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PROPERTIES OF CARNITINE TRANSPORT IN RAT KIDNEY CORTEX SLICES

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Summary

The properties of carnitine transport were studied in rat kidney cortex slices. Tissue:medium concentration gradients of 7.9 for L-[*methyl*-^{14}C]carnitine were attained after 60-min incubation at 37°C in 40 μM substrate. L- and D-carnitine uptake showed saturability. The concentration curves appeared to consist of (1) a high-affinity component, and (2) a lower affinity site. When corrected for the latter components, the estimated K_m for L-carnitine was 90 μM and V = 22 nmol/min per ml intracellular fluid; for D-carnitine, K_m = 166 μM and V = 15 nmol/min per ml intracellular fluid. The system was stereospecific for L-carnitine. The uptake of L-carnitine was inhibited by (1) D-carnitine, γ-butyrobetaine, and (2) acetyl-L-carnitine. γ-Butyrobetaine and acetyl-L-carnitine were competitive inhibitors of L-carnitine uptake. Carnitine transport was not significantly reduced by choline, betaine, lysine or γ-aminobutyric acid. Carnitine uptake was inhibited by 2,4-dinitrophenol, carbonyl cyanide *m*-chlorophenylhydrazone, N_2 atmosphere, KCN, *N*-ethylmaleimide, low temperature (4°C) and ouabain. Complete replacement of Na^+ in the medium by Li^+ reduced L- and D-carnitine uptake by 75 and 60%, respectively. Complete replacement of K^+ or Ca^{2+} in the medium also significantly reduces carnitine uptake. Two roles for the carnitine transport system in kidney are proposed: (1) a renal tubule reabsorption system for the steady-state maintenance of plasma carnitine; and (2) maintenance of normal carnitine levels in kidney cells, which is required for fatty acid oxidation.

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Introduction

Carnitine serves as an essential cofactor for the transport of acyl groups across the inner mitochondrial membrane [1—4]. High rates of fatty acid oxidation by a carnitine-dependent mechanism have been demonstrated in the renal cortex and outer medulla; and in isolated kidney mitochondria [5,6]. In vivo studies have shown that renal fatty acid oxidation was reduced by specific inhibition of palmitoylcarnitine acyltransferase and this was accompanied by significantly lower sodium, phosphate and glucose reabsorption from the glomerular ultrafiltrate [7,8]. Thus, carnitine-dependent fatty acid oxidation seems to be of importance for the maintenance of normal energy requirements in the kidney.

Carnitine biosynthesis in the rat occurs primarily in the liver from the amino acids, lysine and methionine [9—11]. Nevertheless, most extraheptic tissues contain carnitine concentrations much higher than those found in the plasma [12]. In the kidney, tissue:plasma carnitine gradients range from 7 to 8 in vivo [13]. Like heart and skeletal muscle, the kidney of the rat contains most of the enzymes necessary for carnitine biosynthesis, but lacks the final enzyme in the pathway which converts γ-butyrobetaine to carnitine [14,15]. Because of this, the kidney must obtain its entire supply of carnitine from the blood or by reabsorption from the glomerular ultrafiltrate. Under normal conditions, over 95% of the plasma carnitine appearing in the glomerular ultrafiltrate is reabsorbed by the kidney [16,17] and appears to be reabsorbed across the renal tubule at the level of the proximal region [10].

A carrier system for carnitine, exhibiting active transport properties, has been described in rat liver cells [19], human heart cells [20] and in isolated skeletal muscle [21]. Although the properties of carnitine transport in the kidney are not well understood, evidence in a previous study suggested the presence of an active transport system for carnitine in rat kidney cortex slices [22]. The present study was undertaken to describe more thoroughly the characteristics of D- and L-carnitine transport in rat kidney cortex slices.

Materials and Methods

Animals. Male Holtzman rats (200—250 g) were used in all experiments. The rats were given water and a commercial rat chow (Taklad, Sprague Dawley, Madison, WI) ad libitum and were housed in individual stainless-steel cages with wire mesh bottoms in a room with a 14- and 10-h natural light-dark cycles.

Materials. L-Carnitine and acetyl-L-carnitine were gifts from the Sigma Tau Corp., Rome, Italy. D-Carnitine was purchased from ICN Pharmaceuticals, Inc., Cleveland, OH. Carnitine acetyltransferase and all other chemicals used were purchased from Sigma Chemical Co., St. Louis, MO. DL-[*methyl*-^{14}C]Carnitine was obtained from the Radiochemical Center, Amersham Corp., Arlington Heights, IL. [*carboxyl*-^{14}C]Inulin, [*carboxyl*-^{14}C]aminoisobutyric acid and D-[U-^{14}C]glucose were purchased from the New England Nuclear Corp., Boston, MA.

Preparation of 4-N-trimethylaminobutyrate (γ-butyrobetaine). Synthesis of 4-*N*-trimethylaminobutyrate (γ-butyrobetaine) was accomplished by methyla-

tion of the parent amine, γ-aminobutyric acid, using an excess of CH_3I in a solution of CH_3OH, H_2O and $Ba(OH)_2$ [23]. The compound produced by this procedure migrated as a single spot on thin-layer chromatographic plates (Sil-G, Brinkman Instruments, Inc., Westburg, NY (detected by iodine vapor)). The synthesized product also migrated different from the parent compound and co-chromatographed with a pure sample of 4-*N*-trimethylaminobutyrate.

Preparation of D-[*methyl*-^{14}C]- *and* L-[*methyl*-^{14}C]*carnitine.* The procedure described herein is slight modification of that described by Ramsey and Tubbs [24]. Enzymatic acetylation of L-[*methyl*-^{14}C]carnitine from 0.98 μmol DL-[*methyl*-^{14}C]carnitine (specific activity 51 mCi/mmol) was carried out at 30°C for 2 h in a reaction mixture containing 1.0 μmol acetyl-CoA, 10 μmol 4,4-dithiobispyridine and 31 μg/ml carnitine acetyltransferase in a total volume of 2 ml of 10 mM Tris-HCl buffer, pH 8.0.

Identical non-radioactive reactions were performed simultaneously to determine the amount of L-[*methyl*-^{14}C]carnitine acetylated by acetyl-CoA to acetyl-L-[*methyl*-^{14}C]carnitine in the reaction. This was performed by following the absorbance of reduced 4,4-dithiobispyridine at 324 nm [25]. Assuming an extinction coefficient of $19.8 \cdot 10^3\ M^{-1} \cdot cm^{-1}$ for 4-thiopyridone, comparison of the calculated and the observed absorbance at 324 nm indicated that the reaction was 96—100% complete. After incubation, the radioactive reaction mixture was taken to dryness under a stream of N_2 at 50°C, resuspended in 2 ml of the eluting buffer, pH 4.5, and applied to a 50 × 1.5 cm column (Bio-Rad AG-50X8, 200-400 mesh cation-exchange resin) which was equilibrated with the eluting buffer (0.15 M HCHO adjusted to pH 4.5 with concentrated NH_4OH). Acetyl-L-[*methyl*-^{14}C]carnitine was eluted before D-[*methyl*-^{14}C]-carnitine. The peak fractions were collected and desalted by successive periods of freeze-drying. Acetyl-L-[*methyl*-^{14}C]carnitine was deacylated by incubation at 30°C in 2 M NH_4OH for 1 h and dried at 50°C under N_2. The purity of the radioactive isomers was determined by chromatography on both Whatman No. 1 paper and silica gel G thin-layer chromatographic plates using *n*-butanol/acetic acid/water (50 : 30 : 20, v/v) and methanol/chloroform/water/conc. ammonia/conc. formate (55 : 50 : 10 : 7.5 : 2.5, v/v), respectively [26]. Both D-[*methyl*-^{14}C]- and L-[*methyl*-^{14}C]carnitine migrated as a single peak and in the same area as authentic DL-[*methyl*-^{14}C]carnitine.

Preparation and incubation of rat kidney cortex slices. The animals were killed by decapitation after which the kidneys were quickly removed and bisected transversely. The capsule membrane was stripped off and 0.4-mm cortical slices were made with a Stadie-Rigg's microtome (Arthur H. Thomas Co., Philadelphia, PA) [27]. Groups of three kidney slices (100—130 mg) were preincubated for 10 min in 25-ml Erlenmeyer flasks containing 2 ml of carnitine-free Krebs-Ringer bicarbonate buffer, pH 7.4, at 37°C, and placed in a shaking water bath. Slices were then transferred and incubated in an identical medium containing 5.5 mM glucose, and 40 μM of either D-[*methyl*-^{14}C]- or L-[*methyl*-^{14}C]carnitine (0.09 μCi/flask, specific activity 51 mCi/mmol). The incubation medium was continually gassed with a mixture of O_2 and CO_2 (95:5%). At the end of the incubation period, the slices were removed, dipped in excess cold buffer to remove surface radioactivity, blotted, weighted and placed in test tubes containing 2 ml of 10% trichloroacetic acid. The slices were

centrifuged at 17 000 × *g* for 10 min and the supernatants were used to determine radioactivity. The amount of carnitine uptake was assessed by counting 0.2-ml aliquots each of the radioactive 10% trichloroacetic acid tissue supernatants and the remaining incubation medium. The 0.2-ml aliquots were placed in 4 ml Tritosol counting solution and counted in a Packard Model 3310 liquid scintillation counter [28].

Calculation and expression of results. The concentrations of D- and L-carnitine in the experiments to follow are expressed as dpm [*methyl*-^{14}C]carnitine/ml of intracellular fluid or extracellular fluid. Carnitine transport was expressed as the distribution ratio (DR) [27].

$$DR = \frac{\text{dpm}[\textit{methyl}\text{-}^{14}\text{C}]\text{carnitine/ml intracellular fluid}}{\text{dpm}[\textit{methyl}\text{-}^{14}\text{C}]\text{carnitine/ml extracellular fluid}}$$

The value of dpm [*methyl*-^{14}C]carnitine/ml extracellular fluid was equated to the radioactivity in the final incubation medium. The value of dpm [*methyl*-^{14}C]carnitine/ml intracellular fluid was determined according to the method of Rosenberg et al. [27]. In some experiments, the value of dpm [*methyl*-^{14}C]-carnitine/ml intracellular fluid was converted to nmol/ml intracellular fluid on the basis of the specific radioactivity of [*methyl*-^{14}C]carnitine in the medium [27].

Total tissue water was expressed as a percent of wet tissue weight as determined from the difference in tissue weight before and after drying at 100°C overnight. The average water content was 76.5 ± 0.4% before preincubation and 80.1 ± 0.4% after 2 h of incubation. These values agree with previous studies in rat kidney cortex slices [27].

Extracellular space was measured using [*carboxyl*-^{14}C]inulin according to the method of Rosenberg et al. [29]. The change in extracellular fluid space as a function of incubation time increased from 22.6 ± 0.7% wet tissue wt. after 10 min to 28.2 ± 1.2% after 165 min. These values are in agreement with those reported by others in a similar study [29].

Statistical analysis of the results have been made with the Student's *t*-test, and statistical significance placed at probability values of less than 5%. S.E. and *n* values are presented in each of the tables.

Chromatographic identification of tissue-free carnitine and acid-soluble acylcarnitines. Thin-layer chromatography was used to characterize the radioactive compound present in kidney cortex slices after 60-min incubation with L- or D-[*methyl*-^{14}C]carnitine. The kidney slices were incubated as previously described, rinsed and immediately placed in 10% trichloroacetic acid. The acid was removed from the supernatant fraction by repeated extractions with 2.5 vols. of diethyl ether. The aqueous fraction was evaporated to dryness under vacuum and redissolved in 60 μl of ethanol and water (3 : 1, v/v). Aliquots of the concentrated extract, which containing 5000—15 000 cpm, were spotted on silica gel G thin-layer chromatographic plates along with 5 μg non-radioactive L-carnitine and acetylcarnitine as carriers. Ascending chromatography was performed on the plates using a solvent system consisting of $CH_3OH/CHCl_3/H_2O$/conc. NH_4OH/conc. HCHO (55 : 50 : 10 : 7.5 : 2.5, v/v) [26]. The lanes, to which radioactivity was applied, were cut into 0.5-cm sections. Each section

was placed in a scintillation vial, eluted with 0.5 ml water, covered with 12 ml of Bray's solution and counted. The remaining portion of the plate, which contained non-radioactive carnitine and acetylcarnitine standards, was exposed to iodine vapor. The relative mobilities were calculated from the visualized spots and from radioactivity measurements.

Results

Tissue slice viability studies. Studies were undertaken to verify the duration of viability and plasma membrane integrity of the kidney slice preparation. Metabolic viability, as determined by the rate of glucose oxidation, showed that kidney slices incubated in 5.5 mM [U-^{14}C]glucose oxidized glucose linearly for a period of 160 min at a rate of 2.0 μmol/90 min per g wet wt. This is similar to the rate observed in rabbit kidney cortex slices [30]. These results indicate that rat kidney cortex slices remain metabolically viable for at least 160 min.

Plasma membrane integrity was determined by the uptake of α-aminoisobutyric acid. Kidney slices, incubated in 0.1 mM α-[^{14}C]aminoisobutyric acid, accumulated this compound against a gradient and maintained a steady-state concentration gradient for 160 min. These results are similar to the findings of others for rat kidney cortex slices [31] and indicate that the plasma membrane remains intact for at least 160 min.

Carnitine content of rat kidney cortex slices. The L-carnitine concentration in kidney cortex slices prepared as described in Materials and Methods was 195 ± 12 nmol/g wet wt. When incubated in 2 ml of carnitine-free, Krebs-Ringer bicarbonate buffer, pH 7.4, at 37°C, over 50% of the carnitine was released from the slices in the first 15 min. Thereafter, carnitine continued to be released, resulting in a final carnitine concentration that was 25% (45 ± 5 nmol/g wet wt) of the initial slice content after 60 min incubation. In subse-

TABLE I

UPTAKE AND RECOVERY OF RADIOACTIVITY AFTER INCUBATION OF RAT KIDNEY CORTEX SLICES WITH LABELED D- AND L-CARNITINE

Kidney slices were incubated as described in the text with 40 μM of L- or D-[^{14}C]carnitine for 30 min at 37°C. The value of dpm/ml extracellular fluid (ECF) was equated with final incubation medium radioactivity. Triplicate determinations were averaged. ICF, intracellular fluid.

	L-Carnitine	D-Carnitine
Medium radioactivity		
Initial (dpm/flask)	219 823	228 496
Final (dpm/flask)	167 516	202 453
Total tissue radioactivity at end of study (dpm)	43 163	29 269
Total radioactivity recovered (dpm)	210 680	231 722
Intracellular fluid radioactivity (dpm/ml ICF)	555 482	348 489
$\frac{\text{dpm/ml ICF}}{\text{dpm/ml ECF}}$	6.63 ± 0.7	3.44 ± 0.4
Percent initial radioactivity recovered	95.8 ± 1.1	101.5 ± 1.1

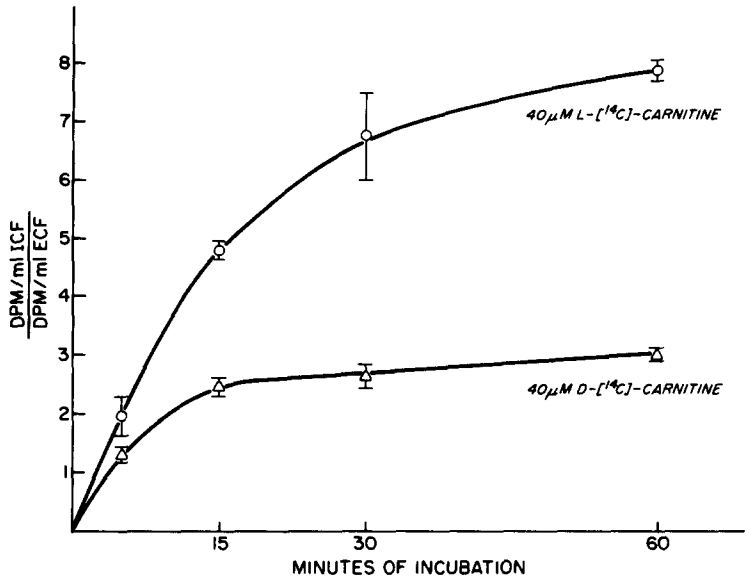

Fig. 1. Time course of L- and D-carnitine uptake by rat kidney cortex slices. Slices were incubated in 2-ml Krebs-Ringer bicarbonate buffer, pH 7.4, at 37°C, 5.5 mM glucose and 40 μM L-[*methyl*-^{14}C]-(○———○) or D-[*methyl*-^{14}C]carnitine, (△———△) for the time periods indicated. Calculation of results is as described in Materials and Methods. Extracellular fluid (ECF) was equated with the final incubation medium radioactivity. No correction was made for carnitine esterification. Each point is the mean ± S.E. (n = 4—5). ICF, intracellular fluid.

quent experiments, a 10 min preincubation step was included to allow this efflux to occur prior to actual incubation in labeled substrate.

The effect of incubation time on the uptake of L- *and* D-*carnitine.* Table I provides data showing carnitine accumulation against a concentration gradient in kidney cortex slices after a 30 min incubation in Krebs-Ringer bicarbonate buffer, pH 7.4, at 37°C containing 40 μM L- or D-[*methyl*-^{14}C]carnitine. The data show that twice as much L-carnitine (DR = 6.6 ± 0.7) was accumulated in 30 min as compared to D-carnitine (DR = 3.4 ± 0.4).

The time course of L-[*methyl*-^{14}C]carnitine uptake is shown in Fig. 1. A rapid period of uptake was observed during the first 15 min of incubation (DR = 5.2 ± 0.5), followed by a gradual period of uptake approaching steady state after 30 min. D-[*methyl*-^{14}C]carnitine was also transported, with a maximum distribution ratio of 3.5 ± 0.5 after 60 min. Slices accumulated L-carnitine twice as fast as D-carnitine. The results of experiments in Fig. 1 are based on the total radioactivity accumulated by the slices and are not corrected for acylcarnitine formation. Table II shows that after a 30 min incubation, the net uptake of L-carnitine, as determined enzymatically (181 ± 3 nmol/ml intracellular fluid), agrees fairly well with the total acid-soluble radioactivity (235 ± 22 nmol/ml intracellular fluid). The excess acid-soluble carnitines in the labeled experiments probably represent acylcarnitine formation. These results provide evidence that carnitine transport in kidney slices results in a net increase in tissue carnitine.

Metabolic conversion of accumulated L-*[methyl*-^{14}*C]carnitine.* The normal in vivo levels of long-chain fatty acylcarnitines (acid-insoluble) and short-chain

TABLE II

NET UPTAKE OF L-CARNITINE BY RAT KIDNEY CORTEX SLICES

Kidney cortex slices were preincubated for 10 min and incubated for 30 min as described in Materials and Methods in 40 μM labeled or unlabeled L-carnitine. At the end of the experiments, slices were extracted with 6% perchloric acid and assayed for L-carnitine content enzymatically [34] and for radioactive carnitine by liquid scintillation spectrometry. Results are mean ± S.E; n = 3—4 experiments.

	Chemically measured	Radioactivity measurement
(1) Total free-carnitine at T = 0	145 ± 6	—
(2) Free carnitine at T = 30 min	326 ± 5	—
(3) Net change in free-carnitine pool	181	—
(4) Total acid-soluble [*methyl*-^{14}C]carnitine at T = 30 min	—	235 ± 22
(5) Acid-soluble acyl[*methyl*-^{14}C]carnitine	—	54 *

* (4) minus (3).

acylcarnitines (acid-soluble) represent approx. 4 and 25%, respectively, of the total intracellular carnitine pool [32,33]. The results, shown in Fig. 1, were not corrected for acylcarnitine formation. We determined, therefore, what portion of the accumulated radioactive carnitine was in the form of long-chain and short-chain acylcarnitines. Table I shows that after a 30 min incubation, 96% of the starting L-[*methyl*-^{14}C]carnitine was recovered from the slices and final incubation medium, in the acid-soluble form, indicating about 4% remaining in the acid-insoluble form. In a previous study, we determined directly by butanol extraction the amount of acid-insoluble acylcarnitines that was formed by kidney slices incubated in 40 μM DL-[*methyl*-^{14}C]carnitine for a total of 60 min [22]. The results showed that 2.4% of the total carnitine accumulated by the kidney slices was recovered in the butanol extraction and agrees well with the normal in vivo level of long-chain acylcarnitines from rat kidney [32].

The amount of accumulated L-[*methyl*-^{14}C]carnitine, converted to acid-soluble acylcarnitines in kidney slices, was determined by subjecting to chromatography acid-soluble extracts of kidney slices incubated for 60 min. Fig. 2A shows that L-[*methyl*-^{14}C]carnitine taken up by kidney slices was moderately

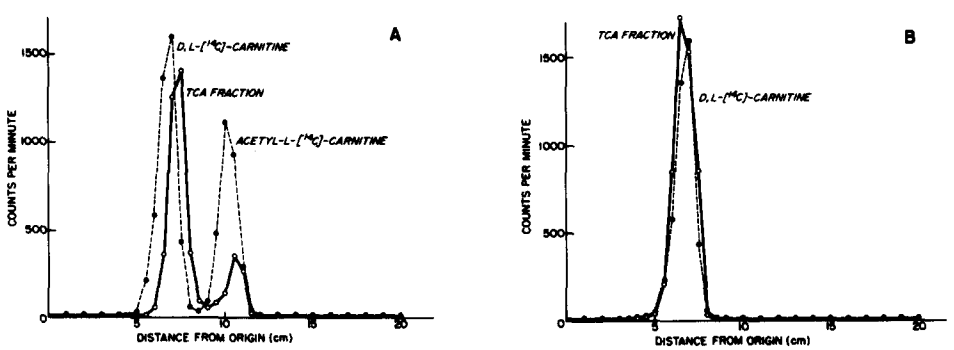

Fig. 2. Thin-layer chromatographic analysis of authentic radioactive compounds (●- - - - -●), and trichloroacetic acid (TCA) extracts from rat kidney cortex slices incubated in 40 μM L-[*methyl*-^{14}C]- (A) or D-[*methyl*-^{14}C]carnitine (B) (○———○). Chromatographic procedures as described in Materials and Methods.

metabolized to acid-soluble acylcarnitines. A peak accounting for 80% of the total applied radioactivity was observed ($R_f = 0.39$) that corresponded to authentic free L-[*methyl*-^{14}C]carnitine ($R_f = 0.38$), while the remaining 20% migrated as a single peak ($R_f = 0.56$) and corresponded to authentic [^{14}C]-acetylcarnitine. Fig. 2B shows that D-[*methyl*-^{14}C]carnitine taken up by kidney slices migrates as a single peak ($R_f = 0.36$). No other peak was observed in this preparation. These results indicate that D- and L-[*methyl*-^{14}C]carnitine accumulated by kidney cortex slices exist primarily in the free form and provide additional evidence that carnitine transport by kidney occurs against a concentration gradient of free carnitine.

The effect of initial substrate concentration. Mediated transport processes are saturable systems and obey Michaelis-Menten kinetics. Fig. 3A and B,

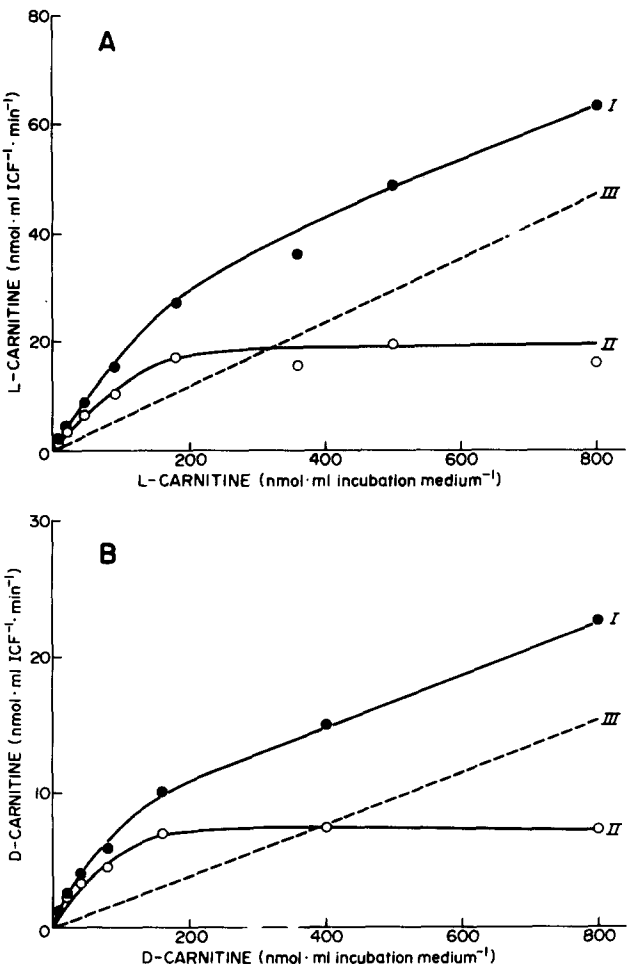

Fig. 3. The concentration curves for (A) L-carnitine and (B) D-carnitine transport into rat kidney cortex slices. The slices were incubated as described in Fig. 1 for 10 min at 37°C with increasing concentrations of D-[*methyl*-^{14}C]- or L-[*methyl*-^{14}C]carnitine. (●———●) Experimental data; (○———○) the theoretical curve II determined by the graphical method of Winter and Christensen [42] representing the low affinity carrier. (- - - - - -) The theoretical curve III obtained by subtraction of the values of curve II from the experimental data. n = 5—8. ICF, intracellular fluid.

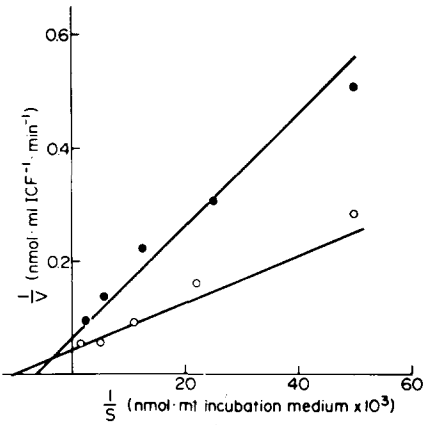

Fig. 4. Lineweaver-Burk plots of the data from concentration curves II for L- and D-carnitine (Fig. 3). The kinetic constants were as follows: L-carnitine (○———○), K_m = 90 μM, V = nmol/min per ml intracellular fluid; D-carnitine (●———●), K_m = 166 μM, V = 15 nmol/min per ml intracellular fluid. ICF, intracellular fluid.

curve I, shows that kidney slices incubated for 10 min with increasing concentrations of D- and L-[*methyl*-^{14}C]carnitine behaved as a saturable process. The concentration curves appear to be the result of two components of uptake: one with a high affinity for the substrate (Fig. 3, curve II), and the other a lower affinity site that may be a mixture of uptake by a nonspecific system and diffusion (curve III). A Lineweaver-Burk plot (Fig. 4) of data from Fig. 3, curve II, yielded an estimated K_m and V of 90 μM and 22 nmol/min per ml intracellular fluir for L-carnitine and 166 μM and 14 nmol/min per ml intracellular fluid for D-carnitine. Thus, the carrier had a markedly higher affinity for L-carnitine, suggesting stereospecificity of carnitine transport.

Effect of structural analogues on L- and D-carnitine uptake. The structural

TABLE III

EFFECT OF STRUCTURALLY RELATED COMPOUNDS ON CARNITINE UPTAKE IN RAT KIDNEY CORTEX SLICES

Kidney slices were incubated as described in the text with 40 μM D- or L-[^{14}C]carnitine at 37°C for 15 and 10 min, respectively. Structural analogue concentrations were 400 μM. Numbers in parentheses indicate number of rats. Results are expressed as per cent of control; mean ± S.E.

Compounds	Carnitine uptake (per cent of control)	
	L-Carnitine	D-Carnitine
None	100	100
L-Carnitine	—	63 ± 12 (3) *
D-Carnitine	64 ± 7 (6) *	—
γ-Butyrobetaine	36 ± 4 (5) **	59 ± 2 (4) **
Acetyl-L-carnitine	59 ± 9 (5) *	67 ± 8 (3) *
Lysine	75 ± 14 (5)	114 ± 8 (2)
Choline	93 ± 17 (3)	96 ± 9 (2)
Betaine	87 ± 19 (3)	—
γ-Aminobutyrate	80 ± 6 (3)	107 ± 17 (2)

* $P < 0.05$.
** $P < 0.01$.

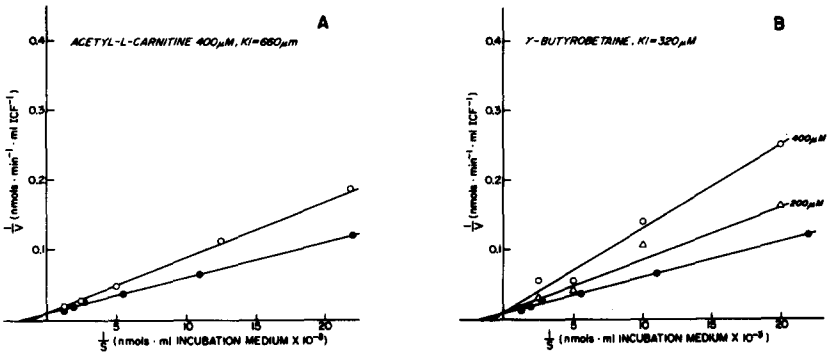

Fig. 5. Lineweaver-Burk plots of L-carnitine uptake by rat kidney cortex slices in the absence of inhibitors (●———●); and in the presence of fixed levels (○———○, △———△) of (A) acetyl-L-carnitine or (B) γ-butyrobetaine. n = 4—5. ICF, intracellular fluid.

analogue experiments reported herine involved incubating kidney cortex slices in 40 μM D- or L-[*methyl*-^{14}C]carnitine plus analogues at 400 μM. Table III shows that carnitine uptake was most effectively inhibited by compounds closely related to the structure of carnitine. D-Carnitine was shown to reduce effectively L-carnitine uptake (64 ± 7% of control), further suggesting that this isomer interacts with the carrier. The uptake of D-carnitine was also reduced by L-carnitine (63 ± 12% of control), although not to the degree expected. γ-Butyrobetaine, the non-hydroxylated precursor of carnitine, was the most effective inhibitor of L-carnitine uptake (36 ± 4% of control). Acetylcarnitine also effectively reduced uptake (59 ± 9% of control). Further studies revealed that γ-butyrobetaine and acetylcarnitine behaved as competitive inhibitors of L-carnitine uptake (Fig. 5A and B). The data suggest that γ-butyrobetaine (K_i = 320 μM *) and L-carnitine (K_m = 330 μM *) have a mututal affinity for the carrier, while acetylcarnitine (K_i = 660 μM *) is approximately one half as efficient.

Compounds that do not contain a four-carbon backbone had the least effect on L-carnitine uptake. Choline, which contains the N-terminal quaternary ammonium and hydroxyl groups of carnitine, did not reduce L-carnitine uptake. Likewise, betaine, the carboxylic acid analogue of choline, was equally ineffective. γ-Aminobutyric acid, which lacks the methylated N-terminal and hydroxyl groups of carnitine, did not significantly reduce uptake. These results suggest that compounds with a four-carbon skeleton containing a 4-*N*-methyl (probably quaternary) and a carboxyl group are necessary for optimal binding to the carnitine carrier.

Lysine caused a moderate but insignificant ($P > 0.05$) inhibition of uptake. Thus, it is unlikely that carnitine uptake is related to this amino acid transport system.

The effect of metabolic inhibitors. In previous studies, an N_2 atmosphere and carbonyl cyanide *m*-chlorophenylhydrazone reduced the uptake of DL-[*methyl*-

* Uncorrected for low-affinity carrier.

TABLE IV

EFFECT OF METABOLIC INHIBITORS ON CARNITINE UPTAKE IN RAT KIDNEY CORTEX SLICES

Kidney slices were preincubated as described in the text for 10 min at 37°C in the presence of the inhibitor, then transferred to an identical medium containing the inhibitor and 40 μM D- or L-[*methyl*-^{14}C]-carnitine for 30 min. Numbers in parentheses indicate numbers of rats. Results are expressed as mean ± S.E.

	% inhibition of carnitine uptake	
	L-Carnitine	D-Carnitine
Control	0	0
Anoxia (95% N_2 : 5% CO_2)	81 ± 2 (2)	73 ± 1 (2)
Carbonyl cyanide *m*-chlorophenylhydrazone (4 μM)	68 ± 3 (3)	62 ± 5 (3)
2,4-Dinitrophenol (1 mM)	82 ± 3 (3)	70 ± 5 (3)
KCN (1 mM)	20 ± 10 (3)	37 ± 10 (3)
n-Ethylmaleimide (1 mM)	87 ± 2 (3)	80 ± 4 (3)
4°C	93 ± 1 (3)	96 ± 2 (3)
Ouabain (0.5 mM)	40 ± 7 (3)	30 ± 9 (3)

^{3}H]carnitine [22]. These studies, therefore, have been repeated and expanded for L- and D-carnitine.

Table IV shows that when kidney slices were incubated in an N_2 atmosphere, L-[*methyl*-^{14}C]carnitine uptake was inhibited 81 ± 2%. Similarly, carbonyl cyanide *m*-chlorophenylhydrazone and 2,4-dinitrophenol caused marked reductions in uptake (82 ± 3 and 68 ± 3%, respectively). In contrast, KCN only moderately inhibited this system (20 ± 10%). L-Carnitine transport was also temperature sensitive as uptake was reduced 93 ± 1% at 4°C. When kidney slices were incubated in the presence of *N*-ethylmaleimide, uptake was reduced 87 ± 2%, supporting previous claims that free sulfhydryl groups may be important for carnitine transport [21]. Ouabain also significantly reduced L-carnitine uptake (40 ± 7%, $P < 0.05$), suggesting that the carrier may be an Na^+-linked uptake process involving the plasma membrane ($Na^+ + K^+$)-ATPase.

Table IV shows that, in general, inhibition of D-carnitine uptake by the afore-mentioned compounds closely paralled those observed with L-carnitine.

Effect of ionic composition of the medium. The results shown above suggest that carnitine transport is dependent on metabolic energy and may be associated with the active movement of Na^+ and K^+ across the membrane. We have, therefore, examined the effects of modifying the ionic composition of the medium on the uptake of L- and D-carnitine.

The effects of the cations of the Krebs-Ringer bicarbonate buffer were studied by incubating brain slices in buffers in which various ions were completely replaced by suitable iso-osmolar substitutes (Table V). In these experiments, both the preincubation and incubation steps were carried out in the modified buffers.

When Na^+ was replaced in the medium with Li^+, a 75% and 60% reduction in L- (DR = 1.65 ± 0.1) and D-carnitine (DR = 1.3 ± 0.1) uptake were observed. Under these conditions, the DR values were only slightly above 1.0, indicating that minimal active transport occurs in the absence of Na^+. This is consistent

TABLE V

EFFECT OF MEDIUM IONIC COMPOSITION ON CARNITINE UPTAKE BY RAT KIDNEY CORTEX SLICES

Kidney slices were incubated as described in the text at 37°C for 30 min in 40 μM D- or L-[^{14}C]carnitine. Composition of control Krebs-Ringer bicarbonate buffer (pH 7.4) was 118 nM NaCl, 4.8 mM KCl, 1.1 mM KH_2PO_4, 2.5 mM $CaCl_2$, 1.2 mM $MgSO_4$ and 25 mM $NaHCO_3$. Numbers in parentheses indicate number of rats. Results are expressed as mean ± S.E. ICF, intracellular fluid; ECF, extracellular fluid.

Salt omitted	L-Carnitine			D-Carnitine	
	Iso-osmolar substitute	ICF/ECF	% of control	ICF/ECF	% of control
Control	None	6.50 ± 0.25 (3)	100	3.29 ± 0.18 (3)	100
NaCl, $NaHCO_3$	LiCl, $KHCO_3$	1.65 ± 0.1 (3) *	25	1.30 ± 0.1 (3) *	40
KCl, KH_2PO_4	LiCl, NaH_2PO_4	4.76 ± 0.15 (4) **	73	2.67 ± 0.1 (3) **	81
$CaCl_2$	LiCl	4.59 ± 0.31 (4) **	71	2.31 ± 0.03 (3) **	70

* $P < 0.001$.
** $P < 0.05$.

with previous observations of an Na^+ dependency for L-carnitine uptake in skeletal muscle [21].

When K^+ was replaced in the medium, a moderate but significant ($P < 0.05$) reduction in L- and D-carnitine uptake was observed (27 and 19%, respectively). These results and the effects observed with ouabain suggest that carnitine transport is coupled directly to the movement of Na^+ and K^+ via the ($Na^+ + K^+$)-ATPase.

Replacement of Ca^{2+} also caused a marked decrease in carnitine uptake (30%, $P < 0.05$). The role of Ca^{2+} in this system is less clear, although Ca^{2+} has been reported to increase the transport of amino acids by kidney [34].

Discussion

In the present study, we have provided evidence that L-carnitine uptake in rat kidney cortex slices occurs against a concentration gradient of free carnitine by a carrier-mediated active transport process associated with the plasma membrane. D-Carnitine was also transported against a concentration gradient by this system. The system showed saturability and was stereospecific in favor of L-carnitine. Kinetic results suggest the presence of a high and lower affinity carrier. The K_m for the high-affinity carrier for L-carnitine was 90 μM and $V = 22$ nmol/min per ml intracellular fluid, whereas for the system uncorrected for the high-affinity carrier, the K_m was 333 μM and $V = 76$ nmol/min per ml intracellular fluid. Assuming a normal blood level of L-carnitine (40 nmol/ml), a glomerular filtration rate of 864 ml/day per 100 g body wt. [35] and a plasma clearance of L-carnitine of 25.5 ml/day [16], the calculated reabsorption rate of L-carnitine would be approx. 23.3 nmol/min per g wet kidney wt. This is compared to 4.0 nmol/min per g kidney wt. taken up by rat kidney cortex slices in vitro, as calculated from the kinetic constants in the present study. These values agree fairly well in spite of the obvious differences between the two preparations [36].

An active transport system for L-carnitine has been reported in isolated liver

cells with a K_m of 5.6 mM and V = 2.4 nmol/min per mg protein [19]. At 40 μM L-carnitine, the calculated rate of uptake (0.34 nmol/min per g wet wt.) is approx. 12-times as fast as in kidney slices. In skeletal muscle [21] and heart cells, carnitine uptake [20] was found to be mediated by a higher affinity system (K_m = 50 and 4.8 μM, respectively), but functions at a slower rate (V = 22 nmol/h per g tissue and 8.7 pmol/h per μg DNA, respectively). Thus, at 40 μM L-carnitine, the calculated rates of uptake (0.14 and 0.015 nmol/min per g tissue, respectively) would proceed at a rate 28- and 266-times as fast as in kidney slices. Brooks and McIntosh [12] have observed a similar trend in vivo for the estimated flux of L-carnitine between blood and individual tissues of the rat. They found the L-carnitine flux in the kidney to be 12-, 33- and 102-times faster than liver, heart and skeletal muscle, respectively. Thus, a rapid uptake system for carnitine exists in the kidney, suggesting the presence of a renal tubule reabsorption system.

The process responsible for the accumulation of L-carnitine in kidney cortex slices had many characteristics of an active transport system. Uptake was markedly inhibited by a variety of metabolic inhibitors and uncouplers, N_2 atmosphere and low temperature (4°C) (Table IV). These results provide evidence indicating a requirement for metabolic energy by this transport system. In keeping with carnitine uptake studies in skeletal muscle and heart cells in vitro [21,37], we observed only moderate inhibition of uptake with 1.0 mM KCN.

The results showing inhibition of uptake by ouabain, an absolute requirement for Na^+ and a need for K^+ for optimal uptake, suggest that carnitine transport in the kidney is driven by the Na^+ gradient and may involve Na^+-carnitine cotransport by a system, of which the energy requirement is derived from the ionic gradient established by the plasma membrane ($Na^+ + K^+$)-ATPase [38,39]. A similar system has been proposed for renal transport of glucose and amino acids [40]. The presence of Ca^{2+} was necessary for optimal carnitine uptake, although the mechanism of action is not clear. However, an ouabain-insensitive Ca^{2+}-stimulated ATPase has been described in rat kidney cortex for the transtubular transport of Ca^{2+} [41].

Analogue-inhibition experiments suggested that carnitine carrier in rat kidney cortex transports compounds containing a four-carbon chain with a terminal quaternary ammonium group and a terminal carboxyl group. These results are similar to those found in isolated heart cells [37]. In addition, results in this study showing competitive inhibition of carnitine uptake by γ-butyrobetaine and acetylcarnitine have also been observed in liver cells [19], skeletal muscle [21] and heart [20]. Thus, the carnitine carrier in kidney cortex and the afore-mentioned tissues exhibit very similar structural specificity. Previous in vivo studies suggest that the kidney also transports acetylcarnitine and γ-butyrobetaine [16,17,19].

In the present study, these compounds were shown to have an affinity for the carrier similar to that of carinitine. Thus, it is considered likely that acetylcarnitine and γ-butyrobetaine are transported in kidney cortex, however, more direct evidence is needed on this subject. Although direct electrochemical gradient measurements were not made, these studies are consistent with the hypothesis that carnitine is transported against an electrochemical gradient.

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