Intestinal Calcium Transport in Spontaneously Hypertensive Rats (SHR) and Their Genetically Matched WKY Rats (43049)

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Abstract. **Calcium transport across the basolateral membranes of the enterocyte represents the active step in calcium translocation. This step occurs by two mechanisms, an ATP-dependent pump and a Ca^{2+}/Na^{+} exchange process. These studies were designed to investigate these two processes in jejunal basolateral membrane vesicles (BLMV) of the spontaneously hypertensive rats (SHR) and their genetically matched controls, Wistar-Kyoto (WKY) rats.**

The ATP-dependent calcium uptake was stimulated several-fold compared with no ATP condition in both SHR and WKY, but no differences were noted between rate of calcium uptake in SHR and WKY. Kinetics of ATP-dependent calcium uptake at concentrations between 0.01 and 1.0 μM revealed a V_{max} of 0.67 ± 0.03 nmol/mg protein/20 sec and a K_m of 0.2 ± 0.03 μM in SHR and V_{max} of 0.69 ± 0.12 and a K_m of 0.32 ± 0.14 μM in WKY rats.

Ca^{2+}/Na^{+} exchange in jejunal BLMV of SHR and WKY was investigated in two ways. First, sodium was added to the incubation medium (*cis*-Na^{+}). Second, Ca^{2+} efflux from BLMV was studied in the presence of extravesicular Na^{+} (*trans*-Na^{+}). Both studies suggest a decreased exchange of calcium and Na^{+}. Kinetic parameters of Na^{+}-dependent Ca^{2+} uptake at concentrations between 0.01 and 1.0 μM exhibited V_{max} of 0.05 ± 0.01 nanmol/mg protein/5 sec and a K_m of 0.21 ± 0.13 μM in SHR and V_{max} of 0.11 ± 0.02 nanmol/mg protein/5 sec and a K_m of 0.09 ± 0.05 in WKY, respectively. These results confirm that the intestinal BLMV of SHR and WKY rats have two mechanisms for calcium extrusion, an ATP-dependent Ca^{2+} transport process and a Na^{+}/Ca^{2+} exchange process. The ATP-dependent process appears to be functional in SHR; however, the Ca^{2+}/Na^{+} exchange mechanism appears to have a marked decrease in its maximal capacity. These findings suggest that calcium extrusion via Ca^{2+}/Na^{+} is impaired in the SHR, which may lead to an increase in intracellular calcium concentration. These findings may have relevance to the development of hypertension. [P.S.E.B.M. 1990, Vol 194]

Calcium homeostasis in humans with essential hypertension has been shown to be altered. An inverse relationship between dietary intake of calcium and hypertension has been well documented (1–4). Decreased serum ionized calcium and increased serum parathormone levels have been documented in patients with hypertension (5–7). The spontaneously hypertensive rat (SHR) was developed by Okamoto and Aoki (8) and currently is the most widely used animal model for the study of human essential hypertension. SHR has been reported to have several abnormalities in calcium metabolism. These abnormalities include reduced serum ionized calcium levels, increased serum parathormone levels, hypercalciuria, and abnormalities in intestinal calcium transport and vitamin D metabolism (9–15). Intestinal calcium transport in SHR was reported to be decreased (15, 16), unchanged (12, 17), or increased (14) compared with that of its genetically matched control. Wistar-Kyoto (WKY) rats. These studies utilized *in vivo* perfusion and *in vitro* everted gut sacs and Üssing chambers to study intestinal calcium transport (12–17).

On the other hand, calcium transport across the enterocyte represents an entry process down concentration gradient (18) and an uphill exit process against

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concentration gradient (19). The exit process is the active step in intestinal calcium transport.

The current studies were designed to characterize calcium transport across the intestinal basolateral membrane of the SHR and their genetically matched control, WKY rats. Because calcium transport at the basolateral membrane occurs by two mechanisms, an ATP-dependent pump and a Na^+/Ca^{2+} exchange system, we investigated in detail these two processes in SHR and WKY, respectively.

Materials and Methods

SHR and WKY were obtained from Taconic Farms (Germantown, NY). Adult (12- to 14-week-old) SHR and WKY rats were used to prepare the jejunal basolateral membrane vesicles. All rats of both strains were fed a regular chow diet that contained 1.2% calcium, 0.8% phosphate, and 1700 units/100 g of ergocalciferol (Teklad Diets, Madison, WI).

^{45}Ca (10–40 mCi/mg) was obtained from New England Nuclear Corp. (Boston, MA). Valinomycin, oligomycin, enzymes, and substrates for leucine aminopeptidase were obtained from Sigma Chemical Co. (St. Louis, MO). Cellulose nitrate filters, 0.45-μm pore size, were obtained from Sartorius Filters, Inc. (Hayward, CA). All other chemicals were of the highest purity available.

Preparation of BLMV in SHR and WKY. Jejunal BLMV were prepared using a modified centrifugation technique followed by separation on a percoll gradient (20). Two rats were used for each preparation. The jejunum was removed from each rat and extended from the ligament of Treitz to 40 cm aborally. The segments were flushed with ice-cold lactated Ringer's solution and filled with warmed buffer solution (37°C) containing 1.5 m*M* KCl, 96 m*M* NaCl, 8 m*M* KH_2PO_4, 27 m*M* Na_3 citrate, 5.6 m*M* Na_2PO_4, and 2 m*M* dithiothreitol (pH 7.4, Buffer 1). The segments were then clamped and incubated for 15 min in a shaking water bath at 37°C. The clamps were removed and the contents emptied. The segments were then filled with ice-cold buffer containing 250 m*M* mannitol and 12 m*M* Hepes-Tris (pH 7.4, Buffer 2) and gently palpitated by fingers for 5 min to release epithelial cells. The contents were drained into a beaker on ice and the volume was brought up to 150 ml in Buffer 2 and then centrifuged at 270*g* for 15 min. The pellet was homogenized in 120 ml of Buffer 2 in an Omni mixer for 3 min. The homogenate was brought up to 150 ml with Buffer 2 and centrifuged at 2500*g* for 20 min. The supernatant was collected and centrifuged at 22,000*g* for 25 min. The supernatant was discarded and the resulting fluffy layer of the pellet was resuspended in 95.5 ml of Buffer 2 and then homogenized in a glass-Teflon homogenizer (20 strokes). The resultant homogenate was mixed with percoll (Pharmacia, Piscataway, NJ) at a concentration of 15% and centrifuged at 48,000*g* for 75 min. A whitish band of BLMV was noted at the upper one third of the percoll gradient. The band was aspirated by a needle and diluted with Buffer 3 (containing 100 m*M* mannitol, 100 m*M* KCl, and 12 m*M* Hepes-Tris, pH 7.4). The diluted BLMV band was centrifuged at 48,000*g* for 20 min. The pellet was resuspended in the appropriate transport buffer (usually Buffer 3) and then centrifuged again at 48,000*g* for 20 min.

Transport Measurement. Uptake of calcium was measured by a rapid filtration technique (21). All experiments were performed at 25°C. Transport was initiated by adding 20 μl of the vesicle suspension to 60 μl of incubation medium. The amount of protein added is approximately 150 μg. The composition of the incubation medium for each individual experiment is described in Results. After the desired time intervals, the reaction was stopped by adding 1 ml of ice-cold "stop solution" containing 300 m*M* mannitol, 100 m*M* KCl, 5 m*M* $MgCl_2$, 20 m*M* Hepes-Tris (pH 7.4), and 1 m*M* EGTA. The vesicles were immediately collected on a cellulose nitrate filter (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 calcium to the filter was determined by filtration of incubation medium without vesicle protein and was considered as background and used in the calculation. The binding was less than 10% of total uptake. Results are expressed as nanomoles of calcium uptake per milligram of vesicle protein. In each experiment two rats were used to prepare BLMV. The experiments were repeated at least three times and run in triplicate. Figures 1–6 depict mean ± SE. In certain figures, the standard errors do not appear because it was too small to be plotted by the computer program.

Statistical Analysis. All data were statistically analyzed as mean ± SE and the statistical evaluation was carried out with a nonparametric approach utilizing the Mann-Whitney *U* test. A probability of <0.05 was considered statistically significant. K_m and V_{max} were calculated according to a computer model for estimation of kinetic parameter (22).

Purity of Vesicle Preparation. Purity of the basolateral membrane preparation was determined by marker enzyme activity. Leucine aminopeptidase activity was measured using a kit from Sigma Chemical Co. Na^+-K^+-ATPase was measured according to the method of Scharschmidt *et al.* (23). Cytochrome oxidase and NADPH-cytochrome *c* reductase were measured as described by Beaufay *et al.* (24).

Results

Animals. SHR and WKY rats were obtained from Taconic Farms at 6 weeks of age with mean body

weights of 150 ± 10 g. Mean body weight at the time of sacrifice (12–14 weeks) was 270 ± 10 g. There were no differences in the mean body weight of SHR and WKY rats.

Validation Studies. *Marker enzyme studies.* Table I depicts the specific activities of enzyme markers in crude homogenate and in basolateral membranes of SHR and WKY rats. As seen, the specific activity of Na^+-K^+-ATPase, a marker of basolateral membranes, was enriched 10.9 ± 0.2 and 11.6 ± 0.3 times as compared with mucosal homogenate in SHR and WKY, respectively; leucine aminopeptidase (brush border membrane marker) was impoverished 0.8 ± 0.1 times, cytochrome *c* oxidase (mitochondrial membrane marker) and NADPH-cytochrome *c* reductase (endoplasmic reticulum marker) were impoverished 0.6 ± 0.1 and 0.4 ± 0.1 times.

D-Glucose transport. D-Glucose transport was studied under both Na^+ and K^+ gradient conditions. As seen in Figure 1, no overshoot phenomenon was noted with Na^+ gradient and there were no differences between uptake values under Na^+ or K^+ gradient conditions. Equilibrium values were similar, indicating similar vesicular volume in SHR and WKY rat preparations.

ATP-Dependent Calcium Uptake by the Intestinal BLMV in SHR and WKY Rats. Figure 2 depicts calcium uptake in the presence and absence of ATP in both SHR and WKY, respectively. As seen in Figure 2, calcium uptake in the presence of ATP was stimulated compared with no ATP condition in both SHR and WKY, respectively ($P < 0.05$, 1–180 min). Mean calcium uptake values in the presence and absence of ATP were not significantly different between SHR and WKY rats.

Kinetics of ATP-Dependent Ca^{2+} Uptake in SHR and WKY Rats. Kinetics of calcium uptake were examined in the presence of 3 m*M* ATP at free Ca^{2+} concentrations between 0.01 and 1.0 μM. Micromolar concentrations of calcium were achieved by a EGTA buffering system as described by Pershadsingh and McDonald (25). Figure 3 depicts kinetic parameters for calcium uptake by intestinal BLMV in both SHR and WKY. K_m and V_{max} for both groups of rats were calculated according to a computer model for estimation of kinetic parameter (22). V_{max} and K_m values in SHR were 0.67 ± 0.03 nmol/mg protein/20 sec and 0.2 ± 0.03 μM and in WKY were 0.69 ± 0.12 nmol/mg protein/20 sec and 0.32 ± 0.14 μM, respectively. There were no significant differences between V_{max} and K_m values of SHR and WKY rats.

Na^+/Ca^{2+} Exchange System by Intestinal BLMV in SHR and WKY Rats. The process of Na^+/Ca^{2+} exchange system was measured by two methods in both SHR and WKY. First, sodium was added to the incubation medium (*cis* · Na^+). As seen in Figure 4, influx of Ca^{2+} in the presence of Na^+ was greater in SHR compared with WKY rats, suggesting limited exit of intravesicular Ca^{2+} in exchange with sodium in the extravesicular space. Second, as seen in Figure 5, Ca^{2+} efflux from BLMV in the presence of extravesicular Na^+ was more rapid in WKY compared with SHR.

Kinetics of the Na^+-Dependent Ca^{2+} Uptake by Intestinal BLMV of SHR and WKY Rats. The rate of Na^+-dependent Ca^{2+} uptake at concentrations between 0.01 and 1.0 μM in the absence of ATP was determined at 5 sec. This parameter was calculated using a computer model of Michaelis-Menten kinetics (22). As seen in Figure 6, V_{max} values were 0.05 ± 0.01 and 0.11 ± 0.02 nanmol/mg protein/5 sec for SHR and WKY rats, respectively ($P < 0.05$). K_m values were 0.21 ± 0.13 μM and 0.09 ± 0.05 μM, respectively ($P > 0.05$).

Discussion

These studies utilized a well-validated technique to investigate the process of calcium exit at the basolateral membrane of the enterocytes of the SHR and WKY rats. Validation studies utilizing marker enzyme enrichment and lack of effective sodium gradient on D-glucose uptake demonstrated biochemical and functional purity of the BLMV in both SHR and WKY rats. Previous studies have shown the presence of ATP-dependent and

Table I. Specific Activities of Enzyme Markers in Basolateral Membrane Vesicles and Crude Homogenate of SHR and WKY Rats[a]

	SHR		WKY	
	Crude homogenate	BLMV	Crude homogenate	BLMV
Na^+-K^+-ATPase (μmol P_i/hr/mg protein)	0.6–0.9	8–9.5	0.8–0.9	9–11
Leucine aminopeptidase (μg BNA hydrolyzed/μg protein)	0.58–0.8	0.45–0.6	0.51–0.72	0.32–0.59
Cytochrome *c* oxidase (μmol/mg protein/min)	0.09–0.14	0.048–0.093	0.12–0.18	0.08–0.098
NADPH cytochrome *c* reductase (μmol/mg protein/min)	8–14	0.038–0.048	9–14	4.0–4.9

[a] Values represent range of values on three separate determinations.

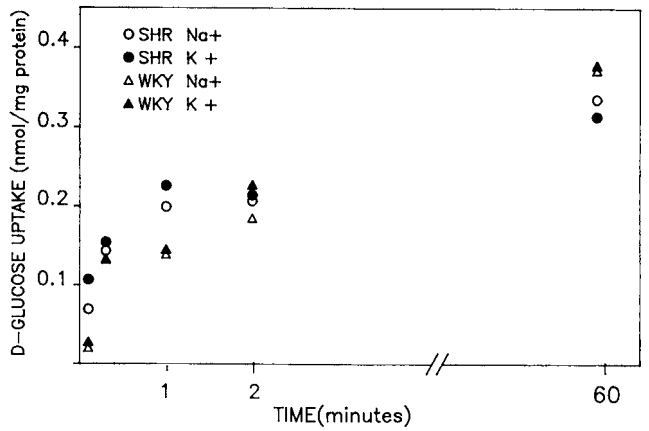

Figure 1. D-Glucose uptake by jejunal BLMV of SHR and WKY rats. BLMV were prepared in 300 m*M* mannitol, 5 m*M* $MgCl_2$, and 20 m*M* Hepes-Tris buffer (pH 7.4). Incubation medium contained either 100 m*M* NaCl or 100 m*M* KCl as well as 100 m*M* mannitol, 20 m*M* Hepes-Tris buffer, 0.1 m*M* D-glucose, and tracer [^{3}H]glucose. Reaction was stopped at desired time points. Each point represents mean ± SE of three separate experiments on different membrane preparations.

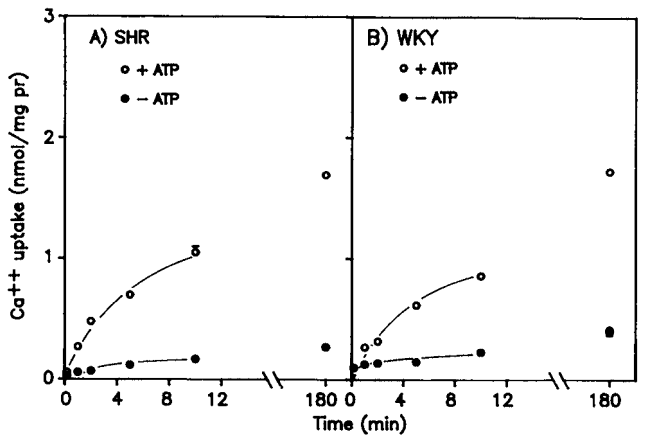

Figure 2. ATP-driven transport of Ca^{2+} into jejunal BLMV of SHR and WKY rats. BLMV were prepared in 100 m*M* mannitol, 100 m*M* KCl, 5 m*M* $MgCl_2$, and 20 m*M* Hepes-Tris buffer (pH 7.4). Incubation medium contained some solutes and also 1 μg/ml oligomycin, 1 m*M* ouabain, 0.1 m*M* $CaCl_2$, 3 m*M* Mg-ATP, and tracer ^{45}Ca. Reaction was stopped at desired time points. Each point represents mean ± SE of three separate experiments on different membrane preparations.

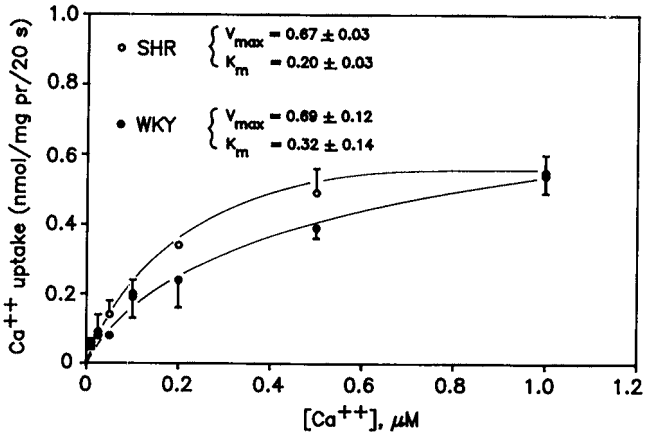

Figure 3. Kinetics of calcium uptake by jejunal BLMV of SHR and WKY rats. BLMV were incubated in a medium containing 100 m*M* mannitol, 100 m*M* KCl, 5 m*M* $MgCl_2$, and 20 m*M* Hepes-Tris buffer (pH 7.4) in the presence and absence of 3 m*M* Mg-ATP and different calcium concentrations (0.01–1 μ*M*) with tracer ^{45}Ca. Micromolar concentrations of calcium were achieved by EGTA buffering system as described by Pershadsingh and McDonald (25). Kinetic parameters were calculated from uptake values with ATP minus uptake in absence of ATP, using a computerized model of Michaelis-Menten kinetics. Reaction was stopped at 20 sec. Each point represents mean ± SE of three separate experiments on different membrane preparations.

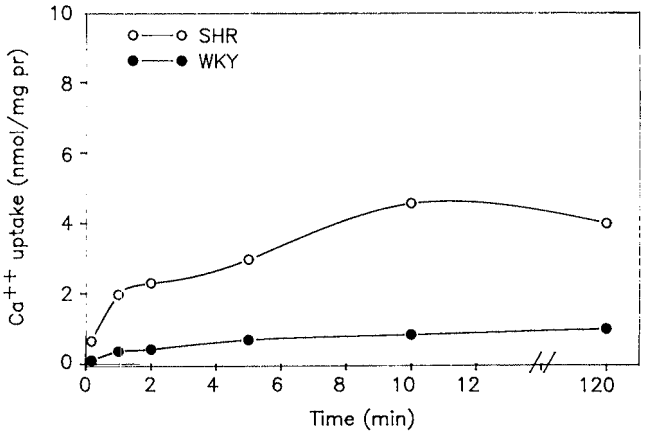

Figure 4. Effect of *cis*-Na^+ on ATP-driven Ca^{2+} uptake by jejunal BLMV of SHR and WKY rats. BLMV were preincubated in a medium containing 100 m*M* mannitol, 100 m*M* KCl, 5 m*M* $MgCl_2$, and 20 m*M* Hepes-Tris (pH 7.4). Incubation solution contained 100 m*M* mannitol, 100 m*M* NaCl, 20 m*M* Hepes-Tris, 3 m*M* Mg-ATP, 0.1 m*M* $CaCl_2$, and tracer ^{45}Ca. Reaction was stopped at desired time points.

Ca^{2+}/Na^+ exchange processes in rat jejunal basolateral membranes (26–28).

The results of the present studies demonstrate marked reduction in the process of Ca^{2+}/Na^+ exchange located at the basolateral membrane of the spontaneously hypertensive rats compared with that of their genetically matched controls. This reduction is secondary to a decrease in V_{max} rather than in K_m, indicating that the number and/or activity of the Ca^{2+}/Na^+ exchanger is decreased in the SHR compared with WKY rats. Several studies have suggested an increase in intracellular calcium concentration in tissues of hypertensive humans and in SHR (29, 30). The mechanisms underlying the increase in intracellular calcium is not apparent from these studies. Two mechanisms have been documented to be responsible for movement of intracellular calcium to the extracellular compartment, an ATP-dependent mechanism requiring the hydrolysis of ATP by Ca^{2+}-Mg^{2+}-ATPase and a carrier-mediated Ca^{2+}/Na^+ exchange (19). The contribution of the Ca^{2+}/Na^+ and the ATP-dependent Ca^{2+} transport to the overall transport of Ca^{2+} is not known because there are no specific inhibitors for each of these processes. The V_{max} of each of these processes is quite similar. Both mechanisms have been described in a variety of tissues including red blood cells (31), human placenta

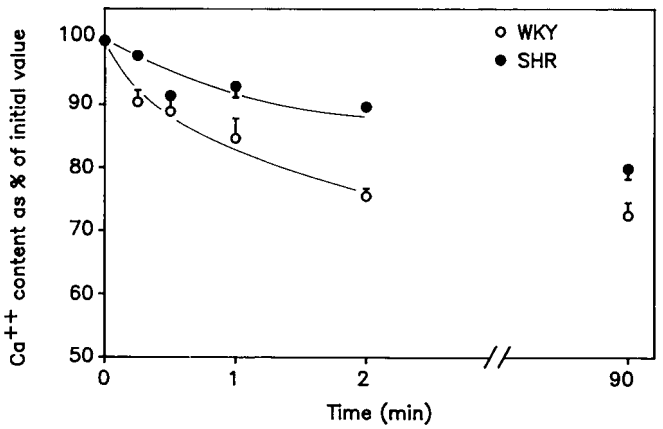

Figure 5. Efflux of calcium from jejunal BLMV of SHR and WKY rats. BLMV were loaded for 60 min with 0.1 m*M* $CaCl_2$ and tracer ^{45}Ca in the presence of 100 m*M* mannitol, 100 m*M* KCl, 5 m*M* $MgCl_2$, and 20 m*M* Hepes-Tris (pH 7.4). Reaction was started by diluting loaded vesicles in a medium containing 100 m*M* mannitol, 100 m*M* NaCl, and 20 m*M* Hepes-Tris (pH 7.5). Reaction was stopped at desired time intervals. Each point represents mean ± SE of three separate experiments on different membrane preparations.

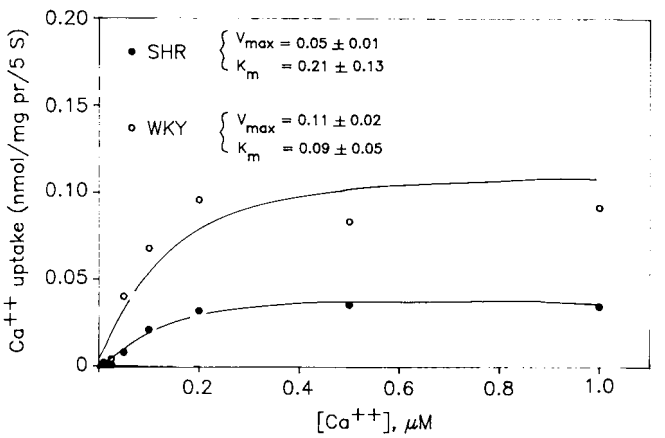

Figure 6. Kinetics of Na^+-Ca^{2+} exchanger in jejunal BLMV of SHR and WKY rats. BLMV were preincubated either in 100 m*M* NaCl, 100 m*M* KCl, and 20 m*M* Hepes-Tris (pH 7.4) or 100 m*M* choline chloride, 100 m*M* KCl, and 20 m*M* Hepes-Tris buffer (pH 7.4) for 30 min. Incubation medium contained per liter: 100 m*M* KCl, 100 m*M* mannitol, 5 m*M* $MgCl_2$, 20 m*M* Hepes-Tris (pH 7.4), valinomycin 10 μg/mg protein, and different Ca^{2+} concentrations. Uptake was determined at 5 sec. Kinetic parameters were calculated by subtracting uptake values of Na^+-choline using a computerized model of Michaelis-Menten kinetics. Each point represents mean ± SE of three separate experiments on different membrane preparations.

(32), adipocyte (25), and cardiac sarcolemma (33), and enterocytes (26, 27). A 33-kDa protein has been purified from the cardiac sarcolemma which represents the Ca^{2+}/Na^+ exchanger (33), whereas a 70-kDa protein was purified from the synaptic sarcolemma (34). The Ca^{2+}/Na^+ exchanger is thought to contribute to the efflux of calcium from the intracellular compartment of polarized cells with an inwardly directed Na^+ gradient. A defect in this exchanger may lead to a rise in the intracellular concentration of calcium. Although our studies have defined abnormalities in calcium handling by the enterocyte, these changes may represent a generalized phenomenon in other cell systems. Of particular importance is the observation that elevated intracellular calcium may lead to functional changes in vascular smooth muscle cell resulting in increased peripheral resistance with the development of hypertension (35, 36).

The results of the current studies may have relevance to the development of hypertension in the SHR.

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