Kidney International, Vol. 32 (1987), pp. 754–759

Effects of L-carnitine on sodium transport in erythrocytes from dialyzed uremic patients

WALTER D. LABONIA, OSCAR H. MORELLI, JR., MARIA I. GIMENEZ, PATRICIA V. FREULER, and OSCAR H. MORELLI

Nephrology Institute, French Hospital, Buenos Aires, Argentina

Effect of L-carnitine on sodium transport in erythrocytes from dialyzed uremic patients. Red blood cell (RBC) sodium transport systems were studied by cation flux methodology, measuring both the ouabain–sensitive Na-K pump and the ouabain–insensitive Na-K cotransport (CoT), as well as the Na-lithium (Li) countertransport (CTT), in eight patients on chronic hemodialysis and a control group of eight normal individuals. Intracellular sodium content and passive Na permeability were also determined. The effect of L-carnitine on RBC sodium transport in the uremic group was evaluated by supplementation with oral (1 g/day) and i.v. (1 g post-hemodialysis) L-carnitine for 60 days. Mean Na efflux through the ouabain–sensitive Na-K pump was 30.7% lower in uremic patients than in controls (3.49 ± 1.52 vs. 5.04 ± 0.72 mmol/liter RBC×hr; $P < 0.025$). Intracellular Na content was higher in uremic patients (11.57 ± 3.38 vs. 8.86 ± 0.88 mEq/liter RBC; $P < 0.05$), but no differences were found in Na-K CoT, Na-Li CTT or passive Na permeability. L-carnitine treatment increased the ouabain–sensitive Na efflux in uremic patients (4.76 ± 1.6 vs. 3.49 ± 1.52 mmol/liter RBC×hr; $P < 0.05$), with no change in CoT, CTT, intracellular Na content or passive Na permeability. We conclude that L-carnitine deficiency may play a major role in uremic Na-K pump disfunction.

Cationic transport systems in the red blood cell (RBC), the most commonly employed cell model, have been clearly defined and differentiated by several authors, as follows: Na-K adenosintriphosphatase (Na-K ATPase) inhibited by ouabain, Na-K cotransport (CoT) inhibited by furosemide, and Na-Na or Na-Li countertransport (CTT) inhibited by phloretin [1–4].

During the last few years attempts have been made to elucidate the effect of uremia on these transport systems. Although the Na-K pump mechanism has been thoroughly studied, it is only recently that Na-K CoT and Na-Li CTT functions in end–stage renal disease (ESRD) have been investigated [5, 6].

The most frequent finding is that erythrocytes from uremic patients present deficient Na-K ATPase activity due to causes as yet unclear. In this regard, the existence of a circulating Na-K pump inhibitor was suggested by the depression observed in pump activity after incubation of normal RBCs with uremic plasma [7, 8]. As regards the chemical nature of this inhibitor(s), it is noteworthy that Tamura et al have recently reported that circulating free fatty acids (FFA) are endogenous inhibitors of the Na-K ATPase [9]. The importance of L-carnitine in fatty acid metabolism has hinted that its deficiency may play a major role in uremic Na-K pump disfunction.

Accordingly, this study evaluates the various sodium transport systems (Na-K pump, CoT, and CTT), together with intracellular Na content and passive Na permeability immediately prior to dialysis, in a group of uremic patients on chronic hemodialysis, and compares findings with a sex– and age–matched control group. The response of erythrocyte transport systems in uremic patients to 60 days oral and intravenous supplementation with L-carnitine was subsequently evaluated.

Methods

Patients

Eight patients, five male and three female, with ESRD of varying etiologies were studied. Their ages ranged from 28 to 64 years, with a mean of 50.5 ± 13.8, and they had been on four hour, thrice–weekly intermittent hemodialysis for 25 to 101 months, with a mean of 50.25 ± 23.03 months. All patients were normotensive with predialysis mean systolic and diastolic BP of 125 ± 14 and 81 ± 9 mm Hg, respectively. All were stable and ambulatory, with no intercurrent diseases. None were on digital or diuretics or had received transfusions within the last six months. The control group consisted of eight normotensive individuals, with mean systolic and diastolic BP of 126 ± 10 and 78 ± 6 mm Hg, respectively, with no significant medical problems, five male and three female, whose ages ranged from 31 to 65 years (50.7 ± 13.3 years).

Study design

At day zero (0) of the study, 20 ml of blood were taken with a heparinized syringe to determine ouabain–sensitive Na efflux (Na-K pump), Na-K CoT, Na-Li CTT, intracellular Na content and passive Na permeability. Blood samples were obtained before dialysis, in the morning and after fasting. At immediate post-dialysis on day 0, uremic patients were put on L-carnitine (ALBICAR™, Casasco, ingestible 1 g vials) at the rate of 1 g/day by oral route plus 1 g i.v. every post-dialysis (1 g i.v. vials). L-carnitine supplementation was given for 60 days.

During the two weeks prior to day 0, baseline determinations of various humoral parameters were carried out, and then repeated at day 62.

Received for publication October 2, 1986
and in revised form February 25 and June 15, 1987

Table 1. Sodium transport systems, sodium content and passive sodium permeability in RBC from normal controls (N) ($N = 8$); and in dialysis patients ($N = 8$) before (Pre) and after (Post) L-Carnitine administration

	N	Pre	Post
Na-K pump			
mmol/liter RBC × hr	5.04 ± 0.72	3.49 ± 1.52[a]	4.76 ± 1.6[b]
Na-K C_oT			
mmol/liter RBC × hr	0.3 ± 0.08	0.31 ± 0.23	0.33 ± 0.2
Na-Li CTT			
mmol/liter RBC × hr	0.33 ± 0.05	0.33 ± 0.11	0.36 ± 0.19
Na content			
mEq/liter RBC	8.86 ± 0.88	11.57 ± 3.38[c]	11.25 ± 2.12
Na permeability			
per hr	0.026 ± 0.012	0.025 ± 0.011	0.026 ± 0.013

[a] $P < 0.025$ (vs. N); [b] $P < 0.05$ (vs. Pre); [c] $P < 0.05$ (vs. N)

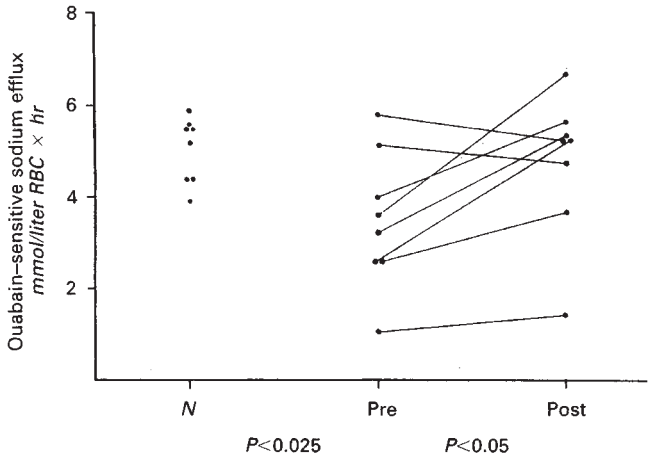

Fig. 1. *Values for ouabain-sensitive sodium efflux (Na-K pump) in sodium loaded RBC from normal controls (N) (N = 8); and in dialysis patients (N = 8) before (Pre) and after (Post) L-carnitine administration.*

Blood samples from the control group were also taken in the morning after fasting to determine ouabain-sensitive Na-efflux, CoT, CTT, intracellular Na content and passive Na permeability.

Methods

Blood sampling was performed before hemodialysis in two heparinized tubes: one was for CTT, while the other was for CoT and Na-K pump evaluation.

After spinning for 10 minutes at 6000 g at 4°C in a cooled centrifuge, the RBC pellet was washed four times with the following solutions: for CTT, (mM) $MgCl_2$ 75, sucrose 85, and Tris-MOPS 10 pH 7.4 at 4°C; for CoT, (mM) choline chloride 149, $MgCl_2$ 1 and Tris-MOPS 10 pH 7.4 at 4°C.

Na-Li CTT measurement. This was done according to the methods described by Canessa et al [10] and Adragna et al [11].

1. For Li loading, 2 ml of washed RBC (hematocrit 20%) were incubated for three hours at 37°C in a loading solution (mM: LiCl 150, Tris-MOPS 10, pH 7.4 at 37°C, glucose 10). Li was removed by washing six times with a solution of (mM) $MgCl_2$ 75, sucrose 85, and Tris-MOPS 10, pH 7.4 at 4°C. A 50% cell suspension in washing solution was used for measurements of hematocrit, hemoglobin, intracellular Na content and fluxes.

2. For flux measurement, Li efflux was measured in Mg and Na medium (4% to 5% hematocrit). Na medium contained (mM): Na 150, glucose 10, Tris-MOPS 10, pH 7.4 at 37°C, and ouabain 0.1. Mg medium contained (mM): $MgCl_2$ 75, Tris-MOPS 10, pH 7.4 at 37°C, glucose 10, sucrose 85, and ouabain 0.1. The 50% suspension was diluted to a 4 to 5% hematocrit with the cooled (4°C) Mg and Na media, and the resultant cell suspension divided into three portions and incubated for 60 minutes at 37°C. Li concentration was determined in the Mg and Na media using an atomic absorption spectrophotometer.

Na-K pump and CoT maximum Na efflux rate measurement. Measurements were carried out with Na loaded RBC by the nystatin method as described by Canessa et al [10].

1. For Na loading, 2 ml of washed RBC were added to 10 ml of loading solution (mM: NaCl 70, KCl 70, sucrose 55, Tris-MOPS 10, pH 7.4 at 4°C) containing 150 λ of nystatin solution (10 mg in 2.5 ml dimethylsulfoxide), and incubated for 20 minutes at 4°C. Nystatin was removed by centrifuging at room temperature and washing four times at 35°C with (mM) NaCl 70, KCl 70, Tris-MOPS 10, pH 7.4 at 4°C, sucrose 55, K_2HPO_4 buffer 1, pH 7.4, glucose 10, and albumin 0.1%. In the first wash the cells were equilibrated for 10 mintues in the water bath, and for four minutes in the last three washes. The cells were subsequently washed with cold (4°C) choline washing solution (mM: choline chloride 149, $MgCl_2$ 1, Tris-MOPS 10, pH 7.4 at 4°C). A suspension at 50% hematocrit was made for measurements of hemoglobin, hematocrit, intracellular Na content and fluxes.

2. For flux measurement to measure Na-K pump function, 0.2 ml of the 50% suspension were added to 7 ml of medium (mM: choline chloride 140, KCl 10, $MgCl_2$ 1, Tris-MOPS 10, pH 7.4 at 37°C, glucose 10) without and with ouabain (0.1 mM). To measure CoT, 0.8 ml of the 50% suspension were added to 7 ml of medium (mM: choline chloride 148, $MgCl_2$ 1, Tris-MOPS 10, pH 7.4 at 37°C, glucose 10, ouabain 0.1) without and with furosemide, 1 mM (33 mg in 100 μl of Tris base 1 M—furosemide 1 M—, and then made up to 1 mM with medium). After the addition of the cell suspension to each flask, 1.5 ml of the flux medium was put into each of five tubes. Duplicate tubes were incubated at 37°C for five minutes, and triplicate tubes for 25 minutes for pump medium and 65 minutes for CoT medium. Cellular Na content was measured by means of suitable standards. All solutions employed for flux measurement required adjustment to 298 ± 5 mOsm/liter. Two normal controls for Na-K pump and CoT were repeated five times, obtaining a variation coefficient lower than 10%. Hemoglobin concentration was measured throughout the experiment, discarding flux measurements when variations were greater than 3% versus the original cells.

In vitro incubation with L-carnitine. In four uremic patients L-carnitine effect on RBC Na-K pump was tested in vitro. In this study, 0.5 mg of free L-carnitine were added to 10 ml of blood (to get 300 μmol/liter, blood concentration usually obtained with the carnitine dosage used in this study [12]), and incubated for 60 minutes. Ouabain-sensitive Na efflux post-incubation was then compared with basal values in the same patients.

Other measurements

Baseline determinations of hematocrit, urea, uric acid, creatinine, natremia, kalemia, calcemia, and phosphatemia, as well

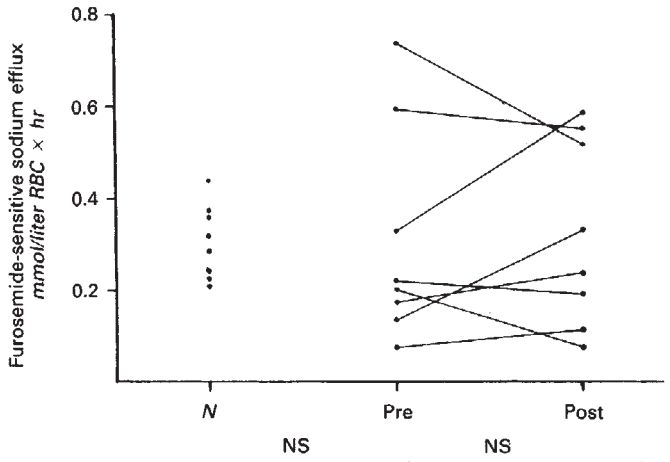

Fig. 2. *Values for ouabain–insensitive, furosemide–sensitive sodium efflux (cotransport) in sodium–loaded RBC from normal controls (N) (N = 8); and in dialysis patients (N = 8) before (Pre) and after (Post) L-carnitine administration.*

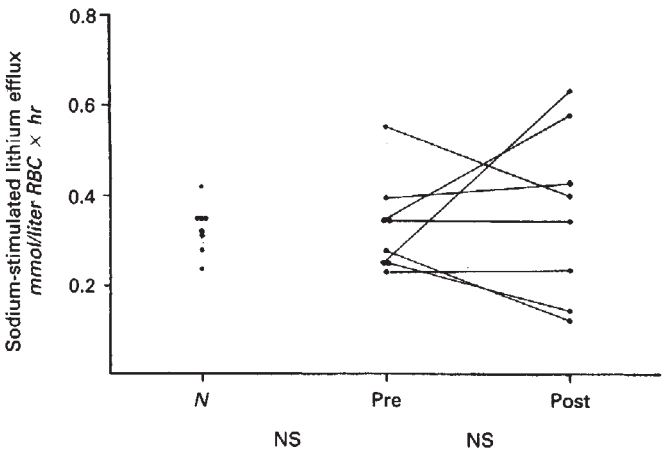

Fig. 3. *Values for sodium–stimulated lithium efflux (countertransport) in RBC from normal controls (N) (N = 8); and in dialysis patients (N = 8) before (Pre) and after (Post) L-carnitine administration.*

as triglycerides, total cholesterol, HDL-cholesterol, and total lipids, were carried out and repeated at day 62 of the study. Blood samples were obtained before dialysis, in the morning, and after fasting.

Hemodialysis

All patients underwent four–hour hemodialysis sessions employing the same cuprophane filters (Travenol 23.08) having 8 μ membrane thickness and 1.4 m^2 surface. Continuous heparinization was employed, with an initial mean dose of 2250 ± 250 IU and 1750 ± 250 IU hourly. Hemodialysis was carried out employing the usual technique, adjusting ultrafiltration to the patient's clinical needs. Bath preparation was automatic throughout, keeping Na concentration at 137 to 139 mEq/liter.

Statistical analysis

Student's *t*-test was employed to evaluate statistical significance. The various correlations were evaluated by linear regression (Pearson's *r*). All results are expressed as mean ± SD.

Results

Na-K pump, Na-K CoT, Na-Li CTT, intracellular Na content, and passive Na permeability results are outlined in Table 1.

Na-K pump

Mean value for ouabain-sensitive Na efflux in uremic patients was 30.7% lower than mean value in controls (3.49 ± 1.52 vs. 5.04 ± 0.72 mmol/liter RBC×hr; $P < 0.025$). If the uremic patient with the lowest value is excluded (Fig. 1), mean Na efflux becomes 3.85 ± 1.24 mmol/liter RBC×hr, which is still significantly lower than normal mean value ($P < 0.05$).

L-carnitine administration to the uremic patients led to a 36.2% Na efflux increase as regards baseline values (3.49 ± 1.52 vs. 4.76 ± 1.6 mmol/liter RBC×hr; $P < 0.05$). Post-L-carnitine mean value was only 5.5% below the control group mean (4.76 ± 1.6 vs. 5.04 ± 0.72 mmol/liter RBC×hr; NS) (Fig. 1).

Na-K CoT

No significant differences were found in ouabain–insensitive, furosemide–sensitive Na efflux for uremic patients versus controls (0.31 ± 0.23 vs. 0.3 ± 0.08 mmol/liter RBC×hr; NS). L-carnitine treatment failed to modify Na-K CoT in uremic patients (0.31 ± 0.23 vs. 0.33 ± 0.2 mmol/liter RBC×hr; NS) (Fig. 2).

Na-Li CTT

There was no significant difference between Na-stimulated lithium efflux in uremic patients and controls (0.33 ± 0.11 vs. 0.33 ± 0.05 mmol/liter RBC×hr; NS) and L-carnitine administration had no effect on the former group (0.33 ± 0.11 vs. 0.36 ± 0.19 mmol/liter RBC×hr; NS) (Fig. 3).

Intracellular Na content

Mean intracellular Na content in uremic patients was 30.5% higher than the control group mean (11.57 ± 3.38 vs. 8.86 ± 0.88 mEq/liter RBC; $P < 0.05$), but L-carnitine administration failed to modify values in the uremic group (11.57 ± 3.38 vs. 11.25 ± 2.12 mEq/liter RBC; NS) (Fig. 4).

Passive Na permeability

Na permeability was measured as the efflux rate constant for Na from Na-loaded cells in the presence of ouabain and furosemide. The efflux rate in mmol/liter RBC×hr was factored by the initial intracellular Na concentration and expressed as a rate constant. Passive Na permeability was not different between uremic patients and normal subjects (0.025 ± 0.011 vs. 0.026 ± 0.012 per hr; NS), and L-carnitine treatment did not modify passive Na permeability in uremic patients (0.025 ± 0.011 vs. 0.026 ± 0.013 per hr; NS).

In vitro incubation with L-carnitine

No significant differences were found in ouabain–sensitive Na efflux in uremic RBCs after in vitro incubation with L-carnitine, when compared with basal values in the same pa-

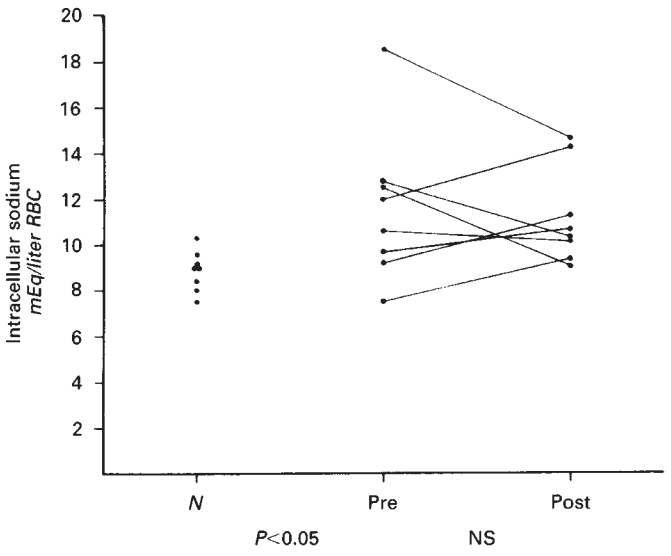

Fig. 4. *Values for intracellular sodium concentration in RBC from normal controls (N) (N = 8); and in dialysis patients (N = 8) before (Pre) and after (Post) L-carnitine administration.*

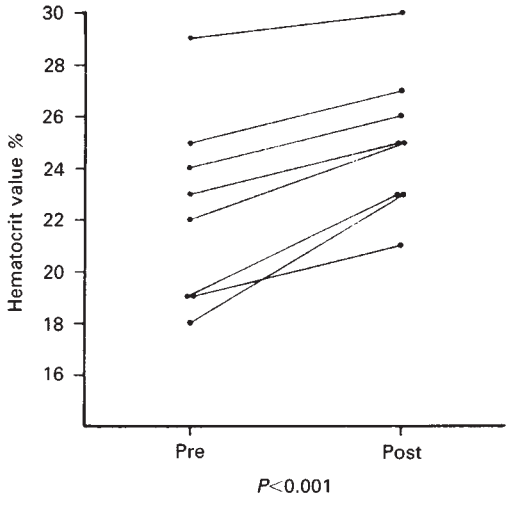

Fig. 5. *Hematocrit values from dialysis patients (N = 8) before (Pre) and after (Post) L-carnitine administration.*

tients (3.37 ± 0.91 vs. 3.46 ± 0.85 mmol/liter RBC×hr; NS) ($N = 4$).

General measurements

Following L-carnitine administration there was a very significant increase in all eight uremic hematocrit values (24.9 ± 2.7 vs. 22.25 ± 3.6%; $P < 0.001$) (Fig. 5), but no changes were observed in the remaining parameters (Table 2).

Statistical correlations

Using linear regression no significant correlations were found for: ouabain–sensitive Na efflux versus intracellular Na (in both uremic and normal subjects); Na-K pump versus hematocrit; Na-K CoT versus kalemia; intracellular Na versus CoT or CTT; or Na pump, CoT and CTT versus total cholesterol, HDL-cholesterol, triglycerides or total lipids.

Table 2. Laboratory parameters in dialysis patients ($N = 8$) before (Pre) and after (Post) L-carnitine administration

	Pre	Post
Hct %	22.25 ± 3.6	24.9 ± 2.7[a]
Serum urea *mg/dl*	155 ± 28	145 ± 43
Serum creatinine *mg/dl*	11.9 ± 3	11.1 ± 2.8
Serum Na *mEq/liter*	141 ± 0.2	142 ± 2.1
Serum K *mEq/liter*	5.3 ± 0.5	5.5 ± 0.2
Serum Ca *mg/dl*	8.6 ± 0.6	8.8 ± 0.6
Serum P *mg/dl*	5.6 ± 1.6	5.3 ± 1.8
Serum uric acid *mg/dl*	8.4 ± 0.9	7.5 ± 1.5
Total serum cholesterol *mg/dl*	176 ± 48	189 ± 36
Serum HDL-cholesterol *mg/dl*	27.2 ± 20	30 ± 19
Serum triglycerides *mg/dl*	175 ± 130	191 ± 107
Total serum lipids *mg/dl*	772 ± 284	787 ± 135

[a] $P < 0.001$

Discussion

A deficient Na-K ATPase function in patients with chronic renal failure was first reported by Welt, Sachs and Manus over 20 years ago [13]. These authors detected in 25% of the uremic patients examined an increase in intracellular Na content, together with a decrease in RBC Na-K pump activity [13]. In 1973, Cole demonstrated a decrease in erythrocyte Na-K ATPase activity in most uremic patients tested [14]. Later, several authors reported similar findings [7, 15, 16], although recently Corry et al failed to detect this defect in erythrocyte Na-K ATPase activity in a group of 36 patients on chronic dialysis (hemodialysis and chronic ambulatory peritoneal dialysis) [5]. Likewise, it is worthwhile pointing out that alterations have been detected in Na-K pump activity in leucocytes [17], brain [18], intestine [19] and muscle cells [20] from uremic subjects.

The cause of this deficient Na-K pump activity in chronic renal failure is far from clear. A deficiency in the pump's energy substrate, the ATP itself, may perhaps be ruled out, since the pump is saturated with ATP under physiological conditions [21], and moreover, ATP concentration is usually increased in uremic erythrocytes [22].

The existence of a circulating Na-K ATPase inhibitor was suggested by the depression observed in pump activity after incubation of normal RBCs with uremic plasma [7, 8]. As regards this "endogenous–ouabain" model, Bohan, Potter and Bourgoignie have detected a low molecular weight substance in urine from uremic dogs, which displaced ouabain from cellular receptors in renal cortical membranes [23], and Graves, Brown and Valdes have reported the existence of a digoxin–like substance in serum from uremic patients [24]. Although the chemical nature of these Na-K ATPase inhibitors has not been exactly defined [25, 26], it is noteworthy that several authors have shown that unsaturated fatty acids inhibit Na-K ATPase and also displace ouabain from the enzyme [27, 28]. In this regard, Tamura et al have recently demonstrated that plasma linoleic and oleic acids are endogenous Na-K pump inhibitors [9]. Moreover, plasma levels of these fatty acids markedly increased during acute volume expansion [9].

With respect to L-carnitine (L-3-hydroxy-4-N-trimethylaminobutyric acid), it is well known that this substance is the

natural carrier of fatty acids from the cellular cytoplasm to the mitochondrial matrix, where they undergo β-oxidation [29]. Patients on chronic hemodialysis present severe serum and tissue L-carnitine deficiency due to a combination of inadequate intake, impaired synthesis and excessive loss during dialysis (L-carnitine molecular wt: 161 dalton) [30, 31].

By decreasing FFA oxidation, carnitine deficiency appears to play an important role in the multifactorial pathogenesis of hyperlipidemia in uremic patients [32]. In fact, several authors have reported an improvement in plasma lipid profile following L-carnitine administration in dialyzed uremic patients [12, 33, 34]. Besides, it has been observed that during hemodialysis the drop in serum L-carnitine levels is accompanied by a rise in serum FFA [35]. This peak in FFA levels is markedly reduced after L-carnitine supplementation for 1 week [36].

Under normal conditions the kidney metabolizes circulating fatty acids, and FFA oxidation provides 10 to 20% of the renal energy requirements [37, 38]. The decrease in renal FFA metabolism may contribute to the increased levels of these Na-K pump inhibitors in uremia.

Since in vitro incubation with L-carnitine did not modify Na-K ATPase activity, we may rule out any direct effect of the drug on RBC Na-K pump. Therefore, we conclude that L-carnitine administration might increase fatty acid transport and oxidation in uremic patients, thus decreasing the plasma concentration of these Na-K ATPase inhibitors. The removal of these "ouabain–like" factors might explain the enhanced Na-K pump activity observed in the present study following L-carnitine supplementation in dialyzed uremic patients.

In the present study a significant increase in hematocrit value after L-carnitine administration was detected in all cases, in agreement with previous literature [12]. Vacha et al suggested that L-carnitine might improve the lipid metabolism in erythrocyte membrane, thus explaining the hematocrit increase [12]. This hypothetical variation in RBC membrane composition after L-carnitine treatment could also lead to an increase in the number of Na-K pump units per RBC. In addition to the FFA model, this alternative hypothesis could explain the partial recovery of RBC Na-K pump activity after L-carnitine administration in uremic patients. In this regard, it is noteworthy that Cheng, Kahn and Kaji have recently proposed that a decreased synthesis of Na-K pump sites in erythroid precursors might account for the uremic Na-K ATPase defect [39].

In our study L-carnitine treatment increased Na-K ATPase activity but failed to decrease intracellular Na content, thus suggesting that the higher Na content in uremic RBCs is not related to the Na-K pump defect. Regarding that CoT, CTT and passive Na permeability were not affected by uremia or L-carnitine treatment, a different Na transport system might be responsible for the increased intracellular Na in uremia.

In addition to the ouabain–sensitive Na-K pump, the RBC contains ouabain–resistant transport systems, such as the Na-K CoT and the Na-Na or Na-Li CTT. The Na-K CoT transports Na and K in the same direction through the RBC membrane, either inwards or outwards, depending on ionic transmembrane chemical gradients [3, 40, 41]. Recently, marked Na-K CoT depression has been reported in dialyzed uremic patients, which the authors partly attribute to the moderate hyperkalemia observed in the group under study [5]. In our study, Na-K CoT was not different between uremic patients and normal subjects, in spite of the former's moderate hyperkalemia (5.3 ± 0.5 mEq/liter). L-carnitine treatment did not modify uremic Na-K CoT activity.

The Na-Li CTT removes intracellular lithium from lithium–loaded erythrocytes, in a 1:1 exchange ratio with extracellular Na [2]. In a recent report, Woods, Parker and Watson have demonstrated that Na-Li CTT maintains normal activity in uremia by means of the stimulating action exerted by a suspected dialyzable plasma factor [6]. The removal of this factor leads to a fall in RBC Na-Li CTT immediately after dialysis. In our study Na-Li CTT was not different between uremic subjects and controls, with no change in uremic Na-Li CTT activity after L-carnitine treatment.

Conclusions

A deficient Na-K ATPase activity was detected in our patients on chronic hemodialysis, in close agreement with most published findings. Besides, intracellular Na content proved significantly higher in uremic patients as compared to normal controls.

L-carnitine administration significantly improved erythrocyte Na-K ATPase activity, but failed to modify Na-K CoT, Na-Li CTT, intracellular Na content or passive Na permeability. A highly significant increase in hematocrit was also observed in all the uremic patients following L-carnitine administration.

Although the number of patients under study was hardly enough to draw any definitive conclusions, our work does support L-carnitine deficiency as a causative factor in erythrocyte Na-K ATPase defect in uremia, as well as its contributory role in the development of anemia in chronic renal failure.

Acknowledgments

This work was presented in abstract form at the VIth Argentine Congress of Nephrology, Buenos Aires, October 1986. The authors appreciate Mr. Alberto Fontana's technical assistance and Mr. Roderick Muir's help in translating the manuscript. L-carnitine was kindly provided by Laboratorios Casasco S.A.I.C., Buenos Aires, Argentina.

Reprint requests to Dr. Walter D. Labonia, Instituto de Nefrologia, Hospital Frances, La Rioja 951, (1221) Buenos Aires, Argentina.

References

1. Dunham PB, Hoffman JF: Na and K transport in red blood cells, in *Physiology of Membrane Disorders*, edited by Andreoli TE, Hoffman JF, Fanestil DD, New York, Publishing Corp., 1978, pp. 255–272
2. Haas M, Schooler J, Tosteson DC: Coupling of lithium to sodium transport in human red cells. *Nature* 258:425–434, 1975
3. Wiley JS, Cooper RA: A furosemide–sensitive cotransport of sodium plus potassium in the human red cell. *J Clin Invest* 53:745–755, 1974
4. Chipperfield AR: The (Na-K-Cl) co-transport system. *Clin Sci* 71:465–476, 1986
5. Corry DB, Tuck ML, Brickman AS, Yanagawa N, Lee DBN: Sodium transport in red blood cells from dialyzed uremic patients. *Kidney Int* 29:1197–1202, 1986
6. Woods JW, Parker JC, Watson BS: Perturbation of sodium–lithium countertransport in red cells. *N Engl J Med* 308:1258–1261, 1983
7. Izumo H, Izumo S, De Luise M, Flier JS: Erythrocyte Na-K pump in uremia. Acute correction of a transport defect by hemodialysis. *J Clin Invest* 74:581–588, 1984
8. Cole CH, Balfe JW, Welt LG: Induction of ouabain–sensitive

ATPase defect by uremic plasma. *Trans Assoc Am Physiol* 81:213–221, 1968
9. TAMURA M, KUWANO H, KINOSHITA T, INAGAMI T: Identification of linoleic and oleic acids as endogenous Na-K ATPase inhibitors from acute volume–expanded hog plasma. *J Biol Chem* 260:9672–9677, 1985
10. CANESSA M, BIZE I, ADRAGNA N, TOSTESON DC: Cotransport of sodium and potassium in human red cells. *J Gen Physiol* 80:149–168, 1982
11. ADRAGNA N, CANESSA M, SLATER E, TOSTESON DC: Red cell Li-Na countertransport and Na-K cotransport in patients with essential hypertension. *Hypertension* 4:795–804, 1982
12. VACHA G, GIORCELLI G, SILIPRANDI N, CORSI M: Favorable effects of L-carnitine treatment on hypertriglyceridemia in hemodialysis patients: Decisive role of low levels of HDL-cholesterol. *Am J Clin Nutr* 38:532–540, 1983
13. WELT LG, SACHS JR, MANUS TJ: An ion transport defect in erythrocytes from uremic patients. *Trans Assoc Am Physiol* 77: 169–181, 1964
14. COLE CH: Decreased ouabain–sensitive ATPase activity in the erythrocyte membrane of patients with chronic renal disease. *Clin Sci Mol Med* 45:775–784, 1973
15. KRAMER HJ, GOSPODINOV D, KRUCK F: Functional and metabolic studies on red blood cell sodium transport in chronic uremia. *Nephron* 16:344–358, 1976
16. SWAMINATHAN R, CLEGG G, CUMBERBATCH M, ZAREIAN Z, MAC KENNA F: Erythrocyte sodium transport in chronic renal failure. *Clin Sci* 62:489–494, 1982
17. EDMONDSON RPS, HILTON PJ, JONES NF, PATRICK J, THOMAS RD: Leucocyte sodium transport in uremia. *Clin Sci Mol Med* 49:213–216, 1975
18. MINKOFF L, GAERTENER G, DARAB M, MERCIER C, LEVIN ML: Inhibition of brain sodium–potassium ATPase in uremic rats. *J Lab Clin Med* 80:71–78, 1972
19. KRAMER HJ, BOCKER A, KRUCK F: Inhibitor of intestinal Na-K ATPase in experimental uremia. *Clin Chim Acta* 50:13–18, 1974
20. COTTON JR, WOODMAN T, CARTER NW, KNOCHEL JP: Resting skeletal muscle membrane potential as an index of uremic toxicity. *J Clin Invest* 63:501–506, 1979
21. MUJAIS SK, SABATINI S, KURTZMAN NA: Pathophysiology of the uremic syndrome, in *The Kidney*, edited by BRENNER BM, RECTOR FC, (3rd ed) Philadelphia, WB Saunders, 1986, p. 1590
22. GRIMES AJ, MANSEL M: Annotation: Red and white blood cell abnormalities in chronic renal failure. *Br J Hematol* 42:169–174, 1979
23. BOHAN T, POTTER L, BOURGOIGNIE JJ: Ouabain radioreceptor assay for natriuretic factor, in *Hormonal Regulation of Sodium Excretion*, edited by LICHARDUS B, SCHRIER RW, PONEC J, Elsevier/North Holland, Biomedical Press, 1980, pp. 393–397
24. GRAVES SW, BROWN B, VALDES R: An endogenous digoxin–like substance in patients with renal impairment. *Ann Int Med* 99:604–608, 1983
25. KELLY RA, O'HARA DS, CANESSA ML, MITCH WE, SMITH TW: Characterization of digitalis–like factors in human plasma. Interactions with Na-K ATPase and cross–reactivity with cardiac glycoside–specific antibodies. *J Biol Chem* 260:11396–11405, 1985
26. DE WARDENER HE, CLARKSON EM: Concept of natriuretic hormone. *Physiol Rev* 65:658–759, 1985
27. AHMED K, THOMAS BS: The effects of long chain fatty acids on sodium plus potassium ion–stimulated adenosintriphosphatase of rat brain. *J Biol Chem* 246:103–109, 1971
28. BIDARD JN, ROSSI B, RENAUD JF, LAZDUNSKI M: A search for an "ouabain–like" substance from the electric organ of electrophorus electricus which led to arachidonic acid and related fatty acids. *Biochim Biophys Acta* 769:245–252, 1984
29. SILIPRANDI N: Carnitine as a "drug" affecting lipid metabolism: General considerations, in *Hypolipidemic Drugs: Therapeutic Selectivity and Risk/Benefit Assessment*, edited by PAOLETTI R, New York, NY, Raven Press, 1982, pp. 265–268
30. BARTEL L, HUSSEY J, SHRAGO E: Perturbation of serum carnitine levels in human adults by chronic renal disease and dialysis therapy. *Am J Clin Nutr* 34:1314–1320, 1981
31. RUMPF K, LESCHKE M, EISENHAUER T, BECKER K, KOTHE U, SCHELER F: Quantitative assessment of carnitine loss during hemodialysis and hemofiltration. *EDTA Proc* 19:298–301, 1982
32. CHAN MK, VARGHESE Z, MOORHEAD JF: Lipid abnormalities in uremia, dialysis and transplantation. *Kidney Int* 19:625–637, 1981
33. GUARNIERI G, RAINIERI G, TOIGO A, VASILE A, CIMAN M, RIZZOLI V, MORACCHIELLO M, COMPAMACCI T: Lipid lowering effect of carnitine in chronically uremic patients treated with maintenance hemodialysis. *Am J Clin Nutr* 33:1489–1492, 1980
34. LACOUR B, CHANARD J, HAGUET M, BASILI C, ASSAN R, DI GIULIO N, CIANCIONI C, LEBKIRI B, DRUEKE T, FUNK–BRETANO J: Carnitine improves lipid anomalies in hemodialysis patients. *Lancet* II (8198):763–765, 1980
35. BARTEL L, HUSSEY J, EWART R, SHRAGO F: Serum carnitine and free fatty acid levels during hemodialysis. *Fed Proc* 37:1237–1242, 1978
36. SUZUKI I, NARITA Y, YAMAZAKI N: Effects of L-carnitine on arrhythmias during dialysis. *Jpn Heart J* 23:349–359, 1982
37. BARAC–NIETO M, COHEN JJ: The metabolic fates of palmitate in the dog kidney in vivo: Evidence for incomplete oxidation. *Nephron* 8:488–493, 1971
38. PARK HC, LEAL PINTO E, MAC LEOD MB, PITTS RF: CO_2 production from plasma free fatty acids by the intact functioning kidney of the dog. *Am J Physiol* 227:1192–1199, 1974
39. CHENG JT, KAHN T, KAJI DM: Mechanism of alteration of Na-K pump of erythrocytes from patients with chronic renal failure. *J Clin Invest* 74:1811–1820, 1984
40. HILTON PJ: Cellular sodium transport in essential hypertension. *N Engl J Med* 314:222–229, 1986
41. GARAY R, ADRAGNA N, CANESSA M, TOSTESON D: Outward sodium and potassium cotransport in human red cells. *J Membr Biol* 62:169–174, 1981