Magnesium Uptake by Intestinal Brush-Border Membranes of Spontaneously Hypertensive Rats (43489)

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Abstract. **Magnesium uptake by intestinal brush-border membranes (BBM) was studied in duodenal and jejunal vesicles of the spontaneously hypertensive rat (SHR) and normotensive control, the Wistar-Kyoto (WKY) rat. In the duodenum, no statistical difference was evidenced between the two types of rats. By contrast, initial rates of magnesium uptake in jejunal vesicles were lower in SHR (5.4 ± 2.1 nmol/mg protein × 10 sec) in comparison to WKY rats (11.0 ± 2.5 nmol/mg protein × 10 sec) at a magnesium concentration of 1 m*M* ($P < 0.01$). In jejunal BBM, kinetic analysis of magnesium uptake showed three components in WKY rats, with one being diffusional. In SHR, only two components were seen, with the diffusional one being absent. The two saturable components showed V_{max} of 6.5 ± 1.3 and 26.2 ± 6.0 nmol/mg protein × 10 sec and apparent K_m of 0.22 ± 0.12 m*M* and 1.9 ± 0.4 m*M* in WKY rats, and V_{max} of 10.9 ± 3.5 and 14.8 ± 5.9 nmol/mg protein × 10 sec and apparent K_m of 0.43 ± 0.23 m*M* and 1.3 ± 0.2 m*M* in SHR. Only the component with the lowest apparent affinity appeared statistically different in SHR as compared with WKY rats for both V_{max} and apparent K_m ($P < 0.05$). Time course evolution of magnesium uptake in jejunal BBM indicated, by extrapolation at zero time, that 2.5 and 5.1 nmol magnesium/mg protein in SHR and WKY rats, respectively, would be in the bound state. The study of the influence of medium osmolarity on 60-min magnesium uptakes was also indicative of a smaller binding compartment in jejunal BBM of SHR (3.70 and 8.26 nmol/mg protein in SHR and WKY rats, respectively); at the four osmolarities assayed, the 60-min uptakes were significantly lower in SHR as compared with WKY rats ($P < 0.01$). From 60-min glucose uptakes, a smaller volume of jejunal BBM vesicles was determined for SHR as compared with WKY rats (0.34 ± 0.06 and 0.63 ± 0.17 μl/mg of protein in SHR and WKY rats respectively, $P < 0.05$), this volume being significantly augmented by the presence of 1 m*M* $MgCl_2$ (0.48 ± 0.05 and 1.27 ± 0.02 μl/mg of protein in SHR and WKY rats respectively, $P < 0.01$). These results suggest that magnesium uptake and binding by jejunal BBM are altered in SHR in comparison to WKY rats, implying a possible role of the small intestine in the abnormalities of magnesium metabolism in genetic hypertension.**

[P.S.E.B.M. 1992, Vol 201]

In genetic hypertension, abnormalities in Ca^{2+} and Mg^{2+} metabolism have been reported repeatedly (1–4) and an inverse relation between Ca^{2+} or Mg^{2+} daily intake and blood pressure, as well as a reduction of hypertension with Ca^{2+} or Mg^{2+} supplementation, has been documented in the human (5–7). In the spontaneously hypertensive rat (SHR), the apparition of hypertension can also be corrected by supplement in dietary Ca^{2+} or Mg^{2+} (8, 9), thus suggesting a possible intestinal malabsorption of these ions.

Intestinal Ca^{2+} transport across the whole mucosa of the SHR, studied by various techniques, has led to conflicting results (for references, see [10]). However, a reduced uptake of Ca^{2+} by duodenal mucosa (11), by isolated duodenal enterocytes (8), and by duodenal and jejunal brush-border membranes of SHR (12, 13), together with an impaired Ca^{2+} extrusion via the Ca^{2+}/

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Received January 15, 1992. [P.S.E.B.M. 1992, Vol 201]
Accepted April 28, 1992.

0037-9727/92/2011-0119$3.00/0

Na^+ exchanger by jejunal basolateral membranes, has been evidenced in SHR, the ATP-dependent pump being unaffected (10). A lower intracellular Ca^{2+} concentration found in duodenal cells of young SHR (8) and in jejunal cells of adult SHR (14) also points to an alteration of Ca^{2+} handling by the SHR intestine. Abnormal vitamin D metabolism and its relation to intestinal Ca^{2+} malabsorption (11) as well as deficiencies in two intestinal vitamin D-dependent proteins, alkaline phosphatase (15, 16) and calbindin-D_{9k} (16), indicate a defect in the vitamin D-dependent pathway for Ca^{2+} absorption in SHR.

Regarding intestinal Mg^{2+} transport in SHR and even in normal rats, little is known up to now. There is some evidence that Mg^{2+} absorbed in the small intestine would mainly follow a paracellular route (17). Nonetheless, the cellular pathway via the brush border seems to exist (17, 18) and could be efficient, possibly when dietary supplies are limited, as it would be the case for Ca^{2+} (19). A possible influence of vitamin D on Mg^{2+} absorption is also suspected (18).

On the other hand, structural abnormalities have been recorded in duodenal microvilli of SHR (20), and sound modifications of duodenal and jejunal brush-border-bound enzymes have been reported (14, 15, 21). These alterations could also participate in Ca^{2+} and Mg^{2+} malabsorption.

With uptake studies by intestinal brush-border membrane (BBM) vesicles currently being designed to investigate the entry process in transcellular absorption, the aim of the present work was to evaluate Mg^{2+} uptake by intestinal brush-border membrane vesicles of SHR and of their genetically matched control, Wistar-Kyoto (WKY) rats. Preliminary results have been published in an abstract (22).

Materials and Methods

Animals. Male SHR of the strain developed by Okamoto and Aoki (23) and age-matched normotensive male rats of the parent strain WKY were supplied by IFFA-CREDO (Les Oncins, France). The animals were received at the age of 3 weeks and housed at constant temperature (22°C) with free access to food (UAR 103, Animalabo) and water. Twelve-week-old animals were used in this study. Their systemic blood pressure was 15.6 ± 1.3 and 11.8 ± 1.6 mm Hg for SHR and WKY rats, respectively ($P < 0.01$), in agreement with previously published values (15). They were starved for 18 hr before they were sacrificed by cervical dislocation and decapitation; the digestive tract was excised and washed with an ice-cold solution composed of 142 m*M* NaCl, 1.1 m*M* $MgCl_2$, 0.9 m*M* $CaCl_2$, 1.5 m*M* KH_2PO_4, and 4.2 m*M* K_2HPO_4 (pH 7.2).

Preparation of BBM Vesicles. Mucosal scrapings of the duodenum and jejunum were collected on ice and BBM vesicles were prepared by Ca^{2+}-precipitation and differential centrifugation (24). Purification of the BBM was evaluated by their enrichment factor in alkaline phosphatase, which was close to 10 for all the membrane preparations used in this study. Electron microscope examination of BBM from SHR and WKY rats was also performed and the results were shown and discussed in another paper on brush-border alkaline phosphatase that was carried out with the same batch of rats and that has already been published (21). The last pellets of BBM were resuspended in the incubating medium further used for uptake measurements. The incubating medium was composed of 200 m*M* mannitol and 10 m*M* Tris-Hepes (pH 7.4). The samples were aliquoted, immediately frozen in a mixture of dry ice and cold methanol, and stored at −30°C.

Transport Studies. Uptake of radiolabeled Mg^{2+} was measured by a rapid filtration technique. Briefly, thawed vesicles were forced through a tuberculin syringe and preincubated for 30 min at 28°C. To initiate uptake, 10 μl of vesicle suspension containing 30–60 μg of protein were mixed with 90 μl of uptake medium containing ^{28}Mg (±1,5 μCi/uptake) and different concentrations of unlabeled $MgCl_2$ (as specified for each set of experiments), preincubated at 28°C. After the desired incubation time interval, the uptake was abruptly stopped by dilution in 2 ml of an ice-cold solution of the same composition as the uptake medium without ^{28}Mg and by immediate filtration of the sample on prewetted (with uptake medium + 1 mg/ml of bovine serum albumin) filters (Millipore, Type HA, 0.45 μm) under suction; then, the filter was rinsed quickly with 2 × 2 ml of the ice-cold solution and its radioactivity was counted by gamma scintillation. Radioactivity obtained by filtration of the same incubation medium without vesicles was substracted from the value of uptake. Radioactivity of the starting incubation medium without filtration was determined. The three values were obtained in duplicate within 10 min of counting, which allowed us to neglect the decay of the isotope (half-life: 20.3 hr) and to calculate the amount of magnesium that had been taken up. The volume of the vesicles was estimated from equilibrated glucose uptakes (60 min), using ^3H-D-glucose (1 μCi/uptake) and 0.1 m*M* unlabeled D-glucose.

^{28}Mg and [^3H]Glucose. ^{28}Mg was produced at the isochronous cyclotron of Louvain-la-Neuve (University of Louvain, Belgium) as tracer-free $^{28}MgCl_2$ once every 2 weeks, and transported to Liège University by our own means. D-[6-^3H(*N*)]Glucose (30.2 Ci/mmol) was from NEN research products (DuPont).

Protein Assay. Protein determination was carried out by the method of Bradford (25) using bovine serum albumin as standard.

Statistical Analysis. All results are expressed as means ± SD. Statistical differences between SHR and

WKY rats were analyzed using unpaired Student's *t* test.

Results

Magnesium uptakes were evaluated at 10 sec, as routinely done for Wistar rats, in BBM from duodenum and jejunum of SHR and WKY rats. As shown in Figure 1, for duodenal BBM from SHR and WKY rats, similar values were obtained. But in the case of jejunum, values at 10 sec, taken as representative of initial rates of uptake, were significantly lower in SHR compared with WKY rats ($P < 0.01$). A time course study of magnesium uptake was carried out in jejunal BBM from SHR and WKY rats. Clearly, the uptake was almost linear during the first minute, with a zero intercept and a slope much lower in SHR compared with WKY rats (Fig. 2); equilibrium was then quickly reached in SHR BBM, whereas in WKY rats, the uptake increased up to 30 min and then decreased (Fig. 3). In duodenal BBM vesicles, the uptake was rather linear during the first 20 sec, with a zero time intercept close to zero, and values at 30 and 60 min at equilibrium (data not shown).

Equilibrated values of glucose uptake at 60 min were determined in order to evaluate the volume of BBM vesicles from duodenum and jejunum of SHR and WKY rats. As shown in Figure 4, vesicles from the duodenum were larger than the jejunal ones ($P < 0.01$ in all cases), and vesicles from WKY rats were larger than the ones from SHR ($P < 0.05$), especially in the presence of Mg^{2+} ($P < 0.01$). Besides, the addition of 1 m*M* $MgCl_2$ to the medium increased the volume of vesicles only slightly in SHR (not significantly), but more importantly in WKY rats ($P < 0.01$).

Since in the first set of experiments, a difference between SHR and WKY was only recorded in the Mg^{2+}

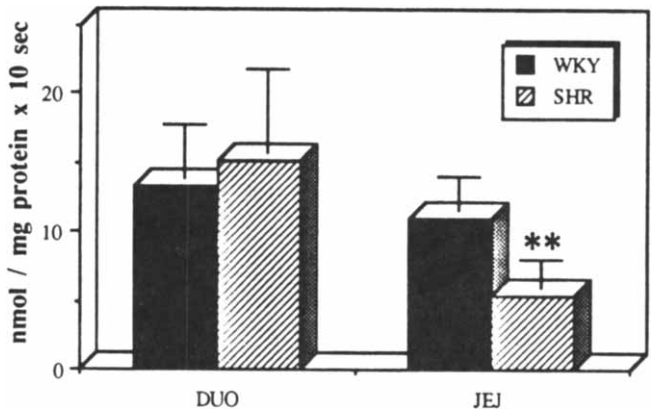

Figure 1. Mg^{2+} uptake by duodenal and jejunal BBM of SHR and WKY rats. Uptake was determined at 10 sec in the presence of 1 m*M* $MgCl_2$. DUO, duodenum; JEJ, jejunum. Values are means ± SD. $n = 6$ for each group of rats (six membrane preparations coming from six different animals) in the case of the duodenum and $n = 10$ in the case of the jejunum. Assays were done in duplicate. **SHR significantly less than WKY rats, $P < 0.01$.

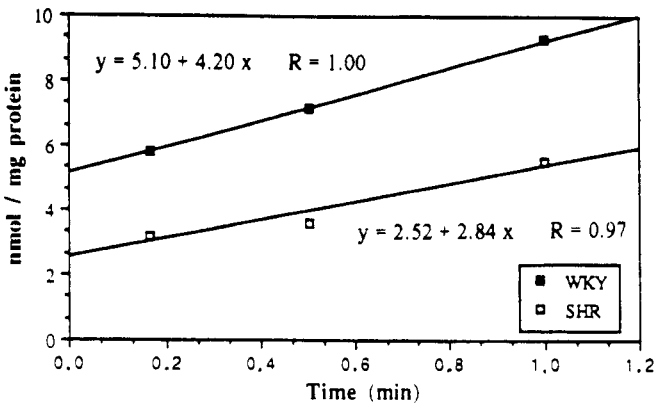

Figure 2. Initial rate of Mg^{2+} uptake in jejunal BBM of SHR and WKY rats. The $MgCl_2$ concentration was 1 m*M*. A single membrane preparation issued from four rats was assayed in duplicate for each group of rats. *y*, regression line; *R*, correlation coefficient.

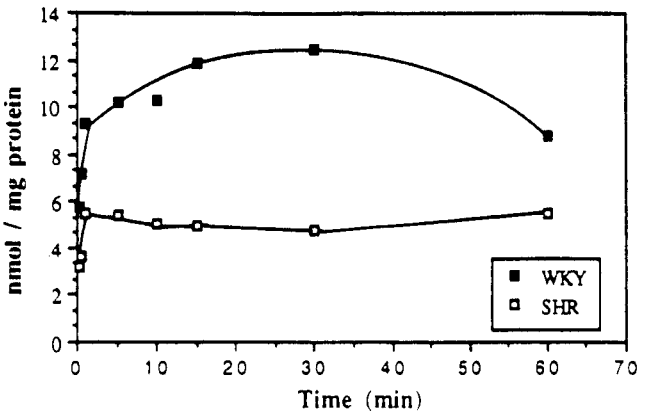

Figure 3. Time course of Mg^{2+} uptake in jejunal BBM of SHR and WKY rats. The $MgCl_2$ concentration was 1 m*M*. This experiment is the same as that in Figure 2.

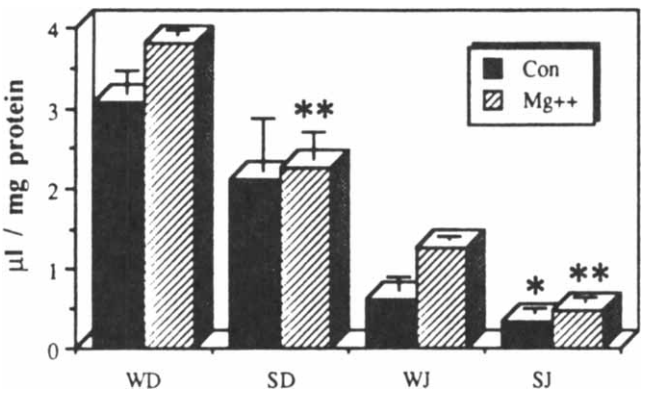

Figure 4. Volume of duodenal and jejunal BBM of SHR and WKY rats, as determined from 60-min glucose uptakes. The glucose concentration was 0.1 m*M*. Con, control. Mg^{2+}, in the presence of 1 m*M* $MgCl_2$. Values are means ± SD. $n = 4$ for each group of rats (four membrane preparations issued from four different rats). All assays were done in duplicate. WD, duodenal BBM from WKY rats; SD, duodenal BBM from SHR; WJ, jejunal BBM from WKY rats; SJ, jejunal BBM from SHR. *SHR significantly less than WKY rats, $P < 0.05$. **SHR significantly less than WKY rats, $P < 0.01$.

uptake by jejunal vesicles, the investigation was further pursued in the jejunum. The influence of $MgCl_2$ concentration was studied (Fig. 5). In SHR BBM, a saturation kinetics was clearly evidenced, whereas in WKY rats, a combination of saturation and diffusional processes seemed to be involved. Computerized linearization of the 10-sec uptakes at 10 concentrations of $MgCl_2$ up to 5 mM was undertaken for each animal preparation of BBM. Apparent K_m and V_{max} are shown in Table I. They were obtained from Hanes-Woolf plots (S/v as a function of S) corresponding to S-multiplied Lineweaver-Burke plots and considered as more reliable than the classical Lineweaver-Burke plots. Two components were identified, both of rather low affinity. Although no statistical difference between SHR and WKY rats could be evidenced for the first one, an apparent higher affinity ($P < 0.05$) but a lower capacity ($P < 0.05$) were recorded for the second one in SHR vesicles as compared with WKY ones.

The positive intercept of the initial rate of uptake observed in jejunal vesicles gave an estimate of instantaneous binding to the membrane (Fig. 2). A half smaller value was observed in SHR as compared with WKY rats (2.52 and 5.10 nmol/mg of protein, respectively). An important binding component was also assessed in the 60-min uptakes by the study of the influence of medium osmolarity. Indeed Mg^{2+} accumulation was shown to decrease as osmolarity was increased (Fig. 6). Zero intercept, corresponding to infinite osmolarity and to zero vesicular volume, is usually considered as representative of the membrane-bound fraction. As compared with WKY jejunal vesicles, SHR ones had a much lower binding component. Besides, the total uptakes at 60 min were significantly lower in SHR at all osmolarities ($P < 0.01$). Finally, the slopes of the regression lines were different in SHR and WKY rats, also indicative of larger volumes in the latter.

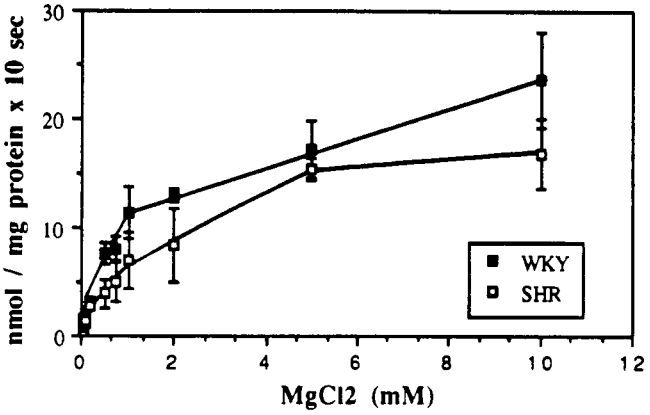

Figure 5. Kinetics of Mg^{2+} uptake by jejunal BBM of SHR and WKY rats. Mg^{2+} uptake was determined at 10 sec. Values are means ± SD. $n = 4$ for each group of rats (four membrane preparations issued from four different rats). The whole range of concentrations was assayed for each rat. All assays were done in duplicate. Slope of linear component in WKY rats is 1.32 ± 0.24 nmol/10 sec × mg protein.

Table I. Apparent K_m and V_{max} of 10-sec Mg^{2+} Uptake by Jejunal BBM Vesicles from SHR and WKY Rats[a]

	WKY	SHR
First component		
K_m (mM)	0.22 ± 0.12	0.43 ± 0.23
V_{max} (nmol/mg protein × 10 sec)	6.5 ± 1.3	10.9 ± 3.5
Second component		
K_m (mM)	1.9 ± 0.4	1.3 ± 0.2[b]
V_{max} (nmol/mg protein × 10 sec)	26.2 ± 6.0	14.8 ± 5.9[b]

[a] Results are means ± SD. The whole range of concentrations was assayed in four BBM preparations issued from four different animals for each type of rats. All assays were performed in duplicate. All values are calculated from regression analysis of Hanes-Woolf plots.
[b] SHR significantly less than WKY, $P < 0.05$.

Discussion

^{28}Mg is a short-lived isotope (20.3 hr) produced rarely, in only a few cyclotrons in the world, at a very high cost; frozen BBM vesicles are routinely used to get the experiments started as soon as the isotope reaches the laboratory. On occasion (when the radioactivity of the isotope production is sufficiently high), duplicate measurements of 10-sec and 60-min magnesium uptakes by fresh and frozen (for 24 hr) duodenal and jejunal BBM vesicles of the same Wistar rat could be carried out. Provided that the frozen BBM are thawed, resuspended through a tuberculin syringe, and preincubated for 30 min at 28°C, values of uptakes at 10 sec as well as at 60 min are about 20% smaller than those in the freshly prepared vesicles. But a 24-hr, 2-week, or 1-month freezing period, for the same membrane preparation, gave similar results of uptake (data not shown).

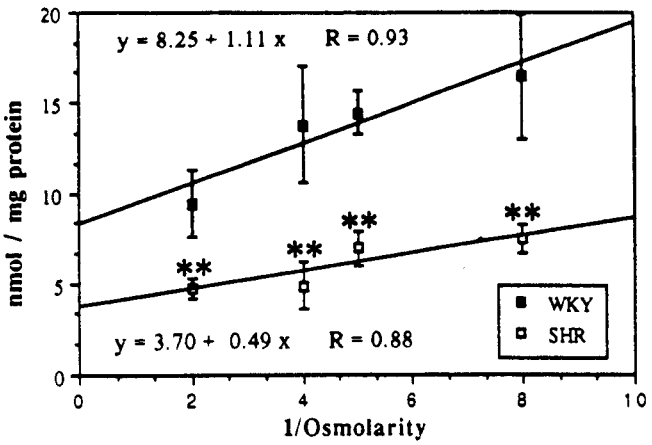

Figure 6. Influence of medium osmolarity on Mg^{2+} uptake by jejunal BBM of SHR and WKY rats. Vesicles were prepared in the usual medium. Uptake values at 60 min were determined in the presence of increasing concentrations of mannitol + 1 mM $MgCl_2$. Values are means ± SD. $n = 4$ for each group of rats (four membrane preparations issued from four different rats). All assays were done in duplicate. **SHR significantly less than WKY rats, $P < 0.01$.

The possibility that freezing differently affects the vesicles of SHR and WKY rats cannot be discarded.

It is shown here that Mg^{2+} uptake at 10 sec is significantly lower in jejunal BBM vesicles of SHR than in the ones of WKY rats (Fig. 1). In WKY rats, this uptake results from the operation of three mechanisms; one is diffusional and the other two are saturable. In SHR, the diffusional component tends to disappear and the saturable mechanism with a low apparent affinity site has a lower V_{max} as well as a higher affinity than in WKY rats (Fig. 5 and Table I). These different characteristics of the SHR initial rate of uptake in jejunal BBM could be responsible, at least in part, for Mg^{2+} malabsorption. It is, however, worth noting that, especially at the level of the jejunum, there is an important fraction of the 10-sec uptake that represents bound Mg^{2+}. A smaller binding component in SHR as compared with WKY rats is also assessed from the 60-min uptakes at different osmolarities (Fig. 6). From 60-min glucose uptakes, a lower vesicle volume is found in SHR as compared with WKY rats (Fig. 4). But different vesicle volumes do not affect the 10-sec uptake, as is the case here in duodenal vesicles.

Whether or not the bound fraction recorded in the 10-sec Mg^{2+} uptakes by jejunal vesicles plays a role in transport remains an unanswered question. Important external binding of Mg^{2+} has been evidenced in Wistar jejunal BBM vesicles by microdialysis (unpublished data) but do not significantly interfere in the radioactive measurements of uptake by the washout procedure in the presence of unlabeled Mg^{2+}. Binding to a membrane protein responsible for mediated uptake could also be invoked, but given the size of the binding component, it seems more reasonable to postulate that binding occurs at the internal side of the vesicle after transport has taken place, and, along this latter hypothesis, apparent kinetic data reported in Table I would characterize transport rather than binding.

No differences between SHR and WKY rats are seen in 10-sec uptakes by duodenal BBM vesicles. In contrast, Ca^{2+} uptakes by both duodenal and jejunal BBM are significantly lower in SHR in comparison to WKY rats (12, 13). A defect in intestinal vitamin D-dependent calbindin-D_{9k} (16) and in alkaline phosphatase (14–16, 21) recorded in SHR has been associated with Ca^{2+} malabsorption. Moreover, bound and purified alkaline phosphatase from duodenal and jejunal BBM of SHR and WKY rats has been shown to be stimulated by $MgCl_2$, but the apparent affinity for Mg^{2+} is smaller by half in SHR (21). However, it has never been ascertained whether alkaline phosphatase plays a part in Mg^{2+} absorption.

It is interesting to point out that values of initial Ca^{2+} uptakes, at 1 m*M* $CaCl_2$ in the incubating medium, are within the same range as the ones of Mg^{2+} uptakes, with slightly higher values in the duodenum. But in the case of Ca^{2+}, the kinetic study suggests one single mediated component with different characteristics in the two types of rats (13).

In a preliminary report on Mg^{2+} uptakes (22), similar values have been obtained for duodenal BBM but much higher ones have been obtained in the case of the jejunum. In those experiments, 1 m*M* EGTA was used in the stop solution, but here, it was replaced by 1 m*M* $MgCl_2$, which importantly reduced the binding to the filters; also, thawed vesicles were not resuspended through a tuberculin syringe before use, as done here. But a lower uptake in jejunal vesicles of SHR was similarly obtained.

Experiments performed with Wistar rats as well as those from the present work show that the vesicular volume is systematically increased by the presence of Mg^{2+}, especially in jejunal BBM vesicles. In WKY rats, this volume is doubled in the presence of 1 m*M* $MgCl_2$ (Fig. 4). This result would suggest either alterations of water transport by Mg^2 or an influence of Mg^{2+} on the contractile activity of the vesicles. Indeed the latter suggestion could find some support in the work on brush-border myosin I, which is considered a mechanoenzyme that can be subtly regulated by physiological levels of Ca^{2+} and Mg^{2+} (26). On the other hand, the fact that SHR jejunal vesicles are systematically smaller than those of WKY rats is indicative of a different fluidity of the brush border and, therefore, possibly of a different lipid composition, as has been suggested (27).

Studies concerning Mg^{2+} intestinal absorption are scarce and rather old. Conflicting results as to the site and mechanisms of absorption have been published, as reviewed recently (18). So far, it is not easy to predict the relative importance of the entry process at the brush border involving a transcellular pathway versus an intercellular route. However, it has been suggested that the cellular absorption could play a role, especially at low dietary Mg^{2+} intakes or in pathological conditions, as would be the case for Ca^{2+} (19).

In the present study, we showed that Mg^{2+} initial uptake is lower in the jejunal brush border of SHR compared with WKY rats. This alteration would result from (i) a suppression of a diffusional component, (ii) a modication of apparent K_m and V_{max} of a mediated transport process, and (iii) a decrease in the amount of bound Mg^{2+}. Together with other abnormalities evidenced in the brush border of SHR small intestine (see Introduction), our results are consistent with a role of the small intestine in the altered Mg^{2+} metabolism evidenced in genetic hypertension.

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