The Renal Handling of Carnitine in Patients with Selective Tubulopathy and with Fanconi Syndrome

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ABSTRACT. Fractional tubular reabsorption (FTR) of free and acyl carnitine was measured in 15 patients with various selective tubular transport defects and in 19 patients with more generalized tubular dysfunction (Fanconi syndrome). FTR of free carnitine was normal in all patients with a selective tubulopathy, FTR of acyl carnitine was normal in most, and plasma carnitine levels were normal without exception. In these patients, there was no evidence for the existence of a defective renal transport mechanism shared by carnitine. In the patients with Fanconi syndrome, mean FTR of free and acyl carnitine was low; their plasma carnitine levels were lowered and correlated with the FTR. In individual patients, FTR of free and acyl carnitine also correlated with the severity of the disease. In the group of Fanconi syndrome patients, FTR of free and acyl carnitine correlated linearly with that of valine. We concluded that the lowering of plasma carnitine in the patients with Fanconi syndrome was caused by excessive loss of carnitine in urine. Its pathophysiological significance remained to be established. (*Pediatr Res* 21: 201-204, 1987)

Abbreviation

FTR, fractional tubular reabsorption

Carnitine is required for the transport of fatty acids across the inner mitochondrial membrane for β -oxidation and eventual energy production (2, 3). Cardiac and skeletal muscle, and to a lesser extent liver and kidney, contain relatively large amounts of carnitine. Tissues not synthesizing carnitine, e.g. muscles, receive it from the blood. Carnitine homeostasis depends on dietary intake, enteral absorption, endogenous synthesis, distribution to tissues, degradation, and on renal handling. Systemic carnitine deficiency leads to myopathy, cardiomyopathy, and poor fasting tolerance with nonketotic hypoglycemia. The biochemical characteristics are those of poor ketogenesis: high concentration of free fatty acids, inappropriately low ketone bodies, and in some instances dicarboxylic aciduria during fasting.

Carnitine (β -hydroxy- γ -trimethylaminobutyric acid) is a metabolite of the amino acid lysine. It exists either in the free or in the acyl ester form. Normally, carnitine is filtered in the glomerulum and reabsorbed in the proximal renal tubule, at a rate which is similar to that of most amino acids (4). Only little is

known about its reabsorption in renal tubular dysfunction (5-7). The purpose of this investigation was to measure tubular reabsorption of carnitine in patients with various selective tubular transport defects and in patients with more generalized tubular dysfunction (Fanconi syndrome). We also wondered whether we could identify in the proximal renal tubule a carrier mechanism for carnitine shared by another urinary compound which is lost in urine in any of these conditions.

MATERIALS AND METHODS

Subjects (Table 1). Patients with selective tubulopathies and Fanconi syndrome were compared with a reference group consisting of inpatients and outpatients not suffering from kidney dysfunction. Those with metabolic disorders were under good metabolic control. The reference group was validated by comparison with healthy adult controls. In one group of controls, adult blood donors, only plasma carnitine concentrations were measured. In all other subjects of this study, the following measurements were made in plasma and urine: free and total carnitine, creatinine, amino acids, phosphate, and uric acid (organic acids were measured in urine only). None of the subjects received carnitine medication prior to sampling. All subjects except some patients with Fanconi syndrome had normal plasma creatinine.

Patients with selective tubulopathies received appropriate treatment (sodium bicarbonate, low protein diet, phosphate, indomethacin, and sodium chloride). Patients with Fanconi syndrome were treated with one or more of the following: sodium bicarbonate, sodium/potassium phosphate, or vitamin D. Plasma creatinine was normal or somewhat elevated (up to 156 $\mu\text{mol/liter}$) in most patients except in one patient with cystinosis before transplantation (625 $\mu\text{mol/liter}$) and in one with inflammatory tubulopathy (1127 $\mu\text{mol/liter}$).

Sampling. Heparinized blood and a urine portion were correlated in time and collected at random during the day. Plasma and urine were frozen at once and stored at -20°C until analysis.

Analytical procedures. Total and free carnitine were measured in plasma and urine as described (8) and acyl carnitine was calculated from the difference. Creatinine was measured by an automated Jaffé reaction, and amino acids in plasma and urine were measured by ion exchange chromatography (Biotronics 7000, Munich, Germany). Organic acids in urine were measured by gas chromatography and identified by coupled mass spectrometry (9). Phosphate and uric acid were determined by routine procedures.

FTR was calculated by the following formula:

$$\text{FTR (\%)} = \left(1 - \frac{U_x}{P_x} \cdot \frac{P_{\text{creat.}}}{U_{\text{creat.}}} \right) \times 100$$

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Table 1. *Subjects of study*

Subjects	Condition	n	(M, F)	Age
Controls	Blood donors	29		
	Healthy fasting adults	14	(8, 6)	24–49 yr
Reference group	Mental retardation, brain injury, muscle hypotonia, phenylketonuria, galactosemia, tyrosinosis type I, vitamin D-dependent rickets, non-ketotic hyperglycinemia, argininosuccinic aciduria, and lactic acidosis with myopathy and cataract	24		3 day–33 yr
Selective tubulopathy	Cystinuria	3	(2, 1)	9, 14, 16 yr
	Lysinuric protein intolerance	4	(3, 1)	1.5, 6, 10, 15 yr
	Phosphate diabetes	5	(1, 4)	11, 13, 14, 18, 20 yr
	Proximal tubular acidosis	1	(1)	15 yr
	Bartter syndrome	2	(2)	12, 13 yr
Fanconi syndrome	Idiopathic Fanconi syndrome	6	(3, 3)	2.5, 9, 15, 21, 35, 51 yr
	Oculo-cerebro-renal syndrome (Lowe)	3	(3)	15, 20, 20 yr
	Glycogenosis with Fanconi syndrome (Fanconi-Bickel)	2	(1, 1)	0.5, 24 yr
	Infantile cystinosis	6	(1, 5)	1.5, 1.5, 3, 6, 8, 12 yr
	Galactosemia	1	(1)	5 day
	Inflammatory tubulopathy	1	(1)	15 yr

Table 2. *Plasma carnitine levels and tubular reabsorption of carnitine in patients with selective tubulopathy, Fanconi syndrome, and in reference group*

	Plasma carnitine (μ mol/liter)		FTR of carnitine (%)	
	Free	Acyl	Free	Acyl
Reference group (n = 24)				
Mean	25	22	98.1	94.8
Range	3–50	5–29	91.8–99.8	89.1–99.7
Median	24	13	99.4	95.0
Controls, blood donors (n = 29)				
Mean	33	17	ND*	ND
Range	18–48	7–26		
Median	33	17		
Controls, fasting (n = 14)				
Mean	21	19	97.1	93.6
Range	13–27	13–25	90.1–99.9	84.2–99.9
Median	21	19	98.5	93.7
Selective tubulopathy (n = 15)				
Mean	25	13	97.0	90.1
Range	11–36	3–19	89.7–99.9	71.4–98.0
Median	26	14	98.1	91.9
Fanconi syndrome (n = 19)				
Mean	12	10	76.6	56.1
Range	3–28	3–30	31.0–95.2	0–90.8
Median	12	8	77	72

* Not done.

RESULTS

The reference group had plasma carnitine levels which were comparable with those of fasting adults (Table 2). Yet, values were in a wider range, the lowest ones belonging to newborns and to one small child treated for phenylketonuria with a formula low in carnitine. Plasma carnitine of nonfasting adults was slightly higher and corresponded well with reported findings (10,

11). FTR of free and acyl carnitine in the reference persons was in the range documented for healthy controls by the majority of available sources (5, 7, 12, 13) and by our own controls (Table 2, Fig. 1). FTR of phosphate and uric acid was normal, and amino acid excretion was normal (results not shown). Thus, the reference group represented a valid comparison group for the study of FTR in the two patient groups to be investigated.

In the patients with selective tubulopathy, FTR of free carni-

Fig. 1. Histogram depicting fractional tubular reabsorption (FTR %) of free and acyl carnitine in the reference group, the patients with selective tubulopathy, and with Fanconi syndrome. Each FTR class covers 3%.

Fig. 2. FTR of free and acyl carnitine in relation to their plasma concentrations in the reference group (ranges are represented by the dashed boxes) and in patients with Fanconi syndrome (symbols) (see also Table 2). The numbers affixed to the symbols represent two or three successive determinations in individual patients: Δ cystinosis, interval $\frac{1}{2}$ yr; $\blacksquare$ galactosemia on diagnosis at age 5 days, at 15 days after 10 days of diet, and at 10 wk after treatment. *cystinosis before and $3\frac{1}{2}$ yr after kidney transplantation.

tine was normal without exception (Fig. 1). FTR of acyl carnitine was also normal in most, but somewhat lower than normal in two girls with phosphate diabetes and one with Bartter syndrome. However, the three other patients with phosphate diabetes (including one hemizygote boy) and one boy with a most severe

form of Bartter syndrome had normal FTR for acyl carnitine. In the whole group, plasma carnitine was normal.

We were thus unable to detect a defect of renal tubular carnitine transport in the patients with selective tubulopathy. In a complementary experiment we overloaded the renal tubular transport of a healthy fasting volunteer by infusing 12 g of L-carnitine (200 mg/kg) in 20 min and collected plasma and urine portions for 24 h while food was withheld. FTR for phosphate, uric acid, and all amino acids remained normal. Thus, these compounds did not seem to share a carrier mechanism with carnitine.

In the group of patients with Fanconi syndrome, mean FTR of free and acyl carnitine was low, and in some patients was markedly low (Table 2, Fig. 1). Plasma carnitine was lower than in all other groups and correlated with FTR—the smaller the FTR, the lower the plasma carnitine (Fig. 2). Such a correlation did not exist in the reference group. There was no correlation between FTR of carnitine and plasma creatinine (not shown).

In order to assess whether in the Fanconi syndrome patients the tubular transport of carnitine was affected to the same degree as that of other compounds, we compared fractional excretion of carnitine with that of valine. Valine was chosen for comparison as its concentration in plasma and excretion in urine normally do not fluctuate much, as its FTR is high, and because it is reliably detected after column chromatography. We found a good linear correlation between both variables (Fig. 3).

It seemed of special interest to follow longitudinally plasma carnitine and FTR of carnitine in individual patients with Fanconi syndrome. Such unprecedented observations were possible in three children. First, in one 2-yr-old cystinotic girl, FTR of free and acyl carnitine dropped, as did plasma free and acyl carnitine within 6 months while the disease took its natural course (Fig. 2). The drop of FTR of carnitine (*i.e.* the rise of the fractional excretion) was paralleled by that of valine suggesting progression of the generalized tubular dysfunction (Fig. 3). In this patient, plasma carnitine depletion probably reflected an increased urinary loss. Second, a 12-yr-old cystinotic boy received a kidney graft. At age 15 yr FTR of carnitine and plasma carnitine had become normal (Fig. 2). The rise of FTR of

Fig. 3. Correlation between the fractional excretion (FE) of free carnitine and of valine in patients with Fanconi syndrome. Symbols as in Figure 2. For FE of acyl carnitine and of valine, the following correlation was calculated: $Y = 1.76 + 0.40X$, $r = 0.6190$, $n = 23$, $p < 0.01$.

carnitine, paralleled by that of valine (Fig. 3), obviously reflected the activity of the donor kidney. Third, a galactosemic boy was diagnosed on day 5 and treated since the diagnosis. FTR of carnitine was low and rose to subnormal within 10 days and to normal after 10 wk (Fig. 2). Generalized hyperaminoaciduria regressed within 10 days, as exemplified by the fractional excretion of valine (Fig. 3). Despite the rapid resolution of his Fanconi syndrome, his plasma carnitine fell under dietary treatment with a soybean formula, which, however, was not carnitine supplemented.

Traces of dicarboxylic acids were discovered in a few of the urine samples collected from the patients with Fanconi syndrome, while, not surprisingly (14), most patients had considerable lacticaciduria.

DISCUSSION

In the majority of our 15 patients with selective tubulopathy, FTR of free and acyl carnitine and plasma carnitine were normal. Thus, we have been unable to find evidence for the existence of a defective transport mechanism shared by carnitine. It is noteworthy that the excretion of lysine in lysinuric protein intolerance and in cystinuria was not paralleled by loss of carnitine as the structural similarities of the compounds might have suggested. Carnitine overloading of an adult did not result in the overexcretion of any of the compounds measured in urine, thus again no evidence of a shared carrier mechanism was produced.

Our study of patients with selective tubulopathy seems to be the first of its kind. Nevertheless, our results are in agreement with those of *in vitro* studies using rat kidney cortex slices (15) and renal brush border membrane vesicles (16). It was shown that lysine, choline, betaine, and γ -aminobutyric and α -hydroxybutyric acids were unable to reduce carnitine transport.

To date, only two groups have reported what appeared to be a specific carnitine transport defect in kidney (13, 17) and possibly in intestine (13) causing systemic carnitine deficiency. None of the patients had excessive amino acid excretion which could have suggested a common transport mechanism. Since the four patients of Engel *et al.* (17) were later reported as having medium chain acyl-CoA dehydrogenase deficiency (18; Engel A, personal communication), the existence of a specific defect of tubular carnitine transport presently rests on the evidence collected in only one child (13) who does not have this enzyme defect (Valle D, personal communication).

The renal handling of carnitine by patients with Fanconi syndrome had been studied earlier. In 18 cystinotic patients (5) and in five other patients with proximal renal tubular dysfunction (5–7), findings were comparable with those in our own 19 patients. FTR of free and acyl carnitine was abnormally low in most patients and varied considerably, and plasma free and acyl carnitine was low in the majority of patients. This was different in the reference group who had normal FTR of carnitine, phosphate, and uric acid and no hyperaminoaciduria even at low plasma carnitine concentrations. We therefore concluded that, first, a low plasma carnitine concentration does not lead per se to a renal loss of carnitine (*e.g.* by limitation of energy supply), amino acids, phosphate, or uric acid. Second, the lowering of plasma carnitine in the patients with Fanconi syndrome was caused by excessive loss of carnitine in urine. As the fractional excretion of carnitine and of valine correlated positively and since they improved or declined conjointly in individual patients, we concluded that both impairments were but the expression of a more generalized tubular dysfunction in the Fanconi syndrome.

Are patients with Fanconi syndrome at risk for systemic carnitine deficiency? A normal rise of plasma ketones was demonstrated in two children with cystinosis after a 24-h fast, but in two others a muscle biopsy revealed mild myopathic changes in one and a somewhat lowered tissue carnitine and increased lipid droplets in both (5). Similarly, one child with idiopathic Fanconi syndrome had muscle weakness, low muscle carnitine, and lipid excess in type I muscle fibers (6) and reportedly improved muscle strength after carnitine supplementation (19). Although in our

patients with Fanconi syndrome we did not find significant dicarboxylic aciduria (an indicator of ω -oxidation owing to lack of β -oxidation), systemic carnitine deficiency may have prevailed in some but was not detected without subjecting the patients to a metabolic stress, *e.g.* prolonged fasting. Therefore, the pathophysiological significance of low plasma carnitine and the need for carnitine supplementation still remain to be established.

Note added in proof: We recently studied a patient with Hartnup disease (courtesy of Dr. B. Hadorn, Munich) *i.e.* yet another selective tubulopathy. He had a normal FTR of free (98.2%) and acyl (90.5%) carnitine while the FTR of valine was abnormally low (95.6%), corresponding to a fractional excretion (log FE, %) of 1.25 for free carnitine and of 1.64 for valine (for comparison, see Fig. 3). We thus concluded that carnitine did not share the tubular transport mechanism for certain neutral amino acids, affected in this disorder.

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