Intestinal Brush Border Calcium Uptake in Spontaneously Hypertensive Rats and Their Genetically Matched WKY Rats (43163)

HARUMICHI SHIBATA AND FAYEZ K. GHISHAN[1]
Department of Pediatrics, Division of Gastroenterology, Vanderbilt University, Nashville, Tennessee 37232

Abstract. **The current studies were designed to characterize calcium transport by intestinal brush border membrane in the spontaneously hypertensive rat (SHR) and normotensive control, the Wistar-Kyoto (WKY) rat. The biochemical and functional purity of the intestinal brush border membranes in SHR and WKY rats was validated by marker enzymes and the ability to transiently transport D-glucose in the presence of Na^+ gradient. Calcium transport into duodenal and jejunal vesicles represented a minor binding component and transmembrane movement as evident by initial rate studies, A23187 studies, and lanthanum displacement experiments. Initial rate and time course of calcium uptake was lower in SHR compared with WKY rats. Kinetic analysis of calcium uptake by the jejunum (total uptake minus binding component) showed a V_{max} of 6.98 ± 0.2 and 1.8 ± 0.2 nmol/mg protein/7 sec in WKY rats and SHR, respectively ($P < 0.001$), whereas K_m values were 0.76 ± 0.04 and 0.87 ± 0.1 m*M* for WKY rats and SHR, respectively. Similar kinetic analysis of calcium uptake by the duodenal segments showed a V_{max} of 10.3 ± 0.8 and 2.8 ± 0.2 nmol/mg protein/7 sec in WKY rats and SHR, respectively ($P < 0.01$). K_m values were 0.7 ± 0.2 and 0.3 ± 0.06 m*M* ($P > 0.05$). V_{max} of calcium uptake in the 2-week-old rats (prehypertensive period) was 6.0 ± 0.3 and 3.53 ± 0.3 nmol/mg protein/7 sec in WKY rats and SHR, respectively ($P < 0.001$), whereas K_m values were 0.60 ± 0.07 and 0.5 ± 0.01 m*M*, respectively. These results suggest that calcium binding and uptake by duodenal and jejunal intestinal brush border membranes of SHR is significantly decreased compared with WKY rats. The decrease in transmembrane calcium uptake is secondary to decrease in V_{max} and is present before the appearance of hypertension, implying a genetically determined defect in calcium uptake in intestinal brush border membranes of the SHR.** [P.S.E.B.M. 1991, Vol 196]

Several epidemiologic studies have established an inverse relationship between dietary intake of calcium and hypertension (1–4). Oral calcium supplementation in humans resulted in a reduction of blood pressure (5). Moreover, a low serum-ionized calcium level has been reported in patients with hypertension (6). These combined studies suggest a close relationship between calcium homeostasis and hypertension.

The spontaneously hypertensive rat (SHR), which was developed by Okamoto and Aoki (7), is the most widely used animal model to study human essential hypertension. Several *in vivo* and *in vitro* studies have suggested the presence of altered calcium metabolism in SHR when compared with its genetically matched control Wistar-Kyoto (WKY) rats (8–11). These studies showed a low serum-ionized calcium, elevated serum parathormone level, and decreased 1,25-$(OH)_2$ vitamin D_3 level in SHR compared with WKY rats. However, intestinal calcium transport in SHR has been reported to be increased (12, 13), unchanged (14), and decreased (15, 16) compared with WKY rats. The previous studies on calcium transport, however, utilized *in vivo* perfusion and *in vitro* gut sacs in which the entry step at brush border membrane (BBM), cytosolic movement, and exit at the basolateral membranes were studied together. In an effort to delineate each of these steps, we designed the present studies to investigate calcium uptake by intestinal brush border vesicles of suckling and adult SHR and WKY rats.

[1] To whom reprint requests should be addressed at Division of Pediatric Gastroenterology and Nutrition, Vanderbilt University, Nashville, TN 37232.

Received April 17, 1990. [P.S.E.B.M. 1991, Vol 196]
Accepted July 31, 1990.

0037-9727/91/1961-0054$2.00/0

Materials and Methods

Animals. Male SHR and WKY rats were obtained from Taconic Farms (Germantown, NY). Adult rats (12–14 weeks old) and suckling rats (14- ± 1-day old) were used to prepare the intestinal BBM vesicles. Adult rats were fed a regular chow diet that contained 1.2% calcium, 0.8% phosphate, and 1700 units/100 g of ergocalciferol (Teklad Diet, Madison, WI). Suckling rats were allowed to suckle freely from their mother. ^{45}Ca (10–40 mCi/mg) was obtained from New England Nuclear. Enzymes and substrates were obtained from Sigma Chemical Co. Cellulose nitrate filters were obtained from Sartorius Filters (Hayward, CA). Blood pressure measurements were carried out as described previously by us (17). Briefly, a tail cuff was used, which is connected to a recorder in adult rats, whereas a carotid cannula was used in the suckling rats.

Preparation of BBM Vesicles. The intestinal BBM vesicles were prepared from the duodenum and jejunum of two to four adult and 12 to 24 suckling rats. Rats were killed by cervical dislocation. The duodenal segments extended from the pylorus to the ligament of Treitz. The jejunal segments extended from the ligaments of Treitz to 20 cm and 50 cm aborally in suckling and adult rats, respectively. The segments were removed, rinsed with ice-cold saline, everted, and placed on a glass plate resting on ice. The mucosa was scraped off with a glass microscope slide. BBM vesicles were isolated at first by sequential precipitation with 0.01 *M* $MgCl_2$ and differential centrifugation as described previously (18–20). To separate plasma membranes from adhering terminal web and microvillus core, we used potassium thiocyanate at the final step, according to the method of Hopfer *et al.* (21). Briefly, mucosal scrapings were placed in 30 ml of Buffer I containing 300 m*M* mannitol, 5 m*M* EGTA, and 12 m*M* Tris-HCl (pH 7.1), and 120 ml of ice-cold water were then added. The scrapings were then homogenized for 3 min at full speed in a Waring Blendor. The homogenate was then treated with 1.5 ml of 1 *M* $MgCl_2$ in 150 ml of Buffer I to yield final concentration of 10 m*M* $MgCl_2$. The homogenate was left for 15 min at 0°C and then centrifuged at 3000*g* for 15 min. The supernatant was centrifuged at 27,000*g* for 30 min and the pellets were resuspended in 30 ml of Buffer II containing 60 m*M* mannitol, 5 m*M* EGTA, and 12 m*M* Tris-HCl (pH 7.1), and homogenized by 10 strokes. The homogenate was treated with 0.3 ml of 1 *M* $MgCl_2$ and left to stand for 15 min at 0°C, and then centrifuged at 3000*g* for 15 min. The supernatant was centrifuged at 27,000*g* for 30 min and the resulting pellet was suspended in 10 ml of mannitol buffer containing 0.1 *M* mannitol, 1 m*M* Tris-HCl (pH 7.4). Potassium thiocyanate was then added at 0.52 *M* concentration and homogenized for 10 strokes, then diluted to 80 ml with mannitol buffer, and left to stand for 10 min at 0°C. The homogenate was then centrifuged at 6000*g* for 10 min and the resulting supernatant was centrifuged at 34,000*g* for 25 min.

Purification of BBM vesicles was assessed by measurement of marker enzyme activities of brush border (leucine aminopeptidase and disaccharidases), basolateral membranes (Na^+-K^+-ATPase), and subcellular organelles (cytochrome *c* oxidase and NADPH cytochrome *c* reductase) as described previously (22, 23). Protein was measured according to the method of Lowry *et al.* (24).

Transport Measurements. Uptake of radiolabeled D-glucose and calcium was measured by a rapid filtration technique. Typically, incubation was initiated by the addition of 20 μl of vesicle suspension to 60 μl incubation solution. Experiments were conducted at 25°C unless otherwise stated. After the desired incubation time interval, transport was terminated by abrupt dilution in 1 ml of ice-cold "stop solution" (100 m*M* mannitol, 150 m*M* $MgCl_2$, and 10 m*M* Hepes-Tris buffer, pH 7.4). The stop solution for D-glucose con-

Table I. Specific Activities of Brush Border Enzyme Markers in Crude Homogenate and in BBM Vesicles of SHR and WKY Rats

	Sucrase (units/mg protein)[a]		Lactase (units/mg protein)[a]		Leucine aminopeptidase (μg BNA hydrolized/pg protein)	
	Crude homogenate	BBM	Crude homogenate	BBM	Crude homogenate	BBM
SHR	—[b]	—	7 ± 1	75 ± 10 (11)[c]	0.6 ± 0.1	5.1 ± 1 (10)
Suckling						
Adult	9.3 ± 1	196 ± 12 (21)	1.7 ± 0.3	30 ± 3 (17)	0.7 ± 0.1	10 ± 2 (14)
WKY	—	—	8 ± 1	83 ± 7 (10)	0.5 ± 0.1	5.2 ± 1 (10)
Suckling						
Adult	14.3 ± 2	204 ± 10 (14)	1.5 ± 0.2	30 ± 2 (20)	0.6 ± 0.1	11 ± 1 (19)

[a] Each unit is 1 μmol of substrate hydrolyzed/min/mg of protein.
[b] Minimal amounts present ($n = 6$).
[c] Numbers in parentheses, enrichment factors.

tained 100 m*M* mannitol, 100 m*M* NaCl, 10 m*M* K_3PO_4, and 0.2 m*M* phlorizin. The reaction mixture was then immediately pipetted onto a prewetted filter (cellulose nitrate 0.45-μm pore size; Sartorius Filters, Inc.) and kept under suction. The filter was then rinsed with 5 ml of ice-cold stop solution and prepared for scintillation counting. Binding of the radiolabeled D-glucose and calcium to the filter was determined by filtration of incubation media without vesicle protein and was considered as background and subtracted from vesicle uptake. Results are expressed as nmol of calcium uptake per mg of vesicle protein. Each experiment was repeated three times on different membrane preparations.

Statistical Analysis. All data were statistically analyzed as mean ± SE, and the significance of differences was determined using unpaired Student's *t* test or analysis of variance for multiple comparisons, K_m and V_{max} were determined according to a computer model for estimation of Michaelis-Menten kinetic parameters (25).

Results

Blood Pressure Measurements. Mean systolic blood pressure was similar in suckling SHR and WKY rats (100 ± 3 and 103 ± 4 mm Hg, respectively, n = 6). Mean systolic blood pressure in adult SHR was 210 ± 8 mm Hg compared with 120 ± 10 mm Hg in WKY rats ($P < 0.001$, $n = 6$).

Enzyme Marker Studies. Brush border enzyme marker studies showed a 10- to 20-fold enrichment of disaccharidase and leucine aminopeptidase in both SHR and WKY rats, whereas marker enzymes for basolateral membranes (Na^+-K^+-ATPase), mitochondria (cytochrome *c* oxidase), endoplasmic reticulum (NADPH cytochrome *c* reductase) were impoverished at 0.8 ± 0.1, 0.1 ± 0.1, and 0.6 ± 0.1-fold, respectively. Table I depicts specific activities of disaccharidase and leucine aminopeptidase in both age groups of SHR and WKY rats.

Table II. Latency of Sucrase and Leucine Aminopeptidase in BBM of Intestinal SHR and WKY Rats

	Sucrase activity (units/mg protein)[a]		Leucine aminopeptidase (μg BNA hydrolized/μg protein)	
	Intact vesicles	Disrupted vesicles	Intact vesicles	Disrupted vesicles
SHR	196 ± 12	200 ± 10	10 ± 2	10.2 ± 0
WKY	204 ± 10	210 ± 8	11 ± 1	9.8 ± 0.8

[a] Each unit is 1 μmol of substrate hydrolyzed/min/mg protein (n = 3).
[b] n = 6.

Orientation of the Vesicle Preparation. To determine the orientation of the vesicle preparation in both groups of rats studied, the latency of sucrase and leucine aminopeptidase was determined. The specific activities of both enzymes were determined in intact vesicles and in vesicles disrupted by freeze-thawing. As seen in Table II, no change in the activity of either markers was noted, indicating that BBM of both groups are oriented right-side out.

D-Glucose Studies. To provide functional evidence for our BBM, we investigated D-glucose uptake in the presence of inwardly directed sodium and potassium gradient conditions in both BBM of SHR and WKY rats. As seen in Figure 1, a typical "overshoot" phenomenon was obtained under Na^+ gradient condition in both SHR and WKY rats, whereas no overshoot was seen under K^+ gradient condition. Equilibrium values were similar in both groups of rats, indicating similar intravesicular size.

Effect of Calcium Ionophore A23187 on Calcium Influx. As seen in Table III, calcium ionophore A23187 induced a greater initial rate of duodenal calcium influx compared with no A23187, suggesting that the ionophore form lipid-soluble complexes with calcium by virtue of their polar nature, and can cross the membranes (26).

Effect of Lanthanum (La^{3+}) on Calcium Displacement from Intact and Disrupted Vesicles. The experiment was designed to determine whether calcium is present in the intravesicular space and therefore inaccessible to La^{3+} displacement or in the external surface,

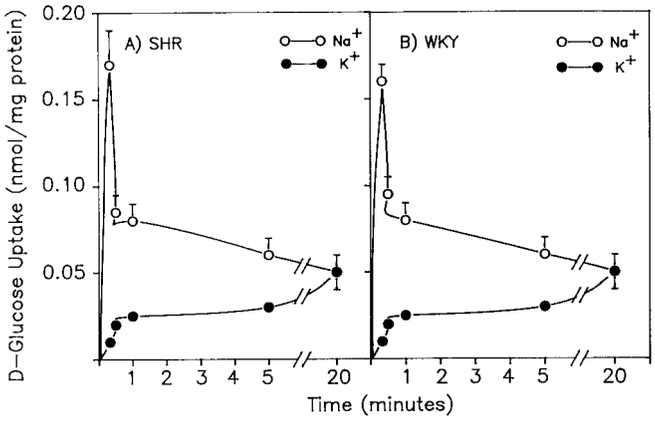

Figure 1. D-Glucose uptake in the presence of Na^+ and K^+ gradient in SHR and WKY rats. Intestinal jejunal BBM from adult SHR and WKY rats were preloaded with 280 m*M* mannitol and 20 m*M* Hepes-Tris buffer (pH 7.4). Incubations were conducted at 25°C in a medium containing 100 m*M* NaCl or 100 m*M* KCl and 100 m*M* mannitol, 20 m*M* Hepes-Tris buffer (pH 7.4), and 0.1 m*M* [^{14}C]D-glucose. At the desired time point, the reaction was stopped by adding a stop solution containing 100 m*M* mannitol, 100 m*M* NaCl, 10 m*M* K_3PO_4, and 0.2 m*M* phlorizin. Values are mean ± SE of three separate experiments on different membrane preparations.

Table III. Effect of Calcium Ionophore A23187 on Initial Rate of Calcium Uptake by Duodenal BBM Vesicles of SHR and WKY Rats[a]

Time (sec)	SHR (nmol calcium/mg protein)		WKY (nmol calcium/mg protein)	
	−A23187	+A23187	−A23187	+A23187
3	0.25 ± 0.002	0.43 ± 0.1[b]	0.32 ± 0.02	0.5 ± 0.01[b]
6	0.3 ± 0.1	0.53 ± 0.1[b]	0.38 ± 0.01	0.68 ± 0.03[b]
9	0.42 ± 0.1	0.58 ± 0.09[b]	0.54 ± 0.03	0.75 ± 0.04[b]
12	0.53 ± 0.04	0.68 ± 0.1[b]	0.63 ± 0.03	0.84 ± 0.05[b]
15	0.58 ± 0.03	0.88 ± 0.2[b]	1.25 ± 0.07	1.9 ± 0.1[b]

[a] BBM were preloaded with 280 m*M* mannitol and 20 m*M* Hepes-Tris buffer (pH 7.4). Uptake was initiated by the addition of the vesicles to a medium containing 100 m*M* mannitol, 100 m*M* NaCl, 20 m*M* Hepes/Tris (0.1 m*M*), $^{45}CaCl_2$, and 10 μg of A23187/mg protein. Values are mean ± SE (n = 3).
[b] Mean values at both SHR and WKY rats with A23187 are significantly greater compared with mean values without A23187 ($P < 0.05$–0.001).

which should be accessible to La^{3+} displacement. In this setting, calcium-preloaded vesicles were diluted in a buffered solution containing 1 m*M* La^{3+}. As seen in Table IV, La^{3+} displaced a minimum amount of calcium from intact vesicles. However, when the vesicles were disrupted by freeze-thawing, significant displacement occurred by La^{3+}, suggesting that opening the vesicles allows access of La^{3+} to displace calcium transported into the inside of vesicles.

Initial Rate of Calcium Uptake in the Presence of Na^+ and K^+ Gradients. Figure 2 depicts initial rate of jejunal calcium uptake in the presence of inwardly directed Na^+ and K^+ gradients in both SHR and WKY rats. There were no differences in calcium uptake in the presence of Na^+ or K^+ gradients in both SHR and WKY rats. However, at each time point studied, calcium uptake was significantly decreased in SHR compared with WKY rats under both Na^+ and K^+ gradient condition ($P < 0.05$–0.001). Calcium uptake was linear until 15 sec under both Na^+ and K^+ gradients in SHR ($Y = 0.19x + 0.089$, correlation coefficient (cc) = 0.85 and $Y = 0.03x + 0.09$, cc = 0.86, respectively) and WKY rats ($Y = 0.07x + 0.2$, cc = 1.0 and $Y = 0.08x + 0.29$, cc = 0.99, respectively). Initial rate uptake of calcium by duodenal BBM of SHR and WKY rats under Na^+ gradient condition was similarly lower in SHR compared with WKY rats as depicted by the equation $Y = 0.34x$ 0.25, cc = 0.99 and $Y = 0.25x$ 0.19, cc = 0.99 for WKY and SHR, respectively. The intercept representing the binding component (27) was significantly lower in duodenal and jejunal BBM of SHR compared with WKY rats ($P < 0.05$–0.01).

Calcium Uptake with Time. Jejunal calcium uptake was determined under Na^+ gradient condition in

Table IV. Effect of La^{3+} on Calcium Displacement from Intact and Disrupted Vesicles[a]

Time (min)	SHR (nmol calcium/mg protein)		WKY (nmol calcium/mg protein)	
	Intact vesicles	Disrupted vesicles	Intact vesicles	Disrupted vesicles
0	17.5 ± 1.2	17.5 ± 1.2	31.2 ± 3	31.2 ± 3
0.5	16.5 ± 1.3	12.9 ± 1.0	30.8 ± 2	30 ± 3
1	14.6 ± 2	12 ± 1.0	29.7 ± 1.5	23 ± 3
5	15.02 ± 2	10 ± 0.8	28.9 ± 1.0	20 ± 1.6
45	16.0 ± 2	8 ± 0.6	28.7 ± 1.2	15.3 ± 1.0

[a] Values represent mean ± SE of three experiments. Duodenal BBM vesicles were preloaded with 1 m*M* $^{45}CaCl_2$. Vesicles were then either left intact or were subjected to freeze-thawing in an effort to rupture them. Vesicles were then diluted into an incubation medium containing 280 m*M* mannitol, 20 m*M* Hepes-Tris, and 1 m*M* lanthanum oxide. At desired time points, membranes were removed and filtered over a millipore system, and remaining radioactivity was counted.

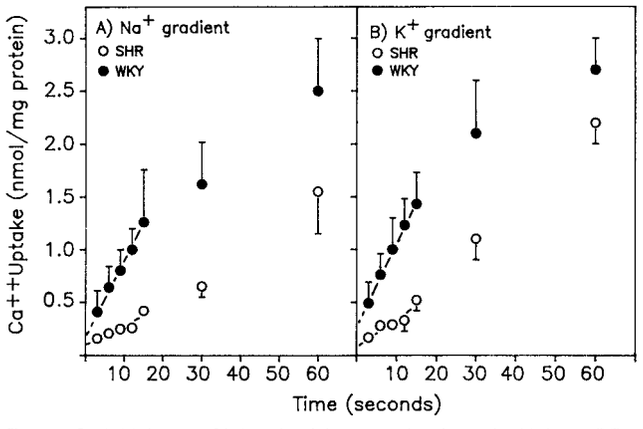

Figure 2. Initial rate of jejunal calcium uptake. Intestinal jejunal BBM vesicles WKY and SHR were preloaded with 280 m*M* mannitol and 20 m*M* Hepes-Tris (pH 7.4). Incubations were conducted at 25°C in a medium containing either 100 m*M* NaCl or 100 m*M* KCl and 100 m*M* mannitol, 20 m*M* Hepes-Tris (pH 7.4), and 0.1 m*M* $CaCl_2$ and tracer ^{45}Ca. Values are mean ± SE of three separate experiments on different membrane preparations. There were no differences in jejunal uptake values under Na^+ or K^+ gradients in SHR and WKY rats. Calcium uptake in SHR and WKY rats appears linear up to 15 sec, however, the slope of the line representing calcium uptake in the presence of Na^+ or K^+ gradient was significantly greater in jejunal WKY rats compared with SHR ($P < 0.05$).

both SHR and WKY rats. As seen in Figure 3 at each time point studied, calcium uptake was markedly decreased in SHR compared with WKY rats ($P < 0.01$–0.001).

Kinetics of Calcium Uptake. Calcium uptake was determined in both SHR and WKY rats at 7 sec, well within the linear phase of uptake at calcium concentrations between 0.01 and 1.0 m*M*. The binding component was subtracted from total uptake as determined from the intercept of the initial rate of uptake (27). Kinetic parameters of duodenal and jejunal transmembrane uptake were calculated from a computerized model of Michaelis-Menten kinetics. Kinetic parameters in the duodenum showed a V_{max} of 10.3 ± 0.8 and 2.8 ± 0.2 in adult WKY and SHR rats, respectively ($P < 0.01$); K_m values were 0.7 ± 0.2 and 0.3 ± 0.06 m*M*, respectively ($P > 0.05$) (Fig. 4). Kinetic parameters in the jejunum showed a V_{max} of 6.98 ± 0.2 and 1.8 ± 0.2 nmol/mg protein/7 sec in both WKY rats and SHR, respectively ($P < 0.001$). K_m values were 0.76 ± 0.04 and 0.87 ± 0.1 m*M*, respectively (Fig. 5). To determine whether these changes are present before the appearance of hypertension, kinetic parameters were conducted in suckling (2-week-old) SHR and WKY rats. As seen in Figure 6, V_{max} was 6.0 ± 0.3 and 3.53 ± 0.3 nmol/mg protein/7 sec in suckling WKY rats and SHR, respectively ($P < 0.01$). K_m values were 0.6 ± 0.07 and 0.5 ± 0.01 m*M* ($P > 0.05$), respectively.

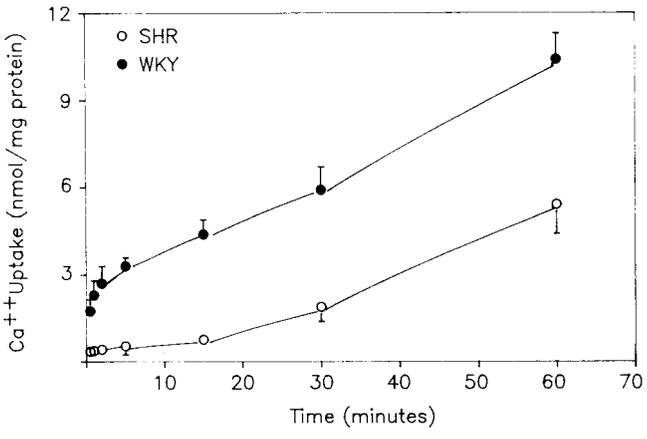

Figure 3. Time course of calcium uptake in SHR and WKY rats. Jejunal BBM from adult SHR and WKY rats were preloaded with 280 m*M* mannitol and 20 m*M* Hepes-Tris buffer (pH 7.4). Incubations were conducted at 25°C in a medium containing 100 m*M* KCl, 100 m*M* mannitol, 20 m*M* Hepes-Tris buffer (pH 7.4), and 0.1 m*M* $CaCl_2$ and tracer ^{45}Ca. Values are mean ± SE of three separate experiments on different membrane preparations.

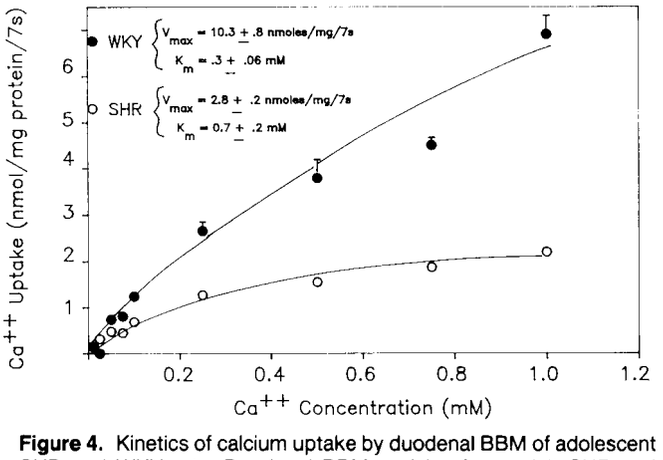

Figure 4. Kinetics of calcium uptake by duodenal BBM of adolescent SHR and WKY rats. Duodenal BBM vesicles from adult SHR and WKY rats were brought up in mannitol buffer (pH 7.4). Calcium uptake was determined at 7 sec. The binding component was subtracted from total uptake. Calcium concentration in the media ranged from 0.01 to 1.0 m*M*. Kinetic parameters were obtained using a computerized model of the Michaelis-Menten kinetics.

Discussion

The present studies characterize calcium transport across duodenal and jejunal BBM vesicles of SHR and WKY rats. The biochemical and functional integrity of the membrane preparation was validated by marker enzyme studies showing marked enrichment with brush border enzymes and the ability to transport glucose with an overshoot phenomenon under sodium gradient condition. The vesicle preparation was right-side out in both membranes of SHR and WKY rats as evidenced by studies of the latency of brush border enzyme markers. Therefore, the current studies utilized a well-validated BBM vesicle to determine the entry process of calcium across the enterocyte. Previously, we and others have validated the utility of BBM vesicles for the study of calcium transport in humans and rats (18, 20, 28, 29). The transmembrane movement of calcium is well-documented by the A23187 and La^{3+} displacement studies. A minor binding component is evident by the positive intercept of the initial rate uptake studies.

The current studies clearly demonstrate a decrease in initial calcium binding to the BBM as well as in the transmembrane movement of calcium in the duodenal and jejunal segments of SHR compared with WKY rats. The decrease in the transmembrane movement is secondary to a decrease in V_{max} rather than K_m, suggesting that the transport carrier activity or number is decrease in SHR membranes compared with WKY rats. Previous studies on intestinal calcium absorption in adult SHR rats have been reported to be increased (12, 13), unchanged (14), and decreased (15, 16) compared with WKY rats. The previous studies measured calcium absorption by several experimental procedures such as the recirculation perfusion method (16), *in situ* duodenal uptake (12), or everted gut sac *in vitro* (13, 14, 16). The discrepancy in these results has remained unexplained. However, the level of serum ionic calcium has been reported to be decreased in SHR compared with WKY rats under the conditions of normal intake of calcium and vitamin D (9, 11, 30, 31). Thus, lower levels of serum ionic calcium in SHR compared with

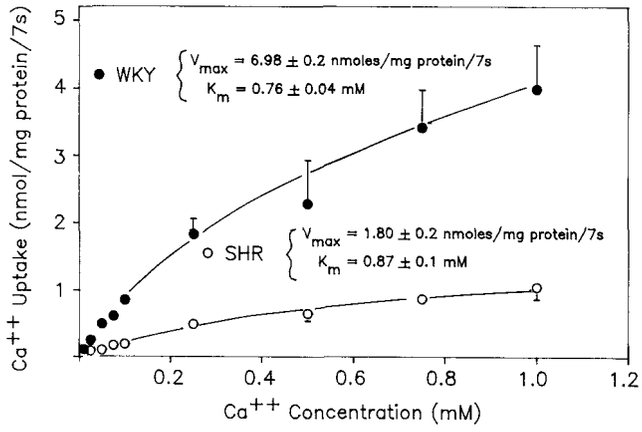

Figure 5. Kinetics of calcium uptake by jejunal BBM of adolescent SHR and WKY rats. Jejunal BBM vesicles from adult WKY rats and SHR were brought up in mannitol buffer (pH 7.4). Calcium uptake was determined at 7 sec. The binding component was subtracted from total uptake. Calcium concentration in the media ranged from 0.01 to 1.0 m*M*. Kinetic parameters were obtained using a computerized model of the Michaelis-Menten kinetics.

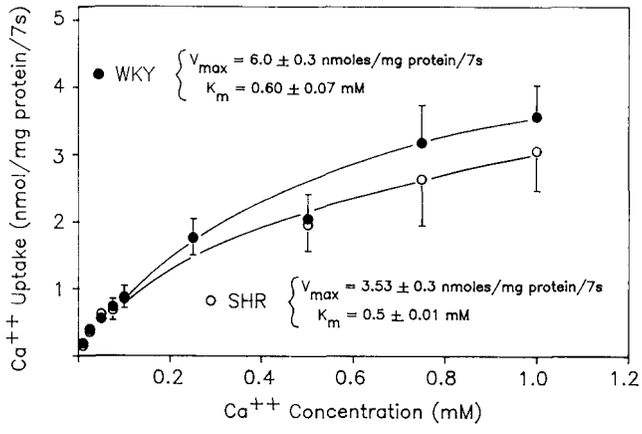

Figure 6. Kinetics of calcium uptake in suckling SHR and WKY rats. Jejunal BBM vesicles from suckling SHR and WKY rats were brought up in mannitol buffer, (pH 7.4). The binding component was subtracted from total uptake. Calcium uptake was determined at 7 sec. Calcium concentration in the media ranged from 0.01 to 1.0 m*M*. Kinetic parameters were obtained using a computerized model of the Michaelis-Menten kinetics.

WKY rats could be attributable to a decrease in intestinal calcium absorption.

The relationship between blood pressure and age in SHR has been reported by several investigators (7, 16, 32). Blood pressure was normal in SHR before 4 weeks of age (32). After 5 weeks of age, systolic blood pressure became significantly higher in SHR compared with WKY rats (17). After 10 weeks of age, hypertension in SHR was well established (7, 16). Our studies confirm these findings relating development of blood pressure to age in SHR. Intestinal calcium absorption was reported by several laboratories before detectable hypertension in SHR (16, 25, 32). At 5 weeks of age in SHR, duodenal calcium absorption by the everted gut sac *in vitro* was found to be unchanged (25), decreased (16), and increased (32). As seen in Figure 6, V_{max} of calcium uptake was significantly lower at 2 weeks of age in SHR compared with WKY rats. These results suggest that the abnormality in calcium uptake by BBM in SHR was present before the development of hypertension. The mechanism for the decrease in V_{max} of calcium uptake by BBM is not known. Abnormalities in vitamin D_3 metabolism may explain some of our results. A low 1,25-$(OH)_2$ vitamin D_3 was reported in SHR compared with WKY rats (33–35). Future studies are underway to examine the role of 1,25-$(OH)_2$ vitamin D_3 in calcium uptake by BBM of SHR and WKY rats. These studies may shed light on the mechanisms of lower calcium uptake in SHR.

The decrease in calcium binding and transmembrane movement of calcium across the intestine, coupled with the observation that a similar defect is also present is erythrocyte (36), adipoctye (37), hepatocyte, and synaptosomes (38) of SHR compared with WKY rats suggest that the permeability of cell membranes to calcium is altered in hypertensive rats. These findings strongly suggest that cell membrane permeability to calcium is related to the development of hypertension.

This study was supported by Grant RFA-86-HL-8-P.

1. Harlan WR, Hull AL, Schmouder RL, Landis JR, Thompson FE, Larkin FA. Blood pressure and nutrition in adults. Am J Epidemiol **120**:17–28, 1984.
2. Harlan WR, Hull AL, Schmouder RL, Landis JR, Larkin FA, Thompson FE. High blood pressure in older Americans: The first national health and nutrition examination survey. Hypertension **6**:802–809, 1984.
3. McCarron DA, Morris CD, Cole C. Dietary calcium in human hypertension. Science **217**:267–269, 1982.
4. McCarron DA, Morris CD, Henry HJ, Stanton JL. Blood pressure and nutrient intake in the United States. Science **224**:1392–1397, 1984.
5. Belizan JM, Villan J, Pineda O. Reduction of blood pressure with calcium supplementation in young adults. JAMA **249**:1161–1165, 1983.
6. McCarron DA. Low serum concentrations of ionized calcium in patients with hypertension. N Engl J Med **307**:226–228, 1982.
7. Okamoto K, Aoki K. Development of a strain of spontaneously hypertensive rats. Jpn Circ J **27**:282–293, 1963.
8. Ayachi S. Increased dietary calcium lowers blood pressure in the spontaneously hypertensive rat. Metabolism **28**:1234–1238, 1979.
9. McCarron DA, Yung NN, Ugoretz BA, Krutzik S. Disturbances of calcium metabolism in the spontaneously hypertensive rat. Hypertension **3**(suppl I):I-162–I-167, 1981.
10. Noon JP, Rice PJ, Baldessarini RJ. Calcium leakage as a cause of the high resting tension in vascular smooth muscle from the spontaneously hypertensive rat. Proc Natl Acad Sci USA **75**:1605–1607, 1978.
11. Wright GL, Rankin GO. Concentrations of ionic and total calcium in plasma of four models of hypertension. Am J Physiol **243**:H365–H370, 1982.

12. Lau K, Zikos D, Spirnok J, Eby B. Evidence for intestinal mechanism in hypercalciuria of spontaneously hypertensive rats. Am J Physiol **247**:E625–E633, 1984.
13. Toraason MA, Wright GL. Transport of calcium by duodenum of spontaneously hypertensive rat. Am J Physiol **241**:G344–G347, 1981.
14. Stern N, Lee DBN, Silis V, Beck FWJ, Deftos L, Manolagas SC, Somers JR. Effects of high calcium intake on blood pressure and calcium metabolism in young SHR. Hypertension **6**:639–646, 1984.
15. McCarron DA, Lucas PA, Shneidman RJ, LaCour B, DrÜeke T. Blood pressure developments of the spontaneously hypertensive rat after concurrent manipulations of dietary Ca^{2+} and Na^{+}. J Clin Invest **76**:1147–1154, 1984.
16. Schedl HP, Miller DL, Pope JM, Horst RL, Wilson HD. Calcium and sodium transport and vitamin D metabolism in the spontaneously hypertensive rat. J Clin Invest **73**:980–986, 1984.
17. Shibata H, Ghishan FG. Intestinal calcium transport in spontaneously hypertensive rats (SHR) and their genetically matched (WKY) rats. I: Endoplasmic reticulum. J Hypertens **8**:473–477, 1990.
18. Ghishan FK, Arab N, Nylander W. Characterization of calcium uptake by brush border membrane vesicles of human small intestine. Gastroenterology **96**:122–129, 1989.
19. Borowitz SM, Ghishan FK. Maturation of jejunal phosphate transport by rat brush border membrane vesicles. Pediatr Res **19**:1308–1313, 1985.
20. Ghishan FK, Leonard D, Pietsch J. Calcium transport by plasma membranes during development: Role of 1,25-$(OH)_2$ vitamin D_3. Pediatr Res **24**:338–341, 1988.
21. Hopfer U, Crowe TD, Tandler B. Purification of brush border membrane by thiocyanate treatment. Anal Biochem **131**:447–452, 1983.
22. Beaufay H, Costesec AA, Feyman E, Thines-Sempoux T, Wibo M, Robie M, Berther J. Analytical study of microsomes and isolated subcellular membranes from rat liver. J Cell Biol **6**:88–98, 1974.
23. Scharschmidt BF, Keefe ED, Blankenship N, Okner R. Validation of a recording spectrophotometric method for measurement of membrane associated Mg and Na-K-ATPase activity. J Lab Clin Med **93**:790–798, 1979.
24. Lowry OH, Rosebrough NH, Farr AL, Randal RJ. Protein measurement with the Folin phenol reagent. J Biol Chem **193**:265–275, 1951.
25. Vaughn WK, Neal RA, Anderson AJ. Computer estimation of the parameter of the sigmoid kinetic model. Comput Biol Med **6**:1–5, 1976.
26. Pressman BC. Biological applications of ionophores. Annu Rev Biochem **45**:501–530, 1976.
27. Nord EP, Hafezi A, Wright E, Fine LG. Mechanisms of Na^{+} uptake into renal brush border membrane vesicles. Am J Physiol **247**:F548–554, 1984.
28. Ghishan FK, Arab N. Developmental maturation of calcium transport by rat brush border membrane vesicles. Pediatr Res **22**:173–176, 1987.
29. Schedl HP, Wilson HD. Calcium uptake by intestinal brush border membrane vesicles. J Clin Invest **76**:1871–1878, 1985.
30. Hsu CH, Patel S, Young EW. Calcemic response in parathyroid hormone in spontaneously hypertensive rat. J Lab Clin Med **110**:682–689, 1987.
31. Wright GL, Toraason MA, Barbe JS, Crouse W. The concentrations of ionic and total calcium in plasma of the spontaneously hypertensive rat. Can J Physiol Pharmacol **58**:1494–1499, 1980.
32. Lau K, Langman CB, Grafter U, Dudeja PD, Brasitus TA. Increased calcium absorption in prehypertensive spontaneously hypertensive rat. J Clin Invest **78**:1083–1090, 1986.
33. Kurtz TW, Portale AA, Morris RC. Evidence in vitamin D metabolism between spontaneously hypertensive and Wistar-Kyoto rats. Hypertension **8**:1015–1020, 1986.
34. Lucas PA, Brown RC, TrÜeke T, Metz JA, McCarron DA. Abnormal vitamin D metabolism, intestinal calcium transport and bone calcium status in the spontaneously hypertensive rat compared with its genetic control. J Clin Invest **78**:221–227, 1986.
35. Schedl HP, Miller DL, Horst RL, Wilson HD, Natarajan K, Conway T. Intestinal calcium transport in the spontaneously hypertensive rat: Response to calcium depletion. Am J Physiol **250**:G412–G419, 1986.
36. Orlov SN, Pokudin NI, Postnov YuV. Calmodulin-dependent Ca^{2+} transport in erythrocytes of spontaneously hypertensive rats. Pflugers Arch **397**:54–56, 1983.
37. Postnov YuV, Orlov SN. Evidence of altered calcium accumulation and calcium binding by the membranes of adipocytes in spontaneously hypertensive rats. Pflugers Arch **385**:85–89, 1980.
38. Devynck MA, Pernollet MG, Nunez AM, Meyer P. Calcium binding alteration in plasma membrane from various tissues of spontaneously hypertensive rats. Clin Exp Hypertens **3**:797–808, 1981.