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Magnesium Deficiency Produces Spasms of Coronary Arteries: Relationship to Etiology of Sudden Death Ischemic Heart Disease

Abstract. *Isolated coronary arteries from dogs were incubated in Krebs-Ringer bicarbonate solution and exposed to normal, high, and low concentrations of magnesium in the medium. Sudden withdrawal of magnesium from the medium increased whereas high concentrations of magnesium decreased the basal tension of the arteries. The absence of magnesium in the medium significantly potentiated the contractile responses of both small and large coronary arteries to norepinephrine, acetylcholine, serotonin, angiotensin, and potassium. These data support the hypothesis that magnesium deficiency, associated with sudden death ischemic heart disease, produces coronary arterial spasm.*

Several recent investigations point to a causal relation between decreased magnesium ion (Mg^{2+}) content of cardiac muscle and coronary arteries and mortality from (nonocclusive) sudden-death ischemic heart disease (SDIHD), the incidence of which is highest in geographic areas with soft drinking water or magnesium-poor soil (*1-5*). Of the minerals that are deficient in soft water, magnesium is the only element that has been found to be lowered in the cardiac muscle of SDIHD victims (*1-3, 5*). Acute hypomagnesemia in animals and man is often associated with increases in blood pressure and in peripheral vascular resistance in several regional circulatory systems (*6, 7*). Artificial lowering of the Mg^{2+} content of isolated (noncardiac) vessels from rats, rabbits, piglets, and dogs induces rapid, potent contractile responses (*8-10*). Acute hypermagnesemia inhibits the spontaneous tone of arteries and veins (*8-10*). Thus, there is evidence that extracellular Mg^{2+} plays a critical role in the regulation of vasomotor tone.

A positive correlation between mortality rates from SDIHD and the estimated high ratio of calcium to magnesium in myocardial tissue has been demonstrated (*3, 4*). Although many tissues of the body have been shown to be resistant to Mg^{2+} depletion, heart tissue and coronary vessels have a significantly reduced Mg^{2+} content in cases of SDIHD (*1, 3, 5*). It was therefore suggested recently that SDIHD mortality could be due to the direct effects of a hypomagnesemic state on coronary vascular tone (*11*). The hypomagnesemia could produce progressive vasoconstriction, vasospasm, and ischemia, which, given time, would lead to SDIHD. To investigate the possibility that vasospasm can be produced by Mg^{2+} deficiency, we determined the influence of sudden magnesium withdrawal and hypermagnesemia (4.8 m*M*) on vascular tone and vasoactive drug-induced responses in isolated coronary arteries of the dog.

Mongrel dogs of either sex weighing 10 to 20 kg were anesthetized with pentobarbital sodium (30 mg/kg). The hearts were removed immediately and coronary arteries were isolated. Because of possible segmental differences in coronary arterial reactivity (*12*), we examined four different coronary inflow vessels: left coronary [outer diameter (O.D.), 1 to 2 mm], circumflex (O.D. 1 to 2 mm), left coronary branch (O.D. < 1 mm), and right coronary branch (O.D. < 1 mm). Helical strips, cut from segments of these coronary arteries, were 20 to 25 mm long by 0.5 to 1.0 mm wide. These were suspended isometrically under 1 g of tension (circumflex, left coronary arteries) or 0.5 g of tension (left and right coronary branch arteries) and incubated in 20-ml muscle chambers containing Krebs-Ringer bicarbonate solution (composition in millimoles per liter: NaCl, 118; KCl, 4.7; $CaCl_2$, 2.5; KH_2PO_4, 1.2; $MgSO_4$, 1.2; glucose, 10; and $NaHCO_3$, 25) at 37°C through which a mixture of O_2 (95 percent) and CO_2 (5 percent) was bubbled. Force of contraction was measured with Grass FT-03 force-displacement transducers and recorded on a Grass model 7 polygraph. Two hours after the preparations were incubated, under tension, the effects of extracellular Mg^{2+} concentration ($[Mg^{2+}]_o$) and vasoactive drugs were examined. The arteries were sequentially exposed to normal (1.2 m*M*), low (0 m*M*), and high (4.8 m*M*) concentrations of magnesium.

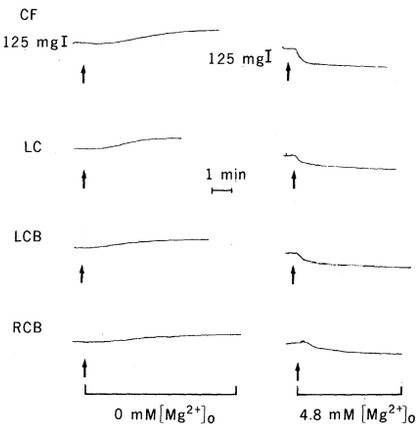

Fig. 1. Influence of extracellular Mg^{2+} on basal tension of canine circumflex (*CF*), left coronary (*LC*), left coronary branch (*LCB*), and right coronary branch (*RCB*) arterial strips. The left panel shows results obtained in a Mg^{2+}-free environment; the right panel indicates results obtained in 4.8 m*M* $[Mg^{2+}]_o$. The vertical bar represents tension (125 mg); time marker, 1 minute. Arrows indicate points at which the normal Krebs-Ringer medium containing 1.2 m*M* $[Mg^{2+}]_o$ was switched to the experimental concentration of $[Mg^{2+}]_o$.

Sudden withdrawal of extracellular Mg^{2+} resulted in rapid, increased tension development in all coronary vessels tested (Fig. 1 and Table 1). In contrast, a sudden increase in extracellular Mg^{2+} (4.8 m*M*) resulted in rapid relaxation of basal tension in all coronary arteries (Fig. 1 and Table 1). Thus, extracellular Mg^{2+} appears to be able directly to alter coronary arterial baseline tension or tone. A similar influence of $[Mg^{2+}]_o$ on vasomotor tone was previously demonstrated in isolated rat and piglet arteries, rabbit aortas, and rat arterioles and portal veins (*8-10*). These effects of $[Mg^{2+}]_o$ cannot be attributed to osmolarity differences, inhibition of Na^+- and K^+-dependent adenosinetriphosphatase activity, or release (or inhibition of release) of endogenous neurohumoral agents from the arterial wall (*7, 8, 10, 13, 14*).

In addition to increasing tone, the withdrawal of Mg^{2+} potentiated the constrictor actions of vasoactive substances such as angiotensin, serotonin, norepinephrine, acetylcholine, and potassium in all coronary arteries (see Fig. 2, Table 2). The order of increase in tension to the vasoactive agents was acetylcholine > norepinephrine > serotonin > K^+ > angiotensin in all types of coronary vessels studied. These enhanced responses,

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in the absence of extracellular Mg^{2+}, were much greater in magnitude than those observed previously in other blood vessels of rats, rabbits, and dogs exposed to Mg^{2+}-free medium (*8, 13, 15*). In contrast, elevated magnesium (4.8 m*M*) decreased the contractile tensions developed in response to these vasoactive agents compared to tensions obtained in 1.2 m*M* or 0 m*M* Mg^{2+} (Fig. 2 and Table 2). All the agents tested are known to be circulating vasoconstrictor substances in and around the coronary vasculature. Thus, the absence of magnesium appears to exert a greater influence on the effects of vasoconstrictors on coronary arteries than on other blood vessels.

It has been suggested that the vascular effects of reduction or elevation in $[Mg^{2+}]_o$ are reflections of this divalent cation's influence on calcium permeability, binding and translocation, as well as membrane stability (*7–10, 13–16*). Recent, direct studies in which radioactive calcium was used revealed that extracellular Mg^{2+} significantly influenced Ca^{2+} content, uptake, and distribution in both arterial and venous smooth muscles (*7, 13, 15, 17*). Lowering of Mg^{2+} in the medium increases total exchangeable and intracellular calcium fractions in blood vessels (*17*). Furthermore, there is evidence that in a hypomagnesemic state, Ca^{2+}, but not Na^+ and K^+, are selectively accumulated by rat and rabbit blood vessels (*7, 13*). Collectively, these findings indicate that when Mg^{2+} is lowered, Ca^{2+} influx is increased and thereby causes contraction. The increased

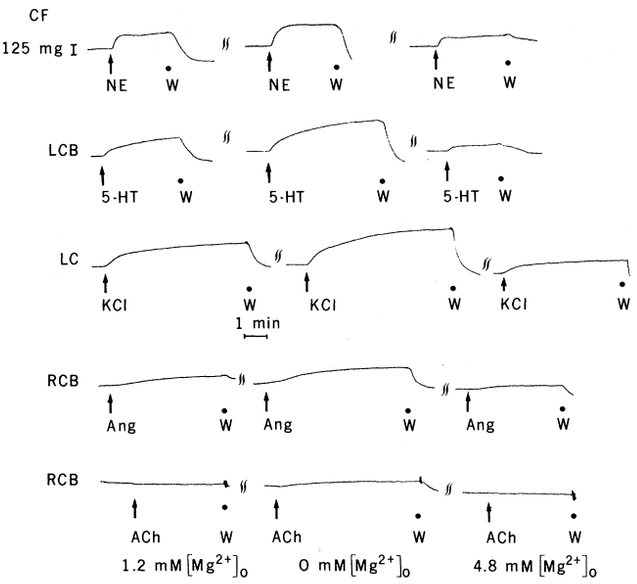

Fig. 2. Responses of canine circumflex (*CF*), left coronary branch (*LCB*), left coronary (*LC*), and right coronary branch (*RCB*) arterial strips to stimulation with norepinephrine bitartrate (*NE*, 50 ng/ml), serotonin creatinine sulfate (*5-HT*, 10 ng/ml), potassium chloride (*KCl*, 20 m*M*), angiotensin II amide (*Ang*, 5 ng/ml), and acetylcholine chloride (*ACh*, 0.4 mg/ml) in Krebs-Ringer bicarbonate containing 1.2, 0, and 4.8 m*M* Mg^{2+}. The *W* indicates the point at which strips were washed and relaxed in normal Krebs-Ringer bicarbonate solution.

Table 1. Influence of extracellular Mg^{2+} on the basal tone of canine circumflex (CF), left coronary (LC), left coronary branch (LCB), and right coronary branch (RCB) arteries. The data are presented as means ± standard error of the means. The number of animals in each group is given in parentheses. All tissues were first incubated in Krebs-Ringer bicarbonate solution containing 1.2 m*M* Mg^{2+}. All the values are significantly different from tensions in 1.2 m*M* Mg^{2+} ($P < .001$).

Mg^{2+} (m*M*)	Tension (mg)			
	CF	LC	LCB	RCB
0	198.0 ± 22.0 (25)	163.0 ± 20.0 (25)	132.5 ± 17.0 (23)	110.0 ± 13.5 (20)
4.8	− 141.0 ± 13.0* (25)	− 114.0 ± 10.0 (25)	− 93.0 ± 7.0 (23)	− 98.0 ± 11.5 (20)

*Minus sign indicates relaxation.

Table 2. Influence of extracellular Mg^{2+} on vasoactive drug-induced contractions of canine circumflex (CF), left coronary (LC), left coronary branch (LCB), and right coronary branch (RCB) arteries. The vasoactive drug concentrations used were: angiotensin II, 5 ng/ml; serotonin, 10 ng/ml; KCl, 20 m*M*; and norepinephrine, 0.05 μg/ml. Norepinephrine contractions were obtained after the tissues were treated with propranolol (0.5 μg/ml), a β-adrenergic blocking agent, for 5 minutes. The data (presented as means ± standard error) were analyzed by paired *t*-tests. The number of animals in each group is given in parentheses.

Concentration of Mg^{2+} (m*M*)	Tension (mg)			
	CF	LC	LCB	RCB
		Angiotensin II		
0	137.5 ± 62.5* (8)	292.5 ± 73.0* (9)	167.5 ± 42.5† (9)	175.0 ± 27.5‡ (9)
1.2	87.5 ± 41.5 (8)	195.7 ± 58.5 (9)	120.0 ± 35.0 (9)	132.5 ± 30.0 (9)
4.8	51.5 ± 32.5† (8)	152.8 ± 67.5 (9)	80.0 ± 22.5§ (9)	90.0 ± 22.5§ (9)
		Serotonin		
0	325.0 ± 57.5‖ (10)	447.5 ± 67.5‖ (10)	335.0 ± 50.0‖ (10)	246.2 ± 41.2‖ (10)
1.2	180.0 ± 42.5 (10)	235.0 ± 47.5 (10)	182.5 ± 30.0 (10)	122.5 ± 24.2 (10)
4.8	82.5 ± 25.0† (10)	142.5 ± 37.5‖ (10)	72.5 ± 15.0‖ (10)	67.5 ± 14.0† (10)
		KCl		
0	625.0 ± 67.5† (8)	462.5 ± 55.0‡ (8)	465.0 ± 75.0‡ (7)	482.5 ± 60.0‖ (8)
1.2	382.5 ± 62.5 (8)	270.0 ± 30.0 (8)	270.0 ± 42.5 (7)	300.0 ± 45.0 (8)
4.8	252.5 ± 67.5‖ (8)	135.0 ± 18.7‖ (8)	117.5 ± 17.5‖ (7)	142.5 ± 27.5‖ (8)
		Norepinephrine		
0	177.5 ± 42.5‡ (9)	190.0 ± 40.0‖ (8)	82.5 ± 25.0* (5)	82.5 ± 15.0‖ (7)
1.2	87.5 ± 20.0 (9)	100.0 ± 23.7 (8)	32.5 ± 7.5 (5)	37.5 ± 7.5 (7)
4.8	55.0 ± 15.0‡ (9)	90.5 ± 29.5‡ (8)	15.0 ± 4.5‖ (5)	23.2 ± 4.2* (7)

*$P < .02$. †$P < .005$. ‡$P < .01$. §$P < .05$. ‖$P < .001$, compared to the effect in physiological Mg^{2+} concentration (1.2 m*M*).

contractile responses to vasoactive agents in the absence of Mg^{2+} are probably also due to enhanced influx or translocation of Ca^{2+} into the vascular muscle cells. It is known that all of these vasoactive agents utilize calcium (that is, extracellular, membrane-bound, or intracellular) for eliciting contractile responses (*18*). This is consistent with the finding of a decreased ratio of magnesium to calcium in the heart muscle of SDIHD groups (*2-4*).

It is very unlikely that changes in receptor affinities could cause the increased or decreased contractile tensions in response to extracellular Mg^{2+}, since similar changes in tension in response to a nonspecific vasoactive agent, KCl, were also obtained. Potassium-induced responses were shown in our studies to be mediated directly rather than indirectly through the release of any known neurotransmitter substance, since a variety of specific antagonists did not modify the response (*19*).

The influence of Mg^{2+} could also, possibly, be explained in terms of an effect on adenosine 3′,5′-monophosphate (cyclic AMP) formation within the cells; Mg^{2+} being an activator of adenylate cyclase (*20*), an enzyme involved in the synthesis of cyclic AMP. There is experimental evidence to suggest that increased and decreased cyclic AMP concentrations participate in coronary vasodilatation and constriction, respectively (*21*). A decrease in cyclic AMP in the absence of Mg^{2+} could result in an increased concentration of free calcium ions within the cytoplasm because there would be less cyclic AMP–mediated calcium sequestration. Thus, this mechanism could, in part, be responsible for the increased tone and reactivity of coronary arteries obtained in the absence of Mg^{2+}. An alternative and contributing mechanism could be an inhibition of a Ca^{2+}-dependent adenosinetriphosphatase at the membrane that is Mg^{2+}-dependent and that presumably extrudes Ca^{2+} (*22*).

Thus our results, which demonstrate that reduced magnesium in the coronary vasculature environment exerts profound influences on coronary vascular tone and reactivity, support the hypothesis that hypomagnesemia could produce progressive vasoconstriction, resulting in coronary arterial spasm and, finally, SDIHD (*11*).

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Faster Cholinergic REM Sleep Induction in Euthymic Patients with Primary Affective Illness

Abstract. *Arecoline, a cholinergic muscarinic receptor agonist, induced rapid eye movement sleep significantly more rapidly in patients with primary affective illness in remission than in normal control subjects matched for age and sex. These results, and others, suggest that patients with primary affective illness may have a supersensitive cholinergic system both when they are ill and when their symptoms are in clinical remission.*

Sleep disturbance is one of the most characteristic biological findings in patients with primary affective illness. The sleep of depressed patients is normally short, shallow, and fragmented; REM latency [the time from sleep onset to the first rapid eye movement (REM) period] is short (*1*). Since cholinergic mechanisms are involved in both arousal and the induction of REM sleep (*2*), we previously suggested that activation of central cholinergic neurons or supersensitive cholinergic receptors may be implicated in the pathophysiology of sleep disturbance in depression (*3*). This interpretation is consistent with the hypothesis that an increased ratio of cholinergic to noradrenergic activity underlies depression (*4*).

To test the hypothesis further, we compared patients with primary affective illness whose symptoms were in remission with normal control subjects on the cholinergic REM-induction test. In this test we measure the speed with which REM sleep is induced by arecoline, a cholinergic muscarinic agonist administered intravenously during non-REM sleep. We had previously used this technique to provide evidence that muscarinic supersensitivity develops in normal volunteers who receive scopolamine, a cholinergic muscarinic receptor blocker, in the morning for two or more consecutive days (*5*).

The control subjects were 16 paid normal volunteers [nine males, seven females, mean age ± standard deviation (S.D.) = 28.3 ± 5.4 years]. They were compared with two groups of patients with primary affective illness whose symptoms were in remission. The initial group (group 1) of 13 patients (four males, nine females, 12 bipolar and 1 unipolar, mean age = 28.9 ± 6.9 years) were tested after all their regular medica-

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