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Carnitine absorption: Effects of sodium valproate and sodium octanoate in the Caco-2 cell culture model of human intestinal epithelium

Charles J. Rebouche

Department of Pediatrics, University of Iowa College of Medicine, Iowa City, IA USA

The Caco-2 cell culture system was used as a model to investigate the mechanism of carnitine absorption in human small intestinal epithelium, and to determine if valproic acid inhibits this process in the model system. The hypotheses tested were: Carnitine is absorbed by a mechanism not involving carrier-mediated transport; and valproic acid specifically inhibits carnitine absorption. Results of the investigation were consistent with exclusively passive, paracellular absorption of carnitine across the Caco-2 cell monolayer. Saturable and structure-specific intracellular accumulation of carnitine from the apical medium was observed, but was independent of the process of absorption. At high concentration (10 mmol/L), both sodium valproate and its straight-chain analog sodium octanoate inhibited cellular accumulation of carnitine from the apical medium, but enhanced transmonolayer passage of carnitine from the apical to the basal medium. Lower concentrations of these organic acid salts (0.1 or 1 mmol/L) did not affect cellular accumulation of carnitine, but at 1 mmol/L concentration, they slightly enhanced transmonolayer flux. Paradoxically, cells cultured for 5 days in the presence of sodium valproate or sodium octanoate accumulated carnitine at a faster rate than cells cultured in the absence of these compounds. It is concluded that carnitine is absorbed across the Caco-2 monolayer by a passive, paracellular route that is not inhibited by sodium valproate. (J. Nutr. Biochem. 9:228–235, 1998)

Keywords: carnitine; absorption; Caco-2 cells; valproic acid; cell culture

Introduction

L-Carnitine is available in the diet of omnivorous humans, and is to a large extent absorbed. The mechanism(s) by which this process occurs, however, has not been elucidated clearly. Aspects of the absorptive process in mammals have been studied in a variety of tissue, cellular, and subcellular preparations (reviewed in [1,2]). Whereas some investigators concluded that carnitine was absorbed by a passive process, data from other studies suggested that active transport process(es) may account for at least a part of carnitine absorbed from the intestinal lumen. Most recently, McCloud et al.[3] observed that Caco-2 cells grown on an impermeable surface accumulate carnitine by a saturable and structure-specific process. The Caco-2 cell culture model has been used extensively for investigation of absorptive capability and capacity for a variety of nutrients, pharmaceuticals and model compounds (reviewed in [4,5]). Although this cell line was derived from a human colon adenocarcinoma, when grown on a permeable surface in vitro it differentiates and expresses, at least qualitatively, many of the enzymatic and absorptive functions of the human small intestinal epithelium. Comparisons between Caco-2 cell monolayers in vitro and properties of absorption in vivo suggested that the cell culture model can be used to predict in vivo human absorption of compounds regardless of mechanism (e.g., active or passive;[6]). In this investigation, the Caco-2 cell culture model has been used to investigate the mechanism of L-carnitine absorption. The working hypothesis was: Carnitine is absorbed across the

Address correspondence and reprint requests to Dr. Charles J. Rebouche, Department of Pediatrics, Oakdale Research Park, 2501 Crosspark Road, Coralville, IA 52241 USA.
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Caco-2 cell monolayer by a passive mechanism, involving both transcellular and paracellular processes.[a]

A second question relating to the effect of sodium valproate on carnitine absorption was explored. Chonic administration of valproic acid, a commonly prescribed anticonvulsant medication, to humans in vivo sometimes results in carnitine depletion.[7] Several mechanisms for this effect were proposed: (a) attenuation of the efficiency of carnitine reabsorption, (b) reduced capacity for carnitine biosynthesis, and/or (c) inhibition of carnitine absorption.[b] In this investigation, direct and indirect effects of sodium valproate (and its unbranched analog, sodium octanoate) on carnitine absorption across the Caco-2 cell monolayer were examined. The working hypothesis was: Sodium valproate, but not sodium octanoate, specifically depresses the rate of carnitine absorption.

Methods and materials

Seed cultures of the Caco-2 cell line (passages 35 to 47) were obtained from the University of Iowa Atherosclerosis SCOR core tissue culture facility. Cells for subsequent passage were cultured in 75 cm^2 flasks (Corning-Costar; Cambridge, MA, USA). Cells were subcultured when cultures were approximately 80% confluent. For subculture, cells were detached from the flask surface by incubation in 0.05% trypsin, 0.53 mmol/L EDTA · 4Na for 3 to 5 min. For all experiments cells were cultured on 4.7 cm^2 polycarbonate membrane inserts in 6-well Transwell® cluster plates (Corning-Costar). The growth medium consisted of Dulbecco's Modified Eagle's Medium with 4.5 g/L glucose (Sigma, St. Louis, MO USA), supplemented with 10% fetal bovine serum (defined; Hy-Clone, Logan, UT USA), 2 mmol L-glutamine/L, 10^5 units penicillin/L, 100 mg streptomycin/L, and 50 mg gentamycin/L. Growth medium was changed every 2 days. Cells were grown in a humidified atmosphere maintained at 37°C, and 10% CO_2 in air.[8]

Cells at passage 37 to 69 were used for experiments. Cell monolayers on membranes in Transwell clusters were used for experiments 13 to 15 days after seeding. Transepithelial electrical resistance (TEER) was measured across cell monolayers on Transwell membranes using a Millicell®-ERS (electrical resistance system) with $Ag/AgCl_2$ electrodes (Millipore, Milford, MA USA). Transwell membranes in growth medium but not seeded with cells were used for blank measurements. All cell monolayers used in experiments had a net TEER $\geq$ 150 Ω (705 $\Omega \cdot cm^2$). In preliminary experiments with cells cultured on Transwell membranes it was found that TEER plateaued by Day 12, protein concentration plateaued by Day 9, and flux of lucifer yellow[9] across the monolayer was minimal by culture Day 6. Average protein content of confluent cell monolayers was 1.3 mg. Protein content was measured for all Transwell monolayers used in experiments. After completion of flux measurements, membranes were excised from the Transwell cup, and protein was solubilized in 5 mL of 50 mmol/L KOH. After 16 to 24 hr at room temperature, protein was quantified by the method of Lowry et al.[10]

For solute flux experiments, monolayer cultures were washed three times in Dulbecco's Phosphate Buffered Saline (DPBS; Sigma), and incubated for 1 hr (37°C, humidified atmosphere, 10% CO_2 in air) in Hanks' Balanced Salts (HBSS) with 25 mmol glucose/L and 10 mmol HEPES/L, pH 7.4 (HBSS/7.4). Solute flux experiments were performed at 37°C in a shaking water bath. Test substances in incubation medium (see below) pre-warmed to 37°C were added to the apical and/or basal media (1.5 and 2.6 mL, respectively) and incubations were performed for 5 to 120 min. Incubation media consisted of HBSS/7.4, HBSS with 25 mmol glucose/L and 10 mmol MES/L, pH 6.0 (HBSS/6.0), or HBSS/7.4 with sodium chloride and sodium phosphate replaced with the corresponding Li salts or choline chloride.

After incubation for the appropriate time interval, apical and basal media were sampled, and the Transwell cup containing the cell monolayer was washed in three successive 100-mL volumes of ice-cold DPBS. The cell monolayer and polycarbonate membrane were excised and placed in 5 mL of 50 mmol/L KOH to solubilize the cell monolayer. After 16 to 24 hr at room temperature, aliquots of the resulting solution were taken for radioactivity determination and protein analysis. Radioactivity in the apical and basal media, and the solubilized cell monolayer, was quantified by liquid scintillation counting.

Test substances synthesized for this study included [*methyl*-^{3}H]L-carnitine (0.83 Ci/mol),[11] N,N,N-trimethylamino-β-alanine, δ-N,N,N-trimethylaminovaleric acid, and ϵ-N,N,N-trimethylaminocaproic acid [prepared by the general method of Mazzeti and Lemmon,[12,13] starting with the corresponding primary amines]. ϵ-N-Trimethyl-L-lysine was prepared as described previously.[14] [3-^{3}H]L-Alanine (76.9 Ci/mmol) was obtained from DuPont New England Nuclear, Boston, MA USA. All other chemicals were reagent grade, obtained from commercial sources.

Data were analyzed using either the paired *t*-test, or repeated measures analysis of variance with post-hoc Tukey's Studentized Range test of significance.[15] Extreme observations within data sets were tested as outliers using the maximum normed residual statistic.[16] Data identified as outliers (5% level) were excluded from subsequent analyses.

Results

Time course and kinetics of carnitine accumulation in the cell monolayer and transmonolayer flux of carnitine

At concentrations of carnitine in the apical medium of 2 μmol/L or 0.1 mmol/L, cellular accumulation of carnitine was linear for 60 min, and continued to increase for at least 120 min (*Figure 1A*). By contrast, accumulation of L-alanine from the apical medium was maximal by 20 min, and subsequently declined (*Figure 1B*). The accumulation of alanine at 20 min was more than 10 times greater than that for carnitine. Transmonolayer flux of both carnitine (*Figure 2A*) and alanine (*Figure 2B*) from the apical to basal compartments was linear for 30 min. Rates for alanine flux were approximately 10 times greater than those for carnitine, at apical solute concentrations of 20 or 1000 μmol/L and initial basal concentrations of 0.

Carnitine accumulation by the cell monolayer from the apical medium exhibited both saturable and non-saturable components (*Figure 3*). Above 50 μmol carnitine/L in the

[a]Definitions of terms, used in relation to Caco-2 monolayers: transcellular flux, rate of movement of solute from the apical medium to the basal medium via the intracellular compartment; paracellular flux, rate of movement of solute from the apical medium to the basal medium via an extracellular route (e.g., across tight junctions); transmonolayer flux, movement of solute from the apical medium to the basal medium, without regard to route; absorption, movement of solute across the cell monolayer, without regard to route; cellular uptake, movement of solute from the apical medium into the intracellular compartment across the brush-border membrane.

[b]Stadler, D.D., Bale, J.F., Jr., Chenard, C.A., and Rebouche, C.J., submitted.

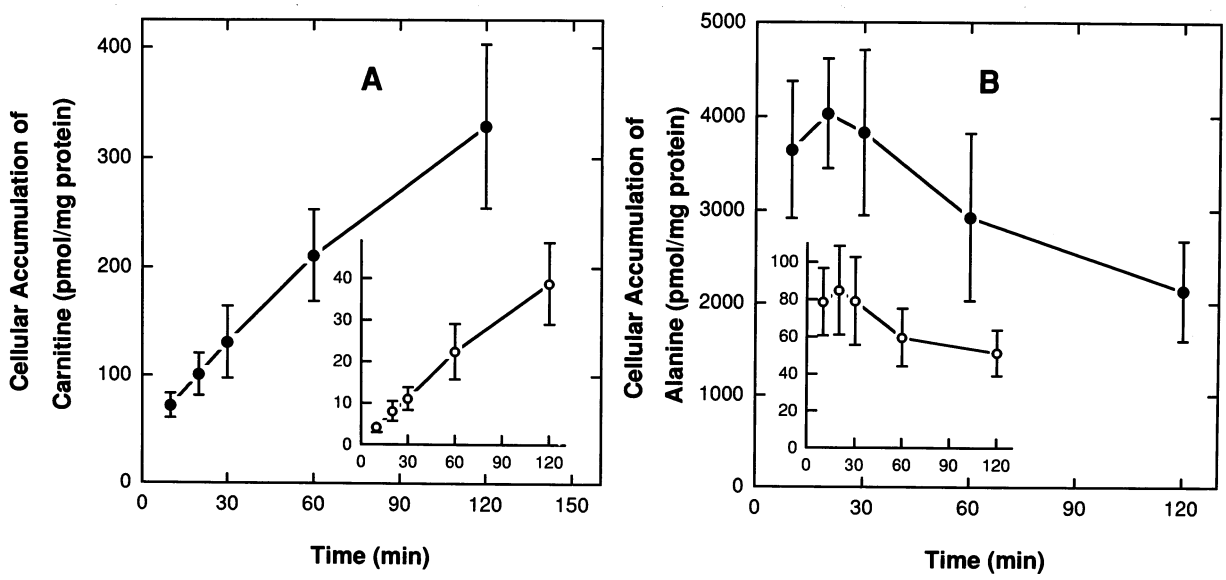

Figure 1 Time course of cellular accumulation of L-carnitine and L-alanine from the apical medium. Cell monolayers were grown to confluency on a permeable surface for 13 to 15 days. After washing and incubation for 1 hr in HBSS/7.4, cells were used for carnitine flux experiments. 1.5 mL of prewarmed (37°C) 2 μmol/L (open symbols, inset) or 100 μmol/L [*methyl*-^{3}H]L-carnitine in HBSS/7.4 (panel A) or [3-^{3}H]L-alanine in HBSS/6.0 (panel B) was placed in the apical chamber of the Transwell, and 2.6 mL of HBSS/7.4 (containing no L-carnitine or L-alanine) was placed in the basal chamber. After incubation at indicated times, cell monolayers were washed and excised, and cell protein was solubilized in 50 mmol/L KOH. Radioactivity and protein in the resulting solution were quantified. All data are means of four observations. Vertical lines indicate standard deviation.

apical medium, carnitine accumulation in the monolayer was linear with increasing extracellular (apical compartment) carnitine concentration. For the saturable component of cellular accumulation of carnitine from the apical medium, K_T and V_{max} calculated from an Eadie-Hofstee plot (*Figure 3*, inset) were 7.3 μmol/L and 1.04 pmol/min/mg protein, respectively. Transmonolayer flux of carnitine from the apical to basal compartments was linear over the apical carnitine concentration range 1 μmol/L to 10 mmol/L (initial basal medium carnitine concentration = 0; *Figure 4*). There was no evidence for a saturable component even at the lowest apical medium carnitine concentrations tested.

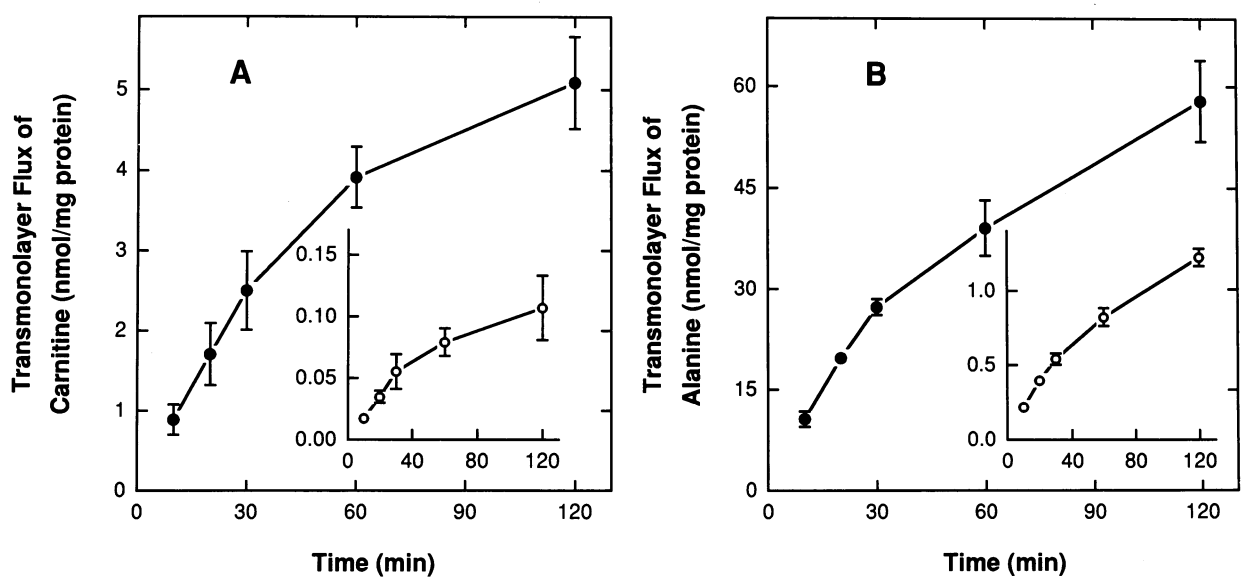

Figure 2 Transmonolayer flux of L-carnitine and L-alanine from the apical medium to the basal medium. Experiments were carried out as described in the legend to *Figure 1*. Panel A, transmonolayer flux of L-carnitine; panel B, transmonolayer flux of L-alanine; open symbols (inset), 2 μmol/L of solute in apical medium; closed symbols, 100 μmol/L of solute in apical medium. All data are means of four observations. Vertical lines indicate standard deviation.

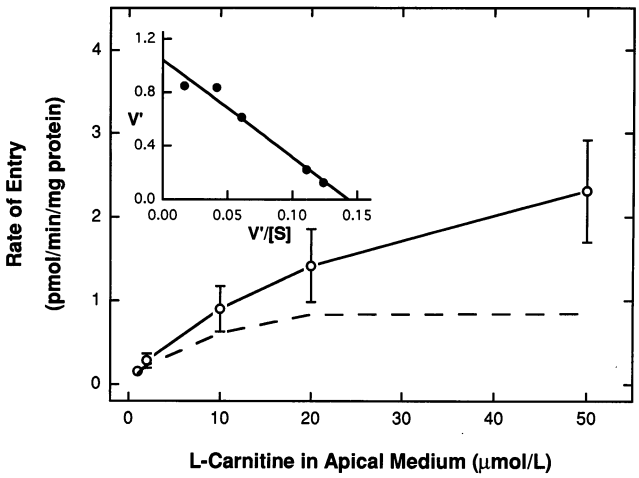

Figure 3 Kinetics of L-carnitine accumulation from the apical medium by Caco-2 cell monolayers. Experiments were carried out as described in the legend to *Figure 1*, except that the L-carnitine concentration in the apical medium was varied from 1 μmol/L to 10 mmol/L (data shown only for concentrations 1 μmol/L to 50 μmol/L). Incubation times were 20 min. Above 50 μmol/L, L-carnitine accumulation in the cell monolayer was linear with increasing L-carnitine concentration in the apical medium. Saturable portion of cellular uptake (dashed line) was calculated by subtraction of linear portion of curve extrapolated to 0. Inset: Eadie-Hofstee plot of saturable portion (V′) of uptake rates. All data are means of five observations. Vertical lines indicate standard deviation.

Cellular accumulation of 20 μmol/L carnitine from the apical medium was unaffected by an inwardly-directed H^+ gradient, established using transport medium buffered with MES, pH 6.0, in the apical chamber (*Table 1*). However, transmonolayer flux of carnitine was significantly reduced by imposition of the H^+ gradient across the monolayer.

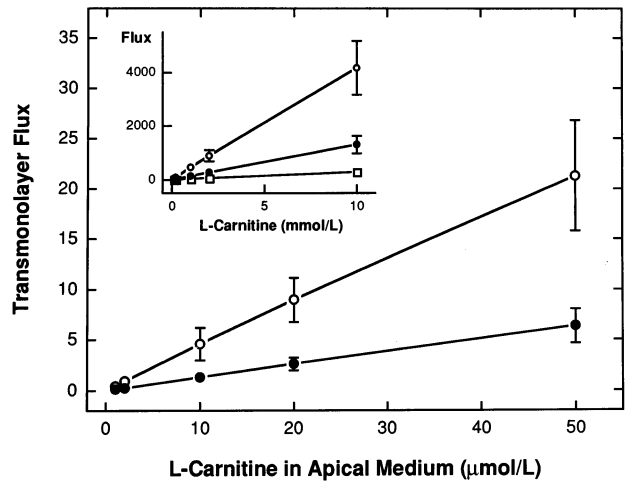

Figure 4 Kinetics of transmonolayer flux of L-carnitine from the apical medium to the basal medium. Conditions as described in the legend to *Figure 3*. Open symbols, flux expressed as pmol/min/mg protein; closed symbols, flux expressed as pmol/min/cm^2. Inset: expanded range of observations, apical L-carnitine concentrations from 0.1 to 10 mmol/L. All data are means of five observations. Vertical lines indicate standard deviation.

Replacement of Na^+ in the apical transport medium with Li^+ also had no effect on cellular accumulation of carnitine, but slightly increased transmonolayer flux of carnitine. On the other hand, replacement of Na^+ with choline in the apical transport medium resulted in a significant decrease in the rate of cellular accumulation of carnitine from the apical medium, and a significant increase in transmonolayer flux of carnitine from the apical to the basal compartments. By contrast to transmonolayer flux of carnitine, alanine absorption across the cell monolayer was stimulated 27% by an apical, inwardly-directed H^+ gradient (data not shown), consistent with earlier observations of Thwaites et al.[17]

Effects of structural analogs on accumulation of carnitine by the cell monolayer and transmonolayer flux of carnitine

Structural analogs of carnitine that contained both quaternary ammonium and carboxyl moieties joined by a carbon chain were effective inhibitors of carnitine accumulation by the cell monolayer from the apical medium, but not of transmonolayer flux of carnitine from the apical to the basal compartments (*Table 2*). A series of carbon chain homologs (two to six carbons) of carnitine, but without the hydroxyl group, all were effective inhibitors of carnitine accumulation by the cell monolayer. Betaine (2-carbon homolog) was the least effective (27% inhibition at 50-fold higher concentration of analog to carnitine), and γ-butyrobetaine (four carbons like carnitine, but without the hydroxyl group) was most effective (61% inhibition; approximating the saturable component of total cell monolayer accumulation of carnitine). D-Carnitine, the non-natural enantiomer of L-carnitine, was slightly less effective than γ-butyrobetaine. Compounds with a quaternary nitrogen but without a carboxyl moiety (e.g., choline) were ineffective, as were carboxylic acids without a quaternary nitrogen (e.g., γ-aminobutyric acid). ε-N,N,N-Trimethyl-L-lysine, although meeting the structural requirements proposed above, was ineffective as an inhibitor of carnitine accumulation by the cell monolayer.

Of 10 structural analogs tested, only N,N,N-trimethyl-β-alanine (the 3-carbon homolog) at 1 mmol/L in the apical medium significantly inhibited transmonolayer flux of carnitine (20 μmol/L) from the apical to the basal compartments (*Table 2*).

Efflux of carnitine from the cell monolayer

If carnitine is absorbed across the Caco-2 cell monolayer by a process involving cellular uptake from the apical medium and release across the basal and lateral membranes (transcellular flux), it is reasonable to assume that carnitine in the apical medium would displace carnitine in the intracellular compartment, driving the latter into the basal medium, and that increasing the concentration of carnitine in the apical medium would accelerate this process. To test the effectiveness of carnitine in the apical medium in displacing or driving carnitine from the intracellular compartment, confluent cell monolayers were loaded for 24 hours with 50 μmol/L [*methyl*-^{3}H]L-carnitine from the apical medium (basal medium contained 50 μmol unlabeled carnitine/L).

Table 1 Effect of an inwardly directed H^+ gradient and replacement of sodium in the apical medium on carnitine uptake and transmonolayer flux of carnitine from the apical medium

Treatment	Cellular uptake[1]	Transmonolayer flux[1]	Transmonolayer flux[2]
H^+ gradient[3]	108	67.1*	53.1*
Na^+ replaced with Li^+ [4]	91.0	111*	106
Na^+ replaced with choline[4]	48.5*	139*	133*

L-Carnitine concentration in the apical medium was 20 μmol/L. Incubation time was 20 min. All values are percent of control. Mean ± SD for controls were: cellular uptake, 1.01 ± 0.289 pmol/min/mg protein; transmonolayer flux, 1.80 ± 0.847 pmol/min/mg protein or 0.479 ± 0.197 pmol/min/cm^2. All values are means of 5 comparisons.
[1]Values calculated from data expressed as pmol carnitine/min/mg protein.
[2]Values calculated from data expressed as pmol carnitine/min/cm^2.
[3]Apical medium was buffered with MES, pH 6.0.
[4]Na^+ in the apical medium was replaced with equimolar Li^+ or choline.
*Significant difference from control ($P < 0.05$).

Cell monolayers were washed in DPBS at 37°C and used immediately for efflux experiments. Transwell inserts containing the cell monolayer were placed in cluster wells containing 2.6 mL of 50 μmol/L L-carnitine in HBSS/7.4 (basal medium), and apical medium (1.5 mL of HBSS/7.4 containing 1, 50, or 1000 μmol carnitine/L) was added. Apical and basal media were sampled at timed intervals and percent efflux of total radiolabeled carnitine from the monolayer was calculated. In the apical medium 6% to 11% of the labeled carnitine appeared from 5 to 120 min (*Figure 5*, left panel). In the basal medium, 1% to 5.5% of the labeled carnitine appeared over the same time interval (*Figure 5*, right panel). The amount of radiolabeled carnitine appearing in the basal compartment at any time interval between 5 and 120 min was independent of the concentration of carnitine initially in the apical medium, suggesting that it was unable to displace intracellular carnitine into the basal extracellular compartment. Exchange of carnitine between the apical medium and intracellular compartment was modestly stimulated by increasing apical carnitine concentration, but only after 1 hr of exposure.

Effects of sodium valproate and sodium octanoate on accumulation of carnitine by the cell monolayer and transmonolayer flux of carnitine

Both sodium valproate and sodium octanoate at 10 mmol/L in the apical medium inhibited accumulation of carnitine by the cell monolayer from the apical medium (*Table 3*). Inhibition by sodium valproate but not sodium octanoate was more pronounced when the carnitine concentration in the apical medium was 20 μmol/L than at 1000 μmol/L, suggesting that the effect of sodium valproate was specific for the saturable component of carnitine accumulation. Neither sodium valproate nor sodium octanoate at 0.1 or 1 mmol/L in the apical medium had any effect on carnitine accumulation by the cell monolayer.

By contrast to cellular accumulation of carnitine, transmonolayer flux of carnitine from the apical to basal extracellular compartments was modestly stimulated by 10 mmol/L sodium valproate and sodium octanoate (*Table 3*). These organic acid salts at a concentration of 0.1 mmol/L had no effect on transmonolayer flux of carnitine.

Table 2 Effect of carnitine analogs on carnitine uptake and transmonolayer flux of carnitine from the apical medium

Test substance	Cellular uptake[1]	Transmonolayer flux[1]	Transmonolayer flux[2]
D-Carnitine	44.7*	114	122
Betaine	73.3*	93.3	89.8
N,N,N-Trimethyl-β-alanine	46.7*	67.3*	65.2*
γ-Butyrobetaine	38.7*	94.5	90.8
δ-N,N,N-Trimethylaminovaleric acid	49.0*	108	108
ϵ-N,N,N-Trimethylaminocaproic acid	53.4*	91.8	94.5
ϵ-N,N,N-Trimethyl-L-lysine	97.2	115	113
Choline	94.6	131	123
Taurine	111	96.9	109
γ-Aminobutyric acid	98.1	105	103
β-Hydroxy-γ-aminobutyric acid	102	103	105

L-Carnitine concentration in the apical medium was 20 μmol/L and test substance concentration in apical medium was 1 mmol/L. Incubation time was 20 min. All values are percent of control. Mean ± SD for controls were: cellular uptake, 1.11 ± 0.193 pmol/min/mg protein; transmonolayer flux, 4.18 ± 2.64 pmol/min/mg protein or 1.39 ± 0.977 pmol/min/cm^2. All values are means of at least 5 comparisons.
[1]Values calculated from data expressed as pmol carnitine/min/mg protein.
[2]Values calculated from data expressed as pmol carnitine/min/cm^2.
*Significant difference from control ($P < 0.05$).

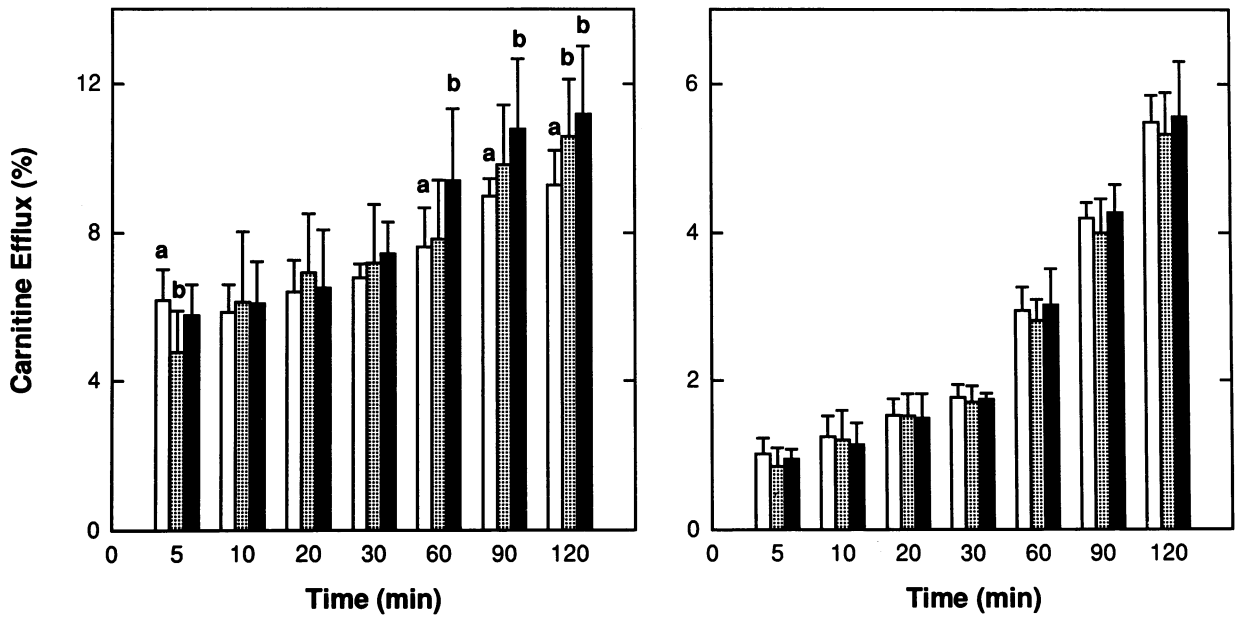

Figure 5 Efflux of [*methyl*-^{3}H]L-carnitine from Caco-2 cell monolayers. Confluent cell monolayers (12 to 14 days in culture) were loaded with [*methyl*-^{3}H]L-carnitine (50 μmol/L) from the apical medium (and 50 μmol/L unlabeled carnitine in the basal medium) for 24 hr under otherwise normal culture conditions. Cells were washed in pre-warmed (37°C) DPBS and used immediately for efflux experiments. For efflux experiments, basal media (HBSS/7.4) contained 50 μmol/L L-carnitine, and apical media (HBSS/7.4) contained 1 (open bars), 50 (stippled bars), or 1000 (filled bars) μmol/L L-carnitine. After incubation at 37°C for the times indicated, apical and basal media were sampled for radioactivity determination, and the cell monolayers were washed and prepared for quantification of radioactivity and protein content. Left panel, efflux into apical medium; right panel, efflux into basal medium. All data are means of five observations. Vertical lines indicate standard deviation. Different letters above bars indicate significant difference ($P < 0.05$) among treatments within the same time interval. No letter or same letter above bars indicates no significant difference among treatments within the same time interval.

To assess the long-term or indirect effects of exposure of Caco-2 cells to sodium valproate and sodium octanoate, cell monolayers were grown to confluency for 9 days, then 2 mmol/L of sodium valproate or sodium octanoate was included in the apical and basal media for 5 days. Carnitine accumulation from the apical medium and transmonolayer flux were then measured in the absence of these modifiers in the incubation media. Cell monolayer accumulation of carnitine from the apical medium was enhanced by culture with sodium valproate, at concentrations of carnitine favor-

Table 3 Effect of sodium valproate and sodium octanoate on carnitine uptake and transmonolayer flux from the apical medium

	Test substance concentration (mmol/L)	20 μmol/L Carnitine concentration in apical medium		1000 μmol/L Carnitine concentration in apical medium	
		Valproate	Octanoate	Valproate	Octanoate
Cellular uptake[1]					
	0.1	100	108	103	101
	1	96.3	103	103	88.0
	10	52.8*	65.2*	87.8*	71.1*
Transmonolayer flux[1]					
	0.1	102	105	98.8	107
	1	111	109	116*	104
	10	147*	134*	132*	131*
Transmonolayer flux[2]					
	0.1	103	107	101	106
	1	110	108*	116*	107*
	10	143*	137*	137*	137*

Sodium valproate and sodium octanoate were included in the apical medium only, at the concentrations indicated. Incubation time was 1 hr. All values are percent of control (no sodium valproate or sodium octanoate added to incubation). Mean ± SD for controls were: cellular uptake, 1.10 ± 0.158 pmol/min/mg protein; transmonolayer flux, 4.56 ± 2.49 pmol/min/mg protein or 1.42 ± 0.594 pmol/min/cm^2. All values are means of five to seven comparisons.

[1]Values calculated from data expressed as pmol carnitine/min/mg protein.

[2]Values calculated from data expressed as pmol carnitine/min/cm^2.

*Significant difference from untreated cells ($P < 0.05$).

Table 4 Effect of growth for 5 days with 2 mmol/L sodium valproate or sodium octanoate on carnitine uptake and transmonolayer flux of carnitine from the apical medium

	Carnitine concentration in apical medium (μmol/L)		
	1	20	1000
Cellular uptake[1]			
Sodium valproate	144	166*	141*
Sodium octanoate	109	137*	120*
Transmonolayer flux[1]			
Sodium valproate	101	102	97.5
Sodium octanoate	153*	159*	134*
Transmonolayer flux[2]			
Sodium valproate	113*	108*	110*
Sodium octanoate	125	131*	114

Sodium valproate or sodium octanoate was present in culture media (both apical and basal chambers) for 5 days before analysis of carnitine flux. No sodium valproate or sodium octanoate was included in the incubation media for flux experiments. Incubation time for flux experiments was 1 hr. Means of five comparisons are expressed as percent of control mean. Mean ± SD for controls were: cellular uptake (pmol/min/mg protein), 0.139 ± 0.027 (1 μmol/L carnitine in apical medium), 1.01 ± 0.217 (20 μmol/L carnitine in apical medium), and 17.1 ± 3.03 (1 mmol/L carnitine in apical medium); transmonolayer flux (pmol/min/mg protein), 0.300 ± 0.169 (1 μmol/L carnitine in apical medium), 6.08 ± 3.59 (20 μmol/L carnitine in apical medium) and 315 ± 187 (1 mmol/L carnitine in apical medium); transmonolayer flux (pmol/min/cm^2), 0.0818 ± 0.0419 (1 μmol/L carnitine in apical medium), 1.69 ± 0.878 (20 μmol/L carnitine in apical medium) and 85.1 ± 46.3 (1 mmol/L carnitine in apical medium).
[1]Values calculated from data expressed as pmol carnitine/min/mg protein.
[2]Values calculated from data expressed as pmol carnitine/min/cm^2.
*Significant difference from untreated cells ($P < 0.05$).

ing both saturable and non-saturable accumulation (*Table 4*). Growth in the presence of sodium octanoate stimulated carnitine accumulation from the apical medium to a lesser extent. Transmonolayer flux of carnitine from the apical to basal compartments was not significantly affected by culture in the presence of sodium valproate, if the rates were indexed to protein content of the monolayer, but were slightly enhanced when indexed to surface area. Culture in the presence of sodium octanoate significantly enhanced transmonolayer flux, when rates were indexed to protein content (*Table 4*). The presence of 2 mmol/L sodium valproate in the growth medium during the final 5 days of culture resulted in a greater protein content of the cell monolayer (1.52 ± 0.138 mg for valproate-treated cells, $n = 15$; 1.33 ± 0.129 mg for untreated cells, $n = 21$; $P < 0.005$). By contrast, cells treated with sodium octanoate had a lower monolayer protein content (1.08 ± 0.066 mg for octanoate-treated cells, $n = 15$; 1.33 ± 0.129 for untreated cells, $n = 21$; $P < 0.005$). No differences were observed in TEER among treated and untreated cells.

Discussion

L-Carnitine in the diet of humans is absorbed to the extent of about 65 to 75%.[11] Unabsorbed carnitine is mostly degraded by microorganisms in the large intestine, and therefore, relatively little normally is excreted in feces. The efficiency of carnitine absorption is less than that of protein-derived amino acids. The latter are generally absorbed by active transport mechanisms. The disparity in extent of absorption of carnitine and α-amino acids in vivo is reflected in the differences in the rates of transmonolayer flux of L-carnitine and L-alanine across Caco-2 monolayers observed in this investigation. Whereas L-alanine is absorbed via an active process characterized by stimulation of transport by an inwardly directed apical H^+ gradient,[17] L-carnitine flux across the cell monolayer proceeds at approximately one-tenth of the rate observed for L-alanine, and is stimulated neither by H^+ or Na^+ gradients.

Results of this investigation are consistent with those of McCloud et al.[3] who observed carrier-mediated uptake of carnitine into Caco-2 cells grown on a solid surface. Moreover, the results are comparable to those obtained by other investigators using in vivo and in vitro preparations of rat small intestine. Gross and Henderson[18] and Gudjonsson et al.[19,20] demonstrated in rats in vivo that carnitine is rapidly taken up by the small intestinal mucosa (in a process that was saturable and structure-specific) but appeared in the circulation much more slowly. Shaw and coworkers[21] observed active transport of carnitine into rat everted duodenal and jejunal sacs and rings. These results all are consistent with carrier-mediated transport of carnitine at the apical membrane, but these studies do not clearly delineate a correlation between this apical membrane process and absorption of carnitine across the intestinal mucosal layer. Results obtained in this investigation using the Caco-2 model system in vitro offer a plausible explanation for the seemingly dichotomous results obtained by other investigators regarding the active and/or passive nature of carnitine absorption in vivo.

Several lines of evidence obtained in this study suggest that cellular accumulation of carnitine from the apical medium is not associated with absorption of carnitine across the Caco-2 cell monolayer. Cellular accumulation was observed to contain a saturable and structure-specific component, whereas transmonolayer flux did not, even at very low carnitine concentrations in the apical medium. Imposition of a H^+ gradient in the apical-to-basal direction had no effect on cellular accumulation of carnitine, but decreased transmonolayer flux. Replacement of Na^+ in the apical medium with choline significantly inhibited cellular accumulation of carnitine from the apical medium, but enhanced transmonolayer flux from the apical to basal compartments. The rate of intracellular carnitine movement into either the basal or apical media was not affected by a wide range of apical carnitine concentrations. Ten mmol/L sodium valproate or sodium octanoate in the apical medium depressed the rate of cellular accumulation of carnitine but stimulated transmonolayer flux from the apical to basal compartments. Lastly, when cells were treated for 5 days with 2 mmol/L sodium valproate or sodium octanoate, the rate of cellular accumulation of carnitine was subsequently enhanced, but only sodium octanoate treatment stimulated transmonolayer flux of carnitine. All of these observations are consistent with passive, paracellular movement of carnitine across the cell monolayer in the apical-to-basal direction. Though clearly present, carrier-mediated uptake of carnitine across the apical

membrane appears to have no significant role in carnitine absorption across the cell monolayer.

To elucidate the etiology of valproic acid-induced carnitine depletion, we tested the hypothesis that valproic acid, as the sodium salt, decreases the rate of carnitine absorption in Caco-2 cells grown on a permeable surface. The hypothesis was rejected, based on the following observations: (a) Although 10 mmol/L sodium valproate (but not lower concentrations) decreased the rate of carnitine uptake into Caco-2 cells, it stimulated carnitine flux across the cell monolayer; and (b) chronic exposure (5 days in culture) of Caco-2 cells to sodium valproate resulted in a stimulation of carnitine uptake into the cell monolayer, without affecting carnitine flux across the monolayer. Valproic acid is an 8-carbon, branched chain organic acid. Its unbranched analog, octanoic acid, as sodium octanoate, produced similar effects on these parameters. Results of this study do not support an effect of valproic acid on carnitine absorption in the etiology of carnitine depletion associated with chronic valproic acid administration.

Two other hypotheses for the mechanism of valproate-induced carnitine depletion have been proposed and investigated. Another study from this laboratory[c] demonstrated that in normal male adults the efficiency of carnitine reabsorption was increased after 28 days of valproic acid (as divalproex sodium) administration. On the other hand, Farkas et al.[22] have shown an inhibitory effect of valproic acid on conversion of γ-butyrobetaine to carnitine in rats in vivo. Although valproic acid did not inhibit γ-butyrobetaine hydroxylase in vitro, it was observed that the concentration of α-ketoglutarate, a necessary cosubstrate for this enzyme, was reduced by 28% in livers of valproic acid-treated rats, compared with controls. Inasmuch as the concentration of α-ketoglutarate in rat liver in vivo is not saturating with respect to γ-butyrobetaine hydroxylase, its lower availability could reduce the rate of carnitine biosynthesis and lead to decreased body stores of carnitine if the supply from diet is limiting as well.

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