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Positive Renal Response to Intravenous Acetazolamide in Patients with Carbonic Anhydrase II Deficiency

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ABSTRACT. Carbonic anhydrase II (CA II) is the only soluble isozyme of CA which is known to be expressed in kidney. We recently identified a deficiency of this enzyme as the basis for the autosomal recessive syndrome of osteopetrosis with renal tubular acidosis and cerebral calcification. In order to explore the physiological importance of CA II in the kidney, we studied the renal response to intravenously infused acetazolamide in two CA II-deficient patients and two control subjects. Following acetazolamide infusion, the CA II-deficient patients exhibited a prompt rise in urinary pH and HCO_3^- excretion similar to the response seen in control subjects. These findings indicate that CA II-deficient patients, who lack detectable CA II in their erythrocytes, still expressed an acetazolamide-inhibitable CA activity in their kidneys. These results can be explained in three ways: 1) the CA II deficiency which is profound in the erythrocytes of these patients may not be expressed in their kidney. 2) An acetazolamide-sensitive CA other than CA II, such as CA I and CA III, which is not normally expressed in kidney, is expressed in kidneys of CA II-deficient patients. 3) The CA II deficiency is expressed in kidney in these patients but the acetazolamide response is due to inhibition of the luminal, membrane-bound CA which is the product of a different gene and unaffected by the CA II deficiency mutation. We favor the third possibility. On this basis, we suggest that patients with CA II deficiency provide an experiment of nature which complements existing pharmacological evidence that distinguishes the contributions of CA II and the membrane-bound CA to HCO_3^- reclamation by the human kidney. A model to explain their roles in proximal and distal acidification is presented. (*Pediatr Res* 19: 1033–1036, 1985)

Abbreviations

CA, carbonic anhydrase
RTA, renal tubular acidosis

CA II deficiency was recently identified as the primary defect in the autosomal recessive syndrome of osteopetrosis with RTA and cerebral calcification (1). This CA isozyme, which was formerly called CA C, is normally widely distributed in man and has been identified (immunologically or by purification) in erythrocytes, brain, kidney, eye, cartilage, liver, lung, skeletal muscle, pancreas, gastric mucosa, and anterior pituitary body (2, 3). The other CA isozymes, whose activities toward CO_2 and HCO_3^- are lower than those of CA II in the order of CA II>CA IV>CA I>CA III (4–6), appear to have a more limited tissue distribution. CA I is found primarily in erythrocytes and CA III mainly in skeletal muscle (5). CA IV differs from CA I, CA II, and CA III, the soluble cytosolic carbonic anhydrases, in being a membrane-bound glycoprotein and is found in the lung (4). Identification of a quantitative defect in CA II in patients with this new inborn error of metabolism provides us with a unique opportunity to assess the importance and function of CA II in humans.

We report herein studies of the renal response to intravenously infused acetazolamide (a CA inhibitor) in CA II-deficient patients. The renal response to infused acetazolamide in these patients is of great interest since CA II is the only soluble CA isozyme identified in the kidney (7–9), and its absence apparently is responsible for the mixed or hybrid-type RTA in CA II-deficient patients (10–15). However, there have been recent reports of a less well-characterized, membrane-bound luminal CA in the brush border of the proximal tubule of the kidney (16, 17). The luminal CA appears to play an important role in the reclamation of filtered HCO_3^- and is known to be sensitive to inhibition by acetazolamide (18, 19). If the membrane-bound, luminal CA is the product of a different gene than CA II, and if this gene is expressed normally in CA II-deficient patients, infused acetazolamide would be expected to inhibit the luminal CA and produce an HCO_3^- diuresis in CA II-deficient patients. In the experiment described below, we compare the renal response to intravenous infusion of acetazolamide in two of the patients with CA II deficiency that we described previously (1, 11) with the responses of two control subjects.

METHODS

Experimental subjects. Two sisters (25 and 32 yr of age) homozygous for CA II deficiency (1) and two healthy men (22 and 24 yr of age) were studied. Urine acidification has been assessed previously in the CA II-deficient sisters following the oral administration of 1.5 g of ammonium chloride four times daily for 3 days (11). The pH of their urine collected under oil (Radiometer pH meter) was 5.58 and 5.40 while the plasma pH was 7.17 and 7.12, respectively. These findings demonstrated a distal RTA. Although a low Tm for HCO_3^- has been documented in other patients with this syndrome, studies to demonstrate this abnormality in these two patients were not successfully completed (11).

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None of the subjects was taking medication. The men were of comparable weights to the patients. Following informed consent, an aliquot was aspirated immediately from a timed urine collection into a 10-ml syringe and heparinized venous blood was collected without stasis into a 3-ml syringe. Syringes were immediately placed on ice. Each subject then received a single intravenous bolus of 125 mg of acetazolamide (Diamox, Lederle Laboratories, Division of American Cynamid Co., Pearl River, NY) dissolved in 1.25 ml normal saline, and the same studies were repeated at intervals during the subsequent 4-h period.

Biochemical studies were performed in the Clinical Chemistry Laboratory, Barnes Hospital, St. Louis, MO. Plasma and urine electrolytes (Na^+, Cl^-, K^+, total CO_2) were assayed with an Astra-8 automated analyzer (Beckman Instruments, Louisville, KY). Urine pH was determined with a Radiometer pH meter.

RESULTS

Results are summarized in Tables 1 and 2. Basal venous pH and measured total CO_2 values in the patients with CA II deficiency (Table 1) were in keeping with their previously documented RTA (1, 11). Intravenous infusion of acetazolamide was well tolerated, both in patients and controls and was followed by a slight but progressive decrease in venous pH. Since the plasma HCO_3^- did not decrease, the acute decrease in pH following acetazolamide infusion must be entirely due to an increase in pCO_2. From data shown in Table 2, it is clear that both the patients and controls demonstrated an abrupt increase in urine pH which was associated with increased urinary HCO_3^- excretion. Elevation of pH persisted even longer in patients than in controls. Urinary Na^+/creatinine and K^+/creatinine ratios both increased with the bicarbonaturia. However, the Cl^-/creatinine ratio did not show a corresponding increase.

DISCUSSION

Two principal types of osteopetrosis have been described in man (20, 21). One is the dominantly inherited, relatively benign condition which is often detected radiologically in asymptomatic adults. A second type is the recessive, lethal, malignant form of osteopetrosis. This form is usually present at birth, becomes symptomatic early in infancy, and leads to death by early childhood from infection or bleeding. Since 1972, a number of families such as the one studied here have been reported with a recessively inherited syndrome of osteopetrosis with RTA and cerebral calcification (11–15) that is compatible with long survival. We recently presented evidence from studies in one kindred that a deficiency of CA II is the basic defect in this syndrome. We have since extended this finding to many other similarly affected families (22, 30). The renal abnormalities in this syndrome are still incompletely understood. Most patients reported have a mixed or hybrid-type RTA (10–15). A proximal component was suggested by HCO_3^- wasting at normal plasma concentrations (*i.e.* a low Tm for HCO_3^-) (12–25). A distal defect, profound in some patients, was evident from their inadequate ability to acidify their urine, even while quite acidotic (13–15).

Table 1. *Response of venous pH and total CO_2 to intravenous infusion of acetazolamide in carbonic anhydrase II-deficient patients and controls*

		Time After Acetazolamide		
	Baseline	1 Hour	2 Hours	4 Hours
pH				
P1	7.22	7.20	7.18	7.17
P2	7.23	7.22	7.20	7.19
C1	7.34	7.32		7.32
C2	7.32	7.30		7.28
Total CO_2 (measured) (mM)				
P1	17	22	20	20
P2	19	20	21	20
C1	27	26		26
C2	28	30		29

* P1 and P2 are CA II-deficient patients III-1 and III-6 from the family we reported previously (1), and C1 and C2 are control subjects BG and PL.

Table 2. *Response of urine pH and electrolytes to intravenous infusion of acetazolamide in carbonic anhydrase II-deficient patients and controls**

		Time after acetazolamide	
	Baseline	0–2 H	2–4 H
pH			
P1	5.9	7.8	8.0
P2	5.4	7.7	8.1
C1	6.1	7.8	7.5
C2	6.7	7.7	7.3
Measured HCO_3^- (mM)			
P1	<5	>40	
P2	<5	>40	
C1	1.7	>40	60
C2	5.2	>40	41
Na^+			
P1	94	299	266
P2	55	248	237
C1	107	324	223
C2	213	397	327
K^+			
P1	32	104	164
P2	37	120	136
C1	90	218	174
C2	93	149	127
Cl^-			
P1	116	129	96
P2	80	135	84
C1	152	188	108
C2	283	254	177

* Na^+, K^+, and Cl^- excretion are expressed as mmol/g creatinine. P1 and P2 are CA II-deficient patients III-1 and III-6 from the family we reported previously (1), and C1 and C2 are control subjects BG and PL.

CA II is normally present in both proximal and distal tubules (9, 23). Lönnerholm (23) recently compared histochemical and immunohistochemical staining of human kidney preparations and confirmed the earlier findings of Spicer *et al.* (9) that the distal tubule and the collecting duct have a more intense immunohistochemical reaction for CA II than the moderate to light staining found in the cytoplasm of cells in the proximal tubule. Lönnerholm also found that the CA in the brush border of the proximal tubule (the luminal or membrane-bound CA) stains histochemically, but does not react with antisera to either CA II or CA I. Lack of cross-reactivity could result from failure of the histochemical reagents to get access to CA I or CA II in membranes. However, when this observation is taken together with the evidence that the luminal enzyme is an integral membrane glycoprotein and much larger than the soluble isozymes (53K *versus* 29K) (24), the more likely interpretation is that the luminal enzyme is immunologically distinct and the product of a different gene than that which specifies CA II.

We have no direct evidence on the status of CA II in the kidney of patients with the syndrome of osteopetrosis with RTA and cerebral calcification. Recent studies of CA activity in whole

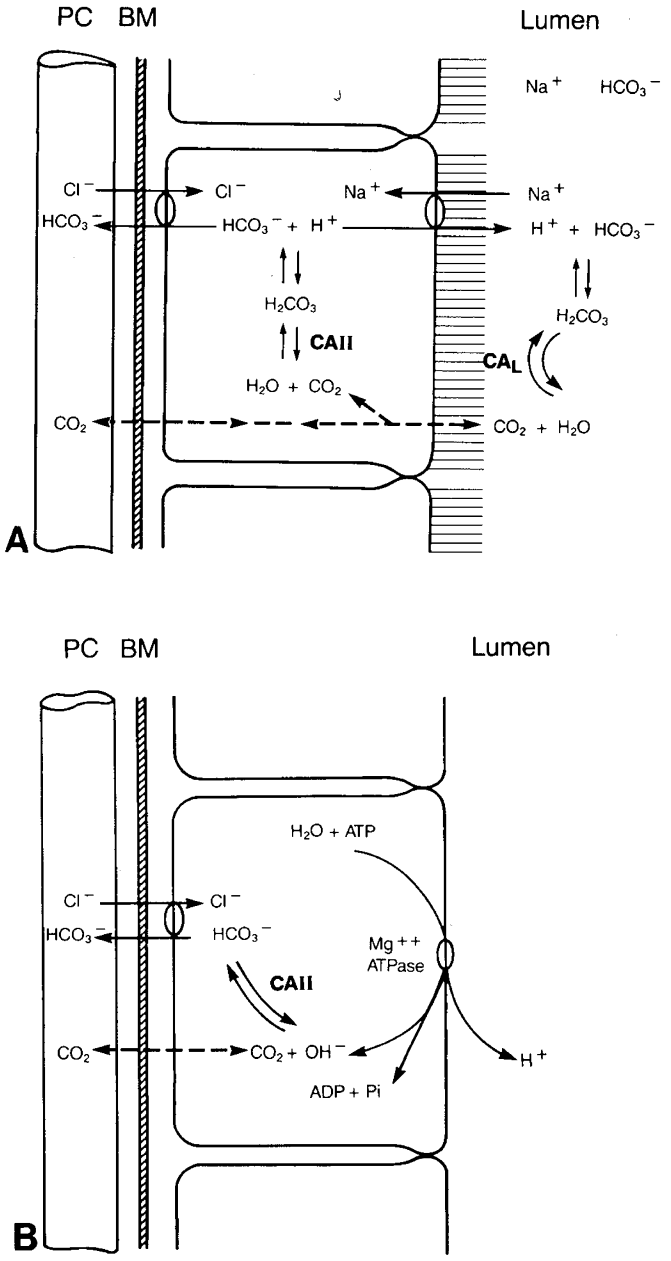

Fig. 1. *A*, proposed roles of carbonic anhydrases in bicarbonate reclamation in the proximal tubule. Na^+ and HCO_3^- enter the lumen of the proximal tubule. H^+ is secreted in exchange for Na^+, and H^+ and HCO_3^- are converted to CO_2 and H_2O in a reaction catalyzed by the luminal CA (CA_L). We propose that this enzyme functions normally in CA II-deficient patients, and its inhibition explains the positive response to acetazolamide. CO_2 diffuses freely into the proximal tubular cell [and across the basement membrane (*BM*) and into the peritubular capillary (*PC*)], and is exposed to cytosolic CA II which catalyzes its rehydration to form HCO_3^- and H^+. The HCO_3^- is transported from the contraluminal surface of the proximal tubular cell (probably in exchange for Cl^-) and the H^+ generated by CA II is secreted in exchange for Na^+ to initiate another cycle of HCO_3^- reabsorption. Loss of CA II-mediated regeneration of H^+ is suggested as the cause of HCO_3^- wasting in CA II-deficient patients. *B*, proposed role of CA II in distal urinary acidification. We propose that H^+ is secreted into the lumen by a proton = translocating Mg^{++} ATPase, as in amphibians, which produces OH^- in the cytosol. CO_2 can condense with OH^- to form HCO_3^- in a CA II-catalyzed reaction, and HCO_3^- can be transported across the basement membrane and into the peritubular capillary. We suggest that failure to titrate the OH^- limits the ability to secrete H^+ and acidify the urine appropriately in CA II-deficient patients.

erythrocytes from the two patients described herein, using a ^{18}O-exchange method, demonstrated 55% of normal CA activity. This activity was attributed to the CA I isozyme, which is not diminished in these patients (22). These studies suggest that the CA II-deficient erythrocytes are not functionally impaired in CO_2 transport *in vivo*. Thus, the RTA is more likely to be the direct result of a deficiency of the CA II isozymes in the kidneys than a secondary consequence of CA II deficiency in erythrocytes.

In Figure 1 we present a model which can explain both the renal abnormalities in CA II-deficient patients and their positive response to acetazolamide. In this model both the luminal (membrane-bound) CA and the soluble, cytosolic CA contribute to HCO_3^- reabsorption in the proximal tubule in normal subjects (Fig. 1*A*). Studies with CA inhibitors proved that enzymatic conversion of HCO_3^- to CO_2 is normally crucial for HCO_3^- reabsorption by the proximal tubule (25). That the CAs act at different sites and in different ways was suggested by experiments of Lucci *et al.* (19). When the luminal enzyme was selectively inhibited, more than 80% of the HCO_3^- reabsorption by proximal tubule was blocked (18, 19). Inhibition of the cytosolic enzyme, in addition, did not further inhibit HCO_3^- reabsorption, although it did reduce H^+ secretion into the proximal tubule (19). There is widespread agreement that HCO_3^- reabsorption depends on secretion of hydrogen ions (25–27), and that an acetazolamide-sensitive intracellular CA plays a role in generating H^+ for HCO_3^- reclamation (19, 16). Since CA II is the only soluble CA in kidney (7–9, 23, 24), we attribute this function to CA II. We suggest that it is loss of the ability to regenerate H^+ enzymatically which explains the impaired reabsorption of HCO_3^- in CA II-deficient patients. Such patients can waste up to 25% of the filtered load of HCO_3^- when their HCO_3^- concentrations are raised to normal levels by infusion (12–15). However, HCO_3^- wasting is not seen when patients are severely acidotic, suggesting that reabsorption of low filtered loads of HCO_3^- does not require CA II.

Most Ca II-deficient patients are unable to acidify their urine appropriately when acidotic, suggesting a role for CA II in distal acidification as well. Immunohistochemical evidence indicates that certain cells of the distal tubule and the collecting ducts of the human kidney are "CA rich" like their possible analogs in amphibians (9, 23). In the turtle bladder, these specialized cells secrete H^+ through a proton-translocating Mg^{++}ATPase, and are capable of generating a steep pH gradient (28, 29). The CA rich cells are sensitive to acetazolamide, possibly because CA is needed to titrate the OH^- produced in the cytosol by the proton-translocating Mg^{++}-ATPase. In the model presented (Fig. 1*B*), we suggest a similar role for CA II in the distal tubules and collecting ducts of the human kidney. Such a role for CA II could explain the distal defect reported in many CA II-deficient patients.

An experiment related to that reported herein was reported by Guibaud *et al.* (12) in 1972. When they observed a response to acetazolamide indicating an acetazolamide-sensitive CA in kidney in their patients with this syndrome, they concluded that CA deficiency must now be the basis of their osteopetrosis with RTA. These observations must now be reinterpreted. We recently collaborated with Professor Guibaud to measure CA II levels in erythrocytes from his patients and found in them the same profound deficiency of CA II (22) that we reported earlier (1) in the two sisters studies in this report. We propose that in his patients as in our patients, the bicarbonaturia following acetazolamide can be explained by inhibition of the luminal carbonic anhydrase which is not affected by the mutation which produces the CA II deficiency syndrome.

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