pool involved in spontaneous and 5-HT-induced contractions.

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