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Neonatal rabbit proximal tubule basolateral membrane Na^+/H^+ antiporter and Cl^-/base exchange

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Abstract

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The present in vitro microperfusion study examined the maturation of Na^+/H^+ antiporter and Cl^-/base exchanger on the basolateral membrane of rabbit superficial proximal straight tubules (PST). Intracellular pH (pH_i) was measured with the pH-sensitive fluorescent dye 2 , 7 -bis(2-carboxyethyl)-5(6)-carboxyfluorescein in neonatal and adult superficial PST. Na^+/H^+ antiporter activity was examined after basolateral Na^+ addition in tubules initially perfused and bathed without Na^+. Neonatal Na^+/H^+ antiporter activity was ~40% that of adult segment (9.7 ± 1.5 vs. 23.7 ± 3.2 pmol·mm^{-1}·min^{-1}; $P < 0.001$). The effect of bath Cl^- removal on pH_i was used to assess the rates of basolateral Cl^-/base exchange. In both neonatal and adult PST, the Cl^-/base exchange activity was significantly higher in the presence of 25 mM HCO_3^- than in the absence of HCO_3^- and was inhibited by cyanide and acetazolamide, consistent with Cl^-/HCO_3^- exchange. The proton flux rates in the presence of bicarbonate in neonatal and adult tubules were 14.1 ± 3.6 and 19.5 ± 3.5 pmol·mm^{-1}min^{-1}, respectively (P = NS), consistent with a mature rate of Cl^-/HCO_3^- exchanger activity in neonatal tubules. Basolateral Cl^-/base exchange activity in the absence of CO_2 and HCO_3^-, with luminal and bath cyanide and acetazolamide, was greater in adult than in neonatal PST and inhibited by bath DIDS consistent with a maturational increase in Cl^-/OH^- exchange. We have previously shown that the rates of the apical membrane Na^+/H^+ antiporter and Cl^-/base exchanger were approximately fivefold lower in neonatal compared with adult rabbit superficial PST. These data demonstrate that neonatal PST basolateral membrane Na^+/H^+ antiporter and Cl^-/base exchanger activities are relatively more mature than the Na^+/H^+ antiporter and Cl^-/base exchangers on the apical membrane.

Cl^-/HCO_3^- exchanger; Cl^-/OH^- exchanger; Na^+-bicarbonate cotransporter; renal development; intracellular pH; microperfusion

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INTRACELLULAR pH (pH_i) plays an important role in many biological activities (32). Various cellular processes affected by pH_i include transepithelial solute transport, enzyme function, and cell proliferation (3, 15, 20). Steady-state pH_i of epithelial cells is determined by the balance between the rates of intracellular acid loading and acid extrusion. Intracellular acid loading occurs by passive movement of protons into the cell, cellular metabolism, and fluxes of acids and bases (32). There are a number of transport mechanisms involved in the regulation of epithelial cell pH_i (3, 10, 21, 28). The Na^+/H^+ antiporter and Cl^-/HCO_3^- exchangers are nearly ubiquitous among mammalian cells and play an important role in pH_i regulation in a number of cells (1, 23, 25, 26, 30, 33, 38). Basolateral Na^+/H^+ antiporter and Cl^-/HCO_3^- exchange activity in the kidney has been demonstrated in the proximal tubule, thick ascending limb of Henle, medullary collecting duct, and glomerular mesangial cells (11–13, 16, 18, 19, 25, 27, 31, 35, 40). Na^+/H^+ and Cl^-/base exchange are on the basolateral membrane of developing nephrons (4). Parallel Na^+/H^+ and Cl^-/HCO_3^- exchange activity on the basolateral membrane play a role in cell volume regulation by mediating cellular NaCl uptake and thereby preventing cell shrinkage when exposed to a hypertonic extracellular milieu (9, 36, 37).

We have recently demonstrated that the neonatal rabbit superficial proximal straight tubule (PST) has a lower rate of active and passive NaCl transport than the adult segment. The rates of apical Na^+/H^+ antiporter and Cl^-/base exchanger, which mediate net NaCl transport across the apical membrane, were approximately fivefold lower in the neonatal segment compared with the adult segment (34). In the present in vitro microperfusion study, we examined the rates of basolateral membrane Na^+/H^+ antiporter, Cl^-/base exchange, and $Na^+ - HCO_3^-$ cotransporter activity in neonatal and adult superficial PST. We find that there is a maturational increase in basolateral membrane Na^+/H^+ antiporter and $Na^- - HCO_3^-$ cotransporter activity but that basolateral Cl^-/base exchange in the presence of 25 mM HCO_3^- is the same in adult and neonatal PST.

METHODS

Isolated segments of adult and neonatal (14–21 days of age) rabbit superficial PST (S_2 segments) were perfused as previously described (5, 14, 34). Briefly, tubules were dissected in Hank's balanced salt solution containing (in mM) 137 NaCl, 5 KCl, 0.8 $MgSO_4$, 0.33 Na_2HPO_4, 0.44 KH_2PO_4, 1 $MgCl_2$, 10 tris(hydroxymethyl)aminomethane hydrochloride, 0.25 $CaCl_2$, 2 glutamine, and 2 lactate at 4°C. Tubules were transferred to a 0.2-ml chamber, in which the bathing solution was preheated to 38°C. The tubules were perfused with concentric glass pipettes.

The solutions used in these experiments are shown in Table 1. The fluorescent dye 2 ,7 -bis(2-carboxyethyl)-5(6)-carboxyfluorescein (BCECF) was used to measure pH_i as described previously (2, 5, 27, 34). We measured pH_i with a Nikon inverted epifluorescent microscope attached to a PTI Ratiomaster at a rate of 30 measurements/s. A variable diaphragm was placed over the area to be measured. To calculate pH from the ratio of fluorescence (F_{500}/F_{450}), a nigericin calibration curve was performed as previously

described (2, 5). There was no difference in the calibration curves of adult and neonatal PST.

Tubules were incubated with the initial luminal and bathing solutions for ≥10 min after loading with 5×10^{-6} M acetoxymethyl BCECF and had a constant pH_i for several minutes before the measurement of the transporter activity. The bathing fluid was changed at a rate of ≥5 ml/min. We measured dpH_i/dt from the slope of the change in pH_i immediately after a bathing fluid change. Steady-state pH_i values were present within 90 s after a bathing fluid exchange but were followed for several minutes to ensure a steady-state pH_i was achieved.

Apparent buffer capacity was measured as previously described with $NH_3 - NH_4^+$ (5, 26, 32, 34). Solutions (*B* and *D*) used in the experiments for measurement of apparent buffer capacity did not contain Na^+ or Cl^- to inhibit all acidification mechanisms caused by Na^+- and Cl^--dependent transporters. In the absence of HCO_3^-, buffer capacity was 28.1 ± 5.0 mM/pH in neonatal PST and 43.0 ± 6.6 mM/pH in adult PST (n = 6 for both groups, P = NS). Buffer capacity in the presence of HCO_3^- was estimated as the sum of the above buffer capacity and the HCO_3^- buffer capacity. The latter was calculated as $2.3 \cdot \left[HCO_{3\,\mathrm{i}}^-\right]$ (27, 32), where $\left[HCO_{3\,\mathrm{i}}^-\right]$ is the intracellular bicarbonate concentration. The buffer capacities in the presence of HCO_3^- were 80.4 ± 5.8 and 94.4 ± 4.6 mM/pH_i in neonatal and adult PST, respectively (P = NS).

Tubular volume was calculated from the measured inner and outer tubular diameters at ×400 magnification with an eyepiece reticle. The tubular volumes of neonatal and adult PST were $5.3 \pm 0.2 \times 10^{-10}$ and $10.2 \pm 0.4 \times 10^{-10}$ l/mm, respectively ($P < 0.001$).

Proton flux rates[1] (J_H, in pmol·mm^{-1}·min^{-1}) resulting from a bathing fluid change were calculated with the following formula

$$J_{\mathrm{H}} = \frac{dpH_{\mathrm{i}}}{\mathrm{d}t} \cdot \frac{\mathrm{V}}{mm} \cdot \beta$$

where dpH_i/dt is the rate of initial change in pH_i after a bathing fluid change, V is the tubular volume in liters, and β is the buffer capacity.

Data are expressed as means ± SE. ANOVA and the Student's *t*-test for paired and unpaired data were used to determine statistical significance.

RESULTS

We first examined the rate of basolateral Na^+/H^+ antiporter activity in neonatal and adult proximal straight tubules (PST). We measured the net J_H in response to addition of 140 mM Na^+ to the bathing fluid (*solution C*) in tubules initially perfused and bathed without Na^+ (*solution B*). These experiments were performed in absence of Cl^- to prevent the Cl^-/base

[1]All proton fluxes are presented as absolute values and expressed as J_H in pmol·mm^{-1}·min^{-1}.

exchanger from attenuating pH_i changes during the bathing fluid change. The steady-state pH_i for neonatal and adult tubules are shown in Table 2. The neonatal PST pH_i was somewhat lower than that of the adult segment ($0.10 > P > 0.05$). Despite the lower pH_i, J_H in the neonatal PST was ~40% that of the adult segment as shown in Fig. 1 ($P < 0.001$).

We next examined Cl^-/base exchange in neonatal and adult tubules in the presence of 25 mM HCO_3^- (*solutions E* and *F*). The initial pH_i were comparable in neonatal and adult segments (Tables 3 and 4). As shown in Fig. 2, there was no significant difference between the J_H of neonatal (14.1 ± 3.6 pmol · mm^{-1} · min^{-1}) and adult PST (19.5 ± 3.5 pmol · mm^{-1}·min^{-1}). As shown in Fig. 2, in both neonatal and adult tubules, 0.1 mM bath DIDS inhibited Cl^-/base exchange ($P < 0.05$).

In the next series of experiments, we examined the rate of basolateral Cl^-/base exchange in neonatal and adult PST in absence of exogenous HCO_3^-. Tubules were initially perfused and bathed in a HEPES-buffered Cl^--containing solution without Na^+ (*solution A*). In the experimental period, Cl^- was removed from the bathing fluid (*solution B*) and J_H was measured. Tables 3 and 4 show the pH_i values in neonatal and adult tubules. There was no significant difference in the initial pH_i of neonatal and adult tubules. As shown in Fig. 3, J_H was significantly lower in neonatal compared with adult PST ($P < 0.05$). However, J_H was significantly higher in both neonatal and adult tubules in presence of HCO_3^- compared with that in HEPES-buffered solutions ($P < 0.05$), consistent with a Cl^-/HCO_3^- exchanger on the basolateral membrane of both neonatal and adult superficial PST.

We next examined the activity of Cl^-/base exchange in the presence of 2 mM cyanide and 0.1 mM acetazolamide to assess the relative contribution of endogenous CO_2 and HCO_3^- in mediating the Cl^-/base exchange. Tubules were initially perfused and bathed in HEPES-buffered high-Cl^- solution (*solution A*) with 2 mM cyanide and 0.1 mM acetazolamide, and during the experimental period bath Cl^- was removed. As shown in Tables 3 and 4, the initial pH_i were comparable between the neonatal and adult groups (P = NS). As shown in Fig. 3, J_H was about 70% lower in neonatal ($P < 0.001$) and 55% lower in adult tubules ($P = 0.01$) compared with that in absence of cyanide and acetazolamide, suggesting that endogenous CO_2 and HCO_3^- were contributing significantly to the Cl^-/base exchange on the basolateral membrane of both neonatal and adult PST.

The residual Cl^-/base exchange with 2 mM cyanide and 0.1 mM acetazolamide could be a result of continued endogenous CO_2 production or of a Cl^-/OH^- exchanger. We next examined the effect of 5 mM cyanide and 0.1 mM acetazolamide on the Cl^-/base exchange in neonatal and adult PST. The J_H in neonatal and adult proximal straight tubules were 1.2 ± 0.1 and 5.4 ± 0.1 pmol·mm^{-1}·min^{-1}, respectively. These rates were not different from the J_H in the presence of 2 mM cyanide and 0.1 mM acetazolamide.

We next examined if the residual Cl^-/base exchange in the presence of 2 mM cyanide and acetazolamide was inhibited by 0.1 mM DIDS. As seen in Tables 3 and 4, bath DIDS almost totally abolished the pH_i change with removal and addition of bath Cl^-. As shown in Fig. 3, bath DIDS resulted in a significant decrease in Cl^-/base exchange. These data suggest that there is a $CO_2 - HCO_3^-$-independent, DIDS-inhibitable anion exchanger on the basolateral

membrane consistent with a Cl^-/OH^- exchanger. The rate of Cl^-/base exchange on the basolateral membrane in the presence of cyanide and acetazolamide was greater in adults than in neonates, consistent with a maturational increase in Cl^-/OH^- exchange.

In the final series of experiments, we examined the activity of the $Na^+ - HCO_3^-$ cotransporter. PST were initially perfused and bathed in bicarbonate-containing solutions in the presence of 50 mM ethylisopropylamiloride (EIPA) without Na^+ (*solution F*) as previously described (22). EIPA was added to inhibit the basolateral Na^+/H^+ exchanger. We then added 140 mM Na^+ in the presence of EIPA, and the effect on pH_i was examined. As shown in Table 5, the initial pH_i was comparable in adult and neonatal tubules. Figure 4 shows that $Na^+ - HCO_3^-$ cotransporter activity was significantly less in neonatal PST than in adult tubules. In both groups the effect of Na^+ addition was inhibited by 0.1 mM bath DIDS ($P < 0.05$).

DISCUSSION

In this study we examined the rates of basolateral Na^+/H^+ antiporter, Na^+/H^+ cotransporter, and Cl^-/base exchange in neonatal PST and compared these to the adult segment. There was a twofold maturational increase in basolateral membrane Na^+/H^+ antiporter activity and a similar maturational increase in $Na^+ - HCO_3^-$ cotransporter activity. In the presence of bicarbonate, the rates of basolateral Cl^-/base exchange were comparable in the neonatal and adult segments.

Two isoforms of the Na^+/H^+ antiporter have been localized to the proximal tubule. NHE3 is the apical Na^+/H^+ antiporter and is responsible for most of the luminal proton secretion in this segment (7, 39). NHE1 is localized to the basolateral membrane of the proximal tubule and has a wide distribution in mammalian tissue (8, 39). We have previously examined the maturation of apical membrane Na^+/H^+ antiporter activity in both proximal convoluted tubules and PST (5, 34). In both segments, there is a maturational increase in antiporter activity. In the PST, there is a fivefold increase in Na^+/H^+ antiporter activity during postnatal maturation (34). Consistent with these findings is the fourfold increase in renal cortical NHE3 mRNA and protein abundance during postnatal maturation (6).

The maturation of proximal tubule basolateral Na^+/H^+ antiporter activity has not previously been examined. However, we have previously demonstrated that rabbit renal cortical NHE1 mRNA and protein abundance, the proximal tubule basolateral Na^+/H^+ exchanger (8), does not change significantly during postnatal maturation (6). Thus there is a clear discordant maturational pattern between NHE1 and NHE3. In the PST we find that there is a 2.4-fold increase in Na^+/H^+ antiporter activity on the basolateral membrane compared with the fivefold increase on the apical membrane. A previous study in rabbit myocardial cells demonstrated comparable Na^+/H^+ antiporter activity in newborns and adults (29).

The rate of $Na^+ - HCO_3^-$ cotransporter was assessed by measuring the effect of bath Na^+ addition in presence of bicarbonate (22). Under these conditions the Na^+/H^+ antiporter plays a minor role on J_H in comparison to the $Na^+ - HCO_3^-$ cotransporter (22). We added EIPA

to the solutions to inhibit any small contribution of the Na^+/H^+ exchanger to the J_H as previously described (22). The rate of $Na^+ - HCO_3^-$cotransporter activity was approximately two- to threefold greater in adults than in neonates. In both segments the $Na^+ - HCO_3^-$ cotransporter was inhibited by DIDS. These results agree well with the maturational changes in $Na^+ - HCO_3^-$ cotransporter activity we have previously found in the rabbit proximal convoluted tubule (5). The rate of $Na^+ - HCO_3^-$ cotransporter activity was comparatively greater than the other basolateral membrane transporters studied here because this transporter plays a major role facilitating basolateral membrane bicarbonate exit.

There is a profound difference in the maturational pattern of the apical and basolateral Cl^-/base exchangers in this nephron segment. We have previously shown that the apical membrane Cl^-/base exchange activity in neonatal rabbit superficial PST was about sixfold lower than in the adult segment. Apical membrane Cl^-/base exchange activity was not augmented by 25 mM HCO_3^- or 0.5 mM formate, consistent with a Cl^-/OH^- exchanger (34). In the present study, we found that the Cl^-/base activity on the basolateral membrane of both neonatal and adult superficial PST was significantly higher in the presence of 25 mM HCO_3^- and was inhibited by cyanide and acetazolamide. These data are consistent with Cl^-/HCO_3^- exchange mediating a significant portion of basolateral Cl^-/base exchange. Kurtz et al. (27) had previously demonstrated that in PST from adult rabbit, apical Cl^-/base exchange was via a Cl^-/OH^- exchange, whereas basolateral exchange was mediated predominantly by a Cl^-/HCO_3^- exchanger.

In the present study, the neonatal Cl^-/HCO_3^- exchange activity was comparable to the adult segment, suggesting a relatively mature Cl^-/HCO_3^- exchange activity on the basolateral membrane of the neonatal superficial PST. In similar experiments, others have shown that the neonatal mammalian myocardium had a fully functional Cl^-/HCO_3^- exchange activity, which played an important role in pH_i regulation (24, 29).

Our results show that there is a significant difference in the Cl^-/base exchange activity between neonatal and adult PST in absence of HCO_3^-. This may, in part, be a result of the higher rate of aerobic metabolism in the adult compared with the neonatal segments (17). To determine the rates of Cl^-/base exchange in the absence of endogenously produced CO_2 and bicarbonate, we added 2 and 5 mM cyanide and acetazolamide to the luminal and bathing solutions. This produced a significant reduction in both neonatal and adult PST Cl^-/base exchange activity consistent with Cl^-/HCO_3^- exchange fueled by metabolically derived CO_2. The residual Cl^-/base exchange activity in the presence of 2 mM cyanide and 0.1 mM acetazolamide was not likely caused by continued CO_2 generation, inasmuch as 5 mM cyanide did not produce a greater inhibition in Cl^-/base exchange activity. Cl^-/base exchange activity in the presence of cyanide and acetazolamide was almost entirely inhibited with DIDS, consistent with a basolateral membrane Cl^-/OH^- exchanger. The residual Cl^-/base exchange activity in the presence of cyanide and acetazolamide was significantly greater in adult than in neonatal PST, consistent with a maturational increase in

a basolateral Cl^-/OH^- exchanger. However, the rate of basolateral membrane Cl^-/OH^- exchange in the presence of HCO_3^- was comparable in neonatal and adult PST.

Perspectives

Basolateral membrane Na^+/H^+ and Cl^-/HCO_3^- exchangers have been shown to play an important role in cell volume and pH regulation. These functions are necessary for cell homeostasis in both neonatal and adult proximal tubule. These studies are consistent with basolateral Na^+/H^+ and Cl^-/base exchangers being relatively more mature than those present on the apical membrane in the PST. The applicability of these observations to other nephron segments and other species will have to be investigated. In addition, the effect of neonatal and adult transporters on the apical and basolateral membrane to defend against changes in pH_i and intracellular volume will need to be investigated in future studies.

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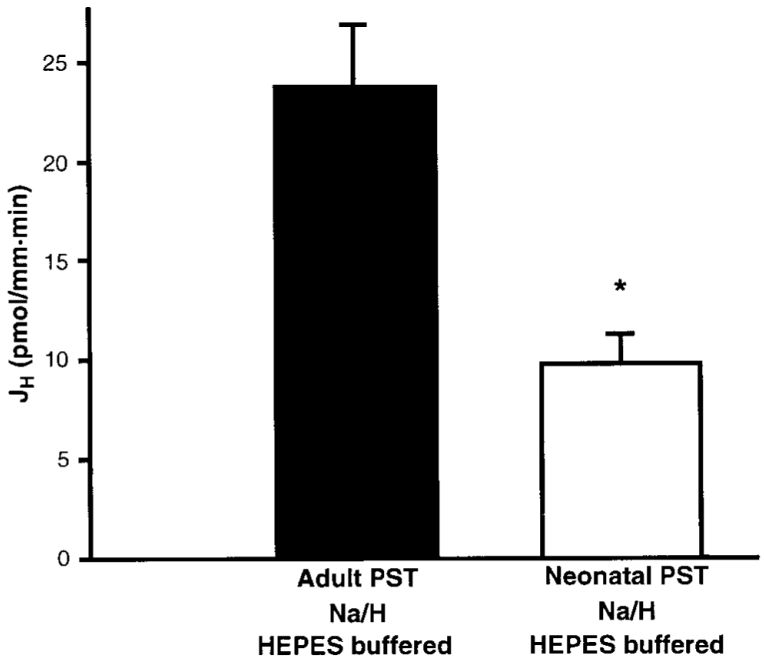

Fig. 1.

Proton flux rates (J_H) in response to addition of bath Na^+ in adult and neonatal proximal straight tubules (PST) initially perfused and bathed in absence of Na^+. *$P < 0.001$ vs. adult PST.

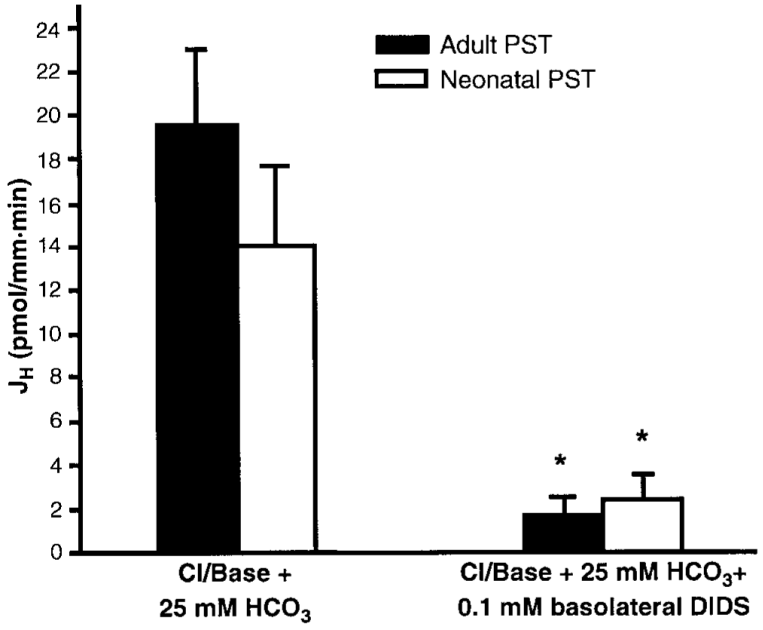

Fig. 2.

J_H in adult and neonatal PST in response to bath Cl^- removal in bicarbonate-containing solutions and in presence of 0.1 mM basolateral DIDS. *$P < 0.05$ vs. corresponding value of Cl^- base + 25 mM HCO_3^-.

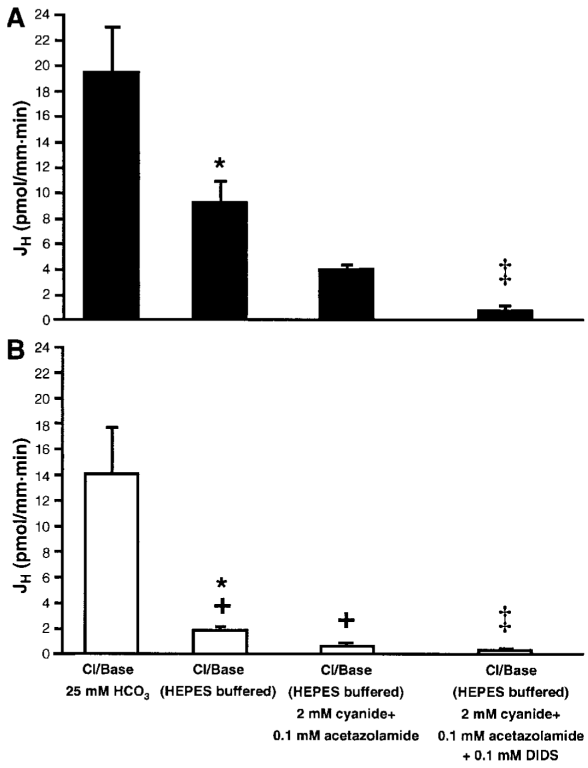

Fig. 3.

J_H in adult (*A*) and neonatal (*B*) PST in response to bath Cl^- removal in presence of 25 mM HCO_3^-, HEPES-buffered solutions (no HCO_3^-, and in presence of luminal and bath cyanide and acetazolamide (± 0.1 mM bath DIDS). **P* < 0.05 vs. Cl^- base + 25 mM HCO_3^- and Cl^-/base + 2 mM cyanide + 0.1 mM acetazolamide; ^+P < 0.05 vs. corresponding adult value; ‡*P* < 0.05 vs. Cl/base + 2 mM cyandide + 0.1 mM acetazolamide.

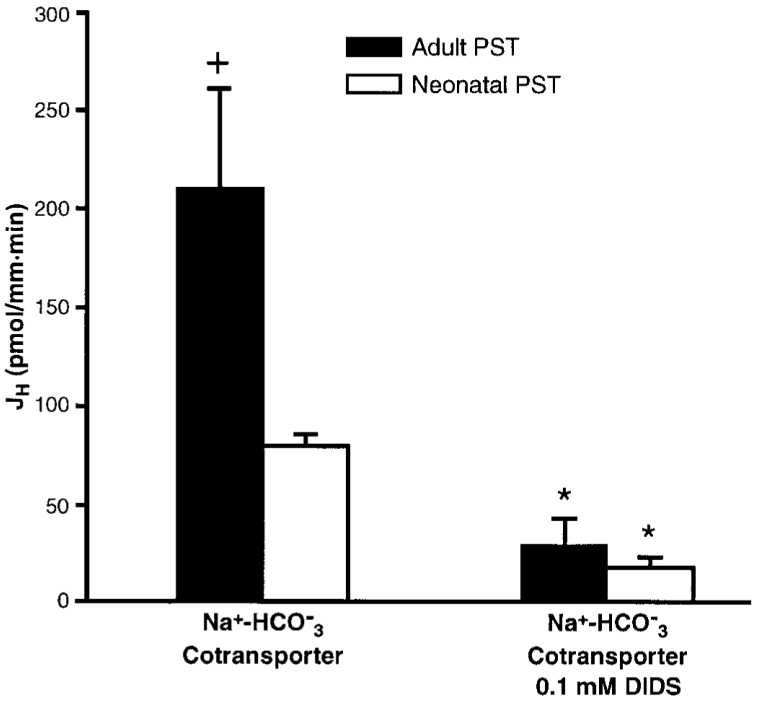

Fig. 4.

J_H in adult and neonatal PST in bicarbonate-buffered solutions in response to bath Na^+ addition in the presence of 50 mM ethylisopropylamiloride ± 0.1 mM DIDS. *P < 0.01 vs. corresponding value of $Na^+ - HCO_3^-$ cotransport activity without DIDS; ^+P < 0.05 vs. corresponding neonatal value.

Table 1

Solutions used in pH_i studies

	Solutions						
	A **(high Cl^-, 0 Na^+)**	***B*** **(0 Cl^-, 0 Na^+)**	***C*** **(0 Cl^-, high Na^+)**	***D*** **(0 Cl^-, 0 Na^+, 20 mM NH_4^+)**	***E*** **(high Cl^-, 0 Na^+, 25 mM HCO_3^-)**	***F*** **(0 Cl^-, 0 Na^+, 25 mM HCO_3^-)**	***G*** **(0 Cl^-, high Na^+, 25 mM HCO_3^-)**
TMA-Cl^-	140				115		
TMA-OH		140		120		115	
$TMA-HCO_3^-$					25	25	
NH_4OH				20			
Na^+ Gluconate			140				115
$NaHCO_3$							25
Gluconic acid lactone		140		140		115	
K_2HPO_4	2.5	2.5	2.5	2.5	2.5	2.5	2.5
$MgCl_2$	1				1		
Mg Gluconate		1	1	1		1	1
$CaCl_2$	1				1		
Ca Gluconate		12.5	12.5	12.5		10	10
Glucose	5	5	5	5	5	5	5
L-Alanine	5	5	5	5	5	5	5
HEPES	5	5	5	5			

All values are expressed in mM. All solutions were adjusted to an osmolality of 295 mosmol/kgH_2O. HCO_3^--containing solutions were bubbled with 95% O_2–5% CO_2 and had pH of 7.4. Non-HCO_3^--containing solutions were bubbled with 100% O_2 and had pH of 7.4. pH_i, Intracellular pH; TMA, tetramethylammonium.

Table 2

Effect of basolateral Na^+ addition on pH_i in adult and neonatal PST

	n	Control 0 Na^+	High Na^+	Recovery 0 Na^+
Neonatal PST	11	7.42±0.05	7.68±0.06*	7.22±0.06
Adult PST	6	7.54±0.03	7.83±0.07*	7.36±0.10

Values are means ± SE; *n* = no. of experiments.

* $P < 0.001$ vs. 0 Na^+-containing control and recovery.

Table 3

Effect of basolateral Cl^- removal on pH_i in adult PST

	n	Control Cl^-	0 Cl^-	Recovery Cl^-
No HCO_3^-	9	7.35±0.05	7.64±0.09*	7.43±0.07
25 mM HCO_3^-	9	7.36±0.04	7.64±0.08*	7.40±0.05
25 mM HCO_3^- + 0.1 mM DIDS (bath)	5	7.21±0.05	7.21±0.06	7.15±0.04†
2 mM Cyanide+0.1 mM acetazolamide	15	7.21±0.07	7.36±0.06*	7.27±0.05
2 mM Cyanide+0.1 mM acetazol-amide+0.1 mM DIDS	7	7.34±0.05	7.37±0.05	7.35±0.06

Values are means ± SE; *n* = no. of experiments.

*$P < 0.001$ vs. Cl^--containing control and recovery;

†$P < 0.05$ different from control and Cl^--containing experimental period.

Table 4

Effect of basolateral Cl^- removal on pH_i in neonatal PST

	n	Control Cl^-	0 Cl^-	Recovery Cl^-
No HCO_3^-	8	7.29±0.05	7.61±0.11*	7.36±0.04
25 mM HCO_3^-	8	7.36±0.05	7.71±0.12*	7.37±0.07
25 mM HCO_3^- + 0.1 mM DIDS (bath)	5	7.25±0.11	7.31±0.14	7.24±0.15
2 mM Cyanide+0.1 mM acetazolamide	10	7.02±0.13	7.13±0.11†	7.13±0.10
2 mM Cyanide+0.1 mM acetazol-amide+0.1 mM DIDS	5	7.04±0.06	7.08±0.05‡	7.08±0.05

Values are means ± SE; *n* = no. of experiments.

*$P < 0.01$ vs. Cl^--containing control and recovery;

†$P < 0.01$ vs. Cl^--containing control;

‡$P < 0.05$ vs. Cl^--containing control.

Table 5

Basolateral $Na^+ - HCO_3^-$ cotransporter activity: effect of basolateral Na^+ addition on pH_i in presence of EIPA and EIPA + DIDS

	n	0 Na^+	High Na^+	0 Na^+
Neonatal PST				
0.05 mM EIPA	6	7.41±0.06	7.97±0.04*	7.46±0.04
0.05 mM EIPA+0.1 mM DIDS		7.34±0.10	7.55±0.08*	7.31±0.10
Adult PST				
0.05 mM EIPA	5	7.43±0.14	7.92±0.11*	7.48±0.18
0.05 mM EIPA+0.1 mM DIDS		7.28±0.13	7.47±0.13†	7.38±0.14

Values are means ± SE; *n* = no. of experiments.

*$P < 0.001$ vs. 0 Na^+-containing control and recovery;

†$P < 0.05$ vs. 0 Na^+-containing control.