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## Maturation of rabbit proximal straight tubule chloride/base exchange

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### Abstract

The present in vitro microperfusion study compared the mechanism and rates of NaCl transport in neonatal and adult rabbit proximal straight tubules. In proximal straight tubules perfused with a late proximal tubular fluid and bathed in a serumlike albumin solution, the rate of volume absorption ( $J_V$ ) was  $0.54 \pm 0.10$  and  $0.12 \pm 0.05$   $\text{nl}\cdot\text{mm}^{-1}\cdot\text{min}^{-1}$  in adults and neonates, respectively ( $P < 0.05$ ). With the addition of  $10^{-5}$  M bath ouabain,  $J_V$  decreased to  $0.27 \pm 0.07$  and  $-0.03 \pm 0.04$   $\text{nl}\cdot\text{mm}^{-1}\cdot\text{min}^{-1}$  in adult and neonatal tubules, respectively ( $P < 0.05$ ), consistent with lower rates of active and passive NaCl transport in the neonatal proximal straight tubule. The effect of luminal sodium and chloride removal on intracellular pH was used to assess the relative rates of  $\text{Na}^+/\text{H}^+$  and  $\text{Cl}^-/\text{base}$  exchange. The rates of  $\text{Na}^+/\text{H}^+$  and  $\text{Cl}^-/\text{base}$  exchange were approximately fivefold less in neonatal proximal straight tubules than adult tubules. In both neonatal and adult proximal straight tubules, the rate of  $\text{Cl}^-/\text{base}$  exchange was not affected by formate, bicarbonate, or cyanide and acetazolamide, consistent with  $\text{Cl}^-/\text{OH}^-$  exchange. These data demonstrate an increase in proximal straight tubule NaCl transport during postnatal renal development.

### Keywords

renal development; intracellular pH; sodium chloride transport; sodium/proton antiporter

The proximal convoluted tubule preferentially reabsorbs organic solutes and bicarbonate, leaving the luminal fluid with a higher chloride concentration than that in the peritubular plasma (15, 16). This chloride concentration gradient provides a driving force for passive chloride diffusion across the para-cellular pathway. In addition to passive chloride transport, there is active electroneutral transcellular NaCl transport by the proximal tubule (2, 7). In the adult rabbit proximal convoluted tubules perfused with a late proximal tubular fluid, approximately two-thirds of NaCl transport is active and one-third is passive (7).

The parallel operation of the  $\text{Na}^+/\text{H}^+$  antiporter and  $\text{Cl}^-/\text{base}$  exchange is thought to mediate net NaCl transport across the apical membrane of the proximal tubule (3, 5). There is

evidence for  $\text{Cl}^-/\text{OH}^-$  or  $\text{Cl}^-/\text{HCO}_3^-$  exchange on brush-border membrane vesicles (25); however, others have not found these exchangers (21). Brush-border membrane vesicles have a  $\text{Cl}^-/\text{formate}$  exchanger (11, 12). Thus the parallel operation of  $\text{Cl}^-/\text{formate}$  exchange and the  $\text{Na}^+/\text{H}^+$  antiporter with formic acid recycling could mediate net  $\text{NaCl}$  transport (3, 12). Indeed, formate has been shown to stimulate  $\text{NaCl}$  transport when added to the apical and bathing solutions of proximal convoluted and straight tubules perfused in vivo and in vitro (14, 18, 19, 23, 24).

The rate of bicarbonate transport by the neonatal proximal tubule and apical membrane  $\text{Na}^+/\text{H}^+$  antiporter activity are less in the neonatal proximal tubule than in the adult (4, 6, 20). Nonetheless, the luminal concentration of bicarbonate decreases along the length of the proximal tubule (20). The mechanism of  $\text{NaCl}$  transport in the neonate is unknown. The present in vitro microperfusion study examined the relative rates of active and passive  $\text{NaCl}$  transport in neonatal and adult proximal straight tubules. We also examined the rates of  $\text{Na}^+/\text{H}^+$  antiporter and  $\text{Cl}^-/\text{base}$  exchange in neonatal and adult proximal straight tubules.

## METHODS

Isolated segments of superficial proximal straight tubules were perfused as previously described (6, 7, 9). Briefly, tubules were dissected in Hanks' balanced salt solution containing (in mM) 137  $\text{NaCl}$ , 5  $\text{KCl}$ , 0.8  $\text{MgSO}_4$ , 0.33  $\text{Na}_2\text{HPO}_4$ , 0.44  $\text{KH}_2\text{PO}_4$ , 1  $\text{MgCl}_2$ , 10 Tris hydrochloride, 0.25  $\text{CaCl}_2$ , 2 glutamine, and 2 L-lactate at 4°C. Tubules were transferred to a 1.2 ml temperature-controlled bath for flux studies and a 0.2-ml chamber in which the bathing solution was preheated to 38°C in intracellular pH ( $\text{pH}_i$ ) studies. The tubules were perfused using concentric glass pipettes.

### In vitro microperfusion flux studies

Tubules were perfused at ~10 nl/min with a high-chloride solution simulating late proximal tubular fluid containing (in mM) 140  $\text{NaCl}$ , 5  $\text{NaHCO}_3$ , 5  $\text{KCl}$ , 4  $\text{Na}_2\text{HPO}_4$ , 1  $\text{CaCl}_2$ , and 1  $\text{MgSO}_4$ . The bathing solution was a serumlike albumin solution containing (in mM) 115  $\text{NaCl}$ , 25  $\text{NaHCO}_3$ , 2.3  $\text{Na}_2\text{HPO}_4$ , 10 sodium acetate, 1.8 mM  $\text{CaCl}_2$ , 1  $\text{MgSO}_4$ , 5  $\text{KCl}$ , 8.3 glucose, 5 alanine, and 6 g/dl bovine serum albumin. The osmolalities of these solutions were adjusted to 295 mosmol/kg $\text{H}_2\text{O}$ . The pH and osmolality of the bathing solution were maintained constant by continuously changing the bath at a rate of 0.5 ml/min.

Net volume absorption ( $J_V$ , in  $\text{nl}\cdot\text{mm}^{-1}\cdot\text{min}^{-1}$ ) was measured as the difference between the perfusion ( $V_O$ ) and collection ( $V_L$ ) rates (in nl/min) normalized per millimeter of tubular length ( $L$ ). Exhaustively dialyzed [methoxy- $^3\text{H}$ ]inulin was added to the perfusate at a concentration of 75  $\mu\text{Ci}/\text{ml}$  so that the perfusion rate could be calculated. The collection rate was measured with a 50-nl constant-volume pipette. The length (in mm) was measured with an eyepiece micrometer.

The transepithelial potential difference (PD, in mV) was measured using the perfusion pipette as the bridge into the tubular lumen. The perfusion and bath solutions were connected to the recording and reference calomel half-cells, via bridges containing perfusion and an ultrafiltrate of the bathing solution, respectively, in series with a 3.6 M  $\text{KCl}/0.9$  M

KNO<sub>3</sub> agarose bridge. This arrangement avoided direct contact of KCl/KNO<sub>3</sub> agarose bridges with the solution that bathed the tubule. The recording and reference calomel half-cells were connected to the high- and low-impedance side, respectively, of an electrometer (model 602; Keithley Instruments, Cleveland, OH).

Tubules were incubated for at least 30 min before initiation of the control period. There were at least three collections in each period for measurement of volume absorption. The mean rate was used as the rate of volume absorption for that tubule. Ouabain (10<sup>-5</sup> M) was then added to the bathing solution to inhibit active transport and repeat collections were performed after incubation for at least 10 min.

### Measurement of pH<sub>i</sub>

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<sub>i</sub> as described previously (1, 4, 6, 23). Measurements of pH<sub>i</sub> were made using a Nikon inverted epifluorescent microscope attached to a PTI Ratiometer at a rate of 30 measurements per second. A variable diaphragm was placed over the area to be measured. To calculate pH from the ratio of fluorescence (F<sub>500</sub>/F<sub>450</sub>), a nigericin calibration curve was performed as previously described (1, 6). There was no difference in the calibration curves of adult and neonatal proximal straight tubules.

Tubules were incubated with the initial luminal and bathing solutions for at least 10 min and had a constant pH<sub>i</sub> for several minutes prior to measurement of transporter activity. dpH<sub>i</sub>/dt was measured from the slope of the change in pH<sub>i</sub> immediately after a luminal fluid exchange. Steady-state pH<sub>i</sub> values were achieved within 1 min after a luminal fluid exchange but were followed for several minutes to ensure a steady-state pH<sub>i</sub> was achieved.

Apparent buffer capacity (β) was measured as previously described using NH<sub>3</sub>/NH<sub>4</sub><sup>+</sup> (14, 17, 23). In the absence of HCO<sub>3</sub><sup>-</sup>, buffer capacity was 28.1 ± 5.0 mM/pH unit in neonatal proximal straight tubules and 43.0 ± 6.6 mM/pH unit in adult proximal straight tubules. Buffer capacity in the presence of HCO<sub>3</sub><sup>-</sup> was estimated as the sum of the above buffer capacity and the HCO<sub>3</sub><sup>-</sup> buffer capacity. The latter was calculated as 2.3·[HCO<sub>3</sub>]<sub>i</sub> (14, 17, 23), where [HCO<sub>3</sub>]<sub>i</sub> is the intracellular bicarbonate concentration. The buffer capacities in the presence of HCO<sub>3</sub><sup>-</sup> were 59.9 ± 7.4 and 71.4 ± 3.4 mM/pH<sub>i</sub> (not significant) in neonatal and adult proximal straight tubules, respectively.

Proton flux rates<sup>1</sup> ( $J_H$ , in pmol·mm<sup>-1</sup>·min<sup>-1</sup>) resulting from a luminal fluid change were calculated using the following formula  $J_H = dpH_i/dt \cdot V/mm \text{ tubule} \cdot \beta$ . V was the tubular volume in liters, and β was the buffer capacity. Tubular volume was calculated from the measured inner and outer tubular diameters at ×400 magnification using an eyepiece reticle. The tubular volumes of neonatal and adult proximal straight tubules were 5.2 ± 0.2 × 10<sup>-10</sup> and 10.5 ± 0.3 × 10<sup>-10</sup> l/mm, respectively ( $P < 0.001$ ).

<sup>1</sup>All proton fluxes are presented as absolute values and expressed as  $J_H$ , in pmol·mm<sup>-1</sup>·min<sup>-1</sup>.

## Statistics

Data are expressed as means  $\pm$  SE. Analysis of variance and the Student's *t*-test for paired and unpaired data were used to determine statistical significance.

## RESULTS

In the first series of experiments, we perfused neonatal and adult proximal straight tubules with a high-chloride solution simulating late proximal tubular fluid and bathed the tubules with a serumlike albumin solution. The tubular length in neonatal and adult tubules were  $0.8 \pm 0.1$  mm. As shown in Fig. 1, the rates of volume absorption were significantly greater in adult proximal straight tubules than those measured in neonates. In both groups,  $10^{-5}$  M ouabain was then added to the bathing solution so that the rate of passive transport could be determined. In adult proximal straight tubules, there was a 50% reduction in transport. However, in neonatal tubules the rate of volume absorption after addition of bath ouabain was not different from zero. These data are consistent with lower rates of both active and passive NaCl transport in neonatal compared with adult proximal straight tubules.

In tubules perfused with a high-chloride solution and bathed with a serumlike albumin solution, the transepithelial PD reflects the sum of any electrogenic sodium and proton transport and the chloride-bicarbonate diffusion potential. The PD was  $3.0 \pm 0.6$  mV the control period and  $3.1 \pm 0.5$  mV after the addition of ouabain (not significant). The PD of the neonatal tubules was only  $0.2 \pm 0.5$  mV ( $P < 0.01$  vs. adults) and increased significantly to  $0.6 \pm 0.5$  mV after the addition of ouabain ( $P < 0.05$ ).

In adult proximal tubules, active NaCl transport is mediated by parallel  $\text{Na}^+/\text{H}^+$  antiporter and  $\text{Cl}^-/\text{base}$  exchange. To examine whether the lower rate of NaCl transport in neonates was due to a lower rate of  $\text{Na}^+/\text{H}^+$  antiporter activity, we measured  $J_{\text{H}}$  in response to addition of 140 mM luminal sodium in tubules initially perfused and bathed without sodium. The  $\text{pH}_i$  for adult and neonatal tubules are shown in Table 2. As shown in Fig. 2,  $J_{\text{H}}$  was significantly less in neonatal proximal tubules compared with that measured in adults. These data are consistent with a lower rate of  $\text{Na}^+/\text{H}^+$  antiporter activity in neonatal compared with adult proximal straight tubules.

We next examined the rate of  $\text{Cl}^-/\text{base}$  exchange in neonatal and adult proximal straight tubules. Tubules were initially perfused and bathed in a HEPES-buffered chloride containing solution without sodium. In the experimental period, luminal chloride was removed and  $J_{\text{H}}$  was measured.  $\text{pH}_i$  values are shown in Tables 3 and 4. As shown in Fig. 3,  $J_{\text{H}}$  was significantly less in neonatal proximal straight tubules than in adult proximal straight tubules. In both adult and neonatal proximal straight tubules,  $\text{Cl}^-/\text{base}$  exchange was inhibited by luminal 1.0 mM DIDS. As shown in Fig. 3, the rate of  $\text{Cl}^-/\text{base}$  exchange was not affected by the addition of 0.5 mM formate to the luminal and bathing solutions. These data are consistent with a DIDS-inhibitable  $\text{Cl}^-/\text{base}$  exchanger that was not affected by formate.

In the next series of experiments, we examined whether  $\text{Cl}^-/\text{base}$  exchange in the neonatal and adult segment were mediated by  $\text{Cl}^-/\text{HCO}_3^-$  or  $\text{Cl}^-/\text{OH}^-$  exchange. In these

experiments, luminal chloride was removed in tubules perfused and bathed in the presence of 25 mM  $\text{HCO}_3^-$ . As shown in Fig. 4,  $J_{\text{H}}$  was not significantly different in the presence and absence of  $\text{CO}_2/\text{HCO}_3^-$ . Since proximal tubules can potentially generate bicarbonate and  $\text{CO}_2$  in HEPES-buffered solutions, we examined the effect of cyanide and acetazolamide on  $J_{\text{H}}$  in tubules perfused and bathed in HEPES-buffered solutions. As shown in Fig. 4, the rate of  $\text{Cl}^-/\text{base}$  exchange was comparable in the presence and absence of acetazolamide and cyanide in the neonate and adult proximal straight tubules. While some production of  $\text{CO}_2$  and  $\text{HCO}_3^-$  cannot be ruled out, these results are consistent with  $\text{Cl}^-/\text{OH}^-$  mediating  $\text{Cl}^-/\text{base}$  exchange in both adult and neonatal proximal straight tubules. The rate of  $\text{Cl}^-/\text{OH}^-$  exchange increases severalfold during postnatal maturation.

## DISCUSSION

In this study the rate of volume absorption from a high-chloride solution simulating late proximal tubular fluid was significantly less in neonatal proximal straight tubules than that measured in adults. Passive transport made up 50% of the total transport in adult tubules, but was not different from zero in neonatal tubules. The lumen-positive PD was consistent with a chloride diffusion potential in adult proximal straight tubules. The fact that there was no change in the transepithelial PD with bath ouabain is in accord with an electroneutral  $\text{NaCl}$  transport in this segment as has been found in the proximal convoluted tubule (7). In the neonatal segment, there was a small lumen-positive potential which was not different from zero mV. Addition of bath ouabain resulted in a small increase in the transepithelial PD. The significance of this change in PD is unclear.

Previous studies had predicted that neonates would have a higher rate of passive proximal tubular transport than adults (13). Injection of inulin and sucrose into early proximal tubular segments resulted in the recovery of 100% of these compounds in the urine in both neonatal and adult guinea pigs. Only 92% of microinjected mannitol, a smaller molecule, was collected in the urine of neonates compared with the total recovery in adults (13). The reabsorption of mannitol by the neonatal proximal tubule was explained by a greater paracellular permeability due to the shorter length of the proximal tubular paracellular pathway in the neonate. However, there was no maturational difference in the width or length of the zonula occludens. We have previously examined the chloride permeability of adult and neonatal proximal convoluted tubules (22). Neonatal proximal convoluted tubules have a very low chloride permeability. The chloride permeability was higher in the adult juxtamedullary proximal convoluted tubules compared with that in the neonate. The data in the proximal straight tubule are in agreement with these results. The rate of volume absorption in the presence of a chloride gradient and bath ouabain provides an estimate of the importance of passive paracellular chloride transport in this segment. While 50% of transport remained in adult proximal straight tubules in the presence of ouabain, neonatal tubular transport was not different from zero.

We have previously compared the rate of  $\text{Na}^+/\text{H}^+$  antiporter activity in neonatal and adult juxtamedullary proximal convoluted tubules (4, 6). The rate of  $\text{Na}^+/\text{H}^+$  antiporter activity was approximately one-third that of adult proximal tubules (6). The abundance of NHE-3 mRNA and protein increases in concordance with that of the  $\text{Na}^+/\text{H}^+$  antiporter (8). In the

present study, we found that  $J_H$  in response to luminal sodium addition was fivefold less in neonatal proximal straight tubules than that of the adult segment. These data demonstrate that there is maturation of proximal tubule  $\text{Na}^+/\text{H}^+$  along the length of the proximal tubule.

A previous study examined the rate of renal  $\text{Cl}^-/\text{formate}$  and  $\text{Na}^+/\text{H}^+$  exchange activity in brush-border membrane vesicles of late fetal, 3- to 5-day-old newborn and adult guinea pigs (10). This study found low rates of  $\text{Na}^+/\text{H}^+$  antiporter activity and  $\text{Cl}^-/\text{formate}$  exchange in the fetal kidney. However, the rates of both transporters were comparable in the neonatal guinea pig as in the adult. The difference between this study and ours is likely due to the different rates of postnatal renal maturation in the rabbit and the guinea pig.

We have previously examined  $\text{Cl}^-/\text{base}$  exchange on the apical membrane of adult proximal convoluted tubules using similar solutions and techniques as that in this study (23). We found that in both superficial and juxtamedullary proximal convoluted tubules, there was a significant rate of DIDS-inhibitable  $\text{Cl}^-/\text{base}$  exchange in the absence of formate. There was no difference in  $J_H$  upon luminal chloride removal in the absence of and presence of  $\text{CO}_2/\text{HCO}_3^-$ , consistent with  $\text{Cl}^-/\text{OH}^-$  exchange in both segments. In superficial but not juxtamedullary proximal convoluted tubules,  $J_H$  was significantly higher in the presence of formate, consistent with  $\text{Cl}^-/\text{formate}$  exchange. In tubules perfused with a high-chloride solution and bathed with a serumlike albumin solution, addition of formate to the lumen and bathing solution increased the rate of volume absorption. In juxtamedullary proximal convoluted tubules, addition of formate did not stimulate volume absorption. These data are consistent with the presence of a  $\text{Cl}^-/\text{formate}$  exchanger on the apical membrane of superficial, but not juxtamedullary proximal convoluted tubules.

The mechanism of apical membrane  $\text{Cl}^-/\text{base}$  exchange has previously been examined in adult rabbit proximal straight tubules perfused in vitro (14). This study found that luminal chloride removal in the absence of lumen and bath sodium resulted in comparable rates of  $\text{Cl}^-/\text{base}$  exchange in the present study. Addition of formate had no effect on the rate of  $\text{Cl}^-/\text{base}$  exchange in their studies, consistent with the absence of a  $\text{Cl}^-/\text{formate}$  exchanger in this segment. Our results in neonatal and adult proximal straight tubules are in agreement with their findings. Neither addition of 25 mM bicarbonate nor the addition of acetazolamide and cyanide in the presence of HEPES-buffered solutions affected  $J_H$ , consistent with  $\text{Cl}^-/\text{OH}^-$  exchange in this segment. Our studies in adult proximal straight tubules are also consistent with these findings. However, the  $\text{pH}_i$  changes in adult and neonatal proximal straight tubules could also be explained by  $\text{HCl}$  cotransport. The previous findings of  $\text{Cl}^-/\text{formate}$  exchange in the superficial proximal convoluted tubule and the present findings showing that the rate of  $\text{Cl}^-/\text{base}$  exchange was not affected by formate in the proximal straight tubule suggest that there is axial heterogeneity for the mechanism of  $\text{Cl}^-/\text{base}$  exchange. The physiological significance of these findings is unclear.

The present study examined the mechanism of  $\text{Cl}^-/\text{base}$  exchange in neonatal proximal straight tubules. We found that, as in the adult,  $\text{Cl}^-/\text{base}$  exchange is mediated by  $\text{Cl}^-/\text{OH}^-$  exchange. Unlike our findings in the superficial proximal convoluted tubule, there was no evidence for  $\text{Cl}^-/\text{formate}$  exchange in the proximal straight tubule. The rate of  $\text{Cl}^-/\text{OH}^-$

exchange was approximately sevenfold less in neonatal proximal straight tubules compared with adult proximal straight tubules.

In summary, the present in vitro microperfusion study compared the rates of NaCl transport in neonatal and adult proximal straight tubules. In tubules perfused with a high-chloride solution simulating late proximal tubular fluid, the rate of active and passive NaCl transport was less in neonatal proximal tubule than in the adult segment. Our data demonstrate that parallel  $\text{Na}^+/\text{H}^+$  and  $\text{Cl}^-/\text{OH}^-$  exchange mediate apical membrane NaCl transport in the neonate and the adult proximal straight tubule. The rates of both transporters are significantly less in the neonate.

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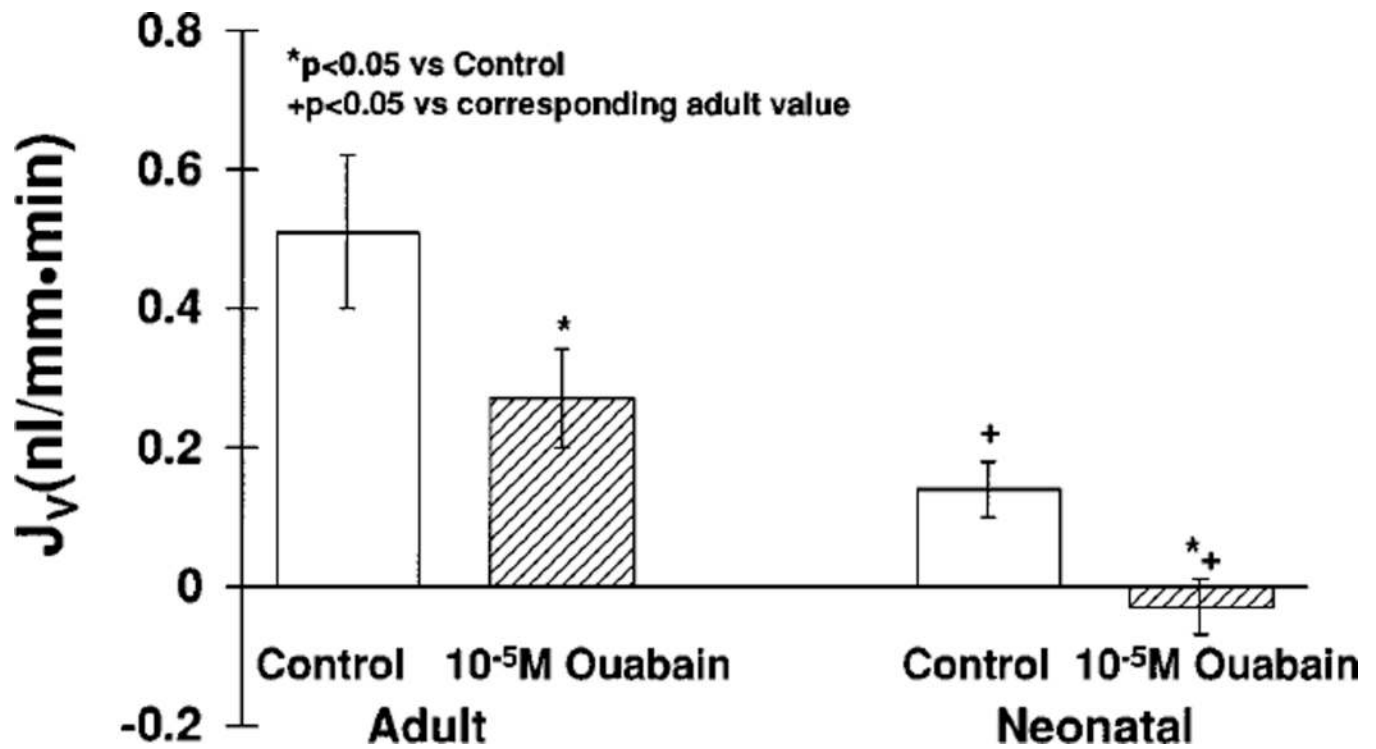
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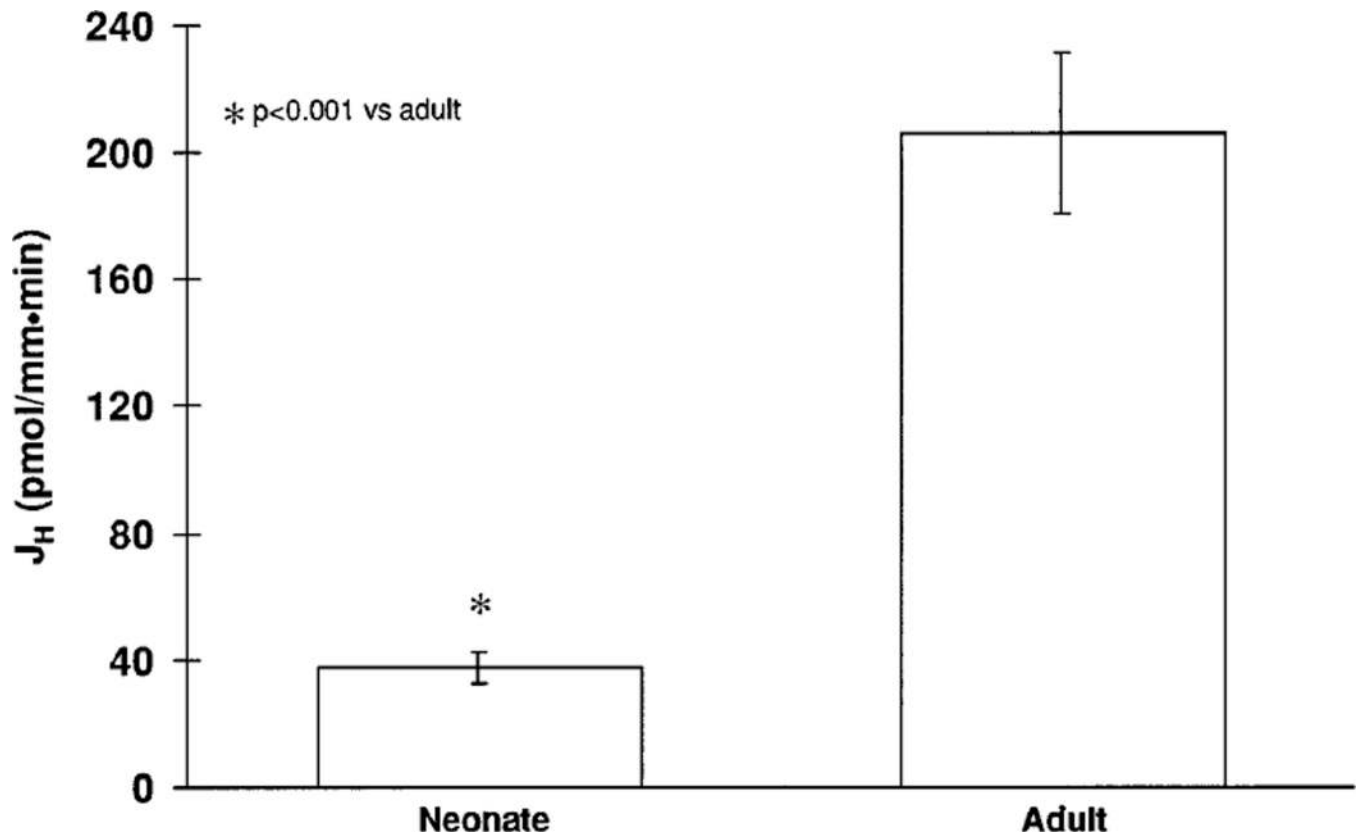
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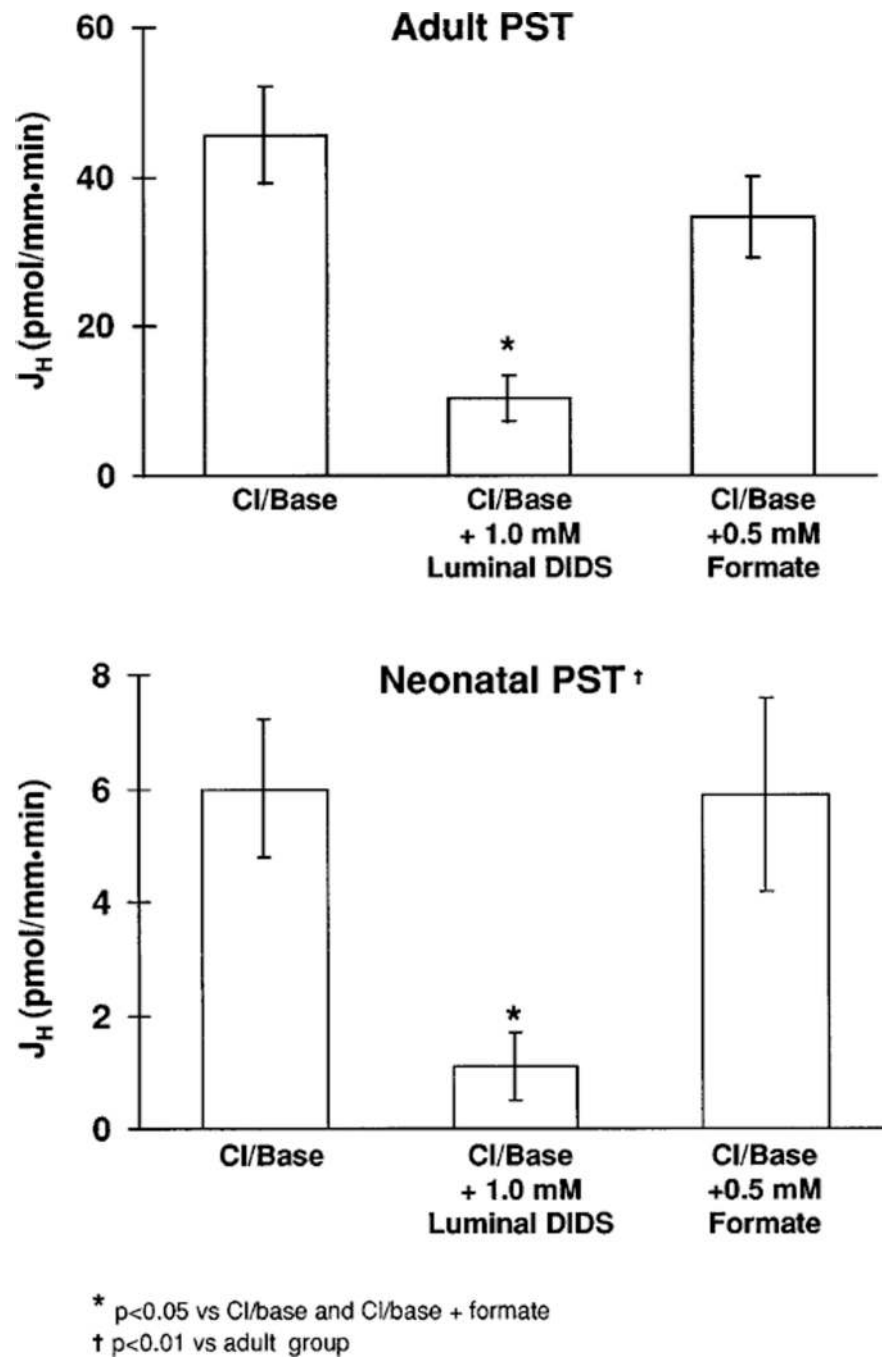




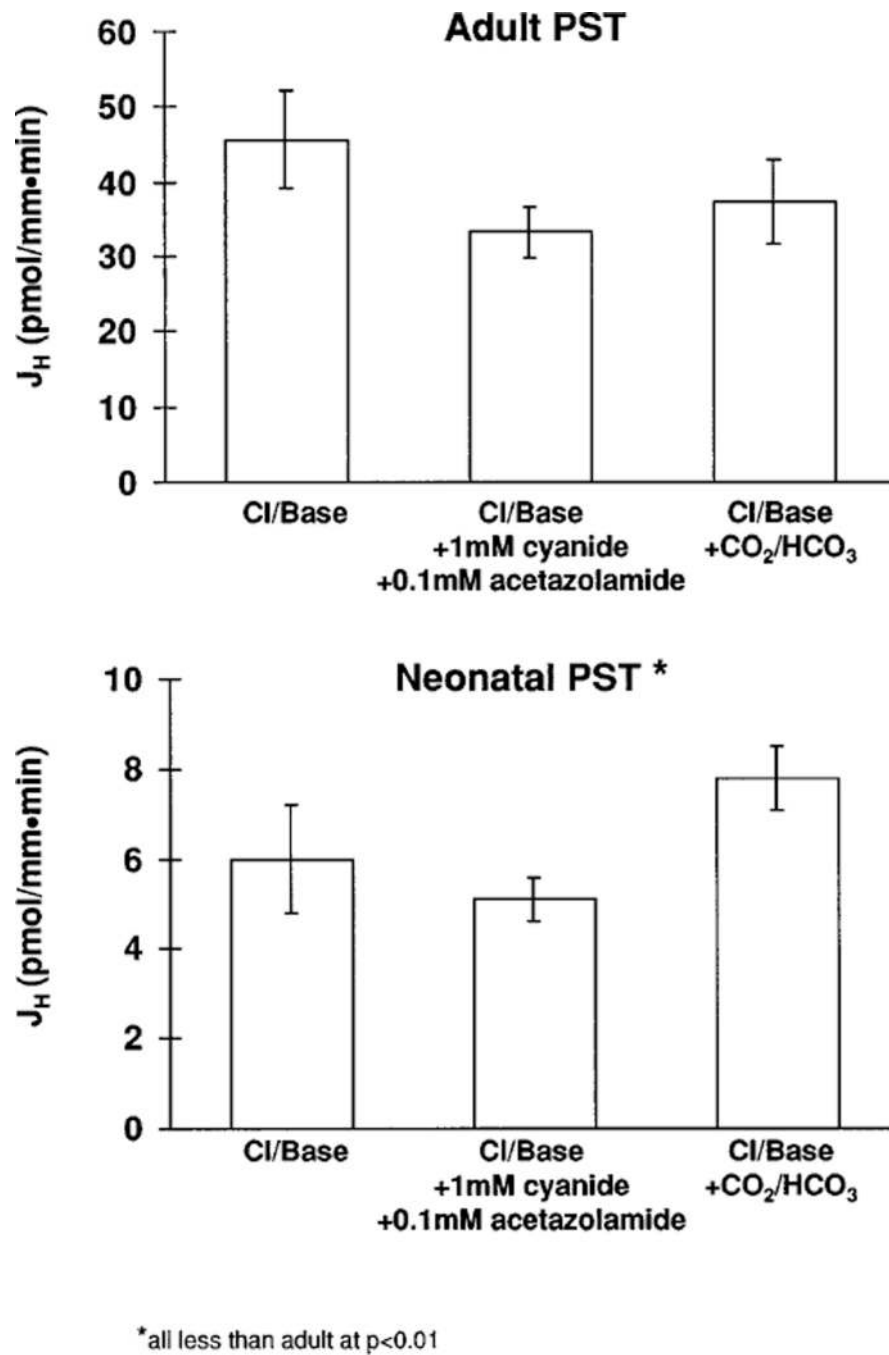
**Fig. 1.** Effect of  $10^{-5}$  M bath ouabain on adult and neonatal proximal straight tubule NaCl transport. Proximal straight tubules were perfused with a high-chloride solution simulating late proximal tubular fluid and bathed in a serumlike albumin solution. After measurements of volume absorption ( $J_v$ ) in the control period,  $10^{-5}$  M bath ouabain was added to the bathing solution to inhibit active transport.



**Fig. 2.** Proton flux rates ( $J_H$ ) in response to addition of luminal sodium addition in neonatal and adult proximal straight tubules.



**Fig. 3.**  $J_H$  values in response to luminal chloride removal in adult and neonatal proximal straight tubules. These experiments were performed using HEPES-buffered solutions in the absence of sodium.



**Fig. 4.**  $J_H$  values in response to luminal chloride removal with HEPES-buffered solutions, in absence and presence of lumen and bath cyanide and acetazolamide, and with bicarbonate-buffered solutions.

Table 1

Solutions used in pH<sub>i</sub> studies

	High Cl <sup>-</sup> , 0 Na	0 Cl <sup>-</sup> , High Na <sup>+</sup>	0 Cl <sup>-</sup> , 0 Na <sup>+</sup> , +20 NH <sub>4</sub> <sup>+</sup>	High Cl <sup>-</sup> , 0 Na <sup>+</sup> , +25 HCO <sub>3</sub> <sup>-</sup>	0 Cl <sup>-</sup> , 0 Na <sup>+</sup> , +25 HCO <sub>3</sub> <sup>-</sup>
TMA-CI	140			115	
TMA-OH	140		120		115
TMA-HCO <sub>3</sub>				25	25
NH <sub>4</sub> OH			20		
Na gluconate		140			
Gluconic acid lactone	140		140		115
K <sub>2</sub> HPO <sub>4</sub>	2.5	2.5	2.5	2.5	2.5
MgCl <sub>2</sub>	1			1	
Mg gluconate		1	1		1
CaCl <sub>2</sub>	1			1	
Ca gluconate	12.5	12.5	12.5		10
Glucose	5	5	5	5	5
L-Alanine	5	5	5	5	5
HEPES	5	5	5		

All constituents are in mM. All solutions were adjusted to an osmolality of 295 mosmol/kgH<sub>2</sub>O. HCO<sub>3</sub><sup>-</sup>-containing solutions were bubbled with 95% O<sub>2</sub>-5% CO<sub>2</sub> and had a pH of 7.4. Non-HCO<sub>3</sub><sup>-</sup>-containing solutions were bubbled with 100% O<sub>2</sub> and had a pH of 7.4. pH<sub>i</sub>, intracellular pH; TMA, tetramethylammonium.

**Table 2**Effect on luminal Na<sup>+</sup> on pH<sub>i</sub> in PST

	<i>n</i>	0 Na <sup>+</sup>	Na <sup>+</sup>	0 Na <sup>+</sup>
Adult PST	6	7.32±0.12	7.81±0.10*	7.23±0.18
Neonatal PST	8	7.26±0.16	7.66±0.15*	7.13±0.16

Values are means ± SE; *n* = no. of experiments. PST, proximal straight tubule.

\* Na<sup>+</sup> significantly different from 0 Na<sup>+</sup> containing control and recovery (*P*<0.01).

**Table 3**Effect of luminal  $\text{Cl}^-$  on  $\text{pH}_i$  in adult PST

	<i>n</i>	$\text{Cl}^-$	0 $\text{Cl}^-$	$\text{Cl}^-$
No formate	10	7.00±0.06	7.32±0.06*	7.07±0.05
Formate (0.5 mM)	9	7.16±0.05	7.47±0.05*	7.28±0.05
Luminal DIDS (1 mM)	7	7.16±0.05	7.29±0.05 <sup>†</sup>	7.24±0.05
$\text{HCO}_3^-$ (25mM)	14	7.09±0.04	7.32±0.05*	7.17±0.05
Cyanide (1 mM) + acetazolamide (0.1 mM)	7	6.76±0.07 <sup>‡</sup>	7.35±0.05*	7.20±0.03

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

\* 0  $\text{Cl}^-$  significantly different from  $\text{Cl}^-$  containing control and recovery ( $P < 0.01$ ).

<sup>†</sup> 0  $\text{Cl}^-$  significantly different from  $\text{Cl}^-$  containing control ( $P < 0.001$ ).

<sup>‡</sup> Initial  $\text{pH}_i$  significantly different from formate, luminal DIDS, and  $\text{HCO}_3^-$  groups ( $P < 0.05$ ).

**Table 4**Effect of luminal  $\text{Cl}^-$  on  $\text{pH}_i$  in neonatal PST

	<i>n</i>	$\text{Cl}^-$	0 $\text{Cl}^-$	$\text{Cl}^-$
No formate	8	7.05±0.11	7.33±0.14*	7.09±0.13
Formate (0.5 mM)	8	7.31±0.10	7.52±0.12*	7.27±0.12
Luminal DIDS (1 mM)	8	7.27±0.04	7.34±0.05 <sup>†</sup>	7.31±0.04
$\text{HCO}_3^-$ (25mM)	8	7.08±0.10	7.36±0.10*	7.20±0.09
Cyanide (1 mM) + acetazolamide (0.1 mM)	9	6.81±0.06 <sup>‡</sup>	7.22±0.06 <sup>†</sup>	7.17±0.05

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

\* 0  $\text{Cl}^-$  significantly different from  $\text{Cl}^-$  containing control and recovery ( $P < 0.01$ ).

<sup>†</sup> 0  $\text{Cl}^-$  significantly different from  $\text{Cl}^-$  containing control ( $P < 0.05$ ).

<sup>‡</sup> Initial  $\text{pH}_i$  significantly different from formate and luminal DIDS groups ( $P < 0.01$ ).