Magnesium Deficiency in Drinking Water Modulates Blood Pressure, Ca and Mg Distribution in Tissues, and Compartmentalization of Membrane-Bound Calcium in Platelets of Normotensive WKY Rats

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Magnesium deficiency in drinking water increases calcium-accumulating capacity of the aortic and myocardial walls in normotensive WKY rats, induces Ca resorption from bones, and impairs compartmentalization of membrane-bound Ca in platelets, resulting in accumulation of Ca in the external plasma membrane without changing blood pressure. Increased systolic blood pressure was characteristic of rats in Ca-deficient groups.

Key Words: *magnesium; drinking water; arterial pressure; calcium*

New data on magnesium (Mg) metabolism and its role in the organism helps us to determine the diseases caused by Mg deficiency. Mg is the second intracellular cation, a cofactor of at least 300 intracellular enzymatic energy-dependent processes, and the only physiological antagonist of calcium (Ca). Drinking water, an important source of bioavailable physiologically active mineral pool, supplies up to 30% of daily Mg requirement. Almost complete absence of bivalent Mg and Ca cations in soft natural water in some regions (North-Eastern Europe — Karelia, Kola peninsula, and Scandinavia) correlates with high incidence of cardiovascular diseases and risk of sudden cardiac death [3,7,9,11-14].

The effect of chronic Mg deficiency on the development of cardiovascular and other diseases is poorly studied due to methodological difficulties (99% total Mg in the organism is located intracellularly).

However, it is physiologically, clinically, and therapeutically important to distinguish between the effects caused by Mg and Ca deficiency in drinking water in order to differentially correct the symptoms caused by them.

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MATERIALS AND METHODS

Normotensive male Wistar-Kyoto rats aged 14-16 weeks (250-280 g) were used. The animals were fed standard rations with Ca and Mg content of 0.6 and 0.2%, respectively, and had free access to water. The animals were divided into 4 groups (5 per group). Group 1 rats (control) were given drinking water with normal Ca and Mg concentrations (80 and 30 mg/liter, respectively), prepared according to WHO recommendations. Group 2 rats received Mg-depleted water (3 mg/liter) with normal Ca content (80 mg/liter) and group 3 animals Ca-depleted (8 mg/liter) water with normal Mg content (30 mg/liter). Group 4 rats drank water with low concentrations of Ca and Mg (8 and 3 mg/liter, respectively).

The concentrations of Ca and Mg in drinking water were measured using an atomic absorption spectrophotometer AAS-3 (Karl Zeiss). Systolic blood pressure (SBP) was measured on the tail artery by the cuff method at 2-week intervals.

By the end of the 16th week the animals were decapitated and Ca and Mg concentrations in the aortic arch, myocardium, tibial diaphyses (mg/g tissue), and serum (mmol/liter) were measured.

Platelet membrane-bound Ca was measured by the fluorescent probe chlortetracycline (CTC, 50 μM)

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[4,6]. The fluorescence kinetics was recorded by an MPF-3 spectrofluorimeter (Hitachi) at excitation and emission wavelengths of 380 and 510 nm, respectively, and 10-nm slits. The contribution of $(Ca\text{-}CTC)^+$ complex associated with the external plasma membrane to the total fluorescence was determined by adding 3 mM EGTA. As a specific Ca chelating agent, this substance selectively binds plasma membrane Ca^{2+}, thus quenching the fluorescence of $(Ca\text{-}CTC)^+$ complex in the plasma membrane and decreasing the total fluorescence of the sample. The residual fluorescence was apparently due to intracellular membrane structures (endoplasmatic reticulum and mitochondria). All operations with cells were carried out at 18-20°C. The kinetic curves of CTC binding to membrane Ca were corrected for the intrinsic platelet fluorescence and light scattering.

The results were statistically processed using Student's *t* test and nonparametric Mann-Whitney's test.

RESULTS

At the beginning of the experiment SBP in rats was 100±4 mm Hg. The differences in SBP appeared by the 8th week and became significant by the end of experiment. After 16 weeks SBP in groups 3 and 4 was significantly higher than in the control (135±8 and 158±8 mm Hg, respectively, $p<0.05$), vs. 123±13 mm Hg in controls). The lowest SBP (111±5 mm Hg) was observed in Mg-deficient animals.

Significant ($p<0.05$) differences in Ca tissue distribution were noted in groups 2 and 4: an increased Ca content in the myocardium and aortic wall and a decreased content in the tibial diaphyses in comparison with the control (Table 1). An increased Ca concentration in the myocardium was observed in Mg-deficient animals drinking water with normal (group 2) and low (group 4) Ca concentrations; Mg levels in tissues virtually did not change.

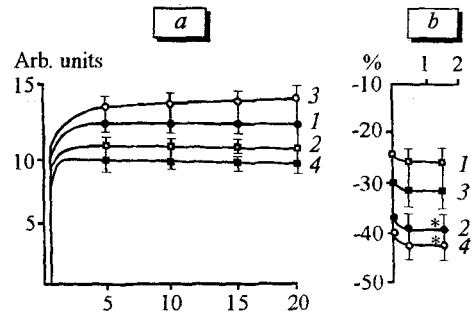

Fig. 1. Kinetic curves of binding (*a*) and fluorescence quenching by EGTA (*b*) 20 min after addition of chlortetracycline to platelet suspension. Numbers of the curves correspond to group numbers. Abscissa: time, min. *$p<0.05$ *vs.* the control.

The study of Ca-binding capacity of platelet membranes with CTC showed appreciable differences between the groups.

Platelet saturation with CTC are characterized by a biphasic kinetics (Fig. 1). The first linear portion of the curve (rapid increase in the fluorescence intensity) represents binding of the $(Ca\text{-}CTC)^+$ complexes to the outer platelet membrane surface (the rate of this process is determined by linear diffusion of the probe to the membrane surface) and the start of CTC binding to intracellular membranes. Further exponential increase in fluorescence corresponds to CTC entry into the cell and its binding to intracellular membranes (tubular system and mitochondria). The plateau corresponds to the end of binding of the fluorescent probe to the platelet membrane structures.

In Mg-deficient animals, the intensity of $(Ca^{2+}\text{-}CTC)^+$ fluorescence immediately after addition of CTC and during the subsequent 15 min was lower than in the control ($p<0.01$, $p<0.05$, respectively).

Addition of EGTA to CTC-loaded platelets rapidly decreased fluorescence in all groups: by 25±4, 40±6, 32±4, and 43±7%, respectively. It should be noted that the drop of fluorescence was most pronounced in Mg-deficient groups, while in the Ca-deficient group it was virtually the same as in the control.

TABLE 1. Relationship between Ca and Mg Dificiency in Drinking Water and Their Blood and Tissue Levels in Normotensive WKY Rats (*M±m*)

Group	Body weight, g	Myocardial weight, mg	Mg and Ca concentrations in tissues (mg/g wet tissue) and serum (mg/liter)							
			aorta		myocardium		tibial diaphysis		serum	
			Mg	Ca	Mg	Ca	Mg	Ca	Mg	Ca
1	342±26	990±81	1.90±0.1	3.20±0.25	0.23±0.01	0.23±0.01	25.7±1.9	443±57	1.1±0.05	2.00±0.20
2	305±22	923±75	1.15±0.09	2.88±0.12*	0.27±0.02	0.35±0.01*	14.6±1.6	278±22*	1.3±0.1	2.3±0.21
3	286±15	997±82	1.4±0.11	2.5±0.15	0.17±0.02	0.18±0.02	29.1±2.5	426±62	1.6±0.1	2.2±0.24
4	278±21	956±102	1.58±0.20	3.2±0.2*	0.25±0.02	0.34±0.01*	23.0±1.8	294±31*	1.6±0.09	1.58±0.20

Note. *$p<0.05$ *vs.* group 1.

Mg-deficient animals characterized by increased Ca-accumulating capacity of the aortic wall and myocardium, enhanced Ca resorption from bones, disturbed compartmentalization of membrane-bound Ca in platelets, particularly its accumulation in the external plasma membrane and, hence, depletion of intracellular compartments of Ca.

Increased SBP was characteristic of the Ca-deficient groups. Previously we showed that elevated SBP in some animals assigned to a low Ca ration [2] was associated with the appearance of a parathyroid hypertensive factor [8] in the blood. The function of erythrocytic ion-transporting systems genetically related to the development of arterial hypertension and of the systems involved in the regulation of intracellular Ca^{2+} concentrations are impaired in these animals [2]. Moreover, physical properties of the myocardium and vessel walls are changed, which depend on the connective tissue status (decreased elasticity of the aortic wall, altered elastic and passive characteristics of the myocardium with a longer relaxation phase, etc.) [14].

Our experiments confirm that SBP is decreased in Mg-deficient animals [5]. The mechanisms of hypotension in chronic Mg deficiency differ from the mechanisms of hypotension in drug therapy and intravenous infusion of therapeutic doses of Mg [5]. It was assumed that hypotension in chronic Mg deficiency results from decreased production of angiotensin-converting enzyme (angiotensin) and increased production of vasodilators (histamine) [5]. The rise of SBP at the later stages of chronic Mg deficiency can be attributed to activation of the renin-angiotensin system (in response to hypotension) in parallel with increased receptor sensitivity to vasoconstrictors, on the one hand, and secondary changes in cell Na and Ca concentrations against the background of reduced activities of Na,K- and Mg-ATPase, on the other [10]. This second phase is not Mg-dependent and cannot be corrected by exogenous Mg.

Generalized disorders in the cation-transporting function of cell membranes in arterial hypertension are associated with marked functional and structural changes of vascular smooth muscle cell membranes, specifically, with disturbances in Ca binding to the membranes. The membrane regulation of the cytosol Ca concentration can be insufficient in vascular smooth muscle cells determining the vascular tone and vascular resistance because these cells are electrically excitable, possess the intracellular second messenger system (cyclic nucleotides, phosphoinositides, and intracellular Ca), and express receptors to various activating factors.

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