Carbonic anhydrase II and IV mRNA in rabbit nephron segments: stimulation during metabolic acidosis

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Tsuruoka, Shuichi, Ann M. Kittelberger, and George J. Schwartz. Carbonic anhydrase II and IV mRNA in rabbit nephron segments: stimulation during metabolic acidosis. Am. J. Physiol. 274 (Renal Physiol. 43): F259–F267, 1998.—Carbonic anhydrase (CA) facilitates renal bicarbonate reabsorption and acid excretion. Cytosolic CA II catalyzes the buffering of intracellular hydroxyl ions by CO₂ whereas membrane-bound CA IV catalyzes the dehydration of carbonic acid generated from the secretion of protons. Although CA II and IV are expressed in rabbit kidney, it is not entirely clear which segments express which isoforms. It was the purpose of this study to characterize the expression of CA II and CA IV mRNAs by specific segments of the nephron using semiquantitative reverse transcription-polymerase chain reaction (RT-PCR) and to determine the effect of chronic metabolic acidosis on CA expression by those segments. Individual nephron segments (usually 1–2 mm) were isolated by microdissection and subjected to RT-PCR. Amplification was performed simultaneously for CA IV, CA II, and malate dehydrogenase (MDH), a housekeeping gene. The intensities of the PCR products were quantitated by densitometry. CA IV mRNA was expressed by S1 and S2 proximal tubules and by outer medullary collecting duct from inner stripe (OMCDi) and outer stripe and initial inner medullary collecting duct (IMCDi). CA II mRNA was expressed by S1, S2, and S3 proximal tubules, thin descending limb, connecting segment (CNT), and all collecting duct segments. Acid loading induced CA IV mRNA expression in S1 and S2 proximal tubules and in OMCDi and IMCDi. CA II mRNA was induced by acidosis in all three proximal segments and nearly all distal segments beginning with CNT. No upregulation of MDH mRNA expression occurred. These adaptive increases in CA II and IV mRNAs are potentially important in the kidney’s adaptation to chronic metabolic acidosis.

reverse transcription-polymerase chain reaction; microdissected tubule segments; ribonucleic acid; Southern analysis; malate dehydrogenase

THE KIDNEY PLAYS a major role in regulating acid-base homeostasis (1). Urinary acidification is believed to be facilitated by the enzyme carbonic anhydrase (CA), which exists in two renal isoforms. More than 95% of the activity is located in the cytosol as CA II, whereas up to 5% is membrane bound and corresponds to CA IV (5, 22, 45). Cytosolic CA II facilitates H⁺ secretion by catalyzing the formation of HCO₃⁻ from cellular OH⁻ generated from the secretion of protons in the presence of CO₂ (20, 23, 24). CA II also facilitates the diffusion of CO₂ through aqueous solutions at physiological pH (10, 13), and this function may help mediate renal H⁺ secretion. It is likely that CA II is important in renal acidification, because patients and mice with CA II deficiency exhibit renal tubular acidosis (3, 29, 34, 38).

CA IV catalyzes the dehydration of intraluminal carbonic acid that results from the secretion of protons into the lumen (8, 20). Membrane-bound CA IV activity has been detected in the brush-border and basolateral membranes of the proximal tubules (22, 28, 44) and in the apical membranes of intercalated cells (17, 26). Functional studies have identified luminal CA activity in rat proximal convoluted tubules (19), along the inner stripe of rabbit outer medullary collecting duct (OMCDi) (36) and in the initial segment of rat inner medullary collecting duct (IMCDi) (42). It is likely that CA IV facilitates renal H⁺ secretion, because, in CA II-deficient patients and mice, inhibition of CA activity diminished renal acid excretion (3, 33). Furthermore, inhibition of luminal CA reduced bicarbonate reabsorption in the proximal tubule by 80% (19).

During metabolic acidosis the kidney responds by increasing H⁺ secretion in the proximal tubule and collecting duct (1). The renal response to acidosis is complex, involving increases in protein and DNA and RNA synthesis (18). Despite its high turnover rate, CA II hydratase activity, as well as mRNA, has also been found to be increased during chronic metabolic acidosis (4, 30). Moreover, sodium dodecyl sulfate (SDS)-resistant CA hydratase activity (presumably CA IV activity) was increased in the cortex of acidotic rabbits (5), while CA IV mRNA was increased in the cortex and outer medulla of similarly treated rabbits (43). Because of the regulation of these CA isoforms, it is likely that they play an important role in the adaptive response to acidosis. Particularly for CA IV, which is much less abundant in the kidney, increased membrane-bound enzyme might be necessary to mediate the dramatically increased rate of H⁺ secretion.

Thus the purpose of the present study was to investigate the expression of CA II and IV mRNA expression along specific isolated tubule segments of the rabbit nephron using the reverse transcription-polymerase chain reaction (RT-PCR). We also examined tubules obtained from acid-treated rabbits in semiquantitative studies to determine whether CA II or IV mRNA expression is regulated in specific segments of the nephron.

METHODS

Animals. Female New Zealand White rabbits weighing 1.5–3 kg and maintained on normal laboratory chow (Purina lab diet no. 5326; Purina Mills, Richmond, IN) plus free access to tap water were used as normal control rabbits in this study. An additional group of rabbits of comparable weight was acid treated for 3 days by providing 75 mM NH₄Cl added to a 7.5% sucrose drinking solution, which yielded an acid equivalent load of 10–15 meq·kg⁻¹·day⁻¹; acidosis was
The animals were killed by intracardiac injection of 130 mg pentobarbital sodium after premedication with ketamine (44 mg/kg) and xylazine (5 mg/kg). Heparinized blood was taken from the ear artery after premedication but prior to the time of death. Urine was obtained by postmortem bladder aspiration. A kidney was removed and placed in chilled dissection solution containing (in mM) 145 NaCl, 2.5 K$_2$HPO$_4$, 2 CaCl$_2$, 1.2 MgSO$_4$, 5.5 α-glucose, 1 trisodium citrate, 4 sodium lactate, and 6L-alanine, pH 7.4, 290 ± 2 mosmol/kgH$_2$O (31). Coronal sections of 1–2 mm were made from the center of each kidney and stored in chilled dissection solution containing 10 mM vanadyl ribonucleoside complex (VRC; 5 Prime → 3 Prime, West Chester, PA) to inhibit RNA degradation (31).

In some cases, the kidneys were perfused through the renal arteries first with chilled dissection medium until they blanched and then with the same solution to which 1 mg/ml bovine serum albumin (BSA) and 1 mg/ml collagenase (type I; Sigma Chemical, St. Louis, MO) were added. The kidney was removed, cut coronally, and 1-mm slices were placed in prewarmed dissection solution containing BSA and collagenase-digested with gentle bubbling for 30 min at 37°C. The incubated tissue was washed with cold dissection solution and stored in chilled dissection solution containing 10 mM VRC to inhibit RNA degradation (31).

Tubule isolation. Tubule segments were isolated under a dissecting microscope using sharpened forceps (40). They were microdissected in chilled dissection medium plus 10 mM VRC (5 Prime → 3 Prime) to inhibit RNA degradation (31). The segments were generally cut to 1 mm in length, as measured using a calibrated eyepiece micrometer. Each segment was rinsed in phosphate-buffered saline (PBS) and then twice in PBS containing 5 mM dithiothreitol (DTT) and 0.7 U/µl ribonuclease inhibitor (RNAsin; Promega, Madison, WI). The washed segment was transferred to a 0.5-ml microcentrifuge tube containing 10 µl of Trition X-100 mix (composed of 2% Triton X-100, 0.7 U/µl RNAsin, and 5 mM DTT). Segments <1 mm were pooled to obtain 1–2 mm in total length; descending and ascending thin limbs were pooled to provide ≥3 mm in total length, and five glomeruli were pooled for each batch. The samples were frozen at −80°C until used (31) to facilitate comparisons with segments along the nephron and with tubules from acidotic rabbits obtained at different times. Standard nomenclature and abbreviations were used for describing the tubule segments (16). From the non-collagenase-treated kidneys, we obtained a proximal straight tubule from the outer cortex (S2 segment), a cortical collecting duct (CCD), and an OMCD. From the collagenase-treated kidneys were obtained a proximal straight tubule from the outer cortex (S2 segment), a cortical collect-

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Gene expression by RT-PCR. Reverse transcriptase was accomplished by adding to the lysed segments or total RNA in the Triton X-100 mix 4 µl of 5× RT buffer (Promega), 2 µl deoxynucleotide mixture (1.25 mM stock of each nucleotide) (Pharmacia LKB Biotechnology, Piscataway, N.J.), 1 µl (40 U) RNAsin, 2 µl oligo(dT) (80 pmol; Pharmacia), and 1 µl (8 U/µl) avian myeloblastosis virus reverse transcriptase (Promega) and incubating 1 h at 42°C (31). The reverse transcriptase was inactivated by heating to 90°C for 5 min, and the contents were cooled to 4°C before allocating for PCR.

The conditions of PCR were as follows: 20 µl of RT product were divided into three aliquots, 12 µl of which was amplified for CA IV, 3 µl for CA II, and 5 µl for malate dehydrogenase (MDH), a housekeeping gene (31), which does not vary much during chronic metabolic acidosis (30). To the RT product was added 10 µl of 10× PCR buffer, 2 µl of deoxynucleotide mixture (10 mM stock of each nucleotide), 1 µl of each sense and antisense primers (~60 pmol), and sterile water with variable amounts of MgCl$_2$, resulting in a total reaction volume of 99.5 µl plus 0.5 µl (2.5 U) Taq polymerase. The final Mg$^{2+}$ concentration was 1.2 mM for the CA IV amplification and 1.5 mM for the CA II and MDH reactions. Amplification was performed on a thermal cycler (M Research, Watertown, MA) using the following program: initial melt at 94°C for 3 min, 40 cycles at 94°C for 1 min (melt), 52°C for 1 min (anneal), and 72°C for 3 min (extend), followed by a final 7-min extension at 72°C and storage at 4°C.

Each RT-PCR run was composed of several tubules from control rabbits, sometimes accompanied by similar segments from acidic animals, with the range of signal defined by using 5 and 500 ng of an aliquot of total RNA from kidney cortex. We consistently observed increased expression over this range of RNA in titrations, or else the experiment was discarded. Tubules from acid-treated animals were always run in parallel with those from control animals to ensure that interassay variation was not causing the observed differences. Controls included tubules, 500 ng of total RNA without reverse transcriptase (RT−), and no RNA template.

The sequence of the rabbit CA IV primers was 5′-ACA GGG GCT CGG AGA ACA G 3′ (sense, bases 362–380, counting from the initial translated ATG) and 5′-GGA AGC CAC ACC ATC AGA GG 3′ (antisense, bases 961–980) (43); this primer was in the 3′-untranslated region (G. J. Schwartz and C. A. Winkler, unpublished observations). The expected size of the CA IV RT-PCR product was 618 bp. The sequence of the rabbit CA II primers was 5′-ATG TCC CAT CAC TGG TGG TAC 3′ (sense, bases 1–21) and 5′-TGG CTC CTC AGG TTC CGC 3′ (antisense, bases 978–998) (30). The expected size of the RT-PCR product was 738 bp. The sequence of the rat MDH primers was 5′-CAA GAA GCA TGG CGT ATG CAA CCC 3′ (sense, bases 466–489) and 5′-TTT CAG CTC AGG GAT GGC CTC G 3′ (antisense, bases 952–973) (12). The expected size of the MDH RT-PCR product was 507 bp.

Twenty microliters of each sample was size fractionated by electrophoresis on 2% agarose gels (one each for CA II, CA IV, and MDH), and the single-banded products were visualized by ultraviolet fluorescence after ethidium bromide staining and photographed. Many of the gels were transferred to Nylon filters (Zeta-; Probe; Bio-Rad, Richmond, CA) and hybridized...
ized at 60°C overnight in a Southern analysis to cDNA probes specific for rabbit CA II, CA IV, or MDH, as previously described (30, 43). To ensure adequate assessment of the signal, somewhat underexposed autoradiograms and photographs were used for densitometry.

Analysis and statistics. The intensity of each RT-PCR signal in the autoradiograms and in photographs of ethidium bromide-stained agarose gels was quantitated by densitometry (SigmaGel; Jandel, San Rafael, CA). The nephron segments were examined in 18 separate RT-PCR runs. The signals were quantitated by densitometry, which was performed on both the Southern analyses and the ethidium bromide fluorescence photographs in 14 of 18 studies; these results were averaged. In four studies for which we performed no Southern analysis, the densitometric data from ethidium bromide fluorescence photographs were used alone. The densitometric data for each nephron segment were expressed per millimeter tubular length as a percentage of that generated from 500 ng of total kidney cortex RNA, with the latter being set to 100%; data from pairs of segments from control and acid-treated animals were expressed per segment, since the tubule lengths were nearly identical and the amplification factor was likely to be nonlinear.

Data are presented as means ± SE. Comparisons among tubules were analyzed by one-way analysis of variance (ANOVA) plus the Tukey-Kramer test for multiple comparisons. Comparisons between tubules from control vs. acid-treated animals were analyzed by unpaired t-tests. Statistical software was used (NCSS, Kaysville, UT). Significance was asserted when P < 0.05.

RESULTS

Animal data. Rabbits ingesting ammonium chloride in the drinking water sustained a moderate metabolic acidosis with blood pH 7.20 ± 0.03 and bicarbonate 17.1 ± 1.4 mM, compared with controls with 7.34 ± 0.02 pH and 25.4 ± 1.2 mM, respectively (P < 0.01 for both comparisons). Concomitant with the acidosis, the urine was acidic with pH 4.9 ± 0.3 compared with controls of 7.8 ± 0.2 (P < 0.01). There was no difference in weight between control animals (2.5 ± 0.1 kg, n = 13) and acid-loaded rabbits (2.4 ± 0.1 kg, n = 8).

RNA titration. An increased ethidium bromide fluorescent signal was observed for increasing amounts of total kidney RNA that were reverse transcribed and amplified for the PCR product of CA IV (Fig. 1A), CA II (not shown), and MDH (Fig. 1B), thus indicating adequate sensitivity of our RT-PCR system. Similar findings were observed after transferring the PCR products to a filter, hybridizing with one of the cDNA probes, and analyzing the autoradiogram, as seen in the Southern analysis of CA II in Fig. 1C. The densitometric values in the titrations increased but not in a linear manner; the densitometric values for the RNA titration in the CA II Southern shown in Fig. 1C were 240, 1,275, 1,675, 2,512, and 5,640 arbitrary units for 5, 10, 25, 50 and 500 ng RNA, respectively. The mean densitometric values for 5 ng total RNA, analyzed in four tubule studies after setting 500 ng to 100%, were 8.3 ± 2.1, 14.0 ± 3.1, and 17.5 ± 2.7% for CA IV, CA II, and MDH, respectively (n = 4, each).

Tubules from control rabbits. The lengths and numbers of segments examined are shown in Table 1. All segments expressed MDH mRNA at approximately one-third the signal observed with 500 ng total kidney cortex RNA (Fig. 2A). There were significant differences among the segments in the one-way ANOVA (F = 3.3, P < 0.01). The highest expression per millimeter tubule length was observed in DCT, OMCD, CNT, MTAL, IMCD, and IMCD, whereas the lowest was seen in DTL and ATL (for which 3 mm of segment was used for RT-PCR). The five glomeruli gave an intermediate signal (26 ± 5% per glomerulus). Because we performed concurrent amplifications of the reverse
transcribed template for CA II, CA IV, and MDH, the lack of expression of MDH was used as a criterion for elimination of a tubule or glomerular segment from further analysis.

Only five segments expressed CA IV mRNA (Fig. 2B): S1 and S2 proximal tubules and OMCDo, OMCDi, and IMCD segments (ANOVA, F = 17.2, P < 0.01). The highest level was observed in IMCDi, reaching 64% of that from 500 ng total RNA. There were slightly lower signals in S2, OMCDo, S1, and OMCDi, reaching 25–41% of 500 ng total RNA. Glomeruli and other segments showed undetectable CA IV mRNA expression under the conditions used in this RT-PCR.

Most of the proximal and collecting duct segments expressed CA II mRNA (ANOVA, F = 5.5, P < 0.01; Fig. 2C). Levels of CA II RT-PCR product attaining 29–50% of the 500 ng total RNA sample were detected in OMCDo, IMCDi, IMCDt, OMCDo, S2, CNT, and S1. Approximately 5–17% of the 500 ng total RNA RT-PCR signal was detected in CNT, S3, and DTL segments. No CA mRNA was detected under these conditions in glomeruli, thick and thin ascending limbs, or DCT.

Effect of metabolic acidosis. Some tubule segments taken from acidotic animals expressed more CA mRNA than those taken from controls. Figure 3A shows two OMCDi segments, each of comparable size (1 mm) from a control and acid-treated animal. It can be seen that the OMCDi from the acid-treated rabbit expressed more CA IV RT-PCR product than that from the control animal, whereas there was no difference in the expression of MDH mRNA. Note also that there was no signal in the absence of reverse transcriptase and that the 5 and 500 ng samples of total RNA bracketed the range of intensities of the RT-PCR signal expressed by each tubule for CA IV and MDH.

Table 1. Numbers and lengths of segments

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<td>Glomerulus</td>
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<td>S1 segment</td>
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<td>S2 segment</td>
<td>19</td>
<td>1.6 ± 0.1</td>
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<td>S3 segment</td>
<td>9</td>
<td>1.4 ± 0.1</td>
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<td>DTL</td>
<td>5</td>
<td>3.2 ± 0.2</td>
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<td>ATL</td>
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<td>3.0 ± 0.2</td>
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<td>MTAL</td>
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<td>1.0 ± 0.1</td>
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<td>CTAL</td>
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<td>DCT</td>
<td>5</td>
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<tr>
<td>CNT</td>
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<td>CCD</td>
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Values are means ± SE; n = no. of tubules. Five glomeruli comprised a segment. DTL, descending thin limb; ATL, ascending thin limb; MTAL, medullary thick ascending limb; CTAL, cortical thick ascending limb; DCT, distal convoluted tubule; CNT, connecting tubule; CCD, cortical collecting duct; OMCDo, outer stripe of outer medullary collecting duct; OMCDi, inner stripe of OMCD; IMCDi, initial inner medullary collecting duct; IMCDt, terminal IMCD. None of the differences between tubules from control vs. acidotic animals reached statistical significance by unpaired t-test.

Fig. 2. Expression of mRNA per millimeter length of nephron segments. Individual nephron segments were isolated by microdissection, rinsed, lysed in Triton X-100, and frozen in Eppendorf tubes. Each segment was reverse transcribed, and the template was divided for simultaneous amplification by PCR, using primers specific for each of the genes as described in METHODS. Intensity of the PCR product derived from each segment was expressed per mm tubular length as a percentage of 500 ng total RNA, which was set to 100%. Average tubule length was 1–2 mm, except for thin limbs, which were 3–4 mm. Glomeruli numbered 5/batch, but are not shown here. A: MDH mRNA was expressed by each segment at approximately one-third the level observed from 500 ng total RNA. Glomeruli expressed MDH at 26% of 500 ng RNA or 5%/glomerulus (not shown). Failure to express MDH resulted in exclusion of the segment. B: CA IV mRNA was expressed by S1 and S2 proximal tubules and by the medullary collecting duct, with the highest levels observed in initial inner medullary collecting duct (IMCDi). No expression was observed in limbs, distal convoluted tubule (DCT), or glomeruli. C: CA II mRNA was expressed by cortical proximal segments and all collecting duct segments at levels of 29–50% of 500 ng total RNA, with the highest level in outer stripe of outer medullary collecting duct (OMCDi). Lesser expression was observed in S3, descending thin limb from loop of Henle (DTL), and connecting segment (CNT). No expression was observed in the ascending limbs, glomeruli, or DCT. S1, proximal convoluted tubule; S2, cortical proximal straight tubule; S3, proximal straight tubule from the outer medulla; ATL, ascending thin limb; MTAL, medullary thick ascending limb; CTAL, cortical thick ascending limb; CNT, connecting tubule; OMCDo, outer stripe of outer medullary collecting duct; OMCDi, inner stripe of OMCD; IMCDi, initial inner medullary collecting duct; IMCDt, terminal IMCD.
CA IV also appeared upregulated in S1, S2, OMCDi, and IMCDi segments from an acid-treated animal, compared with comparable segments from a control rabbit (Fig. 3B). CA II appeared upregulated in S1, S2, S3, CNT, CCD, OMCDo, OMCDi, and IMCDi segments from an acid-treated animal, compared with control segments. Acid treatment did not appear to induce CA IV or CA II in segments that did not express these genes under basal conditions. Glomeruli and tubules each expressed MDH in approximately comparable amounts (bottom).

CA IV mRNA expression was significantly increased by metabolic acidosis in all proximal segments and nearly all collecting duct segments (Fig. 4B). The increase associated with acidosis was from 38 ± 6 to 79 ± 10% in S1, 38 ± 5 to 90 ± 13% in S2, 20 ± 4 to 43 ± 3% in S3, 17 ± 3 to 50 ± 13% in CNT, 39 ± 5 to 73 ± 16% in CCD, 49 ± 2 to 88 ± 14% in OMCDo, 39 ± 5 to 71 ± 6% in OMCDi, 33 ± 8 to 70 ± 11% in IMCDi, and 35 ± 10 to 75 ± 15% in IMCDt (P < 0.05 for each comparison, except for that of IMCDt, which reached 0.06). The change in DTL did not reach significance. A signal for CA II mRNA was not detected in segments from acidotic animals for which the control tubules had been negative (see Fig. 4B).

MDH mRNA expression was not significantly changed in any tubule segment by metabolic acidosis (Fig. 4C).

DISCUSSION

CA IV. This is the first study to report the expression of CA mRNA in single segments along the nephron. Using semiquantitative RT-PCR methodology, we have shown that CA IV mRNA was expressed in H+-secreting segments of the nephron, the proximal tubules, and medullary collecting ducts. The association of CA IV mRNA with H+ secretion suggests a role for CA IV in mediating bicarbonate reabsorption and acid excretion in the kidney. Indeed, selective inhibition of membrane-bound CA IV activity by a dextran-bound CA inhibitor inhibited at least 80% of bicarbonate reabsorption by the in vivo microperfused proximal tubule (19). Other functional studies have shown evidence for luminal CA in the rabbit OMCD (36) and IMCD of the rat (42); no functional evidence for membrane-bound CA activity was detected in the rabbit CCD (37) or OMCD (36) or in the rat CCD (15) or OMCD (11).

Histochemical studies in CA II-deficient mice have detected CA hydratase activity (presumably CA IV activity) along the membranes of cortical proximal tubules, initial thin descending limb, thick ascending limb in cortex and medulla, late distal tubule, cortical and outer medullary intercalated cells, and medullary collecting duct cells around the inner stripe and initial
inner medulla (26). No comparable studies in CA II-deficient rabbits have been reported, but species differences in CA IV expression are likely.

Histochemical studies in rabbit have shown the presence of apparent membrane-bound CA activity in S1, S2, and S3 proximal tubule segments, cortical and medullary intercalated cells, and in collecting duct principal cells in the region of the inner stripe and initial inner medulla (9, 25, 27). Thus there is good agreement among the functional studies, histochemical localization of membrane-bound CA, and our RT-PCR data. In addition, our laboratory has recently shown by in situ hybridization CA IV mRNA over inner medullary collecting duct cells (43). The major disagreement among the studies is the histochemical finding of CA on apical membranes of cortical intercalated cells, a finding that was not corroborated by previous functional studies or by our RT-PCR data from CCD and CNT. Perhaps the histochemical label cannot clearly distinguish cytosolic from membrane-bound CA activity, because little or no mRNA coding for CA IV was detected in CCDs or CNTs. Also, we did not confirm the presence of CA IV mRNA in S3 segments that stained positively in the medulla (9, 27); interestingly, the S3 segment in mouse and dog was negative (9, 27). Indeed, Rosen (27) noted in some preparations that “generally the finalFig. 4. Expression of mRNA by nephron segments. A: CA IV mRNA expression was increased in S1 and S2 proximal tubules and in OMCDi and IMCDi of acid-treated rabbits. Induced levels approached 100% of 500 ng total RNA sample run with each batch of segments. B: CA II mRNA expression was increased in all proximal tubule segments, in CNT, and in most collecting duct segments of acid-treated rabbits. Increase in IMCDt reached P = 0.06 level of significance. After acid treatment, expression of CA II mRNA approached 70–90% of the 500 ng total RNA sample in most segments. Acid-treated S3 segments reached one-half of that level. C: MDH mRNA expression was not statistically changed in any segments from acid-treated rabbits. Solid bars, tubules from controls; hatched bars, tubules from acid-treated animals. *P < 0.05, significant increase in segment RT-PCR product from acid-treated animals vs. from control rabbits.
reaction product was distributed too diffusely to allow precise intracellular localization.”

An examination of the immunocytochemical localization of CA IV in rabbit kidney has thus far not been published. Studies in rat using an affinity-purified antibody raised against the 39-kDa CA IV from rat lung localized CA IV to plasma membranes of cortical proximal tubules (with S2 > S1) and of cortical and medullary thick ascending limbs (7). No label was detected in intercalated cells or in cells of outer medullary and inner medullary collecting ducts. Thus there is substantial disagreement between our findings in the thick limb, OMCD, and IMCD and those of Brown et al. (7), who did not immunolocalize CA IV to the apical membranes of medullary collecting ducts but did find label in thick ascending limbs from cortex and medulla. The fact that the functional and histochemical findings support much of our data would indicate that the antibody used by Brown et al. (7) does not recognize CA IV epitopes in the medullary collecting duct.

During metabolic acidosis, CA IV mRNA was increased in proximal and medullary collecting duct segments, thus confirming previous studies showing increased CA IV mRNA in cortex and outer medulla (43) and increased SDS-resistant hydratase activity in nephron segments from acid-loaded animals (5). Metabolic acidosis induced a tripling of CA IV mRNA levels in cortex and outer medulla (43). There was too much scatter in the inner medulla (a mixture of initial and terminal regions) to show consistent upregulation of CA IV mRNA with acidosis. In the CA activity study, we demonstrated significant increases in SDS-resistant hydratase activity in proximal convoluted tubules (S1); the increases in proximal straight tubules (S2) and OMCDs did not reach significance. The lack of sensitivity of the hydratase assay might have prevented us from demonstrating the changes induced by acidosis. Also, the OMCD was not subdivided into inner stripe and outer stripe segments, which might have obscured changes in the OMCD, such as were shown here in the present study at the mRNA level.

CA II. Our data at the single nephron mRNA level confirm previous findings of CA II activity and protein in rabbit kidney. We have shown that CA II mRNA was expressed by all proximal tubule segments (S1, S2, S3) and all collecting duct segments including CCD, OMCD, IMCD, and IMCD flow cytometry and food restriction.

Our RT-PCR assay might not have shown linear increases in signal with increasing amounts of template (see Fig. 1); the increases between 5 and 50 ng RNA were larger than those between 50 and 500 ng. Perhaps efforts should have been made to work along a more sensitive curve. We felt it was important to have a wider range of standards (5 to 500 ng RNA) because we knew that some segments would express very little mRNA, whereas others might express the mRNA abundantly. Indeed, the signals for most of the segments...
expressing the CA IV, CA II, or MDH mRNA (see Fig. 2) were ~30–50% of the 500 ng RNA. With acidosis, signals were induced approaching 100% that of the 500 ng RNA (see Fig. 4). Based on an apparent decrease in sensitivity over the 50 to 500 ng range of total RNA, the consistent increases in signals from tubules of acidotic animals should reliably indicate upregulation. Our method was designed to be semiquantitative so that several genes could be screened along the nephron. It did not allow us to derive a percentage increase during upregulation. On the other hand, the amplification of the housekeeping gene, MDH, from an aliquot of reverse transcripted template that was concurrently amplified for CA IV and CA II, allowed us to eliminate degraded samples and to ensure that increases in CA gene expression did not result from errors in the amount of sample subjected to RT-PCR.

Finally, it could be asked why would such an abundant enzyme with such a high rate of catalysis be upregulated in metabolic acidosis. Indeed, Maren (20) has concluded that CA is never rate limiting in the kidney. However, we have reported recent data in the perfused OMCD, which shows that doses of extracellular CA inhibitors that inhibited ~50% of the CA IV activity also comparably inhibited HCO$_3^-$ absorption (H$^+$ secretion) (39). This study showed, at least for CA IV, that inhibition of CAIV in the range of the $K_i$ (~50% of activity) had a measurable effect on OMCD HCO$_3^-$ transport, whereas ~99% inhibition of total kidney CA activity is needed to affect renal HCO$_3^-$ clearance (20). In addition, we found recently that HCO$_3^-$ transport by OMCD tubules from acid-treated rabbits was 100 times less sensitive than controls to extracellular CA inhibition (39). There is at present no clear explanation for this reduction in sensitivity to extracellular CA inhibition by chronic acidosis but clearly an increase in CA IV mRNA might be an expected homeostatic response to metabolic acidosis. It is possible that CA plays other roles in the kidney in addition to hydrating CO$_2$ and dehydrating carbonic acid, and for these other roles CA activity could be regulated. CA may facilitate HCO$_3^-$ flux across membranes (32), diffusion through fluids and proton shunting (13), destruction of nitric oxide (41), removal of CO$_2$ and NH$_3$ from metabolically active tissue (14), and the systemic excretion of NH$_3$ (14) during acidosis. The increases in CA II mRNA occurred in eight or possibly nine segments, whereas those in CA IV mRNA occurred in four segments, all unassociated with increases in MDH mRNA, suggesting that upregulation was rather gene specific. These changes may constitute part of an appropriate adaptation to metabolic acidosis.

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