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Carnitine Transport into Muscular Cells. Inhibition of Transport and Cell Growth by Mildronate

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ABSTRACT. Carnitine is involved in the transfer of fatty acids across mitochondrial membranes. Carnitine is found in dairy and meat products, but is also biosynthesized from lysine and methionine via a process that, in rat, takes place essentially in the liver. After intestinal absorption or hepatic biosynthesis, carnitine is transferred to organs whose metabolism is dependent on fatty acid oxidation, such as heart and skeletal muscle. In skeletal muscle, carnitine concentration was found to be 50 times higher than in the plasma, implicating an active transport system for carnitine. In this study, we characterized this transport in isolated rat myotubes, established mouse C2C12 myoblastic cells, and rat myotube plasma membranes and found that it was Na^+-dependent and partly inhibited by a Na^+/K^+ ATPase inhibitor. L-carnitine analogues such as D-carnitine and γ-butyrobetaine interfere with this system as does acyl carnitine. Among these inhibitors, the most potent was mildronate (3-(2,2,2-trimethylhydrazinium)propionate), known as a γ-butyrobetaine hydroxylase inhibitor. It also induced a marked decrease in carnitine transport into muscle cells. Removal of carnitine or treatment with mildronate induced growth inhibition of cultured C2C12 myoblastic cells. These data suggest that myoblast growth and/or differentiation is dependent upon the presence of carnitine. BIOCHEM PHARMACOL **59**;11:1357–1363, 2000.

KEY WORDS. carnitine; muscle cell; transport; mildronate

Carnitine (β-hydroxy-γ-trimethylaminobutyric acid) is an essential cofactor for the transfer of long-chain fatty acids across mitochondrial membranes for β-oxidation [1]. In rat liver, carnitine is synthesized from lysine and methionine [2]. Carnitine is also found in dietary items such as dairy and meat products and is actively absorbed in the intestine [3]. Exogenous and endogenous carnitine is then released into the plasma from which it is taken up by peripheral tissues dependent on fatty acid oxidation via an active mechanism. Among these tissues, muscle is probably the main target for carnitine transport. Ninety to ninety-five of total carnitine is concentrated in muscle, and the ratio between muscle carnitine concentration and plasma concentration is around 50:1 [4]. In human, a defect in carnitine transport is responsible for a syndrome known as the carnitine deficiency syndrome [5–7]. This disease is associated with myopathy, cardiomyopathy, and lipid metabolism disorder. Carnitine transport has been mainly studied in isolated organs and cultured cells. In human muscle, the active transport of carnitine appears to have two components [8], one with high activity involved in carnitine transport in the physiological range and the second with low affinity involved in the transport of a high concentration of carnitine.

This study was performed to characterize carnitine transport into isolated rat myotubes, cultured mouse myoblastic cells, and plasma membranes purified from rat muscle myotubes. The kinetic properties of this transport were estimated as well as interaction with compounds that alter carnitine metabolism or energy production. A special effort was made to study the interaction of carnitine analogues (especially mildronate) with this transporter. We also investigated the relationship between extracellular carnitine concentration and cell growth and differentiation.

MATERIALS AND METHODS

Chemicals

All chemicals were purchased from Sigma. Tritiated carnitine (L-[methyl-^{3}H]carnitine, specific activity 80 Ci/mmol) was obtained from Amersham. Culture medium, fetal bovine serum, and other cell culture ingredients were purchased from Eurobio.

Animals and Cells

All studies were performed on adult male Wistar rats (Dépré) weighing between 200 and 250 g. They were given food and water *ad lib*. and were fasted for 18 hr prior to use

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in experiments. C2C12 cells were purchased from ATCC through Biovalley.

Myotube Isolation

Rat muscle culture was established as described by Freshney [9] from soleus and gastrocnemius muscles from Wistar rats. After the removal of muscles, all subsequent steps were carried out at 4°. Muscles were washed with PBS in a Petri dish, and adipose tissue and blood vessels were then carefully removed and finely chopped into small pieces. Muscle fragments were sedimented for 5 min, and the supernatant was removed and replaced by 10 mL of PBS containing 2000 U/mL of type II collagenase. The incubation was performed for 4 hr at room temperature. The lysate was then spun at 1000 *g* for 5 min, and the pellet was washed 3 times, resuspended in 5 mL of PBS, and subsequently used for carnitine uptake measurement.

Preparation of Plasma Membranes from Muscle Cells

Plasma membranes were prepared according to Dombrowski *et al.* [10]. The final pellet was found to be highly enriched in plasma membranes by monitoring 5′-nucleotidase activity [11].

Marker Activity and Protein Concentration

Protein concentration was measured using the bicinchoninic acid procedure (Pierce-Sigma) with BSA as a standard. 5′-Nucleotidase activity was measured according to Arkesteijn [11].

C2C12 Culture

C2C12 cells were cultured according to the ATCC recommendations. The cells were seeded into 100-mm culture dishes at a ratio of 1:3 to 1:6 and grown at 37° in a humidified atmosphere of 5% CO_2. The medium was changed every three days. C2C12 cells were subcultured at 70% of confluence. For incubation of the cells in the absence of carnitine, the fetal bovine serum was dialyzed against PBS (1:1000, twice).

Cell Growth

Viable cells were numbered after trypan blue exclusion coloration.

Carnitine Transport Measurement

Carnitine uptake studies were carried out at 37°. When using C2C12 cells, the incubation was done in 250 μL of Hanks' buffer; for isolated myotubes, the incubation buffer contained 20 mM Tris/HEPES, pH 7.5 and 150 mM NaCl. Both media contained radiolabeled and cold carnitine. After a 1-hr incubation, myotubes were spun down and the radioactive medium removed, the cells were washed 4 times with ice-cold PBS, and the radioactive material was recovered on a DAWP0.65 Millipore membrane and counted for radioactivity. Non-specific carnitine binding or transport was evaluated with blanks containing 10 mM cold L-carnitine. Active carnitine transport was calculated as the difference between total transport activity and specific transport activity. Under our conditions, non-specific binding/transport always represented less than 5% of total activity.

Statistics

Experimental conditions were performed (at least) in triplicate. The result of each experiment is given as the mean ± SE. Statistical analyses were performed using the Student's *t*-test.

RESULTS

Characterization of Carnitine Transport in Isolated Rat Myotubes

NORMAL COURSE IN ISOLATED MYOTUBES. The uptake of tritiated L-carnitine was inhibited in the absence of sodium ions (Fig. 1). When potassium ion was substituted for Na ion, a 41% inhibition was observed, and when osmolarity was kept constant by substituting sodium by choline, a 59% inhibition was found. The role of the counter ion was also investigated. It appears that, in myotubes, sodium chloride and sodium carbonate, did not affect carnitine uptake (Table 1), while sodium glucuronate induced a 46% decrease in carnitine transport. The optimal pH for the transport was found to be 7.5 (Fig. 1). In the presence of 150 mM NaCl, increasing concentrations of carnitine showed 2 asymptotical changes in the curve, suggesting a 2-component system, one with high affinity and a second with low affinity (Fig. 2). The K_m and V_{max} values for the high-affinity system were 6.4 μM and 7.0 fmol/hr/mg protein, while for the low-affinity system the K_m was 43.4 μM and the V_{max} 27.2 fmol/hr/mg protein. Both systems appeared to be saturable. Most of this study was performed on the high-affinity system. Ouabain is a potent inhibitor of Na^+/K^+ ATPase. When ouabain (0.5 mM) was added to the incubation mixture, a 48% decrease in high-affinity transporter activity was observed (Table 1). Both decanoyl and octanoyl carnitine (0.5–1 mM) reduced high-affinity transporter activity (by 82 and 79%, respectively). Carnitine analogues such as D-carnitine or γ-butyrobetaine inhibited high-affinity transporter activity (Table 1). In the presence of γ-butyrobetaine, the transport activity represented less than 8% of the regular transport activity.

EFFECT OF MILDRONATE ON CARNITINE TRANSPORT IN ISOLATED MYOTUBES. Mildronate (3-(2,2,2-trimethylhydrazinium)propionate) is a carnitine analogue known to be a carnitine biosynthesis inhibitor. As shown in Fig. 3, mildronate was found to be a very potent inhibitor of

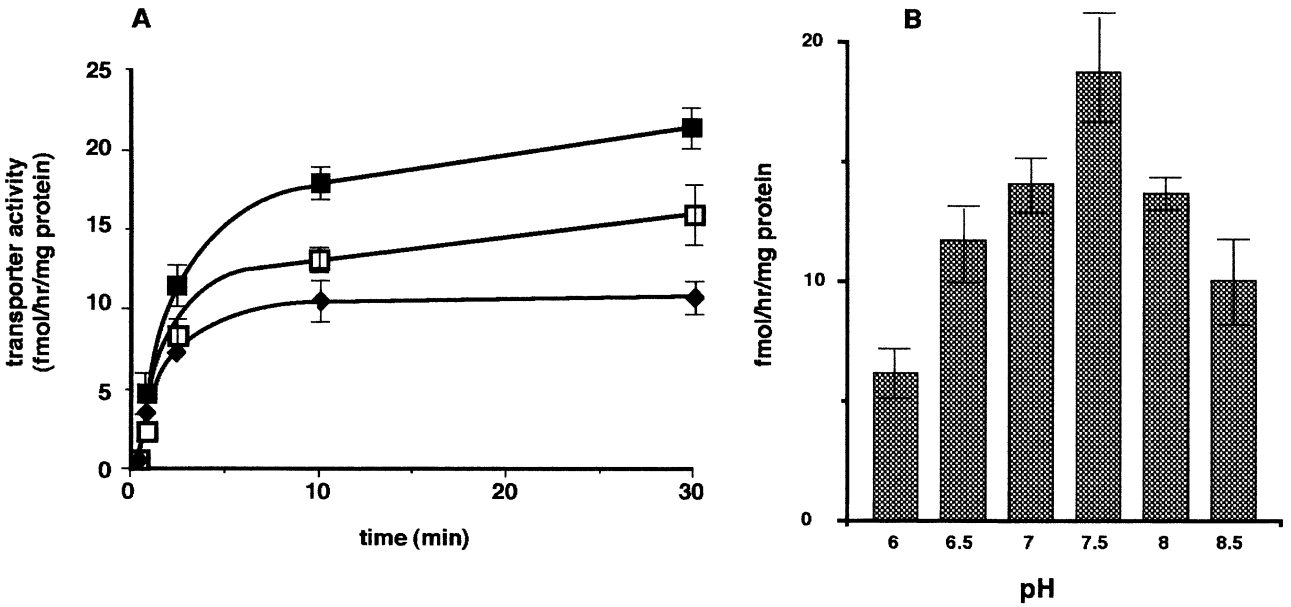

FIG. 1. Normal time–course and influence of pH on L-carnitine uptake by isolated myotubes. (A) Rat myotubes were incubated (from 0 to 30 min) in a reaction mixture containing 150 mM NaCl (■), 150 mM KCl (□), or 150 mM choline (◆). (B) Rat myotubes were incubated at various pH values (6–8.5). The incubation was performed in the presence of 150 mM NaCl buffered with Tris/HEPES. For these assays, carnitine was added at a 60-μM concentration, and the amount of labeled carnitine was at a concentration of 5 nCi. Transporter activity was determined as the amount of carnitine that crosses the membrane and was expressed in fmol/hr/mg protein. Each experiment was done at least three times and the symbols represent the means and the SE.

carnitine transport. It interacted with both the low- and high-affinity transport systems as a non-competitive reagent, since the V_{max} decreased and the K_m remained unchanged. An almost full inhibition of the transport was observed at a mildronate concentration of 0.5 mM.

TABLE 1. Effect of counter ions and acyl carnitine on carnitine transport into isolated myotubes

Compound	Carnitine transport (%) (N)
Sodium chloride	100.0
Sodium carbonate	100.3 ± 5.5 (3)
Sodium glucuronate	54.3 ± 3.2 (3)
Control	100.0
Decanoyl carnitine (0.5 mM)	51.8 ± 2.7 (3)
Decanoyl carnitine (1 mM)	41.9 ± 4.8 (3)
Octanoyl carnitine (0.5 mM)	57.2 ± 4.5 (3)
Octanoyl carnitine (1 mM)	23.0 ± 2.3 (3)
Control	100.0
D-carnitine (0.5 mM)	78.2 ± 6.5 (4)
γ-Butyrobetaine (0.5 mM)	7.3 ± 1.8 (4)
Ouabain (0.5 mM)	52.3 ± 3.3 (4)

Transport activity was determined in isolated rat mytotubes. Ions, acyl carnitine, or ouabain were added to the reaction mixture. Activity of the transporter was determined and expressed in % of the activity of untreated cells. Each value represents the mean and the SE of at least three experiments. The number of experiments is indicated in parentheses.

Characterization of Carnitine Transport in Isolated Membranes

The kinetics of carnitine transport in membranes isolated from rat myotubes was as follows. Carnitine binding and/or transport was not increased when the incubation time was prolonged (Fig. 4). It even slowed down after more than 90 min of incubation.

Characterization of Carnitine Transport in Myoblastic C2C12 Cells

NORMAL COURSE IN CULTURED C2C12 CELLS. C2C12 cells were used in this series of experiments to study the long-term effect of mildronate on muscle cell growth and differentiation. C2C12 cells are myoblastic cells capable of multiplication and differentiation while growing. Carnitine transport activity again showed a dual transport system, one for low concentrations of carnitine and the second for high concentrations of carnitine. Normal kinetic values for carnitine were determined on these cells: a K_m of 7.7 μM and a V_{max} of 11.2 fmol/hr/mg protein for the high-affinity system, and a K_m value of 53.4 μM with a V_{max} of 27.8 fmol/hr/mg protein for the low-affinity system. As in myotubes, the transport was shown to be Na^+-dependent (data not shown).

EFFECT OF MILDRONATE ON CARNITINE TRANSPORT ACTIVITY IN C2C12 CELLS. A 1-hr incubation of C2C12 cells in the presence of mildronate resulted in a significant decrease in carnitine transport activity. As for myotubes, the kinetic

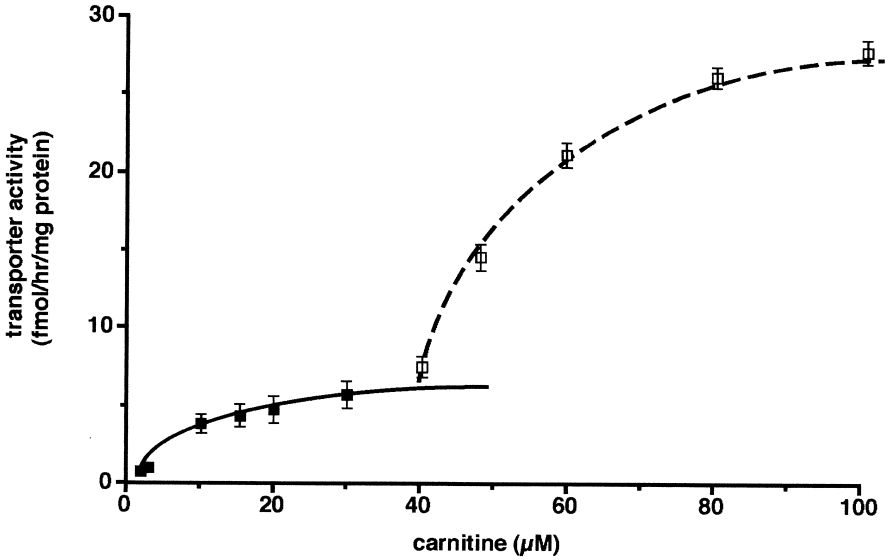

FIG. 2. Kinetics of L-carnitine transport into isolated myotubes. Rat myotubes were incubated in the presence of various concentrations of L-carnitine (1 to 100 μM). The concentration of tritiated L-carnitine in the medium was 12.5 pmol/assay (62.5 fmol/μL). Transporter activity was expressed in fmol/hr/mg protein. The empty squares and the dotted curve represent the low-affinity transport, and the full squares and plain curve the high-affinity transport. Each symbol represents the means and the SE of at least three experiments. For these measurements, the ratio between the specific activity of labeled carnitine and cold carnitine was taken in account.

properties showed a non-competitive inhibition (Fig. 5) for both transport systems. Adding mildronate to the culture medium induced a marked decrease in transport activity. After 72 hr of incubation, high-affinity activity was reduced to 6% of the initial values (Table 2) when 50 mM mildronate was used.

Carnitine and C2C12 Cell Growth

EFFECT OF INCREASING CONCENTRATIONS OF CARNITINE ON C2C12 CELL GROWTH. C2C12 cells were cultured in DMEM supplemented with dialyzed fetal bovine serum and various concentrations of carnitine or mildronate. After one or three days of culture, cells were harvested and counted. Cells cultured in a medium with dialyzed fetal bovine serum showed a slower growth. The number of cells was significantly decreased in a carnitine-free medium. This effect was already seen after one day of treatment. After three days of culture, the number of viable cells in the plate decreased by 20% compared to cells cultured in the presence of total fetal bovine serum. This suggested that carnitine may be required for cell growth. Indeed, the addition of carnitine at a concentration of 60 μM (the physiological concentration) to the medium containing dialyzed fetal bovine serum restored the cell number to that of cells cultured with full fetal bovine serum (Fig. 6).

EFFECT OF MILDRONATE ON C2C12 GROWTH. Mildronate was added to the medium at concentrations ranging from 20 μM to 50 mM. At 20 μM mildronate and after three days of treatment, the cell number was decreased by 22%. This effect was dose-dependent. For instance, at 50 μM, the percentage of inhibition was found to be 35%. At a mildronate concentration of 50 mM, the cell number went down to less than 15%

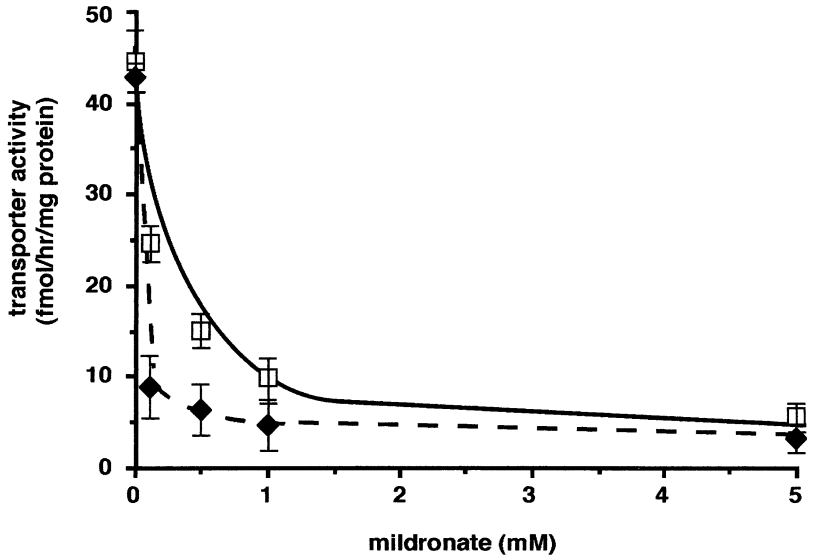

FIG. 3. Effect of mildronate on carnitine transport into isolated rat myotubes. Myotubes were incubated in the presence of various concentrations of mildronate (0 to 5 mM) and at two concentrations of carnitine: 10 μM (□ and plain curve) and 50 μM (◆ and dotted line). Carnitine transport activity was expressed in fmol/hr/mg protein. Means of three experiments are given ± SE.

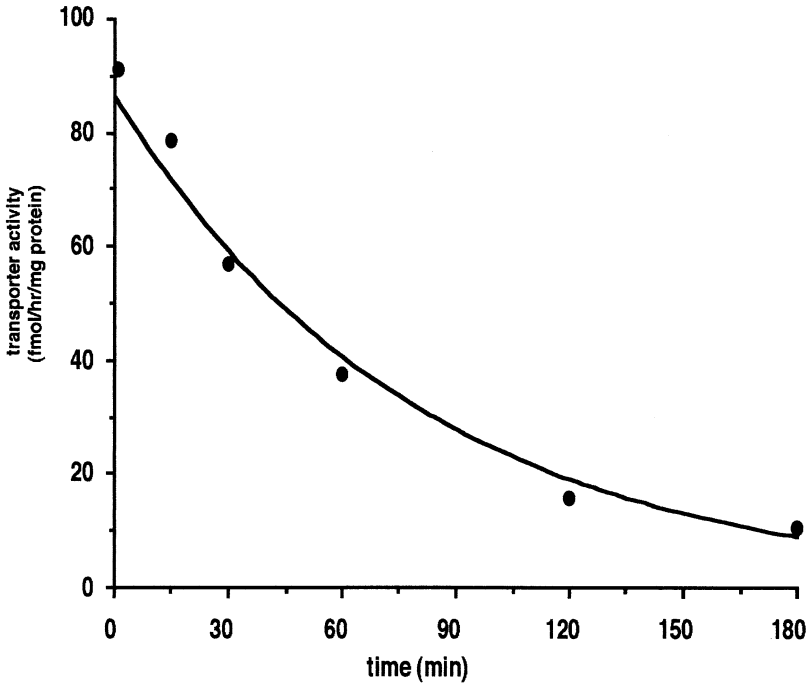

FIG. 4. Time–course of L-carnitine binding and/or transport in plasma membranes isolated from myotubes. For binding and partial transport measurement, the incubation buffer contained 150 mM NaCl and 20 mM Tris/HEPES pH 7.5. Protein (12.5 mg) was added to the mixture, and the amount of radioactive material was determined in these membranes and expressed in fmol/hr/mg protein. In these experiments, the concentration of carnitine was 60 μM. The transporter activity is expressed as fmol of carnitine bound to membrane per mg of protein and per hour.

of that of control (Fig. 6). At that concentration, mildronate could be toxic, since the number of viable cells was lower than the number of cells plated. We found a good correlation ($r > 0.98$) between the log [concentration of mildronate] and cell growth. The IC_{50} was determined after 1 and 3 days of treatment, these values being 920 and 910 μM, respectively.

DISCUSSION

Our results showed that carnitine transport into muscle cells (myotubes and myoblasts) was dependent upon the presence of sodium salt. This Na^+-dependent carnitine transport is not specific for rodent muscle cells, since the

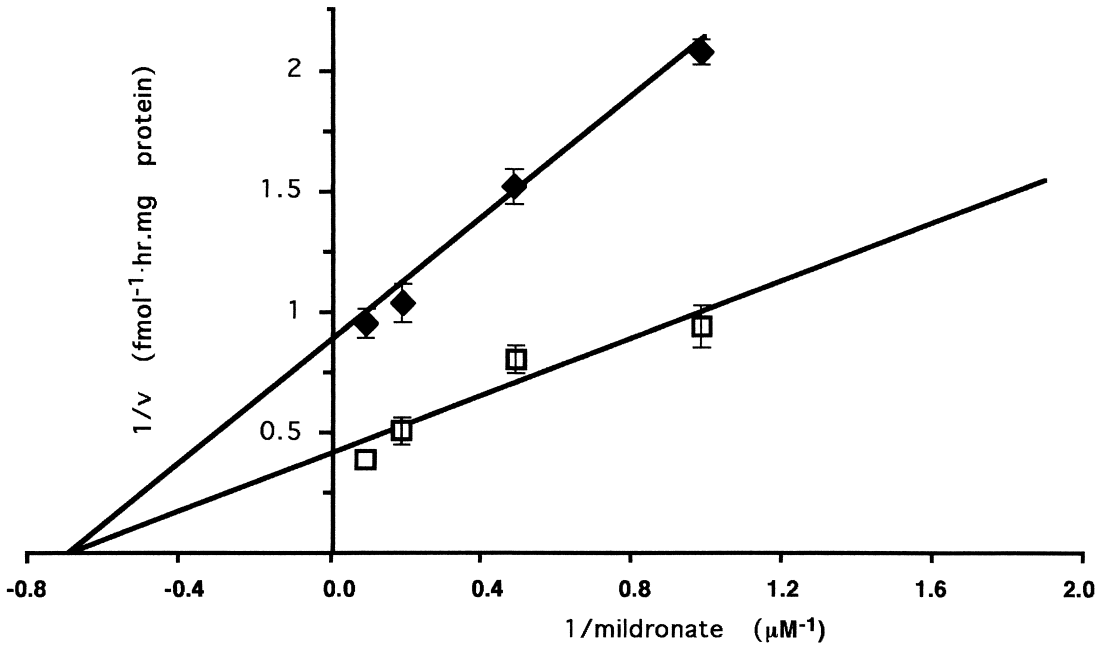

FIG. 5. Effect of mildronate on carnitine transporter activity in C2C12 cells. C2C12 cells were incubated in the presence of various concentrations of L-carnitine (1 to 100 μM) (□). Mildronate was added at a concentration of 20 mM (◆). K_m and V_{max} values were estimated by the double transformation of Lineweaver–Burk. Each symbol represents the means and the SE of at least three experiments.

TABLE 2. Effect of mildronate on carnitine transporter activity

	Transporter activity (%)
Day 1	
Control	100.0
Mildronate 20 μM	102.3 ± 5.5 (4)
Mildronate 50 μM	97.5 ± 6.2 (4)
Mildronate 20 mM	36.8 ± 3.3 (4)
Mildronate 50 mM	8.8 ± 1.6 (4)
Day 3	
Control	100.0
Mildronate 20 μM	99.0 ± 7.7 (4)
Mildronate 50 μM	103.6 ± 2.0 (4)
Mildronate 20 mM	13.8 ± 4.9 (4)
Mildronate 50 mM	5.6 ± 0.9 (4)

Mildronate was added at several concentrations (20 μM–50 mM) to the culture medium. The transporter activity was determined after 1 and 3 days of treatment. Each value is expressed in percentage of reference culture. The means and the SE of independent experiments are indicated in the table. The number of experiments is in parentheses.

same mechanism has been described for various tissues including intestine [12], kidney [13], neuroblastoma NB2a cells [14], and choriocarcinoma cells [15]. We did not find any Na^+-independent carnitine uptake in these cellular models, contrary to what was described in lactating mammary tissue [16]. As had been previously described for cultured muscle cells [8], carnitine uptake showed a dual transport mechanism, one with high affinity, the second at low affinity. Both systems are dependent on the presence of Na^+ ions and are saturable. The same results were found for isolated myotubes and cultured C2C12 cells.

In plasma membranes isolated from myotubes, i.e. in the absence of the cytosolic compounds, carnitine binding appeared to be quickly saturable. The kinetics did not show any time-dependent binding activity. These data suggest that cytosolic or membrane-associated activities are required for full carnitine uptake activity. Among these proteins, the role of tyrosine kinase or Na^+/K^+ ATPase is worthy of attention, since genistein has been described as

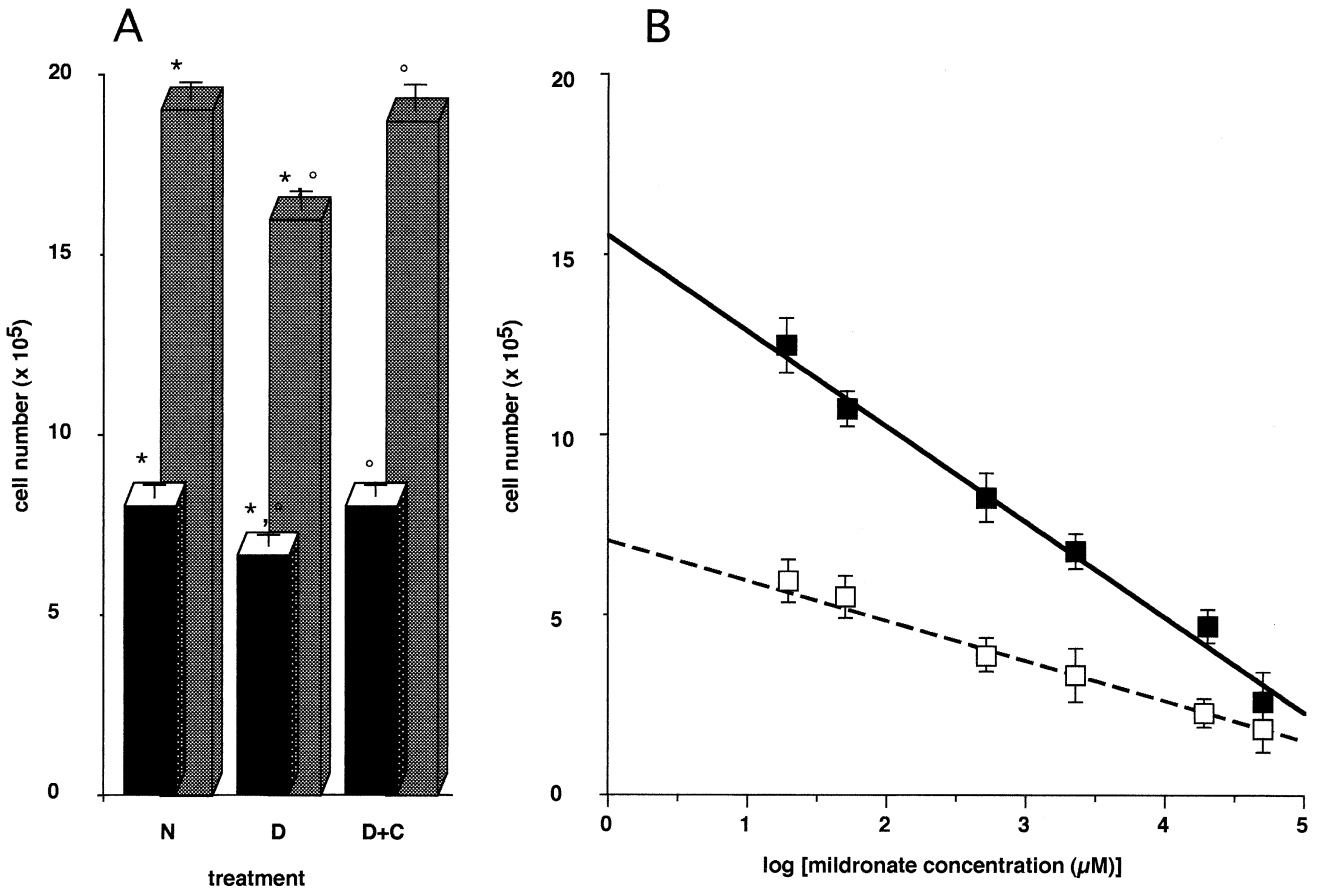

FIG. 6. Effect of carnitine and mildronate on C2C12 cell growth. C2C12 cells (4.10^5 cells) were plated onto 225-mm^2 culture dishes. After attachment, cells were either incubated in the presence of a regular medium containing full fetal bovine serum (N), dialyzed fetal bovine serum (D), or dialyzed serum supplemented with 60 μM carnitine (D + C) (Panel A). Cells were also treated with increasing concentrations of mildronate (20 μM–50 mM) in a medium containing dialyzed fetal bovine serum (Panel B). Both after one or three days of treatment, cells were harvested and viable cells were counted. In Panel A, boxes represent the average, and bars the SE. Black histograms are representative of the data obtained after one day of treatment, while gray histograms correspond to the data collected after three days of treatment. Symbols (* and °) represent significant difference ($P = 0.05$). In panel B, the plain line and full squares represent the action of mildronate on C2C12 growth after 3 days of treatment, and the dashed line (and empty squares), the same effect after only one day of treatment. Bars represent the SE.

inhibiting carnitine transport in S49 cells [17] and ouabain was able to decrease this transport in our experiments. However, the absence of total inhibition after these treatments suggested that other forms of regulation might be involved.

Carnitine transport was also inhibited by carnitine analogues, with mildronate being the most potent, acting as it did as a non-competitive inhibitor of carnitine transport. This inhibitory activity was exerted on both high- and low-affinity transporters. Mildronate is also known as an inhibitor of the enzyme responsible for carnitine synthesis through a non-competitive mechanism [18], but does not compete with carnitine in the carnitine palmitoyl transferase system [19]. Thus, it appears that this compound inhibits both carnitine biosynthesis and carnitine transport into muscle cells.

C2C12 myoblastic cell growth appeared to be dependent upon the presence of carnitine in the medium. In cultured C2C12 cells, the absence of carnitine in the culture medium slowed cell growth. The same effect was observed when these cells were treated with mildronate, which was able to decrease cell growth in a dose-dependent manner. Thus, it appeared that, while reducing carnitine transport, mildronate induced a decrease in the growth rate of these cells. This inhibition is very likely to be related to a decrease in fatty acid oxidation [20].

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References

1. Rebouche CJ and Paulson DJ, Carnitine metabolism and function in humans. *Annu Rev Nutr* **6:** 41–66, 1986.
2. Rebouche CJ and Engel AG, Tissue distribution of carnitine biosynthesis enzymes in man. *Biochim Biophys Acta* **630:** 22–29, 1980.
3. Rebouche CJ, Bosch EP, Chenard CA, Schabold KJ and Nelson SE, Utilization of dietary precursors for carnitine synthesis in human adults. *J Nutr* **119:** 1907–1913, 1989.
4. Siliprandi N, Sartorelli L, Ciman M and Di Lisa F, Carnitine: Metabolism and clinical chemistry. *Clin Chim Acta* **183:** 3–17, 1989.
5. Pons R, Carrozzo R, Tein I, Walker WF, Addonizio LJ, Rhead W, Miranda AF, Dimauro S and De Vivo DC, Deficient muscle carnitine transport in primary carnitine deficiency. *Pediatr Res* **42:** 583–587, 1997.
6. Nezu J, Tamai I, Oku A, Ohashi R, Yabuuchi H, Hashimoto N, Nikaido H, Sai Y, Koizumi A, Shoji Y, Takada G, Matsuishi T, Yoshino M, Kato H, Ohura T, Tsujimoto G, Hayakawa J, Shimane M and Tsuji A, Primary systemic carnitine deficiency is caused by mutations in a gene encoding sodium ion-dependent carnitine transporter. *Nat Genet* **21:** 91–94, 1999.
7. Tang NL, Ganapathy V, Wu X, Hui J, Seth P, Yuen PM, Fok TF and Hjelm NM, Mutations of OCTN2, an organic cation/carnitine transporter, lead to deficient cellular carnitine uptake in primary carnitine deficiency. *Hum Mol Genet* **8:** 655–660, 1999.
8. Martinuzzi A, Vergani L, Rosa M and Angelini C, L-carnitine uptake in differentiating human cultured muscle. *Biochem Biophys Acta* **1095:** 217–222, 1991.
9. Freshney R, *Culture of Animal Cells: A Manual of Basic Technique* (AR Liss, New York, 1987.)
10. Dombrowski L, Roy D, Marcotte B and Marette A, A new procedure for isolation of plasma membranes, T tubules, and internal membranes from skeletal muscle. *Am J Physiol* **270:** 667–676, 1996.
11. Arkesteijn CL, A kinetic method for serum 5′-nucleotidase using stabilized glutamate dehydrogenase. *J Clin Chem Clin Biochem* **14:** 155–158, 1976.
12. McCloud E, Ma TY, Grant KE, Mathis RK and Said HM, Uptake of L-carnitine by a human intestinal epithelial cell, line Caco-2. *Gastroenterology* **111:** 1534–1540, 1996.
13. Rebouche CJ and Mack DL, Sodium gradient-stimulated transport of L-carnitine into renal brush border membrane vesicles: Kinetics, specificity, and regulation by dietary carnitine. *Arch Biochem Biophys* **235:** 393–402, 1984.
14. Nalecz KA, Korzon D, Wawrzenczyk A and Nalecz MJ, Transport of carnitine in neuroblastoma NB-2a cells. *Arch Biochem Biophys* **322:** 214–220, 1995.
15. Prasad PD, Huang W, Ramamoorthy S, Carter AL, Leibach FH and Ganapathy V, Sodium-dependent carnitine transport in human placental choriocarcinoma cells. *Biochim Biophys Acta* **1284:** 109–117, 1996.
16. Shennan DB, Grant A, Ramsay RR, Burns C and Zammit VA, Characteristics of L-carnitine transport by lactating rat mammary tissue. *Biochim Biophys Acta* **1393:** 49–56, 1998.
17. Gustafson B and Ransnas LA, Regulation of carnitine binding to plasma membranes by an ATP-dependent mechanism. *Biochem Biophys Res Commun* **231:** 249–253, 1997.
18. Simkhovich BZ, Shutenko ZV, Meirena DV, Khagi KB, Mezapuke RJ, Molodchina TN, Kalvins IJ and Lukevics E, 3-(2,2,2-Trimethylhydrazinium)propionate (THP)—A novel gamma-butyrobetaine hydroxylase inhibitor with cardioprotective properties. *Biochem Pharmacol* **37:** 195–202, 1988.
19. Tsoko M, Beauseigneur F, Gresti J, Niot I, Demarquoy J, Boichot J, Bezard J, Rochette L and Clouet P, Enhancement of activities relative to fatty acid oxidation in the liver of rats depleted of L-carnitine by D-carnitine and a gamma-butyrobetaine hydroxylase inhibitor. *Biochem Pharmacol* **49:** 1403–1410, 1995.
20. Tang NL, Hui J, Law LK, To KF, Ruiter JP, Ijlst L, Wanders RJ, Ho CS, Fok TF, Yuen PM and Hjelm NM, Primary plasmalemmal carnitine transporter defect manifested with dicarboxylic aciduria and impaired fatty acid oxidation. *J Inherit Metab Dis* **21:** 423–425, 1998.