The role of carbonic anhydrases in renal physiology

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Carbonic anhydrase (CA) catalyzes the reversible hydration of CO_2. CA is expressed in most segments of the kidney. CAII and CAIV predominate in human and rabbit kidneys; in rodent kidneys, CAXII, and CAXIV are also present. CAIX is expressed by renal cell carcinoma (RCC). Most of these isoforms, except for rodent CAIV, have high turnover rates. CAII is a cytoplasmic enzyme, whereas the others are membrane-associated; CAIV is anchored by glycosylphosphatidylinositol linkage. Membrane polarity is apical for CAXIV, basolateral for CAXII, and apical and basolateral for CAIV. Luminal membrane CAs facilitate the dehydration of carbonic acid (H_2CO_3) that is formed when secreted protons combine with filtered bicarbonate. Basolateral CA enhances the efflux of bicarbonate via dehydration of H_2CO_3. CAII and CAIV can associate with bicarbonate transporters (e.g., AE1, kNBC1, NBC3, and SCL26A6), and proton antiporter, NHE1 in a membrane protein complex called a transport metabolon. CAXII and CAXIV may also be associated with transporters in normal kidney and CAIX in RCCs. The multiplicity of CAs implicates their importance in acid–base and other solute transport along the nephron. For example, CAII on the cytoplasmic face and CAIV on the extracellular surface provide the 'push' and 'pull' for bicarbonate transport by supplying and dissipating substrate respectively.

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Carbonic anhydrase (CA) is a zinc metalloenzyme that catalyzes the reversible hydration of CO_2 according to the reaction:

$$H^+ + HCO_3^- \leftrightarrow H_2CO_3 \overset{CA}{\longleftrightarrow} CO_2 + H_2O \qquad (1)$$

CO_2 gas dissolves in water and is in equilibrium with the acid H_2CO_3. The Henderson–Hasselbalch equation relates pH, HCO_3^- concentration, and partial pressure of CO_2 gas in physiologic solutions:

$$pH = 6.1 + \log([HCO_3^-]/[0.03 * pCO_2]) \qquad (2)$$

The uncatalyzed hydration of CO_2 is relatively slow, whereas the turnover number for CAII is of the order of $10^6\ s^{-1}$.[1,2]

At present there are 15 known isoforms of CA, which differ in kinetic properties, susceptibility to inhibitors, and specific tissue distribution. From the reactions 1 and 2 above, it is logical to assume that CA can facilitate renal acidification, because the concentrations of CO_2 and HCO_3^- are interdependent. In addition, CA can modulate acid–base transport in three other ways. First, CA can make dissolved HCO_3^- available for rapid conversion to CO_2, thereby increasing the amount of transported CO_2 by the amount of HCO_3^- present in solution. Second, CA can mobilize HCO_3^- diffusion for CO_2 transport, in a process known as facilitated diffusion, whereby there is HCO_3^- diffusion and simultaneous H^+ transport in the presence of a mobile buffer, such as phosphate or albumin.[3,4] For example, at pH 7.4 there are 20 times more HCO_3^- ions as CO_2 molecules, so that CO_2 diffusion may be increased by 20-fold.[4,5] Third, Enns[5] showed that CA alone, in the absence of HCO_3^- ions, (pH 5.2), directly enhances transport, perhaps by acting as a carrier for CO_2.

CAII accounts for $>95\%$ of CA activity in the kidney and resides in the cytosol. In non-rodent species (e.g. human, rabbit, and bovine) most of the remaining $\sim 5\%$ of renal CA is membrane-associated and comprised of CAIV and CAXII.[6–8] In addition to CAIV and CAXII, CAXIV, and CAXV are expressed in the kidney of rodent species.[9–12] Although CAIX is not expressed in normal kidney, it is commonly found in neoplasms arising from renal tissue, particularly renal clear-cell carcinoma.[13]

In the following sections we will examine the function of CA isoforms in acid/base physiology of the kidney by defining the following parameters for each of the renal CA isoforms: (1) expression along the nephron segments (cell types), (2) subcellular localization, and (3) functional

associations with acid/base transporters. Studies within the last 10 years have demonstrated that CAs associate with acid/base transporters to form H^+/HCO_3^- transport metabolons.[14] Association of CAs with transporters has been shown to markedly enhance H^+/HCO_3^- transport, and thus via mass action CAs provide the 'push' and 'pull' for H^+/HCO_3^- transport by accumulating or dissipating substrate.

CA ISOFORMS

To date 15 CA isoforms have been identified of which twelve are catalytically active; CAs VIII, X, and XI are CA-related proteins that are not catalytically active owing to the absence of one or more of the conserved histidine residues that are required for coordination of Zn, which is essential for CA enzyme catalysis.[11,15] CAs can be divided into three groups based on their domain structure that is comprised of a CA catalytic domain along with other structural features that influence cellular localization (Figure 1). The cytosolic CA isoforms for which CAII serves as a prototype, also include CAs I, III, and VII, which are comprised of a CA domain encompassing the enzyme active site and the three conserved histidine residues found in all catalytically active CA isoforms.

The CAV isoforms are expressed in mitochondria owing to the inclusion of a 33 amino-acid classical mitochondrial leader sequence in the 317 amino-acid precursor peptide containing a CA domain. The CAV isoform includes two homologs, CAVA and CAVB that differ in their tissue distributions: CAVB is expressed in most tissues, whereas CAVA expression is restricted to liver, skeletal muscle, and kidney.[16] Addition of a signal sequence to a CA domain and a C-terminal extension that is rich in hydrophilic amino acid produces CAVI, the lone secreted isoform which is expressed in the salivary gland of a number of mammalian species.[17,18]

There are now five membrane-associated CA isoforms with the recent addition of CAXV to this group,[12] three of which (CAs IX, XII, and XIV) are expressed as single-pass transmembrane proteins.[9,19–21] In addition to a conserved catalytic domain found in all CA isoforms, CAIX contains an N-terminal proteoglycan-like domain that may function in cell adhesion.[22] CAIV and the closely related CAXV,[12] are tethered to the outer leaflet of the plasma membrane via a glycosylphosphatidylinositol lipid anchor (GPI anchor) that typically results in the luminal or apical surface expression of the protein in polarized epithelial cells.[23–25] However, recent studies in our laboratory have revealed an exception to this rule for CAIV expression in proximal tubule epithelial cells, as is discussed in detail below.

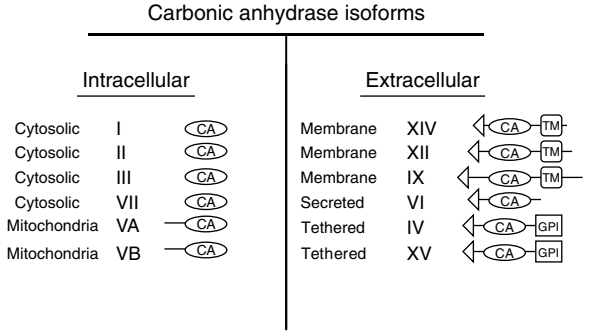


Figure 1 | CA isoforms. CAs are grouped according to their subcellular localization (i.e. cytosolic, mitochondrial, secreted, single-pass transmembrane, and GPI-anchored proteins); the domain structures are shown at the right of each isoform.

ENZYMATIC ACTIVITY

The α class of CAs comprises zinc-containing metalloenzymes that catalyze the reversible hydration of CO_2 according to the reaction (3):

$$CO_2 + H_2O \xleftrightarrow{CA} H_2CO_3 \leftrightarrow H^+ + HCO_3^- \qquad (3)$$

CO_2 hydration occurs in two distinct steps[26] as shown below.

$$\begin{aligned} E - Zn - OH^- + CO_2 &\leftrightarrow E - Zn - HCO_3^- + H_2O \\ &\leftrightarrow E - Zn - OH_2 + HCO_3^- \end{aligned} \qquad (4)$$

First a nucleophilic attack by the zinc-bound hydroxide occurs at the carbonyl carbon of CO_2 to form zinc-bound bicarbonate. For the prototype CAII enzyme the second-order rate constant for CO_2 hydration (K_{cat}/Km) approaches the diffusion control limit. The second step regenerates the active zinc hydroxide species, when a proton is transferred from zinc-bound water to a solvent buffer molecule in two stages via the His[64].

$$\begin{aligned} \mathbf{His(64)}{-}E - Zn - OH_2 &\leftrightarrow H^+{-}\mathbf{His(64)}{-}E - Zn - OH^- \\ &\quad + B \\ &\leftrightarrow \mathbf{His(64)}{-}E - Zn - OH^- \\ &\quad + B - H^+ \end{aligned} \qquad (5)$$

In stage 1 there is an intra-molecular transfer of a proton from zinc-bound water to a protein side chain with a pK_a of 7, then an inter-molecular transfer of the proton to solvent buffer molecule. In reaction (4) E denotes a CA enzyme and B indicates the solvent buffer molecule (5). Histidine residue 64 of the CAII molecule functions as a proton shuttle in the rate-limited step for CO_2 hydration at high substrate and buffer concentrations. Replacement of His-64 with non-ionizable amino acids such as alanine or glutamine markedly reduces hydratase activity.[27,28] Catalytic rates exhibited by CAII are among the highest measured ($10^6\ s^{-1}$)[26] (Figure 2).

Reduced activity of some CA isoforms (CAIII), as well as species differences in CA isoform activity, provides additional insights into the function of other amino-acid residues within the CA catalytic domain. The cytosolic CAIII isoform that is abundantly expressed in skeletal muscle and adipose tissue and to a lesser extent in brain and liver, exhibits the lowest hydratase activity of the CA isoforms (see Figure 2). Although CAII and CAIII have similar backbone conformations (see Figure 1), CAIII is less efficient at proton shuttling due in part to replacement of His with lysine at residue 64.[29]

CA	$k_{cat}/Km(\mu M^{-1}s^{-1})$	$k_{cat}(\mu s^{-1})$
II	100–150	0.8–1.4[a]
III	0.30	0.002[b]
IV	51	1.1[c]
IX	55	1.4[d]
XII	34	0.4[e]

CA	EU/mg protien
II	2000–3000[a]
III	1–5[g]
IV_{Rb}	2495[h]
IV_{Bv}	3024[h]
IV_{Ms}	436[h]
XII	155[f]
XIV	3284[f]
XV	5.3[i]

Figure 2 | Catalytic activities of select CA isoforms. Upper panel: CAs II and III represent the range of hydratase activity from highest to lowest, respectively. CAs expressed in normal kidney include cytosolic CAII, and membrane-associated CAIV and CAXII, which exhibit exceptionally high catalytic activity. CAIX, which is expressed in RCCs, has a catalytic rate comparable to CAIV. Lower panel: a comparison of the specific activity of CAIV isoforms from mouse rodent species with other species reveals that the hydratase activity of rabbit, bovine, and human (not shown) CAIV is comparable to the activity of CAII, whereas a $Gly^{63} \rightarrow Gln$ substitution in rodent CAIV reduces catalytic activity by several fold. The recently described CAXV isoform exhibits low hydratase activity comparable to CAIII. References: [a]Khalifah, *J Biol Chem* 1971; **246**: 2561, Jackman, *et al. Biochemistry* 1996; **35**: 16421, Steiner, *et al. Eur J Biochem* 1975; **59**: 253, [b]Jewell *et al. Biochemistry* 1991; **30**:1484, [c]Baird, *et al. Biochemistry* 1997; **36**: 2669, [d]Wingo, *et al. Biochem Biophys Res Commun* 2001; **288**: 666, [e]Ulmasov, *et al. Proc Natl Acad Sci USA* 2000; **97**: 14212, [f]Whittington, *et al. J Biol Chem* 2004; **279**: 7223, [g]Karhumaa, *et al. J Biol Chem* 2000; **275**: 16044, [h]Tamai, *et al. Proc Natl Acad Sci USA* 1996; **93**: 13647, and [i]Hilvo, *et al. Biochem J* 2005; **392**: 83.

In addition, residue 198 of CAIII contains a phenylalanine phenyl ring about 5 Angstrom from Zn whereas a leucine is present in CAII, perhaps resulting in steric constriction of the CAIII active site. Consistent with this supposition, replacement of Phe with leucine at position 198 increases the rate of CAIII catalysis by at least 10-fold.[30]

The amino-acid residues adjacent to His-64 also influence CAII activity.[31] Introduction of bulky amino acids at position 65 decreases CAII K_{cat} up to 26-fold by disrupting the active site solvent network that facilitates proton transfer. In rodent CAIV (mouse, rat) a glutamine resides at position 63, and as a result the rodent enzyme has 10–20% of the activity of CAIV orthologs with a glycine at position 63 (rabbit, bovine, and human).[32] The presence of a bulkier side chain at position 63 may alter the conformation of His^{64} and thereby decrease the efficiency of the proton shuttling reaction. The importance of CAIV in renal physiology is underscored by the fact that the 80% decrease in CAIV activity in rodents owing to the glutamine residue at position 63 is associated with the compensatory expression of additional CA isoforms in rodent kidney (e.g. CAXIV and CAXV; see below).

The active site of CAII also contains a hydrophobic pocket, which includes valine at residues 121 and 143.[33] Substitution of Val^{143} with amino acids containing large side chains dramatically reduces hydratase activity. For example, a $Val^{143} \rightarrow$ Iso substitution reduces activity eightfold whereas $Val^{143} \rightarrow$ Tyr virtually destroys enzyme activity by reducing K_{cat}/K_m for CO_2 hydrations 3000–10^5-fold.[33] Blockade of the hydrophobic pocket with large amino-acid side chains may hinder the ability of CO_2 to approach the zinc-hydroxide moiety with correct orientation to react. Overexpression of the CAII $V^{143}Y$ mutant displaces endogenous wild-type CAII from putative CAII binding sites and thereby functions as a dominant-negative mutant that may be used to assess the function of CAII interactions in facilitation of ion transport (see below).

CAII

CAII is expressed as a 29 kDa monomeric protein (259 amino acids) in the cytosol of a wide variety of tissues (e.g. bone, brain, eye, stomach, intestine, liver, pancreas, kidney, red blood cells, salivary glands, and uterus),[34–36] and is characterized by its high enzymatic activity and inhibition by exposure to sodium dodecyl sulfate (SDS), owing to the absence of stabilizing disulfide bridges.[7,8,37] As noted above, CAII expression in the nephron accounts for 95% of total CA activity in the kidney, and studies describing the localization of CAII messenger RNA (mRNA) and protein expression in specific nephron segments was recently reviewed in detail.[38] In summary and generalizing from a variety of animals, CAII is expressed in proximal tubule, thin descending limb, thick ascending limb, and intercalated cells of the cortical collecting duct (CCD), outer medullary collecting duct (OMCD), and inner medullary collecting duct (Figure 3). The function of CAII in renal H^+/HCO_3^- transport is perhaps best understood by examining CAII interactions with specific transporters.

Anion exchangers

CAII binding to the carboxyl terminus of human AE1 (band 3) was the first report of direct interaction between CAs and members of the bicarbonate transporter (BT) family.[39] Evidence for CAII interaction with AE1 included: (1) CAII binding to a glutathione *S* transferase (GST)-AE1 C-terminal peptide fusion *in vitro*; (2) blockade of AE1/CAII binding by an antibody to the C-terminal region of AE1; (3) lectin-induced co-clustering of AE1 and CAII; and (4) co-precipitation of CAII with AE1.[39] CAII has since been shown to interact with additional members of the SLC4A family of BTs including three of the anion exchangers (AE1-3).[39,40] Association of CAII with anion exchangers facilitates bicarbonate transport as demonstrated by the acetazolamide-mediated reduction of chloride/bicarbonate exchange activity in human embryonic kidney (HEK)293 cells

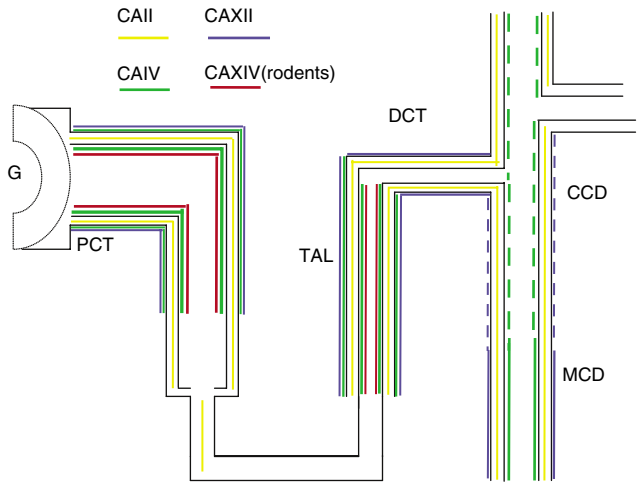


Figure 3 | Localization of CA isoforms along the nephron. CAII (yellow stripe) is expressed within the cytoplasm of virtually all nephron segments with the exception of the loop of Henle and the thin ascending limb. CAIV (green stripe) is both apically and basolaterally expressed in the S1 and S2 segments of the proximal tubule and the thick ascending limb. In the distal nephron CAIV is expressed exclusively on the luminal (apical) surface of α-intercalated cells of the CCD and acid secreting cells of the medullary collecting duct. CAXII (blue stripe) is found on the basolateral membrane of S1 and S2 segments of the proximal tubule and the thick ascending limb. In the distal nephron CAXII is located in distal convoluted tubules, principal cells of the CCD, and the medullary collecting duct of some species. In rodent species CAXIV is expressed on the luminal surface of cells in the S1 and S2 segments of the proximal tubule and the thick ascending limb.

transfected with AE1 complementary DNA.[41] In addition, AE1 mutants, unable to bind CAII, exhibit reduced HCO_3^- transport. Finally, overexpression of a functionally inactive CAII mutant ($V^{143}Y$) inhibits chloride/bicarbonate exchange by anion exchangers by displacing wild-type CAII from its binding site on AE1.[41] Similar results were obtained for AE2 and AE3.

CAII physically associates with anion exchangers (AE1-3) via an acidic motif in the C-terminal region of the transporter.[39] Although the COOH tail of AE1 contains four putative motifs, CAII binding is mediated only by the D^{887}ADD motif.[42] Binding to AE2 requires the homologous sequence, D^{887}ANE.[42] The corresponding interaction motif located in CAII is comprised of a basic patch of amino acids comprised of histidine and lysine in the N-terminal portion of the enzyme.[40]

Most if not all BT family members contain a consensus CAII binding motif consisting of a hydrophobic residue followed by at least two acid residues within the next four amino acids. CAII binding to members of the BT family is pH dependent. *In vitro* binding studies have consistently demonstrated that CAII binding to AE's and NBC3 (see below) is highest at pH 5 and binding affinity is markedly reduced at pH values above 7.[39] The pH dependence of CAII binding most likely reflects increased protonation of basic residues (lysine and histidine) within the transporter binding site of CAII leading to increased electrostatic interactions with acidic residues of the anion transporter. The influence of pH on the interactions between CAII and BT raises the possibility that the pH of the subplasmalemma microenvironment may regulate the stoichiometry of CAII-transporter association in a dynamic manner.

kNBC1

Sodium bicarbonate co-transporters include the kidney isoform, kNBC1 (NBC1a), an electrogenic sodium BT that is expressed on the basolateral membrane of proximal tubule cells.[43] The HCO_3^-: Na^+ stoichiometry is shifted from 3:1 to 2:1 mode in response to phosphorylation of Ser^{982} by protein kinase A, and the stoichiometry shift is accompanied by flux reversal from efflux in the 3:1 mode to influx in the 2:1 mode.[43] An acidic cluster of amino acids (D^{986}NDD), that is homologous to the (D^{887}ADD) CAII binding site in AE1, is required for the phosphorylation-induced stoichiometry shift. CAII binding to kNBC1 was first demonstrated by isotherm calorimetry studies in which CAII bound with high affinity ($K_d = 160 \pm 10$ nM) to a single site on a kNBC1 carboxy-terminus peptide (amino acids 915–1035: $kNBC1^{915-1035}$).[44] In kNBC1 there are two clusters of acidic amino acids, L^{958}DDV and D^{986}NDD, which are involved in binding to CAII, and site-directed mutagenesis studies reveal that the first aspartate residue in each cluster is critical for CAII binding.[45]

Facilitation of kNBC1-mediated bicarbonate transport by CAII was demonstrated in mouse proximal convoluted cell line cell transfectants in which mutations of the two putative CAII binding sites in kNBC1 exhibited a positive correlation between CAII binding and flux inhibition by acetazolamide.[45] Interestingly, acetazolamide reduced bicarbonate flux only when the transporter was in efflux 3:1 mode; acetazolamide inhibited kNBC1-mediated flux by 65% only when the Ser^{982} was not phosphorylated by protein kinase A. Although *in vitro* phosphorylation of a kNBC1 C-terminal peptide containing Ser^{982} located between the two CAII binding motifs increased CAII binding, point mutants in the D^{986}NDD motif of intact kNBC1 dissociated the CAII binding from the loss of the protein kinase A-mediated stoichiometry shift, suggesting that factors other than CAII binding are involved in the transition from 3:1 to 2:1 mode.

NBC3

NBC3 is an electroneutral ethylisopropylamiloride-sensitive and di-isothiocyanatostilbene 2,2′-disulfonic acid disodium salt hydrate-insensitive sodium bicarbonate co-transporter that is expressed in heart, skeletal muscle, salivary glands, and kidney.[46,47] NBC3 is expressed apically by α-intercalated cells in the kidney, where it may directly associate with vacuolar H^+-ATPase[47] and contribute to cell pH regulation. On the other hand, the role of NBC3 in mediating bicarbonate absorption in the OMCD is minor (~15% of total).[48] Evidence for CAII interaction with NBC3 comes from studies by Casey and co-workers showing that in an *in vitro* binding assay CAII binds to a GST fusion peptide containing the

NBC3 cytoplasmic tail.[49] Also in HEK293 cells co-transfected with CAII and NBC3, CAII is recruited to the plasma membrane, whereas in cells transfected with CAII alone or co-transfected with a NBC3 C-terminal deletion mutant, CAII expression is distributed throughout the cytoplasm.[49]

The cytoplasmic tail of NBC3 contains two putative CAII binding motifs LDDLM (CAB1) and LQDDDD (CAB2); however only mutation of aspartic acid residues (D^{1135} and D^{1136} in CAB1) reduced CAII binding, whereas mutagenesis of aspartic acid residues within CAB2 was without effect.[49] These findings indicate that only one site is required for binding. Evidence for facilitation of NBC3-mediated transport by CAII was provided by co-transfection studies in HEK293 cells, in which the dominant-negative CAII ($V^{143}Y$) reduced NBC3-mediated recovery from an intracellular acid load by ~30%, presumably by competing with endogenous CAII for binding to NBC3. Treatment of cells with cyclic adenosine monophosphate agonists also inhibited NBC3 activity (40–50%). However unlike NBC1, protein kinase A did not phosphorylate NBC3 *in vitro*, indicating that phosphorylation by protein kinase A does not regulate CAII binding to NBC3.

SLC26A6

This anion exchanger is expressed in the brush border membrane of proximal tubule epithelial cells, where it may mediate chloride/formate exchange, thereby contributing to NaCl reabsorption.[50] Although SLA26A6 transports a variety of anions including sulfate, formate, oxalate, nitrate, and iodide,[51] the most physiological relevant transport in some tissues may be Cl^-/HCO_3^- or Cl^-/OH^- exchange.[52–54] As the SLC26A6 cytoplasmic tail contains consensus CAII binding sites, Alvarez *et al.*[53] utilized an affinity blotting assay to investigate CAII binding to SLC26A6. GST fusion constructs containing residues Q^{497} to D^{633} of the SLC26A6 cytoplasmic tail were resolved by SDS-polyacrylamide gel electrophoresis, transferred to polyvinylidene fluoride membrane, and then incubated with HEK293 lysate expressing endogenous CAII. CAII binding to GST-SLC26A6-Ct was detected by probing with anti-CAII antibody. The CAII binding motif within the SLC26A6 cytoplasmic tail was defined with an *in vitro* binding assay where plate-immobilized CAII was incubated with GST fusion peptides of the SLC26A6 cytoplasmic tail containing five putative CAII binding sites. Only constructs encompassing the ^{546}DVDF^{549} motif bound CAII. In the HEK293 cell transfection model acetazolamide and dominant-negative CAII reduced Cl^-/HCO_3^- exchange by SLC26A6 ~50 and 40%, respectively. Furthermore, a SLC26A6 mutant lacking the CAII binding site exhibited a ~40–50% reduction in transport activity. Thus, CAII interaction with SLC26A6 facilitates bicarbonate transport.

As the renin–angiotensin system regulates pancreatic bicarbonate secretion via a protein kinase C (PKC)-dependent mechanism,[55] Alvarez *et al.*[56] investigated the effect of angiotensin receptor signaling and PKC activation on SLC26A6 transport activity. AT1aR signaling inhibited bicarbonate transport by SLC26A6, and this effect was blocked by a PKC inhibitor. PKC activation by phorbol ester (phorbol 12-myristate 13-acetate) inhibited transporter activity, and induced dissociation of CAII from SLC26A6 as determined by co-immunoprecipitation. A $S^{553}A$ substitution within SLC26A6 abrogated inhibition of transporter activity by phorbol 12-myristate 13-acetate and blocked the effect of PKC activation on reduction of CAII/SLC26A6 association. These studies indicate that PKC-mediated phosphorylation of S^{553} disrupts CAII binding to SLC26A6, and represents the first report of a kinase-dependent disruption of a CA-transport metabolon.

NHE1

CAII also associates with NHE1, a ubiquitously expressed glycoprotein that protects cells from intracellular acidification by exchanging one intracellular proton for one extracellular sodium.[57] Association between CAII and NHE1 has been demonstrated *in vitro* by affinity blotting and by solid phase binding assays, where GST fusion proteins containing the C-terminal tail of NHE1 bound to immobilized CAII.[58] Interaction between NHE1 and CAII *in situ* was demonstrated by co-immunoprecipitation from cells transfected with NHE1 and CAII.[58] CAII binding to NHE1 involves a motif within the C-terminal tail that is distinct from the consensus acidic cluster that comprises the CAII binding motif found in BT family members.

The region of NHE1 that binds to CAII was localized to a 13 amino-acid peptide (^{790}RIQRCLSDPGPHPE^{803}) using a series of GST fusion peptides containing the C-terminal tail of NHE1 in a variety of binding assays.[59] Site-directed mutagenesis within this 13 amino-acid sequence revealed that the $S^{796}D^{797}$ residues (underlined above) were critical for CAII binding to NHE1. Facilitation of sodium/hydrogen exchange by CAII was demonstrated using AP-1 cells transfected with NHE1, in which co-transfection with CAII increased the rate of cell pH recovery from a bicarbonate-dependent acid-load (in CO_2/HCO_3^- buffer), and this effect was inhibited in the presence of the CA inhibitor acetazolamide.[58] On the other hand, CAII co-transfection did not increase pH recovery from acid-loaded cells expressing the $S^{796}D^{797}$ mutation of NHE1.[59] Finally, co-transfection with a dominant-negative CAII mutant having a $V^{143}Y$ mutation reduced recovery relative to transfection of NHE1 alone, presumably owing to displacement of endogenous CAII from its binding site on NHE1.[58]

Association of CAII with other NHE's, such as NHE3 in the proximal tubule, has not been reported; however, the NHE3 C-terminal tail contains a putative CAII binding motif (rabbit NHE3: ^{713}ERELELSDPEEAP^{725})[59] suggesting that a CAII-NHE3 proton transport metabolon may exist in proximal tubule cells of the kidney. Thus, CAs not only facilitate bicarbonate transport by BT family members, but they can also associate with at least one member of the NHE family of cation transporters and facilitate proton efflux via sodium/hydrogen exchange.

In contrast to SLC26A6, CAII association with NHE1 is positively regulated by phosphorylation. NHE1 activity is regulated by growth factors and *G-P*rotein *C*oupled *R*eceptors through activation of the Ras-extracellular signal-regulated kinase (ERK) pathway and may involve direct phosphorylation of NHE1 by the ERK pathway[60,61] or phosphorylation of NHE1 by kinases functioning upstream of ERK; such as NIK, *N*ck-*I*nteracting *K*inase,[62] or by a downstream kinase, $p90^{rsk}$.[60] Phosphorylation of an NHE1 C-terminal peptide of 182 amino acids by heart cell extracts or ERK, but not by Ca^{2+}/calmodulin-dependent protein kinase II, increased CAII binding.[59] The site of phosphorylation by ERK is probably non-overlapping with the CAII binding site, because an $S^{796}A$ mutation did not affect phosphorylation by ERK2, indicating that ERK2 phosphorylates another residue.[59] The $S^{796}A$ mutation also failed to decrease CAII binding to the phosphorylated peptide, suggesting that phosphorylation of another site in the C-terminal tail of NHE1 induces a conformational shift in NHE1 that favors CAII binding.[59] Thus, mitogenic signal-transduction stimulates NHE1 activity, at least in part, through an ERK pathway-dependent increase in CAII association with NHE1 to form a proton transporting metabolon (Figure 4).

CAIV

CAIV is the most widely distributed of the membrane-associated CA isoforms, with abundant expression observed in several tissues including, kidney, heart, lung, gall bladder, distal small intestine, colon, pancreas, capillary endothelium, and skeletal muscle.[24,63–70] CAIV is one of three membrane-associated CA isoforms, the others being CAXII, and in rodent species CAXIV, that account for the remaining ~5% CA activity in the kidney not contributed by CAII. Studies characterizing CAIV expression in the nephron segments were recently reviewed in detail.[38] In summary, CAIV is expressed in acidifying nephron segments in the proximal tubule (S2 > S1 ≫ S3) and in distal nephron, including outer and inner medullary collecting ducts and α-intercalated cells of the cortical collecting duct (see Figure 3). A major role for CAIV in urinary acidification has been demonstrated in studies where inhibition of luminal CA activity eliminates nearly all net bicarbonate reabsorption in the proximal tubule,[71] inner stripe of the outer medullary collecting duct,[72] and in CAII-deficient patients and mice where inhibition of CA activity diminishes renal acid secretion.[73,74]

CAIV is unique among CA isoforms, because it is tethered to the plasma membrane via a GPI anchor.[23,24] Human and rabbit CAIV are synthesized as 312 and 308 amino-acid precursors, respectively, that include an N-terminal signal peptide, and a COOH-terminal domain containing a putative transmembrane domain that is cleaved upon GPI-anchor attachment in the endoplasmic reticulum.[75,76] CAIV contains two disulfide linkages that confer resistance to denaturation by exposure to the detergent SDS (0.2–5%), which inactivates most other CA isoforms. Thus SDS-resistance has enabled determination of the CAIV contribution to total hydratase activity in tissues containing multiple CA isoforms.[24,77] Rabbit CAIV has a predicted molecular weight of 34 kDa, however CAIV expressed in kidney cortex and medulla exhibit apparent molecular weights of ~46 and 47–60 kDa, respectively, owing to glycosylation.[69] This contrasts with CAIV in human kidney tissue that migrates in SDS–polyacrylamide gel electrophoresis with an apparent molecular weight close to the predicted mass of 35 kDa. Rabbit CAIV contains two putative N-glycosylation sites, and treatment of rabbit CAIV with *N*-glycanase reduces the apparent molecular weight to ~34 kDa, confirming the extensive glycosylation of CAIV in rabbit kidney.[69,76] Differences in the glycosylation patterns of the expressed protein in the kidney cortex and medulla may reflect cell-type specific post-translational modification of CAIV in renal epithelia cells, and might in part explain differences in apparent cellular targeting in proximal versus distal nephron segments (see below).

CAIVs from human, bovine, and rabbit tissues are high-activity enzymes (see Figure 2), whereas CAIV from mouse and rat have only 10–20% as much catalytic activity.[32] Examination of the sequences revealed that human, bovine, and rabbit CAIVs have a glycine residue at position 63, whereas mouse and rat have a glutamine in this position. Indeed, the low activity of mouse CAIV can be improved by the $Q^{63}G$ substitution.[32] The lower activity of the rodent CAIVs is likely due to the Q^{63} substitution, which reduces the efficiency of proton transfer by the adjacent histidine (H^{64}).[32] In previous studies of CAII this histidine was found to be an efficient proton shuttle group transferring protons between the zinc-bound water and surrounding buffer molecules.[27]

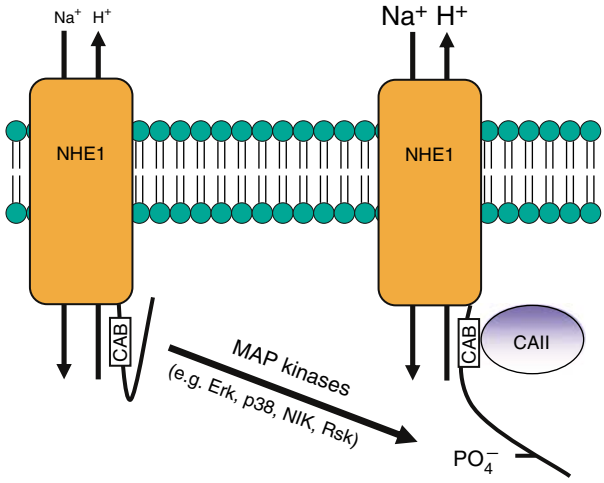


Figure 4 | CAII interaction with NHE1 is regulated by phosphorylation. Mitogenic signaling induces activation of kinase cascades that lead to phosphorylation of NHE1. Phosphorylation of NHE1 by *M*itogen-*A*ctivated *P*rotein (MAP) kinases (e.g. ERK, p38, $p90^{rsk}$, or NIK) increases NHE1 activity, at least in part, by facilitating CAII binding to the transporter.

Most GPI-anchored proteins are targeted to the apical membrane of polarized epithelial cells.[78–81] Nevertheless, CAIV immunoreactivity detected with a variety of different CAIV antibodies has also been observed on the basolateral membranes of proximal tubule segments in human, rabbit, and rodent species.[64,69,82] In distal nephron segments, however, CAIV expression is detected exclusively on the luminal or apical membrane.[69] In a recent study[83] using a Percoll density-gradient separation of apical from basolateral membrane vesicles, we characterized the polarity and the molecular form of CAIV immunoreactivity in rabbit kidney cortex, and found that CAIV was expressed on both the apical and basolateral membranes of proximal tubule epithelia, whereas alkaline phosphatase, which is also expressed in the kidney as a GPI-anchored protein, was expressed exclusively on apical membranes. Indeed, the basolateral membranes enriched from kidney cortex expressed CAIV immunoreactivity that was identical in molecular weight to that in apical membrane fractions[83] (Figure 5). These results ruled out the possibility that antibodies to CAIV were cross-reacting with another CA isoform (e.g. CAXII) expressed in basolateral membranes of the proximal tubule. The amount of CAIV in basolateral membrane fractions comprised a substantial portion (~30%) of the total CAIV protein, suggesting that basolaterally expressed CAIV could facilitate acid/base transport in the proximal tubule.

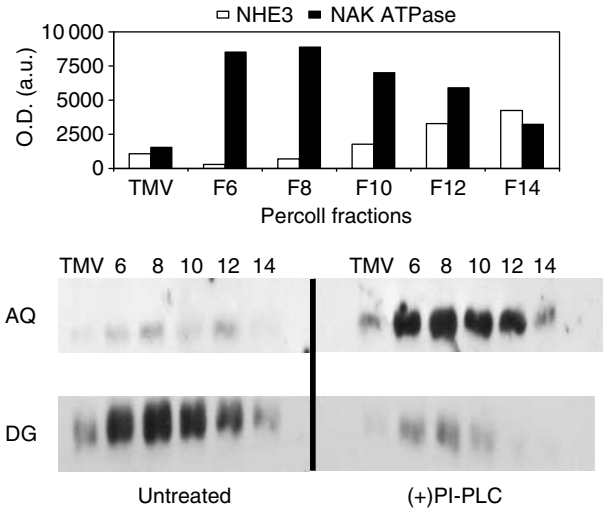


Figure 5 | CAIV is expressed on the apical and basolateral membranes of proximal tubule as a GPI-anchored protein. Membrane vesicles from rabbit kidney cortex were separated into basolateral and apical membrane-enriched fractions by centrifugation through a Percoll density gradient. The levels of NHE3 and Na^+, K^+-ATPase in membrane vesicle fractions determined by densitometry of Western blotting (upper panel). Linkage of CAIV via a GPI anchor was determined by sensitivity to cleavage by PI-PLC. Membrane vesicle fractions were treated with PI-PLC- and GPI-anchored/transmembrane proteins were separated from non-GPI-linked protein by Triton X-114 phase extraction. Lower portion of this panel shows levels of CAIV in the aqueous (AQ) and detergent phases (DG) of control and PI-PLC-treated fractions determined by Western blotting with anti-rabbit CAIV (YDQR). Note that fractions 6 and 8 are basolateral and 12 and 14 are apical.

CAIV expressed in basolateral and apical membranes from kidney cortex were equally sensitive to phosphatidylinositol-dependent phospholipase C (PI-PLC) cleavage (see Figure 5), indicating that CAIV is expressed on both membranes as a GPI-anchored protein.[83] Furthermore, we found no evidence for expression of a ~3 kDa larger molecular weight species corresponding to a putative transmembrane form of CAIV in kidney cortex. Expression of CAIV as a transmembrane protein has been reported in normal pancreatic tissues, where CAIV expressed on the luminal or apical surface of ductal cells was insensitive to PI-PLC cleavage and was detected by a polyclonal antisera raised against the COOH terminal tail that is cleaved upon GPI-anchor attachment.[84] The functional significance of transmembrane CAIV is unclear, as cleavage of the COOH-terminal tail of CAIV has been reported to be necessary for realization of full enzyme activity.[85] Carboxypeptidase M and PrP^C are two other examples of GPI-anchored proteins that are either targeted to both apical and basolateral membranes (carboxypeptidase M)[86] or are preferentially sorted to basolateral membranes (PrP^C)[87] in MDCK cells. In another cell culture model, Fischer rat thyroid epithelial cells, six out of nine detectable endogenous GPI-anchored proteins were localized on the basolateral surface, two were apical and one was not polarized.[88] However, the CAIV study[83] may be the first report demonstrating both apical and basolateral targeting of a GPI-anchored protein in polarized epithelial cells from normal tissue. Thus, CAIV may serve as an excellent model protein for the study of the determinants that control cellular trafficking of GPI-anchored proteins in polarized epithelial cells.

CAIV interacts with members of the BT family of transporters to form the extracellular component of a bicarbonate transport metabolon (Figure 6). Evidence for direct interaction between CAIV and anion exchangers was first reported by Sterling *et al.*[89] who showed that CAIV expressed in HEK293 lysates binds to AE1, AE2, and AE3 immobilized on polyvinylidene fluoride membranes, and that co-transfection of AE1 with CAIV shifts the distribution of CAIV in sucrose-density gradients. Subsequently, the interaction of CAIV with NBC1 was demonstrated by gel overlay assay.[90] The influence of CAIV on bicarbonate transport was demonstrated in HEK293 cells transiently transfected with AE's or NBC1, in which CAII ($V^{143}Y$) reduced the rate of bicarbonate transport, and the transport rate was restored when CAIV was co-transfected with dominant-negative CAII ($V^{143}Y$). Acetazolamide inhibited the effect of CAIV co-transfection on bicarbonate transport rate demonstrating that CAIV hydratase activity was required.[89] Similarly, in HEK293 cells transfected with NBC1, co-transfection of CAIV increased cell pH recovery from an acid load and this effect of CAIV was also inhibited by acetazolamide.[90] Casey and co-workers mapped the CAIV binding region to extracellular loop 4 (EC4) of AE's and NBC1. GST fusion

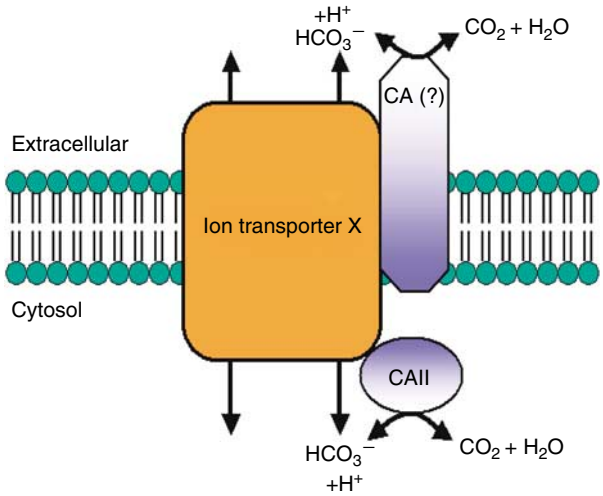


Figure 6 | A bicarbonate transport metabolon. A schematic model illustrating interactions between CAII and membrane-bound CA isoforms with BTs (e.g. *X* = AE1; ? = CAIV). CAs provide the 'push' and 'pull' for bicarbonate transport via mass action by supplying or dissipating substrate.

proteins containing EC4, but not EC3, from either AE1 or NBC1 pulled down CAIV from a cell lysate of transfected HEK293 cells.[89] Alignment of the amino-acid sequence within EC4 of anion exchangers and NBC isoforms reveals highly conserved motifs that putatively bind CAIV.[90] The function of a conserved RGW sequence within EC4, was demonstrated by mutating $G^{767}T$ in the NBC1 EC4; this fusion peptide did not bind CAIV in pull-down assays, and this mutation abrogated the effect of co-expression of CAIV on NBC1-mediated cell pH recovery from an acid load.

CAIV associates with NBC1 to form the extracellular component of a bicarbonate transport metabolon in a non-polarized epithelial cell model.[90] Studies performed in our laboratory have established that a basolaterally expressed membrane-associated CA facilitates bicarbonate and fluid reabsorption in the proximal tubule segments.[91] Evidence for basolateral CAIV expression in kidney cortex suggests that this isoform contributes, at least in part, to the basolateral CA activity that facilitates bicarbonate co-transport by NBC1 in the renal proximal tubule.

CAXIV and CAXV

CAXIV was originally cloned from mouse kidney[9] and subsequently a full-length complementary DNA for the human sequence was obtained from spinal cord mRNA.[10] In both species a ~1.7 kb transcript encodes a 337 amino-acid polypeptide with a predicted molecular mass of 37.5 kDa. CAXIV is most closely related in terms of amino acid similarity to CAXII. The expressed protein is catalytically active and the hydratase activity is inhibited by acetazolamide, but not sulfonamide.[10] The expressed human form appears to be much less active than CAII,[10] whereas the mouse form appears to have activity that is comparable to CAII[92] (see Figure 2). CAXIV is a type I transmembrane protein; the polypeptide contains a hydrophobic N-terminal signal sequence and a putative transmembrane domain at the C-terminus. CAXIV has two cysteine resides that are predicted to form a disulfide bond and are well conserved among membrane-associated CA isoforms.

In mice, human, and rabbit species, CAXIV is abundantly expressed in heart, skeletal muscle, liver, and in mice and humans abundant expression was also observed in brain.[9,10,63] Abundant expression of CAXIV in the kidney is observed only in rodent species.[9] Immunohistochemical studies of expression in mouse kidney reveal that CAXIV is predominantly expressed on the apical membranes of S1 and S2 segments in the proximal tubule, overlapping with CAIV in the S2 segment, suggesting that CAXIV plays an important role in bicarbonate reabsorption in the mouse proximal tubule. CAXIV is also expressed on the luminal side of the thick ascending limb, indicating that in rodent species CAXIV accounts for luminal membrane CA activity in this segment.[92] In contrast to mouse kidney, CAXIV is not abundantly expressed in human kidney[10] and CAXIV mRNA and protein are virtually undetectable in rabbit kidney cortex.[63]

A complementary DNA sequence for a novel CA isoform, CAXV, was submitted to the NCBI in 2000 (GenBank® accession no. AF231122), and Hilvo *et al.*[12] recently reported expression of CAXV mRNA in mouse tissues including kidney and brain. However, in humans the three candidate genes for CAXV are pseudogenes. Whereas CAXV is similar to CAIV in that it is expressed as a GPI-anchored protein, the enzymatic activity is comparable to CAIII (see Figure 2), which is several logs lower than CAII and non-rodent orthologs of CAIV.[12] Thus, CAXIV most likely accounts for much of the apical hydratase activity observed in acidifying nephron segments within the rodent kidney. The expression of CAXIV and CAXV in mice, but not in humans and rabbits, may reflect a compensatory evolutionary response to the reduced activity of CAIV in rodents owing to the $G^{63} \rightarrow Q$ substitution[32] (see Figure 2). Compensatory expression of additional membrane-associated CA isoforms in the proximal tubule emphasizes the importance of membrane-associated CA hydratase activity in the regulation of acid/base transport within the kidney. The high activity of CAIV in humans may have made CAs XIV and XV superfluous in evolution; therefore CAXIV expression would not be conserved in the proximal tubule and CAXV would become a pseudogene in humans.

CAXII and CAIX

CAXII is a type I transmembrane protein of 354 and 355 amino acids in humans and rabbit, respectively, with a predicted molecular mass ~40 kDa. CAXII is a one-pass transmembrane protein expressing an extracellular CA domain with a unique short extracellular segment and a hydrophilic cytoplasmic tail, and one pair of conserved cysteine residues that putatively form one intramolecular disulfide bond.[13,20,21] Human and rabbit CAXII are 83% identical and the expressed proteins (43–44 kDa) are

catalytically active. Human CAXII was initially reported to exhibit 16% of activity of CAIV in COS cell transfectants,[20] whereas the activity of rabbit CAXII expressed in inner medullary collecting duct cell transfectants was comparable to CAIV (with the exception that CAXII hydratase activity was inhibited by 1–2% SDS).[21] Subsequently, using oxygen-18 exchange, Ulmasov *et al.*[93] showed that a recombinant secretory form of human CAXII, as well as membrane-bound CAXII expressed in CHO cells, had catalytic rates of CO_2 hydration that were comparable to human CAIV but less than CAII (see Figure 2). The tissue distribution of CAXII is perhaps the most limited of the CA isoforms with abundant expression of CAXII restricted to kidney and colon of human, mouse, and rabbits,[20,21,94] and the endometrial epithelium and prostate in humans.[13,95]

Immunohistochemical studies of CAXII expression in human kidney revealed abundant expression of CAXII in the basolateral membranes of the thick ascending limb of Henle's loop, distal convoluted tubules, and principal cells of the collecting duct[96] (see Figure 3). Weak basolateral staining was also observed in proximal convoluted tubules. Expression of CAXII mRNA in the rabbit nephron was observed in the proximal convoluted and straight tubules, cortical and medullary collecting ducts, thick ascending limb of Henle, and papillary epithelium, but not in glomeruli or S3 segments.[21] CAXII protein expression in the rabbit kidney has not yet been determined owing to a lack of a suitable antibody. In contrast to results in human kidney, but consistent with expression of CAXII mRNA in rabbits, immunohistochemical studies in mice revealed strong basolateral staining of CAXII in S1 and S2 proximal tubule segments, as well as in H^+-secreting α-intercalated cells of the cortical and medullary collecting ducts.[97] Despite apparent discrepancies for observed CAXII expression in different species, one can conclude that CAXII is expressed basolaterally in H^+-secreting cells in the kidney; its relatively high abundance in rodent kidney cells that transport protons at high rates may be related to the slow catalytic activity of CAIV in these species. Although CAXII is expressed on the basolateral membrane of epithelial cells in the kidney and colon, the association of CAXII with basolaterally expressed transporters to form transport metabolons has not as yet been reported.

CAIX is a 54/58 kDa N-glycosylated single-pass transmembrane protein[19] that, in addition to a conserved extracellular catalytic domain, contains an N-terminal proteoglycan-like domain that functions in cell adhesion and can be shed by stimulus-dependent activation of metalloproteinase activity.[22,98] CAIX is catalytically active and exhibits hydratase activity that is comparable to CAIV[99] (see Figure 2). In cultured cells CAIX expression is density dependent,[100] and thus, in contrast to other CA isoforms CAIX may have dual functional roles in intercellular communication and acid/base physiology.[22,101]

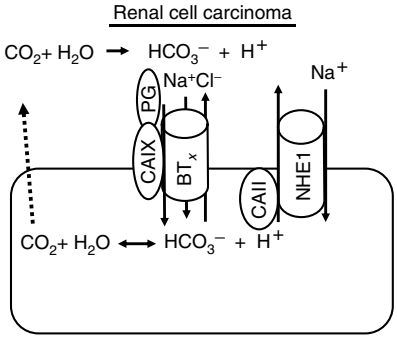
CAIX is abundantly expressed in the proximal gastrointestinal tract including the stomach and gall bladder but not in normal kidney.[63,102,103] CAIX expression is also observed in the basolateral membrane of enterocytes of duodenum and jejunum, with most abundant expression occurring in the crypts in regions where epithelial cells exhibit high proliferative capacity.[102] The function of CAIX in the gastric mucosa has yet to be defined, but basolateral expression of CAIX suggests a role in facilitation of chloride/bicarbonate exchange resulting in bicarbonate extrusion across the basolateral membrane of gastric parietal cells coupled with proton secretion by gastric H^+, K^+ATPase.[104] However, CAIX expression is not restricted to parietal cells, and whereas CAIX-deficient mice exhibit gastric hyperplasia, they do not exhibit marked deficits in parietal cell number or gastric acid secretion.[104] Nevertheless, CAIX function in the proximal gastrointestinal tract is most likely directly or indirectly coupled to gastric acid secretion. In a recent study we demonstrated that induction of CAIX during rabbit maturation coincided with upregulation of the H^+, K^+ATPase α-subunit,.[63] Thus, CAIX likely facilitates H^+/HCO_3^- transport in order to maintain cell pH in gastric and duodenal epithelium exposed to gastric acid in addition to facilitating bicarbonate extrusion in acid-secreting parietal cells.

CAIX and CAXII function in RCC

CAs IX and XII are tumor-associated antigens whose expression is induced by hypoxia,[105] and expression of these two isoforms is consistently observed in clear-cell carcinoma of the kidney. Renal cell carcinoma (RCC) accounts for roughly 3% of adult malignancy and 90–95% of neoplasms arising from the kidney.[106] RCC often presents with few symptoms and has a high incidence of metastatic disease progression at first diagnosis.[106,107] Defects in the von Hippel–Lindau tumor suppressor gene appear to be responsible for approximately 60% of cases of sporadic clear-cell RCC.[107] Ectopic overexpression of CAIX in RCC can result from inactivation of the von Hippel–Lindau tumor suppressor gene complex by frame-shift mutations,[108] leading to stabilization of hypoxia inducible factor-1α which regulates trans-activation of CAIX gene expression.[109,110] CAs IX and XII may promote tumor growth and survival via facilitation of acid/base transport that enables tumor cell growth and survival in hypoxic, acidic environments[105,111]and also by modulating E-cadherin-mediated cell adhesion.[101] Consistent with this hypothesis is the observation that CAIX and CAXII expression is localized to areas of necrotic tumor foci.[105] Robertson *et al.*[112] reported that blockade of hypoxia-mediated CAIX induction by gene silencing with small-interfering RNA slowed growth and reduced survival during hypoxia of breast carcinoma cells, but had no effect on invasiveness. CAIX may also participate in mitogenic signal transduction. Dorai *et al.*[113] recently reported that CAIX is phosphorylated on tyrosine (residue 449 in the cytoplasmic tail domain) and associates with the regulatory p85 subunit of phosphatidylinositol 3′-kinase in an epidermal growth factor-dependent manner in RCC cell lines. In this study

expression of CAIX in RCCs correlated with increased phosphatidylinositol 3′-kinase-dependent Akt phosphorylation. The phosphorylation of CAIX in the midst of these cell signaling systems forms the basis of a vicious cycle in the progression of clear-cell RCC: the CAIX-mediated activation of Akt promotes the expression of hypoxia inducible factor-1α, which in turn promotes the expression of CAIX, which promotes tumor survival under hypoxic conditions.[113]

It has been proposed that CAIX activity facilitates the acidification of extracellular milieu surrounding cancer cells by converting CO_2 that has diffused to the extracellular space back into bicarbonate and protons. This would enable the Na^+-driven bicarbonate-chloride exchanger on the cell membrane to transport these newly generated HCO_3^- ions back into the cytoplasm[113] (Figure 7). This transport process results in the net export of protons and allows cancer cells to buffer their cell pH to neutrality and thereby facilitate their biosynthetic reactions. The resulting extracellular acidic microenvironment is conducive to activating cell surface proteases such as cathepsin B and matrix metalloprotease-9, releasing growth factors, and suppressing the immune function of effector T cells.[113] Consistent with this model is the observation in transfected MDCK cells that CAIX expression facilitated extracellular acidification when cells were exposed to hypoxia.[111] This effect required the CA domain, but not the proteoglycan domain, of CAIX and was reduced by CAIX-selective inhibitors. Interestingly, in this same study a fluorescence tagged CAIX inhibitor bound primarily to CAIX-expressing cells that had been exposed to hypoxia; hypoxia may also stimulate structural changes within the active site of the CAIX molecule.[111] A role for CAIX in tumor cell invasion was also suggested by the observation that acetazolamide, a CA inhibitor, reduces invasiveness of renal cancer cells in an *in vitro* model of metastasis.[114] Additional studies are required to identify associations of CAs IX and XII with acid/base transporters and their role in acid/base homeostasis in normal tissue physiology as well as in tumor cell proliferation, metastases, and survival in hypoxia.



BT_x= Na^+-driven chloride bicarbonate exchanger (NDCBE), SLC4A8)

Figure 7 | Model for CA function in acid/base homeostasis in RCC. CAII facilitates acid secretion by NHE1-mediated sodium/hydrogen exchange that plays a pivotal role in maintaining cell pH of rapidly dividing cell populations. CAIX (and perhaps CAXII) may facilitate extracellular acidification by catalyzing the formation of H_2CO_3 and HCO_3^- from expired CO_2. Juxtaposition of CAIX with an electroneutral sodium bicarbonate co-transporter or Na^+-driven chloride/bicarbonate exchanger may enhance the conversion of CO_2 to bicarbonate, thereby enabling bicarbonate influx to help buffer cell pH during anaerobic metabolism under hypoxic conditions. This would also result in the extracellular accumulation of protons contributing to acidosis.

OVERVIEW OF CARBONIC ANHYDRASE FUNCTION IN REGULATION OF ACID/BASE HOMEOSTASIS IN CELLS OF THE PROXIMAL AND DISTAL NEPHRON AND RCC

Proximal tubule epithelial cell

Co-expression and association of CAs with transporters markedly enhances H^+/HCO_3^- transport;[90] via mass action CAs provide the 'push' and 'pull' for H^+/HCO_3^- transport by accumulating or dissipating substrate (see Figure 6). In the renal proximal tubule filtered bicarbonate and protons secreted by NHE3 activity form carbonic acid (H_2CO_3) that is dehydrated by the CA activity of CAIV (and CAXIV in rodent species) expressed at the brush border of the proximal tubule, thereby enabling passive diffusion of CO_2 into the cell (Figure 8). Although NHE3 contains a putative CAII binding motif,[59] whether CAII and/or CAIV associate with NHE3 to facilitate sodium/proton exchange in the apical membrane of proximal tubule epithelia has not yet been established. CAII may also associate with SLC26A6 in the apical membrane, however the contribution of a CAII/SLC26A6 transport metabolon to H^+/HCO_3^- transport in the renal proximal tubule is unclear; formate rather than bicarbonate may be the physiological substrate for SLC26A6 in the proximal tubule.[50] Furthermore, the intensity of CAII/SLC26A6 interaction may be low owing to AT1aR signaling that disrupts CAII binding

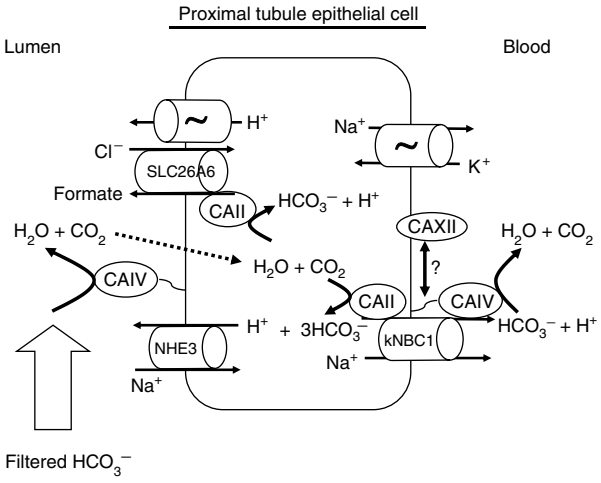


Figure 8 | Model for function of CAs in bicarbonate reabsorption in the proximal tubule. Filtered bicarbonate and protons secreted by NHE3 activity form H_2CO_3 that is dehydrated by CAIV expressed at the brush border of the proximal tubule, and CO_2 passively diffuses into the cell. CAII associated with kNBC1 on the basolateral membrane hydrates CO_2 to provide substrate for electrogenic sodium bicarbonate transport by kNBC1 in the 3:1 mode. Basolaterally expressed CAIV, and perhaps CAXII, associated with kNBC1 facilitate reabsorption by dissipating bicarbonate at the basolateral surface.

to SLC26A6 via a PKC-dependent mechanism.[56] At the basolateral membrane CAII associated with kNBC1[44] hydrates CO_2 to provide substrate for electrogenic sodium bicarbonate transport by kNBC1 in 3:1 mode. Membrane-associated CAs, which include CAIV and CAXII, with CA catalytic domains on the extracellular face of the basolateral membrane may associate with kNBC1 to dissipate bicarbonate or H_2CO_3 at the basolateral surface and thereby facilitate bicarbonate reabsorption.[90]

α-Intercalated cells of the CCD

In the CCD α-intercalated cells contribute to urinary acidification by the secretion of protons via the activity of apically expressed vacuolar H^+-ATPase (Figure 9) and to a lesser extent H^+K^+-ATPase. In addition, α-intercalated cells reabsorb remnants of filtered bicarbonate that is titrated by secreted protons and dehydrated by apically expressed CAIV. Bicarbonate reabsorption is also facilitated by apical NBC3-mediated electroneutral sodium bicarbonate co-transport,[47] which is facilitated CAII on the interior of the membrane.[49] At the basolateral surface CAII also facilitates bicarbonate efflux by AE1-mediated chloride/bicarbonate exchange.[39,41,42] CAXII may contribute to bicarbonate reabsorption mediated by AE1 via dehydration of bicarbonate or H_2CO_3 at the basolateral surface of α-intercalated cells. However, association between CAXII and AE1 to form the extracellular component of a bicarbonate transport metabolon has not been reported (Figure 9).

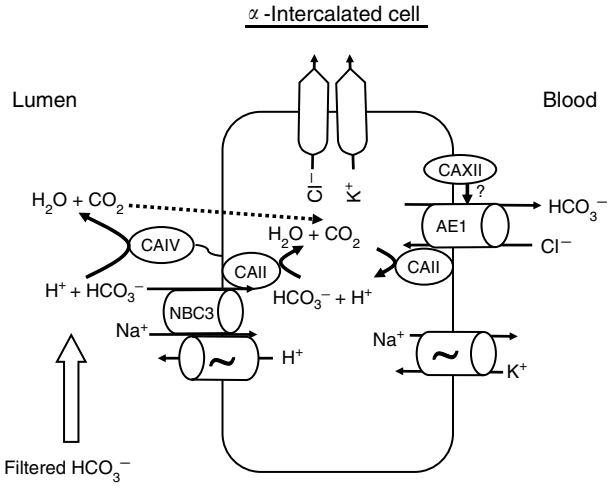


Figure 9 | Model for CA function in acid/base homeostasis in α-intercalated cells of the cortical collecting duct. α-Intercalated cells contribute to urinary acidification by the secretion of protons via the activity of apically expressed vacuolar H^+-ATPase and to a lesser extent H^+K^+-ATPase. In addition, α-intercalated cells reabsorb remnants of filtered bicarbonate via dehydration by apically expressed CAIV as well as by an NBC3-mediated electroneutral sodium bicarbonate co-transport that is facilitated by associated CAII. At the basolateral surface CAII facilitates bicarbonate efflux by AE1-mediated chloride/bicarbonate exchange.

Renal clear-cell carcinoma

Ectopic expression of CAIX in RCC may enable tumor cells to survive and proliferate in hypoxic acidic environments by facilitating H^+/HCO_3^- transport mechanisms that promote extracellular acidification and maintenance of cell pH (see Figure 7). Although the identity of acid/base transporters that associate with CAIX in normal gastric tissue and in neoplasms has not been determined extensively, it is interesting to speculate that CAIX catalyzes hydration of CO_2 generated by respiration and thereby facilitates bicarbonate influx via an associated Na^+-dependent chloride-bicarbonate exchanger.[115] In this scenario bicarbonate influx coupled with proton secretion by the sodium/proton exchange activity of NHE1 results in extracellular acidification and maintenance of cell pH despite acid production by anaerobic metabolism. It is likely that CAII also facilitates tumor cell growth and survival by associating with NHE1 to facilitate sodium/proton exchange.

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