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Effect of Metabolic Acidosis on Hydrogen Ion Excretion in a Pigtail Macaque with Erythrocyte Carbonic Anhydrase I Deficiency[1] (41251)

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Abstract. Metabolic acidosis was induced by NH_4Cl ingestion in a normal pigtail macaque (*Macaca nemestrina*) and a pigtail macaque with an inherited deficiency of red cell carbonic anhydrase I (CA I). After this acid stress, both the normal and CA I-deficient animals were able to acidify their urine, and displayed similar hydrogen ion clearance indexes. These findings suggest that a defective red cell CA I, reported in some cases of renal tubular acidosis in humans, is not directly responsible for the impairment of bicarbonate reabsorption in the kidney tubules.

Carbonic anhydrase (carbonate hydrolyase, EC 4.2.1.1.) is present in mammals in at least three distinct isozymic forms, designated CA I, CA II, and CA III. Characteristically, CA II has the highest specific CO_2 hydrase and esterase (toward *p*-nitrophenyl acetate) activities, the highest binding affinity for heterocyclic sulfonamides, and is found in a wide variety of tissues (including the kidney cortex and medulla). CA I has lower CO_2 hydrase and esterase activities, a lower sulfonamide binding affinity, and is found mainly in red cells, lymphocytes, and certain regions of the gastrointestinal mucosa. CA III, which is found in red skeletal muscle, and rodent liver, possesses very low CO_2 hydrase activity, extremely low esterase activity, and has a very low affinity for sulfonamides. For a general overview see Refs. (1–3).

In the mammalian nephron, carbonic anhydrase appears to facilitate the reabsorption of HCO_3^- by catalyzing the dehydration of carbonic acid in the proximal tubule (lumen) and the hydration of CO_2 in the cytoplasm of the tubular cells (4, 5). Because of these roles, a defective carbonic anhydrase might be expected to impair the reabsorption of HCO_3^- and produce renal tubular acidosis (RTA). RTA can result either from an impairment in the formation of an acid–base gradient in the distal tubules (distal, or gradient variety), or from an impairment of HCO_3^- reabsorption or regeneration in the proximal tubules (proximal, or rate variety). For reviews see Sebastian *et al.* (6), and Steinmetz (7). The only evidence that an abnormal carbonic anhydrase may be causative in certain cases of RTA comes from the reports of Donckerwolcke *et al.* (8), Shapira *et al.* (9), and Kondo *et al.* (10). Donckerwolcke *et al.* (8) presented evidence suggesting that a defective kidney carbonic anhydrase (isozyme unspecified) was responsible for a bicarbonate-losing RTA of the proximal type. In the other reports, an altered (mutant?) form of red cell CA I with reduced CO_2 hydrase activity (9) and esterase activities (9, 10) was described in children with RTA. In the affected individuals, the levels and electrophoretic mobilities of red cell CA I and CA II were not observed to differ from normal. Although the basis for the acidification defect in these two reports was not thoroughly analyzed, the data given suggest that the RTA was the distal variety with marked bicarbonate wasting. In contrast to the above three reports, Kaplan *et al.* (11) found no abnormalities in the levels or activities of red cell CA I and CA II in three unrelated children with distal RTA.

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In the present report, we have examined the possible relationship between red cell CA I deficiency and impaired hydrogen ion excretion, by studying the renal acidification process in a pigtail macaque (*Macaca nemestrina*) which is homozygous for an inherited deficiency of red cell CA I (12). In this individual, CA I was virtually absent (~3000-fold reduction from normal), and the levels of red cell CA II were reduced to about 60% of normal.

Materials and Methods. The acid–base status in the blood (pH and P CO_2) was determined on a Micro Blood Gas Analyzer, Model 313 (Instrumentation Laboratories), and the total serum CO_2 by the method of Van Slyke and Neill (13) using a Natelson Microgasometer. Urine pH was measured with a pH electrode, titratable acid by titration to pH 7.4, and ammonia by the indophenol blue method. The acid-loading test was conducted by oral administration of ammonium chloride after overnight fasting, and urine samples were obtained hourly by a catheter over a 4-hr period. Arterial blood was obtained 2 hr after ammonium chloride dosage. The CA I-deficient pigtail macaque weighed 19 kg and received 5.0 g NH_4Cl. The normal control pigtail macaque weighed 15 kg and was given 4.0 g NH_4Cl.

Surface area was determined from the nomogram of Sendrogy and Cecchini (14).

Results and Discussion. Table I presents the results of a comparison of the normal and CA I-deficient pigtail macaques. The red cell CA I levels as determined by radioimmunoassay (15) were 6.57 and 0.002 μg/mg Hb for the normal and CA I-deficient pigtail macaques, respectively. As can be seen, both the normal and CA I-deficient animals responded in a similar manner to the NH_4Cl loading test. The difference in hydrogen ion clearance index (H^+ C.I.) between the macaque and human probably arises from the application of human surface area calculations of Sendrogy and Cecchini (14) to the macaque. The H^+ C.I. for the normal and deficient macaques are essentially the same despite a 3000-fold difference in their red cell CA I levels.

A condition of red cell CA I deficiency has also been reported in a human family (16) in which three members are apparently homozygous for a CA I deficiency gene. The deficient individuals had less than 1 ng CA/mg Hb compared to 11.57 ± 2.26 mg CA I/mg Hb in normals. They were all apparently healthy, and the one individual in which metabolic acidosis was induced by the administration of NH_4Cl did not show any impairment of his renal acidification.

Obviously, it would be important to know whether CA I is normally present in the primate kidney. The main isozyme which has been isolated or detected in kidney cells of humans (17–19) and rhesus (20) is the high-activity isozyme, CA II. And the small amounts of CA I which have been detected in the same tissues are thought to result from red cell contamination since macaque and human red cells contain about four to seven times more CA I than CA II (cf. Ref. (2)).

The localization of carbonic anhydrase activity has been examined in the nephron of the rhesus macaque (*M. mulatta*) by Rosen (21) who found that the cells of the entire proximal tubule exhibited activity, and that an alternating pattern of inactive and intensely active cells was characteristic of the collecting ducts. It was not possible from these studies (which are based on enzyme activity) to identify which carbonic anhydrase isozymes were present. However, as discussed above, since it appears that the main soluble carbonic anhydrase isozyme of the primate kidney is CA II, this is probably the isozyme that was detected histochemically. A membrane-bound form of CA has been reported from human and canine renal cortex (18, 22) which differs in its properties from CA I and CA II and may represent a fourth isozyme (CA IV?) of carbonic anhydrase.

In view of the findings that urinary acidification proceeds normally in a CA I-deficient macaque and humans, it is difficult to understand the relationship between an inherited low-activity CA I variant and the condition of renal tubular acidosis reported by Shapira *et al.* (9) and Kondo *et al.* (10) in human patients with RTA. Furthermore, we have recently reex-

TABLE I. EFFECT OF AMMONIUM CHLORIDE INGESTION ON THE ACID–BASE STATUS OF THE BLOOD AND URINE IN A NORMAL AND CA I-DEFICIENT PIGTAIL MACAQUE

Weight (kg)	CA I (μg/mg Hb)	NH_4Cl dosage (g)	Time (min)	Blood pH	Blood PCO_2	Blood PO_2	Serum total CO_2 (mmole/liter)	Urine pH	Urine TA[a] (μeq/min/1.73 m^2)	Urine NH_4^+ (μeq/min/1.73 m^2)	Urine H^+ (μeq/min/1.73 m^2)	Urine H^+ C.I.[b]	Urine NH_4^+/H^+ (%)
CA I normal													
15	6.57	4	0	7.41	30.3	89.6	24.2	5.5					
			60					5.8	8	13	21		62
			120	7.37	15.8	158.4	24.2	5.4	12	19	31	0.75	61
			180					5.6	9	13	22		59
			240					5.1	26	25	51		49
CA I deficient													
19	0.002	5	0	7.39	32.6	89.6	19.5	4.9					
			60					5.5	6	18	24		75
			120	7.36	15.2	155.9	19.2	5.1	18	38	56	1.08	68
			180					4.9	10	10	20		50
			240					5.0	16	13	28		44

[a] TA, titratable acidity.

[b] H^+ C.I., hydrogen clearance index [H^+ (meq/min/1.73 m^2) × serum CO_2 in mole/liter]. H^+ C.I. values (means and ranges) for normal adults (Ref. (24)), RTA adults (Ref. (24)), and normal children (Ref. (25)) are, respectively: 2.07 (1.4–3.4), 0.9 (0.6–1.1), and 1.69 (1.25–2.3).

amined one of the individuals with RTA and an inactive red cell CA I originally reported by Shapira *et al.* (9) and found the levels and activities of her red cell CA I to be within normal limits (23). It is possible that the discrepancies between our findings and those of Shapira *et al.* (9) could be due to the presence or absence of inhibitors resulting from differences in the purification procedures.

Thus, the findings of normal renal acidification in humans and a nonhuman primate deficient in red cell CA I through a mutational event (12, 16), the apparent absence of CA I in the primate kidney (17, 18), and normal red cell CA I in a patient previously reported to have an inactive red cell CA I associated with RTA (23) question the importance of CA I activity in the kidney with regard to normal acid–base balance, and indicate the lack of a causal relationship between an abnormal red cell CA I and RTA. It is, of course, possible that chemical abnormalities associated with certain cases of RTA could in some way secondarily alter the structure or activity of CA I.

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