Neurochemical Research, Vol. 14, No. 5, 1989, pp. 489–493

Uptake of Acetyl-L-Carnitine in the Brain

Alessandro P. Burlina,[1,2] Henry Sershen,[1] Edmund A. Debler,[1] and Abel Lajtha[1,3]

(Accepted January 30, 1989)

Analysis in mouse brain slices of the uptake of acetyl-L-[N-*methyl*-^{14}C]carnitine with time showed it to be concentrative, and kinetic analysis gave a K_m of 1.92 mM and a V_{max} of 1.96 μmol/min per ml, indicating the presence of a low-affinity carrier system. The uptake was energy-requiring and sodium-dependent, being inhibited in the presence of nitrogen (absence of O_2), sodium cyanide, low temperature (4°C), and ouabain, and in the absence of Na^+. The uptake of acetyl-L-carnitine was not strictly substrate-specific; γ-butyrobetaine, L-carnitine, L-DABA, and GABA were potent inhibitors, hypotaurine and L-glutamate were moderate inhibitors, and glycine and β-alanine were only weakly inhibitory. In vivo, acetyl-L-carnitine transport across the blood-brain barrier had a brain uptake index of 2.4 ± 0.2, which was similar to that of GABA. These results indicate an affinity of acetyl-L-carnitine to the GABA transport system.

KEY WORDS: Acetyl-L-carnitine; GABA; transport; mouse brain slices.

INTRODUCTION

Recently attention has been focused on the effect of carnitine (3-hydroxy-4-N-trimethylaminobutyric acid) and its naturally occurring derivative acetyl-L-carnitine on the central nervous system (1). Like L-carnitine, acetyl-L-carnitine and the acetyl-L-carnitine synthesizing enzyme carnitine acetyltransferase are present in various cerebral regions (2–4). The importance of carnitine and its ester derivatives in fatty acid metabolism in such tissue as myocardial and skeletal muscle is well established (5, 6), but their function in nervous tissue, which is predominantly dependent on glucose for energy production, is still unknown. Because of its structural similarity to acetylcholine, acetyl-L-carnitine is thought to be involved in cholinergic transmission (7). Microiontophoretic studies of cortical neurons demonstrated cholinergic effects for acetyl-L-carnitine, which were blocked by atropine (8, 9).

Carnitine uptake in rat brain slices has already been investigated (10), but the mechanism by which acetyl-L-carnitine is supplied to the brain is not known. We therefore investigated whether acetyl-L-carnitine uptake is a mediated process and whether acetyl-L-carnitine interacts with the transport of other metabolites in the brain.

In the present study, we describe the specificity and other characteristics of acetyl-L-carnitine uptake in mouse brain slices. Active uptake of acetyl-L-carnitine and inhibition of this uptake by GABA and its analogues are demonstrated. We also measured in vivo acetyl-L-carnitine uptake across the blood-brain barrier in adult rats.

EXPERIMENTAL PROCEDURE

Materials. Acetyl-L-carnitine was a gift from Sigma-Tau Pharmaceutical Co., Pomezia (Rome), Italy. Acetyl-L-[N-*methyl*-^{14}C]carnitine hydrochloride was purchased from Amersham Corporation, Arlington Heights, IL.

Acetyl-L-Carnitine Uptake into Brain Slices. Adult female BALB/cBy mice were killed by decapitation, their brains were quickly removed, and cerebral hemispheres were sliced with a McIlwain tissue chopper. Slices of 0.42-mm thickness were incubated at 37°C in a HEPES-buffered medium containing 119 mM NaCl, 5.0 mM KCl,

[1] The Nathan S. Kline Institute for Psychiatric Research, Center for Neurochemistry, Ward's Island, New York, NY 10035.

[2] Istituto delle Malattie Nervose e Mentali, Universitá di Padova, Italy.

[3] To whom reprint requests should be addressed.

0364–3190/89/0500–0489$06.00/0 © 1989 Plenum Publishing Corporation

0.75 mM $CaCl_2$, 1.2 mM $MgSO_4$, 1.0 mM NaH_2PO_4, 1.0 mM $NaHCO_3$, 25 mM HEPES, 12 mM NaOH, and 10 mM glucose with a pH of 7.4, as previously described (11). After a 15-min preincubation, ^{14}C-labeled acetyl-L-carnitine was added to the medium. The final incubation time was 60 min. The metabolic inhibitors were present during the entire incubation period. Sodium and glucose in the medium where noted were replaced by iso-osmolar amounts of sucrose. The structural analogues and other amino acids were added immediately before the labeled substrate. After the uptake period, the slices were filtered, frozen, weighed, homogenized in perchloric acid (5% w/v), and centrifuged. Radioactivity of the perchloric acid extract was determined.

Acetyl-L-Carnitine Transport Across the Blood-Brain Barrier. Transport of acetyl-L-carnitine across the blood-brain barrier was measured by comparing the passage of [^{14}C]acetyl-L-carnitine to that of a [^3H]water reference from the capillary circulation (12). Adult Wistar rats (170 g) were anesthetized with chloral hydrate (380 mg/kg), the left carotid artery was exposed, and a mixture of 0.5 μCi of [^{14}C]acetyl-L-carnitine and 2 μCi of [^3H]water in 0.2 ml of HEPES-buffered medium was injected into the left carotid artery. The rat was decapitated 15 sec after injection. The left cerebrum was removed and homogenized in 2 ml of perchloric acid (5% w/v), and radioactivity was determined in the clear supernatant.

Calculations. The uptake of the labeled compound was expressed as concentrative uptake (CU), calculated according to the following equation:

$$CU = IC\text{-}CW,$$

where IC is the concentration in the intracellular water and CW is the concentration remaining in the extracellular water (medium) (11). The kinetics of the transport was derived from Lineweaver-Burk plots by a data analysis program (ENZFITTER, Elsevier Biosoft). The statistical significance of differences between two means was assessed by Student's independent two-tailed *t*-test. The brain uptake index (BUI) was calculated as tissue $^{14}C/^{3}H$ ratio divided by the $^{14}C/^{3}H$ ratio of the solution used for injection times 100 (12).

RESULTS

Time Curve of Uptake. The time curve of acetyl-L-carnitine uptake was determined by measuring the uptake of the ^{14}C-labeled compound added to the incubation medium to obtain a final concentration of 1 mM. Uptake was initially linear followed by a steady-state phase, showing that uptake is concentrative (Figure 1).

Kinetics of uptake. Concentrative uptake of acetyl-L-carnitine into brain slices incubated for 60 min with increasing concentrations of Acetyl-L-[N-*methyl*-^{14}C]carnitine from 0.5 to 10 mM obeys Michaelis-Menten kinetics and is apparently a saturable process. Lineweaver-Burk plots of these data show a K_m of 1.92 mM and a V_{max} of 1.96 μmol/min per ml CU (Figure 2).

Effect of Metabolic Inhibitors. Table I shows that Acetyl-L-[N-*methyl*-^{14}C]carnitine uptake was inhibited when brain slices were incubated in an N_2 atmosphere. The metabolic inhibitors sodium cyanide and ouabain

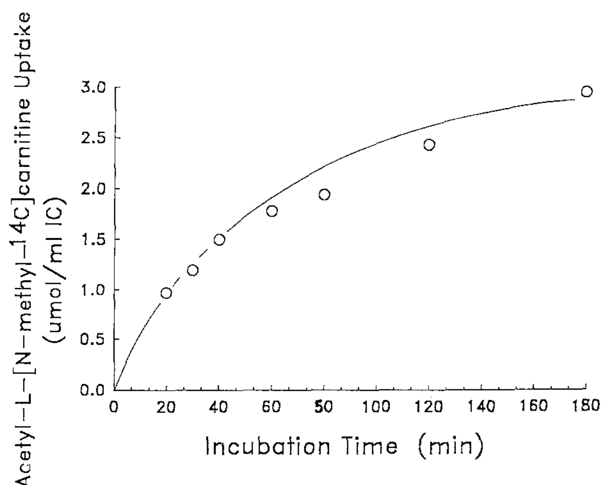

Fig. 1. Time curve of acetyl-L-carnitine uptake in mouse brain cortex slices, expressed as concentration in the intracellular water (IC). Slices were incubated and tissue acetyl-L-carnitine concentration was calculated as described in the text. 1 mM Acetyl-L-[N-*methyl*-^{14}C]carnitine. Each point is mean ± SEM (n = 3).

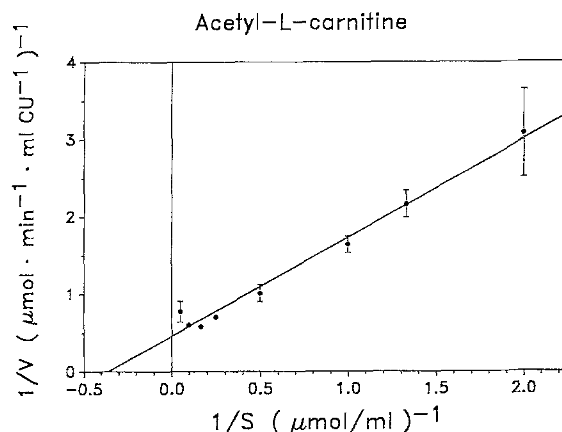

Fig. 2. Lineweaver-Burk plot of acetyl-L-carnitine uptake. The kinetic parameters of transport derived from this plot were as follows: K_m = 1.92 mM, V_{max} = 1.96 μmol/min per ml CU, where CU is the concentrative uptake (concentration in the intracellular water minus the concentration remaining in the extracellular water (medium)).

completely inhibited uptake. The uptake was also temperature dependent, since it was completely inhibited at 4°C. Acetyl-L-carnitine uptake was reduced when slices were incubated in a glucose-free medium.

Effect of Ionic Composition of the Medium. The absence of extracellular sodium ions reduced acetyl-L-carnitine uptake, whereas the increase of calcium ions in the medium did not significantly affect the uptake (Table II).

Table I. Effect of Metabolic Inhibitors on Acetyl-L-Carnitine Uptake in Mouse Brain Slices

Inhibitor or Condition	% Inhibition of acetyl-L-carnitine uptake
Control	–
Anoxia (Nitrogen)	81 ± 10
NaCN (1 mM)	90 ± 1
Ouabain (0.5 mM)	>100
4°C	>100
Minus glucose	28 ± 1

Brain slices were preincubated for 15 min in medium containing the inhibitor; then 50 μM acetyl-L-[N-*methyl*-^{14}C]carnitine was added and the incubation continued for an additional 60 min. Data are expressed as mean ± SEM (n = 3) of inhibition of concentrative uptake (uptake above medium levels).

Table II. Effect of Ionic Composition of the Medium on Acetyl-L-Carnitine Uptake in Mouse Brain Slices

Ionic Condition	% Inhibition of acetyl-L-carnitine uptake
Control	–
No Na^+	60 ± 5
Ca^{2+} (2.5 mM)	20 ± 4

Brain slices were incubated as described in text in HEPES-buffered medium (pH = 7.4) for 60 min at 37°C with 50 μM acetyl-L-[N-*methyl*-^{14}C]carnitine. Sodium cation was replaced by equimolar sucrose. Data are expressed as mean ± SEM (n = 3-6) of inhibition of concentrative uptake.

Table III. Inhibition of Acetyl-L-Carnitine Uptake in Mouse Brain Slices

Effector	% Inhibition of acetyl-L-carnitine uptake
None	–
L-Carnitine	94 ± 5
γ-Butyrobetaine	>100
L-DABA	82 ± 4
GABA	69 ± 4
Hypotaurine	52 ± 8
L-Glutamic Acid	43 ± 4
β-Alanine	32 ± 2
Glycine	25 ± 2
Taurine	15 ± 13
Choline	6 ± 3
L-Lysine	0.5 ± 0.5

Brain slices were preincubated for 15 min; then 5 mM effector and 50 μM acetyl-L-[N-*methyl*-^{14}C]carnitine were added and the incubation was continued for an additional 60 min. Data are expressed as mean ± SEM (n = 3-6) of inhibition of concentrative uptake.

Effect of Compounds With Related Structure (Table III). It is assumed that compounds with affinity to the same carrier interfere with each other's uptake. Of the compounds tested, the greatest inhibition was seen with γ-butyrobetaine, followed by L-carnitine, L-DABA, and GABA. Hypotaurine and L-glutamic acid were moderate inhibitors, β-alanine and glycine rather weak ones. Taurine, L-lysine, and choline were without significant effect.

Acetyl-L-Carnitine Transport Across the Blood-Brain Barrier. Uptake from the circulation through the brain was relatively slow—close to the sensitivity of detection by the method: about 2% of the compound passing through the brain during a single passage was taken up into the brain, resulting in a brain uptake index of 2.4 ± 0.2. (n = 4; data not shown).

DISCUSSION

Previous studies have shown the existence of numerous transport systems in the brain, among them a number of systems for amino acids. In slice preparations, by measuring primarily cellular low-affinity uptake, at least 10 systems could be distinguished for amino acids (11). Recent studies, such as those with synaptic vesicles (13), indicate the presence of several others. Some of the systems are fairly specific for one or a few substrates (11), others have affinity for a family of substrates. The systems are not uniformly distributed—many are absent from cerebral capillaries and do not participate in blood-brain barrier transport (12).

For some neurotransmitter amino acids, such as glutamate, several uptake systems are present: some of low affinity (11, 14) and some of high affinity; some specific for glutamate and aspartate (11), or glutamate alone (15, 16); some Na^+ dependent (17), others Na^+ independent or requiring Cl^- (18).

In the present study we showed the existence of a carrier-mediated active transport system for acetyl-L-carnitine in mouse brain slices. The time curve of acetyl-L-carnitine uptake demonstrated accumulation in the first 120 min, after which it approximated a steady-state phase, and also indicated that the uptake was concentrative. The uptake obeys Michaelis-Menten kinetics indicating saturability. A rather long incubation time (60 min) was used because of the low amount of radioactivity taken up. Values with longer uptake appeared to be more reliable and reproducible. Because of this the true initial uptake rate was not obtained and the V_{max} value was underestimated. The K_m for acetyl-L-carnitine uptake in brain slices (1.92 mM) found in this study indicates the presence of a low-affinity carrier system and was in the range of the K_m reported for L-carnitine uptake (2.85

mM) (10). A high-affinity uptake component was not detected when the concentration range of acetyl-L-carnitine was extended below 0.5 mM (data not shown). The characteristics of acetyl-L-carnitine uptake by brain slices were suggestive of an active transport system. There was uptake against a concentration gradient, and the concentrative uptake component was inhibited by metabolic inhibitors and was abolished at low temperature (4°C).

Acetyl-L-carnitine uptake by brain slices was also abolished in the presence of ouabain, indicating the functional dependency of the (Na^+, K^+) ATPase pump. Moreover, the transport system required the presence of Na^+ in the incubation medium. These properties are similar to those observed for L-carnitine uptake in rat brain slices (10). The uptake was only slightly reduced when glucose was removed from the medium. The explanation is probably that after a long uptake period (i.e., 60 min) brain slices are able to obtain energy from lactate metabolism (19), since uptake is restored in the absence of glucose if other metabolizable substrates, such as lactate, pyruvate, or a mixture of succinate, malate, and pyruvate, are added to the medium (20). Increase of the calcium concentration in the medium did not affect the uptake of acetyl-L-carnitine.

The uptake of acetyl-L-carnitine showed moderate substrate specificity. Lysine, the primary precursor in carnitine synthesis, did not inhibit uptake, whereas L-carnitine completely inhibited it. γ-Butyrobetaine, the non-hydroxylated precursor of carnitine, which also represents one of the possible alternate metabolic pathways for GABA (21), was a good inhibitor. GABA and L-DABA showed strong inhibition of acetyl-L-carnitine uptake, giving some support to the hypothesis of a common carrier.

GABA, L-DABA, and other amino acids tested exhibited a pattern of inhibition similar to that found for uptake of L-carnitine in brain synaptosomes (22). Choline, which lacks the terminal carboxyl group of carnitine, did not have a significant effect on acetyl-L-carnitine uptake, as also reported for L-carnitine uptake in rat brain slices and in mouse brain synaptosomal preparations (10, 22). Since previous findings demonstrated that only the high-affinity transport of choline is specific for cholinergic terminals and is kinetically coupled to acetylcholine synthesis (23), it seems unlikely that the observed low-affinity uptake of acetyl-L-carnitine shares a carrier with choline involved in acetylcholine synthesis. However, this does not rule out the possible involvement of acetyl groups derived from acetylcarnitine in acetylcholine synthesis (24), and a role of acetylcarnitine in cholinergic activity such as acetylcholine release (25).

The low BUI (2.4 ± 0.2) of acetyl-L-carnitine indicates a low penetrance, similar to that of GABA (2.2 ± 0.6) (12), across the blood-brain barrier. Although this indicates slow uptake through the blood-brain interface, it is important to emphasize that compounds can accumulate in the brain even if they are taken up slowly—if they are retained or their rate of exit is lower than their rate of uptake. The uptake inhibition pattern of GABA and L-DABA and the elevation of nigral GABA levels by carnitine and acetyl-L-carnitine (26) further substantiate the involvement of acetyl-L-carnitine in GABAergic systems. The arguement is made, however, that to affect GABA transport relatively high concentrations of carnitine are needed (22), exceeding the normal concentration of carnitine observed in brain (2). If it functions similarly, acetyl-L-carnitine may not have an effect on GABA transport. Although changes in brain GABA and glutathione levels can occur after a high dose of acetyl-L-carnitine (26), these changes can also occur by other mechanisms than an effect on GABA transport. Also, the affinity of acetylcarnitine could be greater at some GABA uptake sites than the average measured in our experiments.

The affinity of acetyl-L-carnitine to GABA transport also indicates that acetylcarnitine may have affinity to the GABA receptor complex. Experiments measuring acetyl-L-carnitine binding specificity are in progress in our laboratory.

ACKNOWLEDGMENT

The grant support of Sigma-Tau Pharmaceutical Company (Rome, Italy) is gratefully acknowledged.

REFERENCES

1. Janiri, L., and Tempesta, E. 1983. A pharmacological profile of the effects of carnitine and acetylcarnitine on the central nervous system. Int. J. Pharm. Res. 3:295–306.
2. Shug, A. L., Schmidt, M. J., Golden, G. T., and Fariello, R. G. 1982. The distribution and role of carnitine in the mammalian brain. Life Sci. 31:2869–2874.
3. McCaman, R. E., McCaman, M. W., and Stafford, M. L. 1966. Carnitine acetyltransferase in nervous tissue. J. Biol. Chem. 241:930–934.
4. Bresolin, N., Freddo, L., Vergani, L., and Angelini, C. 1982. Carnitine, carnitine acetyltranferase and rat brain function. Exp. Neurol. 78:285–292.
5. Bremer, J. 1983. Carnitine—metabolism and functions. Physiol. Rev. 63:1420–1480.
6. Bahl, J. J., and Bressler, R. 1987. The pharmacology of carnitine. Annu. Rev. Pharmacol. Toxicol. 27:257–277.
7. Sass, R. L., and Werness, P. 1973. Acetylcarnitine: on the relationship between structure and function. Biochem. Biophys. Res. Commun. 55:736–742.
8. Falchetto, S., Kato, G., and Provini, L. 1971. The action of

carnitine on cortical neurons. Can. J. Physiol. Pharmacol. 49:1–7.

9. Onofrj, M., Bodis-Wollner, I., Pola, P., and Calvani, M. 1983. Central cholinergic effects of levo-acetylcarnitine. Drugs Exp. Clin. Res. 9:161–169.
10. Huth, P. J., Schmidt, M. J., Hall, P. V., Fariello, R. G., and Shug, S. L. 1981. The uptake of carnitine by slices of rat cerebral cortex. J. Neurochem. 36:715–723.
11. Sershen, H., and Lajtha, A. 1979. Inhibition pattern by analogs indicates the presence of ten or more transport systems for amino acids in brain cells. J. Neurochem. 32:719–726.
12. Oldendorf, W. H. 1971. Brain uptake of radiolabeled amino acids, amines and hexoses after arterial injection. Amer. J. Physiol. 22:1629–1639.
13. Fonnum, F., and Fykse, E-M. 1988. Transmitter amino acid uptake into synaptic vesicles. Trans. Amer. Soc. Neurochem. 19:199.
14. Wheeler, D. D. 1987. Are there both low- and high-affinity glutamate transporters in rat cortical synaptosomes? Neurochem. Res. 12:667–680.
15. Naito, S., and Ueda, T. 1985. Characterization of glutamate uptake into synaptic vesicles. J. Neurochem. 44:99–109.
16. Maycox, P. R., Deckwerth, T., Hell, J. W., and Jahn, R. 1988. Glutamate uptake by brain synaptic vesicles. J. Biol. Chem. 263:15423–15428.
17. Margolis, R., and Lajtha, A. 1968. Ion dependence of amino acid uptake in brain slices. Biochim. Biophys. Acta 163:374–385.
18. Zaczek, R., Balm, M., Arlis, S., Drucker, H., and Coyle, J. T. 1987. Quisqualate-sensitive, chloride-dependent transport of glutamate into rat brain synaptosomes. J. Neurosci. Res. 18:425–431.
19. Schurr, A., West, C. A., and Rigor, B. M. 1988. Lactate-supported synaptic function in the rat hippocampal slice preparation. Science 240:1326–1328.
20. Teller, D. N., Banay-Schwartz, M., DeGuzman, T., and Lajtha, A. 1977. Energetics of amino acid transport into brain slices: effects of glucose depletion and substitution of Krebs cycle intermediates. Brain Res. 131:321–334.
21. Cooper, J. R., Bloom, F. E., and Roth, R. H. 1986. Amino-acid transmitters. Pages 135–136, in Cooper, J. R., Bloom, F. E., and Roth, R. H. (eds.), The Biochemical Basis of Neuropharmacology, Oxford University Press, New York.
22. Hannuniemi, R., and Kontro, P. 1988. L-Carnitine uptake by mouse brain synaptosomal preparations: competitive inhibition by GABA. Neurochem. Res. 13:317–323.
23. Cooper, J. R., Bloom, F. E., and Roth, R. H. 1986. Acetylcholine. Pages 175–178, *in* Cooper, J. R., Bloom, F. E., and Roth, R. H. (eds.), The Biochemical Basis of Neuropharmacology, Oxford University Press, New York.
24. Dolezal, V., and Tucek, S. 1981. Utilization of citrate, acetylcarnitine, acetate, pyruvate and glucose for the synthesis of acetylcholine in rat brain slices. J. Neurochem. 36:1323–1330.
25. Angelucci, L., Imperato, A., Ghirardi, O., Morgan, B., Peschechera, A., Ramacci, M. T., Werrbach-Perez, K., Yang, R., and Perez-Polo, J. R. 1988. Biochemical markers of aging in the rat brain. Trans. Amer. Soc. Neurochem. 19:226.
26. Fariello, R. G., Ferraro, T. N., Golden, G. T., and DeMattei, M. 1988. Systemic acetyl-L-carnitine elevates nigral levels of glutathione and GABA. Life Sci. 43:289–292.