Intralymphocyte Free Magnesium in Patients With Primary Aldosteronism

Aldosterone and Lymphocyte Magnesium Homeostasis

Pietro Delva, Caterina Pastori, Maurizio Degan, Germana Montesi, Paolo Brazzarola, Alessandro Lechi

Abstract—It is known that hyperaldosteronism has been associated with magnesium deficiency, yet there are no data on the intracellular concentration of ionized magnesium ([Mg2+]i) in subjects with primary aldosteronism (PA). We measured intralymphocyte free magnesium ([Mg2+]i) and intralymphocyte free calcium ([Ca2+]i) in 16 patients with PA and 26 normotensive control subjects (NCs). [Mg2+]i and [Ca2+]i were also measured in blood lymphocytes incubated in vitro with aldosterone, according to a fluorimetric method. In subjects with PA, [Mg2+]i was significantly lower than that in NCs (mean±SD; PA 203±56 μmol/L, NCs 291±43 μmol/L, 95% confidence interval 57 to 119, P=0.001). In the patients, [Ca2+]i did not prove to be statistically different from that of NCs (mean±SD; PA 47.2±10.6 nmol/L, NCs 53.2±11 nmol/L). The lymphocytes exposed to the action of aldosterone showed a significant reduction in [Mg2+]i (n=15, NCs 271±28 μmol/L, aldosterone treatment 188±39 μmol/L, P=0.001, 95% confidence interval 57 to 108). The dose-effect curve of aldosterone on [Mg2+]i showed an EC50 value of 0.5 to 1 nmol/L aldosterone. The reduction in [Mg2+]i mediated by aldosterone is antagonized by the receptor inhibitor of aldosterone; it is inhibited by inhibitors of protein synthesis and is not measurable when the lymphocytes are incubated in an Na+-free medium. The data are consistent with the hypothesis that aldosterone affects the cellular homeostasis of magnesium, probably through modification of the activity of the Na+-Mg2+ antiporter. (Hypertension. 2000;35:113-117.)

Key Words: aldosterone ■ ions ■ magnesium ■ calcium ■ lymphocytes ■ hypertension

Primary aldosteronism is a volume-dependent form of hypertension that is the subject of increasing interest. Studies aim to elucidate the cellular events resulting from in vivo aldosterone action. With regard to ion membrane transport systems, an increased activity of membrane Na+-H+ exchanger and increased plasma amounts of an Na+-K+ pump inhibitor have been described in primary aldosteronism. It has long been known that cases of hyperaldosteronism are characterized by magnesium deficiency, yet the data that could explain this phenomenon are extremely limited and contrasting and refer exclusively to levels of plasma and urinary magnesium. This is a limiting factor when we consider the fact that >99% of total body magnesium is located intracellularly.

Aldosterone is believed to influence renal magnesium handling, causing magnesium wasting, although there is disagreement regarding this finding. The acute administration of mineralocorticoids fails to modify magnesium and calcium excretion in humans, but certain clinical and experimental data suggest that the excretion of calcium and magnesium may be affected by the long-term action of mineralocorticoids. It has also been suggested that magnesium operates as a regulator of the production of aldosterone. Indeed, the infusion of magnesium suppresses plasma aldosterone levels in humans. High levels of magnesium decrease aldosterone production in cultures of rat zona glomerulosa cells and experimental magnesium deficiency stimulates aldosterone production.

Very little data are available on plasma magnesium in subjects with primary aldosteronism. In 1954, Mader and Iseri reported the case of a patient with corticosterone-like mineralocorticoid hypersecretion with hypomagnesemia. In 1962, Horton and Biglieri described the cases of 5 patients with primary aldosteronism and an increased renal magnesium clearance despite normal plasma magnesium levels. More recently, Resnick and Laragh described the cases of 10 patients with primary aldosteronism who had normal plasma magnesium values.

There also are no data concerning the possible effects of aldosterone on the cellular homeostasis of magnesium; thus, no data are available so far relating [Mg2+]i in patients with primary aldosteronism and the in vitro cellular effects of aldosterone on Mg2+.
This lack of data has mainly been due to technical limitations in the measurement of intralymphocyte free magnesium concentration ([Mg$^{2+}$]). The synthesis of a new fluorescent magnesium-sensitive dye$^{11}$ has allowed the measurement of [Mg$^{2+}$] in nucleated cells.$^{12}$ The use of this technique has made possible the discovery of a lack of Mg$^{2+}$, in subjects with plurimetabolic syndrome,$^{13}$ suggesting a relationship with peripheral resistance to insulin,$^{14}$ whereas in a group of patients with essential hypertension, any abnormalities in [Mg$^{2+}$] could be detected.$^{12}$

We therefore measured [Mg$^{2+}$] in a group of 16 patients with primary aldosteronism and a group of 26 normotensive control subjects. Lymphocytes are used because of their simple availability. There is no logical relation to the measured parameters, but several studies suggest that the magnesium content of white blood cells represents a reliable indicator of magnesium status. Intralymphocyte free calcium concentration ([Ca$^{2+}$]) was also measured, mainly to exclude substantial Ca$^{2+}$ variations, which theoretically may affect magnesium determinations$^{15}$; in addition, no data on Ca$^{2+}$ in this pathological condition are available.

Finally, we studied the in vitro action of aldosterone on [Mg$^{2+}$] and [Ca$^{2+}$] in human lymphocytes.

**Methods**

[Mg$^{2+}$] and [Ca$^{2+}$] were measured in 16 patients with primary aldosteronism (6 with adrenal adenoma and 10 with adrenal hyperplasia) and in 26 normotensive healthy subjects with no family history of hypertension who were recruited from hospital medical and paramedical staff. Patients with primary aldosteronism and normotensive control subjects were not matched for gender because there is no evidence of gender-dependent [Mg$^{2+}$] and [Ca$^{2+}$] differences.$^{12}$ After hospitalization, the diagnosis of primary aldosteronism was made for all the patients on the basis of a complete clinical, laboratory, and instrumental examination. The diagnosis of primary aldosteronism caused by aldosterone-producing adenomas or bilateral adrenal hyperplasia was made with the use of computed tomography scanning and bilateral adrenal vein sampling. In this way, we sought to detect aldosterone, renin, and cortisol levels; the latter was used to confirm the accuracy of catheter placement in the adrenal vein. Particular care was taken to exclude patients with chronic alcoholism and diabetes because these 2 pathological conditions are associated with magnesium wasting.$^{16,17}$ The study was approved by the local ethics committee, and the subjects gave informed consent.

The blood samples used for the determination of lymphocyte [Mg$^{2+}$] and [Ca$^{2+}$] were taken from patients and control subjects in the morning after overnight fasting. All forms of drug treatment were discontinued ≥3 weeks before the samples were taken. Because diuretics are known to affect Mg$^{2+}$ levels,$^{18}$ patients receiving such treatment at any time were excluded from the study. All subjects were on an unrestricted diet.

**Routine Laboratory Tests**

Total plasma calcium, sodium, and potassium levels were measured with an autoanalyzer (Technicon DAX 96; Miles Inc), as were total plasma magnesium and calcium levels (Hitachi 911 Analyzer; Hitachi Ltd). Total urinary magnesium and calcium, sodium, and potassium levels were measured with the use of flame photometry. Plasma renin, aldosterone concentration, and urinary aldosterone were measured with radioimmunoassay.$^{19,20}$

**Measurement of [Mg$^{2+}$] and [Ca$^{2+}$]**

We used the method previously described.$^{12}$ Briefly, peripheral blood lymphocytes were isolated through Ficoll sedimentation, resulting in cell preparations with a percentage of lymphocytes of >97%. Three separate aliquots of lymphocytes (6 × 10$^6$ each) were treated with 10 μmol/L Furaptra acetoxymethyl ester (Molecular Probes) to measure [Mg$^{2+}$] or with Fura-2 acetoxymethyl ester (5 μmol/L) to measure [Ca$^{2+}$].$^{12,21}$ For [Mg$^{2+}$] determinations, fluorescence emission at 510 nm was measured with alternate excitation at 335 and 370 nm within a thermostatically controlled cuvette holder (31°C) in an Hitachi F-2000 fluorescence spectrophotometer. We used the intracellular cation concentration that was the mean of the concentrations of 3 separate experiments. The transmembrane leak of fluorescence probe was reduced by limiting the temperature to 31°C. Interference with the reading by residual probe leakage was eliminated by the addition of EGTA and EDTA. Calibration was performed after cell lysis with Triton-X. The intra-assay and interassay variability (coefficient of variation) determinations were 5.8 and 3.9 for Mg$^{2+}$ and 4.6 and 12.7 for Ca$^{2+}$, respectively.

**In Vitro Study of Effects of Aldosterone on [Mg$^{2+}$] and [Ca$^{2+}$]**

The lymphocytes used for these tests were from healthy donors chosen from the medical staff. The measurement of [Mg$^{2+}$] and [Ca$^{2+}$] was carried out according to the procedures described earlier. To limit the possibility of artifacts, the hormones tested in vitro on the lymphocytes were not added directly to the test-tube during reading from the spectrofluorometer. We chose another strategy, which involved isolation of the lymphocytes with Ficoll and the subsequent conduction of 2 or more parallel experiments with cells from the same donor, which were incubated with only the vehicle of the hormone (control) or the hormone, respectively.

**Statistical Analysis**

Results are expressed as mean ± SD. Because no evidence of non-normal distribution or inequality of variances was present in the variables considered, comparison between groups was performed with Student’s $t$ test and considered statistically significant when the probability of the null hypothesis was ≤5%. Confidence intervals for differences in mean values were also provided. To determine differences between groups, a 1-way ANOVA was used. Correlations between 2 variables were studied with the use of the linear regression method.

**Results**

**Main Clinical and Metabolic Variables**

Total plasma magnesium, daily urinary magnesium excretion, and total plasma calcium did not differ significantly between patients with primary aldosteronism and normotensive controls (Table). Plasma potassium and plasma renin levels in the upright position were significantly reduced in patients with primary aldosteronism compared with normotensive control subjects. Plasma aldosterone levels in the upright position together with daily urinary aldosterone excretion were significantly increased in patients with primary aldosteronism compared with normotensive control subjects.

**[Mg$^{2+}$] in Patients With Primary Aldosteronism and Normotensive Subjects**

Individual values for [Mg$^{2+}$] in essential hypertensive patients and normotensive control subjects are shown in Figure 1. Mean values are significantly lower ($P=0.000001$) in patients with primary aldosteronism (mean ± SD 203.56 μmol/L) than in normotensive control subjects (291.43 μmol/L; 95% confidence interval 57 to 119). Patients with adenoma had [Mg$^{2+}$] values not statistically different from those of patients with adrenal hyperplasia (mean ± SD; adenoma n = 6, 199.65 μmol/L; hyperplasia n = 10, 204.59 μmol/L).
Urinary aldosterone, m
Normotensive Subjects
Metabolic Variables

*r = 0.544 and 0.540, respectively, plasma total magnesium (r = 0.120), systolic and diastolic blood pressures (r = 0.196) were not statistically different from those of patients with adenoma.

Patients with
Primary
Aldosteronism
Normotensive
Control
Subjects
Parameter

n
16
26
Gender, M/F
3/13
15/11
Age, y
49±14
40±16
SBP, mm Hg
165±23
127±9
DBP, mm Hg
101±8
74±6
Plasma Mg²⁺, mmol/L
0.76±0.08
0.76±0.07
Plasma Ca²⁺, mmol/L
8.7±0.4
8.5±1.5
Plasma K⁺, mmol/L
3.2±0.5†
3.9±0.2
Urine Mg²⁺, mmol/24 h
2.7±1.3
2.8±0.7
Plasma renin, upright, pmol/L
200±315†
592±421
Plasma aldosterone, upright, nmol/L
1.23±0.7¶
0.42±0.19
Urine aldosterone, μmol/24 h
0.043±0.02§
0.02±0.01

*P = 0.0002 vs normotensive control subjects.
†P < 0.05 vs normotensive control subjects.
‡P = 0.00007 vs normotensive control subjects.
§P < 0.02 vs normotensive control subjects.

[Ca²⁺]i in Patients With Primary Aldosteronism and Normotensive Subjects
Mean values for [Ca²⁺]i were not significantly different between patients with primary aldosteronism (mean = 47.2±10.6 nmol/L) and normotensive control subjects (53.2±11 nmol/L). Patients with adenoma had [Ca²⁺]i values that were not statistically different from those of patients with adrenal hyperplasia (adenoma n = 6, 44±4 nmol/L; hyperplasia n = 10, 45±14 nmol/L).

Correlations Between [Mg²⁺]i and Clinical and Metabolic Variables
In univariate regression in patients with primary aldosteronism, [Mg²⁺]i did not correlate with any of the following variables: age (r = 0.120), systolic and diastolic blood pressures (r = 0.544 and 0.540, respectively), plasma total magnesium (r = 0.025), urinary total magnesium (r = −0.347), plasma renin concentration in upright and standing positions (r = 0.360 and 0.338, respectively), plasma aldosterone in upright and standing positions (r = 0.235 and 0.237, respectively), plasma potassium (r = 0.196), and length of history of hypertension (r = 0.007).

[Mg²⁺]i and [Ca²⁺]i in Lymphocytes Exposed In Vitro to Aldosterone and Related Steroid Hormones
Lymphocytes exposed in vitro to the action of aldosterone (1 μmol/L) for 100 minutes showed a significant decrease in [Mg²⁺]i (n = 15, mean = 271±15 μmol/L, aldosterone treatment 188±39 μmol/L; P = 0.0000003; 95% confidence interval 57 to 108). Figure 2 shows the time course of action of aldosterone on these cells. The effect is visible after 30 minutes and reaches a maximum level after ~2 hours, after which it remains more or less stable until the fifth hour. After 5 hours, the fall in [Mg²⁺]i is equal to 50% of the initial level.

Figure 3 shows the effect of aldosterone (1 μmol/L) on the decrease in [Mg²⁺]i is completely antagonized by canrenoic acid (15 μmol/L) and by inhibitors of transcription (50 μg/mL actinomycin D) and protein synthesis (15 μg/mL cycloheximide) (mean ± SD; controls n = 6, 100±8%; aldosterone n = 6, 63±8%; aldosterone and canrenoic acid n = 6, 99±6%; aldosterone and cycloheximide n = 6, 98±7%; aldosterone and actinomycin D n = 6, 98±6%). Figure 4 shows the dose-effect curves of the decrease in [Mg²⁺]i in response to the effect of 3 steroid hormones with 21 carbon atoms: aldosterone, cortisol, and progesterone. The dose-effect curve for aldosterone shows there is a strong dependence of [Mg²⁺]i on aldosterone concentration; and the half-maximal effect of aldosterone (EC₅₀) occurs at a concentration of ~0.5 to 1 nmol/L aldosterone. Progesterone and cortisol (Figure 4) have a negligible effect on [Mg²⁺]i, as shown by its relative EC₅₀ values (~100 nmol/L and ~10 nmol/L, respectively). If we incubate the lymphocytes in a sodium-free medium, the levels of [Mg²⁺]i are much higher because the efflux of Na⁺-dependent magnesium is inhibited. Under these conditions, the decrease in [Mg²⁺]i produced by the aldosterone is not measurable (Na⁺ medium n = 6, 236±18 μmol/L; Na⁺...
medium and 1 μmol/L aldosterone n=6, 161±10 μmol/L [P=0.003]; Na⁺-free medium n=6, 577±147 μmol/L; Na⁺-free medium and 1 μmol/L aldosterone n=6, 587±96 μmol/L [NS]).

Finally, we measured [Ca²⁺] in lymphocytes incubated in vitro with 1 μmol/L aldosterone for 100 minutes. We did not find any statistically significant difference in [Ca²⁺] from the control cells (n=6, controls 47±6 nmol/L, aldosterone treatment 53±6 nmol/L, NS).

Discussion

Primary aldosteronism represents an interesting model for the study of the physiopathology of aldosterone. The in vivo influence of aldosterone on Mg²⁺, has been hypothesized, but cellular measurements have not been performed. Aldosterone is thought to act at renal level through the inducement of renal tubular magnesium excretion, although this is still a matter of debate. This probably occurs secondary to the volume expansion and hypertension associated with hyperaldosteronism because magnesium reabsorption is related to tubular flow rates and sodium reabsorption, both of which are altered in this situation. Horton and Biglieri described significantly increased magnesiuria in patients with primary aldosteronism, but other authors have not confirmed these findings. In our group of patients, daily urinary magnesium excretion was not statistically different from that of control subjects.

Regarding magnesium plasma levels, most data from the literature indicate that they are not significantly altered in patients with primary aldosteronism, and our results are in accordance with previous data. Despite this fact, Resnick and Laragh reported that serum magnesium values in their subjects with primary aldosteronism were within normal limits, they were significantly higher than those in normotensive control subjects. They believe that this is in keeping with the hypothesis that low-renin hypertension has a distinctive magnesium and calcium profile. We must point out that Resnick et al refer to plasma ionized magnesium as opposed to plasma total magnesium, which was measured in the present study. Despite a modest overlap, we found that mean [Mg²⁺] was significantly lower in patients with primary aldosteronism than in normotensive control subjects. Three of 16 patients were characterized by normal [Mg²⁺], but the majority of the patients showed a significant decrease in ionized magnesium. No significant differences were found between patients with adrenal hyperplasia and patients with aldosterone-producing adenomas.

From these data on patients with primary aldosteronism, it is not possible to explain the physiopathological mechanism underlying the Mg²⁺ deficiency. In particular, we were not able to demonstrate any significant correlation between [Mg²⁺] and renal magnesium excretion or with renin or aldosterone plasma and urinary levels, and we can neither confirm nor exclude a cause-effect relationship.

In consideration of the questions raised by these ex vivo results, we studied the effect in vitro of aldosterone on lymphocytes. In vitro, aldosterone decreases the content of basal ionized magnesium by 45% to 50%, which is a highly significant reduction. The effect of aldosterone appears to be mediated by the specific receptor of the hormone in that the decrease in [Mg²⁺] is not observed when canrenoic acid, a specific antagonist of the receptor, is present. Moreover, the effect of the hormone seems to involve the classic genomic pathway of steroid action because it is completely eliminated by actinomycin D and cycloheximide, inhibitors of transcription and protein synthesis, respectively. The effects of aldosterone on the intralymphocyte contents of ionized magnesium appear to be dose dependent with an EC₅₀ value of 0.5 to 1 nmol/L, aldosterone. This value is close the physiological concentration of plasmatic aldosterone (0.5 nmol/L) and suggests that aldosterone may play a role as physiological regulator of the Mg²⁺ content. Cortisol and progesterone, which were tested because of their close biochemical simi-
larity, also reduce \([\text{Mg}^{2+}]\) but only at higher concentrations, as shown by their respective EC\(_{50}\) values, which appear to be higher than that of aldosterone. Finally, the experiments carried out without extracellular sodium suggest a possible mechanism through which the cellular action of aldosterone operates. In fact, in the absence of sodium on the outside of the plasmatic membrane, the activity of the Na\(^+-\text{Mg}^{2+}\) antiporter, the principal known mechanism of magnesium efflux,\(^{23}\) is inhibited. Under these conditions, the action of aldosterone is no longer measurable. The action of aldosterone on \([\text{Mg}^{2+}]\) therefore takes place exclusively in the presence of extracellular sodium, probably through the activation of a transport mechanism that decreases \([\text{Mg}^{2+}]\) by producing a \(\text{Mg}^{2+}\) efflux.

We consider these in vitro data concerning the effects of aldosterone on \(\text{Mg}^{2+}\), to suggest an explanation of the mechanism that leads to the lack of \(\text{Mg}^{2+}\), in patients with primary hyperaldosteronism. It seems probable that aldosterone directly affects the cell and modifies the cellular homeostasis of magnesium, probably through modification of the activity of the Na\(^+-\text{Mg}^{2+}\)antiporter of the plasmatic membrane. There seems to be a different mechanism to explain the negative magnesium balance in cases of hyperaldosteronism, an alternative to the hypothesis that attributes the stimulus to increase renal clearance of the ion to the chronic volume expansion.\(^5\) Moreover, the EC\(_{50}\) values found for the effect of aldosterone on \([\text{Mg}^{2+}]\) could suggest that aldosterone has a role of physiological regulator of the \([\text{Mg}^{2+}]\).

A decrease in \([\text{Mg}^{2+}]\) may play a role in the pathogenesis of arterial hypertension in primary aldosteronism in that it is well known that in vitro magnesium deficiency is followed by an increase in vascular tone and potentiates the pressor effect of angiotensin II.\(^{24}\) Despite this fact, the link between magnesium homeostasis and blood pressure regulation remains controversial.

Finally, although it was not the principal issue of our study, we measured total plasma calcium as well as \([\text{Ca}^{2+}]\). We did not find any statistically significant differences in total calcium plasma levels and \([\text{Ca}^{2+}]\) between patients with primary aldosteronism and normotensive control subjects. Our results, which are limited to total calcium plasma levels, are in agreement with those of Resnick and Laragh.\(^{10}\)

In conclusion, we have shown, for the first time, an \(\text{Mg}^{2+}\) deficiency in patients with primary aldosteronism. Moreover, this hormone shows in vitro a specific effect on the cell model by significantly decreasing \([\text{Mg}^{2+}]\) via a mechanism that is probably genomic. It is therefore possible to hypothesize that the magnesium deficiency found in subjects with primary hyperaldosteronism is related to a direct effect of aldosterone on the cells. Furthermore, the EC\(_{50}\) value for the action of aldosterone on \([\text{Mg}^{2+}]\) suggests that aldosterone may play a role as physiological regulator of the \(\text{Mg}^{2+}\) content.

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References

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