

A Micropuncture Study of HCO_3 Reabsorption by the Hypertrophied Proximal Tubule

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In rats with renal failure produced by excision of one kidney and infarction of large portions of the other kidney, given a low calcium, high phosphorus diet for 2-3 weeks, GFR was reduced by 80 percent, the fractional excretion of sodium increased from 7 to 23 percent, that of bicarbonate from 16 to 23 percent and that of water from 4 to 13 percent. Single nephron GFR in the remaining nephrons was nearly doubled and end-proximal $\text{TF}/\text{P}_{\text{In}}$ was depressed from 2.3 to 1.8, and proximal $\text{TF}/\text{P}_{\text{HCO}_3}$ from 0.52 to 0.35, the latter figure corresponding to an increase of absolute proximal HCO_3 reabsorption from 1.7 to 3.5 nEq/min or from 2.8 to 3.2 Eq/L of single nephron glomerular filtrate. Acute parathyroidectomy had no influence on the fall of GFR or the rise of SNGFR in the remaining nephrons and failed to cause any significant changes in proximal tubular bicarbonate reabsorption. Parathyroidectomy, on the other hand, practically prevented the rise of the fractional excretion of sodium and of water and inverted the rise of the fractional excretion of bicarbonate to a fall. The data are interpreted to indicate that secondary hyperparathyroidism in renal failure impairs distal nephron bicarbonate and sodium reabsorption and, thus, contributes to the maintenance of sodium balance, but could possibly aggravate acidosis.

INTRODUCTION

Several studies in patients with secondary hyperparathyroidism have suggested that parathyroid hormone (PTH) impairs bicarbonate reabsorption by the renal tubules, and thus participates in the genesis of metabolic acidosis [1,2,3]. Almost all studies on the effects of PTH on renal electrolyte excretion in animals have involved acute administration of exogenous hormone derived from a different species. The results of these acute studies have not been entirely consistent, but generally have shown a decrease in tubular reabsorption of bicarbonate which is localized to the proximal tubule in rats [4] and only transient changes in the urine in dogs [5]. In neither of these studies did exogenous PTH lead to metabolic acidosis.

The present micropuncture experiments were designed to study the effect of chronic endogenous hyperparathyroidism, secondary to renal failure, on bicarbonate reabsorption by the kidney. Renal failure was produced in rats by excision of one kidney and segmental infarction of large portions of the other kidney [6]. Parathyroid hyperplasia was potentiated by feeding a low calcium, high phosphorus diet for 2-3 weeks after the renal ablation procedure. Micropuncture and clearance measurements were then performed during infusion of hypertonic NaHCO_3 .

METHODS

White male Sprague-Dawley rats were used, initially weighing 150-200 g. Three groups of animals were studied. All three groups were fed a low calcium, low

phosphorus diet (ICN Pharmaceuticals, Inc., Cleveland, Ohio), providing approximately 0.4 mEq sodium/day. Group C (8 rats) was given 50 ml/day drinking solution containing 90 mg calcium as calcium gluconate and 90 mg phosphorus as neutral sodium phosphate in 10 percent sucrose. The drinking solution provided 5.2 mEq sodium/day. The diet and drinking solution were given 2-3 weeks before study. Group NX (8 rats) underwent ablation of renal tissue by excision of the right kidney and ligation of several branches of the left renal artery [6]. Two to three days after surgery, these rats were started on the same low calcium, low phosphorus diet. Their drinking solution contained 67.5 mg phosphorus/day as neutral sodium phosphate in 10 percent sucrose. They were given 25 ml of this solution/day. The diet and drinking solution were given for 2-3 weeks before the day of experiment. This regimen in uremic rats has been shown to result in marked hypertrophy of the parathyroid glands [7]. Group NX-PTX (7 rats) were prepared in exactly the same manner as the NX rats, including the dietary regimen. In this group, the parathyroid glands were excised on the day of micropuncture study, 60-90 minutes before starting the experiment. The parathyroid glands were weighed on a Cahn gram electrobalance, model G (Ventron Instrument Corp., Paramount, California).

On the day of the experiment, anesthesia was induced with i.p. Inactin® (100 mg/kg body weight in group C, and 75 mg/kg in the NX and NX-PTX animals. Additional anesthesia was given during the course of the experiment, if needed, to stabilize blood pressure and respiration. The surgical preparation for micropuncture was as described in a previous publication [4].

After the surgical preparation was completed, an arterial blood sample was obtained and hypertonic NaHCO_3 solution (750 mEq/L) was then administered continuously IV at a rate of 50 $\mu\text{l}/\text{min}$ throughout the remainder of the experiment. [Carboxyl ^{14}C] inulin was given in a priming dose of 30 μCi and it was added to the IV solution to deliver 30 $\mu\text{Ci}/\text{hr}$. Forty-five minutes were allowed for equilibration of inulin before starting urine or tubular fluid collections. Two tubular fluid collections were made from each of selected end-proximal convolutions, the first for measurement of inulin and the second for measurement of HCO_3^- [4]. Arterial blood samples were collected periodically throughout the experiment for measurement of plasma inulin, pH, pCO_2 and HCO_3^- . The plasma inulin and HCO_3^- concentrations at the midpoint of tubular fluid and urine collections were calculated from a time plot, and these concentrations were used to calculate $\text{TF}/\text{P}_{\text{In}}$, $\text{TF}/\text{P}_{\text{HCO}_3^-}$, $\text{U}/\text{P}_{\text{In}}$ and $\text{U}/\text{P}_{\text{HCO}_3^-}$ ratios.

Single nephron glomerular filtration rate (SNGFR) was calculated from the expression:

$$\text{SNGFR} = \text{TF}/\text{P}_{\text{In}} \times \text{TFR}$$

where TFR is the tubular fluid flow rate in nanoliters per minute. Glomerular filtration rate (GFR) for the whole kidney was calculated from the expression:

$$\text{GFR} = \text{U}/\text{P}_{\text{In}} \times \text{V}$$

where V is the urine flow rate in milliliters per minute.

Percent reabsorption of bicarbonate by the proximal tubule was calculated from

$$[1 - (\text{TF}/\text{P}_{\text{HCO}_3^-} \times \text{P}/\text{TF}_{\text{In}})] \times 100$$

Absolute rate of bicarbonate reabsorption was calculated for each tubule by the following:

$$\text{T}_{\text{HCO}_3^-} = \text{SNGFR} \times (\text{P}_{\text{HCO}_3^-}/\text{nl}) \times [\% \text{HCO}_3^- \text{ reab.}]$$

The data were "normalized" for differences in SNGFR and expressed as

$$[\text{T}_{\text{HCO}_3^-}/\text{SNGFR}] \times 100$$

RESULTS

The pre-infusion and final blood acid-base values for the three groups of rats are presented in Table 1. As can be seen, there were no significant differences in pH, pCO₂, or HCO₃⁻ concentration in the pre-infusion blood samples, all of the values falling within the normal ranges for rats. It is important to note that metabolic acidosis had not developed in either the NX or NX-PTX animals. The final blood samples, at the end of hypertonic NaHCO₃ infusion, showed higher elevations of blood pH and HCO₃⁻ in the NX and NX-PTX groups, but the differences were not significant between C and NX and between NX and NX-PTX. pCO₂ tended to fall slightly during the course of the experiments, but there were no significant differences among the three groups of rats at the end of the experiment.

Urinary electrolyte excretion and whole kidney GFR data are shown in Table 2. Urine flow rate, sodium and bicarbonate excretion and GFR were significantly lower in the NX and NX-PTX rats than in the normal C rats. GFR was reduced by approximately 80 percent in the two renal-ablated groups. Potassium excretion was not statistically significantly impaired in the NX rats, indicating marked adaptation of potassium secretion in the remaining nephrons of NX animals. It is important to note that in the NX-PTX rats, urine flow rate, sodium, potassium, and bicarbonate excretion were all significantly lower than in the hyperparathyroid NX rats. Although the mean GFR value for the NX-PTX group was slightly lower than for the NX group, the difference is not statistically significant. Mean blood pressure in all three groups of rats was comparable, ranging between 110–130 mmHg during the course of the experiments.

The results of the micropuncture data are presented in Figs. 1–3. In Fig. 1 are shown the mean SNGFR data for the three groups of rats, along with whole kidney

TABLE 1
Plasma Acid-Base Values Before and After Hypertonic NaHCO₃ Infusion

Group	pH		pCO ₂ (mmHg)		HCO ₃ ⁻ (mEq/L)	
	1*	2*	1	2	1	2
Control (8)						
Mean	7.38	7.60	38.2	36.5	22.1	37.3
±S.E.	0.02	0.03	1.7	2.2	1.7	1.2
<i>P</i>	NS	NS	NS	NS	NS	NS
NX (8)						
Mean	7.36	7.66	40.9	38.4	22.4	43.4
±S.E.	0.01	0.03	2.1	3.9	0.9	4.8
<i>P</i>	NS	NS	NS	NS	NS	NS
NX-PTX (7)						
Mean	7.38	7.70	46.4	36.0	26.9	43.0
±S.E.	0.06	0.03	3.0	2.7	2.9	2.3

*Column 1 refers to blood collected before starting hypertonic NaHCO₃ infusion. Column 2 shows the final blood values at the end of the experiment.

TABLE 2
Urine Values during Hypertonic NaHCO₃ Infusion

Group	V (ml/min/kg)	U _{Na} V	U _K V (μEq/min/kg)	U _{HCO₃⁻} V	GFR (ml/min/kg)	HCO ₃ ⁻ Reab (%)
Control (8)						
Mean	0.33	84.1	8.6	52.8	10.2	83.7
±SE	0.03	8.6	1.4	4.1	0.8	1.3
P	<.005	<.005	NS	<.005	<.005	NS
NX (8)						
Mean	0.18	48.1	5.5	16.5	2.07	78.9
±SE	0.03	7.2	1.7	4.2	0.29	3.2
P	<.005	<.005	<.05	<.025	NS	<.005
NX-PTX (7)						
Mean	0.07	15.7	2.3	6.7	1.68	88.9
±SE	0.01	1.9	0.3	1.2	0.20	1.5

GFR values. SNGFR in the C rats averaged 59.4 nl/min ± 2.3 S.E., as compared with 113 nl/min ± 5.4 in NX and 107 nl/min ± 5.7 in NX-PTX. The latter two means are significantly higher than C ($p < 0.005$) indicating a marked adaptational increase in SNGFR in the surviving nephrons. The NX and NX-PTX values are not statistically different from one another.

Fig. 2 shows the data for end-proximal TF/P_{In} and TF/P_{HCO₃⁻} ratios in the three groups. Fractional water reabsorption (and presumably Na⁺ reabsorption) fell significantly in the NX group. Thus, TF/P_{In} was 2.31 ± 0.13 S.E. in C, and 1.93 ± 0.09 in NX ($p < 0.01$). In the NX-PTX group, TF/P_{In} was 2.01 ± 0.10, a value slightly higher but not significantly different from the NX group ($p > 0.10$). End-proximal TF/P_{HCO₃⁻} was significantly lower in the NX and NX-PTX rats than in the controls:

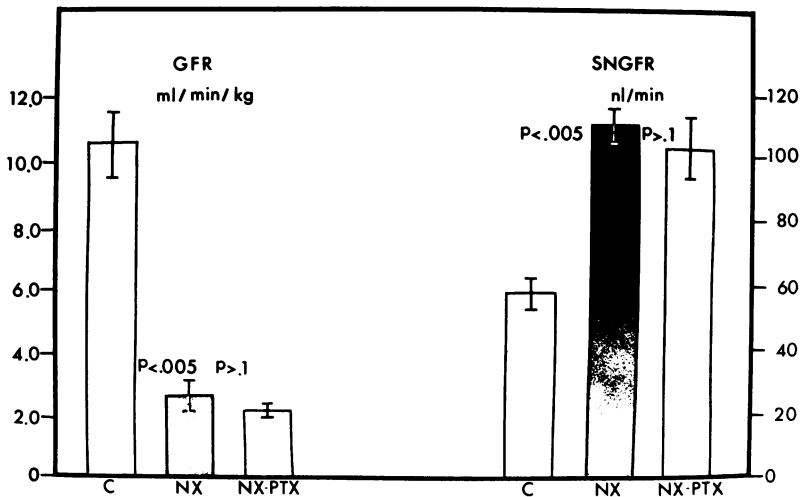


FIG. 1. Whole kidney glomerular filtration rate (GFR) and single nephron filtration rate (SNGFR) in control (C), partially nephrectomized (NX) and parathyroidectomized partially nephrectomized (NX-PTX) rats. The vertical insert lines represent ± S.E.

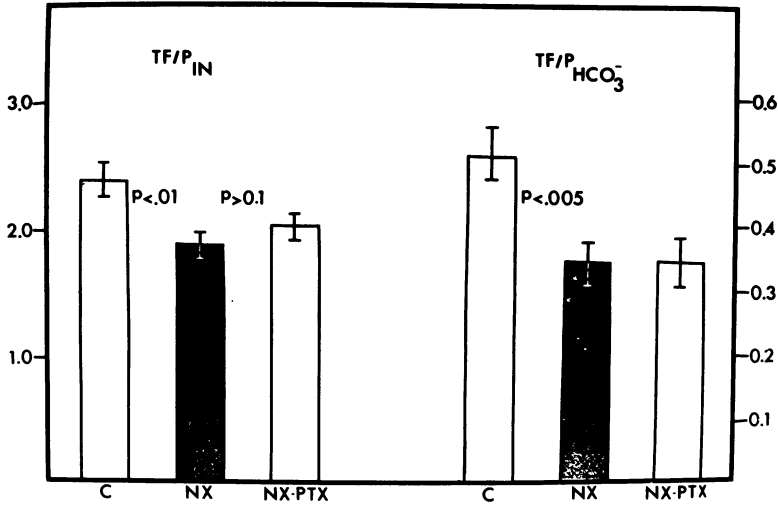


FIG. 2. End proximal tubular fluid/plasma inulin (TF/P_{IN}) and bicarbonate (TF/P_{HCO₃⁻}) ratios in C, NX, and NX-PTX rats during hypertonic NaHCO₃ infusion.

0.52 ± 0.04 S.E. in C; 0.34 ± 0.03 in NX; 0.34 ± 0.04 in NX-PTX (*p* < 0.005). Absolute rates of HCO₃⁻ reabsorption by the proximal tubule, expressed as nEq/min and nEq/100 nl SNGFR, are shown in Fig. 3. The mean values are 1.63 nEq/min ± 0.08 S.E. in C, 3.49 ± 0.23 in NX, and 3.64 ± 0.29 in NX-PTX (*p* < 0.005 for C vs. NX and C vs. NX-PTX). There is no significant difference between NX and NX-PTX. Bicarbonate reabsorption was also increased after “normalization” for differences in SNGFR. Thus, in C the mean value was 2.76 nEq/100 nl ± 0.10 S.E., in NX it was 3.13 ± 0.20, and in NX-PTX it was 3.34 ± 0.19. The *p* values are < 0.05 (C vs. NX), < 0.01 (C vs. NX-PTX) and > 0.10 for NX vs. NX-PTX. Percent HCO₃⁻ reabsorption for the proximal tubule was 75.9 percent ± 2.1 S.E. in C, 81.9 percent ± 1.7 in NX, and 81.5 percent ± 2.4 in NX-PTX. There is no significant difference between NX and

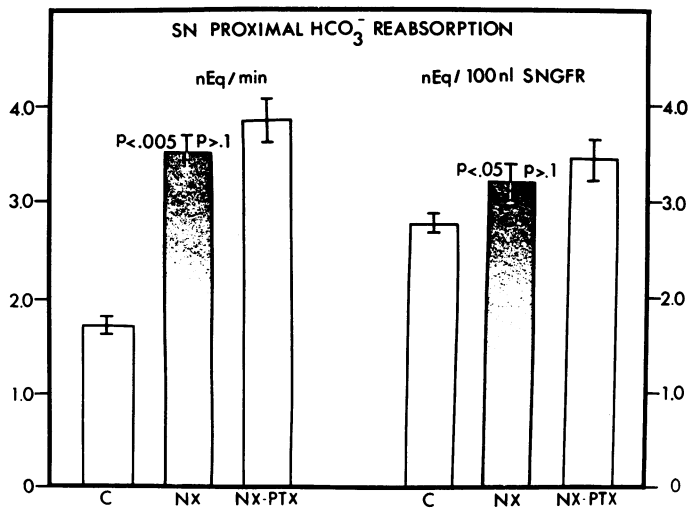


FIG. 3. Absolute rates of HCO₃⁻ reabsorption by proximal convoluted tubules of C, NX, and NX-PTX rats during hypertonic NaHCO₃ infusion. Data on the right have been normalized for differences in SNGFR.

NX-PTX ($p > 0.1$) but both are significantly higher than C ($p < 0.025$). Thus, in the hypertrophied nephrons of the NX and NX-PTX rats, proximal tubular HCO_3^- reabsorption was increased in absolute and fractional amounts, despite a decrease in fractional sodium and water reabsorption.

In Fig. 4 is shown fractional excretion of sodium, bicarbonate, and water in the final urine of the three groups of rats. The normal C rats excreted 6.2 percent \pm 1.2 S.E. of filtered sodium, 16.2 percent \pm 0.9 of filtered HCO_3^- , and 3.9 percent \pm 0.5 of filtered water. The NX rats, with chronic secondary hyperparathyroidism, excreted 22.4 percent \pm 4.8 S.E. of filtered sodium, 21.9 percent \pm 3.3 of filtered HCO_3^- , and 12.1 percent \pm 1.7 of filtered water. All of these values are significantly higher than those for the C rats ($p < 0.01$). In sharp contrast, the NX-PTX rats excreted only 7.7 percent \pm 0.8 S.E. of filtered sodium, 10.7 percent \pm 1.6 of filtered HCO_3^- , and 4.4 percent \pm 0.5 of filtered water. These values are significantly lower than in the NX group ($p < 0.01$), and are as low as or lower than in the C group. Thus, acute parathyroidectomy in rats with chronic secondary hyperparathyroidism sharply reduced fractional excretion of sodium, bicarbonate, and water under these experimental conditions.

DISCUSSION

The results of the present study show that the hypertrophied proximal tubule, resulting from ablation of a large fraction of total renal mass, has a greatly enhanced capacity for reabsorption of bicarbonate, in spite of the presence of secondary hyperparathyroidism. The increase in HCO_3^- reabsorption was evident in terms of absolute nEq/min, percent reabsorption of filtered load, and reabsorption/unit of SNGFR. In sharp contrast, sodium and water reabsorption was increased in absolute amounts, but was decreased when expressed as a percentage of the filtered load. The reason for the dissociation between fractional sodium and bicarbonate reabsorption in the NX rats is not certain. Because the same IV load of hypertonic NaHCO_3 was

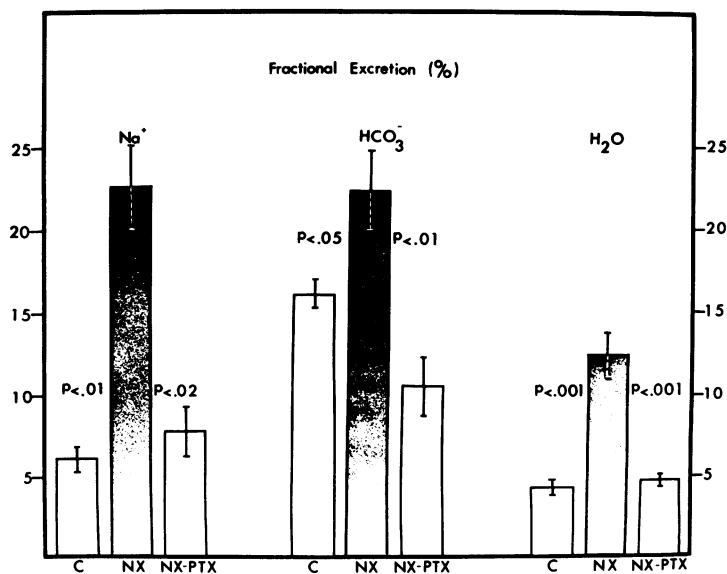


FIG. 4. Fractional excretion of Na^+ , HCO_3^- , and H_2O in the final urine of C, NX, and NX-PTX rats during NaHCO_3 infusion.

given to the C and NX rats, and the absolute excretion rates of electrolytes and water were considerably lower in the NX rats (Table 2), a greater degree of extracellular volume expansion undoubtedly occurred in the NX rats during the course of the experiment. This volume expansion might account for the reduction in proximal fractional sodium and water reabsorption in the NX rats. However, volume expansion obviously did not have an equivalent effect on proximal HCO₃⁻ reabsorption, although it is well established that volume expansion does depress tubular HCO₃⁻ reabsorption in normal rats and dogs [8,9,10]. Our observations suggest a disproportional *enhancement* of HCO₃⁻ reabsorption in the hypertrophied proximal tubule, which was evident in spite of relative extracellular volume expansion and chronic secondary hyperparathyroidism. These observations are essentially in agreement with clearance studies in renal-ablated dogs, recently published by Arruda, Carrasquillo et al. [11]. They found in volume-expanded bicarbonate loaded animals that the ratio of absolute HCO₃⁻ reabsorption/absolute sodium reabsorption was significantly higher after induction of chronic renal failure. This was unaffected by PTX. In contrast, Lubowitz, Purkerson et al. [12] found a disproportional *reduction* in fractional HCO₃⁻ reabsorption in the proximal tubule of unexpanded renal-ablated rats. The reasons for the difference between the findings of Lubowitz et al. [12] and those of the present study are not clear.

It is important to note that the NX rats did not manifest metabolic acidosis, in spite of an 80 percent reduction in GFR and hypertrophy of the parathyroid glands. Arruda et al. [11] similarly found normal acid-base values in dogs with chronic renal failure of approximately the same degree. In contrast, Espinel [13] found a rather severe metabolic acidosis (mean serum [HCO₃⁻] = 10.1 mEq/L) in rats with ablation of approximately 85–90 percent of total renal mass, and related this to impaired HCO₃⁻ reabsorption associated with extracellular volume expansion. Morrin, Gedney et al. [14] found impaired HCO₃⁻ reabsorption in dogs with unilateral pyelonephritis or aminonucleoside nephritis, as compared with a normal contralateral kidney. Slatopolsky, Hoffsten et al. [15] studied patients with bilateral intrinsic renal disease, and found that HCO₃⁻ reabsorption became progressively impaired with lower GFR values and higher fractional excretion of sodium.

From these several studies, it seems that a number of different factors may determine tubular HCO₃⁻ reabsorption in the presence of reduced renal mass. If the surviving nephrons are intact and hypertrophied, it is probably necessary that renal mass be reduced by 90 percent or more before gross impairment becomes evident [13]. Intrinsic renal disease might in some way specifically impair HCO₃⁻ reabsorption, unrelated to the overall amount of functioning renal mass [14]. Extracellular volume expansion in uremia can exaggerate a defect in HCO₃⁻ reabsorption [15]. The results of the present study suggest that PTH might be an additional factor, acting on the distal nephron to impair HCO₃⁻, and Na⁺ reabsorption. Although acidosis did not develop in our rats, presumably because of greatly increased HCO₃⁻ reabsorption in the proximal tubule, it seems possible that the observed distal effect of PTH might lead to urinary HCO₃⁻ wasting if renal mass were reduced further.

It is not clear from our study whether PTH acted primarily to inhibit Na⁺ or HCO₃⁻ in the distal nephron. However, Husted, Nolph et al. [16] recently reported that patients with advanced renal disease excrete sodium more readily if given as NaHCO₃ rather than NaCl. Their observations, taken together with those of the present study, are consistent with the view that chronic secondary hyperparathyroidism inhibits HCO₃⁻ reabsorption in the distal nephron, and that the rejected HCO₃⁻ obligates excretion of proportional amounts of sodium and water.

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