Expression of membrane-associated carbonic anhydrase isoforms IV, IX, XII, and XIV in the rabbit: induction of CA IV and IX during maturation

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Purkerson, Jeffrey M., and George J. Schwartz. Expression of membrane-associated carbonic anhydrase isoforms IV, IX, XII, and XIV in the rabbit: induction of CA IV and IX during maturation. Am J Physiol Regul Integr Comp Physiol 288: R1256–R1263, 2005; doi:10.1152/ajpregu.00735.2004.—Several carbonic anhydrase (CA) isoforms are associated with plasma membranes. It is probable that these enzymes interact with anion transporters to facilitate the movement of HCO₃⁻ into or out of the cell. A better knowledge of CA isofrom expression in a given tissue would facilitate a systematic examination of any associations with such transporters. We examined the expression of CAs IV, IX, XII, and XIV mRNAs in rabbit tissues, including kidney, heart, lung, skeletal muscle, liver, pancreas, gall bladder, stomach, small intestine, colon, and spleen, using quantitative real-time reverse transcription polymerase chain reaction (RT-PCR). CA IV mRNA was mainly in kidney, heart, lung, colon, and gall bladder. CA IX mRNA was restricted to stomach, gall bladder, duodenum, and early jejunum. CA XII mRNA was found in kidney and colon. CA XIV mRNA was localized to heart, lung, skeletal muscle, and liver. The data indicate that there are different patterns of CA expression in various tissues: CA IX was expressed in the proximal gastrointestinal tract, whereas CA XII and CA IV were more distal. CA IV and CA XII are important kidney isoforms. CA XIV was abundant in metabolically active tissues such as liver, heart, lung, and skeletal muscle. Some significant species differences were noted in the expression of some of these isoforms; for example, CA XIV is not expressed in rabbit kidney, despite being abundant in mouse kidney. Maturational studies showed that the expression of CA IX mRNA and protein increased markedly with weaning (∼3–4 postnatal wk) and was well correlated with the maturational expression of the α-subunit of the gastric H⁺,K⁺-ATPase, suggesting that function of CA IX in the gastric H⁺ pump might be linked in the digestion of adult foods. The unique pattern of membrane-bound CA isoforms suggests different functional associations with transporters, depending on the physiological demands on the tissue.

METHODS

Animals

New Zealand white rabbits were purchased from Hazelton-Dutchland farms (Denver, PA). Adult rabbits (1.8–3.1 kg) were euthanized by intramuscular injection with xylazine (5 mg/kg) and ketamine (44 mg/kg) followed by an intracardiac injection of pentobarbital sodium (100 mg/kg). Tissues were harvested immediately and snap-frozen in liquid nitrogen and then stored at −80°C until use. For studies of CA expression during neonatal maturation, pregnant rabbits were purchased from Hazeltan and two litters of 8–10 rabbits were used for tissue harvest at ages ranging from 2–37 days.

Antibodies

Goat anti-rabbit CA IV (KDNV) was produced in our laboratory as previously described (23). The M75 monoclonal antibody recognizing the N-terminal domain of MN/CA IX (21) was kindly provided by Drs. J. Pastorek and S. Pastorekova (Institute of Virology, Slovak Academy of Sciences Bratislava, Slovak Republic). Rabbit antimouse CA XIV polyclonal antiserum was kindly provided by Dr. W. S. Sly (St. Louis University School of Medicine, St. Louis, MO). Goat anti-actin polyclonal antibody (SC-1616) and Goat anti-rabbit CA IV (KDNV) were purchased from Santa Cruz Biotech (Santa Cruz, CA). Monoclonal antibody HK 12.18
directed against the alpha-subunit of porcine H⁺,K⁺ ATPase was purchased from EMD Bioscience (San Diego, CA). Horseradish peroxidase (HRP)-conjugated secondary antibodies utilized in this study included horse anti-mouse (Vector Labs, Burlingame, CA), donkey anti-rabbit (Jackson ImmunoResearch, West Grove, PA) and horse anti-goat (Vector).

Rabbit Isoform Clones

Full-length cDNA clones for rabbit CA IV and CA XII were cloned previously (24, 34). Partial cDNA clones of rabbit CA XIV (647 bp) and CA IX (547 bp) were obtained by RT-PCR amplification from regions of conserved nucleic acid sequences within the human and mouse genes: CA XIV sense primer 5'- TTGGATCCCTGGCTGCAGATGG, CA XIV anti-sense primer 5'- CAACGTACCTTCGAGATGGTGCGA 3', CA IX sense primer 5'- GTTCCCCCTGCAGATCCACCTGGATGTTCA, CA IX anti-sense primer 5'- GAGGGCCAAAAC-CAGGGCTAGATGTACACCCG 3'. In those cases where mismatches between human and mouse sequences were observed, the CA IX primers were based on the human CA IX sequence. The rabbit CA IX fragment was 86% and 89% identical, whereas the CA XIV sequence was 81% and 80% identical to the corresponding mouse and human sequences, respectively.

Analysis of Steady State mRNA Levels by Quantitative Real-Time, RT-PCR

Total RNA was isolated from 20–50 mg of rabbit tissues utilizing either the Tri-Reagent (Molecular Research Center, Cincinnati, OH) or the RNeasy Mini kit (Qiagen, Valencia, CA) according to protocols recommended by the manufacturer. For isolation of total RNA from fibrous tissues, including heart and skeletal muscle, digestion with protease K was incorporated into the RNeasy procedure according to the manufacturer’s recommended protocols. Digestion with RNase-free DNase was also included in the RNeasy protocol to minimize DNA contamination of RNA preparations.

To examine CA mRNA isoforms along the gastrointestinal tract, we used specific sites to reproducibly obtain tissue from specific segments. Stomach tissue samples were taken from the body (fundus), whereas duodenum samples spanned the pyloric sphincter to the duodenal ligament of Treitz and 70 cm proximal to the cecum. Samples from the ileum were taken from the proximal 70 cm point to the cecum. Colon samples were taken from ascending, transverse, and descending colon, excluding the distal colon or rectum. Tissue was also isolated from the appendix.

Complementary DNA (cDNA) was synthesized from 1–2 µg of total RNA using the SuperScript First-strand synthesis system for RT-PCR with the Superscript II reverse transcriptase enzyme (Invitrogen, Carlsbad, CA), utilizing either oligo dT or random primers, according to the manufacturer’s recommended protocol. Partial or complete cDNAs for rabbit CA s and β-actin sequences were used to design specific primer sets and fluorogenic probes (TaqMan, fluorescent 5’ nucleic chemistry) using the PrimerExpress (Applied Biosystems, Foster City, CA) software (Table 1). The relative abundance of CA isoforms and β-actin mRNA was determined by quantitative real-time-RT-PCR using appropriate primer/probe sets and the Sequence Detection Systems (SDS) 7000 instrument and software (Applied Biosystems). Serial dilutions of linearized plasmid DNA containing CA isoform or β-actin sequence were used to generate a standard curve from which the relative mRNA copy number was calculated.

Analysis of Carbonic Anhydride Isoform and H⁺, K⁺-ATPase α-subunit Protein Expression

Total membrane preparations. 25–50 mg of snap-frozen tissue was homogenized in ice-cold 1× total membrane protein (TMP) buffer (25 mM Tris-SO₄ pH 7.5, 150 mM NaCl, 1 mM EDTA) supplemented with protease inhibitor cocktail (One Complete tablet/25- to 50-mL buffer, Roche Applied Science, Penzberg, Germany) and then incubated on ice for 30 min. Cellular debris was pelleted by centrifugation at 1,000 g for 10 min. at 4°C. Supernatants were recovered and then total membranes were pelleted by centrifugation at 110,000 g for 1 h at 4°C. The supernatants were decanted, and pelleted membranes were resuspended in 100- to 200-µL TMB buffer. Protein concentrations in membrane preparations were determined by the Bio-Rad Protein Assay (Bio-Rad Laboratories, Hercules, CA ) using BSA as a standard.

Tissue homogenates. Lysates were prepared from 50 to 200 mg of tissue in pAMBS buffer: 1% NP-40, 0.5% deoxycholate, 0.5 mM EDTA, 25 mM Tris-SO₄ pH 8.7, 150 mM NaCl, 0.1 M Na₂SO₄ containing a protease inhibitor cocktail. Tissues were homogenized in ice-cold pAMBS buffer with two 30-s bursts of a Tekmar TK10 homogenizer. After incubation on ice for 30 min, homogenates were clarified by centrifugation at 14,000 g. Aliquots of tissue homogenates (lysates) were added to equal volume 2× SDS-PAGE sample buffer (100 mM Tris-HCl, pH 6.8, 200 mM DTT, 4% SDS, 20% glycerol, bromophenol blue) incubated at 100°C for 5 min.

p-aminomethylbenzenesulfonylamide (pAMBS) precipitation. Carbonic anhydrases were precipitated from tissue homogenates by incubation with 50-µL packed beads of pAMBS-agarose (6.8 mg/mL packed gel, Sigma Chemical, St. Louis, MO) for 4–16 h at 4°C with agitation. Agarose beads were then washed three times with pAMBS buffer followed by addition of 2× SDS-PAGE sample buffer.

Immune-precipitation. Tissue samples taken from the fundus of the stomach were homogenized in immune-precipitation (IP) buffer (IP buffer, 1% NP-40, 0.5% deoxycholate, 0.5 mM EDTA, 25 mM Tris-HCl pH 7.4, 150 mM NaCl) and clarified by centrifugation at 14,000 g. For immune-precipitation, lysates were preclared with protein A/G-agarose beads (50–100 µL of packed beads; Pierce Biotechnology, Rockford, IL) and then incubated with 1 µg of HK12.18 antibody for 2–4 h at 4°C with agitation, followed by addition of 50 µL of protein A/G-agarose and incubation for an addition 12–16 h at 4°C. Beads were then washed three times with IP buffer followed by two washes with high-salt wash buffer (0.1% Triton-X-100, 500 mM NaCl, 50 mM Tris-HCl pH 7.4, 5 mM EDTA), and one final wash with 10 mM Tris-HCl, pH 7.4. Samples were then heated at 100°C for 5 min after the addition of equal volume of 2× SDS-PAGE sample buffer.

Western blotting. Total membrane proteins, tissue homogenate, or pAMBS-precipitated proteins were resolved by SDS-PAGE (8% 37.5:1 acrylamide/bis-acrylamide; Tris-glycine, Bio-Rad) and were

Table 1. Primers and probes used for quantitative real-time PCR

<table>
<thead>
<tr>
<th>mRNA</th>
<th>Forward Primer</th>
<th>Reverse Primer</th>
<th>TaqMan Probe</th>
</tr>
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<tr>
<td>β-Actin</td>
<td>GACGACTACCTGATGAGATGCTCT</td>
<td>TACAGCTCCACGGCGACGCCG</td>
<td>TACAGCTCCACGGCGACGCCG</td>
</tr>
<tr>
<td>CAIV</td>
<td>CATCACTACCTGACCCAGAACA</td>
<td>CCTGACTCCCCTGGTTGATGA</td>
<td>ACCACACGTGGCCAGGTTTAC</td>
</tr>
<tr>
<td>CAIX</td>
<td>CAGGCTCACTCAGACATTGAAA</td>
<td>GACGACTACCTGACCCAGAACA</td>
<td>TCGAAAGGGCATGACAAAGATTCCC</td>
</tr>
<tr>
<td>CAXII</td>
<td>GAGGCCTCCGCGGTAGACAGAAGA</td>
<td>CAGGCTCACTCAGACATTGAAA</td>
<td>TGACTCAACATTCCCAGGCCCATC</td>
</tr>
<tr>
<td>CAXIV</td>
<td>CAGCACTACCTGACCCAGAACA</td>
<td>CACGCTGAGCTGAGCAGATTGTGAT</td>
<td>TACAGCTCCACGGCGACGCCG</td>
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transferred to nitrocellulose membranes (Bio-Rad) using a Panther (HEP-1) semidy electroblotter (Owl Scientific, Cambridge, MA). Membranes were then blocked with 5% nonfat dry milk (Bio-Rad) in TBST (Tris-buffered saline supplemented with 0.05% Tween 20). Incubations with primary antibodies were carried out in heat-sealable bags with 2- to 5-ml blocking buffer for 2–4 h at room temperature (RT) or 16 h at 4°C, followed by 3 × 10 min washes with TBST. Incubations with HRP-conjugated secondary antibodies were carried for 2–4 h at RT and followed by 3 × 10 min washes with TBST and one wash in TBS. Western blots were developed with ECL chemiluminescent substrate (Amersham Biosciences, Piscataway, NJ), and chemiluminescence was detected by exposure to Hyperfilm (Amersham) for 5–60 s.

RESULTS

Tissue Distribution of Membrane-Associated CA Isoforms in the Rabbit

Rabbits have been used extensively to study the regulation of acid-base physiology. To better understand the contributions of membrane-associated CA isoforms to \( H^+/HCO_3^- \) transport in rabbit tissues, steady-state levels of carbonic anhydrase IV, IX, XII, and XIV mRNA and protein expression were examined by quantitative real-time RT-PCR and Western blotting, respectively. As shown in Fig. 1, each of the CA isoforms examined exhibited a distinct tissue expression pattern. The expression of CA IV was most widely distributed with abundant mRNA expression occurring in the gall bladder, colon, heart, lung, and kidney cortex. The CA IV mRNA expression pattern determined by measuring relative mRNA number copy by quantitative real-time RT-PCR was confirmed by detection of CA IV protein by Western blotting of pAMBS-precipitated proteins with an antibody specific for rabbit CA IV (Fig. 2A). Although steady-state levels of CA IV mRNA expression were highest in gall bladder, CA IV protein expression was most abundant in lung but also present in heart, kidney cortex, gall bladder, and colon.

In contrast to CA IV, CA IX exhibited a much more restricted expression pattern with very abundant CA IX mRNA and protein levels occurring in the stomach and gall bladder (Figs. 1 and 2B). CA XII mRNA expression was restricted to kidney cortex and colon, and CAs IV and XII comprised all of the membrane-associated CA isoforms expressed in these tissues. CA XIV mRNA was detected in the heart, lung, skeletal muscle, and liver, and despite the relatively low mRNA copy number of CA XIV mRNA (Fig. 1), expression of CA XIV protein was readily detected in these same organs by Western blotting of proteins precipitated by pAMBS-agarose beads using CA XIV specific antisera (Fig. 2B). Thus CA XIV is the predominant integral membrane CA isoform in rabbit liver and skeletal muscle and is coexpressed in the heart and lung with CA IV. The variability in CA isoform expression suggests different modes of acid-base and anion transport mediated via tissue-specific associations with different \( H^+/HCO_3^- \) transporters; that is, there may be a variety of transport metabolons (29) in these respective tissues.

Transition of CA Isoform Expression Along the Cranial-Caudal Axis of the Gastrointestinal Tract

Results presented in Tissue Distribution of Membrane-Associated CA Isoforms in the Rabbit suggest that different CA isoforms are expressed proximally vs. distally in the rabbit gastrointestinal tract; CA IX was expressed abundantly in the stomach, whereas CAs IV and XII mRNAs were expressed in the colon (Figs. 1 and 2). The apparent shift in CA isoform expression was further characterized by measuring steady state levels of CA mRNA by quantitative real-time RT-PCR in tissue samples isolated from two to three regions along the cranial-caudal axis of the rabbit gastrointestinal tract (Fig. 3). Results presented in Fig. 3 are representative of two independent experiments performed with tissue isolated from two different rabbits. CA IX mRNA was abundant in the stomach, duodenum, and proximal jejunum, beyond which steady-state levels of CA IX decreased by 10- to 100-fold. As CA IX mRNA expression decreased in the distal jejunum, CA IV mRNA abundance increased, remained steady through the ileum, and reached peak levels in the colon. Analysis of CA isoform protein expression by pAMBS-precipitation and Western blotting confirmed quantitative real-time results and thus demonstrated that CA IV was abundantly expressed in the colon (Fig. 4). Expression of CA XII mRNA was restricted to the colon. Although CA IX mRNA levels were comparable in the stomach and duodenum (Fig. 3), CA IX protein expression...
was less abundant in the duodenum (Fig. 4) and further decreased in the mid-jejunum and beyond (Fig. 4).

CA IX Expression Is Induced During Rabbit Maturation

Abundant expression in the stomach and duodenum indicates that CA IX may facilitate H⁺/HCO₃⁻ transport that is directly or indirectly coupled to gastric acid secretion. Since H⁺,K⁺-ATPase expression and gastric acid secretion are up-regulated during fetal development and neonatal maturation (4, 36), we examined the maturation of CA IX expression in the proximal gastrointestinal tract. In two separate rabbit litters, tissue was isolated from two to three rabbits at each time point, and tissue from one of the rabbit pairs was used for examination of CA IX mRNA abundance by quantitative real-time RT-PCR, whereas the other was used for analysis of protein expression by Western blotting. In both rabbit litters, CA IX mRNA abundance was low in the stomach of 1- to 2-wk-old rabbits (Fig. 5A). In the first litter, CA IX mRNA expression was induced up to 15-fold between the second and third week, whereas in the second litter, a 10-fold induction of CA IX mRNA expression was observed between the third and fifth weeks. Overall, CA IX mRNA abundance was induced 34–84-fold during neonatal maturation. Interestingly, by 3–4 wk, rabbits were consuming solid food and thus had reached weaning age.

CA IX mRNA maturational expression was similarly up-regulated (seven- to nine-fold) in the gall bladder (Fig. 5A) and duodenum (40- to 77-fold) (not shown). Whereas CA IX expression was markedly induced, expression of CA IV in the gall bladder was relatively unchanged (Fig. 5B). CA IV mRNA was expressed at high levels in 1-wk-old neonates, and differ-
ences between 1-wk-old neonates and 5- or 13-wk-old rabbits were no more than two- to three-fold. Consistent with results of a previous study (32), CA IV mRNA abundance in kidney cortex was induced from 9- to 31-fold between 1 and 5 wk of age in the two litters of rabbits examined sequentially. Changes in CA IV mRNA abundance during neonatal rabbit maturation in other tissues, such as colon (not shown) and lung (Fig. 5B), were of the order of 2- to 3-fold. CA IV mRNA abundance also increased in the stomach 5–20 fold, concomitant with changes in CA IX expression (Fig. 5B). However, it is important to note that CA IV mRNA and protein were not abundantly expressed in the stomach (Figs. 1, 2, 3, and 5). Other than the maturation of CA IX and CA IV mRNA, there were no major differences in any tissues between neonatal and adult rabbits with regard to CA isoforms XII and XIV (not shown).

Consistent with results obtained by quantitative real-time RT-PCR, expression of CA IX protein, as detected by pAMBS precipitation and/or Western blotting, was induced during rabbit maturation. In both litters, CA IX protein expression was below the limit of detection in stomach tissue isolated from 1- to 2-wk-old rabbits (Figs. 6 A and B). As was the case for CA IX mRNA abundance, induction of CA IX protein expression in the first litter of rabbits preceded that of the second litter; CA IX protein was relatively abundant in membrane preparations prepared from the stomach of a 3-wk-old rabbit from litter 1 (Fig. 6A), whereas CA IX expression was barely detectable even by pAMBS precipitation in 3-wk-old rabbits from the second litter (Fig. 6B).

To ascertain whether the timing of CA IX expression is related to changes in gastric acid secretion, we examined the expression of the H⁺,K⁺-ATPase α-subunit in tissue homogenates from the stomach during maturation. Levels of H⁺,K⁺-ATPase α-subunit were below the limit of detection in the stomachs of 1- to 2-wk-old neonates, and in each of the two litters the maturational upregulation of the α-subunit correlated with upregulation of CA IX (Figs. 6, A and B). Thus CA IX expression correlated with the maturation-induced expression of the H⁺,K⁺-ATPase catalytic subunit, suggesting that the increase in CA IX was linked to the maturation of gastric acid secretion.

DISCUSSION

To date, there are no systematic studies in the same animal model of the expression of the four membrane CAs, particularly in so many different tissues. The purpose of this study was to examine the presence of membrane-associated CA
isoforms in a large variety of tissues in rabbit. Rabbit physiology has been extensively studied due in part to the relatively large size of the mature animal, which facilitates examination of blood, gastric, intestinal, and urine samples, and administration of various pharmaceutical agents. The size of the rabbit makes it possible to obtain protein and mRNA from a large number of different tissues in the same animal; there is no need for pooling and losing independent data points. In addition, the relatively large size of the immature rabbit permits comparative studies of newborn physiology, making this animal an important model for examining the maturation of these CA isoforms. Documentation of the pattern of expression of CA isoforms in a single species should enable investigators to determine whether there are specific functional interactions with anion and H\(^{+}/\text{HCO}_3\)\(^{-}\) transporters, such as would be expected for a transport metabolon (29).

**CA IV**

CA IV, the first membrane-associated isoform to be studied, is expressed in a wide variety of tissues including kidney, heart, lung, gall bladder, distal small intestine, colon, and skeletal muscle (3, 5, 7, 8, 18, 23, 26, 37). We confirmed nearly all of these findings at the mRNA level using quantitative real-time RT-PCR, pAMBS pull-downs, and Western blotting of homogenates; expression in skeletal muscle was barely detectable only after pAMBS pull-down. Indeed, the low level of expression in skeletal muscle, as well as some of the signal in heart and lung, and perhaps regions of the GI tract may reflect the expression of CA IV in capillary endothelia (7, 8, 26, 27). Thus, at the whole tissue analysis, there is good agreement with previous studies performed in rat, mouse, and human tissues, as well as with our previous study in rabbit (23). Because CA IV has been shown to interact with anion transporters, such as AE1 (28) and NBC1 (1), it is likely that such transport metabolons may be present in the aforementioned tissues.

**CA IX**

CA IX is an isoform whose expression has been associated with human tumors (19), but it is also found in the normal gastrointestinal tract, primarily in the stomach, gall bladder, duodenum and jejunum, but weakly in the ileum and large intestine (10, 20, 22). The high expression of CA IX in the proximal gastrointestinal tract is compatible with its being expressed at sites of rapid cell proliferation (22). However, there are some differences between rat and human expression in ileum and colon (20). In the rabbit, we found definite mRNA expression in gall bladder, stomach, duodenum, and jejunum, with the more distal intestine showing less than significant expression. At the protein level the expression was abundant in stomach and duodenum, and weaker in jejunum; CA IX was detected in ileum and colon only after pAMBS pull-down. Thus there is good agreement with previous findings in mouse, rat, and human gastrointestinal tract and the present results in rabbit.

If there is a functional interaction of CA IX with anion transporters, it likely involves transporters that are different from those interacting with CA IV, not only because CA IV and CA IX are expressed in different tissues, but, unlike the predominant apical expression of CA IV (23), CA IX is expressed basolaterally (3, 20, 23) and would face a different set of proteins.

**CA XII**

CA XII was originally identified because its mRNA was overexpressed in renal cell cancers (31). Whereas its mRNA transcription is subject to regulation by the von Hippel-Lindau tumor suppressor gene (11), CA XII mRNA is normally expressed in colon and kidney, as well as in some other tissues that were not examined in the present study (11, 31). CA XII protein is found in human kidney cortex and colon (12, 17). Our real-time RT-PCR data confirm these findings at the mRNA level in rabbit kidney cortex, but because our available antibodies did not work in rabbit, we were not able to document protein expression by Western blotting.

**CA XIV**

CA XIV expression shows the largest variation according to species. CA XIV mRNA is abundantly expressed in mouse kidney (16) and protein in mouse and rat kidney (6). Other tissues expressing CA XIV in mouse include heart, followed by skeletal muscle, liver, brain, and lung (16). In human tissues CA XIV is expressed in heart, followed by brain, skeletal...
Regulation of CA Expression During Maturation

We have recently shown that CA IV expression in kidney cortex increased five-fold at the mRNA level (35) and 3- to 5-fold at the protein level (25) during postnatal maturation. A previous study showed a 19-fold increase in CA IV mRNA expression in rat lung between fetal day 20 and postnatal day 6, and a 40% postnatal increase to day 17, with no further increase (7). The postnatal increase was nearly comparable to what we have observed in rabbit lung (Fig. 5B). However, the increase in CA IV mRNA in kidney cortex is at least 10-fold and likely to account for the postnatal increment in protein (24). With most of the increase occurring during postnatal weeks 3–5, it is likely that the increase reflects the change in eating habits and a shift to an alkaline ash diet that requires a renal adaptation in transport. The large increase in CA IV may allow the kidney to handle the maturational increase in filtered load of bicarbonate and its proximal reabsorption.

Detailed studies of CA IX expression during maturation have not been published; CA IX is weakly expressed by newborn mice and increases markedly by postnatal week 4 (10). A CA IX-deficient mouse has been developed by targeted gene disruption (10). Whereas such mice did not show overt deviations from normal littermates, their stomachs showed remarkable hyperplastic changes of the glandular stomach epithelium. Importantly, CA IX-deficient mice had no abnormalities of gastric pH, acid secretion, or number of H⁺,K⁺-ATPase-positive cells (10). Our studies show significant maturational changes in CA IX expression in stomach (Figs. 5 and 6). Despite no reported decrease in gastric acid secretion in CA IX-deficient mice compared with wild-type controls, our studies (Fig. 6) showed an excellent correlation between the maturation of gastric CA IX protein expression and that of the gastric H⁺,K⁺-ATPase α-subunit. There was virtually no detectable CA IX or H⁺,K⁺-ATPase expression in neonatal rabbit stomachs, similar to what has been reported for CA IX in mice (10). An abundance of expression occurs around the time of weaning of the rabbit (3–5 wk postnatal), and this is seen for both CA IX and H⁺,K⁺-ATPase. A previous study showed expression of the α-subunit of the H⁺,K⁺-ATPase in microsomal protein by the late fetal rabbit stomach (36), suggesting the probability that neonates would also express the H⁺,K⁺-ATPase. However, in our system using only 1 and 10 μg of gastric membranes, we did not see expression of the H⁺,K⁺-ATPase (or CA IX) until 3–5 wk postnatally. In addition, another study showed that gastric pH does not drop to 3wk postnatally (4), around the time when the H⁺,K⁺-ATPase became easily detectable in our rabbit stomach preparations. The absolute differences in H⁺,K⁺-ATPase notwithstanding, our data show a concurrent expression of CA IX with the α subunit of H⁺,K⁺-ATPase in rabbit stomach around the time of weaning. This finding suggests that the process of weaning programatically induces the expression of the H⁺,K⁺-ATPase and CA IX, or that a change in diet somehow induces these proteins. Further studies are needed to clarify this issue.

Perspectives

There have been no previous studies of multiple membrane CAs in rabbit tissues. This systematic examination permits the further investigation of anion and cation transporters that might be associated with these CAs in functional metabolons. These studies have also revealed minor differences in tissue-specific expression of some membrane CAs, particularly for CA XIV (not in rabbit kidney or human lung). Otherwise, there was good agreement with previous studies in mouse, rat, and human tissues, as well as some substantial new details in the rabbit tissues. Maturational studies confirmed the changes in CA IV in maturing rabbit kidney and also revealed the novel finding of coordinate regulation of CA IX and H⁺,K⁺-ATPase expression in the developing stomach. Understanding the mechanism for CA IX’s function in proximal GI transport and the parallel increase of CA IX and the gastric H⁺ pump require further investigation.

ACKNOWLEDGMENTS

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