AE2 isoforms in rat kidney: immunohistochemical localization and regulation in response to chronic NH4Cl loading

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Frische, Sebastian, Alexander S. Zolotarev, Young-Hee Kim, Jeppe Praetorius, Seth Alper, Søren Nielsen, and Susan M. Wall. AE2 isoforms in rat kidney: immunohistochemical localization and regulation in response to chronic NH4Cl loading. Am J Physiol Renal Physiol 286: F1163–F1170, 2004. First published January 28, 2004; 10.1152/ajprenal.00409.2003.—Three splice variants of anion exchanger (AE)2 (AE2a, b, and c) have been described in the rat, but their relative distribution in rat kidney is not known. The purpose of this study was to describe the segmental and cellular distribution of the AE2 isoforms in the rat kidney and to evaluate whether the expression levels of these AE2 isoforms are regulated independently in response to chronic NH4Cl loading. Two polyclonal antibodies were generated, respectively, recognizing a NH2-terminal peptide unique to AE2a and an amino acid sequence common to AE2a and AE2b. Antibody specificities were tested using cells transfected separately with the AE2a, AE2b, and AE2c isoforms. Immunohistochemistry on sections of paraffin-embedded rat kidneys showed a distribution of AE2a/AE2b labeling in the kidney similar to the distribution of AE2 in the rat kidney reported previously. AE2 is highly expressed in the medullary thick ascending limb, cortical thick ascending limb (cTAL), and macula densa. The pattern of AE2a-specific labeling differed from the pattern of AE2a/AE2b labeling in that relatively more of the total immunolabel was observed in the terminal inner medullary collecting duct. NH4Cl loading (0.033 mmol NH4Cl/g body wt for 7 days) did not change the labeling of AE2 isoforms in the medulla, whereas the labeling in the cortex was intensified and included more distal parts of the cTAL. Immunoblotting confirmed upregulation of AE2a/b expression in the cortex. These results indicate that AE2a and AE2b are differentially expressed and regulated in the rat kidney. The regulation following NH4Cl loading of AE2b in the cTAL suggests a role for AE2 in transepithelial bicarbonate reabsorption in this segment.

immunohistochemistry; collecting duct; nephron segments

Electroneutral Cl-/HCO3- exchange is a ubiquitous process that serves a number of cellular functions. The first Cl-/HCO3- exchanger cDNA cloned was erythroid anion exchanger (AE)1 also known as band 3 protein. In the kidney, AE1 is a truncated splice variant of the red cell band 3 and is critical in the process of net acid secretion along the collecting duct (CD) (8). Subsequently, three other Cl-/HCO3- exchanger cDNAs, encoding proteins of similar molecular structure (AE 2–4), have been reported. In the kidney, AE3 is observed in the afferent arteriole (6), whereas AE4 is expressed in renal intercalated cells (17, 27). However, the physiological role of AE3 and AE4 in the kidney is unknown.

AE2 is thought to be constitutively expressed in almost all mammalian cells and has accordingly been detected by RT-PCR in all rat kidney nephron segments (5). Functionally, AE2 exhibits unique properties relative to AE1. First, AE2 exchange activity increases with both increasing intracellular pH and extracellular pH (23, 24). Second, the anion exchange activity of AE2 is increased by hypertonic extracellular conditions. In Xenopus laevis oocytes, hypertonicity increases AE2-mediated Cl-/HCO3- exchange, which acts in tandem with increased Na+/H+ exchange to generate a regulatory volume increase (14). Third, AE2-mediated anion exchange is regulated by extracellular ammonium (13).

In addition to its functions related to cellular pH and volume regulation, AE2 may play a role in transepithelial bicarbonate transport and urinary acidification in rat kidney. Immunohistochemical studies showed AE2 immunoreactivity in varying amounts in basolateral plasma membrane domains of epithelial cells in all rat kidney nephron segments distal to the proximal tubule, with the strongest labeling in the medullary thick ascending limb (mTAL), inner medullary collecting duct (IMCD), and macula densa (2). Functional studies confirmed the presence of AE2 in the mTAL (10). AE2 mRNA has been shown to be present in rat proximal tubules by RT-PCR. However, by immunohistochemistry, AE2 protein expression is low in this segment (2). Thus AE2 likely plays a minor role in bicarbonate absorption in the proximal tubule.

In contrast to the strongly labeled basolateral regions of macula densa cells, AE2 is weakly detected in the cortical TAL (cTAL) and weakly detected or absent in distal convoluted tubule, connecting tubule, and the cortical portion of the CD (2). As the CD extends into the medulla, immunolabeling increases gradually and is most intense in the most terminal portion of the IMCD (tIMCD) (2). Across the apical membrane of the rat terminal IMCD, secretion of chloride and absorption of bicarbonate (28) occur in tandem with Cl-/HCO3- efflux across the basolateral membrane (electroneutral Na+/HCO3- exchange) (22). Because the rat tIMCD does not express AE1 (1), and because AE2 is highly expressed in rat tIMCD, AE2 might be the gene product that mediates this electroneutral Cl-/HCO3- exchange (2, 25).

Three splice variants of AE2 (AE2a, b, and c), which differ in tissue and cellular distribution, have been described in the rat (29). By Northern blot analysis, mRNA of all isoforms has...
been detected in the stomach, whereas only AE2a and AE2b mRNA have been detected in the kidney (29). However, AE2c mRNA has been detected in the kidney by RT-PCR (2). In rabbit stomach, AE2 protein expression as well as DIDS-sensitive Cl⁻/HCO₃⁻ exchange are more than 10-fold higher in parietal cells than in mucous cells. Moreover, Northern blot analysis has shown that more AE2a than AE2b mRNA is present in mucous cells, whereas AE2b mRNA is the dominant isoform in parietal cells (21). The cell type-specific expression of each AE2 isoform may reflect the distinct functional regulation of Cl⁻/anion exchange in these gastric cell types. The comparative distribution of AE2a and AE2b protein in rat kidney remains unreported.

The purpose of this study was to describe the segmental and cellular distribution of the AE2a and AE2b polypeptides in the rat kidney and to determine whether their levels are regulated independently in response to chronic NH₄Cl loading. This was achieved by immunoblotting, immunohistochemistry, and immunoelectron microscopy using antibodies specific to the unique NH₂-terminal AE2a and to an amino acid (aa) sequence common to AE2a and b but different from AE2c.

MATERIALS AND METHODS

Antibodies. Two new polyclonal antibodies were produced, anti-AE2a (α-2–22) and anti-AE2a/b (α-101–117). AE2a (α-2–22). Polyclonal antibodies were raised in rabbits against a synthetic peptide CSSAPRPASGADSLHITPEPS that corresponds to aa2–22 of the rat and mouse AE2a isoform. This amino acid sequence is absent from AE2c, and only the latter five aminoacids are present in the amino acid sequences of rat AE2b and mouse AE2b. Anti-AE2a/b (α-101–117). Polyclonal antibodies were raised in rabbits against a synthetic peptide CPGRKRPRGSGPTGET corresponding to aa101–117 of the rat and mouse AE2a isoform and aa88–104 in the rat and mouse AE2b sequences. This peptide sequence is not found in rat AE2c. Affinity purification was performed by binding antibodies from hyperimmune serum to the peptide immunosorbent, eluting with citrate-phosphate, pH 2.2, and neutralizing the eluate with Tris base. Sodium azide was added at 0.1% as an antimicrobial. Antibody specificity was tested on cells overexpressing recombinant mouse AE2a, AE2b, and AE2c.

Anti-AE2a, b, c (α J224–1237, α-COOH-term). An antibody raised against aa1224–1237 of the AE2 sequence (α-COOH-term) previously described (2) detects a shared COOH terminus of all AE2 isoforms and was used to test total AE2 expression in transfected cells.

Immunoblotting of cell lysates of ECr-293 cells expressing mouse AE2a, AE2b1, and AE2c1. ECr-293 cells (Clontech) were grown in DMEM plus 10% FCS with penicillin, streptomycin, and Zeocin and were transiently transfected (SuperFect, Qiagen) with mouse AE2a, AE2b1, and AE2c1, or no vector. The following day, cells were exposed to 5 mM ponasterone. Forty-eight hours later, cells were scraped, extracted with 1% Triton X-100, 75 mM NaC1, 10 mM Na phosphate, and 10 mM Tris-HCl, pH 7.4. Cell protein was quantified by BCA assay (Pierce) before extraction. Proteins were separated by 5–20% SDS-PAGE, transferred to nitrocellulose, and blots were developed with the indicated antipeptide antibodies. Peroxidase-conjugated anti-Ig was from Jackson ImmunoResearch; enhanced chemiluminescence detection reagents were from Pierce.

Immunofluorescence analysis of ECr-293 cells expressing mouse AE2a, AE2b1, and AE2c1. ECr-293 cells grown on plastic dishes were transiently transfected with AE2a cDNA. After 20 h, cells were trypsinized and replated on polylysine-coated multichamber slides. After a 5-h recovery period, cells were induced with 5 mM ponasterone. ECr-293 cells plated on polylysine-coated multichamber microscope slides, with stable expression of AE2b1 or AE2c1 cDNAs, were similarly induced with 5 mM ponasterone. After 24 h of induction, cells were rinsed, fixed in 3% paraformaldehyde for 30 min at room temperature, quenched with 50 mM glycine in PBS, washed with PBS, incubated with 1% SDS for 15 min for epitope unmasking, and subjected to immunostaining. Primary antibody was applied for 2 h at room temperature, cells were washed, and then secondary antibodies were applied for 45 min. Slides were visualized with a Bio-Rad MRC-1024 confocal immunofluorescence microscope.

RESULTS. Male Munich-Wistar rats (280–300 g) from Mollegaard Breeding Centre were kept on a standard rodent diet (Altromin 1320, Lage, Germany) until use. Non-Swiss albino mice were purchased from Harlan ( Ardmore, TX). The use of animals in the experiments in the study complied with institutional and National Institutes of Health standards.

NH₄Cl loading of rats with fixed water and food intake. Rats were assigned randomly to either the control or treated group. Before the treatment, the rats were kept in metabolic cages for 3 days to record baseline values of urine output and pH. Each morning rats were given a fixed amount of ground rat chow (0.068 g/g body wt) mixed with water (0.168 g/g body wt). The experimental group (n = 12) was given 0.033 mmol NH₄Cl/g body wt in the food for 7 days, whereas the control group (n = 12) consumed the same amount of food but without NH₄Cl. The NH₄Cl loading induced a marked drop in urinary pH (5.76 vs. 7.94), but the groups did not differ in plasma acid-base status or change in body weight indicating that the animals were in steady state with respect to acid intake and excretion (11).

HCl loading of rats with fixed water and food intake. For comparison with the NH₄Cl loading, a control experiment using HCl loading was performed. The experiment followed the same protocol as the NH₄Cl-loading experiment, except that HCl was used instead of NH₄Cl. The experimental group was given 0.033 mmol HCl/g body wt in the food for 7 days, whereas the control group consumed the same amount of food but without HCl. Similarly to NH₄Cl loading, HCl loading induced a marked drop in urine pH compared with the control group (6.29 vs. 8.25, n = 14), and no difference was observed in plasma pH (7.33 (n = 9) vs. 7.36 (n = 11)).

Rat kidney membrane fractionation and immunoblotting. Kidneys from normal rats and six rats from the NH₄Cl-loaded group were used. The kidneys were divided into three zones: cortex/outer stripe of the outer medulla (Ctx/OS), inner stripe of the outer medulla (ISOM), and inner medulla (IM). The tissue was homogenized in 0.3 M sucrose, 25 mM imidazole, 1 mM EDTA, pH 7.2, containing 8.5 μM leupeptin and 1 mM phenylmethylsulfonyl fluoride using an ultra-Turrax T8 homogenizer (IKA Labortechnik) at a maximum speed (25,000 rpm) for 30 s, and the homogenates were centrifuged at 10,000 g for 30 s, and the homogenates were centrifuged at 10,000 g for 1 h to remove whole cells, nuclei, and mitochondria. The supernatants were solubilized in Laemmli sample buffer containing 3% SDS (final concentration). SDS-PAGE electrophoresis was performed of 30 μg protein in each lane of 9% polyacrylamide gels (Bio-Rad Mini Protein II) and run for 10 min at 100 V followed by 40 min at 200 V. After transfer by electroelution (0.025 M Tris, 0.19 M glycine, and 20% methanol, pH 8.3, 100 V, 1 h) to nitrocellulose membranes, blots were blocked with 5% milk in PBS-T (80 mM NaH₂PO₄, 20 mM NaH₂PO₄, 100 mM NaCl, 0.1% Tween 20, pH 7.5) for 1 h and incubated overnight at 4°C with anti-AE2 antibodies. The labeling was visualized with horseradish peroxidase (HRP)-conjugated secondary antibodies (P448, DAKO, Glostrup, Denmark, diluted 1:3,000) using the enhanced chemiluminescence system (Amersham International). The chemiluminescence was recorded on film (Amersham), which was subsequently scanned using a flatbed scanner. Two-dimensional rolling ball background subtraction and the Gelplot2 macro in Scion Image (Windows version of National Institutes of Health Image) were used for densitometric analysis.

Mouse kidney membrane fractionation and immunoblotting. Six-week-old non-Swiss albino mice were anesthetized with 100% O₂ at 1 l/min with 4% isoflurane before death. Preparation of kidney

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lysates and immunoblotting were performed as reported previously (9). Kidneys were placed in ice-cold solution containing 250 mM sucrose, 10 mM triethanolamine, 1 µg/ml leupeptin (Sigma), and 0.1 mg/ml PMSF (US Biochemical, Toledo, OH), pH 7.6. Kidney tissue was dissected to separate cortex, outer medulla, and IM. Tissue samples from each of these regions were homogenized with a tissue homogenizer (OmniTech Quest, Warrenton, VA) at 15,000 rpm on ice for 15 s. The homogenization step was repeated twice. Protein content was measured using the method of Lowry et al. (19). Membranes were solubilized for 15 min at 4°C in Laemmli sample buffer. SDS-PAGE was performed on minigels of 8% polyacrylamide. Each lane was loaded with 25 µg protein. The proteins were transferred from the gels electrophoretically onto nitrocellulose membranes. After being blocked with 5 g/ml nonfat dry milk, membranes were probed with the anti-AE2a and anti-AEa/b antibodies at 1:10,000 and 1:50,000 dilutions, respectively. Donkey anti-rabbit IgG conjugated to HRP was incubated overnight at 4°C. After washing, membranes were visualized using luminal-based enhanced chemiluminescence (KPL; Biberfeld, Sunnyvale, CA) or autoradiography on X-OMAT AR (Eastman Kodak, Rochester, NY). Kidneys from normal rats, 12 NH4Cl-loaded rats, and 12 control rats were fixed by 1% paraformaldehyde and 1% glutaraldehyde in 0.1 M cacodylate buffer, pH 7.4, and postfixed for 2 h in similar fixative. Kidneys from 9 HCl-loaded and 9 control rats were fixed similarly, except that 3% paraformaldehyde and 1-h postfixation were applied. Kidney slices containing all kidney zones were dehydrated and embedded in paraplast. The paraffin-embedded tissues were cut at 3-µm thickness on a rotary microtome (Leica). The sections were dewaxed in xylene and rehydrated through 99% ethanol to 96% ethanol. After 30-min incubation in 0.3% H2O2 in methanol to block endogenous peroxidase activity, rehydration was completed through a graded series of alcohol and propylene oxide and embedded in TAAB resin. Ultrathin sections were stained with lead citrate and photographed with a Philips CM100 transmission electron microscope.

**RESULTS**

**AE2a antibody specifically recognizes the AE2a polypeptide, and the AE2a/b antibody specifically recognizes the AE2a and AE2b polypeptides.** ECR-293 cells overexpressing mouse AE2a, AE2b1, and AE2c1 polypeptides were used to test antibody specificity. Immunoblotting with antibody recognizing the shared COOH-terminus of AE2 (α-COOH-term) resulted in bands of the expected sizes: ~180 kDa for AE2a and AE2b and ~160 kDa for AE2c (Fig. 1, A and B). The immunizing peptide AE2a aa2–22 used for the NH2-terminal AE2a antibody included five COOH-terminal amino acid residues also present in AE2b1 as aas4–8. However, using the NH2-terminal AE2a antibody (α-NH2-term), immunoreactivity was detected only in lysates of AE2a-transfected cells (Fig. 1C). In contrast, the AE2a/b antibody (α101–117) detected both recombinant AE2a and AE2b1 (Fig. 1D). Neither of the new antibodies detected AE2c polypeptide (Fig. 1, C and D). Endogenous Ecr-293 cell AE2 polypeptide was evident in nontransfected cells as a very faint band (right lane of each panel).

These immunoblot results were confirmed by immunofluorescent labeling of cells expressing the mouse AE2a (Fig. 2,

![Image](http://ajprenal.physiology.org/)

*Fig. 1. Specificity of antibodies tested with overexpressed recombinant anion exchanger (AE2) polypeptides. Immunoblotting of 1% Triton X-100 extract of nonasterone A (Invitrogen) induced Ecr-293 cells transiently transfected with cDNA encoding mouse AE2a (extract from 30 µg total cell protein), AE2b1 (from 15 µg total cell protein), AE2c1 (from 15 µg cell protein), or untransfected cells (30 µg cell protein). A and B: blots were developed with antibody to AE2a amino acid (aa) 1224–1237 (α-COOH-term), which recognizes all 3 polypeptide variants. C: antibody to AE2a aa2–22 (α-NH2-term) recognizes only the AE2a isoform. D: antibody to AE2a aa101–117 (α101–117) recognizes both AE2a and AE2b1 polypeptides. (A: reprobe of stripped C blot; B: reprobe of stripped D blot.)*
AE2 isoforms in rat kidney

A–C, AE2b1 (Fig. 2, D–F), and AE2c1 polypeptides (Fig. 2, G–I). The AE2 antibody (α-COOH-term) labeled membrane domains in all three cell types (Fig. 2, A, D, G). In contrast, the AE2a antibody (α-NH2-term) only labeled the cells expressing AE2a (Fig. 2B vs. 2, E and H), whereas the AE2a/b antibody (α-101–117) labeled the cells expressing AE2a and AE2b1 (Fig. 2, E and F, vs. 2I). Epitope unmasking was required for detection of AE2 by the AE2a (α-NH2-term) antibody and the AE2a/b (α-101–117) antibody. For the AE2 (α-COOH-term) antibody, epitope unmasking greatly enhanced AE2 plasma-membranial stain while abolishing the variably detected intracellular Golgi pattern staining of endogenous AE2, as reported previously in Madin-Darby canine kidney and other cells (7) and in the kidney (2, 25).

**AE2a and AE2b expression in rat and mouse kidney.** In the rat and mouse kidney homogenates, both the AE2a (α-NH2-term) and AE2a/b (α-101–117) antibodies recognized bands at 180 kDa, the expected mobility of AE2a and AE2b polypeptides (18). AE2a immunoreactivity was high in the ISOM and very weak, in the IM (Fig. 3, A and C). AE2a immunoreactivity was lowest in the cortex (Fig. 3, A and C). In the rat, the distribution of AE2a/b (α-101–117) immunoreactivity was similar to that of AE2a. AE2a/b immunoreactivity in the mouse was greatest in the OM and lower in the IM and cortex (Fig. 3, B and D).

**Immunolocalization of AE2 isoforms in normal rat kidney.** In the rat kidney, labeling with the AE2a/b (α-101–117) antibody was detected in the cortex, outer and inner stripe of outer medulla, and in the IM (Fig. 4, A–G). In the glomerulus and proximal tubule, no labeling was detected (Fig. 4, A–C). Only weak labeling was detected in descending thin limbs (DTL) and ascending thin limbs (ATL) of the loop of Henle (Fig. 4, C, E, F). The TAL of the loop of Henle showed strong labeling in the ISOM (Fig. 4D), whereas labeling was relatively weaker in the TAL of the outer stripe of the outer medulla and cortex (Fig. 4, A–C). Strong labeling was seen in basolateral domains of macula densa cells (Fig. 4A). In the CD, a gradual increase in labeling intensity was seen from the proximal to the distal regions. Very weak labeling was observed in the basolateral domains of principal cells in the ISOM, whereas strong labeling was seen in the basolateral domains of IMCD cells in the inner third of the IM (Fig. 4, E–G).

Labeling with antibodies specifically recognizing the AE2a isoform (α-NH2-term) (Fig. 4, H–N) was not found in any cortical tubule segments, except for occasional very weak labeling in basolateral domains of macula densa cells (Fig. 4, H–J). Very weak labeling was seen in the DTL and ATL. Labeling was noted in the medullary part of TAL (Fig. 4, J–M). Strong labeling was seen in the basolateral portion of IMCD cells in the inner third of the IM (Fig. 4N).

**Immunoelectron microscopic localization of AE2 in IM.** To determine the subcellular localization of AE2, electron microscopy using a preembedding HRP-visualized immunolabeling technique was employed (15). Antigen-antibody interactions are detected as a dark precipitate. AE2a/b immunolabeling was detected in the basolateral plasma membrane in IMCD cells in the inner third of the IM (Fig. 5).

**Kidneys from NH4Cl-loaded rats exhibited increased labeling for AE2 in cTAL.** Compared with controls (Fig. 6A), labeling with the AE2a/b antibody (α-101–117) in the cTAL was markedly increased. Moreover, in NH4Cl-loaded rats,
AE2a/b immunoreactivity extended to more distal portions of the cTAL (Fig. 6B). In the medulla, no differences in the labeling between control rats and NH4Cl-loaded rats could be seen using either AE2a antibody or AE2a/b antibody (not shown). HCl loading resulted in a similar increase in AE2a/b immunolabeling of the cTAL (Fig. 6C).

**DISCUSSION**

AE2a and AE2b are coexpressed in the rat kidney, but the relative amounts of the variant polypeptides vary among nephron segments. In the present study, the distributions of the AE2a and AE2b isoforms were determined by comparing immunoblotting confirmed upregulation of AE2 in the cortex in NH4Cl-loaded rats. The effect of NH4Cl ingestion on AE2a/b protein expression was quantified by immunoblot on samples from rat cortex/outer stripe of outer medulla, ISOM, and IM. By immunoblot, AE2a/b (α-101–117) immunoreactivity increased 103% in the cortex/outer stripe of outer medulla of kidneys from NH4Cl-loaded rats (P < 0.01; Fig. 7). AE2a/b expression did not change in the ISOM and IM following NH4Cl ingestion. Moreover, NH4Cl intake did not change AE2a (α-NH2-term) protein expression in any of these regions of the kidney (not shown).
immunolabeling of antibodies that recognize either AE2a only or both AE2a and AE2b. The distribution of AE2a/b labeling was identical to that described previously, which employed an antibody (H9251-COOH-term) that recognizes all AE2 isoforms (2).

The pattern of AE2a-specific labeling in kidney differed from AE2a/b labeling in that no labeling was observed in the cortex. Moreover, relatively more of the total AE2a immunolabel was observed in the tIMCD than in the TAL. The relatively weak AE2a labeling in the TAL suggests that AE2a alone does not account for the strong labeling seen in the TAL with the AE2a/b (α-101–117) antibody. In the IM, both the AE2a and AE2a/b antibody gave rise to strong labeling in the basolateral domain of IMCD cells. This shows AE2a to be present alone or in combination with AE2b. Immunoelectron microscopy confirmed AE2 localization to the basolateral plasma membrane of renal tubular cells.

Immunoblotting of rat kidney samples confirmed the distribution of the AE2 isoforms observed by immunohistochemistry. In both the rat and mouse, greater AE2a expression was detected in the ISOM than in the IM. At first glance, results of immunoblots appear to differ from studies using immunohistochemistry, which suggest that AE2a expression is greater in IMCD than mTAL. However, basolateral membrane proteins of the mTAL comprise a particularly large fraction of the total proteins in ISOM tissue homogenates due to the deep basolateral invaginations of the mTAL cells (26). In the mTAL of the ISOM, lateral invaginations are numerous and often extend two-thirds or more of the distance from the base of the cell to the luminal border (26). Moreover, the mTAL represents more than 70% of the total protein in the outer medulla (4), whereas the tIMCD represents only 20–30% of the total protein in the IM (16). Taken together, basolateral plasma membrane proteins of the mTAL comprise a much larger fraction of the total protein content in ISOM than the fraction of IM protein from basolateral membranes of the tIMCD. Thus, if equal amounts of total protein from the outer and IM were loaded on a gel, greater basolateral membrane protein would be loaded per lane from the mTAL than from the tIMCD. The same bias may not be present by immunohistochemistry.

Alternatively, the apparent discrepancy between immunoblotting and immunohistochemistry with respect to relative intensities of immunoreactivity using the AE2a antibody may

![Image of immunolocalization of AE2 in kidneys from NH4Cl-loaded rats.](image)

**Fig. 6.** Immunolocalization of AE2 in kidneys from NH4Cl-loaded rats. *A:* labeling with the AE2a/b antibody (α-101–117) in kidney cortex from control rats showed intense labeling in the basolateral domain of MD cells and weaker labeling in the basolateral domains of cells in the cortical TAL (cTAL). *B:* labeling with the AE2a/b antibody (α-101–117) in kidney cortex from NH4Cl-loaded rats was found to be markedly increased, and more cTAL showed immunoreactivity compared with controls. *C:* increased AE2a/b labeling was also observed in the cTAL from rats ingesting HCl relative to controls. Scale bars: 30 μm.
occur because the AE2a-specific epitope, localized at the NH2-terminal of AE2a, is less accessible in the mTAL than in the tIMCD in fixed tissue, perhaps due to interactions with other proteins.

Increased cTAL immunolabeling in NH4Cl-loaded rats is likely due to upregulation of AE2b. By both immunoblotting and immunohistochemistry, AE2a/b immunoreactivity was greatly increased in the kidney cortex from NH4Cl-loaded rats relative to controls. No change in AE2a expression was detected in kidney from rats ingesting NH4Cl. Increased AE2 expression was noted by immunohistochemistry as intensified labeling within cTAL. Because AE2a immunoreactivity did not change with NH4Cl ingestion, we conclude that the increased labeling found with the AE2a/b antibody (α-101–117) is largely due to increased abundance of the AE2b isofrom in cTAL following NH4Cl loading. Similar changes in AE2a/b immunolabeling were seen following 7 days of HCl loading, supporting the conclusion that the changes in AE2b expression in cTAL are involved in the renal compensation of a sustained acid load.

AE2a is widely expressed and is thought to serve general cellular functions such as regulation of intracellular pH and cell volume. However, the tissue distribution of rat AE2b mRNA, present in liver, kidney, stomach, and intestine, appears to be more limited (29). The present study demonstrates that immunolabeling of AE2a/b, but not AE2a, is increased in rat kidney cTAL following oral NH4Cl loading. Our data therefore suggest that AE2a and AE2b polypeptide levels are regulated independently. Rat AE2b mRNA is transcribed from a promoter located in intron 2 of the AE2 gene. A number of consensus binding sites for transcription factors have been identified upstream of the initiation site of rat AE2b (29). Although little is known about the signals that may induce AE2b polypeptide expression in rat cells, the human AE2b promoter can be regulated by HNF1α (20).

Functional role of AE2 in TAL and CD. Rat cTAL absorbs bicarbonate (3). Therefore, AE2 expressed in cTAL might participate in the process of bicarbonate absorption and urinary acidification in this segment, particularly following NH4Cl ingestion. Increased absorption of bicarbonate in the cTAL would help correct the metabolic acidosis that follows NH4Cl ingestion. Alternatively, increased AE2 expression might modulate Cl− excretion following NH4Cl ingestion. Chloride channels expressed on the basolateral plasma membrane differ between the cTAL and mTAL (30). Thus the mechanism of Cl− absorption and its regulation may differ between the cTAL and mTAL. Whether upregulation of AE2 in cTAL helps maintain acid-base or chloride balance (or both) following NH4Cl ingestion remains to be determined.

After ingestion of NH4Cl, net acid secretion increases in rat IMCD, both in vivo and in vitro (28). As described above, strong AE2a immunoreactivity was seen in tIMCD, and AE2a may be the only AE2 isofrom present in IMCD. The results of this study do not allow any conclusions regarding the presence of AE2b in IMCD. Expression of AE2 protein in IM was not seen to change with NH4Cl loading. Thus if AE2 contributes to the increased absorption of HCO3− observed following NH4Cl ingestion, either AE2-mediated transport is not rate limiting or AE2 activity increases in this treatment model through a mechanism that does not involve changes in protein expression. NH4Cl loading is associated with a number of adaptive changes in the kidney, such as an increase in the interstitial concentration of NH4+ in the IM (12). In heterologous expression systems, AE2 is activated by increases in extracellular NH4+ concentration. Thus activation of AE2 by NH4+ might increase AE2-mediated HCO3− efflux across the basolateral membrane of the tIMCD in vivo, following NH4Cl ingestion, in the absence of changes in AE2 protein expression.

In conclusion, in the chronic NH4Cl-loading model of metabolic acidosis, AE2 protein expression is upregulated in the cortex, likely through increased abundance of AE2b polypeptide(s) in the cTAL. In the IM, a region in which the AE2a polypeptide is relatively more abundant, no change in AE2 protein expression was observed. Whether the observed up-regulation of AE2 contributes to the increase in net acid secretion observed in this treatment model remains to be determined.

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