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Importance of magnesium and potassium concentration on basal tone and 5-HT-induced contractions in canine isolated coronary artery

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1 *In vitro* studies were undertaken to investigate the effects of external potassium ($[K^+]_o$) and magnesium ($[Mg^{2+}]_o$) concentration on canine coronary arterial basal tone and on 5-hydroxytryptamine (5-HT)-induced contractions.

2 Acute withdrawal of, or reduction in, $[K^+]_o$ produced relaxation of basal tone in isolated coronary arteries, whereas acute withdrawal (but not reduction) of $[Mg^{2+}]_o$ produced contraction of these blood vessels.

3 The magnitude of coronary contraction obtained on withdrawal of $[Mg^{2+}]_o$ was dependent upon the $[K^+]_o$; the higher the $[K^+]_o$, the greater the contraction.

4 The precise ratio of $[K^+]_o/[Mg^{2+}]_o$ appeared to be important in dictating the degree of contraction (maximum response) and sensitivity (EC_{50}) of canine coronary vascular smooth muscle cells to 5-HT. The EC_{50} to 5-HT was enhanced by increases in the $[K^+]_o/[Mg^{2+}]_o$ ratio, whereas the ability of 5-HT to induce a maximal contraction was attenuated by decreases in the $[K^+]_o$; the latter being modulated by $[Mg^{2+}]_o$. Small changes in $[Mg^{2+}]_o$ could effect large changes in the EC_{50} as $[K^+]_o$ was lowered.

5 These actions took place over patho-physiological ranges of $[K^+]_o$ and $[Mg^{2+}]_o$.

6 Maintenance of a constant $[K^+]_o/[Mg^{2+}]_o$ ratio, irrespective of the exact $[K^+]_o$ and $[Mg^{2+}]_o$, produced similar degrees of maximum tension.

7 Use of intact vascular ring preparations and helically-cut vascular strips produced similar results with varying $[K^+]_o/[Mg^{2+}]_o$.

8 A variety of pharmacological receptor antagonists (phentolamine, propranolol, atropine, diphenhydramine, cimetidine), as well as a prostaglandin cyclo-oxygenase inhibitor, did not modify the altered contractile responses or basal tone evoked by varying $[K^+]_o/[Mg^{2+}]_o$ ratios.

9 These results suggest: (1) that basal tone and contractility of canine coronary vascular smooth muscle cells appear to be exquisitely sensitive to alterations in extracellular K^+ and Mg^{2+}; and (2) 5-HT receptor-operated Ca^{2+} channels, as well as those Ca^{2+} channels involved in generation of coronary arterial basal tone are modulated and controlled by the precise concentrations of $[K^+]_o$ and $[Mg^{2+}]_o$.

Introduction

In the past decade, considerable experimental as well as clinical evidence has accumulated concerning the influence of magnesium (Mg)- and potassium (K)-deficiency on the aetiology and progression of cardiovascular disease (see Altura & Kruck, 1984; Altura, 1986; Whelton *et al.*, 1986; for recent reviews). Mg- as well as K-deficiency have been implicated in aetiology of vasospasm, hypertension, stroke and sudden cardiac death. Combined Mg and K depletion are becoming more and more common in the clinical arena (Whang *et al.*, 1984; Dyckner & Wester, 1987), predisposing to cardiac arrhythmias and sudden cardiac death.

Several *in vivo* and *in vitro* studies have shown that an elevation in external potassium ions ($[K^+]_o$) within a physiological range can produce relaxation

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of arterial smooth muscle (Toda, 1978; Altura & Altura, 1978; Murray & Sparks, 1978; Turlapaty & Altura, 1982), whereas a reduction in $[K^+]_o$ can result in contraction of arterial smooth muscle (Brace *et al.* 1974; Bonaccorsi *et al.* 1977; Altura & Altura, 1978; Toda, 1978). These effects have been hypothesized to be caused by activation or inhibition of a membrane Na^+-K^+ electrogenic pump (see Casteels *et al.*, 1985 for recent review). On the other hand, it has been recognized that magnesium ions (Mg^{2+}) are essential for modulation of Na^+-K^+ transport across cell membranes (Skou, 1965; Allen, *et al.*, 1986). We (Altura & Altura, 1974; Turlapaty & Altura, 1980) and others (see Altura & Altura, 1985a, b, for recent reviews) have found that reduction in the level of $[Mg^{2+}]_o$ can result in enhancement of coronary vascular tone and potentiation of coronary vasoconstrictors.

Using a variety of cells and tissues, including vascular smooth muscle, it has been found that cellular depletion of Mg^{2+} results in concomitant depletion of K^+, which often is followed by an increase in uptake of Na^+ and Ca^{2+} (see Günther, 1983; Altura & Kruck, 1984; Altura & Altura, 1984; Whelton *et al.*, 1986, for recent reviews). Thus, it appears that Mg^{2+} might regulate the cellular and subcellular distribution as well as intracellular concentration of K^+ in vascular muscle.

Since preliminary experiments suggested that the $[K^+]_o/[Mg^{2+}]_o$ ratio could regulate cerebral arterial tone (Murakawa *et al.*, 1986), the present study was designed to investigate the interrelationships of $[K^+]_o$ and $[Mg^{2+}]_o$ on vascular tone and reactivity to a neurohumoral vasoactive substance, viz. 5-hydroxytryptamine (5-HT), in canine isolated coronary arteries. The data presented herein strongly suggest that the precise ratio of $[K^+]_o/[Mg^{2+}]_o$ will dictate the degree of coronary arterial basal tone, as well as the sensitivity (EC_{50}) to and magnitude of contraction induced by 5-HT.

Some of these data were presented at a Meeting of the Federated American Societies for Experimental Biology, held at Washington, D.C., April 1987.

Methods

Animals and coronary artery preparations

Mongrel dogs of either sex, weighing 15–20 kg, were killed after anaesthesia was induced by i.v. injection of pentobarbitone sodium (30 mg kg^{-1}). After thoractomy, the hearts were excised quickly and placed in cold Krebs-Ringer bicarbonate (KRB) solution. Left coronary arteries (o.d. 0.5–1.0 mm) were isolated and cut into helical strips approximately 15 mm long by 1.5 mm wide (Turlapaty & Altura, 1980). The specimens were fixed vertically in 20 ml muscle baths with sutures under standard isometric conditions utilizing resting tensions of 2.0 g. Since endothelial integrity has recently been demonstrated to influence responses of coronary arteries to $[Mg^{2+}]_o$ (Altura & Alura, 1987; Ku & Ann, 1987), we also utilized rings in selected experiments. Irrespective of whether strips or rings were utilized, the data were qualitatively similar. The tissues were incubated in normal KRB solution that was aerated with a mixture of 95% O_2–5% CO_2. The temperature was kept at 37 ± 0.5°C. The composition of the KRB solution was as follows (mM): NaCl 118, KCl 4.7, $CaCl_2$ 2.5, KH_2PO_4 1.2, $MgSO_4$ 1.2, glucose 10 and $NaHCO_3$ 25; pH of the solution was 7.4 ± 0.05. The loading tensions were periodically adjusted and maintained throughout the equilibration time (i.e. 2 h). The incubation media were routinely changed every 10–15 min as a precaution against interfering metabolites (Altura & Altura, 1970). Where appropriate, osmotic adjustment was performed to keep the KRB solution at 317.3 mOsm when the external $[K^+]_o$ and $[Mg^{2+}]_o$ were reduced; both K^+ and Mg^{2+} were substituted with Na^+, using either NaCl or $NaHPO_4$. On the other hand, when the external $[K^+]_o$ and $[Mg^{2+}]_o$ were elevated in the media, Na^+ was reduced by equivalent amounts.

Influence of $[K^+]_o/[Mg^{2+}]_o$ ratios on base-line tone

Examination of the effects of alteration in $[K^+]_o$ and $[Mg^{2+}]_o$ base-line tone was undertaken as follows: the external potassium concentration was altered in a random manner from 0 to 7.1 mM (i.e., the sequence 0, 1.2, 2.4, 4.8 and 7.1 mM was randomized), whereas $[Mg^{2+}]_o$ was altered over the range 0 to 4.8 mM. The modified solutions were kept at 37°C and aerated with 95% O_2 + 5% CO_2. After establishment of basal tone in normal KRB solution, the modified solution was substituted. The tension developed was measured 30 min later.

Drug-induced contractile responses and the effects of pharmacological antagonists

Cumulative contractile concentration-response curves for 5-HT over a wide range of $[K^+]_o/[Mg^{2+}]_o$ ratios were obtained by a stepwise increase in concentration from 1.23×10^{-10} M to 2.06×10^{-6} M after a steady state response had occurred to each preceding dose (Altura & Turlapaty, 1982). EC_{50} values were determined as the concentration of 5-HT that produced half-maximum contraction. These dose-response curves were expressed as a % change of control maximal contraction produced by 5-HT in normal KRB solution. In certain experiments, the influence of pharmacol-

ogical receptor antagonists and a cyclo-oxygenase inhibitor on the responsiveness to 5-HT was examined, in order to determine whether the indirect release or generation of different amines and prostanoids were responsible for the modifications induced by alteration in $[K^+]_o/[Mg^{2+}]_o$. Different preparations were incubated with antagonists for 30 min before commencement of the cumulative dose-response curve for 5-HT. The pharmacological antagonists, including H_1-, H_2-receptor blockers (diphenhydramine HCl, 0.5 $\mu g\,ml^{-1}$ and cimetidine HCl, 1.0 $\mu g\,ml^{-1}$, respectively), α- and β-adrenoceptor blockers (phentolamine, 0.5 $\mu g\,ml^{-1}$; propranolol, 0.5 $\mu g\,ml^{-1}$, respectively), muscarinic cholinoceptor blocker (atropine, 0.5 $\mu g\,ml^{-1}$) and a cyclo-oxygenase inhibitor (indomethacin, 0.5 $\mu g\,ml^{-1}$) were used in concentrations that were sufficient to inhibit the response associated with their respective receptor agonists or generation of prostanoids (in the case of indomethacin).

Drugs

Drugs used were 5-HT creatinine sulphate (Nutritional Biochemical Lab.), diphenhydramine HCl (Benadryl, Parke Davis) cimetidine HCl (SK & F), phentolamine methanesulphonate (Regitine, CIBA-GEIGY), propranolol HCl (Sigma Chemical Co.), atropine sulphate (Mann Res. Lab.) and indomethacin (Indocin, Merck Sharp & Dohme Co.). All drugs were dissolved in distilled deionized water as concentrated stock solutions so that the total volumes added to the 20 ml muscle chamber never exceeded 1.0 ml.

Statistical analyses

Where appropriate means ± s.e. means of the responses were compared for statistical significance by Student's t test, paired t test, one-way analysis of variance, and regression line analysis by method of least squares. Values were considered significant if $P < 0.05$.

Results

Influence of various $[K^+]_o/[Mg^{2+}]_o$ ratios on basal tone of coronary arteries

Figure 1 demonstrates that withdrawal of $[Mg^{2+}]_o$ from the bathing medium caused an increase in basal-tone, irrespective of the external K^+ concentration. However, the higher the $[K^+]_o$, the larger the increase in basal tone as $[Mg^{2+}]_o$ was withdrawn. Surprisingly, irrespective of the $[K^+]_o$ concentration (i.e., 0–7.1 mM), the presence of $[Mg^{2+}]_o$

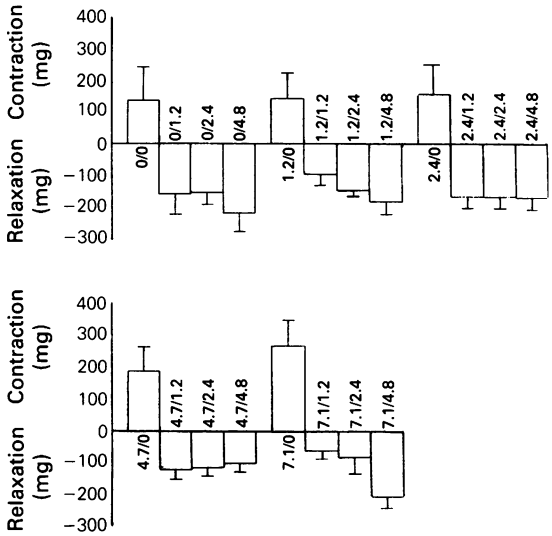

Figure 1 Modification of basal tone in canine coronary arteries by altering the $[K^+]_o/[Mg^{2+}]_o$ ratio. The numbers above and below the columns (mean values) indicate the $[K^+]_o/[Mg^{2+}]_o$ mM. Vertical bars indicate s.e. mean; n = 6–12 each. All mean values were significantly different from normal KRB solution.

resulted in relaxation of the coronary arteries (Figure 1). In 2 of 9 experiments where the $[K^+]_o/[Mg^{2+}]_o$ was 0/1.2 mM, and 3 of 12 experiments where the ratio was 1.2/1.2 mM, substitution of modified solution for normal Krebs-Ringer solution produced contraction, which developed gradually and attained stabilized tensions (i.e., 150–200 mg).

The modifications in basal tone induced by substitution for a variety of $[K^+]_o/[Mg^{2+}]_o$ concentrations were significantly different from that sustained in a normal Krebs-Ringer solution. Alteration in $[Mg^{2+}]_o$ ranging from 1.2 mM to 4.8 mM did not affect the magnitude of the decrease in basal tone when $[K^+]_o$ concentration was constant, irrespective of the $[K^+]_o$ concentration (Figure 1).

Effects of $[Mg^{2+}]_o$ and $[K^+]_o$ on sensitivity of coronary arterial muscle to 5-HT

An examination of the concentration of 5-HT that produces 50% of the maximum contractile responses, at different $[K^+]_o/[Mg^{2+}]_o$ ratios, revealed that an increase in the $[K^+]_o/[Mg^{2+}]_o$ ratio resulted in enhancement of the sensitivity of canine coronary arteries to 5-HT (Figure 2). The lower the $[K^+]_o$, the steeper the slope of the regression line for the relationship between the $[K^+]_o/[Mg^{2+}]_o$ ratio and EC_{50} values. Statistical analysis (i.e., for the regression coefficients of each

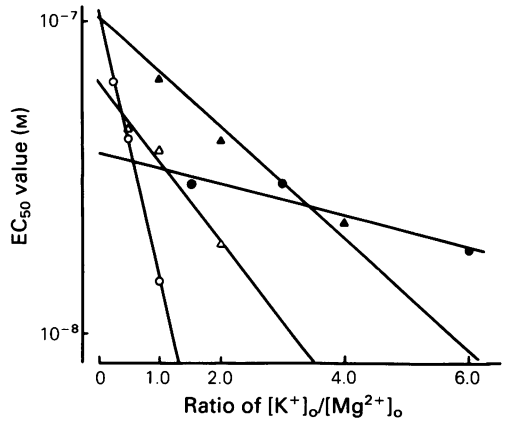

Figure 2 The effect of an increase in $[K^+]_o/[Mg^{2+}]_o$ on the sensitivity (EC_{50}) of canine cornary arteries to 5-hydroxytryptamine (5-HT). Linear regression line equations and correlation coefficients were as follows: $y = -8.710x + 10.40$ ($r = -0.836$), $y = -2.601x + 8.100$ ($r = -0.463$), $y = -1.817x + 10.217$ ($r = -0.677$) and $y = -0.517x + 5.779$ ($r = -0.541$) in 1.2, 2.4, 4.7 and 7.1 mM $[K^+]_o$, respectively. The higher the $[K^+]_o$, the less the slope: (○) 1.2 mM K^+; (△) 2.4 mM K^+; (▲) 4.7 mM K^+; (●) 7.1 mM K^+.

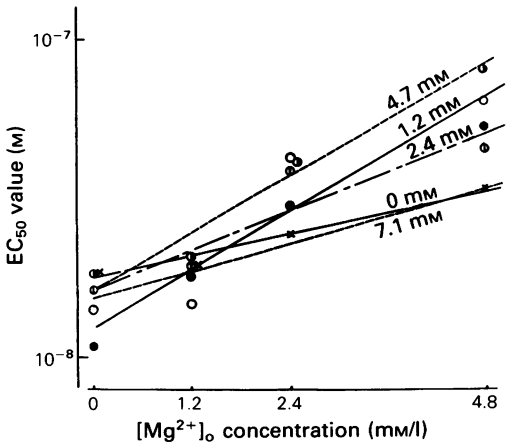

Figure 3 Influence of $[Mg^{2+}]_o$ on the effect of $[K^+]_o$ on the contractile sensitivity of canine coronary arteries to 5-hydroxytryptamine (5-HT). The higher the $[Mg^{2+}]_o$, the less the sensitivity to 5-HT. Linear regression equations and correlation coefficients are as follows: $y = 0.586x + 2.451$ ($r = 0.36$), $y = 1.577x + 0.897$ ($r = 0.797$), $y = 1.1015x + 2.168$ ($r = 0.528$), $y = 1.436x + 2.271$ ($r = 0.737$) and $y = 0.725x + 1.806$ ($r = 0.651$) in 0, 1.2, 2.4, 4.7 and 7.1 mM $[K^+]_o$, respectively. However, there was no significant difference between the EC_{50} values for 5-HT at any one $[Mg^{2+}]_o$ ($P > 0.05$, analysis of variance).

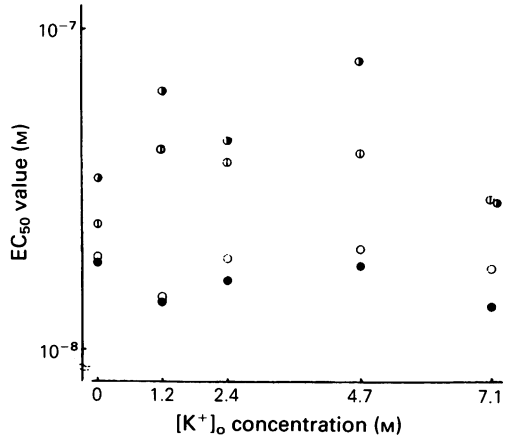

Figure 4 Effect of altering $[K^+]_o$ on the contractile sensitivity to 5-hydroxytryptamine (5-HT) when the $[Mg^{2+}]_o$ was held constant. Under these experimental conditions, the EC_{50} values for 5-HT appeared to be similar at any one, particular $[Mg^{2+}]_o$: (●) 0, (○) 1.2, (Φ) 2.4 and (◑) 4.8 mM $[Mg^{2+}]_o$.

line) indicated that there were significant differences between the slopes of each line ($P < 0.01$). In addition, the y-intercept for each line was different. These findings suggest that the lower the $[K^+]_o$ in the media, the more the EC_{50} value for 5-HT is affected by a small change in $[Mg^{2+}]_o$ (1.2–4.8 mM). In other words, the more $[K^+]_o$ available in the media, the less the EC_{50} value is affected by alterations in $[Mg^{2+}]_o$. Due to the non-parallelism of the regression lines coupled with the varied y- and x-intercepts, it would appear unlikely that a single mechanism can be involved in the marked changes in sensitivity observed with 5-HT. When the $[K^+]_o$ was kept constant as the $[Mg^{2+}]_o$ was altered, we found that the greater the $[Mg^{2+}]_o$, the less sensitive was the tissue to 5-HT (Figure 3). Moreover, there was a linear correlation between the EC_{50} value and change of $[Mg^{2+}]_o$ concentration for a particular constant $[K^+]_o$. Analysis of variance was employed to compare the EC_{50} values obtained from these experiments when the $[Mg^{2+}]_o$ was constant. There were no significant differences in the EC_{50} values, between the values obtained from the dose-response curves for 5-HT, when $[K^+]_o$ was altered from 0 mM up to 7.1 mM, while $[Mg^{2+}]_o$ was maintained constant. When the $[K^+]_o$ concentration was fixed between 0 mM and 7.1 mM and the $[Mg^{2+}]_o$ was altered, no correlation was apparent (Figure 4). Overall, these results suggest that external $[Mg^{2+}]$ modifies, and most likely controls, the effect of $[K^+]_o$ on contractile sensitivity to 5-HT in canine coronary arteries.

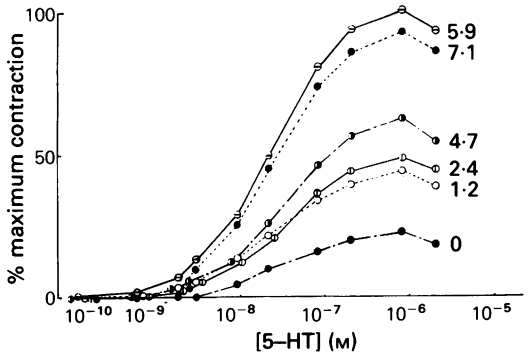

Figure 5 Modification of the contractile concentration-response curves for 5-hydroxytyptamine (5-HT) in canine isolated coronary arteries induced by varying $[K^+]_o$ at a fixed (normal) concentration of $[Mg^{2+}]_o$ (1.2 mM). Maximum contractile responses obtained in normal KRB solution were taken here and in Figures 6–8 to be 100%. Mean value ± s.e. mean of the maximum contraction elicited by 5-HT in normal KRB solution = 1120 ± 160 mg (n = 6–8 each). The numbers beside each curve represent the $[K^+]_o$ in mM.

Effects of $[Mg^{2+}]_o$ and $[K^+]_o$ on contractile responses to 5-HT

Figure 5 demonstrates that the concentration-response curves for 5-HT were attenuated by reduction in the $[K^+]_o$ concentration in a dose-dependent manner when $[Mg^{2+}]_o$ was held constant (e.g. 1.2 mM). The maximum contractions appear to be attenuated in a non-competitive manner with reduction in $[K^+]_o$. On the other hand, varying the $[Mg^{2+}]_o$ modified the contractile response to 5-HT in canine coronary arteries when $[K^+]_o$ was maintained constant (Figures 6 and 7). Figures 6 and 7 clearly demonstrate that an increase in $[K^+]_o$ potentiated the % maximum contraction of the concentration-effect curves for 5-HT in a dose-dependent manner. More than a 100% maximum contraction, compared to that in normal KRB solution (i.e. $[K^+]_o/[Mg^{2+}]_o$ = 5.9/1.2 mM), was obtained when we utilized a $[K^+]_o/[Mg^{2+}]_o$ ratio of 7.1/0 mM. Linear regression line analyses for the % maximum contractions induced by 5-HT, obtained by varying $[K^+]_o$ at fixed $[Mg^{2+}]_o$, revealed good correlations (Figure 8). Statistical analysis revealed that the slopes of the lines were significantly different from each other ($P < 0.01$), except for those between 2.4 mM and 4.8 mM $[Mg^{2+}]_o$ ($P > 0.05$). When the $[Mg^{2+}]_o$ was kept constant, the % maximum contraction increased as the $[K^+]_o$ increased. However, the value of % maximum contractions in an equivalent $[K^+]_o$ medium and different $[Mg^{2+}]_o$ were not significantly different from one another ($P > 0.05$), except for that obtained when the $[Mg^{2+}]_o$ = 0 mM. If the ratio of $[K^+]_o/[Mg^{2+}]_o$ was maintained at unity, irrespective of whether 1.2, 2.4 or 4.8 mM $[K^+]_o$ and $[Mg^{2+}]_o$ was utilized, almost identical %

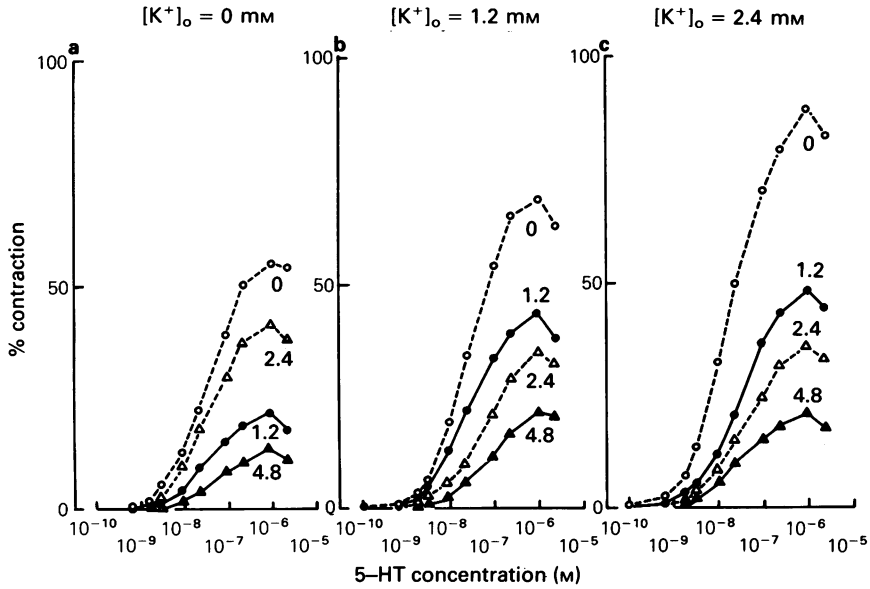

Figure 6 Modification of contractile concentration-response curves for 5-hydroxytryptamine (5-HT) in canine isolated coronary arteries induced by varying $[Mg^{2+}]_o$ at different fixed concentrations of $[K^+]_o$, (a) 0, (b) 1.2 and (c) 2.4 mM. n = 6–8 each. The numbers beside each curve represent the $[Mg^{2+}]_o$ in mM.

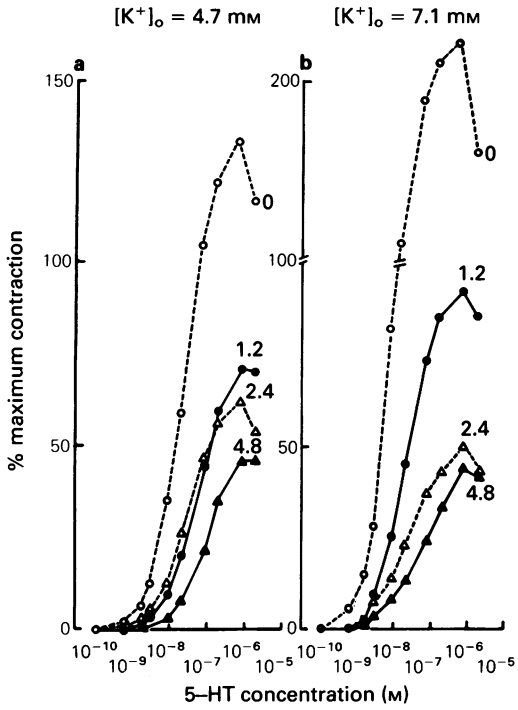

Figure 7 Modification of contractile concentration-response curves for 5-hydroxytryptamine (5-HT) in canine isolated coronary arteries induced by varying $[Mg^{2+}]_o$ at (a) 4.7 or (b) 7.1 mM $[K^+]_o$. $n = 6$–8 each. The numbers beside each curve represent the $[Mg^{2+}]_o$ in mM.

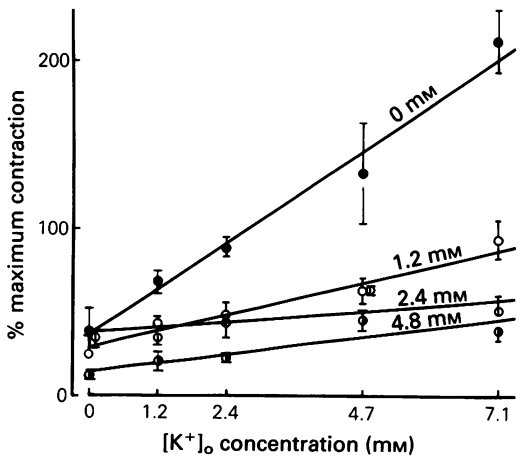

Figure 8 Influence of $[K^+]_o$ on % maximum contractile responses induced by 5-hydroxytryptamine in canine coronary arteries in varying $[Mg^{2+}]_o$. Linear regression equations and correlation coefficients were as follows: $y = 4.425x + 15.020$ ($r = 0.692$), $y = 2.889x + 37.095$ ($r = 0.438$), $y = 8.353x + 28.578$ ($r = 0.751$) and $y = 23.108x + 35.943$ ($r = 0.785$) in 4.8, 2.4, 1.2 and 0 mM $[Mg^{2+}]_o$, respectively. Mean values and s.e. means (indicated as vertical lines) are shown.

Table 1 Influence of constant $[K^+]_o/[Mg^{2+}]_o$ ratio on 5-hydroxytryptamine (5-HT) contractile concentration-effect curves in canine coronary artery

$[K^+]_o/[Mg^{2+}]_o$ (mM)	EC_{50} ($\times 10^{-8}$ M)	% *maximum contraction**
1.2/1.2	1.65 ± 0.11†	43.8 ± 3.8
2.4/2.4	2.31 ± 0.36	44.4 ± 6.9
4.7/4.8	10.02 ± 1.00	45.5 ± 5.9
($n = 7$–10)		

* Relative contractile response to maximum elicited by 5-HT in normal Krebs-Ringer solution. One-way analysis of variance revealed that there was no significant difference for the % maximum contractile responses elicited by 5-HT when the ratio of $[K^+]_o/[Mg^{2+}]_o$ was constant (= 1), irrespective of their concentrations. (F value = 0.031, NS).

† EC_{50} values were significantly different from one another ($P < 0.05$, analysis of variance).

maximum contractile responses were obtained (Table 1). However, EC_{50} values produced by cumulative administration of 5-HT were very different from each other (Table 1).

Failure of pharmacological receptor antagonists and a cyclo-oxygenase inhibitor to modify tone or EC_{50} and maximum responses induced by 5-HT with varying $[K^+]_o/[Mg^{2+}]_o$ ratios

Pharmacological receptor antagonists, including atropine, cimetidine, diphenhydramine, phentolamine and propranolol or the cyclo-oxygenase inhibitor, indomethacin, failed to alter the changes induced by varying the $[K^+]_o/[Mg^{2+}]_o$ ratios on either the EC_{50} or maximal responses produced by 5-HT ($n = 3$–4 each). In addition, these compounds did not influence the altered base-line tone observed after the $[K^+]_o/[Mg^{2+}]_o$ ratios were modified.

Discussion

The results of this study indicate that either acute withdrawal or reduction of $[K^+]_o$ produces relaxation of tone in canine isolated coronary arteries, whereas acute withdrawal, but not reduction, of $[Mg^{2+}]_o$ produces contraction of these coronary blood vessels. In addition, our findings indicate that the degree of coronary contraction observed on

withdrawal of $[Mg^{2+}]_o$ is dependent upon the $[K^+]_o$; the higher the $[K^+]_o$, the greater the vasospasm. The present findings also clearly demonstrate that the precise ratio of $[K^+]_o/[Mg^{2+}]_o$ is important in dictating the magnitude of contraction and sensitivity of canine coronary vascular smooth muscle cells to the agonist and neurohumoral agent 5-HT. Since these actions on basal tone, contractility and sensitivity occur over physiological ranges of these cations, and since 5-HT has been implicated in the aetiology of coronary vasospasm (Turlapaty & Altura, 1980; Toda, 1985), the present findings may be relevant to the regulation of the coronary vascular tree as well as to the aetiology of coronary heart disease and cardiac arrhythmias.

Whereas calcium (Ca^{2+}) is the major cation involved in contractile events in vascular muscle, K^+ and Mg^{2+} have regulatory functions by way of their effects on: (a) membrane potential, (b) Ca^{2+} movements, (c) cell metabolism, and (d) contractile agents (for reviews see Haddy, 1974; Bolton, 1979; Altura & Altura, 1981a, b, Altura & Kruck, 1984). Although it has been known for some time that the potassium ion is a vasodilator substance (Katz & Lindner, 1938; Dawes, 1941) and that a reduction in $[K^+]_o$ can produce increased vascular resistance and constriction of a variety of vascular beds (see Haddy, 1974; Altura & Altura, 1978, for reviews), our data clearly demonstrate that a relaxation or reduction in basal tone is associated with reduction in $[K^+]_o$ (i.e., below 7.1 mM to 0 mM) for canine isolated coronary arteries in the absence of agonists. These results confirm some preliminary findings of other investigators using these blood vessels (Toda, 1978; Altura & Altura, 1982). The data are of interest for at least two different reasons: (1) others have previously observed an increase in coronary vascular resistance due to local hypokalaemia (e.g., 3.4 to 1.6 meq l^{-1}) in the perfused canine heart, hypothesized to be due to an inhibition of the Na^+, K^+ electrogenic pump (Brace *et al.*, 1974); and (2) relaxation of, or reduction in, coronary basal tone was noted in the present study, irrespective of the $[K^+]_o$, provided at least some $[Mg^{2+}]_o$ was present. Since a variety of isolated blood vessels (renal, mesenteric, cerebral, facial, ear as well as aorta and veins) undergo contraction upon reduction of $[K^+]_o$ (Haddy, 1974; Altura & Altura, 1978; Karaki *et al.*, 1978; Toda, 1978; Bolton, 1979; Hayashi & Park, 1984; Casteels *et al.*, 1985), the difference in results observed herein versus the findings on perfused canine hearts (Brace *et al.*, 1974), are most likely attributable to actions of $[K^+]_o$ on large (present study) versus small coronary vessels (Brace *et al.*, 1974) and/or the presence (or release) of vasoactive agents. Unfortunately, no information on the tissue or perfusate Mg levels was given in these studies.

Since the epicardial (large) coronary vessels are thought to play major roles in several types of coronary heart disease (Marcus, 1983), the present findings may be quite important in situations where myocardial deficits in K^+ are observed, e.g., hypertension, congestive heart failure, acute myocardial infarction, diuretic-induced hypokalaemia, and sudden cardiac death among others (Dyckner & Wester, 1987; Wills, 1986).

Our results which demonstrate relaxation, rather than elevation, of basal tone were observed in media containing either normal or raised $[Mg^{2+}]$. Moreover, an alteration in $[Mg^{2+}]_o$ did not affect the changes in basal tone noted when $[K^+]_o$ was modified, regardless of the concentration of the latter. In contrast to these findings, we have recently demonstrated that acute withdrawal of, or reduction in, $[K^+]_o$ elicited contractile responses in canine cerebral arteries which were modulated by the $[Mg^{2+}]_o$ (Murakawa *et al.*, 1986) and seem to be associated with alterations in membrane potential and inhibition of the Na^+, K^+ electrogenic pump. One would be hard-pressed to explain the present findings, however, either on the basis of inhibition of coronary vascular membrane Na^+, K^+-ATPase activity, electrogenic ion transport, or a release of endogenous vasoactive amines (or prostaglandins); a variety of pharmacological antagonists and a cyclo-oxygenase inhibitor failed to affect the changes in tone brought about by alterations in $[K^+]_o$. We suggest, therefore, that the membrane Na^+, K^+ electrogenic pumps in large canine coronary arterial smooth muscle cells may either not be fully inactivated by reduction of external K^+, or the amount of membrane Na^+, K^+-ATPase might be much less in these smooth muscle cells than in small coronary resistance vessels. In this context, it should be noted that: (1) the amount of membrane and microsomal Na^+, K^+-ATPase present in large coronary arteries is indeed much less than that present in most types of peripheral arteries as well as other types of smooth muscle (see Casteels *et al.*, 1985, for recent review); and (2) the ouabain-sensitive Na^+, K^+-ATPase activity characterized from some canine vascular smooth muscles is less than half of the remaining Mg^{2+}-ATPase activity (Allen *et al.*, 1986), thus suggesting a heterogeneity in regional distribution of the membrane Na^+, K^+-pumps.

The fact that the presence of a physiological amount of $[Mg^{2+}]_o$ appears to prevent canine coronary arteries from undergoing contraction or spasm in the face of graded reductions in $[K^+]_o$ (i.e., 7.1–0 mM), suggests that the former divalent cation probably plays an important role in stabilizing the coronary arterial smooth muscle cell membrane (Turlapaty & Altura, 1978; 1980; Altura & Altura, 1984).

In addition to effects on coronary arterial basal tone, our present findings reveal that the exact $[K^+]_o/[Mg^{2+}]_o$ ratio utilized, exerts marked actions on the contractile effects of 5-HT. For example, the sensitivity (i.e. EC_{50}) to this neurohumoral agonist was enhanced by increasing the $[K^+]_o/[Mg^{2+}]_o$ ratio, whereas the ability of 5-HT to induce a maximal contraction was attenuated by decreasing the $[K^+]_o$; the latter being modulated rather precisely by the concentration of Mg^{2+}. It is not surprising that the sensitivity (e.g. Figures 2 and 3) and contractility amplitude (e.g. Figures 6 and 7) of canine coronary arterial muscle to 5-HT is increased as the $[Mg^{2+}]_o$ is reduced, since it has previously been demonstrated that the latter divalent cation exerts a greater influence on membrane channels involved in Ca^{2+} influx activated by receptor-operated agonists, as compared to those activated by membrane depolarization (Altura & Turlapaty, 1982; Altura *et al.*, 1987). However, what should be especially noted is that the lower the $[K^+]_o$, the steeper the slope of the regression line between sensitivity to 5-HT and the $[K^+]_o/[Mg^{2+}]_o$ ratio (e.g. Figure 2). This suggests that a rather small change in $[Mg^{2+}]_o$ effects great alterations in the sensitivity of coronary arterial muscle to 5-HT as the external $[K^+]_o$ is lowered. In addition, this suggests that a rather tight relationship exists between $[Mg^{2+}]_o$ and $[K^+]_o$ in control of sensitivity and contractility of coronary vascular muscle cells to 5-HT and possibly other receptor-mediated agonists. The fact that maintenance of a constant $[K^+]_o/[Mg^{2+}]_o$ ratio produces similar degrees of contractility (i.e., maximum tensions), irrespective of the precise concentrations of K^+ and Mg^{2+}, lends additional support to the latter.

The observed changes in sensitivity to and contractions induced by 5-HT, noted with different $[K^+]/[Mg^{2+}]_o$ ratios, are probably not reflections of either changes in basal tone, presence or absence of intact endothelium and/or release of amines and prostanoids from the vascular wall. Although some elevations in basal tone were observed (e.g. Figure 1), this was only noted upon complete withdrawal of $[Mg^{2+}]_o$; the presence of $[Mg^{2+}]_o$, irrespective of $[K^+]_o$, produced relaxation of tone which was almost equivalent regardless of the $[Mg^{2+}]_o$. Since equivalent results, at least qualitatively, were observed with intact rings, it is unlikely that the intima is playing a role in the results presented here. Furthermore, a variety of amine antagonists and a cyclo-oxygenase inhibitor did not interfere with the observed responses. It is, thus, unlikely that endogenous release and/or synthesis of a variety of vasoactive amines or prostanoids contribute to the results seen on altering $[K^+]_o/[Mg^{2+}]_o$.

Any mechanism proposed to account for the results presented here must take into account the following: (1) irrespective of the direction and magnitude of the change in basal tension, marked increases in the sensitivity and contractility to 5-HT were observed as the $[K^+]_o/[Mg^{2+}]_o$ ratio increased and as the $[Mg^{2+}]_o$ decreased; (2) pharmacological antagonists and a cyclo-oxygenase inhibitor fail to influence these findings; and (3) progressive reductions in $[K^+]_o$ in the presence of normal $[Mg^{2+}]$, i.e., 1.2 mM (e.g., Figure 5), resulted in progressive reductions in contractility to 5-HT. Since progressive reduction in $[K^+]_o$ from 5.9 to 1.2 mM is known to result in increased membrane depolarization of vascular muscle cells (Brace *et al.*, 1974; Haddy, 1974; Bolton, 1979; Harder, 1980; Casteels *et al.*, 1985), thereby reducing the threshold for contraction, one would anticipate an increased sensitivity and contractility of coronary vascular muscle to 5-HT, the opposite of what we observed. One would thus be hard-pressed to equate our present findings solely with alterations in membrane potential. Since 5-HT may induce contractions in some types of vascular smooth muscle by means other than membrane depolarization, *viz.*, pharmacomechanical coupling (Somlyo & Somlyo, 1968; Bolton, 1979), it would seem possible that the latter type of coupling event may be affected by alterations in $[K^+]_o/[Mg^{2+}]_o$ and result in modulation of the 5-HT receptor-operated Ca^{2+} channel (ROC).

In conclusion, our experiments demonstrate that basal tone and contractility of coronary vascular smooth muscle cells appear to be exquisitely sensitive to alterations in extracellular K^+ and Mg^{2+}; this sensitivity appears to take place over physiological concentrations of these cations. A tight coupling between K^+-Mg^{2+} and membrane activation exists in coronary arterial smooth muscle. The precise ratio of $[K^+]_o/[Mg^{2+}]_o$ is critical in dictating the contractile properties of canine coronary vascular muscle cells. The data appear to be most compatible with the idea that 5-HT receptor-operated Ca^{2+} channels as well as those Ca^{2+} channels involved in generation of basal coronary tone, are modulated and controlled by the precise concentrations of $[K^+]_o$ and $[Mg^{2+}]_o$. Lastly, it must be entertained that the cardiac arrhythmias and high incidence of sudden cardiac death seen in patients with concomitant hypokalaemia and hypomagnesaemia (for review see, Whang *et al.*, 1984; Whelton *et al.*, 1986; Wills, 1987, Dyckner & Wester, 1987) may largely be a result of production of coronary vasospasm, as observed in our experimental studies and suggested previously (Altura & Altura, 1984). The high incidence of hypomagnesaemia (e.g., $\cong$40%) observed with hypokalaemia (Wills, 1986; Dyckner & Wester, 1987), and

often seen in patients presenting with hypertension and cardiac arrhythmias, points to the need to extend the present studies.

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