0031-3998/92/3104-0411$03.00/0
PEDIATRIC RESEARCH

Vol. 31, No. 4, 1992
Printed in U.S.A.

Developmental Changes in Rabbit Juxtamedullary Proximal Convoluted Tubule Acidification

MICHEL BAUM

Departments of Pediatrics and Medicine, The University of Texas Southwestern Medical Center at Dallas, Texas 75235-9063

ABSTRACT. The transporters responsible for apical proton secretion were examined in neonatal and adult proximal convoluted tubules (PCT). Transporter activity was assayed from the rate of recovery of cell pH after cell acidification following exposure to NH_4Cl. Cell pH was monitored in *in vitro* perfused tubules using the pH sensitive dye 2′,7′-bis(carboxyethyl)-5,6-carboxyfluorescein. Recovery from an acid load in adult PCT occurred at 0.52 ± 0.09 pH units/min in the presence of sodium and 0.25 ± 0.05 in the absence of sodium ($p < 0.05$). One mmol/L N-ethylmaleimide, an inhibitor of the H^+-ATPase, inhibited the sodium-independent pH recovery from an acid load consistent with a H^+-ATPase on the apical membrane. In neonatal PCT, recovery from an acid load was 0.39 ± 0.08 pH units/min in the presence of sodium and only 0.08 pH units/min in the absence of sodium ($p < 0.05$). Studies using 4 mmol/L luminal amiloride, an inhibitor of the Na^+/H^+ antiporter, were consistent with a larger fraction of pH recovery from an acid load in neonatal PCT being due to the Na^+/H^+ antiporter compared with adult PCT. Thus, maturation of the PCT involves an increase in activity of a sodium-independent proton secretory mechanism, presumably the H^+-ATPase. (*Pediatr Res* 31: 411–414, 1992)

Abbreviations

PCT, proximal convoluted tubule
BCECF, 2′,7′-bis(carboxyethyl)-5,6-carboxyfluorescein
DIDS, 4,4′-diisothiocyanostilbene-2,2′-disulfonic acid
NEM, N-ethylmaleimide
pH_i, intracellular pH
dpH_i/dt, rate of change in intracellular pH

The neonatal juxtamedullary PCT transports bicarbonate at about one third the rate of juxtamedullary PCT from adult animals (1, 2). In the PCT, luminal proton secretion is predominantly mediated by the Na^+/H^+ antiporter (2, 3–6). The driving force for the antiporter is the low intracellular sodium concentration generated by the basolateral Na^+/K^+ ATPase. Bicarbonate exit across the basolateral membrane is via a $Na(HCO_3)_3$ symporter (7–14). The activity of each of these transporters in neonatal PCT is about one third that in mature PCT (2, 15).

There is evidence that proton secretion in the adult PCT may, in part, be mediated by a sodium-independent H^+-ATPase on the apical membrane (16–21). In S_3 proximal straight tubules from adult rabbits, this transporter is present and is an important mechanism for the defense of cell pH from an acid load (19). The importance of sodium-dependent and sodium-independent acidification mechanisms in cell pH defense in neonatal PCT is unknown. The purpose of the present *in vitro* microperfusion study was to examine the relative contribution of the apical membrane sodium-dependent and sodium-independent acidification mechanisms in PCT cell pH defense in neonatal and adult PCT.

MATERIALS AND METHODS

Isolated segments of rabbit PCT were perfused as previously described (2, 22). Briefly, New Zealand White pregnant does were housed at our institution for at least 1 wk before delivery. Newborn animals were cared for by their mothers and studied in the 1st wk of life. Adult animals were obtained from the same vendor. All PCT were dissected from the juxtamedullary cortex and were 0.1 to 0.5 mm in length. No late PCT identified by their attachment to proximal straight tubules were used to avoid problems with axial heterogeneity (21).

Tubules were dissected at 4°C with the same solution that was used as the bathing solution during the control period. The solutions used in these studies are shown in Table 1. Tubules were transferred to a 0.2-mL bath chamber and perfused with concentric glass pipettes. The bathing solution was preheated to 38°C and exchanged at a rate of at least 3 mL/min. In studies in which a change in the bathing solution was performed, the bath was exchanged at 10 mL/min. Tubules were perfused and bathed in symmetrical solutions during a 5- to 10-min equilibration period. The bathing solution was then changed to one that contained 5×10^{-6} mol/L of the acetoxymethyl derivative of BCECF (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 (7). The cells were loaded with the dye for ~5 min, then the bathing solution was changed to the one used in the control period and the tubules were again equilibrated for at least 5 min before any measurements were made. BCECF has a pH-sensitive excitation maximum at 504 nm and 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 (Flouvert; E. Leitz, Wetzlar, Germany) at ×25 magnification. A variable diaphragm was placed over the area to be measured. To calculate pH from the ratio of fluorescence measured at the two excitation wavelengths (F_{500}/F_{450}), a nigericin calibration curve

Received July 24, 1991; accepted December 11, 1991.
Correspondence and reprint requests: Michel Baum, M.D., Department of Pediatrics, UT Southwestern Medical Center, 5323 Harry Hines Blvd., Dallas, TX 75235-9063.
Supported by National Institutes of Health Grant DK 41612-01 and American Heart Association Grant 890664.

was performed. As previously described, there was no difference in the nigericin calibration curve between neonates and adults (2).

To study the transporters responsible for recovery from an acute acid load in neonatal and adult PCT, the NH_4Cl prepulse technique was used (19, 23). After three measurements at 500 and 450 nm to measure steady state pH_i, the control bathing solution (solution I or III) was changed to one containing 20 mmol/L NH_4Cl (solution II or IV) for 5 min. The tubule cells alkalinized as NH_3 diffused into the cells. After 5 min, the bathing solution was changed back to that in the control period, and the cells rapidly acidified. Typical tracings are shown in Figure 1. The rate of recovery of cell pH was determined by continuously monitoring fluorescence at 500 nm, the pH-sensitive wavelength. The initial rate of change (dF_{500}/dt) was used to calculate the initial dpH_i/dt.

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

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 (2).

In experiments in which Na^+/H^+ antiporter activity was measured directly, tubules were perfused with an ultrafiltrate-like solution (solution V). This solution was bubbled with 95% O_2/5% CO_2 and had a pH of 7.4 units. Tubules were bathed in a high chloride, low bicarbonate solution (solution VI). This solution had a pH of 6.8 units and contained 0.5 mmol/L of DIDS. DIDS was present to prevent bicarbonate exit across the basolateral membrane, inasmuch as basolateral membrane transporters are important determinants of cell pH (2, 6). The low bicarbonate, low pH bath was used because Na^+/H^+ antiporter activity is pH-dependent and its activity increased at low pH (4). This approach has been used by myself and others to examine Na^+/H^+ antiporter activity (2, 6, 22). During the experimental period, sodium was removed from the luminal perfusate (choline replacement) and the initial rate of change in cell pH was determined. The data are expressed as a mean ± SEM. The *t* test for paired and unpaired data and analysis of variance were used to determine statistical significance.

RESULTS

The first series of experiments was designed to examine the relative importance of the sodium-dependent and sodium-independent mechanisms of cell pH recovery in response to an acid load. Previous studies have demonstrated that the neonatal and adult PCT have a basolateral $Na(HCO_3)_3$ symporter that is an important determinant of cell pH (2, 6). Thus, to examine transporters on the apical membrane, 0.5 mmol/L bath DIDS was added to inhibit the $Na(HCO_3)_3$ symporter in all experiments that contained sodium. The results are shown in Table 2. In the presence of sodium, pH recovery from an acid load was 0.52 ± 0.09 pH units/min in adult PCT. In the absence of sodium, the rate of recovery from an acid load in adult PCT was 0.25 ± 0.05 pH units/min ($p < 0.05$). Thus, ~50% of pH recovery from an acid load in adult PCT is due to a sodium-independent mechanism.

Table 1. *Solutions**

	I	II	III	IV	V	VI
Na^+	140	120			147	142
K^+	5	5	5	5	5	5
Ca^{++}	1	1	1	1	1.8	1.8
Mg^{++}	1	1	1	1	1	1
$Choline^+$			135	115		
NH_4^+		20		20		
$NMDG^+$			5	5		
Cl^-	139	139	139	139	130.6	145.6
HCO_3^-					25	5
$HPO_4^=$	2.5	2.5	2.5	2.5	1	1
$HEPES^-$	5	5	5	5		
Urea	5	5	5	5	5	5
Glucose	5	5	5	5		5
Alanine	5	5	5	5		5
HEPES	5	5	5	5		
CO_2	0	0	0	0	5%	5%
O_2	100%	100%	100%	100%	95%	95%
pH	7.4	7.4	7.4	7.4	7.4	6.8

* Solutions are in mmol/L. HEPES, *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid.

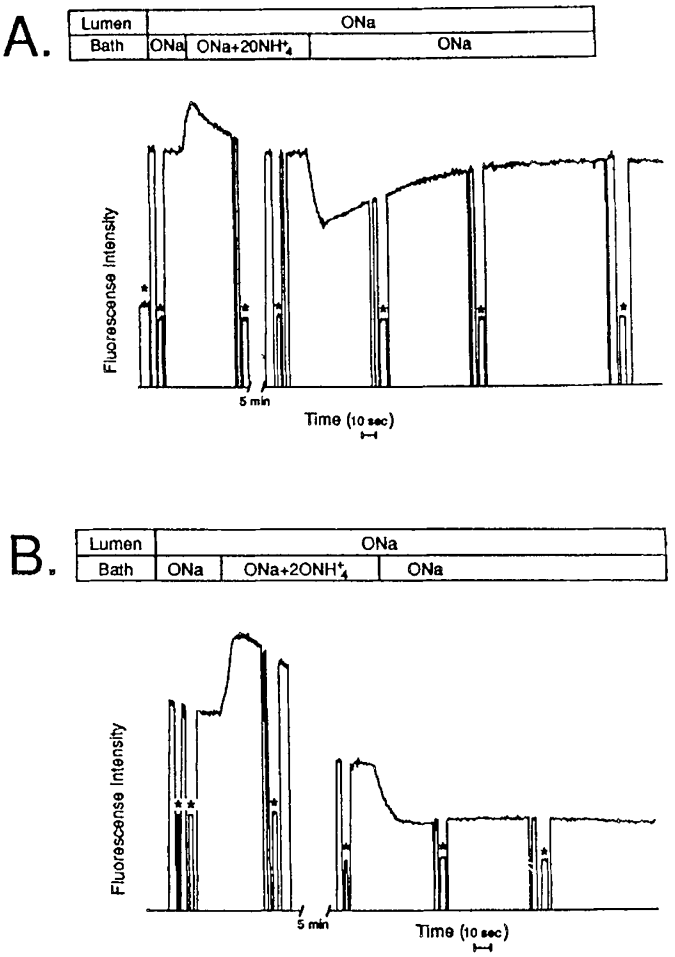

Fig. 1. Typical tracings of the effect of acid loading on pH_i in the absence of sodium. *Tracing A* is from an adult PCT, and *B* is from a neonatal PCT. Excitation is at 500 nm. Measurements at 450 nm are depicted by *.

There is significant evidence for an H^+-ATPase on the apical membrane of the PCT (16–21). In addition, an NEM-sensitive H^+-ATPase has been demonstrated in the PST (19). To examine if the sodium-independent pH recovery was NEM-sensitive, experiments were repeated in the absence of sodium with 1 mmol/L NEM in the luminal perfusate. NEM resulted in cell acidification, as shown in Table 2. Despite a lower cellular pH, which should enhance proton secretion, luminal NEM resulted in an inhibition of pH recovery to 0.14 ± 0.02 pH units/min ($p < 0.05$).

The next series of experiments examined the recovery of cell pH from an acid load in neonatal PCT. The results are shown in Table 3. In the presence of sodium, the rate was 0.39 ± 0.08 pH units/min. In the absence of sodium, pH recovery was only 0.08 ± 0.02 ($p < 0.05$). Thus, recovery from an acid load in neonatal PCT was markedly slowed in the absence of sodium.

The difference in recovery from an acid load in the presence

Table 2. *Effect of cell acidification by NH_4Cl prepulse in adult PCT**

	n	pH_i Baseline	pH_i Minimum	$\frac{dpH_i}{dt}$ (pH units/min)
Adult PCT (140 mmol/L Na)	14	7.33 ± 0.02	6.95 ± 0.05	0.52 ± 0.09
Adult PCT (140 mmol/L Na, 4 mmol/L luminal amiloride)	8	7.29 ± 0.02	6.93 ± 0.03	0.31 ± 0.03
Adult PCT (without Na)	8	7.24 ± 0.09	6.88 ± 0.07	0.25 ± 0.05†
Adult PCT (no Na, 1 mmol/L luminal NEM)	8	6.77 ± 0.06‡	6.42 ± 0.04‡	0.14 ± 0.02§

* $F = 7.76$; $p < 0.01$.
† Different than adult with Na at $p < 0.05$.
‡ Different than adult without NEM at $p < 0.01$.
§ Different than adult without NEM at $p < 0.05$.

Table 3. *Effect of cell acidification by NH_4Cl prepulse in neonatal PCT*

	n	pH_i Baseline	pH_i Minimum	$\frac{dpH_i}{dt}$ (pH units/min)
Neonatal (140 mmol/L)	8	7.18 ± 0.06	6.70 ± 0.10	0.39 ± 0.08
Neonatal (140 mmol/L Na, 4 mmol/L luminal amiloride)	8	7.21 ± 0.03	6.64 ± 0.05	0.18 ± 0.03*
Neonatal (without Na)	7	7.04 ± 0.04	6.76 ± 0.09	0.08 ± 0.02†

* Different than without inhibitor at $0.05 < p < 0.10$.
† Different than bath Na-containing groups $p < 0.05$.

and absence of sodium may be due to the apical Na^+/H^+ antiporter. To estimate the relative importance of the Na^+/H^+ antiporter in the recovery of cell pH from an acid load, two experiments were performed. In the first experiment, the effect of 4 mmol/L luminal amiloride, an inhibitor of the Na^+/H^+ antiporter (24), on pH recovery from an acid load was examined in the presence of sodium with 0.5 mmol/L DIDS in the bathing solution. The results are shown in Table 2 for adult PCT and in Table 3 for neonatal PCT. In adult PCT, pH recovery from an acid load was 0.52 ± 0.09 pH units/min in the presence of sodium and 0.31 ± 0.03 pH units/min in the presence of sodium with 4 mmol/L luminal amiloride. In neonatal PCT, pH recovery from an acid load was 0.39 ± 0.08 pH units/min in the presence of sodium and 0.18 ± 0.03 pH units/min in the presence of sodium and 4 mmol/L luminal amiloride. Thus, 4 mmol/L amiloride inhibited pH recovery from an acid load by 40% in adult PCT and by 54% in neonatal PCT. The contribution of the Na^+/H^+ antiporter to cell pH recovery can only be assessed if the percentage of inhibition of the Na^+/H^+ antiporter by 4 mmol/L amiloride is known. To determine this, Na^+/H^+ antiporter activity was assayed in the presence and absence of 4 mmol/L luminal amiloride by examining the initial dpH_i/dt upon luminal sodium removal in the presence of CO_2 and HCO_3, as previously described in adult and neonatal tubules (2, 6, 22). The results are shown in Table 4.[1] The dpH_i/dt upon luminal sodium removal in adult PCT was 4.72 ± 0.59 pH units/min and 1.78 ± 0.27 pH units/min ($p < 0.001$) in the presence of 4 mmol/L amiloride. Thus, 4 mmol/L amiloride inhibited Na^+/H^+ antiporter activity by 62% in adult PCT. Similarly, there was a 57% inhibition of Na^+/H^+ antiporter activity in neonatal PCT with 4 mmol/L luminal amiloride (2.31 ± 0.24 *versus* 1.00 ± 0.08 pH units/min, $p < 0.001$). From the percentage of inhibition of pH recovery from an acid load by 4 mmol/L amiloride and the percentage of reduction of Na^+/H^+ antiporter activity by 4 mmol/L amiloride, the contribution of the Na^+/H^+ antiporter to pH recovery could be estimated by their quotient. In adult PCT, ~65% of pH recovery from an acid load in the presence of sodium was due to the Na^+/H^+ antiporter, whereas ~95% of pH recovery was due to the antiporter in neonatal PCT.

[1] The dpH_i/dt in adults and neonates were higher than those previously reported by our laboratory (2). The reason for this may be due to the use of bath DIDS rather than 4-acetamido-4′-isothiocyanostilbene-2,2′-disulfonate (SITS) in these protocols.

DISCUSSION

The acidification mechanisms in the PCT provide a mechanism for the vectorial transport of bicarbonate from the tubular lumen into the blood. They also are important in the defense of cellular pH. This *in vitro* microperfusion study used the NH_4Cl prepulse technique to examine the transporters responsible for cellular pH defense from an acid load in neonatal and adult PCT. The data indicate that most of pH recovery from an acid load in neonatal PCT is sodium-dependent, whereas ~50% of pH recovery in adult proximal tubules is sodium-independent.

The neonatal juxtamedullary PCT transports bicarbonate at one third the rate of the adult juxtamedullary PCT (1). Consistent with this observation is the fact that the rate of apical proton secretion by the Na^+/H^+ antiporter in juxtamedullary PCT is one third that measured in adult PCT (2). However, there is increasing evidence that an H^+-ATPase is responsible for a significant portion of proton secretion across the apical membrane (16–21). Kurtz (19) has provided evidence for a H^+-ATPase in S_3 proximal straight tubules by examining the pH recovery from an acid load in the absence of sodium, as was performed in the present studies. In this segment, pH recovery was significantly inhibited by the H^+-ATPase inhibitors 1 mmol/L luminal NEM and 1 mmol/L luminal dicyclohexylcarbodiimide. NEM resulted in ~50% inhibition in pH recovery in the absence of sodium, which was comparable to the inhibition observed in the present study in mature PCT. Of interest is that this study examined pH recovery in the absence of sodium in S_2 proximal straight tubules and found the rate to be 0.10 ± 0.02 pH units/min compared with 0.89 ± 0.15 in S_3 proximal straight tubules. This study demonstrated that the rate of pH recovery in PCT of 0.25 ± 0.05 pH units/min in the absence of sodium was intermediate between these values. Thus, there may be a heterogenous distribution of H^+-ATPase pumps along the mature proximal tubule. pH recovery in the absence of sodium in neonatal PCT was only 0.08 ± 0.02 pH units/min. The present study provides evidence that proximal tubule maturation involves an increase in activity of the H^+-ATPase.

Previous studies in the neonatal PCT have demonstrated that there is an apical Na^+/H^+ antiporter and a basolateral $Na(HCO_3)_3$ symporter (2). However, even at the time of birth, membrane polarity is present and there is no evidence of Na^+/H^+ antiporter activity on the basolateral membrane (2). Thus, to examine the relative importance of the apical Na^+/H^+ antiporter to sodium-dependent recovery from an acid load, amiloride, an inhibitor of the Na^+/H^+ antiporter, was used. Amiloride (4 mmol/L) resulted in ~60% inhibition of Na^+/H^+ antiporter activity in neonatal and adult PCT (Table 4). From the rate of recovery of cell pH in the presence of luminal amiloride and the percentage of inhibition by 4 mmol/L amiloride on the Na^+/H^+ antiporter, the contribution of the Na^+/H^+ antiporter to the recovery of pH_i

Table 4. *Effect of 4 mmol/L amiloride on neonatal and adult Na^+/H^+ antiporter activity*

		Luminal perfusate		$\frac{dpH_i}{dt}$
	n	$UF\bar{s}$ organics*	0 $Na\bar{s}$ organics*	(pH units/min)
Adult	7	7.19 ± 0.03	6.55 ± 0.07	4.72 ± 0.59
Adult + 4.0 mmol/L luminal amiloride	8	7.15 ± 0.08	6.81 ± 0.06†	1.78 ± 0.27‡
Neonate	5	7.40 ± 0.05	6.75 ± 0.02	2.31 ± 0.24§
Neonate + 4.0 mmol/L luminal amiloride	6	7.27 ± 0.04‡	6.93 ± 0.02‡	1.00 ± 0.08‡

* $UF\bar{s}$ organics, ultrafiltrate without organics; 0 $Na\bar{s}$ organics, 0 Na solution without organics.
† Different than age-matched control without amiloride at $p < 0.05$.
‡ Different than age-matched control without amiloride at $p < 0.001$.
§ Different than adult at $p < 0.001$.

from an acid load was estimated. There were striking differences between adult and neonatal PCT. Whereas Na^+/H^+ antiporter contributed to ~65% of pH recovery in the adult, it accounted for ~95% of the recovery in the neonate. In addition, the estimated rates of Na^+/H^+-independent pH recovery in both adult and neonatal PCT compare well with the sodium-independent rates of cellular pH recovery. Thus, this analysis also suggests that proximal tubule maturation involves an increase in a sodium-independent proton secretory mechanism, presumably the H^+-ATPase.

Determination of the relative importance of the Na^+/H^+ antiporter and H^+-ATPase activity to net acidification has been problematic. Inhibitors with variable specificity have been used (17–20). The present analysis examining the relative importance of sodium-dependent and sodium-independent pH recovery also has limitations. The rate of pH recovery is dependent on cellular pH (4). Although the protocol examining the sodium-dependent and sodium-independent pH recovery had quite similar cellular pH in the adult group and the neonatal group, the neonatal PCT cellular pH tended to be lower than adult PCT cellular pH. Thus, a direct comparison of absolute rates between neonatal and adult PCT is not possible. However, the cellular pH in the various protocols using adult PCT and neonatal PCT were quite similar, allowing analysis within each group. It is also possible that the experimental conditions in these studies, such as the absence of CO_2 and bicarbonate, could have affected the activity of the H^+-ATPase. In addition, the relative contribution of the Na^+/H^+ antiporter to sodium-dependent pH recovery was estimated by using amiloride, a compound that is not an entirely specific inhibitor of the Na^+/H^+ antiporter (25).

In conclusion, this *in vitro* microperfusion study examined sodium-dependent and sodium-independent recovery from an acid load in neonatal and adult PCT. Our data demonstrate that ~50% of pH recovery from an acid load in adults is sodium-independent. In neonates, the vast majority of pH recovery from an acid load is sodium-dependent and is due to the apical Na^+/H^+ antiporter.

Acknowledgments. The author thanks Rebecca Aricheta and Ebtisam Shawky for their technical assistance and Janell McQuinn for her able secretarial assistance. Amiloride was a gift of Merck, Sharp and Dohme.

REFERENCES

1. Schwartz GJ, Evan AP 1983 Development of solute transport in rabbit proximal tubule. I. HCO_3^- and glucose absorption. Am J Physiol 245:F382–F390
2. Baum M 1990 Neonatal rabbit juxtamedullary proximal convoluted tubule acidification. J Clin Invest 85:499–506
3. Murer H, Hopfer U, Kinne R 1976 Sodium/proton antiporter in brush-border-membrane vesicles isolated from rat small intestine and kidney. Biochem J 154:597–604
4. Aronson PS, Nee J, Suhm MA 1982 Modifier role of internal H^+ in activating the Na^+-H^+ exchanger in renal microvillus membrane vesicles. Nature 299:161–163
5. Kinsella JL, Aronson PS 1980 Properties of the Na^+H^+ exchanger in renal microvillus membrane vesicles. Am J Physiol 238:F461–F469
6. Alpern RJ, Chambers M 1986 Cell pH in the rat proximal convoluted tubule-regulation by luminal and peritubular pH and sodium concentration. J Clin Invest 78:502–510
7. Alpern RJ 1985 Mechanism of basolateral membrane $H^+/OH^-/HCO_3^-$ transport in the rat proximal convoluted tubule. J Gen Physiol 86:613–636
8. Akiba T, Alpern RJ, Eveloff J, Calamina J, Warnock DG 1986 Electrogenic sodium/bicarbonate cotransport in rabbit renal cortical basolateral membrane vesicles. J Clin Invest 78:1472–1478
9. Biagi BA, Sohtell M 1986 Electrophysiology of basolateral bicarbonate transport in the rabbit proximal tubule. Am J Physiol 250:F267–F272
10. Boron WF, Boulpaep EL 1983 Intracellular pH regulation in the renal proximal tubule of the salamander: basolateral HCO_3^- transport. J Gen Physiol 81:53–94
11. Grassl SM, Aronson PS 1986 Na^+/HCO_3^- co-transport in basolateral membrane vesicles isolated from rabbit renal cortex. J Biol Chem 261:8778–8783
12. Krapf R, Alpern RJ, Rector Jr FC, Berry CA 1987 Basolateral membrane Na/base cotransport is dependent on CO_2/HCO_3 in the proximal convoluted tubule. J Gen Physiol 90:833–853
13. Soleimani M, Grassl SM, Aronson PS 1987 Stoichiometry of Na^+-HCO_3^- cotransport in basolateral membrane vesicles isolated from rabbit renal cortex. J Clin Invest 79:1276–1280
14. Yoshitomi K, Burckhardt BC, Fromter E 1985 Rheogenic sodium-bicarbonate cotransport in the peritubule cell membrane of rat renal proximal tubule. Pflugers Arch 405:360–366
15. Schwartz GJ, Evan AP 1984 Development of solute transport in rabbit proximal tubule. III. Na-K-ATPase activity. Am J Physiol 246:F845–F852
16. Kinne-Saffran E, Beauwens R, Kinne R 1982 An ATP-driven proton pump in brush-border membranes from rat renal cortex. J Membrane Biol 64:67–76
17. Preisig PA Ives HE, Cragge Jr EJ, Alpern RJ, Rector Jr FC 1987 Role of the Na^+/H^+ antiporter in rat proximal tubule bicarbonate absorption. J Clin Invest 80:970–978
18. Bank N, Aynedjian HS, Mutz BF 1985 Evidence for a DCCD-sensitive component of proximal bicarbonate reabsorption. Am J Physiol 249:F636–F644
19. Kurtz I 1987 Apical Na^+/H^+ antiporter and glycolysis-dependent H^+-ATPase regulate intracellular pH in the rabbit S_3 proximal tubule. J Clin Invest 80:928–935
20. Bank N, Aynedjian HS, Mutz BF 1989 Proximal bicarbonate absorption independent Na^+/H^+ exchange: effect of bicarbonate load. Am J Physiol 256:F577–F582
21. Brown D, Hirsch S, Gluck S 1988 Localization of a proton-pumping ATPase in rat kidney. J Clin Invest 82:2114–2126
22. Baum M 1989 Axial heterogeneity of rabbit proximal tubule luminal H^+ and basolateral HCO_3^- transport. Am J Physiol 256:F335–F341
23. Roos A, Boron WF 1981 Intracellular pH. Physiol Rev 61:297–434
24. Kinsella JL, Aronson PS 1981 Amiloride inhibition of the Na^+/H^+ exchanger in renal microvillus membrane vesicles. Am J Physiol 241:F374–F379
25. Soltoff SP, Mandel LJ 1983 Amiloride inhibits the Na^+/K^+-ATPase activity of rabbit kidney proximal tubules. Science 220:957–959