Biochimica et Biophysica Acta, 1066 (1991) 201–207
© 1991 Elsevier Science Publishers B.V. (0005-2736/91/$03.50
ADONIS 000527369100285J

BBAMEM 75293

Localization and activity of renal carbonic anhydrase (CA) in CA-II deficient mice

W.F. Brechue [1], E. Kinne-Saffran [2], R.K.H. Kinne [2] and T.H. Maren [1]

[1] *Department of Pharmacology and Therapeutics, University of Florida College of Medicine, Gainesville, FL 32610 (U.S.A.) and* [2] *Max-Planck-Institut für Systemphysiologie, Dortmund (Germany)*

(Received 12 February 1991)

Key words: Carbonic anhydrase; Urinary electrolyte; Renal acidification; Methazolamide; (Mouse)

A null allele at the mouse *Car 2* locus was induced by ethylnitrosurea; mice homozygous for the new allele lack the carbonic anhydrase (CA)-II isoenzyme. The expression of this genetic lesion was investigated by: (1) using tissue fractionation techniques to determine localization and activity of CA in the kidney, and (2) examining renal response to CA inhibition in CA-II deficient mice (CAD), in normal (N) mice and in heterozygous litter mates (LM). N and LM mice had CA activity in proximal tubule brush border membranes and cytosol. CA activity was also localized to membranes and cytosol of the outer medullary region. CAD mice lacked cytosolic activity but had normal CA activity in all membranes examined. All membrane associated CA had 2–8-fold lower sulfonamide sensitivity than cytosolic CA. These inhibition characteristics suggest that the membrane enzyme is CA-IV. Baseline urinary excretion of Na^+, K^+, and HCO_3^- was similar in all groups. Urine pH and Cl^- excretion were higher and titratable acid output was lower in CAD mice. Inhibition of CA (methazolamide, 25 mg/kg) led in all groups to equivalent increments of urine pH, urine flow, and HCO_3^-, Na^+, and K^+ excretion. Cl^- excretion was unchanged. Thus the extent of the genetic deficiency of CA-II mice extends to the kidney cytosol but does not alter membrane localization or levels of CA, probably CA-IV. The similar response to CA inhibition in CAD mice suggests that CA-IV, the membrane bound isoenzyme is the important isoenzyme in proximal tubule HCO_3^- reabsorption.

Introduction

In 1967, Maren et al. demonstrated carbonic anhydrase (CA) activity in both cytosolic and microsomal fractions of dog renal cortex and suggested that there may be different isoenzymes of CA in the cytosol and in the membrane [1]. In 1977, Wistrand and Kinne provided further evidence that CA activity was associated with highly purified brush border membranes and basal-lateral plasma membranes. Due to its lower sulfonamide sensitivity the membrane-bound enzyme was considered to be an isoenzyme different from the cytosolic form, CA-II [2]. It was tentatively named CA-IV [2]. Recently, this assumption was confirmed when the enzyme CA-IV was purified and shown to have different molecular weight and partial amino acid sequence and different immunological epitopes [3]. The intrarenal distribution of CA-IV beyond the proximal tubule is not known for certainty since histochemical studies can not differentiate between isoenzymes.

In 1988, Lewis, et al. isolated a null mutation in the *Car 2*^{b} allele characteristic of CA-II in DBA mice [4]. In these animals the cytosolic CA-II isoenzyme could not be detected by immunodiffusion analysis in any tissue. CA-II deficiency has also been observed in humans [5]. This disease is associated with osteopetrosis and renal tubular acidosis. These patients, however, have a normal renal response to CA inhibition [6]. This observation suggests that the kidneys of these individuals contained functionally active CA.

In this paper, mice deficient in CA-II (CAD) were used to investigate for the presence of CA in the kidney and if so which type is expressed. In addition, the topic of the intracellular and intrarenal distribution of CA was addressed. Finally, baseline renal function and response to CA inhibition was examined to provide preliminary data for further studies concerning the functional role of membrane-bound CA in the kidney.

Correspondence: W.F. Brechue, University of Florida College of Medicine, Department of Pharmacology and Therapeutics, Box J-267, JHMHC, Gainesville, FL 32610, U.S.A.

A preliminary account of this work was presented in abstract form at the American Physiological Society Meeting, October, 1989 in Rochester, MN.

Methods

Animal model

A null mutation in the *Car* 2^b allele of CA-II was found in F1 progeny of a male DBA/2J that was pre-treated with *N*-ethyl-*N*-nitrosurea and mated to C57BL/6J females. Mice homozygous for this mutation were found to lack completely the CA-II isozyme in all tissues of the body [4]. Litter mates and CA-II deficient animals were the product of crossing heterozygous mice, each parent having one null allele. Homozygous mice do not breed well and were not used in the crossings. Three groups of mice were used in this study, CA-II deficient mice (CAD) weight 21.5 ± 0.8 g, heterozygous siblings (LM) weight 24.0 ± 0.4 g, and normal, Swiss-Webster (N) (Charles River) mice weight 35.3 ± 1.7 g. The CAD mice are quite normal with respect to activity and visual appearance. They are smaller compared to litter mates and normal mice. CAD mice lack the cerebral calcification and osteopetrotic phenotype seen in CAD humans [4]. The CAD and LM mice used in the present studies were kindly supplied by Drs. Richard Tashian and Robert Erikson of the University of Michigan.

Localization studies

Kidney tissue was harvested from mice of either sex and pooled in groups of three animals (six kidneys) to provide enough material to analyze. Mice were killed by cervical dislocation. The kidneys were removed and placed in ice-cold buffer (10 mM mannitol and 2 mM Tris-HCl, pH adjusted to 7.1 with NaOH). All procedures and centrifugation were carried out at 4 °C.

The light brown cortical tissue was completely separated from the red outer medulla by hand, using a razor blade. The remaining tissue was split in half and white papilla was removed thus yielding the outer medullary tissue.

Cytosolic and membrane fractions enriched in brush border membranes were obtained from cortical tissue by a modification of the differential precipitation method described by Booth and Kenny [7]. Tissue (about 0.6 g wet weight from six kidneys) was homogenized by hand, first 10 times with a loose fitting and then 10 times with a tight fitting Dounce homogenizer. The ratio of tissue to medium was 1:6 g wet wt/ml. A small amount of the homogenate was analyzed for enzyme activities and protein content, another small amount was centrifuged for 1 h and 15 min at $100000 \times g$. The resulting supernatant was concentrated in a Centricon 10 microconcentrator (Amicon, Danvars, MA 01923) and represents the cytosolic fraction. The remaining homogenate was brought to a volume of 15 ml and a 1 M $MgCl_2$ solution was added to achieve a final concentration of 5 mM Mg^{2+}. The suspension was gently stirred for 15 min and centrifuged for 12 min at $1500 \times g$. The sediment was discarded and the supernatant centrifuged for 45 min at $20000 \times g$. The resulting pellet was suspended in a small volume of buffer containing 100 mM mannitol and 20 mM Hepes, pH adjusted to 7.4 with Tris base, and homogenized by repeated suction through a 26 gauge needle.

The medullary tissue (about 1.0 g wet wt from six kidneys) was minced in a small amount of ST buffer (250 mmol/l sucrose, 10 mmol/l triethanolamine, pH adjusted to 7.6 with HCl) and then homogenized as described above for cortical tissue. A cytosolic fraction was gained from the medullary homogenate similar to the methods employed for cortical tissue. Membrane fractions were prepared by differential centrifugation. After centrifugation for 10 min at $700 \times g$, the supernatant was centrifuged at $16000 \times g$ for 20 min. The resulting supernatant was saved, the upper white layer of the sediment removed, suspended in 5 ml of ST buffer by homogenization in a loose fitting Potter-Elvejhem homogenizer and spun for 20 min at $16000 \times g$. This step was repeated twice and yielded the plasma membrane fraction [8].

For enzymatic characterization of the different fractions the following enzyme activities were determined at 37 °C as markers: lactate dehydrogenase for cytosol [9], γ-glutamyltranspeptidase for luminal membranes [10], Na,K-ATPase for basal-lateral plasma membranes [11], succinate–cytochrome-*c* reductase for mitochondria [12], acid phosphatase for lysosomes [13], and NADH-dehydrogenase for endoplasmic reticulum [14]. Carbonic anhydrase activity in the various fractions was determined at 25 °C with 8% CO_2, barbital buffer and a 1 ml reaction volume [15]. Inhibition constants were determined for ethoxolamide and methazolamide in terms of I_{50}, the concentration of drug that inhibits 50% of enzyme activity in the assay procedure. Protein content of the fractions was estimated after precipitation with 10% trichloroacetic acid in the cold and resuspension of the precipitate in 1 M NaOH [16]. Unfractionated bovine serum albumin was used as a standard. All determinations were performed at least in duplicate or as otherwise noted.

Renal function studies

Mice were maintained on a 12 h light-dark cycle and fed standard mouse chow and water ad libitum. There were no differences between groups of mice in the amount of food or water consumed or the volume of urine or feces expressed prior to or following experiments. All animals were fasted for 12 h before individual experiments with water ad libitum. For urine collections mice were placed in individual urine collection

cages. All experiments were of 3 h duration with no food or water during the experiment. Prior to each experiment the mouse's bladder was emptied by depressing the abdomen.

Baseline renal function was determined in each group following a 0.03 ml/g body weight i.p. injection of water. Response to CA inhibition was studied following 25 mg/kg methazolamide by i.p. injection. The total volume of fluid injected was the same as in the baseline experiments. These experiments were performed in 10 normal, 5 litter mate and 8 CAD mice.

All urine was collected and stored under oil and on ice until analyzed. Urine Na^+ and K^+ were determined by flame photometry (Eppendorf) and Cl^- by using a chloridometer (Haake). Urine pH was measured with a glass electrode and urine HCO_3^- was calculated from pH and T_{CO_2} measured manometrically. Titratable acid was determined by titrating urine with 0.1 M NaOH to pH 7.4.

Statistics

Unless stated otherwise results are expressed as means ± S.E. Statistical significance was determined by analysis of variance for repeated measures. Post hoc tests were performed using the Neuman-Keuls procedure.

Results

Localization studies

1. Renal cortex. The enrichment of marker enzymes found in the cytosolic and brush border membrane fractions from kidney cortex of the three groups of animals are compiled in Table I. No significant differences between any of the groups were observed indicating that fractions identical in purity were obtained. The cytosolic fractions had, as expected, a high enrichment of lactate dehydrogenase (LDH) and a low activity of all other marker enzymes.

In the brush border membrane fraction activity of γ-glutamyltranspeptidase was enriched by a factor of 6.3, 7.1, and 7.7, respectively. The contamination with marker enzymes for other cell organelles was low with the exception of contamination with acid phosphatase, i.e. lysosomes. This is a well known feature of brush border membrane fractions obtained by divalent cation precipitation [7]. The contamination of membrane fractions with cytosolic enzymes was estimated from LDH enrichment factors. It amounted to 7.5% in N, 6.6% in LM and 9.3% in CAD mice.

Table II gives the activities of CA from the kidney cortex fractions. The highest specific activity was found in the cytosolic fractions derived from normal and litter mates, while cytosolic fractions derived from CAD mice showed no activity. In all three groups CA activity associated with brush border membranes was found. Litter mate and CAD mice had similar activity which was less than that found in brush border membranes from normal mice. Also in Table II the results of studies on the sensitivity of the CA activity to sulfonamides are compiled. The sensitivity found in cytosolic fractions and in membrane fractions was similar when the three groups were compared. However, in each instance the sensitivity of CA in the brush border membrane fractions was lower (2–8-fold) than in the cytosolic fraction.

2. Renal outer medulla. Table III summarizes the enrichment factors of marker enzymes found in the fractions of the renal outer medulla. The crude plasma membrane fractions are enriched in γ-glutamyltranspeptidase and in Na,K-ATPase suggesting a contribution of apical as well as basal-lateral membranes. Again the cytosolic fraction was enriched in LDH. In the medulla the relative contribution of cytosolic proteins

TABLE I

Enrichment factors of marker enzymes in cytosolic and membrane fractions from mouse kidney cortex

Mean values ± S.D. from six experiments (N), respectively, values from one experiment (LM), and mean values from two experiments (CAD) are given. In the latter the difference between the two values was always less than 10%. Specific enzyme activities (μmol substrate/h per mg protein) in the homogenate of normal mice were 82.2 ± 1.7 for lactate dehydrogenase, 16.7 ± 2.2 for succinate dehydrogenase 2.19 ± 0.16 for acid phosphatase, 66.6 ± 7.7 for NADH-dehydrogenase, 57.3 ± 2.9 for γ-glutamyltranspeptidase and 5.12 ± 0.7 for Na,K-ATPase. No significant differences between normal mice, litter mates and CA-deficient mice were observed. Contamination of membrane fractions by cytosolic proteins was evaluated by dividing the LDH enrichment factors in the membrane fraction by those found in the cytosolic fraction. N = normal mice; LM = litter mates; CAD = CA-II deficient mice.

	Cytosolic fraction			Membrane fraction		
	N	LM	CAD	N	LM	CAD
Lactate dehydrogenase (cytosol)	4.5 ± 0.9	4.4	4.9	0.34 ± 0.009	0.29	0.42
Succinate dehydrogenase (mitochondria)	not detectable			0.06 ± 0.001	0.10	0.13
Acid phosphatase (lysosomes)	0.58 ± 0.005	0.58	0.47	2.80 ± 0.540	3.04	2.52
NADH-dehydrogenase (endoplasmic reticulum)	0.66 ± 0.009	0.64	0.75	0.26 ± 0.004	0.30	0.30
γ-Glutamyltranspeptidase (luminal plasma membranes)	0.02 ± 0.000	0.04	0.04	6.30 ± 0.760	7.1	7.7
Na,K-ATPase (contraluminal plasma membranes)	0.07 ± 0.001	0.06	0.07	0.51 ± 0.041	0.69	0.65

TABLE II

Carbonic anhydrase activities and inhibition characteristics in cytosolic and membrane fractions from mouse kidney cortex

Values are means ± S.E. Mean values are from the number of determinations on individual samples, given in the parentheses. These samples were generated from six experiments for N, one experiment for LM and two experiments for CAD (three animals, six kidneys per experiment). One enzyme unit (eu) is defined as the activity yielded when the catalyzed reaction time is one half the uncatalyzed and is given by the equation $(t_{uncat} - t_{cat})/t_{cat}$. N = normal; LM = litter Mates; CAD = CA-II deficient.

	Activity (eu/mg protein)	I_{50} (10^{-8} M)	
		methazolamide	ethoxzolamide
Cytosol			
N	90.0 ± 8.6 (2)	1.9 ± 0.8 (5)	0.4 ± 0.2 (5)
LM	47.8 ± 5.2 (2)	2.7 ± 1.0 (3)	0.6 + 0.1 (2)
CAD	< 1.0 (2)	–	–
Brush border membrane			
N	10.2 ± 0.9 (3)	8.0 ± 0.1 (2)	1.1 ± 0.2 (2)
LM	4.9 ± 0.3 (2)	14.3 ± 2.7 (2)	1.2 ± 0.1 (2)
CAD	5.3 ± 1.1 (2)	9.5 ± 1.8 (3)	1.1 ± 0.4 (3)

TABLE IV

Carbonic anhydrase activities and inhibition characteristics in cytosolic and membrane fractions from mouse outer medulla

Values are means ± S.E. Mean values are from the number of determinations on individual samples, given in the parentheses. These samples were generated from six experiments for N, one experiment for LM and two experiments for CAD (three animals, six kidneys per experiment). One enzyme unit (eu) is defined as the activity yielded when the catalyzed reaction time is one half the uncatalyzed and is given by the equation $(t_{uncat} - t_{cat})/t_{cat}$. N = normal; LM = litter mates; CAD = CA-II deficient.

	Activity (eu/mg protein)	I_{50} (10^{-8} M)	
		methazolamide	ethoxzolamide
Cytosol			
N	39.4 ± 4.3 (3)	2.5 ± 0.7 (2)	0.9 ± 0.1 (2)
LM	26.7 ± 2.8 (2)	2.8 ± 0.3 (2)	0.7 ± 0.2 (2)
CAD	< 1.0 (2)	–	–
Plasma membranes			
N	3.3 ± 0.3 (2)	10.5 ± 1.0 (2)	1.3 ± 0.6 (2)
LM	3.5 ± 0.4 (2)	8.6 ± 1.1 (3)	1.6 ± 0.5 (2)
CAD	2.6 ± 0.9 (3)	14.3 ± 1.5 (2)	1.1 ± 0.5 (2)

to the crude plasma membrane fraction was lower than in the cortex. It accounted for 0.8% in normal, 1.1% in litter mate, and 0.8% in CAD mice.

As shown in Table IV the cytosolic fraction of normal and litter mate mice exhibited significant CA activity although the specific activity was about 50% lower than in the cortical cytosol. The relatively high sulfonamide sensitivity suggests that this represents CA-II activity. The plasma membrane fractions derived from each group showed significant CA activity. The specific activity found in these fractions was not statistically different. The CA present in these membrane fractions exhibited lower sulfonamide sensitivity than the cytosolic CA, similar to comparison made in the cortical fractions.

Renal function studies

Fig. 1 shows the baseline urinary status for the three groups. All groups had similar baseline HCO_3^-, Na^+, and K^+ excretion and urine flow. Urine pH and Cl^- output was similar in N and LM but significantly higher in CAD. Titratable acid output was significantly lower in CAD, 0.47 ± 0.03 (N), 0.46 ± 0.05 (LM), and 0.10 ± 0.01 (CAD) μequiv./min (data not shown in Fig. 1).

The renal response to CA inhibition with methazolamide at 25 mg/kg is presented in Fig. 1. Urinary HCO_3^-, Na^+, and K^+ excretion were significantly and similarly increased in all groups following methazolamide. Cl^- excretion was not affected by CA inhibition. Titratable acid output went to zero following methazolamide in all groups. Urine flow and pH also

TABLE III

Enrichment factors of marker enzymes in cytosolic and membrane fractions from mouse kidney outer medulla

Mean values ± S.D. from six experiments (N), respectively values from one experiment (LM), and mean values from two experiments (CAD) are given. In the latter the difference between the two values was always less than 10%. Specific enzyme activities (μmol substrate/h per mg protein) in the homogenate of normal mice were 211.1 ± 4.6 for lactate dehydrogenase, 42.3 ± 4.1 for succinate dehydrogenase, 3.40 ± 0.26 for acid phosphatase, 50.4 ± 3.8 for NADH-dehydrogenase, 64.2 ± 3.1 for γ-glutamyltranspeptidase and 10.4 ± 2.3 for Na,K-ATPase. No significant differences between normal mice, litter mates and CA-deficient mice were observed. In LM and CAD membrane fractions Na,K-ATPase activity could not be determined due to lack of material. Contamination of membrane fractions by cytosolic proteins was evaluated by dividing the LDH enrichment factors in the membrane fraction by those found in the cytosolic fraction. N = normal mice; LM = litter mates; CAD = CA-II deficient mice.

	Cytosolic fraction			Membrane fraction		
	N	LM	CAD	N	LM	CAD
Lactate dehydrogenase (cytosol)	3.6 ± 0.6	4.1	3.9	0.33 ± 0.001	0.05	0.03
Succinate dehydrogenase (mitochondria)	not detectable			0.23 ± 0.007	0.23	0.55
Acid phosphatase (lysosomes)	0.45 ± 0.006	0.09	0.35	1.00 ± 0.004	1.3	0.90
NADH-dehydrogenase (endoplasmic reticulum)	0.14 ± 0.001	0.10	0.16	0.72 ± 0.006	1.1	0.78
γ-Glutamyltranspeptidase (luminal plasma membranes)	0.06 ± 0.000	0.05	0.05	2.80 ± 0.025	3.8	3.1
Na,K-ATPase (contraluminal plasma membranes)	not detectable	–	–	2.65 ± 0.23	–	–

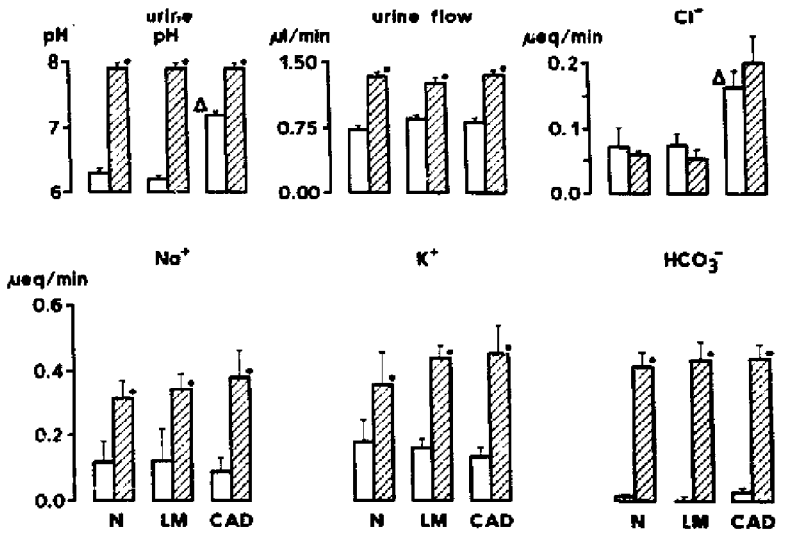

Fig. 1. Baseline renal function and renal response to carbonic anhydrase inhibition following 25 mg/kg methazolamide by i.v. injection. Values are means ± S.E. N = normal mice ($n = 10$); LM = litter mate mice ($n = 5$); and CAD = CA-II deficient mice ($n = 8$). * Significantly different from baseline. ^ Significant difference between groups.

increased equivalently in all groups in response to inhibition.

Discussion

The results presented above provide further information on: (1) the presence of membrane bound CA in the kidney; (2) the cellular distribution of CA-II and CA-IV; (3) the nature of residual CA activity in *Car-2*^{n} homozygous mice; (4) the functional role of membrane bound CA in the absence of cytosolic CA-II.

Localization studies

Previous studies have shown that CO_2 hydration activity of renal cytosolic CA isoenzyme is similar to that of the red cell CA-II and thus the former is denoted CA-II [17]. The membrane bound, CA-IV [3], form is also a high activity form but has some differences compared to CA-II. K_{cat} is 1/4 and K_m 1/2 that of CA-II [1,17], thus there is about a 2-fold difference in specific activity. Both forms have similar *p*-nitrophenylacetate esterase activity [3]. CA-IV differs in amino acid composition, molecular weight, and chromatographic and isoelectric focusing behavior as well [3]. There is no evidence for CA-I or CA-III in the kidney [18,19]. Inhibitors of CA are somewhat more active against CA-II than CA-IV varying 3–15-fold depending on the inhibitor [1,3,20].

In the present study, all cytosolic fractions from normal and litter mate mice had I_{50} values for methazolamide and ethoxolamide similar to red cell CA-II and renal cytosolic CA in dog [20]. Membrane fractions from all groups yielded I_{50} values that were 2–8-fold higher than cytosolic enzyme, in agreement with past studies [1,2,3,17,20]. Thus it appears that the cytosolic and membrane bound forms of CA, in the present study are different isoenzymic forms; the membrane form is CA-IV and the cytosolic form is CA-II.

Tissue fractionation and histochemical studies on the cellular localization of CA in renal cells have always been complicated by local contamination. In tissue sections as well as cell membrane preparations a contribution of cytosolic activity to the activity associated with membranes can not be excluded. This fact is exemplified in the studies with normal and litter mate mice. For example, if one assumes that during purification of renal cortex cytosol, CA and LDH behave identical, the contamination of membrane fractions with cytosol would be 7.5%. Such contamination would result in an increment of specific activity equivalent to more than 50% of the activity found in the membrane fraction. Thus, despite a relatively low contamination with respect to marker proteins, this contamination becomes significant when dealing with a high activity enzyme such as CA. These difficulties do not exist in the *Car 2*^{n} homozygous mice which are devoid of cytoplasmic CA activity. In these mice all enzyme activity found in the membrane fraction can be regarded as membrane associated. Thus, the results obtained with the CAD mice unequivocally establish the presence of CA in the brush border of this species, similar to findings in other species [18,20,21,22,23]. Additionally and importantly, these results show that membrane bound CA is expressed independently from cytosolic CA.

CA activity was found in medullary cytosolic frac-

tions of normal and litter mate mice. It is probably derived from a variety of medullary structures such as collecting ducts (OMCD), thick ascending limb of Henles loop (MTAL) and perhaps the transition zone between S3 and descending thin limb type 2 cells of Henles loop (DLT_2). These stuctures show cytoplasmic staining in histochemical investigations in mice [22].

There seems to be significant CA activity associated with medullary plasma membranes. The cellular origin of this activity is more difficult to delineate. According to histochemical studies in mice the S3 segment of the proximal tubule, which extends into the outer medulla has no CA activity [22]. We therefore can tentatively exclude brush border membranes as the source of the activity. Although, as indicated by the γ-glutamyltranspeptidase activity these membranes contribute to the medullary plasma membrane fraction. Most probably the CA activity originates from MTAL where membrane associated CA activity has been observed histochemically [22]. Thus, in mice, membrane bound CA-IV seems to be present not only in the proximal tubule but also in the MTAL.

CA activity was found to be greater in the cytosol than in membranes of normal and litter mate mice. This agrees with earlier findings, which showed that CA in the cytosol was 4–6-fold greater per mg protein than in membranes [1,17]. In the present case the difference was 6-fold. Thus, a similar relationship for cytosolic/membrane bound activity is seen in mice as well [1,17]. Lack of cytosolic CA-II in CAD does not appear to affect the level of membrane bound enzyme since the activity per mg protein was similar to normals.

Renal function studies

Baseline urine flow, and HCO_3^-, Na^+, and K^+ excretion were similar in all three groups. However, there were important differences between normal and litter mates mice and the CAD mice; titratable acid excretion was less, Cl^- excretion and urine pH were higher. These differences are puzzling and are not consistent with that reported for CA-II deficient humans [6].

If CAD mice and humans have a slight metabolic acidosis as previously reported [4,6] then the CAD mice should be excreting acid, with a low urine pH as with CAD humans [6]. With less titratable acid and higher urine pH, the question is how can CAD mice regulate acid-base status? It is not expected that these mice have a reduced acid load since their diet was similar in composition and quantity, and metabolism appeared to be the same (see Methods) as in normals and litter mates. The possibility exists that CAD mice increase production of ammonia and augment acid secretion via increased ammonium (NH_4^+) excretion. Evidence for this may be the higher CL^- output seen in CAD which could serve as the counter ion for NH_4^+ excretion and the higher urine pH.

A major difference with respect to renal CA localization between mice and humans is the complete lack of distal convoluted tuble (DCT) CA in mice, while humans have both membrane and cytosolic enzyme in this segment [18,22]. Thus, in human CA-II deficiency, the DCT would act like the proximal tubule, no cytosolic CA with viable membrane CA-IV associated with bicarbonate reabsorption (acid secretion). On the other hand, CAD mice would not have the membrane enzyme in this segment and may therefore, rely on an NH_4^+ trapping scheme as has been suggested for other segments where membrane CA-IV is not present [24]. Experiments are in progress to clarify these points.

Renal response to CA inhibition was equivalent in all three groups. The HCO_3^- response, 10–15 μequiv./min per kg, in all groups agrees in magnitude with inhibition responses in man, dog, and rat [25]. Increments in urine pH, flow, and Na^+ and K^+ output without a Cl^- response and the disappearance of titratable acid are also in agreement with past studies [26]. That CAD mice responded to CA inhibition is also in agreement with work done in human CAD patients [6].

The role of membrane bound and cytosolic CA in urinary acidification/HCO_3^- reabsorption in the proximal tubule is greatly debated. In the proximal tubule 80% of the filtered HCO_3^- is reabsorbed [27]. Selective (membrane only) and non-selective (cytosolic and membrane) inhibition of CA results in the same 80–90% suppression of this HCO_3^- reabsorption [27]. Thus, the membrane bound enzyme, which has been shown to be in functional contact with proximal tubule fluid [28,29], is critical for HCO_3^- reabsorption in the proximal tubule and the cytosolic enzyme is not. Our data show the same, that normal response to CA inhibition in CAD mice is completely due to the presence of membrane bound CA in the proximal tubule.

In conclusion, evidence is presented to support the idea that membrane bound, CA-IV, is localized to brushborder membranes of the proximal tubule and to medullary thick ascending limb of Henles loop in mice. Likewise, the cytosolic form, CA-II, is present in the proximal tubule and in medullary collecting ducts, and thin descending and thick ascending limbs of Henles loop. It appears that expression of cytosolic CA-II and membrane bound CA-IV are controlled by separate genes. Expression is independent, CA-IV is expressed in normal amounts and distribution even when CA-II is not, as is the case with CA-II deficient mice. Extrapolating the present data to human CA deficiency it can now be concluded that there is absence of CA-II not only in the red cells but in the kidney as well, and perhaps all tissues of the body. Additionally, response to CA inhibition is due to the presence of CA-IV in

both CAD mice and humans. CA-IV is the isoenzyme of importance in reabsorbing bicarbonate In the proximal tubule; CA-II appears to be unnecessary for this function.

References

1 Maren, T.H. and Ellison, A.C. (1967) Mol. Pharmacol. 3, 503–508.
2 Wistrand, P.J. and Kinne, R. (1977) Pflügers Arch. 370, 121–126.
3 Wistrand, P.J. and Knuuttila, K.G. (1989) Kidney Int. 35, 851–859.
4 Lewis, S.E., Erikson, R.P., Barnett, L.B., Venta, P.J. and Tashian, R.E. (1988) Proc. Natl. Acad. Sci. USA 85, 1962–1966.
5 Sly, W.S., Whyte, M.P., Sundaram, V., Tashian, R.E., Hewett-Emmett, D., Guibaud, P., Vainsel, M., Baluarte, H.J., Gruskin, A., Al-Mosawi, M., Sakati, N. and Ohlsson, A. (1985) N. Engl. J. Med. 313, 139–145.
6 Sly, W.S., Whyte, M.P., Krupin, T. and Sundaram, V. (1985) Pediatr. Res. 19, 1033–1036.
7 Rooth, A.G. and Kenny, A.J. (1974) Biochem. J. 142, 575–581.
8 Heidrich, H.-G., Kinne, R., Kinne-Saffran, E. and Hannig, K. (1972) J. Cell Biol. 54, 232–245
9 Vassault, A. (1983) in Methods of Enzymatic Analysis, Vol. 3 (Bergmeyer, H.U.), pp. 118–126, Verlag Chemie, Weinheim.
10 Orlowski, M. and Meister, A. (1978) Biochim. Biophys. Acta 7, 676–679.
11 Kinne, R., Schmitz, J.E. and Kinne-Saffran, E. (1983) Pflügers Arch. 329, 191–206.
12 Green, D.E. and Ziegler, D.M. (1963) in Methods of Enzymology, Vol. 6 (Colowick, S.P. and Kaplan, N.O., eds), pp. 416–424, Academic Press, New York.
13 Lansing, A.I., Belkhode, M.L., Lynch, W.E. and Lieberman, I. (1967) J. Biol. Chem. 242, 1772–1775.
14 Hoelzl-Wallach, C.F. and Kamat, V.R. (1963) in Methods of Enzymology, Vol. 6 (Colowick, S.P. and N.O. Kaplan, eds), pp. 164–172, Academic Press, New York.
15 Maren, T.H. and Couto, E.O. (1979) Arch. Biochem. Biophys. 196, 501–510.
16 Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. (1951) J. Biol. Chem. 193, 265–275.
17 Sanyal, G., Pessah, N.I. and Maren, T.H. (1981) Biochim. Biophys. Acta 657, 128–137.
18 Lönnerholm, G and Wistrand, P.J. (1984) Kidney Int. 25, 806–898.
19 Wählstrand, T. and Wistrand, P.J. (1980) Uppsala J. Med. Sci. 85, 7–17.
20 Maren, T.H. (1980) Ann. N.Y. Acad. Sci. 341, 246–258.
21 Brown, D., Zhu, X.L. and Sly, W.S. (1990) Proc. Natl. Acad. Sci. USA 87, 7457–7461.
22 Dobyan, D.C., Magill, L.S., Friedman, P.A., Herbert, S.C. and Bulger, R.E. (1982) Anat. Rec. 204, 185–197.
23 Lönnerholm, G. (1971) Acta Physiol. Scand. 81, 433–439.
24 Star, R.A., Kurtz, I., Mejia, R., Burg, M.B. and Knepper, M.A. (1987) Am. J. Physiol. 253 (Renal Fluid Electrolyte Physiol. 22), F1232–F1242.
25 Maren, T.H. (1967) Physiol. Rev. 47, 595–781.
26 Maren, T.H., Wadsworth, B.C., Yale, E.K. and Alonso, L.C. (1956.) Bull. Johns Hopkins Hosp. 98, 159–183.
27 Lucci, M.S., Tinker, J.P., Weiner I.M. and DuBose, T.D., Jr. (1983) Am. J. Physiol. 245 (Renal Fluid Electrolyte Physiol. 14), F443–F449.
28 Rector, F.C., Carter, N.W. and Seldin, D.W. (1965) J. Clin. Invest. 44, 278–290.
29 Viera, F.L. and Malnic, G. (1968) Am. J. Physiol. 214, 710–718.