Exchangeable Magnesium Pool Masses in Spontaneously Hypertensive Rats

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We explored magnesium (Mg) metabolism by determination of exchangeable Mg pool masses and Mg kinetic parameters using stable Mg isotopes in spontaneously hypertensive rats (SHRs). Classical intracellular and extracellular Mg status biomarkers were also measured. Male SHRs and their male Wistar Kyoto (WKY) controls were fed a semipurified diet containing Mg 550 mg /kg for 2 weeks. Each rat received then an intravenous injection of 1.37 mg ^{25}Mg. The plasma ^{25}Mg disappearance curve over the next 7 days was used to measure the mass and fractional transport rate of 3 rapidly exchanging Mg metabolic pools, M1, M2, and M3. In the SHRs, plasma and erythrocyte Mg levels and urinary Mg excretion were not modified compared with their control WKYs, but tibia Mg level was significantly lower in the SHRs. Pool M3, the deep tissue pool, was significantly lower in SHRs compared with WKYs, but pools M1 and M2, the extracellular Mg pools, were statistically similar. The fractional transport rate of Mg from M1 to M2 and from M2 to M1 in the SHRs was higher than in the controls. The half-life of M1 was significantly decreased in SHRs compared with WKYs. In conclusion, this work demonstrates a decrease in intracellular Mg stores in SHRs compared with WKYs and disturbance of Mg exchanges in extracellular Mg, confirming a Mg metabolism disturbance in spontaneously hypertensive rats. Further work is now needed to elucidate the origin of the Mg depletion in SHRs and to explore Mg pools in hypertensive patients.

MAGNESIUM (Mg) is the second most abundant intracellular cation and is involved in numerous biological processes. Abnormalities in Mg metabolism appear to play important roles in ischemic heart disease, congestive heart failure, sudden cardiac death, a number of cardiac arrhythmias, vascular complications in diabetes mellitus and preclampsia-eclampsia, and hypertension.[1,2] The exact cellular mechanisms underlying the modulatory actions of Mg on vascular function are unclear, but experimental studies support the importance of Mg in the contractile and mechanical functions and on the structure of blood vessels, and indicate that Mg can change blood pressure mainly by affecting peripheral resistance.[3,4] In cardiac and vascular cells, Mg has an important regulatory role on potassium and calcium channels, on ion transporters, and on enzymes that utilize adenosine triphosphate (ATP) and that require Mg for substrate formation (Na-K-ATPase, Ca-ATPase). Moreover, Mg is competitive with calcium for intracellular binding sites, and can significantly modulate intracellular signal transduction system. Therefore, a slight change in Mg concentration may influence intracellular free concentrations of sodium, potassium, and calcium, modulating in turn cardiac excitability and vascular contraction.[4] The role of Mg in activation of calcium-activated potassium channel has been recently observed.[5]

Numerous studies demonstrated that patients with hypertension of diverse etiologies can exhibit hypomagnesemia in serum and/or in tissues.[2] The spontaneously hypertensive rat (SHR), a model for studying essential hypertension, also exhibits lower intracellular and extracellular Mg levels in most studies.[6-10] In view of such functions, it was suggested that hypertension could be the consequence of the reduced Mg status. Of particular interest is the observation that long-term Mg supplementation significantly attenuates the development of hypertension in SHR. Furthermore, intracellular Mg deficiency and calcium overlaod in SHR are normalized by Mg treatment.[11] However, some investigators have failed to observe Mg deficiency in the SHR.[12-15] Since the effects of Mg status on blood pressure are still the subject of much debate and remain contradictory, a good assessment of Mg status in this experimental model of essential hypertension seems of particular interest. Classical Mg status parameters are known to have low sensitivity or specificity, and are not informative about intracellular Mg exchange and dysfunction. In the 1960s, the radioisotope ^{28}Mg was used as a research tool to assess Mg metabolism in hypertensive patients, but results were not conclusive.[16,17] Recently, with improvement in analytical techniques, the use of stable isotopes of Mg has been developed.

In this work, we explored Mg metabolism by determination of exchangeable Mg pool masses and Mg kinetic parameters using stable Mg isotopes in SHRs. Classical intracellular and extracellular Mg status biomarkers were also measured.

MATERIALS AND METHODS

Reagents and Equipment

Enriched Mg (96.7% ^{25}Mg, 2.2% ^{24}Mg, and 1.1% ^{26}Mg) as oxide was obtained from Chemagas, (Paris, France). Suprapure HNO_3, suprapure H_2O_2, suprapure HCl, lanthanum oxide, and standard solutions of Mg (1 g/L) were obtained from Merck (Darmstadt, Germany). All other chemicals were of the highest quality available, and demineralized water was used throughout. The Mg isotope ratio measurements were performed using an inductively coupled plasma/mass spectrometry (ICP/MS) instrument (Plasma Quad-II System, Fisons Instruments, Manchester, UK), equipped with a Meinhard nebulizer. A Perkin Elmer 560 atomic absorption spectrometer (Perkin Elmer, St-Quentin-en-Yvelines, France) was used for total Mg measurements.

Animals and Diets

Male SHRs and their male Wistar Kyoto (WKY) controls (IFFA-CREDO, L'Arbresle, France) weighing approximately 320 g and aged

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Submitted July 13, 2002; accepted November 13, 2002.

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0026-0495/03/5205-0016$30.00/0
doi:10.1053/meta.2003.50104

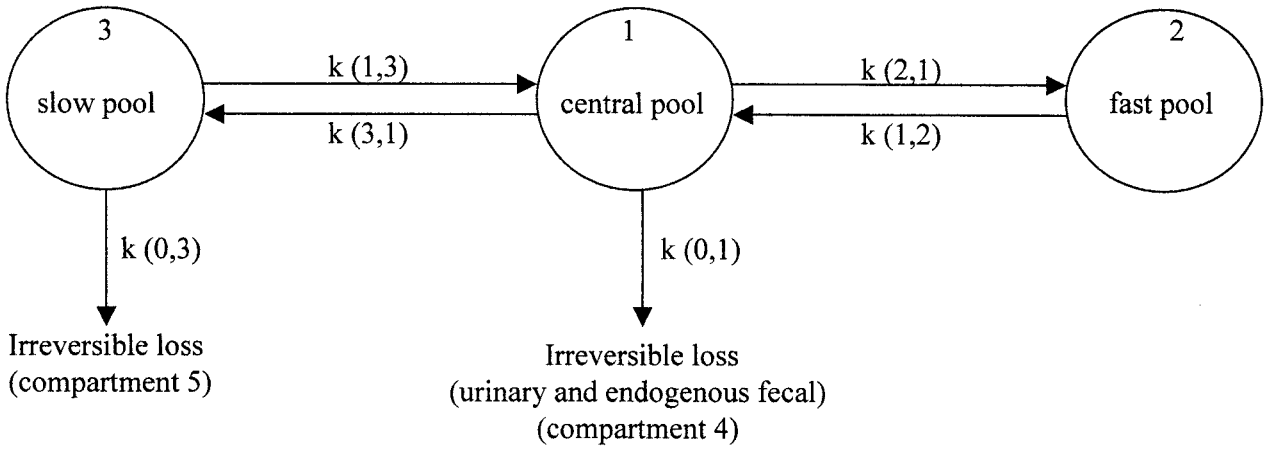

Fig 1. Three-compartmental model of Mg kinetics from Avioli and Berman.[20] Arrows represent intercompartmental movements of the cation as determined by appropriate rate constants and irreversible loss.

15 weeks were used. They were housed under conditions of constant temperature (20 to 22°C) and humidity (45% to 50%) with a standard dark cycle (8 PM to 8 AM). Our institutional guidelines for the care and use of laboratory animals were observed.

The rats were fed a semipurified diet in semiliquid form (water/powder, 1:1 wt/wt) containing 550 mg/kg of Mg for 2 weeks and had free access to distilled water and food. The semipurified diet contained (g/kg): casein 200, starch 650, corn oil 50, alphacel (cellulose) 50, DL-methionine 3, choline bitartrate 2, modified AIN-76 mineral mix 35, and AIN-76A vitamin mix 10 (ICN Biomedicals, Orsay, France).[18] Mg level in the experimental diet was checked by flame atomic absorption spectrometric analysis (Perkin Elmer 560, Norwalk, CT), and 24-hour urine samples were collected the day before isotope injection.

Daily food intake was monitored by weighing the offered meal (30 g powdered fresh diet + 30 mL of distilled water) and the food remaining 24 hours later for each animal. The remaining food and a sample of the offered meal were dried for 72 hours at 80°C to take into account the water proportion present in both samples. This allows determination of the net food intake as dry weight of the used diets. This was determined at least twice for each animal.

Preparation of Stable Isotope Solution

An aliquot of 328 mg of enriched MgO, equivalent to 200 mg of Mg, was moistened with 2 mL of demineralized water, and 2 mL of 12-mol/L HCl (suprapure) was added to convert the oxide into soluble $MgCl_2$. The solution was then neutralized with 2 mL of 1-mol/L NaOH and 25 mL of 1.67-mol/L $NaHCO_3$. The solution was then completed to 100 mL with physiological saline (0.9% NaCl). The pH of the resulting solution was 7.0. The concentration of Mg in this solution was 2.00 mg/mL. Total Mg concentration and isotope ratio were checked before use.

Isotope Injection and Sampling

After 2 weeks on the experimental diets, each rat received an intravenous injection of 1.37 mg ^{25}Mg. Because the number of blood samples obtainable from each rat was limited, it was necessary to use 14 rats per group. Half of each group was sampled at the following times: 15, 30, 60, 90 minutes and 2, 3, 4, and 6 hours after ^{25}Mg injection (short-term kinetics). The other half of each group was sampled at 6 hours and 1, 2, 3, 4, 5, and 7 days after ^{25}Mg injection (long-term kinetics). Rats in both groups were anesthetized at each time point, and blood (0.5 mL) was collected through a catheter in the left carotid for the short-term kinetics and from the retro-orbital sinus for the long-term kinetics. Blood samples were centrifuged and the plasma was separated. Erythrocytes were collected at 6 hours and 7 days, washed with saline solution, and hemolyzed. The anesthetized rats were then euthanized, and the tibia was collected at the end of the short-term and long-term kinetics for total Mg determination.

Analysis

The ^{25}Mg concentration of the plasma samples was determined by ICP/MS.[19] Before analysis, the plasma was diluted in 14 mmol/L HNO_3, and natural Mg and beryllium were used as external and internal standards, respectively. Total Mg concentrations in the analyzed samples were adjusted to about 50 μg/L to ensure reliable measurements. Within- and between-run percentage residual standard deviations were, respectively, 0.45% and 0.71% for $^{25}Mg/^{26}Mg$ of Mg standard solution and 0.58% and 0.86% for plasma dilution.

For total Mg determination, plasma and hemolyzed erythrocytes were simply appropriately diluted in 0.1% of lanthanum chloride. Urine was acidified with pure HNO_3 (final concentration, 14 mmol/L) and then diluted in 0.1% of lanthanum chloride. For tibia, kidney, and heart analysis, the sample was first dried, dry-ashed, taken up with HNO_3 and H_2O_2, heated at 110°C, and then dried down. The dry residue was then taken up with concentrated HNO_3 and appropriately diluted in 0.1% of lanthanum chloride. Mg concentration was determined by atomic absorption spectrophotometry (Perkin Elmer 560) at 285 nm. Appropriate quality controls have been run with each series of measurement. Within- and between-run percentage residual standard deviations were, respectively, 2.5% and 3.71% for Mg standard solution, 3.2% and 5.1% for plasma Mg, 3.7% and 5.6% for erythrocyte hemolysate Mg, and 3.5% and 4.9% for tissue Mg measurements.

Kinetic Analysis

Mg kinetics were determined using a multicompartmental model as described by Avioli and Berman[20] and Sojka et al.[21] The model is schematized in Fig 1. Compartmental modeling of the data was performed with the aid of the SAAM II (Stimulation, Analysis, and Modeling) program (SAAM Institute, Seattle, WA).[22] Plasma data were expressed as tracer/tracee, with tracer = (^{25}Mg from the injection) and tracee = (Mg total − ^{25}Mg from the injection). Because half the rats in each group participated in either the short-term kinetics (0 to 6 hours) or the long-term kinetics (6 hours to 7 days), one rat from the short-term kinetics and one rat from the long-term kinetics were cou-

Table 1. Characteristics of the SHR and WKY Rats

	WKY	SHR
Plasma Mg (mmol/L)	0.751 ± 0.128	0.756 ± 0.126
Erythrocytes Mg (mmol/L)	2.26 ± 0.06	2.30 ± 0.27
Heart Mg (mmol/kg dry weight)	27.6 ± 0.9	26.9 ± 0.7
Urinary Mg (mmol/24h)	0.112 ± 0.023	0.106 ± 0.020
Renal Mg (mmol/kg dry weight)	35.8 ± 2.4	34.2 ± 1.2
Tibia Mg (mmol/kg dry weight)	209 ± 8	189 ± 13*†

NOTE. Results are means ± SD, n = 14.
*$P < .05$.
†$P < .01$.

pled according to their ^{25}Mg plasma values to perform complete kinetic analysis. The mass of the different pools (M1, M2, M3), the fractional transport rate (exchange constant between pools [k1,2; k2,1; k1,3; k3,1] and the irreversible loss of Mg from M3 [k 0,3]) were determined from the model using the SAAM II program. Irreversible loss from M1 (k 0,1) was calculated using the 24-hour urinary excretion values obtained in this experiment. Endogenous fecal Mg losses were not taken into account in this calculation because they have been shown to contribute very slightly to the total Mg pool turnover (<2% in human).[23] The half-life of the 3 exchangeable pools was determined from a 3-exponential curve using the SAAM II program.

Statistical Analysis

Results were expressed as means ± SD. The statistical significance of differences between means was assessed using Student's *t* test. The limit of statistical significance was set at $P < .05$. Statistical analyses were performed using the GraphPad program (V3.00, GraphPad Software, San Diego, CA).

RESULTS

Characteristics of the Rats and Conventional Biomarkers of Mg

The weight of the SHRs and WKY rats was the same at the beginning of the isotope study (370 ±16 g and 362 ±17 g, respectively). Food intake was not different between SHR and WKY (20.1 ± 2.7 mg/d and 17.8 ± 1.9 mg/d, respectively, n = 7, $P = .0977$). Plasma, erythrocytes, and heart and renal Mg levels and urinary Mg excretion did not differ significantly in the 2 rat strains, while tibia Mg contents were significantly lower in SHRs than in WKYs (Table 1).

Compartmental Model of Mg Metabolism

Semilogarithmic plots of the model fitted to plasma data for SHR and WKY are shown in Fig 2. The pattern of the curve was outwardly similar for the 2 experimental groups, showing a rapid disappearance of tracer during the first 10 hours, followed by a slow decline lasting for at least 170 hours. However, we clearly noted that all values of tracer/tracee were greater for SHRs than for WKYs.

Mg exchangeable pools M1 and M2 are primarily composed of extracellular fluid Mg, while M3 represents intracellular Mg.[17] Pool M3 was significantly smaller in SHRs than in WKYs (Table 2) and accounted for 83% of total exchangeable Mg in WKYs and for 81% in SHRs. However, no differences were observed for M1 and M2 between the 2 groups.

Fractional transport rates of Mg from M1 to M2 and from M2 to M1 (k2,1 and k1,2) were significantly increased in SHR compared with WKY (Table 2), while all the other exchange constants measured were statistically similar.

Mg half-life from M1 was significantly lower in SHRs than

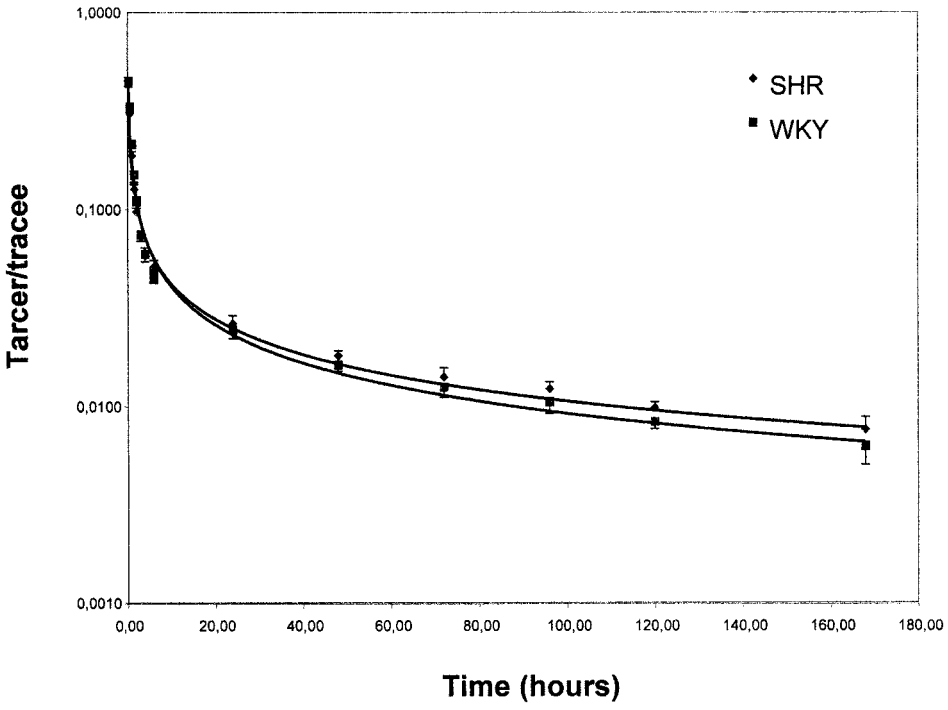

Fig 2. Semilogarithmic plot of the model fit to plasma data for WKY and SHR. Because half of the rats in each group were sampled at either the short-term (0-6 h) or long-term kinetic (6 h-7 d), 1 rat from the short kinetic and 1 from the long kinetic were coupled according to their closed ^{25}Mg plasma values. Plasma data were expressed as tracer/tracee. Compartmental modeling of the data was performed with the aid of the SAAM II) program.[22]

Table 2. Calculated Tricompartmental Model Parameters of the SHR and WKY Rats

	WKY	SHR
Mass of compartment		
M1[a] (mg)	2.54 ± 0.11	2.50 ± 0.26
M2[a] (mg)	8.40 ± 1.96	8.38 ± 2.17
M3[a] (mg)	53.8 ± 6.5	45.4 ± 3.6*
Exchange constant		
k1,2[b] (h^{-1})	0.188 ± 0.029	0.243 ± 0.042*
k2,1[b] (h^{-1})	0.606 ± 0.074	0.816 ± 0.096†
k1,3[b] (h^{-1})	0.017 ± 0.005	0.019 ± 0.004
k3,1[b] (h^{-1})	0.339 ± 0.056	0.375 ± 0.079
Irreversible loss		
k0,1[c] (h^{-1})	0.044 ± 0.005	0.044 ± 0.007
k0,3[d] (h^{-1})	0.006 ± 0.002	0.006 ± 0.001
Half-life		
T1[e] (h)	0.622 ± 0.017	0.494 ± 0.034†
T2[e] (h)	9.77 ± 2.66	8.40 ± 2.08
T3[e] (h)	94.2 ± 18.7	104 ± 18

NOTE. Results are means ± SD, n = 7.

*$P < .05$.

†$P < .001$.

[a]M1, M2, and M3 are the mass of the different exchangeable pools and were determined from the model of Avioli and Berman[20] using the SAAM II program. M1 and M2 are primarily composed of extracellular fluid Mg, while M3 represents intracellular Mg.

[b]k1,2, k2,1, k1,3, and k3,1 are the fractional transport rate and were determined from the model of Avioli and Berman[20] using the SAAM II program.

[c]k 0,1 is the irreversible loss from pool 1 and was approximated using the urinary excretion values obtained from previous experiments in our laboratory (data not shown).

[d]k 0,3 is the irreversible loss of Mg from pool and was determined from the model of Avioli and Berman[20] using the SAAM II program.

[e]T1, T2, and T3 are the half-lives of pools 1, 2, and 3 and were determined from the model of Avioli and Berman[20] using the SAAM II program.

in WKYs (Table 2). Mg half-lives from M2 and M3 were not different between the 2groups of rats.

DISCUSSION

We evaluated in SHRs the mass of the exchangeable Mg pools, the Mg half-life in these pools, and the Mg kinetic parameters using stable Mg isotopes and also the conventional Mg status biomarkers. In the studied SHRs, plasma and erythrocyte Mg levels and urinary Mg excretion were not different from those of their WKY controls. On the other hand, tibia Mg levels were significantly lower in SHRs (−10%). Our results agree with those of Wallach and Verch,[6] who observed no difference in the plasma Mg concentration between SHR and WKY but found a significant 6% to 16% decrease in tissue Mg content in the SHRs for kidney, heart, lung, and tibia. On the contrary, Berthelot et al[7] reported decreased serum Mg levels and decreased urinary Mg excretion in SHRs compared with WKYs, and Henrotte et al[8] described lower erythrocyte Mg levels in SHRs than in WKYs. However, Berthelot et al[7] observed that urinary Mg in SHRs was decreased during the development of hypertension, but when the hypertension was established (10 weeks) there was no difference between SHRs and WKYs. As rats were 17 weeks old in our experiment, no difference in urinary Mg excretion in the studied SHRs was consistent with Berthelot's observation.[7] Even if studies of blood and urinary Mg values yielded conflicting results according to the literature, most of them demonstrated abnormalities of Mg metabolism in SHRs.

Mineral metabolism is ideally explored by compartmental analysis, which allows rates of mineral transfer and sizes of mineral stores to be calculated accurately.[24] In the 1960s, Silver et al[16] explored exchangeable Mg pools in patients suffering from hypertension. They used ^{28}Mg as a tracer, but as he did not compare them with normal individual values, a modification of pool size in hypertension could not be proved. Some years later, Bauer et al[17] observed decreased exchangeable Mg in hypertensive men compared with healthy subjects, but not in hypertensive females. Their results in hypertensive women were difficult to interpret accurately, probably because of obesity in this group. Moreover, compartmental analysis of Mg using ^{28}Mg was imprecise because of the short half-life of ^{28}Mg (21.3 hours) and did not allow Silver et al[16] or Bauer et al[17] to determine turnover rates of Mg in the slowly exchangeable pool, or fractional transport rates between pools. The results of these 2 studies were therefore not conclusive. To our knowledge, no other kinetic analysis on Mg body pools has been performed in hypertension on an experimental animal model or in human patients using ^{28}Mg as a tracer. Recently, with improvements in analytical techniques, the use of the stable Mg isotopes ^{25}Mg and ^{26}Mg has been developed to explore exchangeable Mg pools. However, so far only few studies have been performed in animal models or in humans. Studies in Mg-deficient rats demonstrated that determination of exchangeable Mg pool sizes does appear sufficiently sensitive to assess Mg status in both marginal and severe Mg deficiency.[25-27] Sojka et al[21] and Abrams and Ellis[23] determined Mg pool sizes in children and adolescents and observed that the body fat-free mass positively correlates with the exchangeable Mg pool masses in children, demonstrating the importance of accounting for growth in determining a subject's Mg utilization. The exchangeable Mg pool sizes were also determined in healthy women, before and after 8-week Mg supplementation, and it was observed that Mg pool size was not affected by the supplementation, probably because Mg stores were full before Mg supplementation.[28]

For a better characterization of modifications of Mg metabolism in hypertension, we explored exchangeable Mg pools, using stable Mg isotopes, in SHRs. To analyze Mg kinetics, we used a compartmental model based on the model of Avioli and Berman.[20] In this model, there are 3 exchangeable magnesium pools with varied rates of turnover. Pools M1 and M2 represent pools with a relatively fast turnover. Together, these 2 pools approximate extracellular fluid in distribution. M3 is an intracellular pool, with a slower turnover. There is also a fourth pool, which represents urinary excretion and endogenous fecal loss, and a fifth pool, which is a loss pathway representing deposition into tissues.[21]

We found that pool M3 was significantly (12 %) lowered in SHRs compared with WKYs. On the other hand, pools M1 and M2 were statistically similar. In both SHRs and WKYs, the M3 accounted for more than 80% of exchangeable Mg. The third pool of exchangeable Mg represents the tissue Mg level (mostly bone and muscle Mg) that the conventional biochem-

ical markers of Mg fail to assess. Thus, the size of the exchangeable pools reflects the state of Mg storage in the body better than plasma and erythrocyte levels of Mg. The fractional transport rate of Mg from M1 to M2 and from M2 to M1 in SHRs was a quarter higher than in the control rats. Thus in hypertensive rats, an increase in the fractional flux of extracellular Mg was demonstrated. The half-life of M1 was significantly (21%) decreased in SHRs compared with WKYs, confirming alteration of Mg exchanges in extracellular compartments. As the exchangeable Mg pool M3 was lower in SHRs than in WKYs, while Mg urinary excretion was not modified, alteration of Mg absorption in SHRs can be suspected. Berthelot et al[7] demonstrated that apparent intestinal Mg absorption was not modified in SHRs during development of hypertension or when hypertension was well established, suggesting that there may have been a shift of Mg into tissues. However, our results were not concordant with this conclusion. A decrease in Mg intake might also explain the lower exchangeable Mg pool M3 in SHRs by comparison with WKY. In fact, we demonstrated lower M3 mass in rats fed Mg-deficient diet by comparison to that in rats fed Mg-sufficient diet.[25,26] Moreover, it was shown that diet induced hypomagnesemia aggravated the hypertension of SHR.[15] However, control of food intake in this study failed to reveal any difference between the 2 groups. Therefore, the origin of Mg depletion is still unclear. Further studies need to be conducted with stable isotopes to explore true Mg absorption in SHRs.

In conclusion, this is the first time that kinetic data from compartmental analysis performed with stable Mg isotope have been obtained in SHRs, and more generally in an animal model of hypertension. This work demonstrates a decrease in intracellular Mg stores in SHRs compared with WKY and disturbed Mg exchanges in extracellular Mg, confirming Mg metabolism disturbance in spontaneously hypertensive rats. Studies are now needed to clarify the origin of the Mg depletion in SHRs and to explore Mg pools in hypertensive patients.

ACKNOWLEDGMENT

The authors thank D. Bayle, A. Bellanger and J.C. Tressol for technical assistance.

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