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Lowered Total Intracellular Magnesium Status in a Subgroup of Hypertensives

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Abstract. A new method to determine total Mg^{++} content in lymphocytes was developed, offering advantages for routine measurements as compared to fluorescence methods. Intracellular total Mg^{++} measurements were performed in lymphocytes of 18 healthy subjects and 19 untreated essential hypertensive patients. Mg^{++} content was referred to lymphocytic and membrane protein, which was determined according to Bradford's method. Mg^{++} measurements were performed by atomic absorption spectroscopy using a Video 12 apparatus of Thermo Electron Instrumentation Laboratory, Andover, USA. The results show that in patients with essential hypertension total intralymphocytic Mg^{++} content is significantly lower (0.07 ± 0.05 mmol/g lymphocytic protein, mean ± s.d.) as compared to controls (0.11 ± 0.04 mmol/g lymphocytic protein, mean ± s.d., $p < 0.05$).

Free intracellular Mg^{++} content was measured in lymphocytes by the fluorescent indicator mag-fura-II, showing no significant differences in normotensives and hypertensives (0.30 ± 0.16 versus 0.38 ± 0.17 mmol/l). Additionally, in platelets free intracellular Mg^{++} concentrations were not found of significant difference in normotensives and hypertensives (0.52 ± 0.23 versus 0.47 ± 0.27 mmol/l) using mag-fura-II. In plasma Mg^{++} concentrations there was no significant difference in the normotensive and hypertensive group (0.92 ± 0.07 versus 0.88 ± 0.07 mmol/l). There was no correlation between plasma or free or total cellular magnesium concentrations in both groups. Furthermore this method seems also suitable for routine measurements of total intracellular Mg^{++} concentrations in even larger measurements like mag-fur-II. Lowered total intracellular Mg^{++} concentrations in a subgroup of primary hypertensives may contribute to the development of this disorder, perhaps due to different buffering systems.

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Introduction

Whereas plasma Mg^{++} concentrations in essential hypertensives have often been investigated, comparatively few data on intracellular Mg^{++} concentrations are available [1–2]. Furthermore the role of intracellular Mg^{++} content in essential hypertension is also controversely discussed [3–4]. Decreased intracellular free Mg^{++} concentrations in erythrocytes of essential hypertensive patients have been described, whereas other authors were unable to confirm these findings [5–6]. Therefore it was of interest to develop a new method to determine total Mg^{++} content in lymphocytes of normotensive and essential hypertensive patients, offering advantages for routine measurements as compared to other analytical techniques (e.g. fluorescent dye techniques using mag-fura-II) [7].

Material and Methods

Eighteen healthy subjects and 19 untreated essential hypertensive patients were investigated. The clinical data of the patients are shown in Table 1. In each patient plasma and free (platelet and lymphocytic) and total intracellular (lymphocytic) Mg^{++} contents were determined. The Mg^{++} concentrations in plasma were measured with an electrothermal atomic absorption spectroscope (Thermo Jarrell Ash Video 12, Thermo Jarrell Instrumentation Laboratory, Andover, USA) as described earlier [1]. For each sample a mean value was calculated from 5 measurements. The intraassay variation coefficient was 4.8% in 10 consecutive measurements and the interassay variation coefficient was between 6 and 8%.

Lymphocytes were obtained from 20 ml heparinized blood. Briefly, blood was centrifuged at 240 g for 15 min and the upper two-thirds of the supernatant were aspirated. Lymphocytes were isolated by layering 5 ml of diluted blood (1:1 vol:vol with isotonic NaCl) on 3 ml of Lymphoprep (Boehringer Mannheim, Germany; 5.6% wt/vol Ficoll; density 1.077 g/ml) and centrifuged at 240 g for 20 min. The lymphocyte interphase was carefully aspirated, washed three times in isotonic NaCl by centrifuge at 400 g for 5 min, and resuspended in Hanks' balanced salt solution containing (in mmol/litre): NaCl 136, KCl 5.4, KH_2PO_4 0.44, Na_2HPO_4 0.34, $CaCl_2$ 1.0, D-glucose 5.6, N-2-hydroxyethylpiperazine-N-2-ethanesulphonic acid (HEPES) 10; pH 7.4.

Then the Mg^{++} concentration was determined directly at atomic absorption spectroscopy [1]. Analogously lymphocytic protein content was determined according to Bradford's method as a reference [8].

Table 1. Clinical data of 18 normotensive (NT) and 19 essential hypertensive (EH) (means ± s.d., * = $p < 0.05$).

	NT	EH
Blood pressure (mm Hg, syst./diast.)	115.4 ± 10.1/ 83.8 ± 7.8	178.4 ± 14.3/ 106.9 ± 14.0*
Serum creatinine (mg/dl)	1.02 ± 0.11	0.98 ± 0.12
Age (years)	48.6 ± 13.9	52.8 ± 8.7
Sex (m/f)	9/9	9/10
Renal disease	—	—
Pre-eclampsia	—	—
Diabetic	—	—

Free lymphocytic Mg^{++} content was determined in lymphocytes by the fluorescent dye technique using mag-fura-II as an indicator for Mg^{++}. Lymphocytes were obtained from 20 ml heparinized blood. Cellular free magnesium was calculated according to the standard equation reported by Grynkiewicz et al. as described earlier [10].

Blood platelets were isolated from heparinized human blood by differential centrifuge. The cytosolic free magnesium concentration $_{i}Mg^{++}$ was measured after incubation of platelets with 10 µmol/l mag-fura-II-acetoxymethylester (Cabiochem, Bad Soden, Germany) and 0.1% nonionic detergent Pluronic F-127 (Molecular Probes, Eugene, OR) for 60 min at 37°C [11]. Statistical analysis was performed using ANOVA with Bonferoni post-hoc test, data are mean ± s.d., p values below 0.05 were considered significant.

Results

In 18 normotensive and 19 essential hypertensive plasma and free (platelet and lymphocytic) and total intracellular (lymphocytic) Mg^{++} concentrations were determined.

In plasma Mg^{++} concentrations there was no significant difference in normotensive (0.92 ± 0.07 mmol/l) and essential hypertensive patients (0.88 ± 0.07 mmol/l) (mean ± s.d.). Plasma Mg^{++} concentrations ere found in the normal range between 0.8–1.2 mmol/l (Figure 1).

In patients with essential hypertension total intralymphocytic Mg^{++} content was significantly lower (0.07 ± 0.05 mmol/g lymphocytic protein) as compared to controls (0.11 ± 0.04 mmol/g lymphocytic protein) ($p < 0.05$) (Figure 2).

After quenching of extracellular fluorescence using 1 mmol/l EGTA, resting lymphocyte free Mg^{++} concentrations were measured 0.38 ± 0.17 in controls versus 0.30 ± 0.16 mmol/l in the hypertensives, showing no significant difference (Figure 3). After quenching of extracellular fluorescence using 50 µmol/l $MnCl_2$, resting $_{i}Mg^{++}$ in lymphocytes was 0.40 ± 0.09 mmol/l in patients with essential hypertension and 0.33 ± 0.08 mmol/l in control subjects, also showing no significant difference. In platelets free Mg^{++} concentrations were not found of significant difference in the control and hypertensive group (0.52 ± 0.23 versus 0.47 ± 0.27 mmol/l (Figure 4). Within the two groups, there was no significant correlation between free and total intracellular and plasma magnesium concentrations and blood pressure values.

Discussion

A pathogenetic role of a magnesium deficiency in the development of hypertension is discussed [3–5]. To clarify the nature of the underlying cellular defect in primary hypertension both free and total Mg^{++} concentrations have to be considered. As our results show, it seems likely that a specific defect in Mg^{++} transport or membrane systems seems to be involved in the pathogenesis of primary hypertension. Although it still remains unclear whether lowered membrane or intracellular Mg^{++} concentrations in essential hypertension is a cause or a result of hypertension disorder.

A role for intracellular Mg^{++} concentration in vascular tone has been postulated in primary hypertension [12–17].

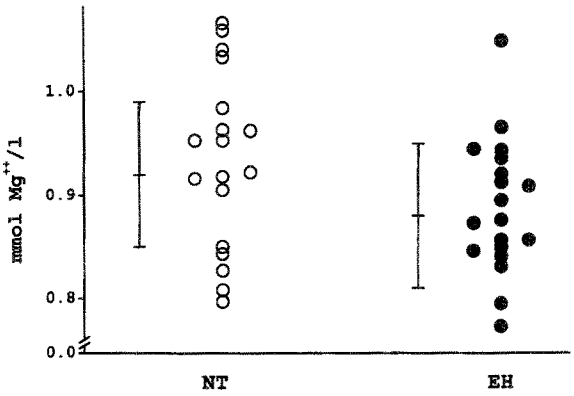

Fig. 1. Normal plasma Mg^{++} concentrations in 18 normotensive controls (NT) and 19 essential hypertensive patients (EH) (mean ± s.d.).

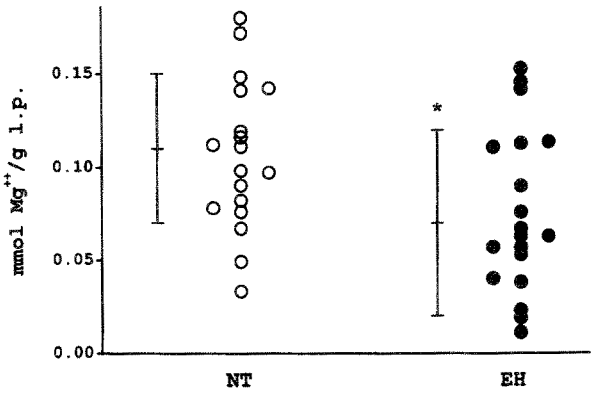

Fig. 2. Total intracellular (lymphocytic) Mg^{++} content in 18 normotensive controls (NT) and 19 essential hypertensive patients (EH) (mean ± s.d., * = $p < 0.05$).

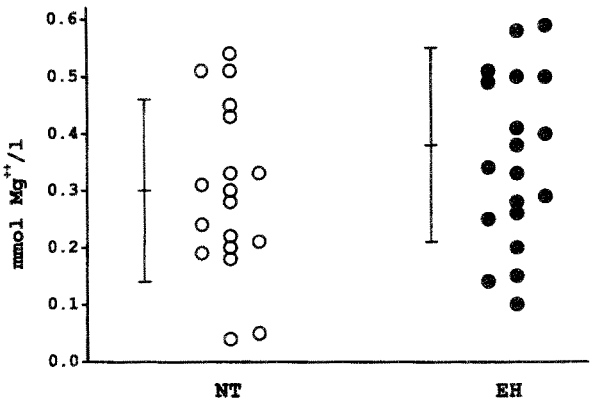

Fig. 3. Lymphocytic free Mg^{++} concentrations in 18 controls (NT) and 19 essential hypertensives (EH) (mean ± s.d.).

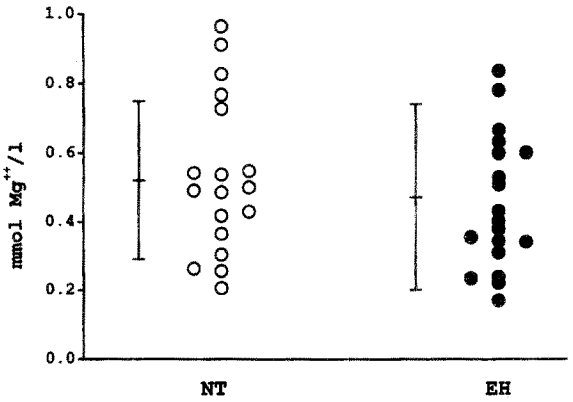

Fig. 4. Free platelet Mg^{++} content in 18 controls (NT) and 19 essential hypertensives (EH) (mean ± s.d.).

In essential hypertensives, Resnick et al. found decreased intracellular free Mg^{++} concentrations in red blood cells as estimated by nuclear magnetic resonance spectroscopy [5]. Analogous findings were reported in the spontaneously hypertensive rat [18]. Thus, an intracellular Mg^{++} deficiency and possibly a defect in cellular Mg^{++} transport could play a pathogenetic role. On the basis of experimental data, the mechanisms underlying the Mg^{++}-induced vasodilation may be: (a) a modification of the response to vasopressor hormones, or (b) an interaction with cellular Ca^{++} handling [19]. These possible mechanisms are supported by 3 findings of recent evidence. First, the extracellular Mg^{++} concentration can influence Ca^{++} metabolism of vascular smooth muscle by changing the Ca^{++} influx through the plasma membrane. Recently, in single myocytes from frog ventricle, the site of interaction between Mg^{++} and Ca^{++} was identified as the Ca^{++} inward current that is dependent on phosphorylation by cyclic adenosine monophosphatase [20]. Second, changes in the extracellular Mg^{++} concentration induced inverse changes in the Ca^{++} content of vascular smooth muscle and in exchangeable Ca^{++} [21–23]. Third, a decrease in the intracellular free Mg^{++} concentration results in diminished membrane Na^+, K^+-adenosine triphosphatase and Ca^{++} ATPase activities, and, as a corollary, increased Na^+–Ca^{++} exchange and increased intracellular Na^+ and Ca^{++} concentrations [24,25].

The results obtained in our study show significantly lowered total intracellular Mg^{++} concentrations in lymphocytes of essential hypertensives as compared to controls ($p < 0.05$). The findings are similar to those in red blood cells of essential hypertensives or in spontaneously hypertensive rats [5,18]. The variation of the Mg^{++} values in the hypertensive group was higher than in the other group. Since methological reasons may not be likely, one explanation may be that the essential hypertensives represent an inhomogeneous group. Possibly, lowered intracellular or membrane Mg^{++} values play a role only in a subgroup of essential hypertensives, which at present cannot be identified by clinical characteristics. For these reasons the determination of intracellular Mg^{++} concentrations is of more use than plasma Mg^{++} concentrations. Furthermore total intracellular Mg^{++} measurements seem to be of more interest than free intracellular Mg^{++} concentrations when classifying this subgroup of "essential" hypertensives.

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