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Carnitine Transport and Its Inhibition by Sulfonylureas in Human Kidney Proximal Tubular Epithelial Cells

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ABSTRACT. The kidney plays an important role in the homeostasis of carnitine by its ability to reabsorb carnitine almost completely from the glomerular filtrate. The transport process responsible for this reabsorption has been investigated thus far only in laboratory animals. Here we report on the characteristics of carnitine uptake in a proximal tubular epithelial cell line derived from human kidney. The uptake process was found to be obligatorily dependent on Na^+ with no involvement of anions. The process was saturable, with a Michaelis–Menten constant of 14 ± 1 μM. The Na^+:carnitine stoichiometry was 1:1. The same process also was found to be responsible for the uptake of acetylcarnitine and propionylcarnitine, two acyl esters of carnitine with potential for therapeutic use in humans. The uptake process was specific for carnitine and its acyl esters. Betaine, a structural analog of carnitine, interacted with the uptake process to a significant extent. The present studies also showed that sulfonylureas, oral hypoglycemic agents currently used in the management of type 2 diabetes, inhibited the carnitine uptake system. Among the sulfonylureas tested, glibenclamide was the most potent inhibitor. The inhibition was competitive. Glibenclamide inhibited the uptake not only of carnitine but also of acetylcarnitine and propionylcarnitine. The inhibition most likely was the result of direct interaction of the compound with the carnitine transporter because the inhibition could be demonstrated in purified rat kidney brush border membrane vesicles. BIOCHEM PHARMACOL **58**;8:1361–1370, 1999. © 1999 Elsevier Science Inc.

KEY WORDS. humans; proximal tubular cell; renal absorption; carnitine uptake; carnitine acyl esters; sulfonylureas

Carnitine is an obligate requirement for fatty acid oxidation. It is synthesized endogenously in humans in the liver as well as in the kidney [1–3]. This biosynthetic process, coupled with dietary intake, is responsible for maintenance of body stores of carnitine. Kidneys absorb >95% of carnitine from the glomerular filtrate via an active transport mechanism [4, 5]. Studies with isolated brush border membrane vesicles from the kidney have shown that a Na^+-dependent transport system is responsible for this active reabsorptive process across this membrane [6, 7]. The renal reabsorptive mechanism for conservation of carnitine is impaired in patients with primary carnitine deficiency [8] and in the juvenile visceral steatosis (*jvs*) mouse with systemic carnitine deficiency [9]. This impairment leads to increased elimination of carnitine in urine, resulting in carnitine deficiency. The clinical symptoms of carnitine deficiency include myopathy, cardiomyopathy, encephalopathy and failure to thrive [8, 10, 11]. These findings underscore the importance of the carnitine transport system in the kidney for the maintenance of carnitine status in humans.

To date, the renal carnitine transport system has been investigated in detail only with isolated brush border membrane vesicles [6, 7]. There are no reports available in the literature on carnitine transport in intact renal proximal tubular cells. An intact cell system may prove to be advantageous in characterizing certain specific aspects of renal absorption of carnitine, especially in studying the potential regulatory mechanisms involved in the modulation of the absorptive process. Rebouche *et al.* [5, 6] have provided strong evidence for renal adaptation of carnitine reabsorption in response to changes in carnitine levels in the circulation. Availability of an intact cell system to study renal carnitine transport will facilitate investigations of the regulation of the renal reabsorptive process for carnitine at the molecular level. Recently, several human kidney proximal tubular cell lines have been developed, and these cell lines maintain the morphological and functional characteristics of the normal renal proximal tubular epithelium [12]. In the present study, we have used one of these cell lines to characterize the transport of carnitine and its acyl deriva-

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tives and also to investigate the interaction of sulfonylureas with the transport process.

MATERIALS AND METHODS

Materials

L-[^{3}H]Carnitine (specific radioactivity, 65 Ci/mmol), [^{3}H]acetyl-L-carnitine (specific radioactivity, 65 Ci/mmol), and [^{3}H]propionyl-L-carnitine (specific radioactivity, 65 Ci/mmol) were purchased from Moravek Biochemicals, Inc. L-[^{3}H]Alanine (specific radioactivity, 62 Ci/mmol) and [^{3}H]taurine (specific radioactivity, 25.6 Ci/mmol) were purchased from DuPont-NEN. Unlabeled carnitine, carnitine esters, amino acids, and sulfonylureas were obtained from the Sigma Chemical Co. The serum-free keratinocyte medium used for the culture of the HPCT* cells was purchased from Life Technologies. Epidermal growth factor and bovine pituitary extract also were obtained from Life Technologies. Fetal bovine serum was purchased from Atlanta Biologicals.

Culture of HPCT Cells

The HPCT cell line used in the present study was developed originally by immortalization of early proximal tubule primary cultures from humans [13]. This cell line forms confluent, electrically resistive monolayers, which is one of the important epithelial cell characteristics. Cells were maintained in a humidified atmosphere of 95% air/5% CO_2 at 37°. Cells were cultured in keratinocyte medium, supplemented with epidermal growth factor (0.2 ng/mL), bovine pituitary extract (30 μg/mL), fetal bovine serum (10%), penicillin (100 units/mL), and streptomycin (100 μg/mL). Trypsin-released cells were seeded in 35-mm Petri dishes at a density of 1.5×10^6 cells/dish and allowed to grow as monolayers. Cells reached confluence on day 3, and transport measurements were made the following day.

Transport Measurements in Cells

All steps involved in transport measurements were carried out at room temperature. The medium was aspirated, and the cells were washed once with the transport buffer. One milliliter of transport buffer containing radiolabeled substrate (L-carnitine, acetyl-L-carnitine, propionyl-L-carnitine, or amino acids) was added to the cells and incubated for the desired time. Transport was terminated by aspirating the buffer and subsequently washing the cells three times with fresh uptake buffer. The cells were lysed with 1 mL of 0.2 N NaOH-1% SDS, and the lysate was transferred to scintillation vials for quantitation of radioactivity. The composition of the transport buffer in most experiments was 25 mM HEPES/Tris, pH 7.5, 140 mM NaCl, 5.4 mM KCl, 1.8 mM $CaCl_2$, 0.8 mM $MgSO_4$, and 5 mM glucose.

* *Abbreviations*: HPCT, human proximal convoluted tubular; OCT, organic cation transporter; and OCTN, novel organic cation transporter.

In experiments dealing with the anion dependence of carnitine transport, the composition of the transport buffer was modified by substituting potassium gluconate for KCl, calcium gluconate for $CaCl_2$, and sodium salts containing the desired anion for NaCl. In experiments dealing with the influence of Na^+ on carnitine transport, the buffers containing 140 mM NaCl or 140 mM *N*-methyl D-glucamine chloride were mixed to give uptake buffers of desired Na^+ composition.

Transport Measurements in Rat Renal Brush Border Membrane Vesicles

Brush border membrane vesicles from rat renal cortical tissue were prepared as described previously [14, 15] by using the Mg^{2+}-aggregation method. The membrane vesicles were preloaded with 10 mM HEPES/Tris buffer (pH 7.5), containing 75 mM potassium gluconate and 150 mM mannitol. Transport was initiated by mixing 40 μL of membrane vesicles with 160 μL of transport buffer containing L-[^{3}H]carnitine [16]. The composition of the transport buffer was 10 mM HEPES-Tris (pH 7.5), containing 150 mM NaCl or 150 mM KCl. The mixture was incubated at room temperature for the desired time. At the end of the incubation, transport was terminated by the addition of 3 mL of ice-cold 5 mM HEPES-Tris buffer (pH 7.5) containing 160 mM KCl, followed by filtration under vacuum on a Millipore filter (DAWP type, 0.65 μm pore size). By this procedure, the brush-border membrane vesicles were retained on the filter. The filter was washed four times with 5 mL of the same KCl buffer, following which the radioactivity associated with the filter was determined by liquid scintillation spectrometry.

Data Analysis

Experiments were done routinely in duplicate or triplicate, and each experiment was repeated two to three times. The results are given as means ± SEM (N = 6–9) Kinetic analyses were done using a commercially available computer program, Fig. P., version 6.0 (BioSoft). Kinetic parameters were calculated using nonlinear regression and confirmed by linear regression methods.

RESULTS

Characteristics of Carnitine Uptake in HPCT Cells

HPCT cells grow as a monolayer. Under the experimental conditions employed in the present study, cells reached confluence on day 3 following the seeding of the cells. We first investigated the influence of culture time on carnitine uptake. The uptake of carnitine was studied in HCPT cells in the presence of NaCl or choline chloride in the uptake medium on different days following confluence (3–9 days). On day 3 (i.e. the day of confluence), the uptake of carnitine (20 nM) in the presence of NaCl was 216 ± 10 fmol/mg protein/30 min). The uptake was markedly lower

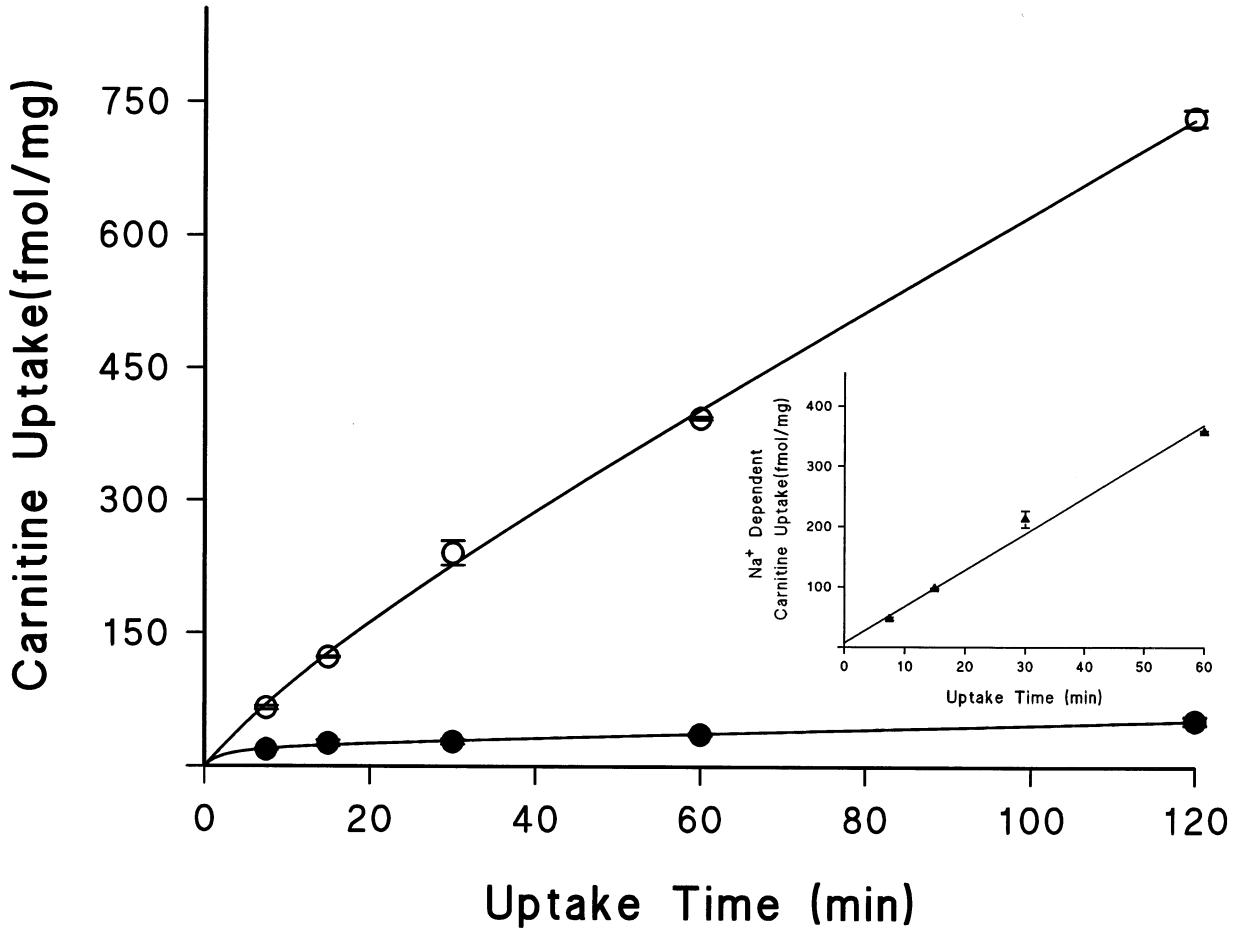

FIG. 1. Time course of carnitine uptake in HPCT cells in the presence of NaCl (○) or choline chloride (●) in the uptake medium. The concentration of carnitine was 20 nM. Inset: Time course of the Na^+-dependent component of carnitine uptake. This component was determined by subtracting the uptake measured in choline chloride-containing medium from the uptake measured in NaCl-containing medium at each time point. Values are means ± SEM (N = 6).

when NaCl in the uptake medium was replaced by choline chloride (26 ± 1 fmol/mg protein/30 min). When studied on different culture days following confluence, there was no significant difference in the uptake of carnitine in NaCl-containing medium or in choline chloride-containing medium compared with corresponding uptake values obtained on the day of confluence. Thus, the uptake of carnitine remained steady at least up to 6 days following confluence. All subsequent experiments, therefore, were done on the fourth day following cell seeding (i.e. the day after confluence).

Figure 1 describes the time course of carnitine uptake in the presence of NaCl or choline chloride in the uptake medium. The uptake measured in the presence of NaCl was several-fold higher than in the presence of choline chloride. With an uptake time of 30 min, the presence of Na^+ stimulated the uptake 8.6-fold. This stimulation was even greater (14.1-fold) when the uptake time was 120 min. The Na^+-dependent uptake (carnitine concentration, 20 nM) was linear with uptake time at least up to 60 min (Fig. 1, inset). The time course of the Na^+-dependent uptake was also studied at 10 μM carnitine. With three time periods examined (10, 20, and 30 min), the uptake was linear up to 30 min (4.13 ± 1.4 pmol/mg protein at 10 min; 9.65 ± 0.14 pmol/mg protein at 20 min; 12.91 ± 1.0 pmol/mg protein at 30 min). All subsequent uptake measurements were made with an incubation time of 30 min to obtain initial uptake rates.

Table 1 describes the dependence of carnitine uptake on

TABLE 1. Influence of monovalent cations and anions on carnitine transport in HPCT cells*

Salt	Carnitine transport (fmol/mg protein/30 min)	(%)
NaCl	281.7 ± 2.7	100
Na gluconate	319.6 ± 3.3	113
NaF	329.6 ± 3.3	117
NaI	206.6 ± 3.0	73
NaSCN	179.2 ± 0.2	64
LiCl	141.3 ± 1.1	50
KCl	54.7 ± 1.1	19
Choline Cl	37.5 ± 1.5	13

*Transport of carnitine (20 nM) in monolayer cultures of HPCT cells was measured with a 30-min incubation in the presence of a 140 mM concentration of the indicated inorganic salts in the extracellular medium. Values are means ± SEM (N = 6).

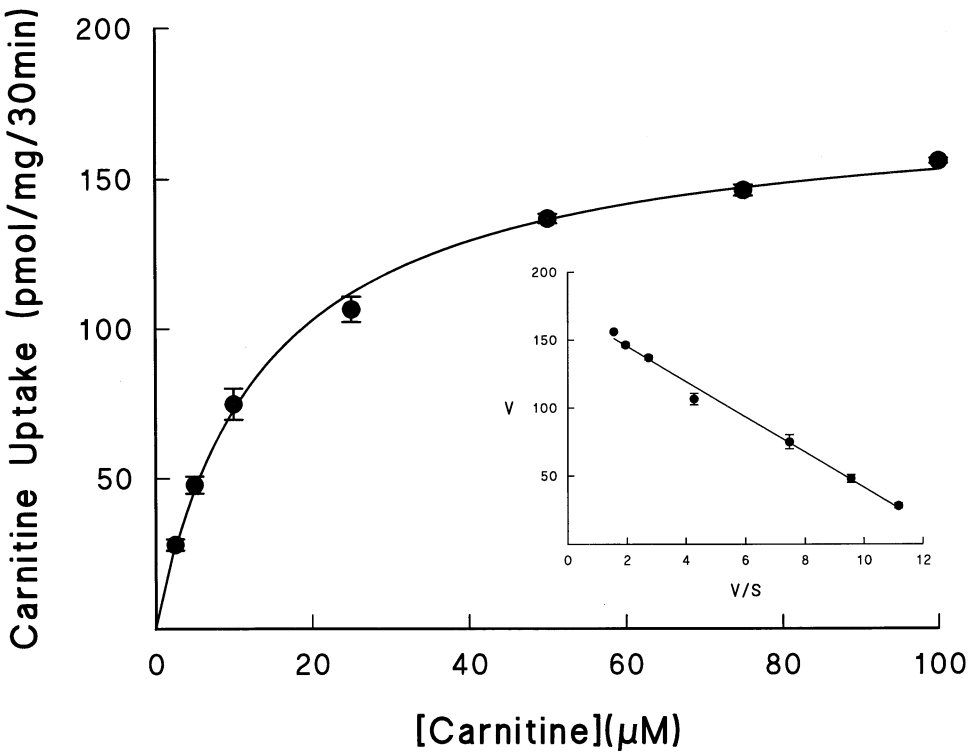

FIG. 2. Saturation kinetics of carnitine uptake in HPCT cells. Uptake of carnitine was measured with a 30-min incubation over a carnitine concentration range of 2.5 to 100 μM. The concentration of [^{3}H]carnitine was kept constant at 20 nM, and unlabeled carnitine was added to obtain the indicated concentrations. Uptake was measured in the presence of NaCl or choline chloride in the uptake medium. Uptake measured in the presence of choline chloride was not saturable, and this value was subtracted from the uptake measured in the presence of NaCl to determine the Na^+-dependent uptake component, which was used in kinetic analysis. Inset: Eadie–Hofstee plot (v/s vs v) where v is the uptake rate in pmol/mg protein/30 min, and s is the carnitine concentration in μM. Values are means ± SEM (N = 9).

different cations and anions in the uptake medium. The uptake values were similar when NaCl in the medium was replaced iso-osmotically with Na gluconate or NaF. Replacement with NaI or NaSCN, however, decreased the uptake to a small extent. Since the uptake remained essentially unaltered in the presence of chloride, gluconate, or fluoride as long as Na^+ was present, the results led to the conclusion that carnitine uptake in HPCT cells was not dependent on anions. The reasons for the decrease in uptake when chloride was replaced by iodide or thiocyanate are not known. The specificity of Na^+ involvement in the uptake process was investigated by measuring the uptake from the medium in which NaCl was iso-osmotically replaced by LiCl, KCl, or choline chloride. The uptake was

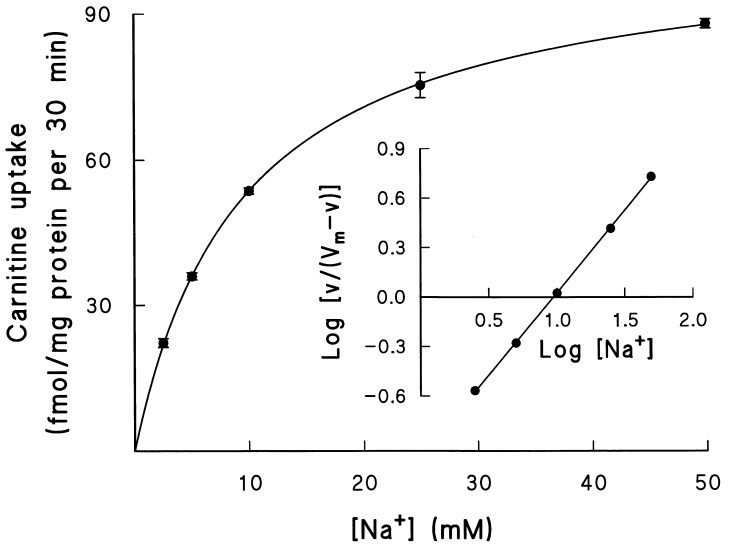

FIG. 3. Kinetics of activation of carnitine uptake by Na^+ in HPCT cells. Uptake was measured with a 30-min incubation. The carnitine concentration was 20 nM. The concentration of Na^+ was varied over a range of 2.5 to 50 mM. This was done by mixing appropriately two uptake buffers, one containing 140 mM NaCl and the other containing 140 mM *N*-methyl-D-glucamine chloride. Uptake measured in the absence of Na^+ was subtracted to determine the Na^+-dependent component, which was used in kinetic analysis. Inset: Hill plot. v, carnitine uptake rate in fmol/mg protein/30 min; V_m, maximal carnitine uptake rate, which was determined from the hyperbolic relationship of Na^+ concentration versus uptake rate using the Michaelis–Menten equation. Values are means ± SEM (N = 9).

TABLE 2. Substrate specificity of the Na^+-dependent carnitine transport system in HPCT cells*

Substrate analog	[^{3}H]Carnitine transport (fmol/mg protein/30 min)	(%)
None	223.8 ± 6.1	100
L-Carnitine	4.5 ± 1.3	2
D-Carnitine	21.4 ± 1.0	10
Acetyl-L-carnitine	22.4 ± 1.1	10
Propionyl-L-carnitine	12.4 ± 4.7	6
Palmitoyl-L-carnitine	19.9 ± 5.7	9
Stearoyl-L-carnitine	92.8 ± 11.5	48
Betaine	87.1 ± 1.6	39
γ-Aminobutyrate	248.8 ± 4.0	111
Alanine	232.7 ± 5.6	104
Lysine	226.2 ± 12.9	101
Proline	235.6 ± 12.5	105

*Transport of [^{3}H]carnitine (20 nM) in monolayer cultures of HPCT cells was measured with a 30-min incubation in the presence of NaCl. When present, the concentration of unlabeled substrate analogs was 500 μM. Transport measured in the absence of Na^+ (i.e. choline chloride-containing transport buffer) was subtracted to calculate Na^+-dependent [^{3}H]carnitine transport. Values are means ± SEM (N = 6).

the lowest in the presence of choline chloride. KCl also failed to support the uptake. The uptake in the presence of LiCl was much higher than in the presence of KCl or choline chloride, but much lower than in the presence of NaCl. These data led to the conclusion that carnitine uptake in HPCT cells was obligatorily dependent on the presence of Na^+. Li^+ was able to substitute for Na^+ to some extent. The obligatory requirement for Na^+ was evident not only at 20 nM carnitine (Table 1) but also at 10 μM carnitine (data not shown).

Carnitine uptake in the presence of Na^+ was saturable (Fig. 2). When only the Na^+-dependent uptake was considered, the relationship between uptake rate and carnitine concentration was hyperbolic. In these experiments, the uptake measured in the presence of choline chloride was subtracted from the uptake measured in the presence of NaCl to calculate the Na^+-dependent uptake. The Eadie–Hofstee transformation of the data for Na^+-dependent uptake yielded a linear plot (Fig. 2, inset), suggesting involvement of a single saturable uptake process. The Michaelis–Menten constant (K_t) for the process was 14 ± 1 μM. The maximal velocity (V_{max}) was 175 ± 3 pmol/mg protein/30 min.

Figure 3 describes the dependence of carnitine uptake on Na^+ concentration. In these experiments, uptake was measured in the presence of various concentrations of Na^+ (2.5 to 50 mM) while maintaining the concentration of Cl^- constant at 140 mM. Na^+ was iso-osmotically replaced by *N*-methyl-D-glucamine. Choline was not used here to substitute for Na^+ because even though choline does not support carnitine uptake on its own, this cation inhibits carnitine uptake in the presence of Na^+ [17]. It appears that choline competes for the Na^+-binding site on the transporter without supporting the uptake. Alternatively, choline might compete with carnitine for the substrate-binding site with low affinity. *N*-Methyl-D-glucamine does not compete with Na^+ or with carnitine. The uptake of carnitine was found to be saturable with respect to Na^+ concentration. When only the Na^+-dependent uptake was considered, the relationship between uptake rate and Na^+ concentration was hyperbolic. In these experiments, the uptake measured in the absence of Na^+ was subtracted from the uptake measured in the presence of Na^+ to determine the Na^+-dependent uptake. The concentration of Na^+ necessary to produce half-maximal stimulation of uptake was 10 ± 1 mM. The Hill plot (Fig. 3, inset) was linear with a slope of 1, indicating the interaction of one Na^+ with the uptake process. In other words, the Na^+:carnitine stoichiometry was 1:1.

The substrate specificity of the carnitine uptake process was then investigated by assessing the ability of various carnitine derivatives and amino acids (500 μM) to inhibit the Na^+-dependent uptake of 20 nM radiolabeled carnitine

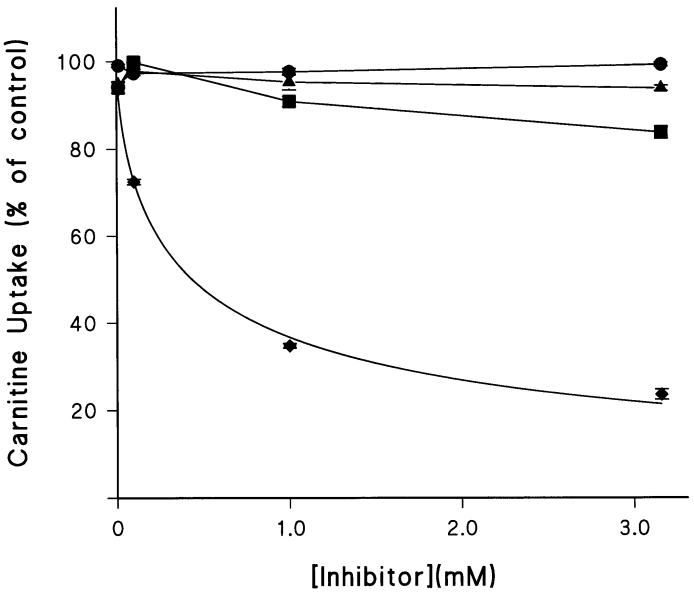

FIG. 4. Inhibition of carnitine uptake by glycine and its *N*-methyl derivatives in HPCT cells. Uptake of carnitine (20 nM) was measured with a 30-min incubation in the presence of NaCl. Glycine and its *N*-methyl derivatives were used over a concentration range of 0.01 to 3.16 mM. Control uptake measured in the absence of inhibitors was taken as 100% (216 ± 10 fmol/mg protein/30 min) Key: (▲) glycine; (●) sarcosine (*N*-methylglycine); (■) *N*-dimethylglycine; and (♦) betaine (*N*-trimethylglycine). Values are means ± SEM (N = 6).

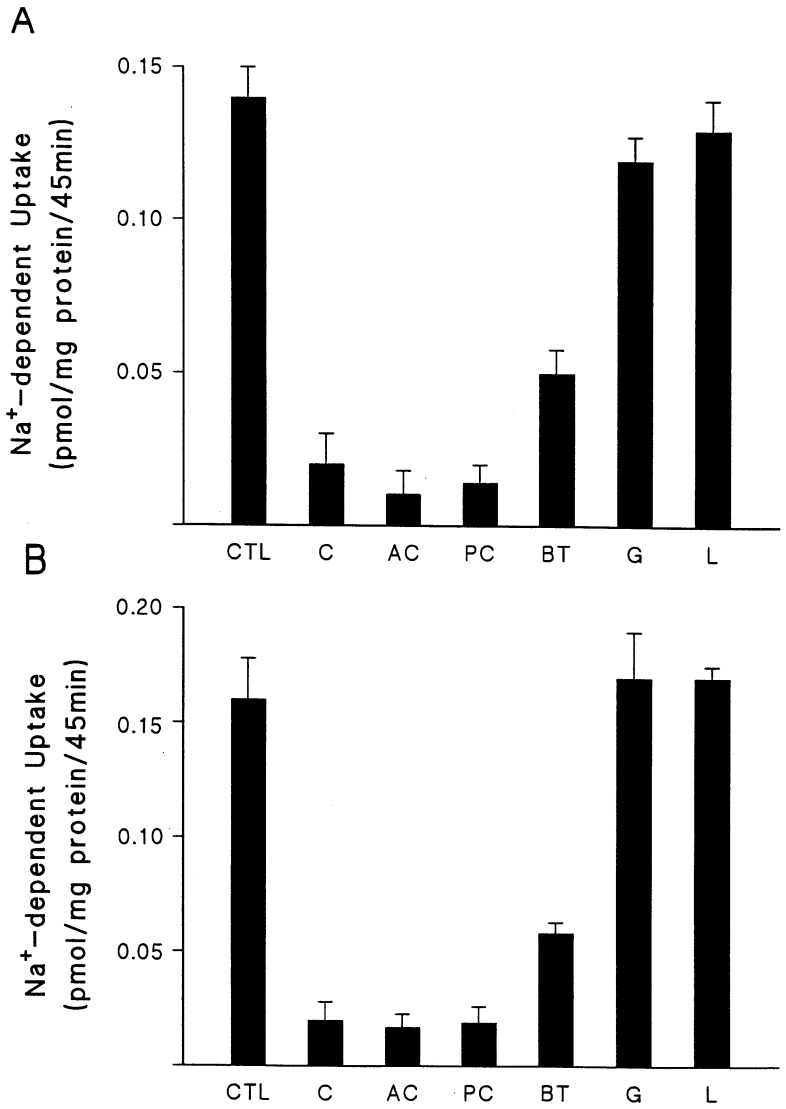

FIG. 5. Uptake of acetyl-L-[^{3}H]carnitine (A) and propionyl-L-[^{3}H]carnitine (B) in HPCT cells. Uptake was measured with a 45-min incubation. The concentration of radiolabeled acetyl-L-carnitine and propionyl-L-carnitine was 50 nM. The uptake measured in the presence of choline chloride was subtracted from the uptake measured in the presence of NaCl to determine the Na^+-dependent uptake. The concentration of unlabeled inhibitors was 500 μM. Inhibitors used were: L-carnitine (C), acetyl-L-carnitine (AC), propionyl-L-carnitine (PC), betaine (BT), γ-aminobutyrate (G), and lysine (L). Values are means ± SEM (N = 6).

(L-isomer) (Table 2). Both L- and D-isomers of carnitine were found to be potent inhibitors. Among the fatty acyl esters of carnitine, acetyl-L-carnitine, propionyl-L-carnitine, and palmitoyl-L carnitine inhibited [^{3}H]carnitine uptake almost completely. Stearoyl-L-carnitine was a moderate inhibitor, causing ~50% inhibition. Betaine, a structural analog of carnitine, also was found to be an inhibitor. In contrast, γ-aminobutyrate, alanine, lysine, and proline were ineffective as inhibitors. Thus, the carnitine uptake process in HPCT cells was specific for carnitine and fatty acyl esters of carnitine.

Since betaine (*N*-trimethylglycine) exhibited moderate inhibitory potency, we studied the ability of glycine and its other methyl derivatives to inhibit [^{3}H]carnitine uptake (Fig. 4). Glycine and sarcosine (*N*-methylglycine) did not inhibit the uptake when studied up to a concentration of 3.16 mM. *N*-Dimethylglycine showed a small inhibition (15% inhibition at 3.16 mM). Betaine was the most potent inhibitor among the methyl derivatives of glycine. The concentration of betaine causing 50% inhibition was 0.44 ± 0.04 mM.

We also studied directly the uptake of acetyl-L-carnitine and propionyl-L-carnitine in HPCT cells using the respective radiolabeled compounds. The uptake of acetyl-L-[^{3}H]carnitine and propionyl-[^{3}H]carnitine was found to be Na^+-dependent. The Na^+-dependent uptake of these radiolabeled acylcarnitine esters was inhibited potently by unlabeled carnitine, acetyl-L-carnitine, and propionyl-L-carnitine as well as betaine (Fig. 5). γ-Aminobutyrate and lysine did not inhibit the uptake of these acylcarnitine esters. Thus, the characteristics of the uptake of acetyl-L-carnitine and propionyl-L-carnitine were similar to those of the uptake of carnitine, demonstrating that these acylcarnitine esters were also taken up into HPCT cells via the carnitine transport system.

Inhibition of Carnitine Uptake System by Oral Hypoglycemic Agents

Sulfonylureas are oral therapeutic agents that increase insulin secretion by pancreatic β cells and reduce blood sugar levels in patients with non-insulin-dependent diabe-

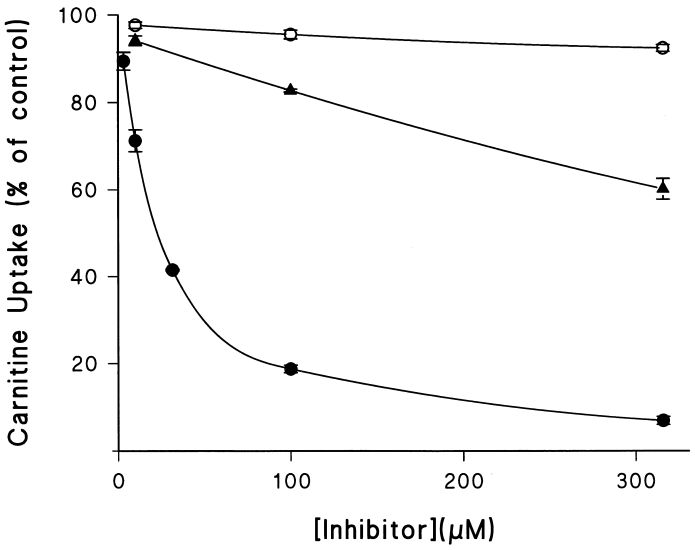

FIG. 6. Inhibition of carnitine uptake by sulfonylureas in HPCT cells. Uptake of carnitine (20 nM) was measured with a 30-min incubation. Data represent only the Na^+-dependent uptake component. Sulfonylureas were used over a concentration range of 3.16 to 316 μM. Control uptake measured in the absence of inhibitors was taken as 100% (212 ± 14 fmol/mg protein/30 min). Key: (○) tolbutamide; (▲) glipizide; and (●) glibenclamide. Values are means ± SEM (N = 6).

tes mellitus (type 2 diabetes) [18]. These compounds produce their therapeutic effects by interacting with a high-affinity sulfonylurea receptor in β cells, which associates with a K_{ATP} channel [19, 20]. A report by Broadway and Saggerson [21] showed that sulfonylureas inhibit carnitine acyl transferases, possibly by interacting with the carnitine-binding sites of these enzymes. Therefore, we investigated whether the carnitine transport system also is influenced by these drugs. The sulfonylureas tested were tolbutamide, glipizide, and glibenclamide. As can be seen in Fig. 6, tolbutamide did not have any noticeable effect on carnitine uptake in HPCT cells even up to 300 μM. Glipizide was found to inhibit carnitine uptake. The inhibition was noticeable at 300 μM (40 ± 3% inhibition) and 100 μM (17 ± 1% inhibition). Glibenclamide was the most potent inhibitor among the three sulfonylureas. Inhibition (29 ± 3%) was observed at 10 μM and increased to 81 ± 1% at 100 μM. The inhibition of carnitine uptake in HPCT cells by glibenclamide was a specific effect because under similar experimental conditions there was no effect by glibenclamide on the uptake activity of two unrelated transport systems, namely Na^+-dependent alanine uptake and Na^+ plus Cl^--dependent taurine uptake (data not shown). Kinetic analysis indicated that the inhibition of carnitine uptake by glibenclamide was competitive (Fig. 7). The presence of 25 μM glibenclamide increased the K_t value for carnitine uptake almost 2-fold, from 12.0 ± 1.0 to 24.7 ± 3.2 μM. The V_{max} was not affected by glibenclamide (161 ± 3 pmol/mg protein/30 min in the absence of glibenclamide and 157 ± 7.0 pmol/mg protein/30 min in the presence of glibenclamide). Glibenclamide also was found to inhibit the uptake of acetyl-L-carnitine and propionyl-L-carnitine (Table 3), as expected because these two acyl esters of carnitine are transported via the carnitine transport system (Fig. 5).

Sulfonylureas are known to increase cytoplasmic Ca^{2+} levels in target cells, an effect believed to be responsible for stimulation of exocytosis in β cells, leading to insulin

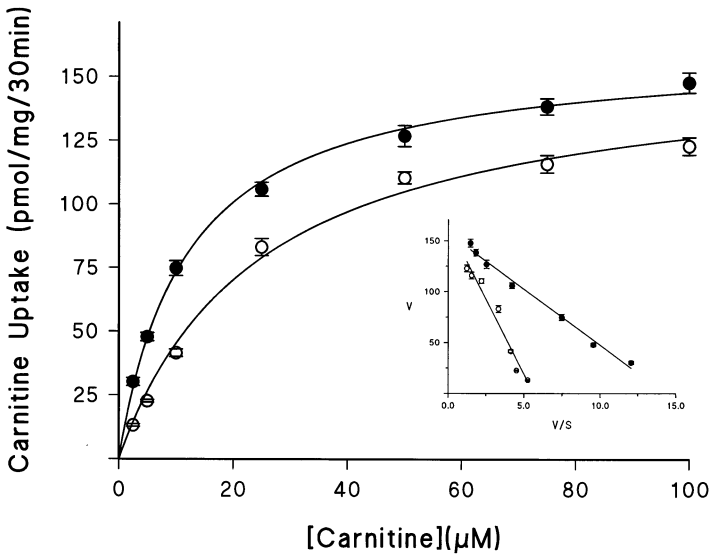

FIG. 7. Kinetics of inhibition of carnitine uptake by glibenclamide in HPCT cells. Uptake measurements were made as described in Fig. 2, except that uptake was measured in the absence (●) as well as in the presence (○) of 25 μM glibenclamide. Inset: Eadie–Hofstee plots. Values are means ± SEM (N = 6).

TABLE 3. Inhibition of the transport of acetyl-L-carnitine and propionyl-L-carnitine by sulfonylureas in HPCT cells*

Sulfonylurea	Acetyl-L-carnitine transport (pmol/mg protein/45 min)	(%)	Propionyl-L-carnitine transport (pmol/mg protein/45 min)	(%)
Control	0.14 ± 0.01	100	0.19 ± 0.02	100
Tolbutamide	0.15 ± 0.01	107	0.17 ± 0.02	90
Glipizide	0.12 ± 0.01	86	0.17 ± 0.02	90
Glibenclamide	0.05 ± 0.01	36	0.03 ± 0.01	16

*Transport of acetyl-L-carnitine (50 nM) or propionyl-L-carnitine (50 nM) in monolayer cultures of HPCT cells was measured with a 45-min incubation. The concentration of sulfonylureas was 100 μM. Values are means ± SEM (N = 6).

secretion [18, 22]. Furthermore, these drugs stimulate protein kinase C without the participation of changes in intracellular Ca^{2+} levels [23] and also inhibit protein kinase A [24]. Since glibenclamide was found to inhibit carnitine uptake in intact cells, any of these signaling pathways may be involved in the observed inhibition. Alternatively, glibenclamide may interact directly with the carnitine transporter by competing with the carnitine-binding site. We found that preincubation of HPCT cells with glibenclamide was not a prerequisite for the inhibition of carnitine uptake by the drug. The inhibition was instantaneous and the magnitude of inhibition was similar whether carnitine uptake was measured for 1 or 30 min in the presence of the drug. These results, together with the observed competitive nature of the inhibition, suggested that the effect of glibenclamide on carnitine uptake is likely due to a direct interaction of the drug with the carnitine transporter. To provide additional supportive evidence for this mechanism, we studied the effect of glibenclamide on carnitine uptake in a cell-free system, using brush border membrane vesicles prepared from rat kidney (Fig. 8). Carnitine uptake in these membrane vesicle preparations was Na^+-dependent and exhibited the typical overshoot phenomenon indicative of transient uphill transport of carnitine into the vesicles. The presence of 25 μM glibenclamide caused a marked inhibition of the initial uptake rate of carnitine. This inhibition was observed only in the presence of Na^+. The uptake measured in the presence of K^+ was not affected. Similarly, the equilibrium uptake, whether measured in the presence of Na^+ or K^+, also was not influenced by glibenclamide. These data strongly suggest that the inhibition of carnitine uptake by glibenclamide is due to a direct interaction of the drug with the Na^+-dependent carnitine transporter.

DISCUSSION

The kidney plays an important role in carnitine homeostasis due to its ability to reabsorb >95% of carnitine in the glomerular filtrate. Impairment of this reabsorptive function can lead to systemic carnitine deficiency, as evident in patients with primary carnitine deficiency [8]. The characteristics of the transport system responsible for renal carnitine reabsorption have been studied thus far only in laboratory animals. There is no information available on the carnitine transport system in the human kidney. We report here for the first time on the characterization of carnitine transport in a proximal tubular epithelial cell line derived from the human kidney.

Uptake of carnitine in human kidney proximal tubular cells occurred via a Na^+-dependent transport process. The transporter exhibited high affinity for carnitine (K_t ~ 10

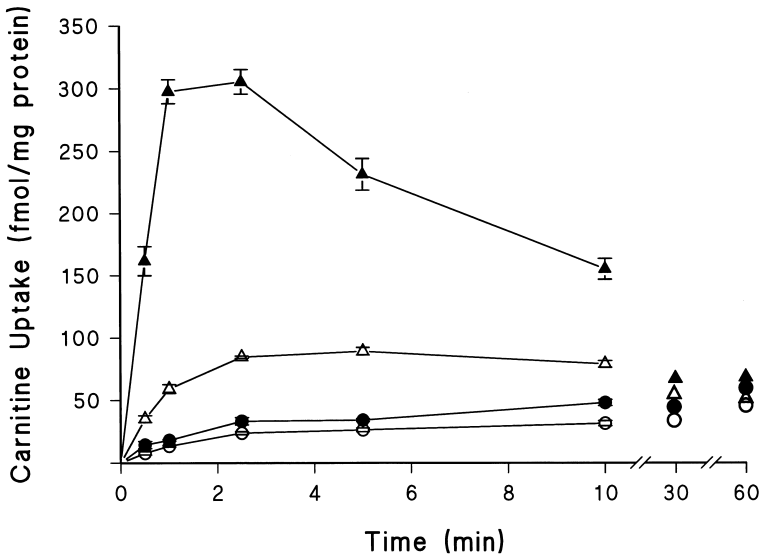

FIG. 8. Inhibition of carnitine uptake by glibenclamide in rat kidney brush border membrane vesicles. Membrane vesicles were preloaded with 10 mM HEPES-Tris buffer (pH 7.5), containing 75 mM potassium gluconate and 150 mM mannitol. Uptake of [^{3}H]carnitine (25 nM) was initiated by mixing 40 μL of membrane suspension (200 μg of membrane protein) and 160 μL of 10 mM HEPES-Tris buffer (pH 7.5), containing [^{3}H]carnitine and either 150 mM NaCl (▲, △) or 150 mM KCl (●, ○). When present (△, ○), glibenclamide concentration was 25 μM. The mixture was incubated for various time periods, and uptake was determined by rapid filtration assay. Values are means ± SEM (N = 6).

μM). There is some evidence that the rat renal brush border membrane may possess at least two different carnitine uptake systems, one with a high affinity and the other with a low affinity for carnitine [7]. We did not find any evidence for the presence of the low affinity carnitine uptake system in HPCT cells. The kinetics of the stimulation of carnitine uptake by Na^+ have not been investigated in any of the earlier studies [6, 7]. We report here on the Na^+ activation kinetics of carnitine uptake in HPCT cells. The Na^+:carnitine stoichiometry was 1:1, and the concentration of Na^+ necessary for half-maximal activation of the uptake process was 10 ± 1 mM. Carnitine exists predominantly as a zwitterion at physiological pH, and therefore a Na^+:carnitine stoichiometry of 1:1 indicates that the Na^+-dependent carnitine transport process is electrogenic. This means that the renal reabsorptive process is energized not only by the transmembrane Na^+ gradient but also by the inside-negative membrane potential. With a $K_{0.5}$ value of 10 ± 1 mM for Na^+ activation of carnitine uptake, the renal carnitine reabsorptive process is activated maximally under physiological Na^+ concentrations known to occur in the glomerular filtrate. The substrate specificity and the cation- and anion-dependence of carnitine uptake in HPCT cells were similar to those of the high-affinity carnitine uptake process in rat renal brush border membrane vesicles. One interesting difference, however, is that carnitine uptake in HPCT cells was inhibited by betaine, whereas in rat renal brush border membrane vesicles betaine stimulates carnitine uptake markedly [7]. The reasons for this difference are not known, but our earlier studies of carnitine uptake in human placental cells also have shown that betaine is an inhibitor of carnitine transport [17]. A Na^+-dependent, high-affinity carnitine transporter has been cloned recently in our laboratory from a human placental cell line [25] and by Tamai *et al.* [26] from the human kidney. This transporter, designated OCTN2, can transport carnitine as well as organic cations. Therefore, OCTN2 is actually an organic cation/carnitine transporter. At the amino acid sequence level, OCTN2 does exhibit homology to other members of the organic cation transporter gene family, namely OCT1 [27], OCT2 [28], OCT3 [29], and OCTN1 [30]. We have shown previously that OCTN2-specific mRNA transcripts are present in the human kidney and in HPCT cells used in the present study [25]. Those findings corroborate the results of the present study that provide evidence for the expression of the carnitine transporter at the functional level.

The present report also provides information suggestive of the involvement of the same transport process for the renal reabsorption of acyl esters of carnitine. The uptake of acetyl-L-carnitine and propionyl-L-carnitine in HPCT cells was Na^+-dependent and was inhibited by carnitine. These findings are of clinical relevance because the acetyl and propionyl esters of carnitine are currently undergoing human trials as potential therapeutic agents in the treatment of Alzheimer's disease [31] and peripheral vascular disease [32]. Our results indicate that these acyl esters of carnitine, when administered to humans, are likely to be conserved very efficiently by renal reabsorption via the Na^+-dependent carnitine transport system.

The findings that sulfonylureas interact with the Na^+-dependent carnitine transport system in HPCT cells are interesting, but the importance of these findings remains to be seen. Among the sulfonylureas tested, glibenclamide was the most potent inhibitor of carnitine uptake. Significant inhibition was observed with concentrations as low as 3 μM glibenclamide. However, the therapeutically effective concentrations of this drug in humans for the control of blood glucose levels in type 2 diabetes are in the range of 1–2 μM [33]. Therefore, whether or not glibenclamide would interfere with carnitine reabsorption *in vivo* in humans at therapeutic doses remains to be seen. A more clinically relevant issue arises if glibenclamide is a transportable substrate for the Na^+-dependent carnitine transport system. The present study demonstrated that this sulfonylurea was a competitive inhibitor of carnitine uptake but did not show whether or not it was a transportable substrate. If glibenclamide is transported by the Na^+-dependent carnitine transporter, this drug is likely to be concentrated in renal absorptive cells as well as in various other cells that are known to possess the Na^+-dependent carnitine transport system. Thus, the carnitine transporter potentially may mediate the entry of glibenclamide into several tissues. The therapeutic target for this drug, namely the sulfonylurea receptor, has been found not only in the pancreas but also in other tissues such as the heart, blood vessels, and brain [34]. Furthermore, the receptor is localized predominantly to intracellular membranes in the pancreas [35]. This also may be true in other tissues expressing the receptor. Therefore, the pharmacological actions of glibenclamide are likely to involve its interaction with the receptor intracellularly. Similarly, sulfonylureas are known to influence the activity of protein kinase A [24] and protein kinase C [23], and these actions would require the entry of sulfonylureas into the target cells. Since the Na^+-dependent carnitine transporter is expressed widely in several tissues, the potential involvement of this transporter in the entry of glibenclamide and other sulfonylureas into cells may be of relevance to the pharmacological actions of these drugs. This work was supported, in part, by a grant from the Sigma-Tau Pharmaceutical Co. We thank Joyce Hobson for excellent secretarial assistance.

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