Br. J. Pharmac. (1982) **77**, 649–659

WITHDRAWAL OF MAGNESIUM ENHANCES CORONARY ARTERIAL SPASMS PRODUCED BY VASOACTIVE AGENTS

BURTON M. ALTURA & PRASAD D.M.V. TURLAPATY

Department of Physiology, State University of New York, Downstate Medical Center, Brooklyn, New York 11203, U.S.A.

1 The influence of external magnesium ions ($[Mg^{2+}]_o$) on the sensitivity (i.e. EC_{50}) and contractility (maximum response) of isolated large and small coronary arteries of the dog, obtained from different regions of the myocardium, to vasoactive agents was studied.

2 Removal of $[Mg^{2+}]_o$ from the physiological salt solution enhanced, while elevation in $[Mg^{2+}]_o$ to 4.8 mM, lowered the contractile sensitivity to three different agents, 5-hydroxytryptamine (5-HT), angiotensin II and KCl.

3 Contractility, of both large and small coronary arteries, to 5-HT and angiotensin II was potentiated and depressed, respectively, by withdrawal and elevation of $[Mg^{2+}]_o$; maximum responses to KCl were not altered by 0 or 4.8 mM $[Mg^{2+}]_o$.

4 Cumulative concentration-contractile effect curves to $CaCl_2$ were shifted leftward on removal of $[Mg^{2+}]_o$; elevation of $[Mg^{2+}]_o$ to 4.8 mM shifted the $CaCl_2$ concentration-effect curves to the right. Maximal contractile responses to $CaCl_2$ were enhanced by removal of, and reduced by elevation of, $[Mg^{2+}]_o$.

5 The calcium channel blocking agent, verapamil (10^{-6} M), inhibited completely contractile responses to KCl; contractile responses elicited by angiotensin II and 5-HT were attenuated by verapamil.

6 A variety of pharmacological antagonists (phentolamine, propranolol, methysergide, atropine, diphenhydramine), as well as use of a prostaglandin cyclo-oxygenase inhibitor, did not modify the altered contractile responses evoked by angiotensin II or KCl in different concentrations of Mg^{2+}.

7 These results suggest: (1) $[Mg^{2+}]_o$ may exert considerably greater influence on receptor-operated rather than membrane-potential sensitive channels involved in Ca^{2+} transport in coronary arterial smooth muscle; (2) Mg^{2+} interferes with the affinity (binding) of certain agonists (5-HT and angiotensin II) for their respective receptors in coronary vascular muscle; and (3) a functional pool of Ca^{2+} which is resistant to Ca^{2+}-depletion, but accessible to activation by 5-HT and angiotensin II is present in canine coronary arterial smooth muscle.

Introduction

Experimental and clinical evidence suggest that magnesium (Mg^{2+}) deficiency, in animals and man, could be a significant factor in the aetiology of several cardiovascular disease states (Seelig & Heggtveit, 1974; Burch & Giles, 1977; Altura, 1979; Altura 1980; Altura & Altura, 1981). In a recent *in vitro* strudy, we demonstrated that acute withdrawal of magnesium induces contractile responses in canine coronary arteries (Turlapaty & Altura, 1980). Furthermore, we also demonstrated that magnesium withdrawal potentiated the contractile responses of coronary arteries to various vasoactive agents which have been implicated, clinically, in induction of coronary vasospasm. However, the mechanism involved in this phenomenon is not clear (Leven & Freeman, 1976; Yasue, Touyama, Kato, Tomaka & Akiyama, 1976; Hansen & Sandøe, 1978).

Recent, previous studies indicate that acute withdrawal of Mg^{2+} from a physiological salt solution can induce contractions in several types of blood vessels which are dependent on extracellular calcium (Altura & Altura, 1971; 1974; 1976; 1978; 1980; 1981; Turlapaty & Altura, 1978). Since it is known that vasoactive agents utilize different sources of calcium (Ca) for their contractile responses (Weiss, 1978), it has been suggested that in a magnesium-deficient state, Ca^{2+} influx might be enhanced, thereby resulting in an increased tone and contractility (Altura & Altura, 1974; 1978; 1981; Altura, 1979; Turlapaty & Altura, 1980). It is also possible that Mg^{2+}-deficiency might increase the sensitivity of postsynaptic receptors, as it has been shown by several investigators that Mg^{2+} can alter the sensitivity of non-cardiac blood vessels to several neurohumoral

0007-1188/82/120649-11 $01.00

substances (Somlyo, Woo & Somlyo, 1966; Altura & Altura, 1971; 1974; 1978; Altura, 1975; Turlapaty, Carrier & Jurevics, 1975; Altura, Altura & Waldemar, 1976; Goldstein & Zsotér, 1978).

The present study, in which isolated coronary arteries of the dog were used, was designed to determine whether withdrawal of magnesium, and elevation in magnesium (4.8 mM): (1) can influence, significantly, both the sensitivity (as determined by threshold concentration and EC_{50}) and contractility (indicated by maximal contractile tensions) of these blood vessels to agents such as 5-hydroxytryptamine (5-HT), angiotensin, and potassium thought to be involved in production of coronary vasospasm; and (2) can alter permeability to Ca^{2+}.

Methods

Animals and coronary artery preparations

Mongrel dogs of either sex weighing 15–20 kg were anaesthetized with pentobarbitone sodium (30 mg/kg, i.v.). After thoracotomy, the hearts were excised quickly and coronary arteries were isolated.

Because of possible segmental differences in coronary arterial reactivity (Altura, 1966), we examined four different coronary arteries: left coronary (LC, with o.d. of 1–1.5 mm), circumflex (CF, with o.d. of 1–2 mm), left coronary branch (LCB with o.d. of 0.3–0.5 mm) and right coronary branch (RCB, with o.d. of 0.5–0.8 mm). Helical strips cut from segments of these coronary arteries (20 to 25 mm in length and 0.3 to 1.0 mm in width), were tied at both ends with sutures and arranged isometrically, *in vitro*, under a resting tension of 1.0 g for large arteries (LC, CF) and 0.5 g for small arteries (LCB, RCB), respectively, as described previously (Turlapaty & Altura, 1980). These were incubated in 20 ml muscle chambers containing Krebs Ringer bicarbonate solution (composition, mmol/l: NaCl 118, KCl 4.7, $CaCl_2$ 2.5, KH_2PO_4 1.2, $MgSO_4$ 1.2, glucose 10, and $NaHCO_3$ 25) and maintained at 37°C through which a mixture of O_2 (95%) and CO_2 (5%) was bubbled. The loading tensions were periodically adjusted and maintained throughout the equilibration time. The incubation media were routinely changed every 10 to 15 min as a precaution against interfering metabolites (Altura & Altura, 1970). The tissues were attached to force transducers (Grass model FT.03) connected to a Grass model 7 polygraph, and isometric tensions of the coronary arterial smooth muscle preparations were recorded.

Types of experiment

After the 2 h incubation period, the following experiments were carried out:

(1) Large and small coronary arteries were exposed to single concentrations (EC_{40}–EC_{60}) of 5-HT (10 ng/ml), angiotensin (5 ng/ml) or potassium chloride (20 mM) in the presence of 0, 1.2 or 4.8 mM Mg^{2+}-containing Krebs-Ringer solution to determine the influence of extracellular Mg^{2+}($[Mg^{2+}]_o$) on the fast and slow components of the contractile responses induced by these agonists.

(2) The influence of $[Mg^{2+}]_o$ on the sensitivity (EC_{50}) and maximal contractile tensions developed to 5-HT, angiotensin and potassium were determined. Coronary arteries were exposed to cumulative concentrations of these agonists in the presence of 0, 1.2 or 4.8 mM magnesium. These cumulative dose-responses were expressed as a percentage of control (1.2 mM Mg^{2+}) maximal agonist-induced tensions.

(3) In certain experiments, coronary arteries were exposed to certain specific pharmacological antagonists 30 min before addition of 5-HT, angiotensin or potassium to determine whether these agonist-induced contractile responses are affected by α-adrenoceptor blockade (phentolamine, 0.1 μg/ml), β-adrenoceptor blockade (propranolol, 0.5 μg/ml), 5-HT receptor blockade (methysergide maleate, 0.5 μg/ml), cholinoceptor blockade (atropine sulphate, 0.5 μg/ml), histamine receptor blockade (diphenydramine HCl, 0.5 μg/ml) or a prostaglandin synthetase inhibitor (indomethacin, 1 μg/ml). All of the pharmacological antagonists were used in concentrations that inhibit responses elicited to EC_{60}–EC_{70} concentrations of their respective agonists (Turlapaty & Altura, 1980).

(4) In some additional experiments, coronary arteries were exposed to Ca^{2+}-free medium or verapamil (1×10^{-6} M) for 30 min and then stimulated with EC_{40}–EC_{60} doses of 5-HT, angiotensin or potassium to determine the influence of extracellular Ca^{2+} on these responses.

(5) In some experiments, the influence of $[Mg^{2+}]_o$ on calcium-induced contractile responses was determined. Coronary arteries were equilibrated in normal Krebs-Ringer solution and then exposed for 30 min to a calcium-free Krebs-Ringer and then for an additional 45 min to a Ca^{2+}-free, high potassium solution, as described previously (Altura & Altura, 1974). The latter solution had 118 mM NaCl iso-osmotically replaced with KCl (total K^+ = 123.9 mM). Such tissues contract in response to added calcium (Altura & Altura, 1974). Cumulative calcium chloride dose-response curves were then obtained in different concentrations of $[Mg^{2+}]_o$ (0, 1.2 or 4.8 mM). Calcium-free Krebs-Ringer and high K^+, Ca^{2+}-free Krebs-Ringer contained either 0, 1.2 or 4.8 mM Mg^{2+}. These results were expressed as a percentage of control (1.2 nM Mg^{2+}) maximal Ca^{2+}-induced contractile responses.

Contractile responses were evaluated by threshold

concentration (concentration necessary to produce the first sign of contraction), EC_{50} (concentration of agonist necessary to produce half maximal tension) and maximal contractile tension.

Drugs

The following drugs and chemicals were used: 5-hydroxytryptamine (serotonin) creatinine sulphate (Sigma Chemical Co.), angiotensin II amide (Hypertensin, Ciba Pharmaceutical Co.), phentolamine HCl (Regitine, Ciba Pharmaceutical Co.), (±)-propranolol HCl (Sigma Chemical Co.), methysergide maleate (Sandoz Ltd), atropine sulphate (Mann Research Laboratories), diphenhydramine HCl (Benadryl, Parke Davis and Company), verapamil (Isoptin, Knoll Pharmaceutical Co.), potassium chloride (Fisher Scientific Company), and magnesium sulphate (Fisher Scientific Company). The concentration of each drug or chemical is expressed as final bath concentration.

Statistical analyses

Where appropriate, the means ± s.e.means of the responses were compared for statistical significance by Student's *t* test or paired *t* test and considered significant if $P < 0.05$.

Results

Influence of extracellular magnesium on the contractile responses of canine large and small coronary arteries to single doses of agonists

Typical contractile responses elicited to angiotensin, 5-HT or potassium of large and small coronary arteries in the presence of different concentrations of $[Mg^{2+}]_o$ (1.2, 0 or 4.8 mM) are illustrated in Figure 1. It is evident that while withdrawal of $[Mg^{2+}]_o$ enhanced the contractile responses to all three agonists, elevation of $[Mg^{2+}]_o$ (4.8 mM) attenuated such contractile responses. The relative order of the increase in contractile tension evoked by these agonists in the absence of $[Mg^{2+}]_o$ was 5-HT > KCl > angiotensin (Table 1). No differences between large and small coronary arteries in the influence of $[Mg^{2+}]_o$ on these agonist-induced contractions could be observed (Table 1).

As anticipated, KCl and 5-HT elicited two-phased contractile responses which consisted of a fast com-

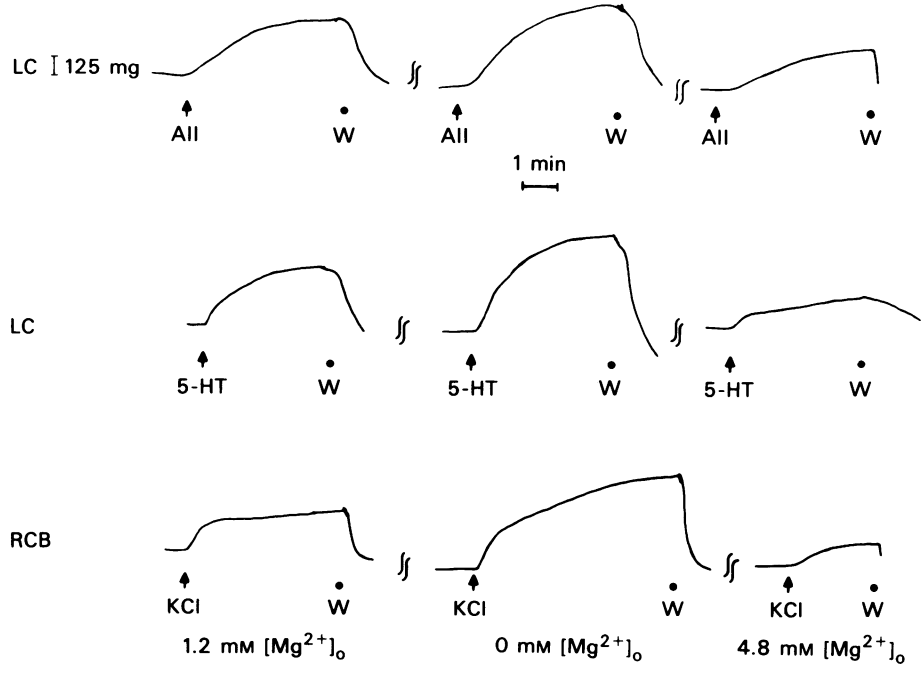

Figure 1 Responses of canine large (left coronary, LC) coronary arteries to stimulation with 5-hydroxytryptamine (5-HT, 10 ng/ml), and angiotensin II amide (AII, 5 ng/ml) and of the small (right coronary branch RCB) coronary artery to potassium chloride (KCl, 20 mM) in Krebs-Ringer bicarbonate containing 1.2, 0 and 4.8 mM Mg^{2+}. W indicates the point at which strips were washed and relaxed in normal Krebs-Ringer bicarbonate solution.

Table 1 Influence of extracellular magnesium on contractile responses* induced by single doses of angiotensin, 5-hydroxytryptamine (5-HT) and potassium chloride of canine large (circumflex, CF; left coronary LC) and small (left coronary branch, LCB; right coronary branch, RCB) coronary arteries

Agonist/Magnesium (mM)	*CF*	*LC*	*LCB*	*RCB*
Angiotensin				
0	+57±6.1†	+50±5.0	+40±4.3	+32±1.8
4.8	−41±5.3‡	−22±4.5	−34±2.1	−32±3.4
5-HT				
0	+80±7.9	+90±9.1	+84±5.3	+100±8.5
4.8	−54±6.0	−40±2.5	−40±3.6	−45±2.4
Potassium				
0	+64±4.6	+71±6.8	+72±3.7	+61±5.0
4.8	−34±2.9	−50±4.2	−57±6.3	−53±7.3

*Contractile responses are expressed as percentage change from contractile tensions obtained in 1.2 mM Mg^{2+}-containing Krebs-Ringer (NKR) and these values are expressed as means±s.e. of 8 different tissues; †plus sign indicates increase; ‡minus sign indicates decrease.

ponent and a subsequent slow component; angiotensin-induced contraction consisted of only a single slow component. Withdrawal of $[Mg^{2+}]_o$ increased both the fast and slow components of the agonists. Elevation in $[Mg^{2+}]_o$ either prevented (KCl) or reduced (5-HT) the fast components and markedly attenuated the slow components (Figure 1).

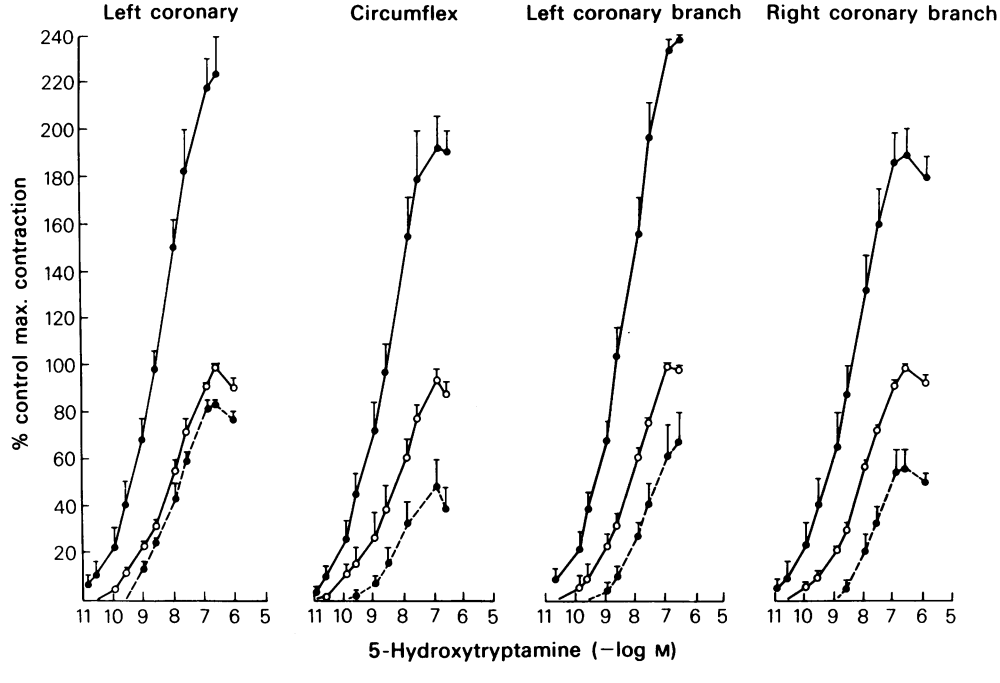

Figure 2 Influence of extracellular magnesium (0 mM ●——●; 1.2 mM ○——○; 4.8 mM ●---●) on concentration-contractile effect curves of canine large (circumflex, left coronary) and small (left coronary branch, right coronary branch) coronary arteries to 5-hydroxytryptamine (5-HT).

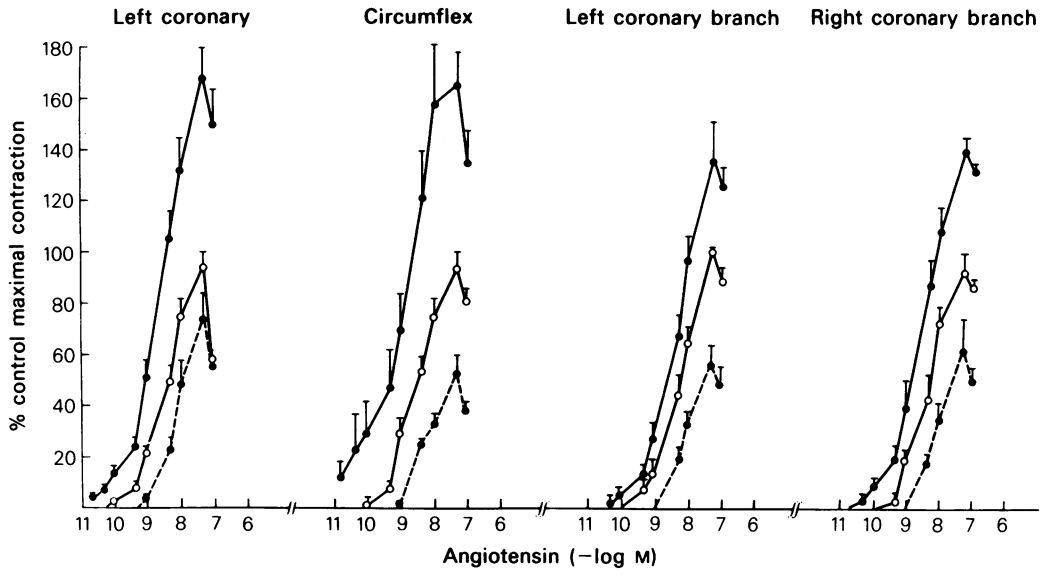

Figure 3 Influence of extracellular magnesium (0 mM ●——●; 1.2 mM O——O; 4.8 mM ●---●) on concentration-contractile effect curves of large (circumflex, left coronary) and small (left coronary branch, right coronary branch) coronary arteries to angiotensin.

Influence of extracellular magnesium on the sensitivity (threshold concentration, EC_{50}) and contractility (maximal contractile tensions) of large and small coronary arteries to agonists

Figures 2 and 3 demonstrate that the withdrawal of magnesium from the medium not only shifts the cumulative concentration-effect curves for 5-HT and angiotensin to the left in a parallel fashion, but also results in significant potentiation of these drug-induced maximal contractions. With potassium as the agonist, only a parallel shift to the left of the dose-response curves (without any effect on maximal contractions) was observed in the absence of $[Mg^{2+}]_o$ (Figure 4). It is also clear from these figures that elevation in $[Mg^{2+}]_o$ decreased the sensitivity of all three agonists (as indicated by parallel shifts to the right of the concentration-effect curves). However, only maximal contractile tensions produced by 5-HT and angiotensin were inhibited significantly in the presence of high magnesium (Figures 2, 3).

Tables 2, 3 and 4 summarize the threshold concentrations, EC_{50}s and maximal contractile tensions of 5-HT, angiotensin and potassium. It is clear from these data that 5-HT and angiotensin require relatively lower threshold concentrations to elicit con-

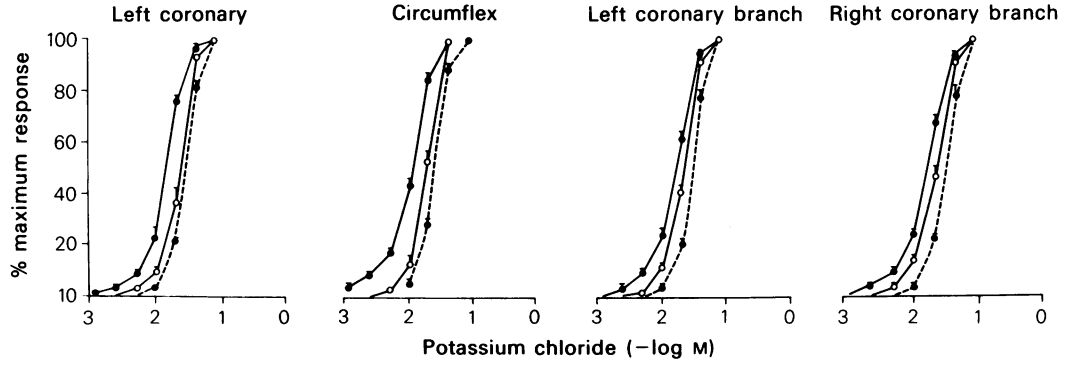

Figure 4 Influence of extracellular magnesium (symbols as in Figures 2 and 3) on concentration-contractile effect curves of canine large (circumflex. left coronary) and small (left coronary branch, right coronary branch) coronary arteries to potassium chloride.

Table 2 Effect of extracellular magnesium on the threshold concentrations of agonists to elicit contractile responses of canine large (circumflex, CF; left coronary, LC) and small (left coronary branch, LCB; right coronary branch, RCB) coronary arteries*

Agonist/Artery	*Magnesium* (mM) 1.2	0	4.8
Angiotensin	$\times 10^{-9}$ M	$\times 10^{-9}$ M	$\times 10^{-9}$ M
CF	0.31 ± 0.07	0.017 ± 0.009*	5.20 ± 1.60**
LC	0.36 ± 0.08	0.018 ± 0.009**	4.40 ± 1.30**
LCB	0.76 ± 0.07	0.04 ± 0.01**	5.58 ± 0.68†
RCB	0.79 ± 0.17	0.04 ± 0.02*	4.90 ± 0.10
5-HT	$\times 10^{-9}$ M	$\times 10^{-9}$ M	$\times 10^{-9}$ M
CF	0.11 ± 0.04	0.01 ± 0.002**	0.78 ± 0.35*
LC	0.15 ± 0.03	0.018 ± 0.004†	1.10 ± 0.41†
LCB	0.69 ± 0.12	0.05 ± 0.02*	4.70 ± 2.62*
RCB	0.44 ± 0.15	0.047 ± 0.01**	6.0 ± 2.50**
Potassium	$\times 10^{-3}$ M	$\times 10^{-3}$ M	$\times 10^{-3}$ M
CF	6.90 ± 1.0	1.38 ± 0.14†	12.80 ± 1.80††
LC	4.72 ± 0.70	1.94 ± 0.42†	12.70 ± 1.80†
LCB	7.70 ± 0.88	2.90 ± 0.55†	16.70 ± 3.30**
RCB	6.38 ± 0.94	2.36 ± 0.38†	12.50 ± 1.50†

*Values are means ± s.e. of 8 different tissues. Significantly different from values obtained in 1.2 mM Mg^{2+} with P values of * < 0.05; ** < 0.025; † < 0.001; †† < 0.005.

tractions in small (LCB, RCB) than in large (CF, LC) coronary arteries suspended in Krebs-Ringer containing 0 mM Mg^{2+}. In contrast, in the presence of high $[Mg^{2+}]_o$, 5-HT, but not angiotensin, required relatively higher threshold concentrations to elicit contractions in small than in large coronary arteries. The relative increase and decrease in threshold concentrations for potassium stimulation, in 0 vs. 1.2 and

Table 3 Effect of extracellular magnesium on the EC_{50} of vasoactive agonists in canine large (circumflex, CF; left coronary, LC) and small (left coronary branch, LCB; right coronary branch, RCB) coronary arteries*

Agonist/Artery	*Magnesium* (mM) 1.2	0	4.8
Angiotensin	$\times 10^{-8}$ M	$\times 10^{-8}$ M	$\times 10^{-8}$ M
CF	0.47 ± 0.09	0.23 ± 0.09*	1.89 ± 0.67**
LC	0.53 ± 0.12	0.33 ± 0.08†	0.90 ± 0.21††
LCB	0.71 ± 0.18	0.45 ± 0.10††	0.99 ± 0.12††
RCB	0.56 ± 0.13	0.36 ± 0.11†	1.01 ± 0.22†
5-HT	$\times 10^{-8}$ M	$\times 10^{-8}$ M	$\times 10^{-8}$ M
CF	0.92 ± 0.10	0.39 ± 0.13††	1.78 ± 0.18††
LC	0.90 ± 0.09	0.43 ± 0.10††	1.53 ± 0.26††
LCB	0.72 ± 0.18	0.49 ± 0.14†	1.70 ± 0.29*
RCB	1.14 ± 0.21	0.53 ± 0.19†	2.08 ± 0.29†
Potassium	$\times 10^{-2}$ M	$\times 10^{-2}$ M	$\times 10^{-2}$ M
CF	1.90 ± 0.13	1.10 ± 0.07**	2.60 ± 0.14**
LC	2.06 ± 0.17	1.42 ± 0.10**	2.71 ± 0.14**
LCB	2.24 ± 0.11	1.69 ± 0.13**	2.94 ± 0.17**
RCB	2.04 ± 0.13	1.55 ± 0.09**	2.86 ± 0.16**

*Values are means ± s.e. of 8 different tissues. Significantly different from values obtained in 1.2 mM Mg^{2+} with P values of: * < 0.025; ** < 0.001, † < 0.005; †† < 0.01.

Table 4 Effect of extracellular magnesium on the maximal contractile tensions developed to vasoactive agonists in canine large (circumflex, CF; left coronary, LC) and small (left coronary branch, LCB; right coronary branch, RCB) coronary arteries*

Agonist/Artery	*Magnesium* (mM)		
	1.2	0	4.8
Angiotensin	mg	mg	mg
CF	208± 78	304± 53*	106± 53**
LC	283± 48	446± 61**	194± 24†
LCB	222± 54	303± 46†	157± 55*
RCB	255± 49	360± 63*	150± 29†
5-HT			
CF	198± 51	371± 78††	113± 48**
LC	456± 98	962±150*	247± 65††
LCB	234± 43	544± 64*	144± 38†
RCB	189± 48	355± 90*	133± 44†
Potassium			
CF	789±150	828±117	836±116
LC	894±150	964±196	930±200
LCB	640±122	733±136	666±128
ICB	592± 98	659±110	608±102

*Values are means ± s.e. of 8 different tissues. Significantly different from values obtained in 1.2 mM Mg^{2+} with P values of: *<0.001, **<0.005, †<0.05, ††<0.01.

0 vs. 4.8 mM $[Mg^{2+}]_o$, were not different between large and small coronary arteries. The increase in maximal contractile tensions seen upon withdrawal of $[Mg^{2+}]_o$ was greater for 5-HT (90 to 130%) than for angiotensin (40 to 60%) in both large and small coronary arteries (Table 4).

Contractile responses of large and small coronary arteries to agonists in calcium-free Krebs-Ringer or in the presence of verapamil

Contractile responses elicited to 5-HT, angiotensin or potassium were completely inhibited when both large and small coronary arteries were incubated in calcium-free Krebs Ringer (Figure 5). On the other hand, verapamil (1×10^{-6} M), an inhibitor of calcium influx into vascular smooth muscle, completely prevented only potassium-induced increases in tension. Although verapamil depressed 5-HT- and angiotensin-induced contractions, it did not completely prevent them (Figure 6). It should be noted that the contractile responses developed to all three agonists could be restored to control level after washing away the verapamil.

Influence of magnesium on calcium-induced contractions of coronary arteries

Figure 7 demonstrates that the absence of $[Mg^{2+}]_o$ in the medium shifted the cumulative calcium dose-response curves leftward in a parallel fashion at the same time as a significant increase in the sensitivity and maximal tensions occurred. On the other hand, elevation in $[Mg^{2+}]_o$ to 4.8 mM significantly decreased both the sensitivity and maximal tensions. These influences of $[Mg^{2+}]_o$ were similar in both large and small coronary arteries. It is also interesting to note that coronary arteries developed greater contractile tensions to calcium when compared to any other agonist studied (Table 5 vs. Table 4).

Influence of pharmacological antagonists on 5-hydroxytryptamine, angiotensin and potassium-induced contractions of coronary arteries

Adrenoceptor (phentolamine, propranolol), 5-HT receptor (methysergide), cholinoceptor (atropine) and histamine receptor (diphenhydramine) antagonists and a prostaglandin synthetase inhibitor (indomethacin) could not modify the angiotensin or potassium-induced contractile responses observed in different $[Mg^{2+}]_o$. 5-HT-induced contractions were selectively inhibited by methysergide in all $[Mg^{2+}]_o$; no other antagonists studied attenuated 5-HT-induced responses.

Discussion

In a previous study (Turlapaty & Altura, 1980), as

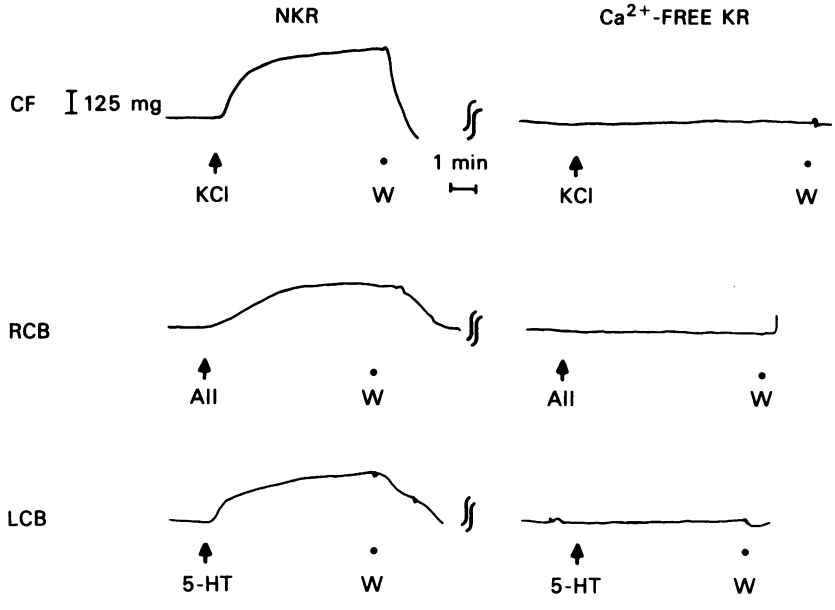

Figure 5 Responses of canine large (circumflex, CF) and small (right coronary branch, RCB; left coronary branch, LCB) coronary arteries to stimulation with potassium chloride (KCl, 20 mM), angiotensin II (AII, 5 ng/ml) and 5-hydroxytryptamine (5-HT, 10 ng/ml) in normal Krebs-Ringer bicarbonate solution (NKR) and calcium-free Krebs-Ringer solution. W indicates the point at which tissues were washed and relaxed in NKR.

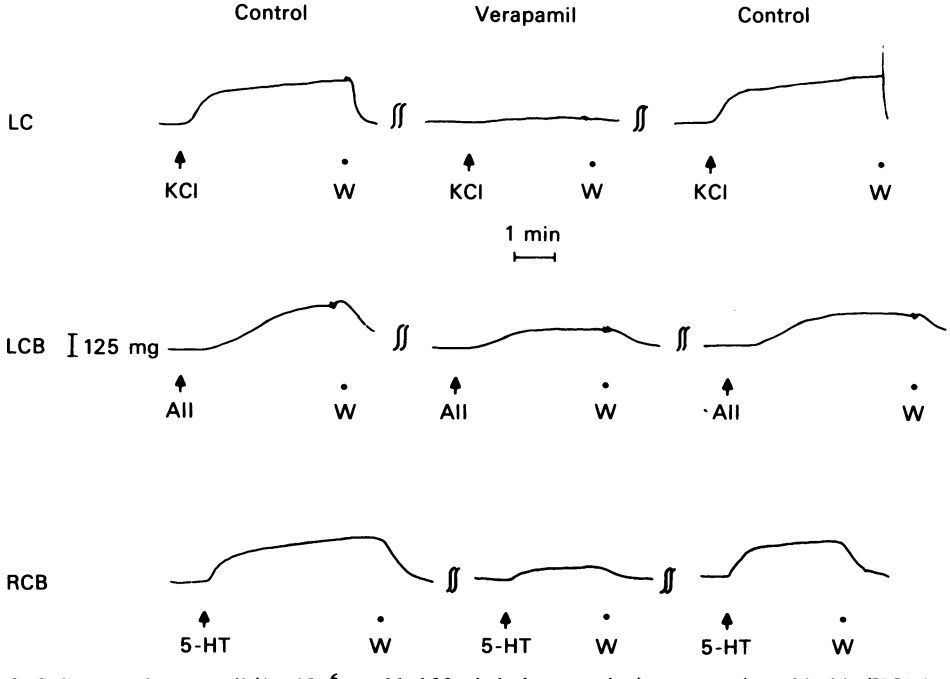

Figure 6 Influence of verapamil (1×10^{-6} M, added 30 min before agonists), on potassium chloride (KCl, 20 mM), angiotensin II (AII, 5 ng/ml) and 5-hydroxytryptamine (5-HT, 10 ng/ml)-induced contractile responses of canine large (left coronary, LC) and small (left coronary branch, LCB; right coronary branch, RCB) coronary arteries in Krebs-Ringer bicarbonate solution. W indicates the point at which tissues were washed and relaxed in NKR.

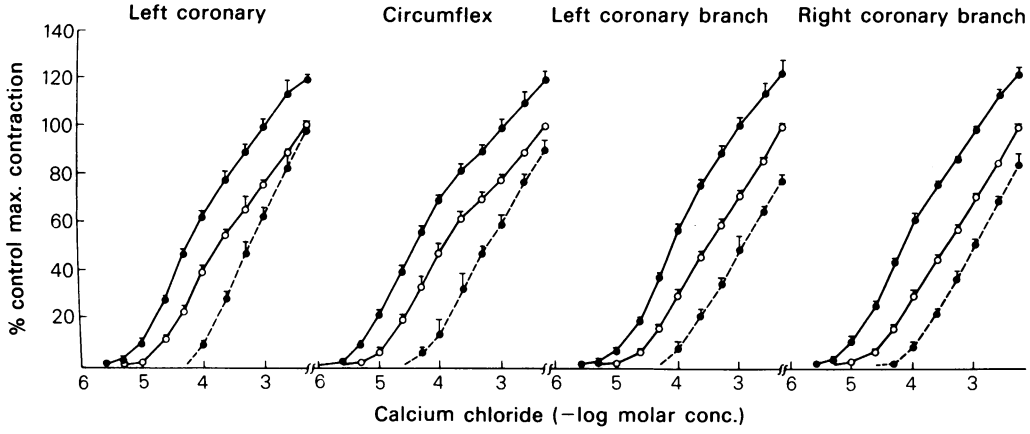

Figure 7 Influence of extracellular magnesium (symbols as in Figures 2 and 3) on calcium-induced concentration-contractile effect curves (in Ca^{2+}-free, high potassium solution) of canine large (circumflex, left coronary) and small (left coronary branch, right coronary branch) coronary arteries.

well as in the present study, we have demonstrated that withdrawal of $[Mg^{2+}]_o$ enhances, while an elevation in $[Mg^{2+}]_o$ depresses, contractile responses of canine large and small coronary arteries elicited to both specific (receptor-mediated) and non-specific (KCl) agonists. This finding prompted us, in the present study, to investigate whether or not extracellular magnesium ions exert these effects by virtue of an influence on: (1) specific agonist-receptor interactions; (2) cellular calcium pools that are differentially utilized by different agonists, and/or (3) smooth muscle cell membrane permeability to Ca^{2+}.

The results obtained in the present study, using cumulative dose-response curves, demonstrate that Mg^{2+} differentially affects contractions induced by receptor-mediated agonists (i.e., 5-HT, angiotensin) and contractions induced by depolarization (potassium). The leftward and rightward parallel shifts of the potassium dose-response curves, without a depression of the maximal contractile tension, in the

Table 5 Influence of magnesium on calcium-induced contractile responses of canine large (circumflex, CF; left coronary, LC) and small (left coronary branch, LCB; right coronary branch, RCB) coronary arteries*

Artery/Magnesium (mM)	*Threshold conc.*	EC_{50}	*Maximum tension*
CF	$\times 10^{-5}$ M	$\times 10^{-4}$ M	mg
1.2	1.42±0.34	1.58±0.39	2775±230
0	0.30±0.02*	0.71±0.14**	3175±200*
4.8	7.50±1.40*	4.50±1.07**	2550±125**
LC	$\times 10^{-5}$ M	$\times 10^{-4}$ M	
1.2	1.53±0.32	2.16±0.41	2567±238
0	0.50±0.07*	0.94±0.11†	2970±230†
4.8	10.0 ±1.0 *	5.58±0.16†	2170± 78††
LCB	$\times 10^{-5}$ M	$\times 10^{-4}$ M	
1.2	3.30±0.61	3.51±0.61	1046± 95
0	0.90±0.30*	1.39±0.14*	1242± 97*
4.8	15.0 ±4.20†	6.50±1.19††	806±196†
RCB	$\times 10^{-5}$ M	$\times 10^{-4}$ M	
1.2	2.50±0.40	3.58±0.46	1387±120
0	0.56±0.06*	1.16±0.12*	1660±120*
4.8	8.0 ±1.20**	6.70±0.52††	1080±260*

*Values are means ± s.e. of 14 different tissues. Significantly different from values obtained in 1.2 mM Mg^{2+} with P values: *<0.001; **<0.05; †<0.005; ††<0.01.

absence and presence of high magnesium, respectively (Figure 4), suggests that Mg^{2+} affects potassium responses in a competitive manner. In contrast, contractile responses to 5-HT and angiotensin revealed that $[Mg^{2+}]_o$ exerts its influence in both a competitive and a non-competitive manner as shown by changes in both sensitivity and contractility (maximal tensions) (Figures 2, 3). These latter data could be interpreted to indicate that Mg^{2+} might have a greater influence on membrane channels involved in Ca^{2+} transport activated by receptor-mediated agonists than those activated by depolarization. It has been proposed that Ca^{2+} enters vascular smooth muscle cells through membrane potential-sensitive channels activated by depolarization and receptor-operated channels by specific agonists (Bolton, 1979).

Several previous studies indicate that magnesium ions can either increase or decrease the affinity of various agonists for their specific receptors in different types of vascular smooth muscle (Somlyo *et al.*, 1966; Altura & Altura, 1971; Altura, 1975; Turlapaty *et al.*, 1975; Goldstein & Zsotér, 1978). In the present study, we demonstrate that sensitivity of coronary arteries to 5-HT and angiotensin is increased by withdrawal of $[Mg^{2+}]_o$ and decreased by elevated levels of $[Mg^{2+}]_o$. These findings suggest, but do not prove, that magnesium ions might interfere with the binding of these two agonists for their receptors in coronary arterial smooth muscle. Binding of various agonists to their receptors has been shown previously to be influenced by magnesium ions in the medium (Takagi, Takayanagi & Liao, 1972).

Since contractile responses elicited to 5-HT, angiotensin and potassium in canine coronary arteries are abolished in Ca^{2+}-free Krebs Ringer (Figure 5), it is tempting to conclude that these blood vessels are dependent entirely on $[Ca^{2+}]_o$ for activation. However, this may not be the case as is shown by some recent findings: (1) 5-HT and angiotensin could still elicit contractions in the presence of the specific extracellular calcium influx blocker, verapamil (Figure 6), when used in a concentration (1×10^{-6} M) that can inhibit by more than 90% calcium-induced contractions of K^+-depolarized canine coronary arteries (Turlapaty and Altura, unpublished findings); and (2) coronary arteries after prolonged incubation (60 to 90 min) in Ca^{2+}-free medium can still exhibit potent contractions when Mg^{2+} is acutely removed from the medium (Turlapaty and Altura, unpublished findings). These findings suggest that coronary arteries are, therefore, not dependent entirely on extracellular calcium. Furthermore, it is also possible that coronary arteries might contain a bound calcium pool that is resistant to calcium depletion but regulated by extracellular magnesium (Altura & Altura, 1974); this functional membrane calcium pool could be accessible to agonists acting through specific receptors, such as 5-HT and angiotensin, but not to agonists acting through membrane depolarization (e.g., potassium).

The present finding that withdrawal of $[Mg^{2+}]_o$ potentiated significantly maximal contractile tensions elicited by 5-HT and angiotensin suggests that, in the absence of magnesium, a release of bound cellular calcium associated with either receptors on the membrane or at some intracellular sites might occur and thus become available for contractile proteins. Support for this hypothesis can be gleaned from previous reports which indicate that: (1) binding of calcium associated with angiotensin receptors in the microsomal fraction of aortic smooth muscle is increased by magnesium (Devynck, Pernollet, Meyer, Fermandrian & Fromageot, 1973); and (2) intracellular lanthanum-resistant calcium content is increased in the absence of $[Mg^{2+}]_o$ in at least two types of vascular smooth muscle (Turlapaty & Altura, 1978; Goldstein & Zsotér, 1978). The decrease in these agonist-induced maximal contractile tensions in the presence of elevated $[Mg^{2+}]_o$ could be due to increased binding and/or sequestration of calcium ions into intracellular storage sites. Another contributory mechanism could be due to activation of Mg^{2+}-dependent Ca-ATPase, located on the membrane that presumably extrudes calcium (Popescu, 1977). It is less likely that high magnesium exerts its influence by interference with functioning of contractile proteins since glycerinated smooth muscles have been shown to contract maximally to 20 mM magnesium even in the absence of calcium (Nakahata, 1979).

Based on the findings in the present study that (1) Ca^{2+}-induced contractions of K^+-depolarized coronary arteries are increased in the absence of $[Mg^{2+}]_o$, (2) contractile responses induced by 5-HT, angiotensin and potassium are inhibited in Ca^{2+}-free Krebs-Ringer, and (3) Ca^{2+}-induced contractions as well as agonist-induced contractions are decreased by elevated magnesium, it is reasonable to assume that the enhancement of vasoactive agent-induced coronary vasospasm in a Mg^{2+}-deficient state is due (at least in part) to an increased influx of $[Ca^{2+}]_o$.

References

ALTURA, B.M. (1966). Differential action of polypeptides and other drugs on coronary in flow vessels. *Am. Heart. J.*, **72**, 709–711.

ALTURA, B.M. (1975). Magnesium-neurohypophyseal hormone interactions in contraction of vascular smooth muscle. *Am. J. Physiol.*, **228**, 1615–1620.

ALTURA, B.M. (1979). Sudden-death ischemic heart disease and dietary magnesium intake: Is the target site coronary vascular smooth muscle? *Med. Hypotheses*, **5**, 843–848.

ALTURA, B.M. & ALTURA, B.T. (1970). Differential effects of substrate depletion on drug-induced contractions of rabbit aorta. *Am. J. Physiol.*, **219**, 1698–1705.

ALTURA, B.M. & ALTURA, B.T. (1971). Influence of magnesium on drug-induced contractions and ion content in rabbit aorta. *Am. J. Physiol.*, **220**, 938–944.

ALTURA, B.M. & ALTURA, B.T. (1974). Magnesium and contraction of arterial smooth muscle. *Microvasc. Res.*, **7**, 145–155.

ALTURA, B.M. & ALTURA, B.T. (1976). Magnesium withdrawal and contraction of arterial smooth muscle. Effects of EDTA, EGTA and divalent cations. *Proc. Soc. exp. Biol. Med.*, **151**, 752–755.

ALTURA, B.M. & ALTURA, B.T. (1978). Magnesium and vascular tone and reactivity. *Blood Vessels*, **15**, 5–16.

ALTURA, B.M. & ALTURA, B.T. (1981). Magnesium ions and contraction of vascular smooth muscle: Relationship to some vascular diseases. *Fedn Proc.*, **40**, 2672–2679.

ALTURA, B.M., ALTURA, B.T. & WALDEMAR, Y. (1976). Prostaglandin-induced relaxations and contractions of arterial smooth muscle. Effects of magnesium ions. *Artery*, **2**, 326–336.

ALTURA, B.T. (1980). Type-A behaviour and coronary vasospasm: A possible role of hypomagnesemia. *Med. Hypotheses*, **6**, 735–738.

ALTURA, B.T. & ALTURA, B.M. (1980). Withdrawal of magnesium causes vasospasm while elevated magnesium produces relaxation of tone in cerebral arteries. *Neuroscience Lett.*, **20**, 323–327.

BOLTON, T.B. (1979). Mechanism of action of transmitters and other substances on smooth muscle. *Physiol. Rev.*, **59**, 606–718.

BURCH, G.E., GILES, T.D. (1977). The importance of magnesium deficiency in cardiovascular disease. *Am. Heart J.*, **94**, 649–657.

DEVYNCK, M-A., PERNOLLET, M-G., MEYER, P., FERMANDRIAN, S., FROMAGEOT, P. (1973). Angiotensin receptors in smooth muscle cell membranes. *Nature, New Biol.*, **245**, 55–58.

GOLDSTEIN, S. & ZSOTÉR, T.T. (1978). The effect of magnesium on the response of smooth muscle to 5-hydroxytryptamine. *Br. J. Pharmac.*, **62**, 507–514.

HANSEN, J.F. & SANDØE, E. (1978). Treatment of Prinzmetal's angina due to coronary artery spasm using verapamil: a report of three cases. *Eur. J. Cardiol.*, **7**, 327–335.

LEVENE, D.L. & FREEMAN, M.R. (1976). α-adrenoceptor-mediated coronary artery spasm. *J. Am. med. Ass.*, **236**, 1018–1022.

NAKAHATA, N. (1979). Magnesium dependent contraction of glycerinated smooth muscle. *Pflugers Arch.*, **382**, 133–136.

POPESCU, L.M. (1977). Cytochemical study of the intracellular calcium distribution in smooth muscle. Surface microvesicles and cellular calcium homeostasis. In *Excitation-Contraction Coupling in Smooth Muscle*. ed. Casteels, R., Godfraind, T. & Rüegg, J.D. pp. 13–23. Amsterdam: Elsevier/North-Holland.

SEELIG, M.S., HEGGTVEIT, H.A. (1974). Magnesium interrelationships in ischemic heart disease: a review. *Am. J. Clin. Nutr.*, **27**, 59–79.

SOMLYO, A.V. WOO, C.Y., & SOMLYO, A.P. (1966). Effect of magnesium on posterior pituitary hormone action of vascular smooth muscle. *Am. J. Physiol.*, **210**, 705–714.

TAKAGI, K., TAKAYANAGI, I. & LIAO, C.S. (1972). The effect of calcium and magnesium ions on drug-receptor interactions. *Eur. J. Pharmac.*, **19**, 330–342.

TURLAPATY, P.D.M.V. & ALTURA, B.M. (1978). Extracellular magnesium ions control calcium exchange and content of vascular smooth muscle. *Eur. J. Pharmac.*, **52**, 421–423.

TURLAPATY, P.D.M.V. & ALTURA, B.M. (1980). Magnesium deficiency produces spasms of coronary arteries: Relationship to etiology of sudden death ischemic heart disease. *Science*, **208**, 198–200.

TURLAPATY, P.D.M.V., CARRIER, O. Jr. & JUREVICS, H. (1975). Effect of magnesium on isoproterenol induced alpha and beta receptor responses of vascular smooth muscle. *J. Pharmac. exp. Ther.*, **192**, 372–380.

WEISS, G.B. (1978). Calcium and contractility in vascular smooth muscle. In *Advances in General and Cellular Pharmacology*. Vol. II. ed. Narahashi, T. & Bianchi, C.P. pp. 71–154. New York: Plenum.

YASUE, H., TOUYAMA, M., KATO, H., TOMAKA, S. & AKIYAMA, F. (1976). Prinzmetal's variant form of angina as a manifestation of *alpha*-adrenergic receptor-mediated coronary artery spasm: Documentation by coronary arteriography. *Am. Heart J.*, **91**, 148–155.

(Received March 18, 1982.)