

## Effect of acute changes in glomerular filtration rate on Na<sup>+</sup>/H<sup>+</sup> exchange in rat renal cortex.

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### Research Article

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# Effect of Acute Changes in Glomerular Filtration Rate on Na<sup>+</sup>/H<sup>+</sup> Exchange in Rat Renal Cortex

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## Abstract

Studies were undertaken in Munich-Wistar rats to assess the influence of changes in filtered bicarbonate (FL<sub>HCO<sub>3</sub></sub>), induced by changes in GFR, on Na<sup>+</sup>/H<sup>+</sup> exchange activity in renal brush border membrane vesicles (BBMV). Whole-kidney and micropuncture measurements of GFR, FL<sub>HCO<sub>3</sub></sub>, and whole-kidney and proximal tubule HCO<sub>3</sub> reabsorption (APR<sub>HCO<sub>3</sub></sub>) were coupled with BBMV measurements of H<sup>+</sup> gradient-driven <sup>22</sup>Na<sup>+</sup> uptake in each animal studied. <sup>22</sup>Na<sup>+</sup> uptake was measured at three Na<sup>+</sup> concentration gradients to allow calculation of V<sub>max</sub> and K<sub>m</sub> for Na<sup>+</sup>/H<sup>+</sup> exchange. GFR was varied by studying animals under conditions of hydropenia, plasma repletion, and acute plasma expansion. The increase in GFR, FL<sub>HCO<sub>3</sub></sub>, and APR<sub>HCO<sub>3</sub></sub> induced by plasma administration correlated directly with an increase in the V<sub>max</sub> for Na<sup>+</sup>/H<sup>+</sup> exchange in BBMV. The K<sub>m</sub> for sodium was unaffected. In the plasma-expanded rats, the V<sub>max</sub> for Na<sup>+</sup>/H<sup>+</sup> exchange was 22% greater than in the hydropenic rats (*P* < 0.025) whereas APR<sub>HCO<sub>3</sub></sub> was 86% greater (*P* < 0.001). These results indicate that increases in FL<sub>HCO<sub>3</sub></sub>, induced by acute increases in GFR, stimulate Na<sup>+</sup>/H<sup>+</sup> exchange activity in proximal tubular epithelium. This stimulation is a mechanism which can, in part, account for the delivery dependence of proximal bicarbonate reabsorption. (*J. Clin. Invest.* 1992. 89:1296–1303.) Key words: bicarbonate reabsorption • brush border • membrane vesicles • micropuncture

## Introduction

The proximal convoluted tubule of the rat nephron normally reabsorbs ~ 80% of the filtered load of bicarbonate (FL<sub>HCO<sub>3</sub></sub>),<sup>1</sup> and the reabsorptive rate in this segment of the nephron is highly dependent on bicarbonate delivery (1, 2). As bicarbonate is reabsorbed, luminal pH and bicarbonate concentration fall along the length of the nephron (1, 3–6). One proposed explanation for delivery dependence of bicarbonate reabsorption is that, as FL<sub>HCO<sub>3</sub></sub> increases, the point at which maximal H<sup>+</sup> and HCO<sub>3</sub><sup>-</sup> gradients are developed is shifted further away from

the glomerulus, thereby effectively increasing the length of the proximal tubule that can participate in bicarbonate reabsorption (1). Delivery-dependent changes in bicarbonate reabsorption occur both in the early and the late proximal tubule, although the capacity to increase reabsorption is much greater in the early proximal tubule (7–11). Although an increase in FL<sub>HCO<sub>3</sub></sub> induced by acute plasma expansion increases both early and late proximal bicarbonate reabsorption, no evidence of a decrease in the fraction of bicarbonate reabsorbed by the early proximal tubule is observed (7, 12, 13), as would be expected if luminal pH and/or bicarbonate concentration gradients were the controlling factor. Results such as these suggest instead that delivery-dependent changes in reabsorption are regulated by changes in epithelial transporter activity. Two epithelial transporters, the Na<sup>+</sup>/H<sup>+</sup> exchanger and the H<sup>+</sup>-ATPase, drive proximal tubular bicarbonate reabsorption. Of these, the Na<sup>+</sup>/H<sup>+</sup> exchanger is probably responsible for the majority of bicarbonate reabsorption under control conditions (14–16). Indirect evidence from nephron deletion studies suggests that this transporter is stimulated by increased delivery under conditions where single-nephron (SN)GFR is increased, but in none of these studies was bicarbonate delivery actually measured (17–19).

The present studies were undertaken to address more directly the question of whether an increase in bicarbonate delivery stimulates Na<sup>+</sup>/H<sup>+</sup> exchange. In hydropenic, euvoletic, and plasma-expanded rats, we have coupled measurements of single-nephron and whole-kidney GFR and bicarbonate delivery with assessment of Na<sup>+</sup>/H<sup>+</sup> exchange activity in renal cortical brush border membrane vesicles (BBMV). Our results show a significant correlation between bicarbonate delivery and/or (SN)GFR and the V<sub>max</sub> of exchanger activity.

## Methods

All studies were carried out in male Munich-Wistar rats obtained from Simonsen Laboratories, Gilroy, CA. The rats had free access to water and to a low-bulk sodium- and potassium-free diet (ICN Nutritional Biochemicals, Cleveland, OH), the latter supplemented with 2.0 mmol NaCl and 2.0 mmol KCl per 12 g. All rats were prepared for micropuncture as described previously from our laboratory (2, 6). They were maintained on a small animal ventilator breathing a mixture of room air and O<sub>2</sub> bubbled through water to maintain arterial PO<sub>2</sub> at ~ 100 mmHg. For determination of GFR, SNGFR, and fluid reabsorption by the proximal tubule, all rats were given a 0.4-ml bolus injection of Ringer solution containing dialyzed [methoxy-<sup>3</sup>H]inulin (100 μCi/ml) followed by a constant infusion at 0.84 ml/h. Inulin infusion was begun ~ 60 min before beginning measurements.

*Experimental protocols.* Experimental protocols were designed to vary SNGFR and hence the filtered load of bicarbonate over a wide range under conditions in which plasma bicarbonate concentration and pH were relatively unchanged. These included hydropenia, euvoletic, and plasma volume expansion. For hydropenia, seven rats were infused only with the Ringer solution containing [methoxy-<sup>3</sup>H]inulin. In five rats, surgically induced plasma losses were replaced to achieve

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1. Abbreviations used in this paper: A<sub>II</sub>, angiotensin II; APR<sub>HCO<sub>3</sub></sub>, proximal tubular bicarbonate reabsorptive rate; AR<sub>HCO<sub>3</sub></sub>, whole-kidney bicarbonate reabsorptive rate; FL<sub>HCO<sub>3</sub></sub>, filtered load of bicarbonate; Hct, hematocrit.

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euvoemia by the infusion of 1.3% body wt isoncotic rat plasma over 1 h during surgical preparation for micropuncture, followed by a maintenance plasma infusion sufficient to maintain hematocrit constant at awake animal levels (20). Plasma volume expansion was achieved in an additional five rats by the infusion of 3% body wt isoncotic rat plasma plus 3% body wt Ringer solution over 90 min. Volume expansion was maintained by the constant infusion of plasma and Ringer solution at 20–25  $\mu\text{l}/\text{min}$  each, adjusted to maintain hematocrit at the postexpansion level. After 20–30 min of equilibration, measurements were begun. In an additional three hydrogenic rats, saralasin ([Sar<sup>1</sup>;Ala<sup>8</sup>]-angiotensin II, Sigma Chemical Co., St. Louis, MO), was infused at 1  $\mu\text{g}/(\text{kg} \cdot \text{min})$  in Ringer solution (0.84 ml/h) beginning at least 60 min before micropuncture. This dose has minimal effects on glomerular filtration rate (21, 22), and inhibits early proximal bicarbonate reabsorption in hydrogenic rats, owing to competitive inhibition of endogenous angiotensin II (A<sub>II</sub>) (22).

**Micropuncture measurements.** Tubular fluid was collected from last surface loops of proximal convoluted tubules for measurement of SNGFR and proximal fluid and bicarbonate reabsorption, as described previously (2, 6). One to three end-proximal collections lasting 5–10 min were obtained in each rat. The sample volumes were measured and an aliquot taken for total CO<sub>2</sub> determination by microcalorimetry (23). The remaining volume was measured and transferred to liquid scintillation vials and counted for <sup>3</sup>H activity. Bicarbonate concentration was taken as equal to total CO<sub>2</sub> concentration (6). Blood and timed urine samples were collected during the experiment for measurement of plasma and urine <sup>3</sup>H activity, plasma protein concentration, blood pH, PCO<sub>2</sub>, and PO<sub>2</sub>. Whole blood, obtained on the day of micropuncture from a donor rat, was used to replace all blood withdrawn for analytical purposes.

**Membrane vesicle preparation.** After completion of micropuncture and clearance studies, BBMV were prepared from both kidneys in each rat studied using a modification of the Mg<sup>++</sup> aggregation and differential centrifugation method (24, 25). The left renal pedicle was ligated and the kidney rapidly removed and placed into an ice-cold isotonic buffered solution (solution A) consisting of mannitol ~ 170 mM, Hepes ~ 85 mM, and Tris (trizma base) ~ 45 mM, pH 7.40, total osmolality = 300 mosmol/kg H<sub>2</sub>O. The right kidney was excised and placed into the buffered media within ~ 30 s after removal of the left kidney. The renal cortices were isolated and weighed, diluted in a volume of solution A equal to four times their weight and homogenized. After removing an aliquot for determination of total protein concentration, alkaline phosphatase activity, and Na<sup>+</sup>-K<sup>+</sup>-ATPase activity, sufficient 1 M MgCl<sub>2</sub> was added to raise the concentration of magnesium to 10 mM and the samples placed on ice. Differential centrifugation was then performed, alternating slow-speed centrifugations (10 min each, saving the supernatant), high-speed centrifugations (20 min each, saving the pellet), and 15-min incubations of the high-speed pellet diluted to ~ 7 ml with solution A (plus 10 mM MgCl<sub>2</sub>). The slow-speed spins were 2,250, 3,250, and 4,300 rpm in a refrigerated (4°C) Sorvall RC-5B centrifuge with an SS-34 rotor (E.I. DuPont de Nemours & Co., Inc., Sorvall Instruments Div., Newtown, CT); the high-speed centrifugations were at 15,000 rpm. The brush border membrane marker enzyme alkaline phosphatase, assayed using *p*-nitrophenyl phosphate as a substrate, was increased 6–10-fold (mean 7.3±0.3) in the final pellet compared to the homogenate. The ratio of Na<sup>+</sup>-K<sup>+</sup>-ATPase specific activity in the vesicles/whole homogenate was = 0.90±0.08. The final purified pellets obtained from the left and right kidneys were combined (total volume ~ 500  $\mu\text{l}$ ) and frozen overnight at -83°C.

**Transport measurements.** Timed uptakes of <sup>22</sup>Na<sup>+</sup> into the brush border membrane vesicles were assayed at 22°C by the rapid filtration technique (26). Vesicles were thawed, and divided into two samples. One was diluted with 13 times its volume with solution A, pH 7.40. The second was diluted 13 times its volume with solution B, pH 5.90, consisting of ~ 182 mM mannitol, ~ 42.5 mM Hepes, ~ 22.5 mM Tris, and ~ 52.5 mM 2-(*N*-morpholino)-ethanesulfonic acid, total osmolality 300 mosmol/kg H<sub>2</sub>O. The two vesicle solutions were then

incubated for 2 h and spun down at 15,000 rpm for 20 min in the Sorvall RC-5B centrifuge, and the final pellets were resuspended in sufficient pH 5.9 or 7.4 buffer to yield protein concentrations of 5–7 mg/ml. Protein concentrations of the final vesicle preparations were performed using the method of Lowry et al. (27). Because the Lowry assay is sensitive to Tris buffer concentrations (28), the blanks and albumin standards used for protein determinations were mixed with pH 5.9 or 7.4 buffer (where appropriate) which were diluted to the same strength as the buffers present in the whole homogenate, original vesicle pellet, or the pH 5.9 and 7.4 final vesicle pellets.

The influx experiments were initiated by addition of 10  $\mu\text{l}$  of vesicle preparation to 40  $\mu\text{l}$  of pH 7.4 buffer (solution A) containing <sup>22</sup>NaCl (0.386  $\mu\text{Ci}/40 \mu\text{l}$ ) and either 1.25, 7.0, or 25.0 mM NaCl (final osmolality always 300 mosmol/kg H<sub>2</sub>O) yielding final [NaCl] = 1.0, 5.6, or 20 mM. For the pH 5.9 vesicles, uptake was measured at 0, 2, 4, 6, 10, and 30 s as well as at 2-h equilibrium for the 1 mM NaCl gradient and at 0, 2, 4, and 10 s plus 2-h equilibrium values for the 5.6 and 20 mM NaCl gradients. Values at 0, 2, and 4 s were generally performed in triplicate or quadruplicate. For uptake rates into pH 7.4 vesicles (i.e., no transmembrane pH gradient), uptakes were determined at 0-, 5-, and 10-s intervals (usually in triplicate) and at 2 h. Timed incubations were terminated by the rapid addition of 1 ml of an ice-cold MgSO<sub>4</sub> solution (~ 14 mM Tris, ~ 14 mM Hepes, ~ 35 mM mannitol, ~ 285 mM MgSO<sub>4</sub>, pH 8.00, total osmolality = 375 mosmol/kg H<sub>2</sub>O). The mixture was immediately filtered through the center of an 0.45- $\mu\text{m}$  filter (DAWP; Millipore Corp., Bedford, MA.) prewetted with MgSO<sub>4</sub> stop solution plus 25 mM NaCl. The filter was quickly washed directly over the vesicle spot with 1.5–2 ml stop-solution followed by four 3-ml rinses. Filters were then dissolved in 3.5 ml Pico-Fluor 15 (Packard Instrument Co., Inc., Downers Grove, IL), and radioactivity was determined by liquid scintillation spectrometry. Nonspecific retention of radioactivity to the filters (determined in triplicate for each sodium concentration) was subtracted from total counts of the sample. Triplicate 1- $\mu\text{l}$  samples of each of the incubation solutions were counted to determine the specific activity of <sup>22</sup>Na<sup>+</sup> in the uptake media.

**Analytical methods, calculations, and statistics.** Blood pH, PCO<sub>2</sub>, and PO<sub>2</sub> were measured using a blood gas analyzer (Radiometer, Copenhagen). Plasma bicarbonate concentration was calculated using the Henderson-Hasselbalch equation. Total osmolalities of the solutions used for membrane vesicle studies were determined by freezing point depression. SNGFR and proximal reabsorption rates were calculated using standard formulas. FL<sub>HCO<sub>3</sub></sub> was calculated from the product of SNGFR and Bowman's space concentration of bicarbonate. Sodium uptake into the vesicles was calculated from the <sup>22</sup>Na<sup>+</sup> uptake and the specific activity of sodium in the incubation medium and plotted versus time. The initial rate of transport was obtained from a linear regression of uptake at 0 and 2 s for the pH 5.9 vesicles and at 0 and 5 s for the pH 7.4 vesicles and expressed as nmol Na<sup>+</sup>/(min × mg protein).

In preliminary experiments, addition of 4 × 10<sup>-3</sup> M amiloride to the pH 7.4 uptake medium, which should inhibit > 90% of the Na<sup>+</sup>/H<sup>+</sup> exchange (29), blocked <sup>22</sup>Na<sup>+</sup> uptake into the pH 5.9 vesicles to values nearly identical to those observed with pH 7.4 vesicles in the absence of amiloride. Therefore, the hydrogen-dependent sodium uptake rate (termed Na<sup>+</sup>/H<sup>+</sup> exchange) was calculated as the difference between nmol sodium uptake/(min · mg protein) for the pH 5.9 vesicles minus the sodium uptake/(min · mg protein) for the pH 7.4 vesicles at each time point. For each animal the kinetic constants ( $V_{\text{max}}$  and  $K_m$ ) of sodium uptake were calculated from a fit of the data to the Michaelis-Menten equation {Na<sup>+</sup>/H<sup>+</sup> exchange =  $a([\text{Na}^+])/(b + [\text{Na}^+])$ }, where  $a = V_{\text{max}}$  for Na<sup>+</sup>/H<sup>+</sup> exchange and  $b = \text{the } K_m \text{ for sodium}$ . The kinetic constants were also calculated from an Eadie-Hofstee transformation of these data, in which hydrogen-dependent sodium uptake rate [nmol Na<sup>+</sup> uptake/(min · mg protein)] was plotted against uptake rate/[Na<sup>+</sup>] for each of the three sodium concentrations used, also yielding the  $K_m$  (negative slope) and  $V_{\text{max}}$  ( $y$ -intercept) values. The kinetic constants were the same using either approach. Statistical analyses of results were carried out by unpaired analysis of variance and by covariance analysis for linear equations (e.g., Eadie-Hofstee data). The nonlinear relation-

Table I. Body Weights and Systemic Arterial Blood Composition

Group	Body wt g	Hct		AP mmHg	pH	PCO <sub>2</sub> mmHg	[HCO <sub>3</sub> ] mM	Plasma [Protein] g/dl
		B <sub>0</sub> vol %	E vol %					
Hydropenia (7)	307±10	51.5±0.8	58.0±0.5 <sup>  </sup>	104±4	7.43±0.01	41.6±1.3	26.8±0.5	5.4±0.1
Euvolemia (5)	324±10	53.0±0.5	52.2±0.3*	109±3	7.45±0.01	45.2±1.0	30.2±0.2*	5.7±0.1*
Plasma expansion (5)	309±15	51.8±0.8	44.7±0.5** <sup>  </sup>	112±3	7.44±0.01	42.7±1.6	27.9±0.7 <sup>‡</sup>	5.8±0.1*
Hydropenia, saralasin-treated (3)	306±9	52.2±0.2 (n = 2)	59.0±1.0 <sup>§§</sup>	89±3 <sup>§§</sup>	7.42±0.01	40.1±0.3	25.5±0.7 <sup>§§</sup>	5.2±0.1 <sup>§§</sup>

Values shown are means±SE. Abbreviations: Hct, hematocrit; AP, mean arterial pressure; B<sub>0</sub>, values obtained immediately after anesthesia and insertion of femoral artery catheter; E, values obtained during micropuncture. Numbers in parentheses indicate numbers of rats. \* *P* < 0.05 vs. hydropenia, ANOVA. <sup>‡</sup> *P* < 0.05 vs. euvolemia, ANOVA. <sup>§</sup> *P* < 0.05 vs. plasma expansion, ANOVA. <sup>||</sup> *P* < 0.05 vs. B<sub>0</sub>, paired *t* test.

ships (e.g., Michaelis-Menten regression curves) were fitted using a nonlinear least squares regression analysis (reference 30, p. 370), and the curves were compared using a residual sum of squares technique (reference 30, p. 372). Results are presented as means±SE.

## Results

**General.** Table I presents the body weights and the systemic arterial blood composition of the hydropenic, euvolemic, and plasma-expanded animals, and the hydropenic rats treated with saralasin. There were no significant differences in body weight among the groups. Hematocrit (Hct) during the micropuncture period was maintained near the B<sub>0</sub> level in the euvolemic group by replacing surgically induced plasma losses (20), whereas Hct rose significantly in the hydropenic groups and was reduced below B<sub>0</sub> levels in the plasma-expanded group. Mean experimental arterial pressure was comparable in all groups except the saralasin-treated rats in which mean arterial pressure was significantly reduced by 15–20 mmHg. No significant differences were noted in arterial pH or PCO<sub>2</sub> among groups, but the calculated plasma bicarbonate concentration was significantly higher in both euvolemic and plasma-expanded rats than in either of the hydropenic groups. Plasma protein concentration remained at euvolemic levels in the plasma-expanded rats but was significantly reduced in the hydropenic animals.

Table II presents clearance and reabsorption data obtained at the whole-kidney and single-nephron level during the micropuncture period. GFR and SNGFR increased progressively as

plasma volume was increased from hydropenic to plasma expanded levels. As shown in Fig. 1, the whole-kidney and single-nephron filtered load of bicarbonate increased with these progressive increases in GFR and SNGFR, yielding proportional increases in whole-kidney and proximal tubular bicarbonate reabsorption (AR<sub>HCO<sub>3</sub></sub> and APR<sub>HCO<sub>3</sub></sub>, respectively).

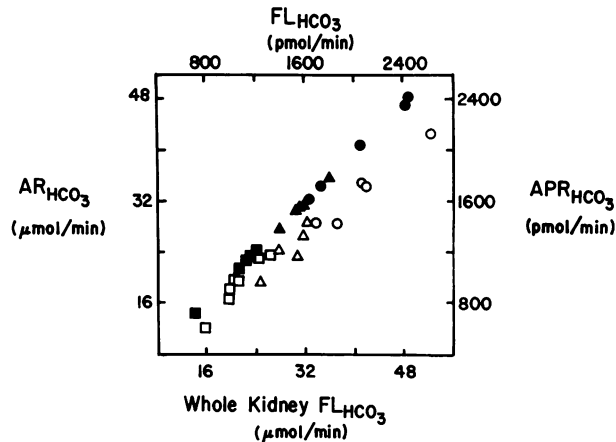
**Na<sup>+</sup>/H<sup>+</sup> exchange.** The left panel of Fig. 2 shows the time course for <sup>22</sup>Na<sup>+</sup> uptake in membrane vesicles obtained from five plasma-expanded rats studied in the presence of a 1 mM [Na<sup>+</sup>] gradient. Sodium entry was accelerated by the presence of a high intravesicular hydrogen ion concentration, but the equilibrium values (2 h) were identical for the pH 5.9 and 7.4 vesicles. In all groups studied, a similar pattern of <sup>22</sup>Na<sup>+</sup> uptake was observed. No differences in <sup>22</sup>Na<sup>+</sup> uptake were noted in the pH 7.4 vesicles among the groups. In addition, no differences were noted in the 2-h equilibrium values in either the pH 5.9 or 7.4 vesicles among groups. The right panel of Fig. 2 shows the early time course for <sup>22</sup>Na<sup>+</sup> uptake in the presence of a 1 mM [Na<sup>+</sup>] gradient in BBMV obtained from a plasma-expanded rat. Each point denotes a single measurement. The initial rate of <sup>22</sup>Na<sup>+</sup> uptake was taken as the slope obtained for the zero and 2-s data points in the presence of an outwardly directed [H<sup>+</sup>] gradient, as depicted by the solid line. In the absence of a pH gradient, the initial rate of <sup>22</sup>Na<sup>+</sup> uptake was determined as the slope of the zero and 5-s data points, shown as the dashed line.

The initial rates of sodium transport via Na<sup>+</sup>/H<sup>+</sup> exchange as a function of Na<sup>+</sup> concentration are shown in the left panel of Fig. 3 for the hydropenic and plasma-expanded rats. Com-

Table II. Whole-Kidney and Single-Nephron Clearance and Reabsorption Data

Group	Whole-kidney GFR	Whole-kidney FL <sub>HCO<sub>3</sub></sub>	AR <sub>HCO<sub>3</sub></sub>	SNGFR	Single-nephron FL <sub>HCO<sub>3</sub></sub>	APR <sub>HCO<sub>3</sub></sub>
	μl/min	μmol/min			nl/min	
Hydropenia	739±62 (5)	21.25±1.75 (5)	21.25±1.75 (5)	34.1±2.1 (7)	1,052±64 (7)	931±76 (7)
Euvolemia	957±45 (5)	31.72±1.26* (5)	31.68±1.26* (5)	42.1±2.4 (5)	1,469±72* (5)	1,197±89 (5)
Plasma expansion	1336±129 <sup>§§</sup> (5)	41.09±3.34 <sup>§§</sup> (5)	40.63±3.22 <sup>§§</sup> (5)	65.7±4.8 <sup>§§</sup> (5)	2,133±168 <sup>§§</sup> (5)	1,734±164 <sup>§§</sup> (5)
Hydropenia, saralasin-treated	738±96 <sup>§</sup> (3)	20.77±2.59 <sup>§§</sup> (3)	20.77±2.59 <sup>§§</sup> (3)	32.4±0.6 <sup>§</sup> (3)	957±24 <sup>§§</sup> (3)	858±38 <sup>§</sup> (3)

Values are means±SE. Whole-kidney data are for one kidney. \* *P* < 0.025 vs. hydropenia, ANOVA. <sup>‡</sup> *P* < 0.025 vs. euvolemia, ANOVA. <sup>§</sup> *P* < 0.025 vs. plasma expansion, ANOVA.



**Figure 1.** Relationship between bicarbonate reabsorption and the  $FL_{HCO_3}$  in hydropenic, euvolemic, and plasma-expanded rats. Whole-kidney values for bicarbonate reabsorption ( $AR_{HCO_3}$ ) and  $FL_{HCO_3}$  are represented by closed symbols, and the values indicated by the left ordinate and the lower abscissa. Proximal bicarbonate reabsorption in individual nephrons ( $APR_{HCO_3}$ ), and single-nephron  $FL_{HCO_3}$  are represented by open symbols, and the values indicated in the right ordinate and the upper abscissa. Data from hydropenia are shown by squares, from euvolemia by triangles, and from plasma expansion by circles.

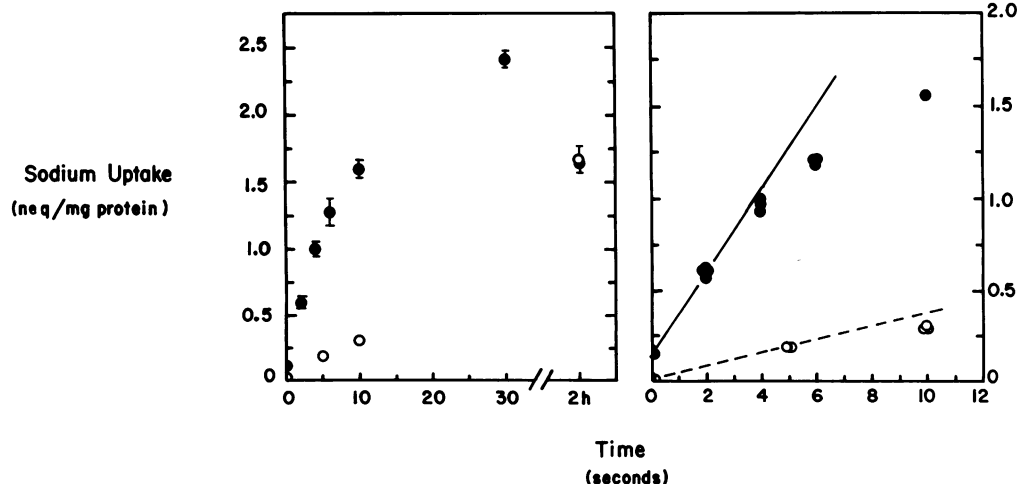
parison of the Michaelis-Menten regression curves for the two groups reveals enhanced  $Na^+/H^+$  exchange in the plasma expanded rats ( $P < 0.005$ , Table III). The right-hand panel of Fig. 3 shows an Eadie-Hofstee transformation of these data which indicates that the enhanced  $Na^+/H^+$  exchange in the plasma expanded rats was the consequence of a significantly higher  $V_{max}$  ( $y$ -intercept,  $P < 0.025$ ) with no significant change in  $K_m$  (negative slope). Table IV summarizes the average values of  $K_m$  and  $V_{max}$  for each group using Eadie-Hofstee transformations for each individual rat. Again, the  $V_{max}$  was higher in the plasma expanded rats than in hydropenia ( $P < 0.05$ ). Values of  $V_{max}$  obtained for euvolemic rats (see Table IV) fell between the data for hydropenic and plasma-expanded rats.

Fig. 4 (left panel) shows the relationship between whole-kidney and single-nephron filtered load of bicarbonate and the  $V_{max}$  of  $Na^+/H^+$  exchange for all rats. A significant correlation between bicarbonate delivery rate and  $V_{max}$  was observed (Table III). The right-hand panel of Fig. 4 demonstrates that a significant correlation between  $V_{max}$  and GFR or SNGFR is also obtained. The lines describing the relationship between the whole-kidney values and  $V_{max}$  shown in each panel of Fig. 4 parallel those for single-nephron values vs.  $V_{max}$ , suggesting that nephrons throughout the cortex are responding in a similar fashion. No significant correlation between the filtered load of bicarbonate or glomerular filtration (either whole kidney or single nephron) with the  $K_m$  for sodium was observed. Neither  $V_{max}$  nor  $K_m$  correlated with body weight, or arterial  $PCO_2$ , pH, or plasma bicarbonate concentrations. No significant correlation was obtained between an index of plasma volume ( $100\text{-Hct}$ ) and  $V_{max}$ .

$A_{II}$  has been shown to stimulate  $Na^+/H^+$  exchange and early proximal bicarbonate reabsorption (10, 22, 31–34), and the volume-contracted hydropenic rats would be expected to have high endogenous  $A_{II}$  levels. The slope of the relationship between  $V_{max}$  and either  $FL_{HCO_3}$  or SNGFR was greatest at low  $FL_{HCO_3}$  values (Fig. 4), representing primarily hydropenic rats. To examine whether the endogenous  $A_{II}$  level was influencing this relationship, we infused the competitive  $A_{II}$  inhibitor saralasin intravenously in three hydropenic rats for at least 90 min before vesicle preparation. Fig. 5 shows the relationship between  $V_{max}$  and either  $FL_{HCO_3}$  (left panel) or SNGFR (right panel) in the hydropenic rats. The rats infused with saralasin, shown by the open circles in Fig. 5, did not show any decrease in  $V_{max}$  as compared to untreated hydropenic rats at comparable values of  $FL_{HCO_3}$  or SNGFR.

## Discussion

The present studies have, for the first time, coupled measurements of single-nephron and whole-kidney bicarbonate delivery and GFR with BBMVs measurements of  $Na^+/H^+$  exchange activity in the same animal. Our results indicate that variations



**Figure 2.** Sodium uptake by BBMVs. (Right) Results in BBMVs obtained from a plasma-expanded rat. Each point denotes a single measurement in the presence of a  $1\text{ mM } Na^+$  gradient. (●) Uptake in the presence of an outwardly directed  $H^+$  gradient; (○) uptake in the absence of an  $H^+$  gradient. The lines drawn through the data were calculated by linear regression using the zero and 2-s data for ● and the zero and 5-s data for the ○. (Left) Mean results from five plasma-expanded rats for uptake in the presence of a  $1\text{ mM } Na^+$  gradient. (●) Data

for vesicles with an outwardly directed  $H^+$  gradient; (○) data from vesicles with no  $H^+$  gradient. Values of  $Na^+$  uptake are shown as mean  $\pm$  SE. For those points lacking standard error bars, the symbols were larger than the spread between the error bars.

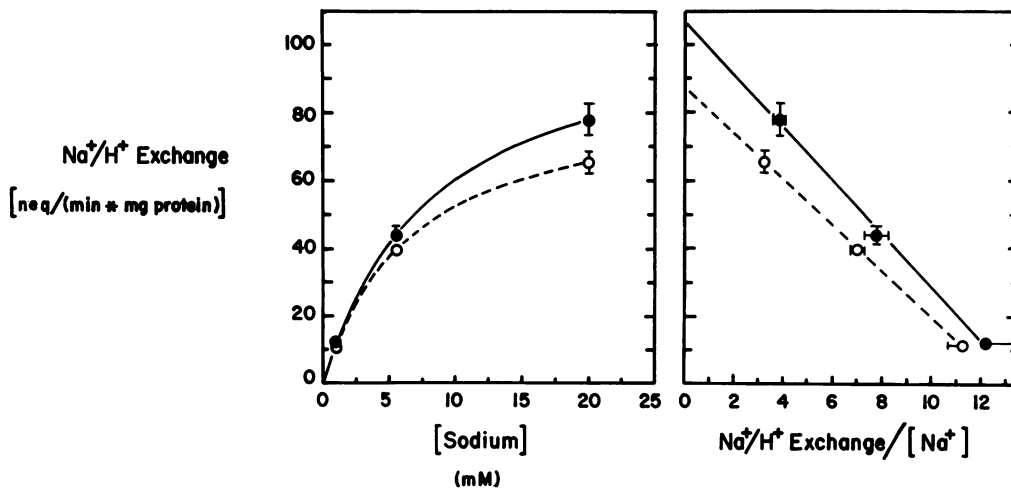


Figure 3. Kinetic analysis of  $\text{Na}^+/\text{H}^+$  exchange activity. (Left) Plot of  $\text{Na}^+/\text{H}^+$  exchange vs. the  $\text{Na}^+$  concentration in the uptake medium for hydropenic ( $\circ$ , ---) and plasma expanded rats ( $\bullet$ , —). (Right) Eadie-Hofstee plot of these data where the  $K_m$  for sodium is equal to the negative slope of the linear regression and the  $V_{\max}$  is the y-intercept. Both lines in the right panel were obtained by linear regression using the three mean values for each group. The calculated  $V_{\max}$  and  $K_m$  using these data are equal to the coefficients  $a$

and  $b$  of the Michaelis-Menton regression equation obtained for the curves in the left panel (see Table III). Data in each panel are shown as means  $\pm$  SE. Where error bars are not shown, the symbol was larger than the error bars. See Table III for regression equations, coefficients, and statistical comparisons.

in bicarbonate delivery, induced by changes in GFR, are accompanied by changes in the  $V_{\max}$  for  $\text{Na}^+/\text{H}^+$  exchange. When SNGFR was increased further by repletion of surgically induced plasma losses (euvoolemia) or by acute plasma expansion, the  $V_{\max}$  for  $\text{Na}^+/\text{H}^+$  exchange increased significantly as well (Figs. 3 and 4). The binding affinity for  $\text{Na}^+$  ( $K_m$ ) was unaffected by these changes (Fig. 3 and Table IV), indicating that the change in transporter function was not due to increased competition between  $\text{Na}^+$  and  $\text{H}^+$  for an external binding site on the transporter.

The observation that  $\text{Na}^+/\text{H}^+$  exchange activity is altered in apical membrane vesicles in these studies indicates that changes in  $\text{FL}_{\text{HCO}_3}$  and/or (SN)GFR induce sustained changes in this epithelial transporter. An increase in delivery or flow leads either to insertion of additional  $\text{Na}^+/\text{H}^+$  exchangers into the apical membrane or to a higher exchange rate in existing transporters (or to both effects). These results provide an explanation for our earlier observations in plasma-expanded rats (7). We showed that bicarbonate reabsorption increased with increasing GFR and  $\text{FL}_{\text{HCO}_3}$  in both the early and late proximal tubule, with no evidence of a shift in reabsorption from the early to later portions of this nephron segment. The increased reabsorptive capacity along the proximal tubule is consistent

with the increase in  $\text{Na}^+/\text{H}^+$  exchange observed in the present study. Our results, however, do not distinguish whether the  $V_{\max}$  for  $\text{Na}^+/\text{H}^+$  exchange is increased uniformly along the entire proximal tubule.

Maunsbach and co-workers (35) demonstrated that an increase in flow rate caused a spreading of apical microvilli in isolated perfused tubules. Such conformational changes could result in the exposure of more membrane transporters to the luminal fluid or could alter local  $\text{H}^+$  and  $\text{HCO}_3^-$  gradients, promoting bicarbonate reabsorption. The results of the present study, however, make it unlikely that the increase in reabsorption in plasma expansion is simply the result of conformational changes in the tubular epithelium induced by changes in flow. Such changes would not be preserved in vesicle preparations and thus should not produce any change in  $\text{Na}^+/\text{H}^+$  exchange activity in isolated vesicles.

Our results are consistent with studies carried out after uninephrectomy, a maneuver known to increase (SN)GFR in the remaining kidney (36). Other laboratories have shown an increase in  $V_{\max}$  for  $\text{Na}^+/\text{H}^+$  exchange 24 h or less after uninephrectomy (17, 19). Although  $\text{FL}_{\text{HCO}_3}$  was not measured in these studies, GFR was measured in one study and was found to have increased significantly by this time (17). One group of

Table III. Regression Equations, Coefficients, and Statistical Comparisons for Data Presented in Figs. 3 and 4

Figure	Panel	Relationship examined		Coefficients				Curve comparisons		
		y	x	a	b	r	n	P value for regression	F	p value
3,PE	Left	$\text{Na}^+/\text{H}^+$ exchange	$[\text{Na}^+]$	110.2	8.42	0.943	15	<0.01	6.55	<0.005
3,H	Left	$\text{Na}^+/\text{H}^+$ exchange	$[\text{Na}^+]$	87.6	6.84	0.994	21			
4	Left	$V_{\max}$	$\text{FL}_{\text{HCO}_3}$	147	754	0.70	17			
4	Left	$V_{\max}$	$\text{WKFL}_{\text{HCO}_3}$	155	18.6	0.71	15	<0.01		
4	Right	$V_{\max}$	SNGFR	158	28.1	0.75	17	<0.01		
4	Right	$V_{\max}$	GFR	177	832	0.77	15	<0.01		

The equation used for all figures was  $y = a \cdot x / (b + x)$ . Abbreviations:  $V_{\max}$ , calculated maximum rate of  $\text{Na}^+/\text{H}^+$  exchange;  $r$ , correlation coefficient of regression;  $n$ , number of observations used in calculating regression coefficients; PE, plasma expansion; H, hydropenia.

Table IV. Kinetic Constants for Na<sup>+</sup>/H<sup>+</sup> Exchange Activity and Vesicular Volumes

Group	$V_{max}$	$K_m$	Vesicle volume
	neq/(min · mg protein)	mM	μl/mg protein
Hydropenia	88.0±6.5 (7)	6.9±0.7 (7)	1.46±0.08 (7)
Euvolemia	93.0±5.6 (5)	6.4±0.5 (5)	1.73±0.12 (5)
Plasma expansion	107.8±5.5* (5)	8.2±0.9 (5)	1.55±0.05 (5)
Hydropenia, saralasin-treated	89.4±0.7 (3)	7.9±1.6 (3)	1.52±0.10 (3)

$V_{max}$  for Na<sup>+</sup>/H<sup>+</sup> exchange and the  $K_m$  for sodium obtained by Eadie-Hofstee transformation of the data for each rat. Vesicle volume calculated from an average of the 2-h equilibrium <sup>22</sup>Na<sup>+</sup> uptake values at each of the three sodium concentrations for each rat. *n*, number of rats.

investigators has shown an increase in Na<sup>+</sup>/H<sup>+</sup> exchange activity within 25 min of ligation of the contralateral renal artery (19). Again, delivery was not measured, however, and it is much less clear that an increase would have occurred over this brief time interval.

Previous studies in our laboratory have indicated that proximal bicarbonate reabsorption increases equally well whether FL<sub>HCO<sub>3</sub></sub> is increased by changes in SNGFR or plasma [HCO<sub>3</sub><sup>-</sup>] (7–9, 37). If HCO<sub>3</sub><sup>-</sup> delivery is the predominant factor influencing Na<sup>+</sup>/H<sup>+</sup> exchange, the data of the present study and those discussed above for the remnant kidney could provide an explanation for the disparity in previous results in animal models of metabolic alkalosis. Na<sup>+</sup>/H<sup>+</sup> exchange activity has been found to be decreased in rabbits when metabolic alkalosis was induced by furosemide (38, 39), unchanged from control in a similar model in rats (40), and increased in rats when metabolic alkalosis was induced by potassium depletion (41). Although the degree of alkalemia was similar in all these studies, the time of experimentation after induction of alkalosis varied widely. In the rabbits, vesicle studies were carried out within 1–2 d of

furosemide treatment, a time when SNGFR and FL<sub>HCO<sub>3</sub></sub> are known to be reduced (2). The rat studies were undertaken 7–10 d after furosemide, at a time when FL<sub>HCO<sub>3</sub></sub> returns to control levels (2). Finally, the potassium depletion studies were undertaken after 3–4 wk, at a time when FL<sub>HCO<sub>3</sub></sub> is known to be at or above control levels (42). In none of these studies was GFR or FL<sub>HCO<sub>3</sub></sub> measured, but the results could be explained by the correlations between  $V_{max}$  and FL<sub>HCO<sub>3</sub></sub> observed in the present study. Further measurements are needed to answer this question.

Although Na<sup>+</sup>/H<sup>+</sup> exchange activity increases in relation to increases in (SNGFR or FL<sub>HCO<sub>3</sub></sub>), the rise appears to be blunted at higher levels of delivery (Fig. 4). At the highest delivery rates the  $V_{max}$  for Na<sup>+</sup>/H<sup>+</sup> exchange increased only 1.5-fold over that seen at the lowest hydropenic rates. In this setting, whole-kidney and proximal tubular bicarbonate reabsorption continue to increase in a linear fashion to values threefold greater than the lowest rates seen in hydropenia (Fig. 1). One must be cautious in extrapolating from membrane vesicle data to the intact nephron, but our results suggest that only part of the increase in reabsorption seen over the range of filtered loads studied can be accounted for on the basis of a change in Na<sup>+</sup>/H<sup>+</sup> exchange activity measured in vesicles. Bank and co-workers (14) have shown that Na<sup>+</sup>-independent H<sup>+</sup> secretion (presumably via H<sup>+</sup>-ATPase) is stimulated as well by increasing bicarbonate delivery to the proximal tubular epithelium in the rat. It is conceivable that H<sup>+</sup>-ATPase plays a larger role in stimulating bicarbonate reabsorption at high filtered loads, but further studies are needed to answer this question.

Although our results indicate that the Na<sup>+</sup>/H<sup>+</sup> exchanger is stimulated by increases in delivery, they provide no insight into the signal which produces this change. Increases in SNGFR increase the delivery of other substrates, hormones, growth factors, etc., as well as bicarbonate, and any of these could potentially provide the signal. A<sub>II</sub> at low concentration has been shown to stimulate Na<sup>+</sup>/H<sup>+</sup> exchange activity (10, 22, 31–34), but it is unlikely that this hormone is the mediator of the response in our studies. A<sub>II</sub> levels would be expected to be reduced by plasma expansion, yet in this setting the  $V_{max}$  increased. Moreover, the administration of saralasin to hydro-

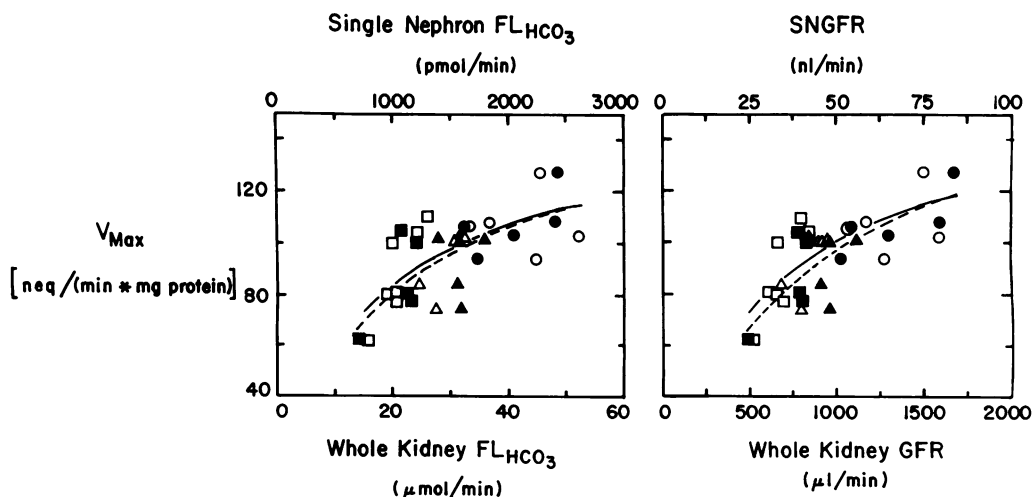


Figure 4. Relationship between filtered bicarbonate (left) or GFR (right) and the  $V_{max}$  for Na<sup>+</sup>/H<sup>+</sup> exchange in BBMVs. In both panels, whole-kidney values are denoted by filled symbols, and the values indicated on the lower abscissa. Single-nephron values are denoted by open symbols and the values indicated on the upper abscissa. Data from hydropenia are denoted by squares, euvolemia by triangles, and plasma expansion, by circles. The lines drawn through the data were obtained by regression analysis. The solid lines

depict the relationships for single-nephron data, and the dashed lines for whole kidney data. Table III gives the regression equations and coefficients for the lines.

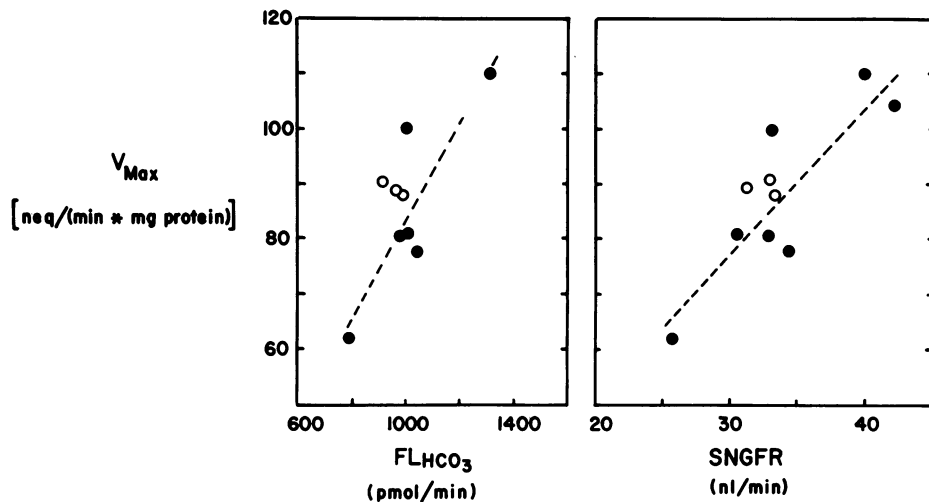


Figure 5. Relationship between single-nephron  $FL_{HCO_3}$  (left) or SNGFR (right) and the  $V_{max}$  for  $Na^+/H^+$  exchange. (●) Data from hydroponia; (○) data from hydroponic rats treated with saralasin. The linear regression equation describing the data from hydroponic rats for the left panel is  $Y = 0.09(X) - 6.5$  ( $r = 0.878$ ,  $P < 0.01$ ). For the right panel,  $Y = 2.69(X) - 3.6$  ( $r = 0.859$ ,  $P < 0.01$ ).

penic rats did not have a notable influence on the relationship between  $FL_{HCO_3}$  and  $V_{max}$  (Fig. 5). Of note, apical membrane  $Na^+/H^+$  exchange activity is increased in cultured opossum kidney cells after increases in the perfusion rate to both their apical and basolateral surfaces (43). These results in isolated cell cultures suggest that at least in this setting flow-induced changes in  $Na^+/H^+$  exchange activity are independent of hormonal effects. The nature of the signal, and whether other transporters are affected, remain subjects for future investigation.

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