The effect of diets adequate and deficient in calcium on blood pressures and the activities of intestinal and kidney plasma membrane enzymes in normotensive and spontaneously hypertensive rats

BY PETER BLAKEBOROUGH, SHEILA G. NEVILLE AND BRIAN A. ROLLS

Department of Human Nutrition, AFRC Institute of Food Research, Reading Laboratory, Shinfield, Reading RG2 9AT

(*Received 10 January 1989 – Accepted 9 August 1989*)

Basolateral and brush-border membranes were prepared from the intestines and kidneys of spontaneously hypertensive (SHR) and normotensive (WKY) rats fed on a calcium-adequate diet and assayed for their enzyme activities. In intestinal basolateral membranes the activities of Na^+ K^+-ATPase (*EC* 3.6.1.37) Ca^{2+}-ATPase (*EC* 3.6.1.38) and alkaline phosphatase (*EC* 3.1.3.1) were lower in SHR rats when compared with WKY rats, whilst 5′-nucleotidase (*EC* 3.1.3.5) (a marker for basolateral membranes) was unaffected. In kidney basolateral membranes all enzymes were similar in activity in SHR and WKY rats. In intestinal brush-border membranes the activities of Ca^{2+}-ATPase and alkaline phosphatase were lower in SHR rats when compared with WKY rats, whilst microvillus aminopeptidase (*EC* 3.4.11.2) (a marker for brush-border membranes) was unaffected. In kidney brush-border membranes all enzymes were similar in activity in SHR and WKY rats. The blood pressures of the SHR rats were considerably higher than those of the WKY rats. When SHR rats were fed on a Ca-deficient diet the activities of Na^+K^+-ATPase, Ca^{2+}-ATPase and alkaline phosphatase in basolateral membranes and Ca^{2+}-ATPase and alkaline phosphatase in brush-border membranes were all increased in the intestine when compared with SHR rats fed on a Ca-adequate diet. The equivalent enzymes in the kidneys of SHR rats, and the intestines and kidneys of WKY rats, were not affected by altering the Ca in the diet. The blood pressures of SHR rats fed on a Ca-deficient diet were higher than in those fed on a Ca-adequate diet. Blood pressures of WKY rats were not affected by altering the diet in this way. The results indicate that the absorption of Ca by active mechanisms may be reduced in SHR rats compared with WKY rats. Changing the level of Ca in the diet modified both blood pressure and the activities of enzymes which catalyse active Ca transport. The implications of these results to the aetiology, and possible nutritional treatment, of essential hypertension are discussed.

Brush-border membrane: Calcium: Hypertension: Rat

There is much evidence that the development of essential hypertension in man may be linked with diets that are high in sodium or low in calcium, or both (for reviews, see McCarron, 1985; MacGregor, 1985). Mechanisms to account for these observations are not fully understood. Diets low in Na or high in Ca, or both, are often proposed to decrease hypertensive symptoms (MacGregor, 1985; McCarron & Morris, 1985). Intestinal Ca transport has been reported to be lower, whilst Na transport was increased, in the spontaneously hypertensive rat (SHR) compared with the normotensive Wistar Kyoto (WKY) rat (Schedl *et al.* 1984).

The present study was designed to examine the activities of enzymes which catalyse active Ca transport across plasma membranes of the intestine and kidney, and to observe whether these enzymes play a role in the development of hypertension. In addition, a study was made of the effect of diets adequate and deficient in Ca on the activities of these enzymes. Homogenates of enterocytes and kidneys, together with preparations of the absorptive

basolateral and brush-border membranes, were assessed for enzyme activities to determine whether they were altered in the hypertensive state. The SHR rat was used as it is considered to be the best available animal model for human essential hypertension (Trippodo & Frohlic, 1981). A preliminary account containing some of these results has been presented (Blakeborough *et al.* 1988).

MATERIALS AND METHODS

Materials

Calmodulin, *p*-nitrophenylphosphate, ouabain, adenosine monophosphate, adenosine deaminase, levamisole, ATP, NADH, 2-(*p*-iodophenyl)-3-(*p*-nitrophenyl)-5-(phenyl) tetrazolium hydrochloride and L-alanine 4-nitroanilide were purchased from Sigma Chemical Co Ltd, Poole, Dorset. Other chemicals were purchased from BDH Chemicals Ltd, Poole, Dorset and were of analytical grade where possible.

Animals and diets

Female SHR and WKY rats were purchased from Olac Ltd (Shaw's Farm, Bicester). Groups of five rats were maintained from 8 weeks of age on diets based on a standard expanded rat and mouse grower–breeder laboratory diet (CRM(X), K & K Greeff Ltd, Croydon, Surrey). Two diets were prepared: one was adequate in Ca, containing (mg/g) 6 Ca, 4 Na (+Ca), and one was deficient in Ca, containing (mg/g) 0·6 Ca, 4 Na (−Ca). Ten rats were used for each of the four groups of rats (WKY + Ca, WKY − Ca, SHR + Ca, SHR − Ca) in each experiment.

From 12 weeks of age the systolic blood pressures were measured by tail-cuff sphygmomanometry, using an IITC model 120 cuff pump and model 159 amplifier (IITC Inc., Woodland Hills, California). At 18 weeks of age the rats were killed using carbon dioxide. At this age animals were of similar body-weight (WKY 289 (SEM 10) g, SHR 297 (SEM 8) g.

Preparation of membranes

The rats were killed and the small intestines and kidneys were excised and stored at −20°. Membranes were prepared from this frozen tissue without previous removal of the mucosal layer. Homogenates were prepared as described by Kessler *et al.* (1978), and the homogenates from three animals were used for each membrane preparation.

Basolateral membranes were prepared from the homogenates after resuspension in 0·25 M-sucrose–10 mM-triethanolamine–0·5 mM-EGTA buffer, pH 7·6, as described by Scalera *et al.* (1980), using Percoll density-gradient centrifugation. The membranes were finally suspended in 160 mM-potassium chloride–20 mM-Hepes, pH 7·4, and stored at −20°.

Brush-border membranes were prepared from the homogenates by the method of Booth & Kenny (1974), using magnesium chloride instead of calcium chloride to precipitate other cell membranes.

Enzyme assays

Na^+K^+-ATPase (*EC* 3.6.1.37) is known to have an ouabain-sensitive K^+-dependent phosphatase activity (Colas & Maroux, 1980). This activity was measured instead of ATP hydrolysis. From the findings reported here the specific activity of the enzyme in basolateral membranes (2·74 μmol/h per mg protein) was similar to that previously reported using this assay (42–54 nmol/min per mg protein (Colas & Maroux, 1980)). 5′-Nucleotidase (*EC* 3.1.3.5) activity was assayed using a coupled reaction where 5′-AMP hydrolysis was monitored at 265 nm with excess adenosine deaminase (*EC* 3.5.4.4) (Colas & Maroux, 1980). Levamisole (1 mM) was added to the incubation to inhibit alkaline phosphatase (*EC* 3.1.3.1) activity. Na^+K^+-ATPase and 5′-nucleotidase are recognized markers for basolateral plasma membranes (Colas & Maroux, 1980).

Microvillus aminopeptidase (*EC* 3.4.11.2) activity was assayed as described by Sjöström *et al.* (1978). Alkaline phosphatase activity was assayed by observing the initial velocity of the conversion of *p*-nitrophenylphosphate to *p*-nitrophenol (Murer *et al.* 1976). These two enzymes are markers for brush-border membranes.

Succinate dehydrogenase (*EC* 1.3.99.1) (a marker for mitochondria) was assayed by the method of Porteous & Clark (1965). Lactate dehydrogenase (*EC* 1.1.1.27) (a marker for cytosol) was assayed as described by Reeves & Fimognari (1966). Acid phosphatase (*EC* 3.1.3.2) (a lysosomal marker) was assayed by the technique of Scalera *et al.* (1980). High-affinity ($Ca^{2+}+Mg^{2+}$)-ATPase (*EC* 3.6.1.38) activity was assayed in a reaction volume of 1 ml, containing 50 mM-Tris–1,4-piperazinediethanesulphonic acid, pH 7·5, 200 μM-EGTA, 200 μM-$CaCl_2$ (free Ca^{2+} concentration 2·7 μM (Chan *et al.* 1986)), 20 mM-sodium azide, 5 mM-$MgCl_2$, 1 μM-calmodulin, 10–20 μg membrane protein. Reactions were initiated with ATP (1 mM final concentration), conducted for 15 min at 37° and terminated with 0·5 ml sodium lauryl sulphate (100 g/l). The method of Fiske & Subbarow (1925) was used to determine the amount of inorganic phosphorus released by the reaction. Mg^{2+}-ATPase activity was defined as that activity obtained in the absence of $CaCl_2$ and calmodulin and in the presence of EGTA. Ca^{2+}-ATPase was defined as the difference between the two activities.

Protein was assayed as described by Lowry *et al.* (1951) after precipitation with sodium deoxycholate and trichloroacetic acid (Bensadoun & Weinstein, 1976).

Experimental

Two experiments were conducted. In the first, the activities of intestinal and kidney enzymes were compared in SHR and WKY rats given +Ca diets. The second experiment compared the activities of certain basolateral and brush-border membrane enzymes in SHR and WKY rats given +Ca (SHR +Ca, WKY +Ca) and −Ca (SHR −Ca, WKY −Ca) diets. With intestinal samples the two experiments were conducted separately. With the kidney samples the two experiments were combined into one.

RESULTS

The blood pressures of SHR and WKY rats fed on +Ca and −Ca diets are shown in Fig. 1. The blood pressures of SHR rats were significantly higher ($P < 0.001$) than those of WKY rats. Also, SHR rats fed on −Ca diets exhibited higher blood pressures ($P < 0.05$) than SHR rats fed on +Ca diets. A similar trend in blood pressures was observed with the WKY rats, but the difference was not significant.

Tables 1–4 compare the enzyme activities in the homogenates and basolateral and brush-border membranes of intestines and kidneys of SHR and WKY rats. Basolateral membranes were purified approximately five- to sevenfold over homogenate values from both intestine and kidney, as assessed by 5′-nucleotidase assay. Brush-border membranes were purified approximately five- to sevenfold (intestine) and 14 to 16-fold (kidney) over homogenate values, as assessed by microvillus aminopeptidase assays. The two membranes were purified to similar extents whether isolated from SHR or WKY rats. The level of contamination by other cell membranes was low.

Preparations of intestinal basolateral membranes from SHR rats showed significantly lower activities of Na^+K^+-ATPase (by approximately 45%), alkaline phosphatase (by approximately 35%) and Ca^{2+}-ATPase (by approximately 30%), whilst the activity of microvillus aminopeptidase was increased (by approximately 25%) compared with the membranes of WKY rats. The other enzymes assayed, and results for homogenate samples, were relatively unaffected by rat strain (Table 1).

Preparations of intestinal brush-border membranes from SHR rats exhibited significantly

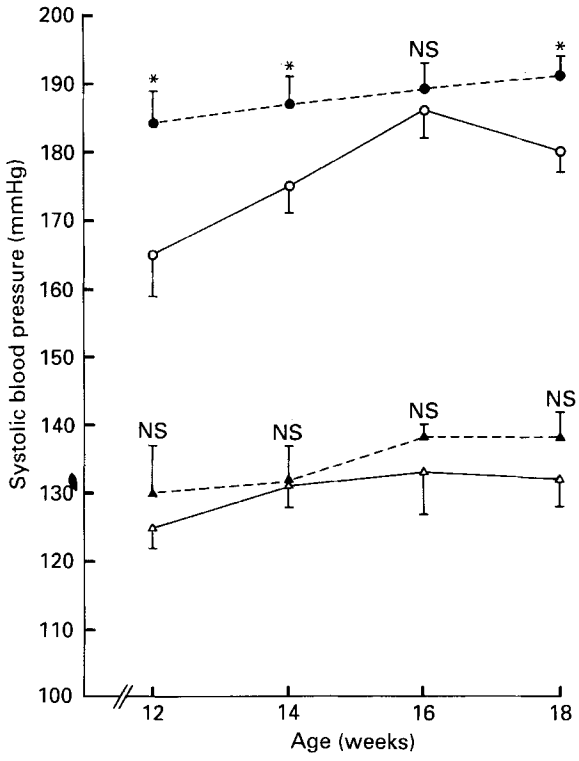

Fig. 1. The effect of calcium adequate (+Ca) and deficient (−Ca) diets on the blood pressures of normotensive (WKY) and spontaneously hypertensive (SHR) rats. WKY and SHR rats were given +Ca and −Ca diets and their blood pressures were measured, as described on p. 66. (△), WKY +Ca; (▲), WKY −Ca; (○), SHR +Ca; (●), SHR −Ca. Values are means with their standard errors, represented by vertical bars, for ten rats. Differences between WKY +Ca and WKY −Ca, and SHR +Ca and SHR −Ca were tested for statistical significance using Student's *t* test. NS, not significant; * $P < 0.05$.

lower activities of alkaline phosphatase (by approximately 55%) and Ca^{2+}-ATPase (by approximately 20%) compared with the membranes of WKY rats. The other enzymes assayed, and results for homogenate samples, were relatively unaffected by rat strain (Table 2).

Preparations of kidney basolateral (Table 3) and brush-border membranes (Table 4) exhibited similar values for all the enzymes tested, irrespective of whether they were isolated from SHR or WKY rats.

Table 5 shows the effect of +Ca and −Ca diets on the activities of some enzymes of basolateral and brush-border membranes of the intestines of SHR and WKY rats. The activities of alkaline phosphatase, Ca^{2+}-ATPase and, for basolateral membranes only, Na^+K^+-ATPase were again lower in SHR rats, compared with WKY rats, when fed on +Ca diets. Activities of these enzymes were significantly higher in basolateral and brush-border membranes of SHR rats given −Ca diets compared with those given +Ca diets. This effect of diet was not observed in WKY rats. However, activities of 5′-nucleotidase in basolateral membranes and microvillus aminopeptidase in brush-border membranes were not affected by strain of rat or diet.

Table 6 shows the effects of +Ca and −Ca diets on the activities of some basolateral and brush-border membrane enzymes of the kidneys of SHR and WKY rats. There were no

Table 1. *A comparison of enzyme activities between intestinal basolateral membranes of spontaneously hypertensive (SHR) and normotensive (WKY) rats*

(Results are means with their standard errors of six membrane preparations. Units for specific activity are all μmol/h per mg protein except for microvillus aminopeptidase, 5′-nucleotidase, alkaline phosphatase and lactate dehydrogenase (μmol/min per mg protein))

	Rat strain	Specific activity: Homogenate Mean	Homogenate SEM	Basolateral membranes Mean	Basolateral membranes SEM	SHR/WKY (activity in basolateral membranes)	Purification Mean	Purification SEM
Marker enzymes								
5′-Nucleotidase (*EC* 3.1.3.5)	WKY	0·18	0·007	1·00	0·15	—	5·61	0·86
5′-Nucleotidase	SHR	0·15^{NS}	0·016	0·8^{NS}	0·019	0·8	5·27^{NS}	0·13
Microvillus aminopeptidase (*EC* 3.4.11.2)	WKY	0·074	0·01	0·119	0·07	—	1·77	0·19
Microvillus aminopeptidase	SHR	0·08^{NS}	0·007	0·152†	0·011	1·28	1·9^{NS}	0·13
Succinate dehydrogenase (*EC* 1.3.99.1)	WKY	1·14	0·28	1·32	0·39	—	1·19	0·11
Succinate dehydrogenase	SHR	1·35^{NS}	0·63	2·51^{NS}	1·14	1·9	1·95††	0·18
Lactate dehydrogenase (*EC* 1.1.1.27)	WKY	7·45	1·61	1·2	0·23	—	0·18	0·06
Lactate dehydrogenase	SHR	6·29^{NS}	1·24	1·27^{NS}	0·2	1·1	0·21^{NS}	0·05
Acid phosphatase (*EC* 3.1.3.2)	WKY	1·99	0·14	2·52	0·5	—	1·25	0·16
Acid phosphatase	SHR	1·86^{NS}	0·32	2·52^{NS}	0·72	1·0	1·3^{NS}	0·14
Calcium transport enzymes								
Ca^{2+}-ATPase (*EC* 3.6.1.38)	WKY	11·57	3·85	136·66	13·74	—	11·67	1·59
Ca^{2+}-ATPase	SHR	6·76^{NS}	1·07	92·85*	18·94	0·68	13·28^{NS}	1·54
Mg^{2+}-ATPase (*EC* 3.6.1.38)	WKY	57·45	13·94	127·8	12·3	—	2·71	0·06
Mg^{2+}-ATPase	SHR	42·04^{NS}	14·79	114·25^{NS}	35·17	0·89	2·57^{NS}	0·4
Alkaline phosphatase (*EC* 3.1.3.1)	WKY	1·8	0·22	6·48	0·4	—	3·85	0·46
Alkaline phosphatase	SHR	1·25^{NS}	0·05	4·06††	0·3	0·63	3·25^{NS}	0·23
Na^+K^+-ATPase (*EC* 3.6.1.37)	WKY	0·43	0·075	2·74	0·32	—	6·93	1·34
Na^+K^+ATPase	SHR	0·38^{NS}	0·044	1·47**	0·23	0·54	3·95^{NS}	0·83

NS, not significant.
Mean values were significantly different from those for WKY rats (Student's *t* test): * $P < 0·05$, † $P < 0·025$, ** $P < 0·01$, †† $P < 0·005$.

Table 2. *A comparison of enzyme activities between intestinal brush-border membranes of spontaneously hypertensive (SHR) and normotensive (WKY) rats*

(Results are means with their standard errors of six membrane preparations. Units for specific activity are all μmol/h per mg protein except for microvillus aminopeptidase, lactate dehydrogenase and alkaline phosphatase (μmol/min per mg protein))

	Rat strain	Specific activity: Homogenate Mean	Homogenate SEM	Brush-border membranes Mean	Brush-border membranes SEM	SHR/WKY (activity in brush-border membranes)	Purification Mean	Purification SEM
Marker enzymes								
Microvillus aminopeptidase (*EC* 3.4.11.2)	WKY	0·086	0·019	0·549	0·14	—	6·95	0·88
Microvillus aminopeptidase	SHR	0·085^{NS}	0·007	0·43^{NS}	0·027	0·78	5·33^{NS}	0·33
Succinate dehydrogenase (*EC* 1.3.99.1)	WKY	0·48	0·18	0·11	0·065	—	0·23	0·14
Succinate dehydrogenase	SHR	0·54^{NS}	0·16	0·018^{NS}	0·018	0·17	0·019^{NS}	0·019
Lactate dehydrogenase (*EC* 1.1.1.27)	WKY	6·64	0·86	0·23	0·04	—	0·037	0·015
Lactate dehydrogenase	SHR	7·4^{NS}	3·9	0·31^{NS}	0·17	1·35	0·041^{NS}	0·002
Acid phosphatase (*EC* 3.1.3.2)	WKY	3·59	1·19	3·91	0·46	—	1·27	0·55
Acid phosphatase	SHR	3·68^{NS}	1·96	3·26^{NS}	1·15	0·83	1·01^{NS}	0·23
Calcium transport enzymes								
Ca^{2+}-ATPase (*EC* 3.6.1.38)	WKY	13·99	5·7	117·1	15·88	—	12·51	3·11
Ca^{2+}-ATPase	SHR	6·84^{NS}	0·41	91·42*	7·06	0·78	13·75^{NS}	1·36
Mg^{2+}-ATPase (*EC* 3.6.1.38)	WKY	58·2	1·75	152·02	10·92	—	2·57	0·15
Mg^{2+}-ATPase	SHR	38·19*	8·21	134·21^{NS}	15·76	0·88	2·66^{NS}	0·31
Alkaline phosphatase (*EC* 3.1.3.1)	WKY	1·78	0·17	14·16	1·68	—	7·95	0·94
Alkaline phosphatase	SHR	1·49^{NS}	0·18	6·26††	0·38	0·44	4·2**	0·25
Na^+K^+-ATPase (*EC* 3.6.1.37)	WKY	0·42	0·14	1·03	0·66	—	2·45	0·94
Na^+K^+-ATPase	SHR	0·3^{NS}	0·23	0·47^{NS}	0·02	0·46	1·58^{NS}	1·27

NS, not significant.

Mean values were significantly different from those for WKY rats (Student's *t* test): * $P < 0·05$; ** $P < 0·01$, †† $P < 0·005$.

Table 3. *A comparison of enzyme activities between kidney basolateral membranes of spontaneously hypertensive (SHR) and normotensive (WKY) rats*

(Results are means with their standard errors of three membrane preparations. Units for specific activity are all μmol/h per mg protein except for microvillus aminopeptidase, 5′-nucleotidase, alkaline phosphatase and lactate dehydrogenase (μmol/min per mg protein))

	Rat strain	Specific activity: Homogenate Mean	Homogenate SEM	Basolateral membranes Mean	Basolateral membranes SEM	SHR/WKY (activity in basolateral membranes)	Purification Mean	Purification SEM
Marker enzymes								
5′-Nucleotidase (*EC* 3.1.3.5)	WKY	0·012	0·002	0·0756	0·01	—	6·37	0·52
5′-Nucleotidase	SHR	0·009^{NS}	0·001	0·063^{NS}	0·008	0·83	7·12^{NS}	0·22
Microvillus aminopeptidase (*EC* 3.4.11.2)	WKY	0·113	0·012	0·344	0·062	—	3·14	0·74
Microvillus aminopeptidase	SHR	0·096^{NS}	0·008	0·255^{NS}	0·064	0·74	2·69^{NS}	0·73
Succinate dehydrogenase (*EC* 1.3.99.1)	WKY	3·92	0·32	1·34	0·28	—	0·34	0·07
Succinate dehydrogenase	SHR	3·3^{NS}	0·45	0·91^{NS}	0·29	0·68	0·31^{NS}	0·13
Lactate dehydrogenase (*EC* 1.1.1.27)	WKY	1·22	0·01	0·11	0·03	—	0·09	0·02
Lactate dehydrogenase	SHR	1·08†	0·05	0·13^{NS}	0·02	1·18	0·12^{NS}	0·02
Acid phosphatase (*EC* 3.1.3.2)	WKY	2·8	0·04	3·77	0·32	—	1·35	0·12
Acid phosphatase	SHR	2·92^{NS}	0·08	3·58^{NS}	0·89	0·95	1·23^{NS}	0·3
Calcium transport enzymes								
Ca^{2+}-ATPase (*EC* 3.6.1.38)	WKY	1·99	0·53	8·81	4·01	—	4·43	1·43
Ca^{2+}-ATPase	SHR	1·61^{NS}	0·61	6·84^{NS}	2·07	0·78	5·21^{NS}	1·39
Mg^{2+}-ATPase (*EC* 3.6.1.38)	WKY	4·51	0·52	27·61	6·61	—	5·96	0·82
Mg^{2+}-ATPase	SHR	4·74^{NS}	0·92	30·16^{NS}	9·21	1·09	6·11^{NS}	0·64
Alkaline phosphatase (*EC* 3.1.3.1)	WKY	0·38	0·04	1·12	0·17	—	3·04	0·62
Alkaline phosphatase	SHR	0·33^{NS}	0·03	0·95^{NS}	0·28	0·85	2·8^{NS}	0·77
Na^+K^+-ATPase (*EC* 3.6.1.37)	WKY	0·38	0·03	2·03	0·24	—	5·37	0·72
Na^+K^+-ATPase	SHR	0·43^{NS}	0·08	2·01^{NS}	0·31	0·99	5·02^{NS}	1·03

NS, not significant.
Mean values were significantly different from those for WKY rats (Student's *t* test): † $P < 0·025$.

Table 4. *A comparison of enzyme activities between kidney brush-border membranes of spontaneously hypertensive (SHR) and normotensive (WKY) rats*

(Results are means with their standard errors of three membrane preparations. Units for specific activity are all μmol/h per mg protein except for microvillus aminopeptidase, alkaline phosphatase and lactate dehydrogenase (μmol/min per mg protein))

	Rat strain	Specific activity: Homogenate Mean	Homogenate SEM	Brush-border membranes Mean	Brush-border membranes SEM	SHR/WKY (activity in brush-border membranes)	Purification Mean	Purification SEM
Marker enzymes								
Microvillus aminopeptidase (*EC* 3.4.11.2)	WKY	0·083	0·05	1·17	0·25	—	14·1	2·81
Microvillus aminopeptidase	SHR	$0·073^{NS}$	0·004	$1·196^{NS}$	0·05	1·02	$16·34^{NS}$	1·09
Succinate dehydrogenase (*EC* 1.3.99.1)	WKY	3·72	1·71	0·78	0·57	—	0·16	0·06
Succinate dehydrogenase	SHR	$2·12^{NS}$	0·34	$0·42^{NS}$	0·06	0·54	$0·24^{NS}$	0·07
Lactate dehydrogenase (*EC* 1.1.1.27)	WKY	1·28	0·17	0·54	0·06	—	0·42	0·01
Lactate dehydrogenase	SHR	$1·09^{NS}$	0·03	$0·38^{NS}$	0·05	0·7	$0·35^{NS}$	0·05
Acid phosphatase (*EC* 3.1.3.2)	WKY	2·63	0·09	3·29	0·45	—	1·24	0·13
Acid phosphatase	SHR	2·94*	0·1	$3·83^{NS}$	0·39	1·16	$1·31^{NS}$	0·14
Calcium transport enzymes								
Ca^{2+}-ATPase (*EC* 3.6.1.38)	WKY	0·8	0·31	11·55	3·74	—	12·07	1·52
Ca^{2+}-ATPase	SHR	$1·35^{NS}$	0·19	$13·97^{NS}$	2·74	1·21	$10·68^{NS}$	2·19
Mg^{2+}-ATPase (*EC* 3.6.1.38)	WKY	3·8	0·7	15·99	5·13	—	3·75	1·06
Mg^{2+}-ATPase	SHR	$4·95^{NS}$	1·35	$15·34^{NS}$	2·01	0·96	$3·58^{NS}$	1·02
Alkaline phosphatase (*EC* 3.1.3.1)	WKY	0·28	0·02	2·66	0·46	—	9·37	1·21
Alkaline phosphatase	SHR	$0·24^{NS}$	0·05	$3·29^{NS}$	0·06	1·24	$15·74^{NS}$	4·65
Na^+K^+-ATPase (*EC* 3.6.1.37)	WKY	0·17	0·05	0·11	0·02	—	0·78	0·14
Na^+K^+-ATPase	SHR	$0·15^{NS}$	0·04	$0·077^{NS}$	0·02	0·7	$0·67^{NS}$	0·34

NS, not significant.
Mean values were significantly different from those for WKY rats (Student's t test): * $P < 0·05$.

Table 5. *The effect of calcium-adequate (+Ca) and Ca-deficient (−Ca) diets on the specific activities of some enzymes of intestinal plasma membranes of spontaneously hypertensive (SHR) and normotensive (WKY) rats*

(Results are means with their standard errors of three membrane preparations)

Strain of rat, and diet...	WKY +Ca		WKY −Ca		SHR +Ca		SHR −Ca	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
Basolateral membranes								
Na^+K^+-ATPase‡ (*EC* 3.6.1.37)	2·18	0·67	2·98^{NS}	0·61	1·13*	0·07	2·0†	0·38
Alkaline phosphatase§ (*EC* 3.1.3.1)	6·73	0·85	7·73^{NS}	1·82	3·79†	0·42	5·7*	0·98
Ca^{2+}-ATPase‡ (*EC* 3.6.1.38)	115·81	8·6	131·1^{NS}	14·89	70·93†	13·0	101·61*	5·21
5′-Nucleotidase§ (*EC* 3.1.3.5)	0·68	0·02	0·73^{NS}	0·12	0·74^{NS}	0·07	0·73^{NS}	0·1
Brush-border-membranes								
Alkaline phosphatase§	11·85	1·58	13·38^{NS}	3·48	6·4†	0·63	8·38*	0·82
Ca^{2+}-ATPase‡	160·25	38·48	120·33^{NS}	20·5	90·22†	5·59	112·65*	2·39
Microvillus aminopeptidase§ (*EC* 3.4.11.2)	0·41	0·03	0·47^{NS}	0·06	0·42^{NS}	0·04	0·36^{NS}	0·04

NS, not significant.

Mean values were significantly different from those for control values (Student's *t* test): * $P < 0·05$, † $P < 0·025$. WKY −Ca *v.* WKY +Ca; SHR +Ca *v.* WKY +Ca, SHR −Ca *v.* SHR +Ca.

‡ μmol/h per mg protein.

§ μmol/min per mg protein.

Table 6. *The effect of calcium adequate (+Ca) and Ca-deficient (−Ca) diets on the specific activities of some enzymes of kidney plasma membranes of spontaneously hypertensive (SHR) and normotensive (WKY) rats*

(Results are means with their standard errors of three membrane preparations)

Strain of rat, and diet...	WKY +Ca		WKY −Ca		SHR +Ca		SHR −Ca	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
Basolateral membranes								
Na^+K^+-ATPase‡ (*EC* 3.6.1.37)	2·03	0·24	1·83^{NS}	0·25	2·01^{NS}	0·31	2·24^{NS}	0·18
Alkaline phosphatase§ (*EC* 3.1.3.1)	1·12	0·17	0·8^{NS}	0·21	0·95^{NS}	0·28	0·79^{NS}	0·17
Ca^{2+}-ATPase‡ (*EC* 3.6.1.38)	8·81	4·01	4·61^{NS}	1·08	6·84^{NS}	2·07	10·66^{NS}	4·61
5′-Nucleotidase§ (*EC* 3.1.3.5)	0·076	0·01	0·063^{NS}	0·013	0·063^{NS}	0·008	0·73^{NS}	0·16
Brush-border-membranes								
Alkaline phosphatase§	2·66	0·46	2·53^{NS}	0·21	3·29^{NS}	0·06	2·86†	0·11
Ca^{2+}-ATPase‡	11·55	3·74	11·66^{NS}	4·47	13·97^{NS}	2·74	11·76^{NS}	3·33
Microvillus aminopeptidase§ (*EC* 3.4.11.2)	1·17	0·25	0·99^{NS}	0·11	1·2^{NS}	0·05	1·23^{NS}	0·18
Na^+K^+-ATPase‡	0·11	0·02	0·11^{NS}	0·04	0·08^{NS}	0·02	0·09^{NS}	0·02

NS, not significant.

Mean values were significantly different from those for control values (Student's *t* test): † $P < 0·025$. WKY −Ca *v.* WKY +Ca; SHR +Ca *v.* WKY +Ca; SHR −Ca *v.* SHR +Ca.

‡ μmol/h per mg protein.

§ μmol/min per mg protein.

significant differences in activities of any of the enzymes tested either between SHR and WKY rats or between +Ca or −Ca diets. In brush-border membranes the activity of alkaline phosphatase was significantly less in SHR rats given the −Ca diet compared with the +Ca diet. However, this activity was not significantly different from that derived from WKY rats, when given +Ca or −Ca diets.

DISCUSSION

The blood pressures of SHR rats are known to be sensitive to dietary Ca: decreasing dietary Ca leads to an exacerbation of hypertension, whilst increasing dietary Ca leads to reduced blood pressure (McCarron *et al.* 1981). This hypertensive effect of low dietary Ca was observed in the present study. The reduction in activities of Na^+K^+-ATPase, Ca^{2+}-ATPase and alkaline phosphatase observed in the intestines of SHR rats compared with WKY rats, and the sensitivities of these enzymes to the alteration of dietary Ca, may give some clues to the underlying molecular mechanisms which lead to essential hypertension.

A high-affinity, calmodulin-dependent Ca^{2+}-ATPase activity has been directly correlated with the active transport of Ca out of cells against a thermodynamic gradient, functioning, for example, in Ca absorption (Nellans & Popovitch, 1981). The activity of this enzyme has been reported to be impaired in erythrocytes of SHR compared with WKY rats (Vezzoli *et al.* 1985) and in the erythrocytes (Postnov *et al.* 1984) and platelets (Resink *et al.* 1986) of hypertensive human subjects compared with normotensive subjects. In the present report the activity of high-affinity Ca^{2+}-ATPase was significantly reduced in the intestinal basolateral and brush-border membranes of SHR compared with WKY rats, whilst Mg^{2+}-ATPase activity (which does not correlate with Ca transport) was unaffected. The presence of a Ca^{2+}-ATPase in intestinal brush-border membranes was confirmed. This activity was not due to contamination by basolateral membranes, because it was purified to a five- to sixfold greater extent than the marker for basolateral membranes, Na^+K^+-ATPase. Ca^{2+}-ATPase activity in intestinal plasma membranes has been shown to be at least a partial expression of the alkaline phosphatase enzyme (Williams *et al.* 1985). Alkaline phosphatase activity was strongly inhibited in the basolateral and brush-border membranes of SHR compared with WKY rats in the present study. The results, therefore, indicate that there is decreased Ca absorption by the active transport mechanism in SHR compared with WKY rats.

Na^+K^+-ATPase is a large plasma membrane enzyme which actively transports Na^+ out of cells whilst K^+ moves in the opposite direction. The enzyme creates an electrochemical gradient across the membrane which facilitates the transport of glucose, amino acids and other metabolites (Ullrich, 1979). The activity of Na^+-K^+-ATPase has been reported to be reduced (Aderounmu & Salako, 1979; Walter & Distler, 1982) or increased (Simon & Conklin, 1986) in erythrocytes of hypertensive patients and similar conflicting results have been observed in tissues of the SHR rat (Semple & Lever, 1986). One theory of hypertension suggests the existence of a circulating inhibitor which results in the accumulation of intracellular Na in many tissues (Blaustein & Hamlyn, 1984). In the present study the activity of Na^+K^+-ATPase was significantly reduced in intestinal basolateral membranes of SHR compared with WKY rats (Na^+K^+-ATPase is not present in brush-border membranes, except as a contaminant). This is likely to be due to a defect in the membrane itself, as no soluble inhibitor molecule would be present in the purified basolateral membranes. The reduction in activity would, following Blaustein & Hamlyn's (1984) hypothesis, result in an accumulation of Na^+ in the enterocytes, which in turn would tend to reduce Ca absorption by way of Na^+–Ca^{2+} exchange (Ghijsen *et al.* 1983). Ca^{2+}-ATPase and Na^+K^+-ATPase belong to a group of energy-transducing ATPases and are

very similar in general structure, show many sequence homologies and have similar reaction mechanisms (MacLennan *et al.* 1985; Shull *et al.* 1985). They have evolved by gene duplication from a common ancestor and provide possible genetic loci for hypertension.

These effects on the activities of plasma membrane ATPases seemed to be tissue-specific, as there were no significant differences between SHR and WKY rats in the corresponding enzymes of the kidney. In addition, Na^+K^+-ATPase and Ca^{2+}-ATPase activities were not affected in the hearts, whilst in diaphragms Na^+K^+-ATPase activity was not affected but Ca^{2+}-ATPase activity was significantly reduced in SHR compared with WKY rats (P. Blakeborough, unpublished results).

Rendering SHR rats Ca-deficient had the effect of increasing the activities of Na^+K^+-ATPase, Ca^{2+}-ATPase and alkaline phosphatase in intestinal basolateral and brush-border membranes, compared with rats given a +Ca diet. The same enzymes of kidney membranes were again unaffected by changing the diet in this way. One possible explanation of these findings is that the partial activation of these ATPases in the −Ca rat may be an adaptation to attempt to increase Ca absorption when the dietary supply of Ca is limiting. If so it is unsuccessful because blood pressures of SHR rats given a −Ca diet were higher than those of SHR rats given a +Ca diet. When the supply of dietary Ca is low absorption of Ca by passive means will certainly be reduced and the stimulation of active mechanisms of Ca absorption may not be sufficient to counteract this hypertensive effect. Further studies will be necessary in order to explain whether this potential mechanism is correct.

In summary, these results support the idea of defects in Ca metabolism contributing to the development of essential hypertension. Intestinal Ca absorption may be reduced by two separate mechanisms in the hypertensive patient. There are indications that modifying the dietary supply of Ca can affect mechanisms of Ca absorption which, in turn, can affect the severity of the hypertension. Additional dietary Ca may be a possible method of treatment, increasing the Ca in the gastrointestinal tract and maximizing the efficiency of active and passive transport mechanisms. It has been postulated that only a subset of hypertensive patients will benefit from Ca supplementation of the diet (Resnick, 1987). The present results lend some support to this, as modifying the dietary Ca of WKY rats had no effect on the activities of intestinal ATPases (Resnick, 1987).

It should be possible to investigate intestinal Ca absorption directly, observing the uptake of ^{45}Ca by SHR and WKY rats using basolateral and brush-border membranes. The effect of manipulating the level of Ca in the diet on these transport processes could also be studied. Similar studies have provided information on the effect of dietary components on zinc absorption (Blakeborough & Salter, 1987).

The authors are pleased to acknowledge the assistance of Ms Beverly Linsey in the initial stages of this research.

REFERENCES

Aderounmu, A. F. & Salako, L. A. (1979). Abnormal cation composition and transport in erythrocytes from hypertensive patients. *European Journal of Clinical Investigation* **9**, 369–375.

Bensadoun, A. & Weinstein, D. (1976). Assay of proteins in the presence of interfering materials. *Analytical Biochemistry* **70**, 241–250.

Blakeborough, P., Neville, S. G. & Rolls, B. A. (1988). The effect of diets adequate and deficient in calcium on the activities of intestinal plasma membrane ATPases and blood pressure in normotensive and spontaneously hypertensive rats. *Proceedings of the Nutrition Society* **47**, 91A.

Blakeborough, P. & Salter, D. N. (1987). The intestinal transport of zinc studied using brush-border-membrane vesicles from the piglet. *British Journal of Nutrition* **57**, 45–55.

Blaustein, M. P. & Hamlyn, J. M. (1984). Sodium transport inhibition, cell calcium, and hypertension. The natriuretic hormone/Na^+–Ca^{2+} exchange/hypertension hypothesis. *American Journal of Medicine* **77**, 45–59.

Booth, A. G. & Kenny, A. D. (1974). A rapid method for the preparation of microvilli from rabbit kidney. *Biochemical Journal* **142**, 575–581.

Chan, K.-M., Delfert, D. & Junger, K. D. (1986). A direct colorimetric assay for Ca^{2+}-stimulated ATPase activity. *Analytical Biochemistry* **157**, 375–380.

Colas, B. & Maroux, S. (1980). Simultaneous isolation of brush border and basolateral membranes from rabbit enterocytes. Presence of brush border hydrolases in the basolateral membrane of rabbit enterocytes. *Biochimica et Biophysica Acta* **600**, 406–420.

Fiske, C. H. & Subbarow, Y. (1925). The colorimetric determination of phosphorus. *Journal of Biological Chemistry* **66**, 375–400.

Ghijsen, W. E. J. M., De Jong, M. D. & Van Os, C. H. (1982). ATP-dependent calcium transport and its correlation with Ca^{2+}-ATPase activity in basolateral plasma membranes of rat duodenum. *Biochimica et Biophysica Acta* **689**, 327–366.

Ghijsen, W. E. J. M., De Jong, M. D. & Van Os, C. H. (1983). Kinetic properties of Na^+/Ca^{2+}-exchange in basolateral plasma membranes of rat small intestine. *Biochimica et Biophysica Acta* **730**, 85–94.

Kessler, M., Acuto, O., Storelli, C., Murer, H., Muller, M. & Semenza, G. (1978). A modified procedure for the rapid preparation of efficiently transporting vesicles from small intestinal brush border membranes. Their use in investigating some properties of D-glucose and choline transport systems. *Biochimica et Biophysica Acta* **506**, 136–154.

Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951). Protein measurement with the folin phenol reagent. *Journal of Biological Chemistry* **193**, 265–275.

McCarron, D. A. (1985). Is calcium more important than sodium in the pathogenesis of essential hypertension. *Hypertension* **7**, 607–627.

McCarron, D. A. & Morris, C. D. (1985). Blood pressure response to oral calcium in persons with mild to moderate hypertension. A randomised double-blind, placebo controlled, crossover trial. *Annals of Internal Medicine* **103**, 825–831.

McCarron, D. A., Yung, N. N., Ugoretz, B. A. & Krutzik, S. (1981). Disturbances of calcium metabolism in the spontaneously hypertensive rat. *Hypertension* **3**, suppl. 1, I-162–I-167.

MacGregor, G. A. (1985). Sodium is more important than calcium in essential hypertension. *Hypertension* **7**, 628–637.

MacLennan, D. H., Brandl, C. J., Korkzak, B. & Green, N. M. (1985). Amino-acid sequence of a $Ca^{2+}+Mg^{2+}$-dependent ATPase from rabbit muscle sarcoplasmic reticulum, deduced from its complementary DNA sequence. *Nature* **316**, 696–700.

Murer, H., Ammann, E., Biber, J. & Hopfer, U. (1976). The surface membrane of the small intestinal epithelial cell. 1. Localisation of adenyl cyclase. *Biochimica et Biophysica Acta* **433**, 509–519.

Nellans, H. N. & Popovitch, J. E. (1981). Calmodulin-regulated, ATPase-driven calcium transport by basolateral membranes of rat small intestine. *Journal of Biological Chemistry* **256**, 9932–9936.

Porteous, J. W. & Clark, B. (1965). The isolation and characterisation of subcellular components of the epithelial cells of rabbit small intestine. *Biochemical Journal* **96**, 159–171.

Postnov, Y. V., Orlov, S. N., Reznikova, M. B., Rjazhsky, G. G. & Podukin, N. I. (1984). Calmodulin distribution and Ca^{2+} transport in the erythrocytes of patients with essential hypertension. *Clinical Science* **66**, 459–463.

Reeves, W. J. Jr. & Fimognari, G. M. (1966). L-Lactic dehydrogenase: heart (H4). In *Methods in Enzymology*, vol. 9, pp. 288–294 [W. A. Wood, editor]. London: Academic Press.

Resink, T. J., Tkachuk, V. A., Erne, P. & Buhler, F. R. (1986). Platelet membrane calmodulin-stimulated Ca-ATPase. Altered activity in essential hypertension. *Hypertension* **8**, 159–166.

Resnick, L. M. (1987). Uniformity and diversity of calcium metabolism in hypertension: a conceptual framework. *American Journal of Medicine* **82**, suppl. 1B, 16–26.

Scalera, V., Storelli, C., Storelli-Joss, C., Haase, W. & Murer, H. (1980). A simple and fast method for the isolation of basolateral plasma membranes from rat small intestinal epithelial cells. *Biochemical Journal* **186**, 177–181.

Schedl, H.P., Miller, D. L., Pape, J. M., Horst, R. L. & Wilson, H. D. (1984). Calcium and sodium transport and vitamin D metabolism in the spontaneously hypertensive rat. *Journal of Clinical Investigation* **73**, 980–986.

Semple, P. F. & Lever, A. F. (1986). Glimpses of the mechanism of hypertension. *British Medical Journal* **293**, 901–902.

Shull, G. E., Schwarz, A. & Lingrel, J. B. (1985). Amino-acid sequence of the catalytic subunit of the $(Na^+ + K^+)$ATPase deduced from a complementary DNA. *Nature* **316**, 691–695.

Simon, G. & Conklin, D. J. (1986). In vivo erythrocyte sodium concentration in human hypertension is reduced, not increased. *Journal of Hypertension* **4**, 71–75.

Sjöström, H., Noren, O., Jeppesen, L., Staun, M., Svensson, B. & Christiansen, L. (1978). Purification of different amphiphilic forms of a microvillus aminopeptidase from pig small intestine using immunoadsorbent chromatography. *European Journal of Biochemistry* **88**, 503–511.

Trippodo, N. C. & Frohlic, E. D. (1981). Similarities of genetic (spontaneous) hypertension: man and rat. *Circulation Research* **48**, 309–319.

Ullrich, K. J. (1979). Sugar, amino-acid and Na^+ co-transport in the proximal tubule. *Annual Review of Physiology* **41**, 181–195.

Vezzoli, G., Elli, A. A., Tripodi, G., Bianchi, G. & Carafoli, E. (1985). Calcium ATPase in erythrocytes of spontaneously hypertensive rats of the Milan strain. *Journal of Hypertension* **3**, 645–648.

Walter, U. & Distler, A. (1982). Abnormal sodium efflux in erythrocytes of patients with essential hypertension. *Hypertension* **4**, 205–210.

Williams, S. A., Culp, J. S. & Butler, L. G. (1985). The relationship of alkaline phosphatase, Ca ATPase, and phytase. *Archives of Biochemistry and Biophysics* **241**, 10–13.

Printed in Great Britain