Na/H Antiporter mRNA Expression in Single Nephron Segments of Rat Kidney Cortex

Reto Krapf and Marc Solioz
With the technical assistance of **Catherine Fehlmann**
Department of Medicine and Institute of Clinical Pharmacology, Insel University Hospital, CH-3010 Berne, Switzerland

Abstract

Renal cortical tubules consist of polarized epithelial cells where Na/H antiport activity has been demonstrated on the apical and/or basolateral membrane. Apical Na/H antiport activity plays an important role in transcellular bicarbonate (HCO_3^-) reabsorption, whereas basolateral Na/H antiport activity could be involved in transcellular HCO_3^- secretion as well as cell volume and pH control.

To determine whether this heterogeneity in both localization and function is due to the existence of more than one Na/H antiporter, we studied the tissue distribution of Na/H antiporter mRNA by use of reverse transcription (RT) and polymerase chain reaction (PCR) in isolated nephron segments from rat renal cortex. The primers used were directed against the rat renal cortical Na/H antiporter cDNA which is homologous to the human growth factor–activatable Na/H antiporter. RT/PCR of beta-actin mRNA were performed as positive controls.

Na/H antiporter mRNA expression in the proximal tubule was not detectable in S1 and S2 segments from superficial and most midcortical nephrons, which exhibit exclusively luminal Na/H antiport activity. It was expressed in S1 and S2 segments from juxtamedullary nephrons which have also basolateral Na/H antiport activity. Beta-actin mRNA was expressed uniformly in all segments of the proximal tubule. Na/H antiporter mRNA was also expressed in cortical thick ascending limb and cortical collecting duct, segments with basolateral Na/H antiport activity as well as in the glomeruli.

In conclusion, at least two different Na/H antiporters exist in the renal cortex, i.e., the proximal tubule. The close correlation between functional localization of basolateral Na/H antiport activity and mRNA expression suggests that the rat kidney Na/H antiporter DNA homologous to the human growth factor activatable Na/H antiporter encodes a basolateral exchanger. The observed expression in a minority of midcortical proximal tubules could reflect a certain heterogeneity in these nephron segments. (*J. Clin. Invest.* 1991. 88:783–788.) Key words: Na/H antiporter • mRNA expression • nephron segments • reverse transcription • polymerase chain reaction

Introduction

In the polarized renal cortical tubule cells, electroneutral, amiloride-sensitive Na/H antiport activity has been demonstrated on either the luminal (apical) and/or the basolateral side. When located on the apical membrane, the Na/H antiport activity effects proton secretion into the luminal fluid. The cellular bicarbonate ions thus generated are transported into the extracellular fluid across an electrogenic $Na/CO_3^{2-}/HCO_3^-$ symporter. This polarized, in series location of a luminal Na/H antiporter and a basolateral $Na/CO_3^{2-}/HCO_3^-$ symporter allows efficient transcellular bicarbonate reabsorption in the proximal tubule and the cortical thick ascending limb (1–3). In the proximal tubule, the luminal Na/H antiporter mediates as much as two-thirds of proximal tubule bicarbonate reabsorption (4).

The physiological role of Na/H antiport activity located on the basolateral membrane is less clear. In tubule segments with the capacity to secrete bicarbonate into the luminal fluid such as the cortical collecting duct, bicarbonate exit across the apical membrane would acidify the cells. This acid load would stimulate a basolateral Na/H antiporter to secrete protons into the extracellular fluid, thus completing the process of transcellular bicarbonate secretion (5). In tubule segments not known to secrete bicarbonate (e.g., cortical thick ascending limb, S1 and S2 segments of juxtamedullary nephrons, see below), a basolateral Na/H antiporter could assume an important role in intrinsic cell regulation such as cell pH defense and cell volume control as well as be involved in signal transduction (growth factor activation).

In the mammalian renal cortex, an exclusive apical location for the Na/H antiporter has been demonstrated functionally for the proximal convoluted tubule of superficial and midcortical nephrons (S1 and S2 segments; 6–9). The cortical thick ascending limb also has a luminal Na/H antiporter (2), but, in addition, shows basolateral Na/H antiport activity (3). Na/H antiport activity restricted to the basolateral membrane was found in the cortical collecting duct (5). In the proximal tubule, the existence and distribution of a basolateral Na/H antiporter is controversial. While Geibel and co-workers described recently that rabbit S1 and S2 segments from juxtamedullary nephrons exhibit basolateral Na/H antiport activity (9), no such evidence was found by Baum (10). In other cortical tubule segments (i.e., the distal convoluted and connecting tubules) no direct information about presence and location of Na/H antiport activity is available. Na/H antiport activity has been demonstrated, however, in the nonpolarized mesangial cells of glomeruli (11).

It is not known, whether the heterogeneity in distribution (location in S1 and S2 proximal tubule segments from superficial and midcortical versus juxtamedullary nephrons), polarity (location on apical versus basolateral membrane), and function (bicarbonate transport versus cell pH and volume control) of Na/H antiport activity in the mammalian renal cortex is due to differential epithelial sorting and regulation of one and the same antiporter or to the existence of more than one antiporter.

Evidence from LLC-PK1 cells, immortalized cells of renal tubule origin, which exhibit apical *and* basolateral Na/H anti-

Received for publication 25 March 1991.

J. Clin. Invest.

0021-9738/91/09/0783/06 $2.00
Volume 88, September 1991, 783–788

port activity, raises the possibility of the existence of two different antiporters. The apical antiporter exhibits low amiloride sensitivity (IC_{50} for the amiloride analogue ethylisopropylamiloride, ~ 10 μM), whereas the basolateral transporter is very sensitive to ethylisopropylamiloride inhibition (IC_{50} ~ 20 nM; 12). Thus, based on these inhibition kinetics, the apical form resembles the proximal tubule brush border Na/H antiporter, whereas the basolateral transporter behaves like the ubiquitous transporter found in many epithelial and nonepithelial cells (13). It was postulated, therefore, that LLC-PK1 cells contain two different Na/H antiporters: a basolateral, "housekeeping" Na/H antiporter involved in cell pH defense and volume regulation, and an apical Na/H antiporter involved in transcellular proton/bicarbonate and possibly sodium transport. In addition, recent preliminary evidence (14) suggests that apical and basolateral antiporters in these cells are regulated differently by Ca^{++}/calmodulin-dependent processes. Immunostaining with polyclonal antibodies against amino acid sequences of a cloned LLC-PK1 Na/H antiporter with ~ 95% homology to the human growth factor activatable Na/H antiporter, revealed exclusive localization of this transporter on the basolateral membrane in LLC-PK1 cells (15). In addition, a human Na/H antiporter probe was noticed to localize a basolateral Na/H antiporter in the polarized intestinal cells, Caco2 and HT29 (Huet, C., D. Louvard, C. Sardet, and J. Pouysségur, personal communication). However, in the bovine kidney, immunostaining of the cortex was predominantly localized apically, in the brush border membrane of proximal tubules (16). These immunohistochemical observations raise the important possibility that the expression of two different Na/H antiporters could be restricted to mutated, immortalized cell lines.

We have recently cloned and sequenced a partial-length rat renal Na/H antiporter cDNA (17) which exhibits ~ 95% homology to the human, growth factor–activatable Na/H antiporter (18) and have shown that mRNA expression of this transporter is enhanced in response to metabolic acidosis in vivo (19). If there are two different antiporters in the renal cortex, our cDNA would be expected to encode for the basolateral antiporter based on the observations from immunohistochemical experiments. Thus, in the case of two different antiporters, mRNA corresponding to our cDNA should not be expressed in S1 and S2 proximal tubule segments of superficial and midcortical nephrons. This mRNA should be expressed, however, in thick ascending limbs, cortical collecting ducts, possibly S1 and S2 proximal tubule segments from juxtamedullary nephrons and glomeruli.

The present studies were therefore designed to investigate the segmental localization of the mRNA expression of our recently cloned rat renal Na/H antiporter in rat renal cortex. We chose the technique of reverse transcription (RT)[1] of specific mRNA in microdissected, isolated nephron segments with subsequent amplification of the cDNA by the polymerase chain reaction (PCR).

Methods

All chemicals and materials were RNAse-free, of molecular biology grade and obtained from Sigma Chemical Co., St. Louis, MO, unless stated otherwise.

Experimental animals and microdissection. Male Wistar rats weighing 80–140 g were obtained from the Animal Breeding Facility at the Insel University Hospital, Berne, Switzerland. The animals had free access to water and commercial rat food.

Rats were anesthetized by intraperitoneal injection of pentobarbital and were sacrificed by decapitation. The aorta was cannulated with polyethylene tubing (PE 50) and the left kidney was selectively perfused with 10 ml of ice-cold dissection solution containing 1.5 mg/ml of collagenase (type 1). The dissection solution was bicarbonate-free and contained (in millimolar): NaCl 135, Na_2HPO_4 1, $MgSO_4$ 1.5, $CaCl_2$ 2, KCl 5, glucose 5, Hepes 5, titrated to pH 7.4 with NaOH (room temperature). After perfusion, the kidney was excised and the renal capsule was stripped off. The kidney was then cut into coronal slices of ~ 1 mm. The slices were transferred to individual tubes containing 1 ml of the same collagenase solution as used to perfuse the kidney and were incubated for 30–45 min at 37°C in a water bath. Before dissection, the slices were washed once with ice-cold collagenase-free dissection solution.

Tubule dissection was performed in ice-cold dissection solution (see above) using dissecting forceps (Dumont No. 5) and a dissecting microscope at 40× magnification and horizontal illumination. Tubule segments were identified according to established criteria (20). S1 and S2 proximal convoluted tubule segments (PCT) were dissected and studied separately according to their origin from superficial, juxtamedullary, or midcortical nephrons.[2] For the dissection of superficial nephrons, glomeruli high in the cortex (within 1 mm of the renal capsule) were located. For the dissection of juxtamedullary nephrons, glomeruli located < 1 mm from the outer edge of the medulla were chosen. Midcortical nephrons were from glomeruli located at least 1 mm away from the renal capsule and at least 1 mm from the outer edge of the medulla. S1 PCT segments were identified by their attachment to a glomerulus. PCT segments extending beyond 1 mm of the glomerulus were considered to be S2. Cortical thick ascending limbs and cortical collecting ducts were dissected from the medullary rays. Distal convoluted tubules and glomeruli were dissected from the cortical labyrinth. All attached tissue (blood vessels, tubule segments) were removed from glomeruli before transfer.

The length of the tubules was between 0.7 and 1 mm. Using a bent Pasteur pipette coated with a 0.1% BSA solution, the tubules were transferred from the dissection dish to fresh dissection solution to rinse the segments free of debris. The tubules were then captured with small glass beads (diameter, 425–600 μm) and transferred as before (new pipette) to the RT-PCR tube. The beads/tubules were rinsed three times with ice-cold dissection solution and finally suspended in 20 μl of dissection solution containing > 1 U/μl of human placental RNAase inhibitor (Promega Biotec, Madison, WI). Glomeruli, after rinsing in a wash dish, were transferred without glass beads and washed like the tubule segments. The reaction tubes were placed on ice until RT was begun (maximum 60 min).

Reverse transcription (RT). Reaction tubes were centrifuged for a few seconds (12,000 rpm) to pellet the beads/tubules and glomeruli. The dissection solution was carefully removed and 10 μl of lysis buffer containing 2% Triton X-100 (Boehringer-Mannheim GmbH, Mannheim, FRG), > 1.5 U/μl of RNAse inhibitor and 5 mM dithiothreitol were added. The contents were gently mixed by tapping and the reverse transcriptase reagents were added (final amounts/concentrations; reaction volume, 20 μl): antisense primer, 40 pmol; deoxynucleotides, 200 μmol; Tris-HCl, 10 mM, pH 8.3; KCl, 50 mM; $MgCl_2$, 2.5 mM; DTT, 1 mM; BSA, 1 mg/ml; reverse transcriptase, 2.5 U/μl (Moloney murine leukemia virus; New England Biolabs, Beverly, MA). Reaction tubes were incubated at 37°C for 60 min. Negative control reactions contain-

1. *Abbreviations used in this paper:* PCR, polymerase chain reaction; PCT, proximal convoluted tubule; RT, reverse transcription.

2. S3 proximal tubules were also studied in preliminary experiments. However, the results are not included here because the cortical portion of S3 segments is ill defined. In four out of four tested S3 segments, Na/H antiporter mRNA could be reverse-transcribed and amplified.

ing all the reagents save the reverse transcriptase were performed in parallel for each cortical tubule segment and glomeruli.

Polymerase chain reaction (PCR). For the Na/H antiporter gene, primer 1 (antisense) was 5'-ATCTGGTTCCAGGCTTCCTCG-TAGG-3' defined by bases 1997–1973 and primer 2 (sense) was 5'-CAAGAGACGAAGCGCTCCATCAACG-3' defined by bases 1534–1558 (base enumeration according to full-length human antiporter cDNA; 19, 20). Thus, the cDNA amplification product was predicted to be 464 bp in length. The amplified region of the rat renal Na/H antiporter cDNA has 98% homology to the growth factor–activated human Na/H antiporter (17, 18). While the intron/exon boundaries of the rat Na/H antiporter gene are not yet defined, the high homology between human and rat cDNA suggests interspecies conservation of intron/exon boundaries. Based on this comparison, the primers used amplify a cDNA segment encompassing three introns (21). An additional oligonucleotide (sense) 5'-GGTTGAGCTTGTCCTTCCAG-3' (defined by bases 1636–1655) was used as an amplification specific probe. RT and PCR of rat cytoplasmic beta-actin served as a positive control. The primers were defined by the following cDNA base sequences (22): primer 1 (antisense), bases 3078–3053, sequence, 5'-ACCTTCAACACCCCAGCCATGTACG-3'; primer 2 (sense), bases 2168–2193, sequence, 5'-CTGATCCACATCTGCTGGAAGGTGG-3'. These primers span two introns and result in a 703-bp cDNA amplification product (22).

All the primers were checked for the absence of fortuitous homology to any sequence in the EMBL data bank.

After RT, 30 μl of PCR reagent mix were added to each tube directly. The PCR reactions (50 μl) contained the following components (final amounts/concentrations): Taq polymerase 2 U (Boehringer-Mannheim GmbH), primers (40 pmol each), deoxynucleotides (200 μM), Tris-HCl (10 mM), pH 8.3 (room temperature), KCl (50 mM), $MgCl_2$ (2 mM), gelatine (1 mg/ml), Triton X-100 (1%). The reaction tubes were overlayered with 50 μl of mineral oil to prevent evaporation. PCR was performed in an automated thermal cycler (Easy Cycler; Ericomp, San Diego, CA) which was programmed as follows. First, incubation at 92°C for 2 min (initial melt), followed by 30 PCR cycles: 92°C for 10 (denature), 55°C for 60 (anneal), and 72°C for 100 s (extend). PCR was completed by a final extension (72°C, 10 min).

RT of Na/H antiporter and actin RNA from different tissues with subsequent amplification by PCR was also performed. Total RNA was isolated from rat kidney cortex, liver, skeletal muscle, heart, lungs, and spleen using guanidinium isothiocyanate (4 M) and cesium chloride gradient centrifugation (19). For RT, 100 ng total RNA were used. Reverse transcriptase concentration was 2 U/tube. All other reagents for RT and PCR were as described above.

For analysis of the PCR products, 40 μl of reaction volume were ethanol-precipitated. DNA was resuspended, and the PCR product size-fractionated on 2% agarose gels and stained with ethidium bromide (23).

For Southern blot analysis of PCR products, gels were denatured (NaOH), electrotransferred, and blotted onto Zeta-probe Nylon membranes (Bio-Rad Laboratories, Inc., Richmond, CA) as recommended by the supplier. To test for rat renal Na/H antiporter specific PCR amplification products, 1 pmol of the amplification product specific primer (20 mer oligonucleotide) was labeled with ^{32}P at the 5' end using T4 polynucleotide kinase (24) and used as a hybridization probe.

The actin-specific amplification product was analyzed on ethidium bromide stained agarose gels. No attempt was made to quantitate RT/PCR.

Results

The presence of Na/H antiporter mRNA was first analyzed by RT/PCR in single S1 and S2 proximal convoluted tubules from juxtamedullary nephrons, a cortical collecting duct segment and six glomeruli. For all these segments, there is functional evidence for the presence of a Na/H antiporter. Fig. 1 shows the 2% agarose gel (*left*) and the corresponding Southern blot analysis (*right*) of the amplification products. An amplification product of the predicted size was evident in the RT/PCR reactions from all tubule segments tested. The Southern blot of

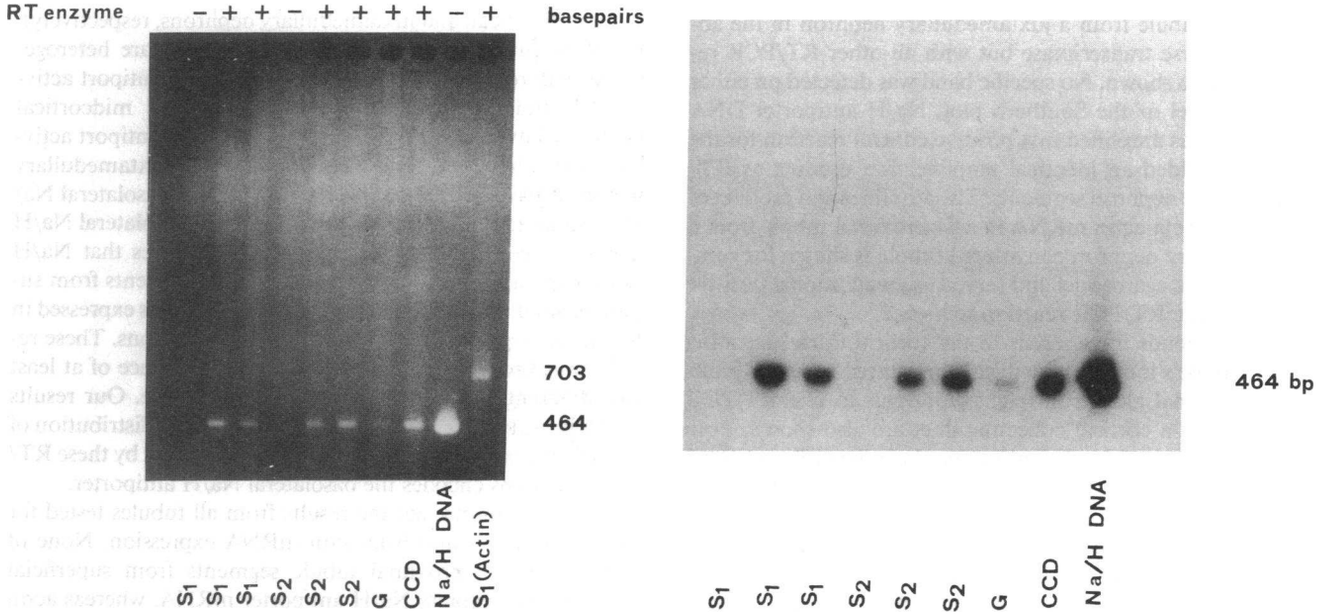

Figure 1. Product analysis of RT/PCR for Na/H antiporter mRNA in the proximal tubule of juxtamedullary nephrons (S1 and S2 segments), glomeruli (G, $n = 6$) and cortical collecting duct (CCD). (*Left*) an ethidium bromide stained 2% agarose gel; (*right*) an autoradiogram of the corresponding Southern blot. Results are shown from experiments performed in the presence (RT-enzyme +) and absence (RT-enzyme –, negative controls) of reverse transcriptase. The amplification product of PCR of Na/H antiporter DNA and the RT/PCR product of beta-actin mRNA served as positive controls.

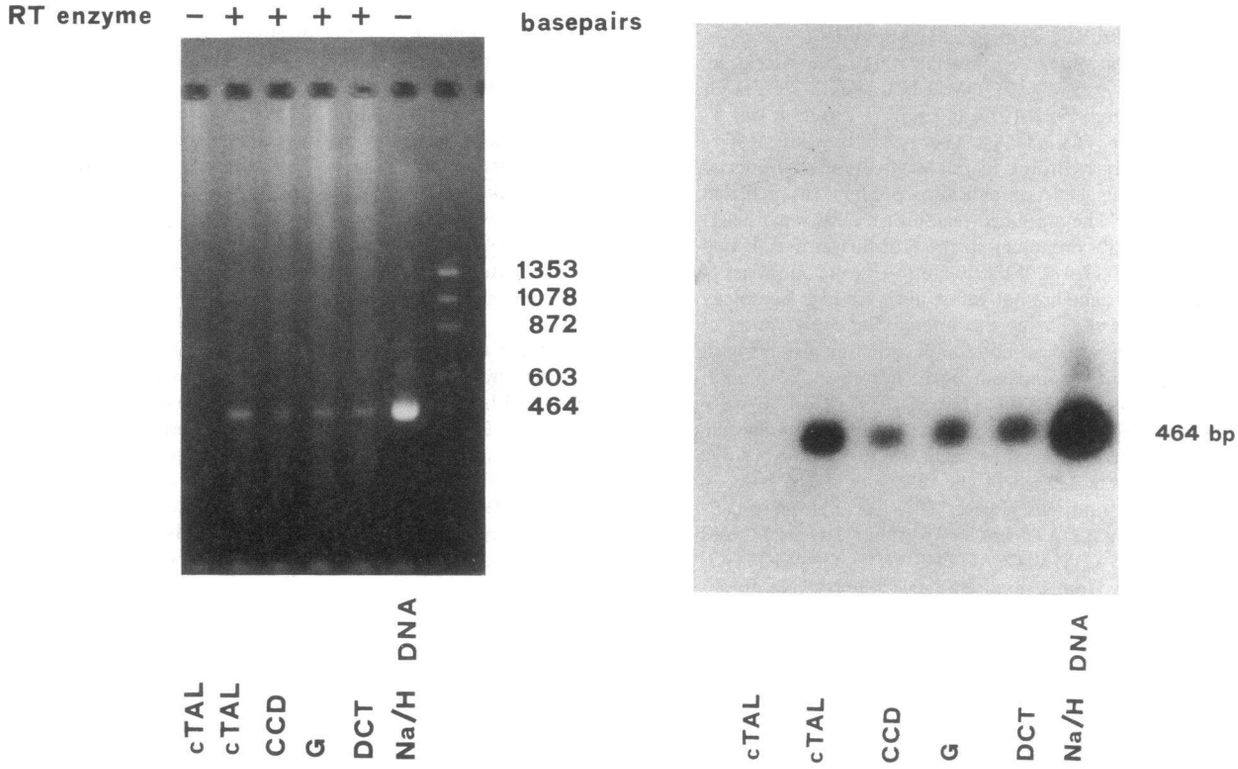

Figure 2. Product analysis for RT/PCR for Na/H antiporter mRNA expression in cortical thick ascending limb (cTAL), cortical collecting duct (CCD), glomeruli (G, $n = 8$) and distal convoluted tubule (DCT). (*Left*) An ethidium bromide stained 2% agarose gel; (*right*) autoradiogram of the corresponding Southern blot. Results are shown from experiments in the presence (RT-enzyme +) and absence (RT-enzyme −, negative control) of reverse transcriptase.

the gel demonstrates specific binding of the independent oligonucleotide to the product, thus confirming its identity. Negative control reactions using either an S1 or an S2 proximal convoluted tubule from a juxtamedullary nephron in the absence of reverse transcriptase but with all other RT/PCR reagents are also shown. No specific band was detected on either the agarose gel or the Southern blot. Na/H antiporter DNA (~ 0.5 ng) was amplified in a positive control reaction for the PCR and yielded an identical amplification product as RT/PCR from the nephron segments. The amplification product of RT/PCR of beta-actin mRNA in a S1 proximal tubule from a juxtamedullary nephron convoluted tubule is shown for comparison on the agarose gel and served as an additional positive control for the RT/PCR reaction sequence.

Fig. 2 extends the analysis to the cortical thick ascending limb of Henle's loop and the distal convoluted tubule. Results from additional glomeruli (eight glomeruli in one RT/PCR reaction) and a cortical collecting duct are also shown. Both apical and luminal Na/H antiporters have been found functionally in the cortical thick ascending limb, whereas there is no direct functional evidence for the presence of a Na/H antiporter in the distal convoluted tubule. Again, amplification products of the predicted length are shown on the agarose gel (Fig. 2, *left*). Their identity was confirmed by highly specific binding of the independent oligonucleotide on a Southern blot (Fig. 2, *right*). The negative control reaction using a cortical thick ascending limb did not show any bands. The identity and specificity of the RT/PCR product is further illustrated by the positive control reactions on Figs. 1 and 2.

The next experiments were designed to specifically analyze the distribution of Na/H antiporter mRNA in the six subsegments of the proximal tubule (S1 and S2 segments from superficial, midcortical, and juxtamedullary nephrons, respectively). Based on functional studies, proximal tubules are heterogeneous with respect to the distribution of Na/H antiport activity. S1 and S2 segments of superficial and midcortical nephrons have apical, but no basolateral Na/H antiport activity (6–9), whereas S1 and S2 segments from juxtamedullary nephrons have been shown to have apical and basolateral Na/H antiport activity, although the presence of basolateral Na/H activity is controversial (9, 10). Fig. 3 illustrates that Na/H antiporter mRNA was absent in S1 and S2 segments from superficial and midcortical nephrons, whereas it was expressed in S1 and S2 segments from juxtamedullary nephrons. These results thus provide strong evidence for the presence of at least two different antiporters in the proximal tubule. Our results and observations in studies about the functional distribution of Na/H antiporter suggest that the mRNA detected by these RT/PCR reactions encodes the basolateral Na/H antiporter.

Table I summarizes the results from all tubules tested for Na/H antiporter and beta-actin mRNA expression. None of the S1 and S2 proximal tubule segments from superficial nephrons did express Na/H antiporter mRNA, whereas actin mRNA expression was uniform. In contrast, all S1 and S2 proximal tubule segments from juxtamedullary nephrons were positive for Na/H antiporter mRNA. The table further illustrates that a clear majority of S1 and S2 segments from midcortical nephrons did not express Na/H antiporter mRNA. How-

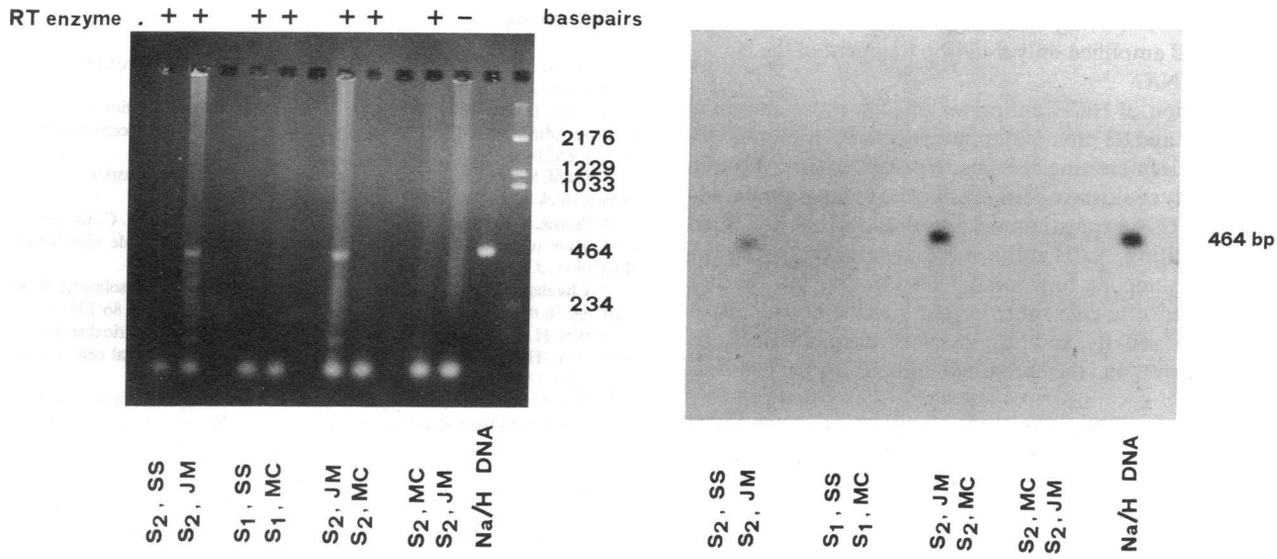

Figure 3. Product analysis of RT/PCR for Na/H antiporter mRNA expression in S1 and S2 proximal tubule segments of superficial (SS), midcortical (MC), and juxtamedullary (JM) nephrons. (*Left*) An ethidium bromide stained 2% agarose gel; (*right*) an autoradiogram of the corresponding Southern blot. Results are shown from experiments performed in the presence (RT-enzyme +) and absence (RT-enzyme −, negative control) of reverse transcriptase. PCR of Na/H antiporter DNA served as a positive control.

ever, a minority of segments (one out of 10 S1 segments and two out of 12 S2 segments) expressed Na/H antiporter mRNA. A false positive result in these tubules is excluded because we performed negative controls in all experiments (see Methods, Figs. 1–3). Thus, if the dissection criteria used (see Methods) reliably identify midcortical nephrons, there is apparent heterogeneity in these midcortical S1 and S2 segments with respect to Na/H antiporter mRNA expression.

In additional experiments, we wished to determine whether the RT/PCR reaction detected Na/H antiporter mRNA in several organs. If the Na/H antiporter mRNA detected by our RT/PCR encoded for the "housekeeping" Na/H antiporter involved primarily in cell pH and volume control, this mRNA would be expected to be expressed ubiquitously in extrarenal tissues. RT/PCR was performed on 100 ng of total RNA isolated from liver, skeletal muscle, lung, heart, and spleen. Amplification products of the predicted sizes were detected for Na/H antiporter mRNA and cytoplasmic beta-actin in all these organs (data not shown).

Table I. Na/H Antiporter and Beta-Actin mRNA Expression in Rat Renal Cortex

	Na/H antiporter (positive/total)	Beta-actin (positive/total)
Proximal tubule		
S1 superficial	0/6	6/6
S2 superficial	0/6	6/6
S1 midcortical	1/10	8/8
S2 midcortical	2/11	8/8
S1 juxtamedullary	6/6	6/6
S2 juxtamedullary	6/6	6/6
Cortical thick ascending limb	7/7	7/7
Distal convoluted tubule	5/5	5/5
Cortical collecting duct	8/8	8/8
Glomeruli	6/6	6/6

Discussion

These studies investigated the tissue distribution of mRNA expression of a rat Na/H antiporter homologous to the human growth factor–activatable antiporter. The main findings were: (*a*) this Na/H antiporter mRNA is expressed in glomeruli and all the cortical tubule segments that show basolateral Na/H antiport activity (i.e., S1 and S2 segments of juxtamedullary nephrons, cortical thick ascending limbs, cortical collecting duct); (*b*) this antiporter mRNA is not expressed in tubule segments with functional evidence only for apical, but not for basolateral Na/H antiport activity (i.e., S1 and S2 proximal tubule segments of superficial and the majority of midcortical nephrons); and (*c*) the antiporter mRNA expressed in segments with basolateral Na/H antiporter activity is indistinguishable from an antiporter mRNA expressed in a variety of nonepithelial, nonbicarbonate transporting tissues (i.e., spleen, heart, lung, skeletal muscle).

The results of our studies therefore provide strong evidence for the existence of at least two different Na/H antiporter proteins within the kidney cortex, i.e., the proximal tubule. Several observations from our study deserve special emphasis. First, in S1 and S2 proximal tubules from superficial and the majority of midcortical nephrons, there was no expression of mRNA corresponding to the Na/H antiporter cDNA with 95% homology to the human growth factor–activatable Na/H antiporter. In these segments, there is unequivocal evidence for a luminal Na/H antiporter that has a quantitatively important role in proximal tubule NaCl and bicarbonate reabsorption, whereas a basolateral Na/H antiporter has not been detected. Thus, this proximal tubule luminal Na/H antiporter is a different transporter than the basolateral Na/H antiporter and does not correspond to the mRNA detected by our method. It remains to be established whether this luminal Na/H antiporter is the product of a different gene or of an isoform of the same gene. Our results exclude posttranslational modification as the mechanism for the production of a distinct luminal Na/H antiporter.

Differential RNA splicing remains a possibility because we transcribed and amplified only a 464-bp fragment of the Na/H antiporter mRNA.

The detection of Na/H antiporter mRNA expression in a minority of S1 and S2 proximal tubule segments from midcortical nephrons is interesting. It is possible that basolateral Na/H antiport activity could have been overlooked in functional studies because mRNA expression was found in only ~ 10% of all tubules. In addition, Na/H antiport activity may be very low in these segments making functional detection difficult. Further studies are needed to gain more insight into the precise distribution, level of activity, and physiological role of a basolateral Na/H antiporter in the proximal tubule of midcortical nephrons.

Second, it is striking that mRNA expression of this Na/H antiporter closely parallels the functional evidence for the tissue distribution of a basolateral Na/H antiporter. In addition, an identical mRNA could be reversely transcribed and amplified in several nonepithelial tissues (heart and skeletal muscles, spleen, lung) and glomeruli. In these tissues, Na/H antiport may be predominantly involved in cell pH defense and cell volume regulation as well as in signal transduction (growth factor activation). Thus, the mRNA detected and amplified with our primers is probably the gene product of renal Na/H antiporter responsible for these "housekeeping" functions within the kidney. It is presently unclear whether housekeeping functions are effected by one or more antiporters. The gene expression of the antiporter detected by our method has been shown to be enhanced in response to in vivo metabolic, but not respiratory, acidosis (19). It remains to be seen whether the luminal Na/H antiporter is regulated similarly.

Third, our results predict the existence of a Na/H antiporter in cells from the distal convoluted tubule. In the cortical thick ascending limb, our results do not permit us to decide whether the Na/H antiporter mRNA studied is translated into the apical or basolateral Na/H antiporter. However, because the apical Na/H antiporter is involved in transcellular bicarbonate transport, it is more likely that the Na/H antiporter mRNA studied here is translated into the basolateral protein.

In conclusion, our study shows that mRNAs from at least two different Na/H antiporters are expressed in the rat renal cortex. A DNA/RNA with 95% homology to the human growth factor activatable Na/H antiporter encodes for a basolateral Na/H antiporter that is probably involved in "housekeeping" functions such as cell pH defense, signal transduction, and volume regulation.

Acknowledgments

The authors thank Arlyn Garcia-Perez, National Institutes of Health, Bethesda, MD, for her help in adapting the technique of RT/PCR to isolated nephron segments.

This work was supported by Swiss National Foundation grants 31-25370.88 to R. Krapf and 31-28577.90 to M. Solioz.

References

1. Alpern, R. J., and M. Chambers. 1986. Cell pH in the rat proximal convoluted tubule. *J. Clin. Invest.* 78:502–510.

2. Good, D. W. 1985. Sodium-dependent bicarbonate absorption by cortical thick ascending limb of rat kidney. *Am. J. Physiol.* 248 (Renal Electrolyte Physiol. 17): F821–F829.

3. Krapf, R. 1988. Basolateral membrane $H^+/OH^-/HCO_3^-$ transport in the rat cortical thick ascending limb. *J. Clin. Invest.* 82:234–241.

4. Preisig, P. A., H. E. Ives, E. J. Cragoe, R. J. Alpern, and F. C. Rector, Jr. 1987. Role of the Na^+/H^+ antiporter in the rat proximal tubule bicarbonate absorption. *J. Clin. Invest.* 80:970–978.

5. Chaillet, J. R., A. G. Lopes, and W. F. Boron. 1985. Basolateral Na-H exchange in the rabbit cortical collecting tubule. *J. Gen. Physiol.* 86:795–812.

6. Ives, H. E., J. V. Yee, and D. G. Warnock. 1983. Asymmetric distribution of the Na^+/H^+ antiporter in the renal proximal tubule epithelial cell. *J. Biol. Chem.* 258:13513–13516.

7. Sabolic, I., and G. Burckhardt. 1983. Proton pathways in the rat renal brush-border and basolateral membranes. *Biochim. Biophys. Acta.* 734:210–220.

8. Krapf, R., R. J. Alpern, F. C. Rector, Jr., and C. A. Berry. 1987. Basolateral membrane Na/base cotransport is dependent on CO_2/HCO_3^- in the proximal convoluted tubule. *J. Gen. Physiol.* 90:833–853.

9. Geibel, J., G. Giebisch, and W. F. Boron. 1989. Basolateral sodium-coupled acid-base transport mechanisms of the rabbit proximal tubule. *Am. J. Physiol.* 257(Renal Fluid Electrolyte Physiol. 26):F790–F797.

10. Baum, M. 1990. Neonatal rabbit juxtamedullary proximal convoluted tubule acidification. *J. Clin. Invest.* 85:499–506.

11. Boyarski, G., M. B. Ganz, E. J. Cragoe, and W. F. Boron. 1990. Intracellular pH dependence of Na-H exchange and acid-loading in quiescent and arginine vasopressin-activated mesangial cells. *Proc. Natl. Acad. Sci. USA.* 87:5921–5924.

12. Haggerty, J. G., N. Agarwal, R. F. Reilly, E. A. Adelberg, and C. W. Slayman. 1988. Pharmacologically different Na/H antiporters on the apical and basolateral surfaces of cultured porcine kidney cells (LLC-PK_1). *Proc. Natl. Acad. Sci. USA.* 85:6797–6801.

13. Friedrich, T., J. Sablotni, and G. Burckhardt. 1987. Species differences between rat and rabbit renal Na^+/H^+ exchangers. *Biochem. Biophys. Res. Commun.* 144:869–875.

14. Burns, K. D., T. Homma, and R. C. Harris. 1990. Calmodulin antagonism inhibits basolateral and activates apical Na^+/H^+ exchange in LLC-PK_1/Cl_4. *JASN.* 1:714. (Abstr.)

15. Reilly, R. F., D. Biemesderfer, F. Hildebrandt, M. Exner, C. Sardet, J. Pouysségur, C. Slayman, and P. S. Aronson. 1990. Cloning and immunolocalization of a Na^+/H^+ exchanger in LLC-PK_1 cells. *JSAN.* 1:743. (Abstr.)

16. Ross, W. R., P. Breen, M. Hammerman, and A. R. Morrison. 1990. Immunohistochemical localization of the bovine Na^+/H^+ exchanger. *JASN.* 1:730. (Abstr.)

17. Pearce, D., R. Krapf, F. C. Rector, Jr., and T. L. Reudelhuber. 1990. Cloning of a partial length rat kidney cDNA homologous to the human Na/H antiporter. *Kidney Int.* 33:233. (Abstr.)

18. Sardet, C., A. Franchi, and J. Pouysségur. 1989. Molecular cloning, primary structure, and expression of the human growth factor-activatable Na/H antiporter. *Cell.* 56:271–280.

19. Krapf, R., D. Pearce, C. Lynch, X.-P. Xi, T. L. Reudelhuber, J. Pouysségur, and F. C. Rector, Jr. 1991. Expression of rat renal Na/H antiporter mRNA levels in response to respiratory and metabolic acidosis. *J. Clin. Invest.* 87:747–751.

20. Kaissling, B., and W. Kriz. 1979. Structural analysis of the rabbit kidney. *Ad. Anat. Embryol. Cell Biol.* 56:1–121.

21. Miller, R. T., R. Lifton, L. Cunillon, G. Pages, C. Sardet, and J. Pouysségur. 1990. Structure of the human Na/H antiporter gene and 5′ flanking regulatory region. *JASN.* 1:742. (Abstr.)

22. Nudel, U., R. Jahut, M. Shani, S. Neuman, Z. Levy, and D. Yaffe. 1983. The nucleotide sequence of the rat cytoplasmic beta-actin gene. *Nucleic Acids Res.* 11:1759–1771.

23. Sambrook, J., E. F. Fritsch, and T. Maniatis. 1989. Molecular Cloning. Second ed. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

24. Chaconas, G., and J. H. van den Sonde. 1980. 5′-^{32}P labeling of RNA and DNA restriction fragments. *Methods Enzymol.* 65:75–88.