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Proline and glucose transport by renal membranes from dogs with spontaneous idiopathic Fanconi syndrome

(brush border vesicles/renal clearance studies/two-component transport systems)

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ABSTRACT **The uptake of proline and glucose by renal brush border membrane vesicles isolated from Basenji dogs exhibiting a spontaneous Fanconi syndrome was examined. The magnitude of the 1-min, Na^+-gradient uptake of proline (0.02 mM) and glucose (1.0 mM) by vesicles from affected dog kidney was lower than normal. Concentration-dependent 15-sec uptake in vesicles from normal and affected dogs reveals a two-component transport system for proline and glucose. Kinetic analysis shows altered K_m and V_{max} values for both proline and glucose transport in the affected dogs. The data suggest that, in this model of Fanconi syndrome, the defective renal reabsorption of proline and glucose is associated with an alteration of luminal transport systems.**

We recently described (1) the occurrence of a spontaneous renal tubular disorder in Basenji dogs that resembled human idiopathic Fanconi syndrome and was manifested by excessive urinary loss of glucose, amino acids, phosphate, bicarbonate, sodium, potassium, and water. Clinical signs, such as polydipsia, polyuria, dehydration, weight loss, and weakness, appear in adult Basenji dogs of both sexes with progression to renal failure after several months or years. The profound glycosuria and hypotonic urine are present in the absence of diabetes mellitus. Initially, plasma electrolytes are normal, but arterial blood gas values suggest a moderate metabolic acidosis.

In previous studies to assess the ability of renal cortical slices from affected Basenji dogs to transport sugars and amino acids, the uptake of α-methyl D-glucoside (MeGlc), a glucose model, and of the amino acids lysine and glycine by renal cortical slices from affected dogs was significantly lower than uptake by kidney slices of unaffected Basenjis (2). The decreased uptake of MeGlc suggests that a transport defect may be present at the luminal surface of the renal tubule because it has been shown by the indicator dilution technique that, in the dog kidney, MeGlc is transported only by the brush border membrane present on the luminal surface (3).

To characterize further the nature of the obvious transport defect as manifested by the defective uptake, the uptakes of proline and glucose were examined in renal brush border membrane vesicles prepared from Basenji dogs. The use of renal brush border membrane vesicles eliminated a number of the disadvantages of the renal cortical slice and isolated tubule preparations. These include the influence of substrate metabolism on transport processes, and the questions of patency of the tubule lumen and whether the basolateral membrane plays a role in the uptake process. Proline and glucose were used because data are available describing the characteristics of their transport by renal brush border membrane vesicles (4–6). Studies were performed with vesicles prepared from six normal and two affected Basenji dogs in which renal clearance studies had been done to confirm the presence and nature of the Fanconi syndrome.

MATERIALS AND METHODS

Eight adult Basenji dogs (males and females) were used for these experiments. Conventional renal clearance studies were performed for creatinine, glucose, phosphate, sodium, potassium, and amino acids on the two affected Basenji dogs (7). The affected dogs were nonuremic and in good health and were initially identified by a urine screening program using paper chromatography. Membrane vesicles were prepared from the two affected dogs at least 14 days after clearance studies were performed. To prepare membrane vesicles, the kidneys were surgically removed, and the dogs were killed by arterial injection of 2 ml of T-61 (Hoechst). The kidneys were perfused at 4°C with buffered saline via a 30-ml syringe in the renal artery to remove as much blood as possible. The renal cortex was excised from the medulla, and cortical slices were made by using a Stadie–Riggs microtome. The cortical slices were then minced with a microtome blade and weighed. Renal brush border membrane vesicles were prepared by using the method of Booth and Kenny (8), modified as described (9). The final membrane pellet was suspended in 2 mM Tris·Hepes/100 mM mannitol pH 7.2 buffer (THM buffer) to a protein concentration of 2–4 mg/ml, as determined by the method of Lowry *et al.* (10). Purity of the luminal membrane preparations from both affected and normal dogs was determined by assaying for alkaline phosphatase (EC 3.1.3.1) and Na^+,K^+-ATPase (EC 3.6.1.3) (11). Alkaline phosphatase enrichment relative to homogenate was 6.29 and Na^+,K^+-ATPase enrichment was 0.86 in preparations from both affected and normal dogs.

The measurement of proline and glucose uptakes, with Millipore filters (HAWP, 0.45 μm), was performed by using the techniques described by McNamara *et al.* (4). All uptake experiments were performed at 22°C. All materials were of the highest quality available. Radioactive compounds were purchased from New England Nuclear: [U-^{14}C]proline (294 mCi/mmol; 1 Ci = 3.7×10^{10} becquerels), D-[U-^{14}C]glucose (310 mCi/mmol), and L-[U-^{3}H]glucose (17.46 mCi/mmol).

RESULTS

Clearance Studies. The fractional reabsorption of proline and glucose in the affected dogs (dogs SM and CR) was significantly decreased compared to normal (Table 1). The clearance studies also showed a decreased tubular reabsorption of phosphate, sodium, potassium, uric acid, and amino acids. Dog SM had the

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Abbreviation: MeGlc, α-methyl D-glucoside.

Table 1. Fractional reabsorption of filtered load (%)

Solute	Normal	Dog SM	Dog CR
Proline	99.7 ± 0.2	75.2 ± 1.7*	89.7 ± 0.9*
Glucose	99.6 ± 0.2	73.5 ± 2.2*	92.0 ± 1.3†

Results shown are the mean ± SEM of three consecutive clearance periods of a clearance experiment.
*$P < 0.001$.
†$P < 0.01$.

more severe defect for all solutes examined. Both affected dogs had normal blood chemistry values and glomerular filtration rates.

Time-Dependent Uptake Studies. In the presence of an inwardly directed 100 mM NaCl gradient the time-dependent uptake of both proline and glucose, in vesicles prepared from normal dogs, showed an overshoot at 0.5 min to values of 0.66 and 2.67 nmol/mg of protein, respectively (Figs. 1 and 2). Equilibrium was attained by 20 min. The magnitude of the overshoot for proline and glucose was decreased in vesicles prepared from the two affected dogs. Equilibrium uptake was similar to that of the vesicles prepared from normal dogs. In addition, intravesicular space, as measured by diffusion of L-glucose, was the same in both preparations.

Concentration-Dependent Uptake Studies. The 15-sec concentration-dependent uptake of proline (0.0208–2.1 mM) and glucose (0.0087–5.87 mM) in the presence of a Na^+ gradient, was measured in vesicles from normal and affected dogs. Analysis of these data by Lineweaver–Burk plots of proline and glucose uptakes revealed a two-limbed curve indicative of two uptake systems for each substrate. Nonlinear regression analysis was used to calculate the kinetic parameters shown in Table 2 by using observed data as initial estimates and finding the best fit to the total observed values in each experiment as described (9). The goodness of fit using the calculated kinetic parameters is indicated by the finding that the calculated and observed uptakes at any substrate concentration of proline and glucose varied by less than 7%.

The 15-sec uptake of proline by vesicles from dogs SM and CR was less than that by vesicles from normal dogs at each substrate concentration used. Proline uptake by vesicles averaged 56% of normal for dog SM and 34% for dog CR. Table 2 shows

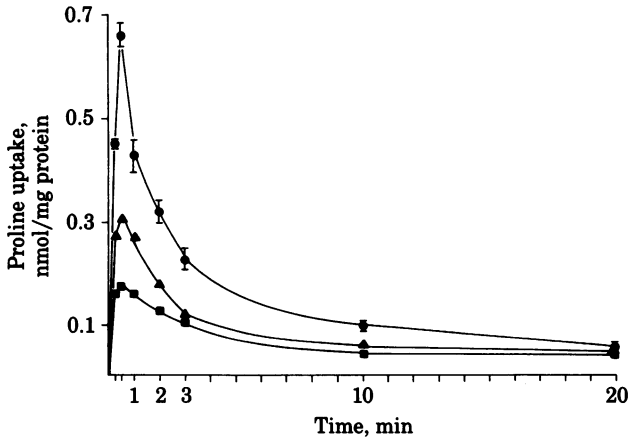

FIG. 1. Uptake of L-proline by renal brush border membrane vesicles isolated from normal Basenji dogs (●) and affected Basenji dogs [dogs SM (▲) and CR (■)]. The vesicles were suspended in THM buffer and were incubated with 0.02 mM ^{14}C-labeled proline and 100 mM NaCl for the times shown. Values shown for normal dogs are the mean ± SEM of four determinations in each of six dogs; those for dogs SM and CR are the mean of four determinations.

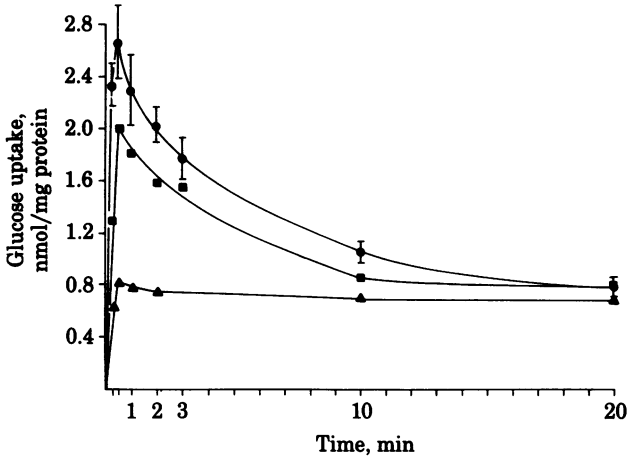

FIG. 2. Uptake of D-glucose by renal brush border membrane vesicles isolated from normal Basenji dogs (●) and affected Basenji dogs [dogs SM (▲) and CR (■)]. The vesicles were suspended in THM buffer and were incubated with 1.0 mM ^{14}C-labeled glucose and 100 mM NaCl for the times shown. Values shown for normal dogs are the mean ± SEM of four determinations in each of six dogs; those for dogs SM and GR are the mean of four determinations.

that, in the high-affinity, low-velocity system, the K_{m_1} values for proline uptake by vesicles from dogs SM and CR were similar to the normal value, whereas the V_{max_1} values of the affected dogs were lower than normal. In the low-affinity, high-velocity system, the K_{m_2} values for dogs SM and CR were higher, indicating a decreased affinity for transport when compared to normal. The V_{max_2} for dog SM was normal; that for dog CR was lower.

The functional defect suggested by the altered kinetic parameters of the affected animals is illustrated in Figs. 3 and 4. These show the relative contributions of the two transport systems to the total uptake of proline and glucose for normal and affected dogs as determined from the equation

$$V_{total} = \frac{V_{max_1}[S]}{K_{m_1} + [S]} + \frac{V_{max_2}[S]}{K_{m_2} + [S]} \quad [1]$$

and the calculated kinetic parameters shown in Table 2. The contribution of each transport system in the affected dogs, for

Table 2. Kinetics of proline and glucose uptakes by renal brush border membrane vesicles from Basenji dogs

	K_{m_1}, mM	V_{max_1}, nmol/mg per 15 sec	K_{m_2}, mM	V_{max_2}, nmol/mg per 15 sec
		Proline		
Normal dogs	0.043 ± 0.008	1.070 ± 0.045	1.12 ± 0.08	3.98 ± 0.42
Dog SM	0.030	0.749	5.84	4.30
Dog CR	0.032	0.390	1.75	1.45
		Glucose		
Normal dogs	0.036 ± 0.006	0.419 ± 0.073	3.25 ± 0.84	7.26 ± 0.62
Dog SM	0.032	0.199	5.20	6.93
Dog CR	0.090	0.412	9.12	7.26

The vesicles were suspended in THM buffer and were incubated with 100 mM NaCl and ^{14}C-labeled proline or ^{14}C-labeled glucose for 15 sec. Values given for normal dogs are the mean ± SEM of four determinations in each of six dogs; for dogs SM and CR, the values are the mean of four determinations.

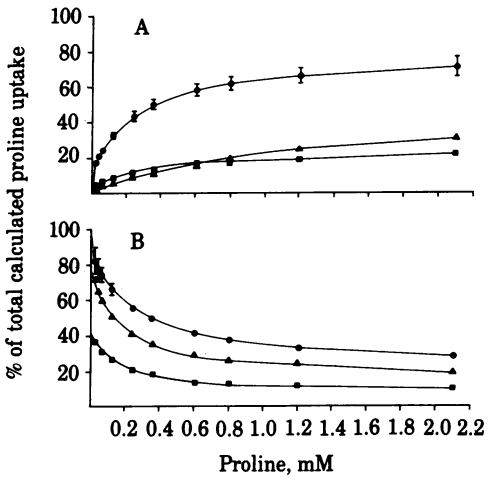

FIG. 3. Relative contribution to total uptake of the low-affinity system (*A*) and the high-affinity system (*B*) calculated for proline uptake, as determined from Eq. 1, for six normal Basenji dogs (●) and the two affected Basenji dogs SM (▲) and CR (■). For the normal dogs the values shown are the mean ± SEM; where no bars are seen, the SEM is within the size of the symbol.

both substrates, is expressed as a percentage of the uptake by the corresponding control transport system. Total uptake of proline and of glucose in dogs SM and CR is lower than uptake in normal dogs, at each concentration examined.

In the normal dogs, 35% of proline was transported by the low-affinity system (Fig. 3*A*) and 65% by the high-affinity system (Fig. 3*B*), at endogenous plasma proline concentrations (0.097–0.223 mM). In the affected dogs, this was decreased to an average of 35% by the high-affinity system and about 10% by the low-affinity system. The low-affinity transport system for proline in the affected dogs showed a greater relative defect than did the high-affinity system.

The 15-sec uptake of glucose by vesicles from dogs SM and CR was lower than uptake by vesicles from normal dogs, at each substrate concentration used. Glucose uptake by vesicles from dog SM averaged 25% of normal glucose uptake and from dog CR, 53%. In the high-affinity system, the K_{m_1} value for glucose uptake by vesicles of dog SM was normal whereas that by vesicles of dog CR was greater, indicating a decreased affinity for transport (Table 2). The V_{max_1} value for dog CR was normal; that for dog SM was lower. In the low-affinity system, the K_{m_2} values for dogs SM and CR were higher; the V_{max_2} values were normal.

In the normal animals, >85% of glucose was transported by the low-affinity system (Fig. 4*A*) and <15% was by the high-affinity system (Fig. 4*B*) at endogenous plasma glucose concentrations (3.22–6.87 mM). In the affected dogs, this was decreased to about 50% by the low-affinity system, but the high-affinity system was little changed.

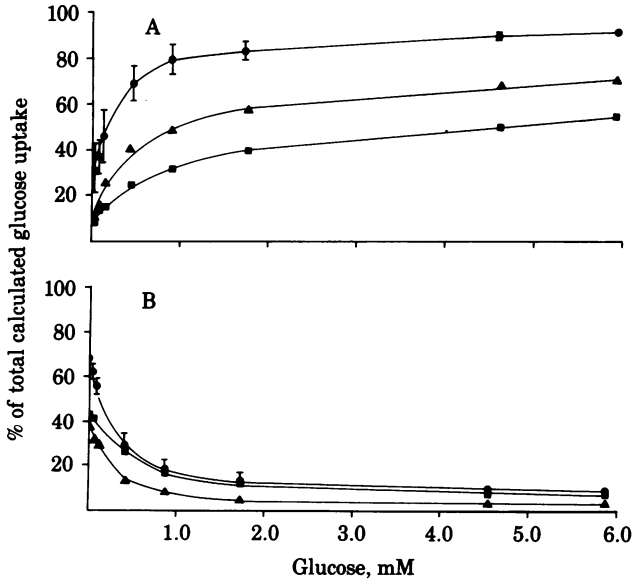

FIG. 4. Relative contribution to total uptake of the low-affinity system (*A*) and the high-affinity system (*B*) calculated for glucose uptake, as determined from Eq. 1, for six normal Basenji dogs (●) and the two affected Basenji dogs SM (▲) and CR (■). For the normal dogs the values shown are the mean ± SEM; where no bars are seen, the SEM is within the size of the symbol.

DISCUSSION

The present studies directly demonstrate decreased renal brush border solute transport in a spontaneously occurring model of human Fanconi syndrome. The decreased magnitude of the overshoot for Na^+-gradient proline and glucose uptakes by vesicles from the affected dogs suggests that the brush border function in these animals is defective. Analysis of the concentration-dependent proline and glucose uptakes shows that the kinetics of substrate transport are altered in the affected dogs, resulting in decreased net substrate transport by these vesicles compared to vesicles from normal dogs. Whether these abnormalities are due to a defect of the membrane carrier for these substrates or to an alteration of the membrane structure or compositon that affects carrier function is unanswered.

Because the Fanconi syndrome in the Basenji breed may be genetically determined (12), expression of a specific gene locus defect would be expected to result in a specific carrier defect, such as an increased K_m or decreased V_{max} for substrate transport, with the same alteration present in all affected subjects. Because the renal transport of sugars, amino acids, uric acid, sodium, and phosphate is largely carrier mediated, it seems unlikely that a defect of the carrier for each solute is present in the Fanconi syndrome to account for the excessive urinary loss of all of these solutes. It is more likely that the altered renal handling of these compounds is due to a defective or altered membrane in which the carriers function. This alteration of membrane structure or composition may express itself variably as a change in the K_m or V_{max} for solute transport dependent on the progression or stage of the membrane abnormality. However, the functional consequences of the described changes are evidenced by decreased *in vivo* fractional solute reabsorption, decreased uptake by renal slices, and decreased uptake by vesicles.

The kinetics of proline transport by vesicles from normal Basenji dogs are similar to those reported for renal brush border vesicles of the rat (4). The possibility of a two-component transport system for glucose in human renal brush border membrane vesicles has been suggested (5). Decreased brush border transport of both proline and glucose is evident in vesicles from the affected dogs. However, in the affected dogs there does not appear to be a direct correlation between the vesicle uptake and clearance data. The magnitude of the transport defects for proline and glucose in vesicles from the affected dogs was greater than that suggested by the clearance data. The vesicle preparation selects for brush border membranes from superficial cortex and primarily reflects the transport characteristics of the proximal convoluted tubule (13). Although amino acids are almost entirely reabsorbed in the first millimeter past the glomerulus (14), amino acid transport has been demonstrated in both

early and late straight tubule segments (15). It has also been suggested that distal tubular glucose transport may be important in the regulation of urinary excretion of glucose in dogs (16). Therefore, as indicated by our clearance data, the kidney may be able to compensate in part for the decreased uptake shown by the vesicles by reabsorbing solute by different processes during the flow of urine through the nephron.

Prior to the description of this spontaneously occurring model of human Fanconi syndrome, the principal animal model involved the administration of maleic acid in intact animals or *in vitro* incubation of the chemical in cortical cell preparations. The administration of maleic acid to rats causes a transient increase in urinary excretion of amino acids, glucose, phosphate, sodium, and bicarbonate (17, 18). Rosenberg and Segal (19) showed that rat renal slices incubated with maleic acid had decreased amino acid uptake; this was thought to be primarily due to accelerated solute efflux. Roth *et al.* (20) reported similar findings for MeGlc in maleic acid-treated rat renal tubules. They also described decreased MeGlc influx with longer periods of tubule incubation. Whereas maleic acid causes a Fanconi syndrome-like condition *in vivo* and defective uptake of amino acids when incubated with metabolically active renal slices and tubules, cortical slices from animals given maleic acid show no defect when studied *in vitro* (19), in contrast to results with cortical slices from affected Basenji dogs (1, 2). Reynolds *et al.* (21) demonstrated that incubation of maleic acid with rat renal brush border membrane vesicles had no effect on amino acid transport. This is consistent with the findings by Angielski (22) and by Gonick (23), which suggest that maleic acid-induced renal transport defects are not due primarily to brush border abnormalities but may be secondary to altered cellular metabolism because the effect is transient and reversible. It is possible that an alteration of cellular metabolism could affect renal solute transport by changing Na^+-linked transport or by exerting as yet undetermined secondary effects on renal tubular membranes that render transport systems ineffectual.

The Fanconi syndrome is seen clinically in patients with a wide variety of metabolic disorders, both inherited and acquired. These include cystinosis, tyrosinemia, galactosemia, hereditary fructose intolerance, Lowe syndrome, multiple myeloma, and heavy metal poisoning; there is an idiopathic form of unknown cause (24). The Fanconi syndrome disappears with the elimination of factors that cause some of the above conditions, such as dietary galactose, fructose, or tyrosine or the offending heavy metal. The reversible nature of the Fanconi syndrome as seen in patients with hereditary fructose intolerance—in which there is a rapid onset of the tubule reabsorptive abnormality and defervesence when fructose is administered and removed—suggests a metabolic origin. This would be akin to the situation in the experimental maleic acid model. The findings in the Basenji dog, with progression of the renal disorder and eventual severe systemic derangement of homeostatic mechanisms, resemble those in the human idiopathic Fanconi syndrome in which the renal abnormality is ever present and relentless.

Our experiments suggest that there is defective function of the brush border membrane in affected Basenji dogs but its nature remains to be determined. Because these dogs become available only sporadically for study, we have initiated a breeding program with the hope of investigating the genetic mode of transmission and making affected animals more readily available for study. Examination of the physicochemical properties of affected brush border membranes, the properties of basolateral membranes, and the metabolism of isolated tubules would then be possible.

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