Neonatal Rabbit Juxtamedullary Proximal Convoluted Tubule Acidification

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Abstract

The present in vitro microperfusion study examined apical membrane Na^+/H^+ antiporter and basolateral membrane $Na(HCO_3)_3$ symporter activity in newborn and adult juxtamedullary proximal convoluted tubules. Proton fluxes were determined from the initial rate of change of intracellular pH after a change in the luminal or bathing solution, buffer capacity, and tubular volume of newborn and adult tubules. Intracellular pH (pH_i) was measured fluorometrically using the pH-sensitive dye (2',7')-bis (carboxyethyl)-(5,6)-carboxyfluorescein (BCECF). Apical Na^+/H^+ antiporter proton flux, assayed by the effect of sodium removal ($147 \rightarrow 0$ meq/liter) on pH_i, was one-third the adult level for the first 2 wk and doubled in the 3rd wk of life. Adult levels were achieved by 6 wk of age. Na^+/H^+ antiporter activity was not detected on the basolateral membrane of 1-wk-old newborns, indicating that polarity of this transporter was already present. Basolateral membrane $Na(HCO_3)_3$ proton flux, assayed by the effect of a bath bicarbonate change ($25 \rightarrow 5$ meq/liter) and by a bath sodium change ($147 \rightarrow 0$ meq/liter) on pH_i, was 50–60% of adult values in 1-wk-old newborns. Basolateral membrane $Na(HCO_3)_3$ proton flux assayed by a bath bicarbonate change ($25 \rightarrow 5$ meq/liter) remained at 50–60% of adult values for the 1st mo of life and increased to adult levels by 6 wk of age. This transporter not only plays a role in net acidification, but is an important determinant of cell pH in newborn juxtamedullary proximal convoluted tubules. (*J. Clin. Invest.* 1990. 85:499–506.) intracellular pH · Na^+/H^+ antiporter · $Na(HCO_3)_3$ symporter · proton flux

Introduction

Examination of acidification in newborn animals has been complicated by several factors. Clearance studies examine the composite of several nephron segments which may each be at different stages of maturation. These studies do not differentiate differences in transport between superficial and juxtamedullary nephrons (1–3). This problem of nephron heterogeneity encountered in adult animals is accentuated in the developing kidney where there is a centrifugal pattern of nephron maturation (4–8). Furthermore, a change in transport during development may be due to tubular maturation or to developmental changes in the peritubular environment. Both peritubular physical forces and the hormonal milieu which

may affect transport change during the first weeks of life (9–14). The problems encountered in studying developmental changes in transport are averted by using in vitro microperfusion where a specific nephron segment can be studied under controlled conditions.

In an in vitro microperfusion study, Schwartz and Evan (15) examined bicarbonate and glucose transport and volume absorption during development in juxtamedullary proximal convoluted tubules (PCT).[1] Volume absorption and bicarbonate transport remained fairly constant for the first 3–4 wk of age at about one-third the adult rate. Between 4 and 6 wk of age there was a dramatic increase in both parameters to values near those seen in adult animals. Glucose transport showed a similar maturational pattern.

Proximal tubule apical proton secretion is primarily via the apical Na^+/H^+ antiporter (16–21) and bicarbonate exit is primarily via the $Na(HCO_3)_3$ symporter (18, 22–29). Developmental maturation of net bicarbonate transport could be dependent on changes in cellular metabolism as well as Na^+/H^+ antiporter, $Na(HCO_3)_3$ symporter, and Na^+-K^+ ATPase activity. Using fluorescent measurement of intracellular pH (pH_i), the present in vitro microperfusion study examined Na^+/H^+ antiporter activity and basolateral $Na(HCO_3)_3$ symporter activity in newborn and adult juxtamedullary PCT. The present study demonstrates that the maturation of apical membrane sodium-dependent proton secretion precedes the increase in net bicarbonate transport measured by Schwartz and Evan (15). The present study also demonstrates that polarity of Na^+/H^+ antiporter activity demonstrated in PCT in adult animals is achieved in newborn juxtamedullary PCT. Surprisingly, basolateral membrane bicarbonate exit is relatively more mature in newborns than apical sodium-dependent proton secretion. This may be due to the fact that the basolateral membrane $Na(HCO_3)_3$ symporter not only plays a role in net acidification, but is an important determinant of cell pH in newborn juxtamedullary PCT.

Methods

Isolated segments of rabbit juxtamedullary PCT were dissected and perfused as previously described (18, 30). Briefly, New Zealand white rabbit pregnant does were housed at our institution. Newborn rabbits aged from 1 (1–7 d) to 6 wk (36–42 d) were cared for by their mothers. Adult animals were obtained from the same vendor. Kidneys from animals sacrificed at specific ages were cut in coronal slices. All PCT were dissected from the juxtamedullary cortex and were 0.1–0.3 mm in length. No late PCT identified by their attachment to proximal straight tubules were employed to avoid problems with axial heterogeneity (18). Individual tubules were dissected in cooled (4°C) ultrafiltrate-like (UF) solution. This solution was bubbled with 95% O_2/5% CO_2 and

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1. *Abbreviations used in this paper:* BCECF, (2',7')-bis (carboxyethyl)-(5,6)-carboxyfluorescein; PCT, proximal convoluted tubule; SITS, 4-acetamido-4'-isothiocyanostilbene-2,2'-disulfonate; UF, ultrafiltrate-like (solution).

Table I. Solutions

	UF	UF s̄ organics	Low-Na, UF	Low-Na, UF±NH₄Cl	ONa c̄ organics	ONa s̄ organics	High-Cl-5HCO₃⁻	OCl, UF	ONa, OCl
				meq/liter					
Na⁺	142*	147	40	40	—	—	142	147	—
K⁺	5	5	5	5	5	5	5	5	5
Choline⁺	—	—	100	80	142.0	147.0	—	—	25
NMG⁺	—	—	—	—	—	—	—	—	122
NH₄⁺	—	—	—	20	—	—	—	—	—
Ca²⁺	1.8	1.8	1.8	1.8	1.8	1.8	1.8	9.3	9.3
Mg²⁺	1	1	1	1	1	1	1	1	1
Cl⁻	123.6	128.6	110.6	110.6	123.6	128.6	143.6	—	—
Gluconate⁻	—	—	—	—	—	—	—	143.6	143.6
HCO₃⁻	25	25	25	25	25	25	5	25	25
SO₄⁻	1	1	—	—	1	1	1	1	1
HPO₄⁻	1	1	1	1	1	1	1	1	1
Urea	5	5	5	5	5	5	5	5	5
Glu	5	—	5	5	5	—	5	5	5
Ala	5	—	5	5	5	—	5	5	5
Hepes	—	—	25	25	—	—	—	—	—

* In solutions where 2 mM amiloride was used, MgCl₂ rather than MgSO₄ was employed to increase the solubility of amiloride.

had a pH of 7.4. The solutions used are listed in Table I. Tubules were transferred to a 0.2-ml bath chamber and were perfused using concentric glass pipettes. The bathing solution was preheated to 38°C and exchanged at a rate of at least 3 ml/min to maintain a constant bath temperature and pH. In studies where changes were made in the bathing solution in the experimental period, the bath was exchanged at 10 ml/min. All solutions were adjusted to an osmolality of 295 mosmol/ kg H₂O with an osmometer (model 3W2, Advanced Instruments, Inc., Needham Heights, MA) by adding H₂O or the predominant salt.

Tubules were initially perfused with an UF solution and bathed in UF solution during a 5–10-min equilibration period. The bathing solution was then changed to one which contained 5×10^{-6} M of the acetoxymethyl derivative of (2′,7′)-bis(carboxyethyl)-(5,6)-carboxyfluorescein (BCECF-AM, Molecular Probes, Eugene, OR). This compound is lipid soluble, permeates into cells, and does not fluoresce. Cytoplasmic esterases cleave the ester groups forming the pH-sensitive dye, BCECF. BCECF has four negative charges and leaves the cell slowly (22). The cells were loaded with the dye for 3–6 min and then the bathing solution was changed to the one employed in the control period where the tubules were again equilibrated for at least 5 min before making any measurements.

Measurement of pH$_i$. The fluorescent dye BCECF was used to measure intracellular pH as has been described previously (18, 22, 27, 30). BCECF has a pH-sensitive excitation maximum at 504 nm is relatively insensitive to pH at 436 nm. Peak emission occurs at 526 nm. In the present study excitation was produced alternately at 500 and 450 nm and emission was measured at 530 nm (filters from Corion Corp., Holliston, MA). Background was measured before loading and was subtracted from all measurements. Measurements were made using an inverted epifluorescent microscope (Fluovert, E. Leitz, Wetzlar, FRG) at ×25 magnification. A variable diaphragm was placed over the area to be measured. The area ranged from 500 to 2,500 μm^2.

To calculate pH from the ratio of fluorescence measured at the two excitation wavelengths (F_{500}/F_{450}), a nigericin calibration curve was performed as previously described (18, 22, 27, 31). There was no developmental difference in the nigericin calibration curve at any pH (6.5, 6.9, 7.3, 7.7) between 1-wk-old ($n = 6$) and adult juxtamedullary PCT ($n = 6$). The slopes of both curves were 1.67 fluorescent units/pH units.

Acidification rate. After three measurements at 500 and 450 nm, either the lumen or the bath was changed to that used in the experimental period. Transporter activity was assayed by continuously monitoring fluorescence at 500 nm, the pH-sensitive wavelength, as either the luminal or bathing solution was changed. The initial rate of change (dF_{500}/dt) was used to calculate the initial rate of change in cell pH, dpH_i/dt[2]:

$$\frac{dpH_i}{dt} = \frac{dF_{500}/dt}{F_{450} \times S}, \tag{1}$$

where F_{450} is the pH-insensitive wavelength interpolated from readings before and after the fluid exchange and S is the slope of the pH calibration curve relating F_{500}/F_{450} to pH. Once a steady state was reached, three measurements at 500 and 450 nm were made. The solution was then changed to that in the initial period, followed by three additional measurements at 500 and 450 nm.

Buffer capacity. Apparent buffer capacity was measured in a similar fashion to that previously described (18, 27, 31). Tubules were initially perfused and bathed in low-Na UF solution (pH = 7.40). The bathing solution was then rapidly changed to one containing 20 mM NH₃/ NH₄⁺ (low-Na UF solution, +NH₄Cl, pH = 7.40). Because of the large NH₃ permeability (32), the cells immediately alkalinize. This is followed by a slower acidification as the cell defends its pH$_i$. The ratio of the cellular base load to the resulting initial cell pH change is the buffer capacity:

$$\beta = \frac{\Delta[NH_4]_i}{\Delta pH_i}, \tag{2}$$

where ΔpH_i was measured after the addition of NH₃/NH₄⁺. $[NH_4^+]_i$ can be calculated from:

$$[NH_4^+]_i = [NH_4^+]_0 \cdot 10^{pH_0 - pH_i}. \tag{3}$$

This equation uses the assumption that the extracellular and intracellular NH₃ concentrations are equal. The apparent buffer capacity should be measured when all acidification mechanisms are inhibited. For this reason, 2 mM luminal amiloride was added to inhibit the apical Na⁺/H⁺ antiporter and 1 mM 4-acetamido-4′-isothiocyanostilbene-2,2′-disulfonate (SITS) was added to all bathing solutions to inhibit basolateral bicarbonate exit mechanisms (18, 27).

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2. The dpH_i/dt and proton fluxes are reported as absolute values.

Table II. Developmental Changes in Juxtamedullary PCT Size

Age (wk)	1	2	3	4	6	Adult	
Tubules (n)	40	19	26	23	31	47	F test
Outer diameter (μm)	40.0±0.6	35.0±1.0	37.4±0.7	38.2±0.6	47.1±0.5	47.8±0.6	67.4 $P < 0.001$
Inner diameter (μm)	15.3±0.8	12.0±0.6	11.8±0.5	11.8±0.7	14.1±0.6	15.4±0.6	6.4 $P < 0.001$
Tubular volume ($\times 10^{-10}$, liters per millimeter)	10.65±0.33	8.58±0.48	9.96±0.41	10.33±0.29	15.82±0.34	16.07±0.41	67.8 $P < 0.001$

Values are means±SEM.

Proton flux rates. The proton equivalent flux rate (J_H, in picomoles per millimeters per minute) is the change in proton flux resulting from an experimental maneuver. It is calculated using the formula:

$$J_H = \frac{dpH_i}{dt} \cdot \frac{V}{mm} \cdot \beta, \qquad (4)$$

where dpH_i/dt is the initial rate of change in cell pH, V is the tubular volume in liters per millimeter, and β is the buffer capacity. Tubular volume per millimeter was calculated from the measured inner and outer tubular diameter at $\times 400$ magnification using an eyepiece reticle.

Statistics. The data are expressed as a mean±SEM. Analysis of variance and the Student's t test for paired and unpaired data were used to determine statistical significance.

Results

Juxtamedullary PCT morphometrics. The proton flux induced by a change in the external milieu is in part dependent on the tubular volume per millimeter. The measured inner and outer diameter and the calculated tubular volume of 186 tubules are shown in Table II. The tubular volume of juxtamedullary PCT remains fairly constant during the 1st mo of life and then increases to near the adult value by 6 wk of age. These results are in excellent agreement with the tubular volumes calculated from the inner and outer diameters reported by Schwartz and Evan (15).

Buffer capacity. Apparent buffer capacity is also a factor influencing the proton flux induced by a change in the external environment. To examine if buffer capacity changes during the course of development, it was measured in 1-wk-old newborns and in adult rabbits in the presence of CO_2 and bicarbonate. The initial pH before NH_3/NH_4^+ addition was 7.24±0.03 and 7.32±0.03 in newborns and adults, respectively ($P = NS$). The apparent buffer capacity was 64.3±14.0 and 66.4±9.9 mmol/liter·pH unit in newborns ($n = 5$) and adults ($n = 6$), respectively. Thus, buffer capacity was similar in adults and newborns and a constant factor for comparison of proton fluxes in juxtamedullary PCT during development.

Apical membrane sodium-coupled proton secretion. Proximal tubule apical membrane proton secretion is predominantly via the Na^+/H^+ antiporter (16–21). To examine if Na^+/H^+ antiporter activity changes during development, juxtamedullary PCT were perfused with a UF solution without organics (UF s̄ organics) and bathed in a high-Cl-5HCO₃ (pH = 6.8) solution containing 1 mM SITS. SITS was present in the bathing solution to inhibit bicarbonate exit across the basolateral membrane. Previous studies have demonstrated that the basolateral membrane transporters are important determi-

nants of cell pH (20). Furthermore, Na^+/H^+ antiporter activity is pH dependent and its activity is increased at lower pH's (17). This approach has been used by myself and others to examine Na^+/H^+ antiporter activity (18, 20). There was no glucose and amino acids in the perfusate in these experiments since the reabsorption of these solutes is sodium-coupled and electrogenic. Reabsorption of glucose and amino acids will depolarize the basolateral membrane and affect the rate of bicarbonate exit, an electrogenic process (20). In the experimental period, the perfusate was rapidly changed to one without sodium (0Na s̄ organics). The initial rate of change in pH_i was measured followed by measurements of steady-state pH_i. The luminal perfusate was then changed to one that contained sodium and measurements of steady-state pH_i were determined to assess recovery.

The steady-state cell pH's are shown in Table III. There was no developmental change in cell pH under the conditions employed. In each case the cells acidified upon removal of sodium and alkalinized to a value not different from control after sodium was added back to the luminal perfusate. To assess Na^+/H^+ antiporter activity, the initial rate of change in cell pH upon sodium removal was calculated in each tubule. The results are shown in Fig. 1. There was a constant initial rate of change in cell pH for the first 2 wk of life in response to a luminal sodium change. By the 3rd wk the rate almost doubled and was not different from that in adult animals.

Since tubular volume also changes during development, the proton flux rate resulting from luminal sodium removal was calculated for each group.[3] The results are shown in Fig. 2. J_H remained fairly constant at about one-third the adult rate for the first 2 wk of life. J_H doubled between the 2nd and 3rd wk ($P < 0.05$) and reached a rate not different from that in adults by 6 wk. Thus, there is significant maturation of apical sodium-coupled proton secretion in juxtamedullary PCT.

Basolateral bicarbonate exit. Bicarbonate exit across the basolateral membrane of the PCT is predominately via the $Na(HCO_3)_3$ symporter (18, 22–29). Two series of experiments were performed to examine if the rate of bicarbonate exit across the basolateral membrane differed in a 1-wk-old newborn as compared to adults. In the first series of experiments the change in pH_i in response to a change in bath sodium concentration was examined. Previous studies have demonstrated that bicarbonate exits the cell by a sodium-dependent mechanism (18, 22–29). The stoichiometry of the transporter is believed to be $Na(HCO_3)_3$ (28, 29). Thus, lowering bath

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3. Since buffer capacity in 1-wk-old and adult juxtamedullary PCT was identical, this value was used in calculation of J_H in 2–6-wk-old rabbits.

Table III. Effect of Luminal Na⁺ Concentration on Steady-State pH_i

Age (wk)	Luminal perfusate		
	UF s̄ organics	ONa s̄ organics	UF s̄ organics
1 ($n = 7$)	7.33±0.07	7.03±0.06*	7.31±0.06
2 ($n = 8$)	7.24±0.07	6.91±0.06*	7.18±0.07
3 ($n = 11$)	7.33±0.03	6.90±0.05*	7.33±0.03
4 ($n = 10$)	7.23±0.04	6.86±0.06*	7.22±0.04
6 ($n = 9$)	7.31±0.03	6.98±0.05*	7.29±0.03
Adult ($n = 7$)	7.33±0.02	7.04±0.04*	7.32±0.03

* Different from control and recovery at $P < 0.01$.

Figure 2. Proton flux (J_H in pmol/mm · min) in response to luminal sodium removal. ($F = 6.68$; $P < 0.001$).

sodium should also acidify the cell. To examine the relative rates in newborn vs. adult juxtamedullary PCT, tubules were perfused and bathed with 0Cl-UF. The Na(HCO₃)₃ symporter is not dependent on chloride, so for these experiments chloride was removed from the perfusate and bathing solution. During the experimental period the bath sodium was rapidly removed (0Na, 0Cl). Measurements of the initial rate of change in pH_i and steady-state pH_i were made and the bathing solution was changed back to the 0Cl-UF in the control period. In these experiments luminal amiloride was not employed since previous studies have demonstrated that measurements of Na(HCO₃)₃ symporter activity is not significantly affected by apical Na⁺/H⁺ antiporter activity (18). The adult PCT steady-state pH_i's in the control period were significantly lower than in the 1-wk-old newborn PCT (7.51±0.03 vs. 7.61±0.03, $P < 0.05$), but this difference was not observed in the recovery period (7.57±0.04 in adult PCT vs. 7.60±0.03 newborn PCT). The magnitude of the pH change in adult PCT in response to a sodium change was greater in adult PCT than in newborn PCT. Upon sodium removal ΔpH_i averaged 0.52±0.03 and 0.39±0.03 pH units in adults and 1-wk-old newborns, respectively ($P < 0.01$). The initial rate of change in pH_i and proton equivalent flux induced by a bath sodium change are shown in Fig. 3. The proton equivalent flux in newborn juxtamedullary PCT in response to a bath sodium change was ~ 50% of that in adult PCT.

In the second series of experiments examining if the rate of bicarbonate exit in newborns differed from that in adults, tubules were perfused with a UF solution (pH = 7.4) and bathed
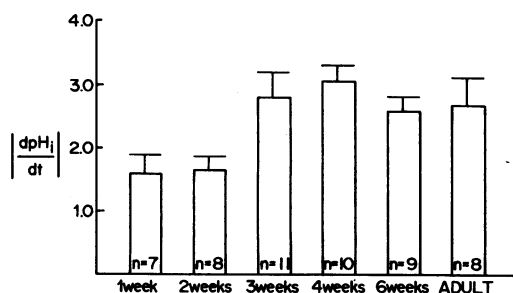
in the same solution in the control period. In the experimental period, the bath bicarbonate concentration was rapidly lowered from 25 to 5 meq/liter (high-Cl-5HCO₃, pH = 6.8) and the rate of change of pH_i was measured. This provided a favorable gradient for bicarbonate to leave the cell resulting in cell acidification. After the pH_i reached a steady state, the pH_i was measured and the bathing solution was rapidly changed back to the UF solution used in the control period. In these studies experiments were also performed in 2-, 3-, 4-, and 6-wk-old animals to examine the rate of maturation of this transporter. The results of the steady-state cell pH's are shown in Table IV. There was no significant maturational difference in steady-state pH_i. In each group the initial rate of change in cell pH upon bicarbonate reduction (25 → 5 meq/liter) was the same as the recovery period (5 → 25 meq/liter). The results were combined and shown in Fig. 4. Surprisingly, there was no significant difference in dpH_i/dt at any age. In Fig. 5 the pro-

Figure 1. Initial rate of change in intracellular pH (dpH_i/dt in pH units per minute) in response to luminal sodium removal. ($F = 3.56$; $P < 0.01$).
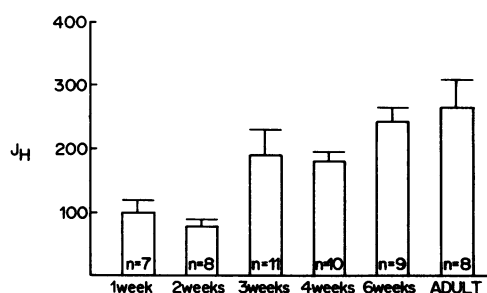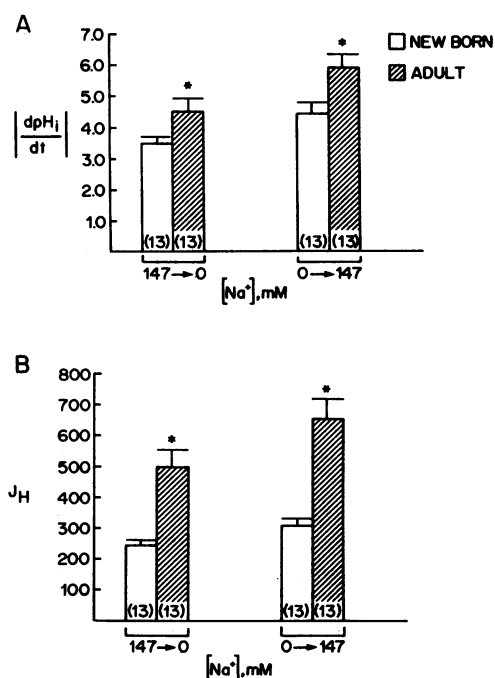

Figure 3. (A) Initial rate of change in cell pH (dpH_i/dt in pH units per minute) in response to a bath sodium change. (*$P < 0.05$). (B) Proton flux (J_H in pmol/mm · min) in response to a bath sodium change. (*$P < 0.0001$).

Table IV. Effect of Bath HCO_3^- Concentration on Steady-State pH

Age (wk)	UF	Bathing solution, high-Cl-5HCO₃⁻	UF
1 ($n = 13$)	7.51±0.03	7.11±0.04*	7.50±0.03
2 ($n = 10$)	7.45±0.02	6.98±0.03*	7.45±0.03
3 ($n = 8$)	7.44±0.03	7.02±0.03*	7.45±0.03
4 ($n = 10$)	7.51±0.02	7.05±0.02*	7.51±0.02
6 ($n = 10$)	7.46±0.02	7.05±0.03*	7.47±0.02
Adult ($n = 12$)	7.49±0.02	7.07±0.02*	7.49±0.02

* Different from control and recovery at $P < 0.01$.

Figure 5. Proton flux (J_H in pmol/mm · min) in response to a bath bicarbonate change. ($F = 7.64$; $P < 0.001$).

ton equivalent flux rates are shown. The newborn rate of proton secretion resulting from a bath bicarbonate change was ~ 60% of that of the adult for the first month of life. J_H reached adult levels by 6 wk of age.

In the above series of experiments, bath sodium removal resulted in cell acidification and readdition resulted in cell alkalinization. These results are consistent with a $Na(HCO_3)_3$ symporter, but could also be explained by a basolateral Na^+/H^+ antiporter. While a basolateral Na^+/H^+ antiporter has not been found in adult PCT (22, 26, 27, 33), the polarization of the Na^+/H^+ antiporter to the apical membrane may be a maturational event. To examine if the changes in cell pH resulting from a bath sodium change was due to the Na^+/H^+ antiporter or $Na(HCO_3)_3$ symporter, 2 mM amiloride, an inhibitor of the Na^+/H^+ antiporter, or 1 mM SITS, an inhibitor of the $Na(HCO_3)_3$ symporter, was added to the bathing solution of the above adult and 1-wk-old newborn PCT and a second measurement of J_H was made. J_H was 326±25.3 pmol/mm · min in newborn juxtamedullary in the absence of bath amiloride and 408.7±60.0 pmol/mm · min in the presence of 2 mM bath amiloride ($n = 5$, NS). J_H in adult PCT was 677.7±67.0 pmol/mm · min in the absence of amiloride and 590.±83.9 pmol/mm · min after addition of bath amiloride ($n = 6$, NS). J_H was 236.2±25.4 pmol/mm · min in newborn juxtamedullary PCT in the absence of bath SITS and 45.1±13.7 pmol/mm · min in the presence of 1 mM bath SITS ($n = 6$, $P < 0.001$). J_H in adult PCT was 436.6±71.2 pmol/mm · min in the absence of bath SITS and 55.9±17.5 pmol/mm · min after the addition of 1 mm bath SITS ($n = 6$, $P < 0.01$). Thus, bath amiloride did not significantly inhibit J_H. However, bath SITS resulted in an 80–90% inhibition in J_H in newborn and adult juxtamedullary PCT. These data are consistent with an asymmetrical distribution of the Na^+/H^+ antiporter in newborn juxtamedullary PCT.
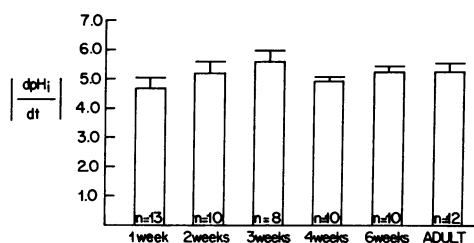

Figure 4. Initial rate of change in cell (dpH$_i$/dt, in pH units per minute) in response to a bath bicarbonate change ($F = 0.98$; NS).
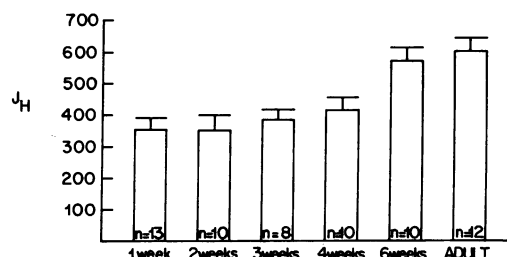
Previous developmental studies have provided evidence that maturation of the apical membrane transporters preceded those on the basolateral membrane (34). The present data are consistent with a relatively mature rate of basolateral bicarbonate exit in comparison to apical Na^+/H^+ antiporter activity. The mechanisms involved in vectorial bicarbonate transport may also be involved in defense of cell pH. To examine if the basolateral membrane was predominantly involved in defense of cell pH$_i$, 1-wk-old newborn juxtamedullary PCT tubules were perfused and bathed with a UF solution. During the experimental period the luminal perfusate was changed to a high-Cl-5HCO₃ (pH = 6.8) and steady-state cell pH was measured. During the recovery period, the perfusate was changed to the UF solution. As is demonstrated in Fig. 6, there was no significant change in cell pH ($n = 5$). Cell pH was 7.51±0.06 in the control period, 7.48±0.06 upon luminal acidification and 7.49±0.05 in the recovery period. However, when 1 mM SITS was added to the bathing solution to inhibit the basolateral bicarbonate transport mechanisms, the same luminal change in cell pH produced a large change in cell pH. Cell pH was 7.35±0.05 in the control period and decreased to 7.19±0.07 upon luminal acidification ($P < 0.01$). Upon increasing the bicarbonate in the luminal perfusate to 25 meq/liter, pH$_i$ increased to 7.36±0.05 ($P < 0.05$).[4] These data are consistent with the basolateral membrane transporters' being an important determinant of cell pH.

Discussion

Epifluorescent measurement of intracellular pH was used to examine transporter activity in newborn and adult juxtamedullary PCT. Apical sodium-coupled proton secretion is approximately one-third the adult value during the first 2 wk of life. The activity doubled by the 3 wk and reached adult values by 6 wk of age. Basolateral $Na(HCO_3)_3$ symporter activity in 1-wk-old animals is one-half to two-thirds that measured in adult animals and remains stable for the 1st mo of life. $Na(HCO_3)_3$ activity reaches adult levels at 6 wk of age. Basolateral $Na(HCO_3)_3$ activity is not only involved in net acidification, but is a major determinant of intracellular pH in newborn juxtamedullary PCT.

The present study measured the initial rate of change in pH$_i$ to assay transporter activity in juxtamedullary PCT. This assay has been used in vivo and in vitro to examine transporter

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4. Bath SITS inhibits $Na(HCO_3)_3$ activity in the rabbit and rat PCT (22, 27). SITS results in the expected cell alkalinization in the rat (20, 22). However, bath SITS does not cause cell alkalinization in the rabbit PCT (27). The reason for this is unclear at present.
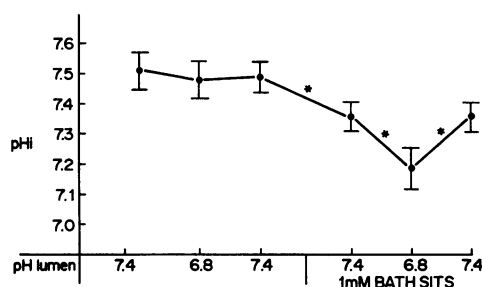
Figure 6. Effect of a luminal pH change on pH_i in the absence and presence of 1 mM bath SITS. (*$P < 0.05$).

activity in the PCT (18, 35, 36). While tubular buffer capacity and tubular volume have not been a factor in previous studies, they are potential variables in examining transporter activity during development (18, 35, 36). If the proximal tubule cellular composition was to change dramatically, buffer capacity could be a factor in comparative measurements of proton flux. Buffer capacity was not different in juxtamedullary proximal convoluted tubules in the 1st wk of life and in adult juxtamedullary PCT. However, tubular volume was a significant factor in these comparisons. Despite the rapid growth of renal size and proximal tubular length during the first month, juxtamedullary PCT volume per millimeter does not change (15). However, tubular volume increases by 50% to adult values between 4 and 6 wk of age. Tubular volume was calculated from the measured inner and outer PCT diameter. This method assumes that measurements from the tip of the brush border to the basolateral membrane are entirely intracellular and that relative inaccuracies in this measurement are constant during proximal tubule development. This method does not explicitly take changes in surface area to tubular volume into account. Changes in transporter activity during development may be due to a constant density of transporters during maturation with membrane amplification or to an increase in transporter density. Evan et al. (4) have previously demonstrated that there are maturational increases in proximal tubule apical and basolateral membrane surface area during development. The current results are expressed to take only estimated maturational changes in cell volume into account. This allows direct comparison of transporter activity, measured as proton flux in picomoles per millimeter per minute, to previously determined flux data in this segment expressed in similar units (15).

Newborn infants have a lower renal threshold for bicarbonate than adults (37). Since 80% of bicarbonate is reabsorbed by the proximal tubule, it is likely that the proximal tubule is a factor to explain the lower bicarbonate threshold in newborns. There are several potential reasons for this altered threshold, including tubular immaturity and developmental changes in peritubular physical forces and in the hormonal environment which could affect bicarbonate transport. Previous studies by Schwartz and Evan (15) have clearly demonstrated there is maturation of the juxtamedullary PCT acidification during development. The low rates of bicarbonate transport in newborn juxtamedullary PCT could be due to several factors. In the proximal tubule apical proton secretion is predominantly via the Na^+/H^+ antiporter and bicarbonate exit is via the $Na(HCO_3)_3$ symporter. Decreased activity of either of these transporters, or of other acidification mecha-

nisms such as the proton pump, could explain the lower rates of transport in newborn proximal tubules. The driving force for the Na^+/H^+ antiporter is a low intracellular sodium generated by the Na^+-K^+ ATPase. Thus, a limitation of Na^+-K^+ ATPase activity or of ATP production could be factors that are responsible for the low rate of bicarbonate transport. Furthermore, each of these factors could be at mature levels but net transport could be low if the bicarbonate permeability were high enough to result in diffusion of transported bicarbonate back into the tubular lumen.

Only one of the above factors has been examined in juxtamedullary PCT. The basolateral membrane surface area is low in newborns, but rises to adult levels by 4 wk (4). Interestingly, however, Na^+-K^+ ATPase maturation lags behind the maturation in bicarbonate transport in juxtamedullary PCT. In the 1st wk of life, Na^+-K^+ ATPase activity is one-third that measured in adults (34). The level increases to 60% of the adult value at 6 wk, a time by which bicarbonate transport was comparable to that measured in adults. Only by 7 wk did Na^+-K^+ ATPase activity reach adult levels (34).

The delayed maturation of the Na^+-K^+ ATPase activity could be due to its induction by increases in cellular sodium concentration resulting from more rapid maturation of transporters on the apical membrane (34). This induction of Na^+-K^+ ATPase by increases in apical membrane transport has been described in other epithelia (38). The present study suggests that this may be the case in the proximal tubule. Apical sodium-coupled proton secretion was at about one-third the adult level for the first 2 wk. By 3 wk the level had doubled. This is at a time preceding a significant increase in net acidification suggesting that other factors may limit transport. The cause for the induction of antiporter activity is unknown. As speculation, it may be related to an increase in GFR in juxtamedullary nephrons. In adult rats hyperfiltration leads to an increase in Na^+/H^+ antiporter activity (36, 39). Both uninephrectomy and high-protein diets result in an increase in GFR and brush border membrane vesicle Na^+/H^+ antiporter activity (39). Whereas an increase in GFR results in an adaptive increase in antiporter activity in adult animals, it may be an inductive factor in developing nephrons.

In PCT from adult animals the Na^+/H^+ antiporter is present on the apical, but not the basolateral membrane (22, 26, 27, 33). This polarity of transporter distribution increases the efficiency of net acidification. It is possible that this polarization is a maturational event. To examine this issue, sodium was removed from the basolateral membrane in the presence and absence of 2 mM amiloride. There was no inhibition of transport by amiloride. These data are not consistent with a basolateral Na^+/H^+ antiporter and suggest that at the time of birth the Na^+/H^+ antiporter is restricted to the apical membrane.

To assess basolateral $Na(HCO_3)_3$ symporter activity I employed both changes in the bath bicarbonate concentration and sodium concentration. Surprisingly, the rate of change in cell pH in response to a change in bicarbonate was the same in newborns and adult juxtamedullary PCT. However, there was a small but statistically significant difference in the rate of change in cell pH_i in response to changes in bath sodium concentration. The $Na(HCO_3)_3$ symporter activity assessed by a decrease in bath sodium was inhibited by SITS in both newborns and adults. Determination of proton flux rates, which also take buffer capacity and tubular volume into account,

demonstrated that $Na(HCO_3)_3$ symporter activity was significantly lower in newborns as assessed by changes in bath bicarbonate and sodium concentration.

In examining data in this nephron segment several striking features are apparent. The rate of both fluid reabsorption and bicarbonate transport in 1-wk-old newborns is one-third that measured in adults (15). In addition, the Na^+-K^+ ATPase activity in newborn juxtamedullary proximal convoluted tubules is also one-third that in the adult (34). This study demonstrates that Na^+/H^+ antiporter activity is also one-third that of the adult animal. Interestingly, however, the basolateral membrane $Na(HCO_3)_3$ symporter activity in newborns was 50–60% of the value measured in adult juxtamedullary PCT. The maturation of $Na(HCO_3)_3$ symporter activity was examined by measuring the proton flux in response to a bath bicarbonate change. The rate was constant for the 1st mo of life. There was a significant increase to adult levels by 6 wk of age. Thus, whereas initiation of maturation of Na^+/H^+ antiporter activity occurred between 2 and 3 wk of age, an increase in $Na(HCO_3)_3$ symporter was not measured until several weeks later. It is unclear at present what induces the maturation of this transporter. It is unlikely to be due to intracellular pH changes for this variable remained stable during development.

Acidification mechanisms may not only be operative in net acidification. Cells must be able to defend changes in intracellular pH. In adult rat superficial PCT the basolateral ·membrane is a more important determinant of cell pH than transporters on the apical membrane (20). To examine this in newborn juxtamedullary proximal convoluted tubules, the effect of changes in luminal pH on cell pH was examined. There was no significant effect on cell pH despite a 0.6 pH unit change in luminal pH. In the presence of basolateral SITS to inhibit the $Na(HCO_3)_3$ symporter, there was a 0.17 pH unit change in cell pH. Thus, in the absence of SITS changes in cell pH were protected by basolateral membrane transporters. This basic and important function may be a factor in the relative maturity of acidification mechanisms on the basolateral membrane.

Acknowledgments

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