JCI The Journal of Clinical Investigation

Adaptation of Na+-H+ exchange in renal microvillus membrane vesicles. Role of dietary protein and uninephrectomy.

R C Harris, … , J L Seifter, B M Brenner

J Clin Invest. 1984;74(6):1979-1987. https://doi.org/10.1172/JCI111619.

Research Article

The ablation of renal mass and institution of a high protein diet both lead to renal cortical hypertrophy and increased glomerular filtration rate (GFR). We studied Na+ transport in rat microvillus membrane vesicles isolated from uninephrectomized or sham operated rats fed 6% (low), 24% (standard), or 40% (high) protein diets. The feeding of high protein, as compared with low protein, was associated with a 50% increase in rates of pH-stimulated 22Na+ transport in isolated vesicles from sham and uninephrectomized animals. Values for the standard protein diet were intermediate to values for high and low protein. At each level of dietary protein intake, vesicular Na+ transport was greater in the uninephrectomized than in sham rats. The high protein diet was also associated with increased vesicular 22Na+ flux inhibitable by 1 mM amiloride. Increases in total and amiloride sensitive flux were also noted in the absence of a pH gradient. Conductive Na+ and H+ transport were not altered, nor were sodium-glucose and sodium-alanine cotransport. Kinetic studies revealed evidence for an increased Vmax of Na+-H+ exchange in uninephrectomized animals fed a 40 vs. a 6% protein diet whereas Km was unchanged. Supplements of NaHCO3 in the 40% protein diet, to adjust for an increased rate of net acid excretion, did not prevent the increased rates of Na+-H+ exchange. However, treatment with actinomycin D […]

Find the latest version:

https://jci.me/111619/pdf

Adaptation of Na^+-H^+ Exchange in Renal Microvillus Membrane Vesicles

Role of Dietary Protein and Uninephrectomy

Raymond C. Harris, Julian L. Seifter, and Barry M. Brenner

Laboratory of Kidney and Electrolyte Physiology and Department of Medicine, Brigham and Women's Hospital, and Harvard Medical School, Boston, Massachusetts 02115

Abstract. The ablation of renal mass and institution of a high protein diet both lead to renal cortical hypertrophy and increased glomerular filtration rate (GFR). We studied Na^+ transport in rat microvillus membrane vesicles isolated from uninephrectomized or sham operated rats fed 6% (low), 24% (standard), or 40% (high) protein diets. The feeding of high protein, as compared with low protein, was associated with a 50% increase in rates of pH-stimulated $^{22}Na^+$ transport in isolated vesicles from sham and uninephrectomized animals. Values for the standard protein diet were intermediate to values for high and low protein. At each level of dietary protein intake, vesicular Na^+ transport was greater in the uninephrectomized than in sham rats. The high protein diet was also associated with increased vesicular $^{22}Na^+$ flux inhibitable by 1 mM amiloride. Increases in total and amiloride sensitive flux were also noted in the absence of a pH gradient. Conductive Na^+ and H^+ transport were not altered, nor were sodium-glucose and sodium-alanine cotransport. Kinetic studies revealed evidence for an increased V_{max} of Na^+-H^+ exchange in uninephrectomized animals fed a 40 vs. a 6% protein diet whereas K_m was unchanged. Supplements of $NaHCO_3$ in the 40% protein diet, to adjust for an increased rate of net acid excretion, did not prevent the increased rates of Na^+-H^+ exchange. However, treatment with actinomycin D (0.12 mg/kg) prevented the increased Na^+-H^+ activity as well as the increased renal mass and GFR noted 24 h after unilateral nephrectomy. Na^+-H^+ exchange rate was closely correlated with GFR (r = 0.961; $P < 0.005$) and renal mass (r = .986; $P < 0.001$). These observations provide evidence for modification of the luminal membrane Na^+-H^+ exchanger in response to changes in dietary protein content and nephron number.

Portions of this work have been published in abstract (1984. *Kidney Int.* 25:302).

Received for publication 11 April 1984.

J. Clin. Invest.
© The American Society for Clinical Investigation, Inc.
0021-9738/84/12/1979/09 $1.00
Volume 74, December 1984, 1979–1987

Introduction

The reabsorption of fluid and solutes by the renal proximal tubule is a dynamic process that can adapt to changes in several physiologic factors, including the prevailing glomerular filtration rate (GFR),[1] and the state of acid-base, electrolyte, and hormonal balance of the animal. That such adaptation might be due to intrinsic changes in the transport function of the epithelial cells has been shown in studies using the isolated perfused proximal tubule (1, 2). On the subcellular level, it has also been possible to demonstrate adaptation of specific carrier mediated transporters located in the brush border membrane of the proximal tubule (3, 4). Cohn et al. found increased Na^+-H^+ exchange in microvillus membrane vesicles isolated from remnant nephrons in a model of renal ablation (5). Since Na^+-H^+ exchange is an important mechanism for acidification of proximal tubule fluid, an increase in activity of this transporter may contribute to the increased reabsorption of $NaHCO_3$ that occurs in this setting (6, 7, 8).

After ablation of renal mass, the remaining tissue undergoes hypertrophy and an increase in filtration rate of the remnant glomeruli. It has been determined that changes in dietary protein intake may modify both the compensatory growth and the increase in GFR that follow partial nephrectomy (9, 10). The purpose of the present study was to characterize the adaptive response of the Na^+-H^+ exchanger in rat microvillus membrane vesicles isolated from remnant renal tissue after unilateral nephrectomy and to correlate altered membrane transport with changes in renal mass, GFR, and variations in dietary protein intake.

1. *Abbreviations used in this paper:* BUN, blood urea nitrogen; GFR, glomerular filtration rate; MES, 2(*N*-morpholino)ethane sulfonic acid; NAE, net acid excretion; SN, single nephron.

Methods

Male Sprague-Dawley rats (Charles River Breeding Laboratories, Inc., Wilmington, MA) initially weighing 325–375 g were used in all studies. Animals were anesthetized by intraperitoneal injection of Brevital (methohexitol) (Eli Lilly and Co., Indianapolis, IN), 50 mg/kg, and then subjected to right nephrectomy or sham surgery, the latter consisting of a dorsal flank incision. The control diet was standard 24% protein rat chow (Ralston Purina Co., St. Louis, MO). A high protein (40% casein) diet was prepared as a 3:1 mixture of 50 and 12% protein, and a low protein (6% casein) diet as a 3:1 mixture of 4 and 12% protein (Teklad, Madison, WI). Unless otherwise noted, animals were studied 14–21 d after surgery and initiation of the appropriate diet. All rats were killed by decapitation, samples for blood urea nitrogen (BUN) were drawn in the morning at the time of sacrifice, and the kidneys were promptly removed and weighed. The renal cortices were isolated, and microvillus membrane vesicles were prepared by a modification of the Mg^{++} aggregation and differential centrifugation method of Booth and Kenny (11) and of Aronson (12). After homogenization, the cortical suspension was centrifuged at alternating slow and fast speeds with a Sorvall RC-5B centrifuge and an SA600 rotor E. I. DuPont de Nemours & Co., Inc., Sorvall Instruments Div., Newtown, CT). The slow speed centrifugations were for 15 min at, successively, 5,000, 6,000, and 6,500 rpm. The high speed centrifugations were 25 min each at 15,000 rpm. The homogenizing buffer contained 200 mM mannitol, 80 mM Hepes, titrated with KOH to give pH 7.50, and 48 mM K^+, 328 mOsm. The brush border membrane marker enzyme alkaline phosphatase was assayed with *p*-nitrophenyl phosphate as substrate (12a) and was purified 6–10-fold in the final pellet as compared with homogenate. The basolateral membrane marker Na^+-K^+ ATPase, assayed as ouabain sensitive K^+-phosphatase, decreased in specific activity (0.4–0.8-fold). The homogenate specific activities and brush border membrane enrichment ratios for alkaline phosphatase and ouabain-sensitive K^+ phosphatase were the same in membranes obtained from each of the different experimental groups. Membrane protein was determined by the method of Lowry et al. (13).

Transport measurements. Timed uptakes of radiolabeled solutes at 20°C were assayed by a rapid filtration technique (14). Preliminary transport measurements of 1 mM $^{22}Na^+$ flux showed that Na^+ uptake in the presence of an inside acid pH gradient was a linear function of time between 2 and 10 s. For 20 mM Na^+, uptake was linear through 5 s. Therefore, 5-s uptakes were used in kinetic studies. In the other studies, which examined 1 mM Na^+ uptake, 5- and 10-s uptakes were used as approximations of initial rates. Timed incubations were terminated by the addition of 4 ml of an ice-cold $MgSO_4$ solution and then filtered immediately through a pre-wetted 0.65 μm filter (DAWP; Millipore Corp., Bedford, MA). The filter was washed with an additional 12 ml of the cold $MgSO_4$ solution. Filters were then dissolved in 4.5 ml of Aquasol (New England Nuclear, Boston, MA), and radioactivity was determined by liquid scintillation spectrometry. Nonspecific retention of radioactivity to the filters was subtracted from total counts of the sample. All incubations were performed in triplicate using fresh membrane preparations.

Fluorescence studies. Proton efflux rates were studied by the use of acridine orange fluorescence quenching as a measure of transmembrane pH gradients (15). A spectrofluorometer was employed (emission 530, excitation 493 nm; model MPF-44B; Perkin-Elmer Corp., Instrument Div., Norwalk, CT). An aliquot of 20 μl (150–300 μg vesicle protein), pH 6.1, was diluted into 3 ml of buffer solution containing 200 mM mannitol, 48 mM K^+, 80 mM Hepes, 8 μM acridine orange, pH 7.5. The addition of vesicles resulted in a rapid quench of fluorescence, followed by a slower recovery. The rate of intravesicular pH dissipation was assessed by the method of Reenstra et al. (15). The steady state fluorescence minus the fluorescence at varied times was plotted as a logarithmic function vs. time. The negative slope of this line represents the rate constant of pH gradient dissipation.

Clearance techniques and acid excretion rates. In separate animals, inulin clearances were performed by techniques described previously (16). Briefly, animals were anesthetized by intraperitoneal injection of inactin (100 mg/kg) and placed on a temperature regulated table, and a tracheostomy was performed. The left femoral artery and both jugular veins were catheterized with indwelling polyethylene catheters (No. 50). The abdomen was incised and the left ureter was catheterized with a polyethylene catheter (No. 10). Animals received plasma replacement infusions to ensure euvolemia (17). Each rat received 0.75 ml of a 10% inulin solution in isotonic NaCl as an intravenous priming dose at least 60 min before the first clearance period. A continuous inulin infusion at the rate of 1.5 ml/h. was then continued throughout the experiment. Each measurement was the mean of three or four collection periods. Titratable acid, ammonium, and net acid excretion were determined for 24-h urine collections (18).

Statistics. Results are expressed as the mean±SEM for at least three separate experiments on different membrane preparations. The unpaired *t* test was used. When more than two groups were compared, one-way analysis of variance and the Bonferroni *t* test were employed. Regression analysis was performed by means of least squares technique.

Materials. $^{22}Na^+$, [^{3}H]D-glucose (31 Ci/mmol), and [^{3}H]L-alanine (108 Ci/mmol) were obtained from New England Nuclear. Amiloride was a gift of Merck Sharp & Dohme (West Point, PA). Acridine orange and valinomycin were obtained from Sigma Chemical Co.

Results

Physiological and metabolic parameters. Dietary protein content significantly influenced renal weight in both the sham and uninephrectomized animals (Table I). In the sham operated groups, left kidney weight averaged 1.91 g in animals on the 40% protein diet and 1.22 g for rats on the 6% protein diet. The average renal weight for the 24% dietary protein group was intermediate to values for high and low protein. Dietary protein content also influenced renal weight in the uninephrectomized animals: 2.48 g for rats on the 40% protein diet, 2.14 g for those on the 24% protein diet, and 1.56 g for those on the 6% protein diet. At each level of dietary protein, the uninephrectomized animals had larger kidneys than did sham operated rats.

In a smaller group of animals whole kidney inulin clearance was measured. As shown in Table I, dietary protein content had an important influence on the level of GFR for both the sham and uninephrectomized animals. As was the case with renal mass, at each level of protein intake, mean values for left kidney GFR were significantly greater in uninephrectomized animals. These results are consistent with previously reported observations (9, 10, 19). Finally, in Table I, mean values for BUN are also given. On the average, uninephrectomized animals had higher BUN values than did sham rats; within

Table I. Body Weight, Kidney Weight, BUN, and Whole Kidney Inulin Clearance of Sham Operated or Uninephrectomized Rats Fed Varying Protein Diets

Dietary protein	Body weight	Kidney weight	BUN	C_{inulin}
	g	*g*	*mg/dl*	*ml/min*
Sham				
40% P (n = 22)	458±8*	1.91±0.04*	33±1.5*	1.87±0.07* (n = 3)
24% P (n = 19)	395±5*	1.41±0.02*	18±1.3*	1.53±0.05* (n = 5)
6% P (n = 24)	365±12	1.22±0.02	6±0.4	1.21±0.07 (n = 4)
Uninephrectomy				
40% P (n = 51)	401±6‡	2.48±0.04‡	53±3.0‡	2.23±0.18‡ (n = 4)
24% P (n = 21)	435±5‡	2.14±0.04‡	24±2.0‡	2.05±0.05‡ (n = 3)
6% P (n = 56)	354±11	1.56±0.03	6±0.4	1.60±0.04 (n = 4)

P, protein. C, clearance. *$P < 0.05$ as compared with sham 6% protein by analysis of variance and Bonferroni t test. ‡ $P < 0.05$ as compared with uninephrectomy 6% protein by analysis of variance and Bonferroni t test.

each group, BUN also varied directly with dietary protein content.

Transport. To determine the relative contributions of variations in dietary protein intake and uninephrectomy on Na^+ transport in isolated microvillus membrane vesicles, the time course of 1 mM $^{22}Na^+$ uptake into vesicles was evaluated for each experimental group. As shown in Fig. 1 *A*, the presence of an inside acid pH gradient gave greater stimulation of Na^+ uptake into vesicles isolated from animals on the 40% protein than from those on the 6% diet. For example, the 10-s Na^+ uptake was greater for the high protein diet in both the sham (2.47±0.10 nmol/mg protein vs. 1.75±0.24; $P < 0.05$) and the uninephrectomized animals (3.17±0.18 vs. 2.26±0.08 nmol/mg protein; $P < 0.001$). Also, at each level of protein intake, the uninephrectomized rats had greater vesicular Na^+ transport than did sham operated animals. Values for equilibrium Na^+ uptakes were the same for each group. Fig. 1 *B* shows the Na^+ uptake data for the non-pH gradient condition ($pH_{in} = pH_{out} = 7.50$). The 10-s rates of Na^+ uptake were again significantly greater for the 40% than for the 6% protein diet in both sham (0.51±0.05 vs. 0.37±0.03 nmol/mg protein; $P < 0.001$) and uninephrectomized (0.66±0.09 vs. 0.39±0.05; $P < .05$) groups. The finding of increased Na^+ transport in the absence of a pH gradient indicated that decreased H^+ leak or buffer permeability could not account for the stimulated uptake shown in Fig. 1 *A*. Studies using acridine orange confirmed that the non-Na^+-dependent pH gradient dissipation rate was the same in vesicles from rats on 40 and 6% protein intakes. The rate constant for pH gradient dissipation averaged 0.0051±0.0009 vs. 0.0061±0.0009 s^{-1}, respectively (n = 3, NS).

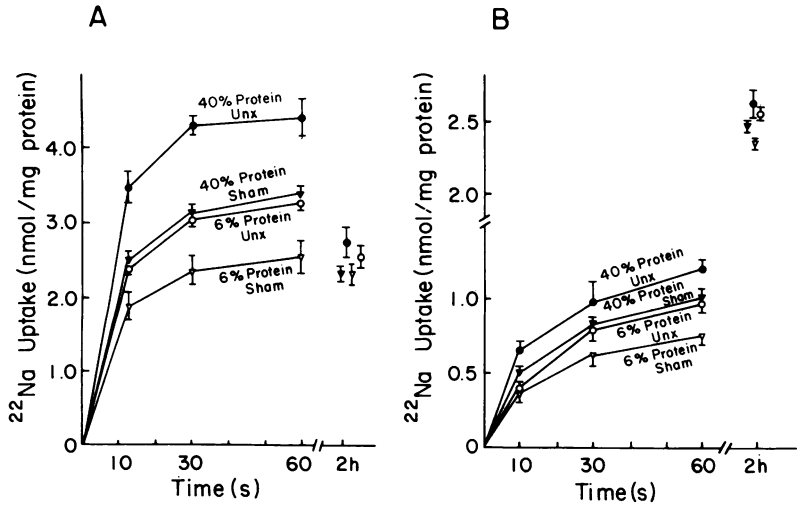

Figure 1. Time course of $^{22}Na^+$ uptake into renal cortical brush border membrane vesicles in the presence (*A*) or absence (*B*) of an inside acid pH gradient. Membrane vesicles were preincubated for 120 min to contain at equilibrium 224 mM mannitol, 24 mM K^+, 40 mM Hepes, 40 mM 2(*N*-morpholino)ethane sulfonic acid (MES), pH 6.1 (A) or 264 mM mannitol, 24 mM K^+, 40 mM Hepes, pH 7.5 (B). Influx of 1 mM $^{22}NaCl$ was assayed at 20°C after the addition of 10 μl of vesicle suspension to 40 μl of an external solution containing 264 mM mannitol 24 mM K^+, 40 mM Hepes, pH 7.5 to give final external pH 7.2 (*A*) and 7.5 (*B*). Values represent the mean±SEM for six or seven experiments in each group. UNx, uninephrectomized.

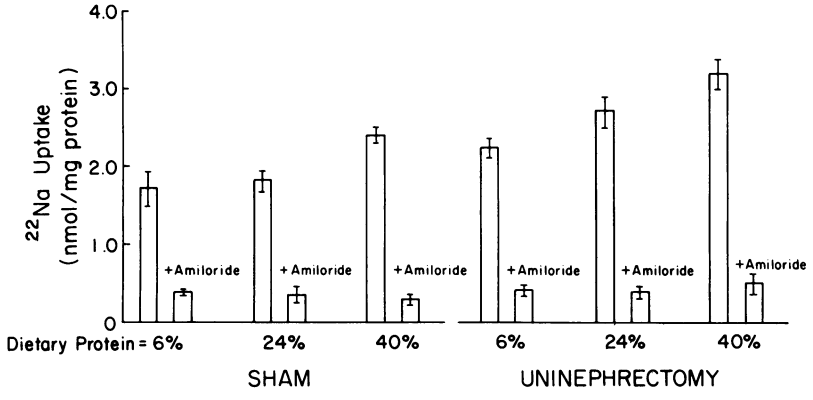

Figure 2. Amiloride sensitive $^{22}Na^+$ transport in membrane vesicles. Vesicles were preincubated for 120 min to contain at equilibrium 224 mM mannitol, 24 mM K^+, 40 mM Hepes, and 40 mM MES, pH 6.1. 10-s uptakes of 1 mM $^{22}NaCl$ were performed after addition of 10 μl of vesicle suspension to 40 μl of external medium containing 1.25 mM $^{22}NaCl$, 264 mM mannitol, 24 mM K^+, 40 mM Hepes, pH 7.5, with or without amiloride (1 mM final concentration). Values represent the mean±SEM for from three to six experiments in each group.

To determine whether the increased Na^+ transport was due to altered Na^+-H^+ exchange, the effect of amiloride, a known inhibitor of Na^+-H^+ exchange in brush border membrane vesicles (20), was tested. Fig. 2 shows that the amiloride sensitive component of Na^+ flux increased with increasing dietary protein content. Thus, in the presence of an inside acid pH gradient, vesicles from sham operated rats had a greater inhibitable component of Na^+ flux if rats were fed the 40 rather than the 6% diet (2.1±0.20 vs. 1.1±0.12 nmol/mg protein; $P < 0.02$), and uninephrectomized rats gave similar increases in amiloride sensitive Na^+ transport in the 40% protein group (3.14±0.08 vs. 1.87±0.07 nmol/mg protein; $P < 0.001$). Renal ablation also gave increased inhibitor sensitive transport at each level of protein intake. Under non-pH gradient conditions (not illustrated) the high protein diet and contralateral uninephrectomy each led to increased amiloride sensitive Na^+ transport.

To confirm that the increment in Na^+ transport was due to electroneutral Na^+-H^+ exchange, the pH gradient-stimulated Na^+ uptakes were repeated in the presence of a clamp of membrane electrical potential by addition of the K^+ ionophore, valinomycin, with $K^+_{in} = K^+_{out}$. The addition of valinomycin was shown to shunt membrane potential under these conditions. In the absence of valinomycin, Na^+-glucose cotransport, known to be potential sensitive (21), was stimulated 65% by a Cl^- diffusion potential when Cl^- replaced gluconate as the 100 mM Na^+ salt. In the presence of valinomycin, Na^+-glucose cotransport was increased only 9% by Cl^- substitution for gluconate, indicating an effective voltage clamp. However, as shown in Table II, Na^+ flux was largely voltage independent in vesicles from uninephrectomized animals on either high or low protein intake, since total Na^+ uptake was unchanged in the presence of the voltage clamp and there was no change in the amiloride insensitive component of Na^+ flux.

In contrast to the increased Na^+-H^+ exchange activity observed in the preceding experiments, neither dietary protein nor uninephrectomy altered rates of Na^+ cotransport with glucose or alanine (Table III). Thus, the adaptive changes were restricted to Na^+-H^+ countertransport and were not observed for these other Na^+-solute cotransport processes.

The effect of dietary protein manipulation on the kinetics of pH gradient-stimulated Na^+ transport was evaluated in uninephrectomized animals on either 40 or 6% protein intakes. The 5-s influx rates at varied Na^+ concentrations are indicated in Fig. 3 in the form of a Woolf-Augustinsson-Hofstee plot. The apparent V_{max} (y-intercept) was significantly increased by the high protein diet (240±18 vs. 173±15 nmol/mg protein per min; $P < 0.05$), whereas the K_m for Na^+, represented as the negative slope of the line, was unchanged (K_m = 6.8 vs. 6.6 mM). These findings are consistent with an increase in the number of carriers inserted into the plasma membrane during adaptation to high protein diet, although an increased turnover or availability of existing transporters in the membrane is also possible.

In order to determine whether increases in Na^+-H^+ exchange accompany the early changes after nephrectomy, animals were studied 1 d after unilateral nephrectomy. The sham operated rats on the 24% protein diet underwent unilateral nephrectomy and at that time were placed on the 40% protein diet. Within 24 h after surgery these animals had increased renal weight

Table II. Amount of Sodium Flux Dependent upon Voltage

	− Valinomycin, $K^+_{in} = K^+_{out}$ (*nmol/mg protein per 10 s*)		+Valinomycin, $K^+_{in} = K^+_{out}$ (*nmol/mg protein per 10 s*)	
	Total uptake	Amiloride insensitive uptake	Total uptake	Amiloride insensitive uptake
40% P UNx	2.48±0.12	0.35±0.04	2.23±0.14	0.35±0.03
6% P UNx	1.95±0.19	0.39±0.006	1.90±0.15	0.38±0.03

Microvillus membrane vesicles were preincubated for 120 min in the presence or absence of valinomycin (50 μg/mg vesicle protein) to contain at equilibrium 132 mM mannitol, 70 mM K^+, 46 mM Cl^-, 40 mM Hepes, 40 mM MES, pH 6.1. Influx of 1 mM $^{22}Na^+$ with or without added amiloride (1 mM) was then assayed after addition of 10 μl of vesicles to 40 μl of a solution containing 172 mM mannitol, 70 mM K^+, 46 mM Cl^-, 40 mM Hepes, pH 7.5. Values represent the mean±SEM for three separate experiments in each group. Within each protein group, the addition of valinomycin did not significantly decrease either total $^{22}Na^+$ flux or amiloride insensitive uptake. P, protein. UNx, uninephrectomized.

Table III. Sodium-Solute Cotransport in the Absence of a Sodium Gradient in Sham and Uninephrectomized Rats Fed Varying Protein Diets

	[^{3}H]D-Glucose uptake (pmol/mg protein)			[^{3}H]L-Alanine uptake (pmol/mg protein)		
Dietary protein	15 s	60 s	2 h	15 s	60 s	2 h
Sham						
40% P (n = 3)	0.20±0.02	0.30±0.03	0.27±0.08			
24% P (n = 5)	0.18±0.02	0.27±0.04	0.34±0.07			
6% P (n = 3)	0.16±0.02	0.23±0.04	0.44±0.05			
Uninephrectomy						
40% P (n = 3)	0.19±0.03	0.30±0.04	0.42±0.11	0.019±0.005	0.042±0.006	0.087±0.023
6% P (n = 3)	0.20±0.02	0.29±0.02	0.29±0.11	0.021±0.005	0.040±0.009	0.090±0.026

P, protein. Microvillus membrane vesicles were pre-equilibrated for 120 min to contain at equilibrium 82.5 mM NaCl, 24 mM K^+, 100 mM mannitol, 40 mM Hepes, pH 7.5. Uptakes were assayed at 20°C in the presence of 82.5 mM NaCl, 24 mM K^+, 100 mM mannitol, 40 mM Hepes, pH 7.5 and either 0.35 μM [^{3}H]D-glucose or 0.1 μM [^{3}H]L-alanine. There were no significant differences noted within or between groups.

(average values of 1.54±0.04 vs. 1.41±0.02 g; $P < 0.05$) and inulin clearance (2.19±0.13 vs. 1.53±0.05 ml/min; $P < 0.05$) (Table IV). At the same time, Na^+ transport was also increased in vesicles isolated from uninephrectomized as compared with sham rats (e.g., 1.91±0.10 vs. 1.22±0.07 nmol/mg protein per 5 s; $P < 0.05$), as shown in Fig. 4. Actinomycin D, an inhibitor of protein synthesis, given at the time of surgery, prevented the increase in renal weight and inulin clearance associated with contralateral nephrectomy (Table IV). Actinomycin D did not decrease vesicle Na^+ transport in the animals that were not nephrectomized but did prevent the increase in Na^+ uptake associated with contralateral nephrectomy. For example, in animals treated with the drug, vesicular Na^+ uptake averaged 1.42±0.09 nmol/mg protein per 5 s in the nephrectomized group as compared with 1.45±0.12 nmol/mg protein per 5 s for the sham rats.

Arterial blood gases were comparable for animals fed either a high or low protein diet (40% protein uninephrectomized vs. 6% protein uninephrectomized: pCO_2, 40±6 vs. 36±3 mmHg; pH, 7.41±0.07 vs. 7.47±0.04; HCO_3^-, 25±2 vs. 25±1 mM (n = 4). However, the effect of dietary protein could be mediated by altered rates of net acid excretion (NAE). In Table V it is apparent that NAE was greater for the 40 than the 6% diet in uninephrectomized rats (5.79±0.56 vs. 2.05±0.28 meq/24 h)

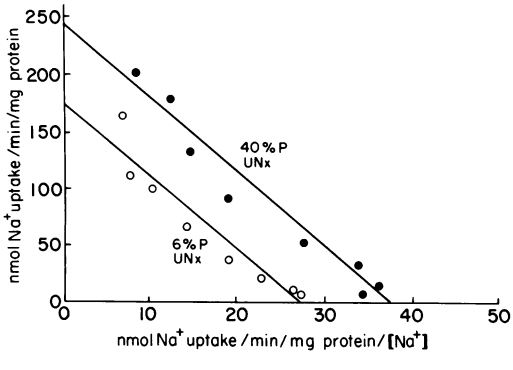

Figure 3. Kinetics of $^{22}Na^+$ uptake in vesicles from uninephrectomized (UNx) rats on high and low protein diets. Vesicles were pre-equilibrated at pH 6.1, as in Fig. 2. 5-s uptakes of $^{22}Na^+$ were performed in the presence of an external solution containing 24 mM K^+, 40 mM Hepes, 8 mM MES, pH 7.2, and 0.25 to 25 mM $^{22}NaCl$, with mannitol added to maintain constant osmolarity. The Woolf-Augustinsson-Hofstee transformation of the data is presented. The V_{max} (y-intercept) averaged 240±18 nmol/mg protein per min for UNx 40% protein and 173±15 for UNx 6% protein rats ($P < 0.05$). The K_m (−slope) averaged 6.8 mM for UNx 40% protein and 6.6 mM for UNx 6% protein rats. Data represent the mean for three separate experiments in each group. P, protein.

Table IV. Body Weight, Kidney Weight, and Whole Kidney Inulin Clearance (C) 24 h after Unilateral Nephrectomy and Institution of 40% Protein Diet

	Body weight	Kidney weight	C_{inulin}
	g	*g*	*ml/min*
Without actinomycin D			
Sham (n)	395±5 (19)	1.41±0.02 (19)	1.53±0.05 (5)
Uninephrectomy (n)	381±6 (9)	1.54±0.04* (9)	2.19±0.13* (4)
With actinomycin D (0.12 mg/kg)			
Sham (n)	390±12 (9)	1.40±0.05 (9)	—
Uninephrectomy (n)	367±7 (8)	1.45±0.05 (8)	1.31±0.17 (5)

* $P < 0.005$ as compared with sham.

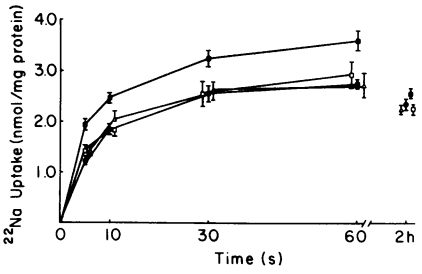

Figure 4. $^{22}Na^+$ transport 1 d after contralateral nephrectomy. At the time of unilateral nephrectomy and institution of 40% protein diet or sham surgery, groups of animals received an intraperitoneal injection of actinomycin D, 0.12 mg/kg body wt. Vesicles were isolated from these animals and from untreated rats 24 h after surgery by methods described in text. Uninephrectomy (■), uninephrectomy + actinomycin D (□), sham (●), and sham + actinomycin D (△). The time course of 1 mM $^{22}Na^+$ uptake was determined in each group of vesicles in the presence of an inside acid pH gradient (pH_{in} 6.1; pH_{out} 7.2) as in Fig. 2. Values represent the mean±SEM for from three to seven separate experiments in each group.

and that this correlated with increased Na^+-H^+ exchange rates in vesicles. As shown in Table V, there were not significant differences in urinary pH or titratable acid. Animals on the 40% protein diet had a significant increase in urinary ammonium excretion. However, although not shown in Table V, there were no significant differences in net acid excretion between uninephrectomized and sham operated animals receiving 40% protein (5.79 vs. 5.40 meq/24 h) or 6% protein (2.05 vs. 2.27 meq/24 h). With supplemental feeding of $NaHCO_3$ to uninephrectomized animals on the 40% protein intake, NAE was reduced to values similar to those observed for the 6% protein diet (2.62±0.44 vs. 2.05±0.28 meq/24 h). These animals had arterial blood gases similar to those of nephrectomized animals not fed $NaHCO_3$: pCO_2, 38±2 mmHg; pH 7.46±0.03; HCO_3^-, 27±1 mM (n = 5). However, Na^+-H^+ exchange rates in vesicles from animals on 40% protein remained significantly greater than for the 6% protein group (2.90±0.16 vs. 2.26±0.08 nmol/mg protein; $P < 0.05$). The Na^+ flux in vesicles from animals on the 40% protein diet supplemented with bicarbonate was not significantly different than Na^+ flux in vesicles from rats fed either the 40% protein diet alone or the 40% protein diet supplemented with NaCl (Table V). Thus, the rates of net acid excretion could be dissociated from the diet-induced increases in Na^+-H^+ exchange activity.

Discussion

After loss of renal mass, the remnant kidney tissue undergoes several important adaptations. There is a compensatory increase in single nephron (SN) GFR and hypertrophy of the remaining nephrons (9, 19). In addition, there occur increases in absolute proximal fluid reabsorption (22) and increased reabsorptive capacity for HCO_3^- (6–8). The increased proximal acidification has been shown to rise in proportiion to SNGFR (23) and to be independent of daily NAE. (7). On the cellular level there is an increased rate of ammoniagenesis in remnant tissue (24). An increase in dietary protein intake can evoke many of the same effects observed after renal ablation in that renal mass and GFR increase (10), as do proximal HCO_3^- reabsorption (25) and renal synthesis of NH_3 and glucose (24, 26).

The present study demonstrates that a high protein diet can induce increased Na^+-H^+ exchange activity in microvillus membrane vesicles isolated from both uninephrectomized and normal animals. Furthermore, the stimulated Na^+-H^+ exchange that accompanies uninephrectomy can be prevented by limiting

Table V. Relationship between Urinary Acid Excretion and Vesicular $^{22}Na^+$ Uptake

Group	UpH	$U_{TA}V$	$U_{NH_4}V$	NAE	$^{22}Na^+$ uptake
		meq/24 h	*meq/24 h*	*meq/24 h*	*nmol/mg protein per 10 s*
6% P UNx (n = 4)	6.48±0.28	0.36±0.26	1.69±0.37	2.05±0.28	2.26±0.08 n = 7
40% P UNx (n = 4)	6.71±0.53	1.19±0.45	5.11±0.92*	5.79±0.56*	3.17±0.18* n = 8
40% P UNx + NaCl (n = 5)	6.49±0.24	1.24±0.37	5.41±0.36*	6.45±0.40*	3.20±0.34* n = 4
40% P UNx + $NaHCO_3$ (n = 4)	6.65±0.80	0.69±0.29	1.93±0.32	2.62±0.44	2.90±0.16* n = 5

24-h urine volumes were collected under oil for measurement of rates of acid excretion in uninephrectomized (UNx) rats fed 6 or 40% protein (P) with or without added NaCl or $NaHCO_3$ (25 meq/100 g diet). After isolation of microvillus membrane vesicles from these animals, 1 mM $^{22}Na^+$ uptake was determined in the presence of an inside acid pH gradient as in Fig. 2. UpH, urinary pH. $U_{TA}V$, urinary titratable acid excretion. $U_{NH_4}V$, urinary ammonium excretion. * $P < 0.05$ as compared with 6% by analysis of variance and Bonferroni t test.

dietary protein intake. Cohn et al. found increased Na^+-H^+ exchange activity in vesicles isolated from remnant renal tissue of dogs with chronic renal failure (5). We find that increased Na^+-H^+ exchange can occur after less severe degrees of nephron loss and in the absence of uremia or systemic acid-base disturbances. Furthermore, we demonstrate the important effect of dietary protein in modulating Na^+-H^+ antiporter activity. The activities of other brush border functions (alkaline phosphatase and Na^+-coupled glucose and alanine cotransport) remain unchanged during the adaptive process. Studies in dogs with chronic renal failure demonstrated decreased Na^+-$PO_4^=$ cotransport in renal microvillus vesicles (27). Phosphate transport was not examined in the present study.

Kinetic analysis reveals that the increased Na^+-H^+ exchange associated with high protein feeding and uninephrectomy corresponds to an increase in the V_{max} for Na^+ transport. It is possible that incorporation of newly synthesized transport proteins into the microvillus membrane occurs during adaptation, although activation of pre-existing transport sites or increased cycling from a cytoplasmic pool of transporters to the plasma membrane cannot be ruled out.

The increased Na^+-H^+ exchange in the remnant kidney was noted within 24 h after contralateral nephrectomy, at a time when increases in GFR, renal mass, cortical RNA/DNA ratio (28), and protein synthesis (29) are known to occur.

In a variety of cell types, Na^+-H^+ exchange has been shown to play an important role in maintenance of intracellular pH (30). Furthermore, in the proximal tubule, luminal Na^+-H^+ exchange is thought to be the most important pathway for H^+ secretion and HCO_3^- reabsorption (31). A number of studies have found that in the presence of metabolic acidosis, microvillus membrane Na^+-H^+ exchange is increased (4, 32, 33). In the present study, none of the experimental groups manifested a systemic acid-base disorder, although the high protein diet did provide an increased acid load. When net acid excretion was normalized by the addition of $NaHCO_3$ to the high protein diet, the increase in Na^+-H^+ exchange activity was not prevented. Therefore, we do not find a close correlation between acid excretion and Na^+-H^+ exchange rates. It remains to be determined whether uninephrectomy or dietary protein alters intracellular pH.

In addition to its role in mediating proximal acidification, Na^+-H^+ exchange may also be one of the more important luminal membrane mechanisms for transcellular Na^+ reabsorption (34). Burg and Green have shown the importance of $NaHCO_3$ transport to absolute fluid reabsorption in the isolated perfused proximal tubule of the rabbit (35). In conditions in which SNGFR is altered chronically, there is evidence for a concordant change in absolute proximal fluid and Na^+ reabsorption (chronic glomerulotubular balance [1, 2, 22]). In the present study, there is a linear correlation between microvillus membrane Na^+ transport and whole kidney clearance when data from all the groups are considered (Fig. 5). It is therefore possible that Na^+-H^+ exchange is modified by prevailing GFR.

Ablation of renal mass and increases in dietary protein,

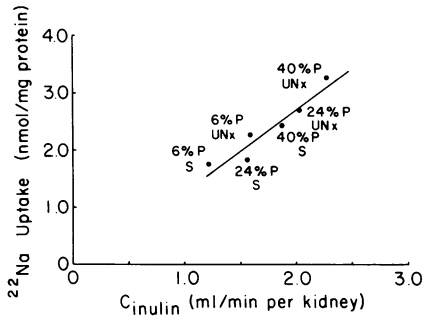

Figure 5. Correlation of Na^+ uptake in vesicles and whole kidney GFR. Data given in Fig. 2 and Table I are correlated to show the relationship between rates of Na^+ uptake and whole kidney inulin clearances of animals from each of the experimental groups ($r = 0.961$; $P < 0.005$). P, protein. UNx, uninephrectomized. S, sham.

manuevers that increase SNGFR, also stimulate increases in renal cortical growth. The close correlation between Na^+ transport rate and renal mass is shown in Fig. 6. It is noteworthy that actinomycin D administration prevented adaptive increases in $^{22}Na^+$ uptake, since in this study, as well as previous studies (28, 36) this agent has been shown to inhibit the compensatory growth and increased GFR that follow unilateral nephrectomy. Whether actinomycin D inhibits synthesis of new transport sites or interferes with other regulators of Na^+-H^+ exchange is not known.

The finding of a correlation between increases in renal mass and Na^+ transport in isolated vesicles raises the possibility that the increase in the Na^+-H^+ exchanger may be a specific manifestation of growth. In this regard, other conditions which induce increases in Na^+-H^+ exchange, such as metabolic acidosis and chronic K^+ deficiency (37), are known to be potent stimuli for renal cortical hypertrophy (29, 36, 38). It is also possible that increased Na^+-H^+ exchange is a prerequisite for renal growth since in other tissues, growth factors and

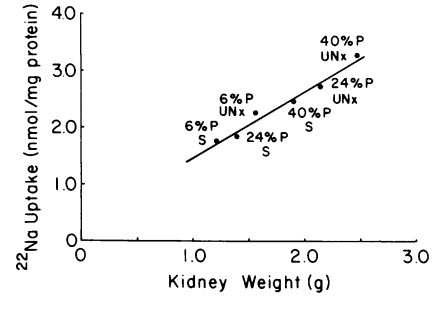

Figure 6. Correlation of Na^+ uptake in vesicles and renal weight. Data given in Fig. 2 and Table I are correlated to show the relationship between rates of Na^+ uptake and the weight per kidney of the experimental animals in each group ($r = 0.986$; $P < 0.001$). P, protein. UNx, uninephrectomized. S, sham.

mitogenic stimuli are known to activate Na^+-H^+ exchange (39–41). The resulting effects on intracellular ion concentration and pH are required for growth to occur.

In conclusion, we find increased activity of the renal microvillus membrane Na^+-H^+ exchanger of rats adapted to high protein diets or to contralateral nephrectomy. These changes in membrane transport are associated with parallel increases in renal mass and GFR.

Acknowledgments

The technical assistance of Robert A. Lufburrow III and the secretarial assistance of Michelle Hardiman are gratefully acknowledged.

This work was supported by a grant from the U. S. Public Health Service (AM19467). Dr. Harris is supported by an institutional National Research Service Award (AM07241). Dr. Seifter is the recipient of a New Investigator Research Award (AM31983).

References

1. Trizna, W., N. Yanagawa, Y. Bar-Khayim, B. Houston, and L. G. Fine. 1981. Functional profile of the isolated uremic nephron. Evidence of proximal tubular "memory" in experimental renal disease. *J. Clin. Invest.* 68:760–767.

2. Tabei, K., D. J. Levenson, and B. M. Brenner. 1983. Early enhancement of fluid transport in rabbit proximal straight tubules after loss of contralateral renal excretory function. *J. Clin. Invest.* 72:871–881.

3. Freiberg, J. M., J. Kinsella, and B. Sacktor. 1982. Glucocorticoids increase the Na^+-H^+ exchange and decrease the Na^+ gradient-dependent phosphate-uptake systems in renal brush border membrane vesicles. *Proc. Natl. Acad. Sci. USA.* 79:4932–4936.

4. Cohn, D. E., S. Klahr, and M. R. Hammerman. 1983. Metabolic acidosis and parathyroidectomy increase Na^+-H^+ exchange in brush border vesicles. *Am. J. Physiol.* 245:F217–F222.

5. Cohn, D. E., K. A. Hruska, S. Klahr, and M. R. Hammerman. 1982. Increased Na^+-H^+ exchange in brush border vesicles from dogs with renal failure. *Am. J. Physiol.* 243:F293–F299.

6. Schmidt, R. W., N. S. Bricker, and G. Gavellas. 1976. Bicarbonate reabsorption in the dog with experimental renal disease. *Kidney Int.* 10:287–294.

7. Arruda, J. A. L., T. Carrasquillo, A. Cubria, D. R. Rademacher, and N. A. Kurtzman. 1976. Bicarbonate reabsorption in chronic renal failure. *Kidney Int.* 9:481–488.

8. Bank, N., W.-S. Su, and H. S. Aynedjian. 1978. A micropuncture study of HCO_3 reabsorption by the hypertrophied proximal tubule. *Yale J. Biol. Med.* 51:275–282.

9. Hostetter, T. H., J. L. Olson, H. G. Rennke, M. A. Venkatachalam, and B. M. Brenner. 1981. Hyperfiltration in remnant nephrons: a potentially adverse response to renal ablation. *Am. J. Physiol.* 241:F85–F93.

10. Meyer, T. W., T. H. Hostetter, H. G. Rennke, J. L. Noddin, and B. M. Brenner. 1983. Preservation of renal structure and function by long term protein restriction in rats with reduced nephron mass. *Kidney Int.* 23:218. (Abstr.)

11. Booth, A. G., and A. S. Kenny. 1971. A rapid method for the preparation of microvilli from rabbit kidney. *Biochem. J.* 142:575–581.

12. Aronson, P. S. 1978. Energy-dependence of phlorizin binding to isolated renal microvillus membranes. *J. Membr. Biol.* 42:81–98.

12a. 1982. Phosphatase, both acid and alkaline. Technical bulletin 104. Sigma Chemical Co., St. Louis, MO.

13. Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall. 1951. Protein measurement with the folin phenol reagent. *J. Biol. Chem.* 193:265–275.

14. Aronson, P. S., and B. Sacktor. 1975. The Na^+-gradient dependent transport of D-glucose in renal brush border membranes. *J. Biol. Chem.* 250:6032–6039.

15. Reenstra, W. W., D. G. Warnock, V. J. Yee, and J. G. Forte. 1981. Proton gradients in renal cortex brush border-membrane vesicles. *J. Biol. Chem.* 256:11663–11666.

16. Brenner, B. M., and J. H. Galla. 1971. Influence of postglomerular hematocrit and protein concentration on rat nephron fluid transfer. *Am. J. Physiol.* 220:148–161.

17. Ichikawa, I., D. A. Maddox, M. G. Cogan, and B. M. Brenner. 1978. Dynamics of glomerular ultrafiltration in euvolemic Munich-Wistar rats. *Renal Physiol.* 1:121–131.

18. Wilcox, C. S., D. A. Cemerikic, and G. Giebisch. 1982. Differential effects of acute mineralo- and glucocorticosteroid administration on renal acid elimination. *Kidney Int.* 21:546–556.

19. Deen, W. M., D. A. Maddox, C. R. Robertson, and B. M. Brenner. 1974. Dynamics of glomerular ultrafiltration in the rat. VII. Response to reduced renal mass. *Am. J. Physiol.* 227:556–562.

20. Kinsella, J. L., and P. S. Aronson. 1981. Amiloride inhibition of the Na^+-H^+ exchanger in renal microvillus membrane vesicles. *Am. J. Physiol.* 241:F374–F379.

21. Beck, J. C., and B. Sacktor. 1975. Energetics of the Na^+-dependent transport of D-glucose in renal brush border membrane vesicles. *J. Biol. Chem.* 250:8674–8680.

22. Hayslett, J. P., M. Kashgarian, and F. H. Epstein. 1968. Functional correlates of compensatory renal hypertrophy. *J. Clin. Invest.* 47:774–782.

23. Buerkert, J., D. Martin, D. Trigg, and E. Simon. 1983. Effect of reduced renal mass on ammonium handling and net acid formation by the superficial and juxtamedullary nephron of the rat. Evidence for impaired reentrapment rather then decreased production of ammonium in the acidosis of uremia. *J. Clin. Invest.* 71:1661–1675.

24. Schoolwerth, A. C., R. S. Sandler, P. M. Hoffman, and S. Klahr. 1975. Effects of nephron reduction and dietary protein content on renal ammoniagenesis in the rat. *Kidney Int.* 7:397–404.

25. Bennett, C. M., P. D. Springberg, and N. R. Falkinburg. 1975. Glomerular-tubular balance for bicarbonate in the dog. *Am. J. Physiol.* 228:98–106.

26. Schoolwerth, A. C., J. Blondin, and S. Klahr. 1974. Renal gluconeogenesis. Influence of diet and hydrogen ions. *Biochim. Biophys. Acta.* 372:274–284.

27. Hruska, K. A., S. Klahr, and M. R. Hammerman. 1982. Decreased luminal membrane transport of phosphate in chronic renal failure. *Am. J. Physiol.* 242:F17–F22.

28. Northrup, T. E., and R. L. Malvin. 1976. Cellular hypertrophy and renal function during compensatory renal growth. *Am. J. Physiol.* 231:1191–1195.

29. Malt, R. A. 1969. Compensatory growth of the kidney. *N. Engl. J. Med.* 280:1446–1459.

30. Roos, A., and W. F. Boron. 1981. Intracellular pH. *Physiol. Rev.* 61:296–434.

31. Warnock, D. G., and F. C. Rector, Jr. 1981. Renal acidification mechanisms. *In* The Kidney. Second ed. B. M. Brenner and F. C. Rector, Jr., editors. W. B. Saunders Co., Philadelphia. 440–495.

32. Kinsella, J., T. Cujdik, and B. Sacktor. 1984. Na^+-H^+ exchange activity in renal brush border membrane vesicles in response to metabolic acidosis: the role of glucocorticoids. *Proc. Natl. Acad. Sci. USA.* 81:630–634.

33. Tsai, C. J., H. E. Ives, R. J. Alpern, V. J. Yee, D. G. Warnock, and F. C. Rector, Jr. 1984. Increased V_{max} for Na^+/H^+ antiporter activity in proximal tubule brush border vesicles from rabbits with metabolic acidosis. *Am. J. Physiol.* 247:F339–F343.

34. Schafer, J. A. 1982. Salt and water absorption in the proximal tubule. *Physiologist.* 25:95–103.

35. Burg, M. B., and N. Green. 1976. Role of monovalent ions in the reabsorption of fluid by isolated perfused proximal renal tubules of the rabbit. *Kidney Int.* 10:221–228.

36. Lotspeich, W. D. 1976. Metabolic aspects of acid-base change. *Science (Wash. DC).* 155:1066–1075.

37. Seifter, J. L., and R. C. Harris. 1984. Chronic K^+ depletion increases Na^+-H^+ exchange in rat renal cortical brush border membrane vesicles. *Kidney Int.* 24:302. (Abstr.)

38. Gustafson, A. B., L. Shear, and G. S. Gabuzda. 1973. Protein metabolism in vivo in kidney, liver, muscle, and heart of potassium-deficient rats. *J. Lab. Clin. Med.* 82:287–296.

39. Moolenaar, W. H., Y. Yarden, S. W. de Laat, and J. Schlessinger. 1982. Epidermal growth factor induces electrically silent Na^+ influx in human fibroblasts. *J. Biol. Chem.* 257:8502–8506.

40. Pouyssegur, J., J. C. Chambard, A. Franchi, S. Paris, and E. Van Obberghen-Schilling. 1982. Growth factor activation of an amiloride-sensitive Na^+/H^+ exchange system in quiescent fibroblasts: coupling to ribosomal protein S6 phosphorylation. *Proc. Natl. Acad. Sci. USA.* 79:3935–3939.

41. Benos, D. J., and V. S. Sapirstein. 1983. Characteristics of an amiloride-sensitive sodium entry pathway in cultured rodent glial and neuroblastoma cells. *J. Cell Physiol.* 116:213–220.