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Action of L-Acetylcarnitine on Different Cerebral Mitochondrial Populations from Hippocampus and Striatum During Aging

Roberto F. Villa[1] and Antonella Gorini[1]

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The maximum rates (V_{max}) of some mitochondrial enzyme activities related to energy transduction (citrate synthase, malate dehydrogenase, NADH cytochrome c reductase, cytochrome oxidase) and amino acid metabolism (glutamate dehydrogenase) were evaluated in non-synaptic (free) and synaptic mitochondria from rat hippocampus and striatum. Three types of mitochondria were isolated from control rats aged 4, 8, 12, 16, 20 and 24 months and treated ones with L-acetylcarnitine (100 mg·kg $^{-1}$, i.p., 60 min). Enzyme activities of non-synaptic and synaptic mitochondria are different in hippocampus and striatum, confirming that a different metabolic machinery exists in various types of brain mitochondria. During aging, enzyme activities behave quite similarly in both areas. In vivo administration of L-acetylcarnitine decreased the enzyme activities related to Krebs' cycle mainly of synaptic mitochondria, suggesting a specific subcellular trigger site of action. The drug increased cytochrome oxidase activity of synaptic and non-synaptic mitochondria, indicating the specificity of molecular interaction with this enzyme.

KEY WORDS: Hippocampus; striatum; energy metabolism; enzymes; aging; L-acetylcarnitine.

INTRODUCTION

Carnitine and/or its esters are the requisite carriers for most of the transmembrane movements of long chain fatty acids. The rate of cerebral long-chain fatty acid oxidation is enhanced when carnitine is added to brain homogenates (1); cerebral mitochondria oxidize acetylcarnitine at rates comparable to other tissues (2, 3), the acetyltransferase activity being present also in the nervous tissue (4).

Pronounced changes in the tissue contents of carnitine and its esters occur in response to a variety of alterations in the metabolic state, especially at mitochondrial level in normal, pathological and aged brain (5–16).

[1]Institute of Pharmacology, Faculty of Science, University of Pavia, Piazza Botta, 11, 27100, Pavia, Italy.

During posthypoglycemic recovery following severe hypoglycemia with isoelectric EEG (17, 18), L-acetylcarnitine increases the reduction in the individual and total phospholipids and fatty acids, concomitant with a reduction of the glycolytic flux by the activity of mitochondrial carnitine acetyltransferases and the operation of a translocase system (12, 15, 19).

These data contribute to the understanding of the action of L-acetylcarnitine at cerebral level. However, the evidence exists for a direct action of L-acetylcarnitine after in vivo administration on the mitochondrial enzyme activities related to energy transduction, but only at selected ages (20). Acute and sub-chronic treatment with various doses of L-acetylcarnitine decreases citrate synthase and glutamate dehydrogenase activities on mitochondria obtained from hippocampus synaptosomes of 8 and 16 weeks-old animals.

Therefore, the aim of the present research was to

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study the effect of acute **in vivo** treatment with L-acetylcarnitine on the maximum rate of some representative cerebral enzymes of the tricarboxylic acid cycle, of the electron transfer chain and of the glutamate metabolism during aging, extending the observations to the 4-24 month life-span of rats.

Because of the heterogeneity of brain mitochondria (20,21,28), these enzyme activities were evaluated in three different mitochondrial populations: (a) non-synaptic mitochondria, namely the population from the perikaryon of the neural cells; (b) synaptic mitochondria, namely the two populations obtained by lysis from the synaptosomes; (c) of the hippocampus and striatum because of the well-known vulnerability to aging of these areas and the effect of L-acetylcarnitine on the hippocampus (20).

EXPERIMENTAL PROCEDURE

Animal Care and Treatments. The experiments were performed on female Sprague-Dawley rats (Cobs-Charles River) aged 4, 8, 12, 16, 20, and 24 months. The animals were selected randomly, kept from birth under standard cycling and caging conditions (temperature: 22 ± 1 °C; relative humidity 60 ± 3%; lighting cycle: 12 hr light and 12 hr darkness; low noise disturbance), fed with a standard pellet diet and water ad libitum and housed three, and subsequently two, per cage. The time course of pharmacological treatment was established by permutation tables.

The animals were treated with intraperitoneal injections of: (a) vehicle only ($NaHCO_3$ 0.8–1.0 M; control animals); (b) single dose of 100 $mg \cdot kg^{-1}$ of L-acetylcarnitine (acute treatment). The substance was injected at 9:00 a.m. and the 24 hr fasted animals were sacrificed 60 min after dosing (10:00 a.m.).

Preparation of Synaptic (Free) and Non-Synaptic (Two Types) Mitochondria. Non-synaptic and synaptic (two types) mitochondria were prepared from hippocampus and striatum by an adaptation to single animals (20) of the basic method of Lai et al. (21). The female rats aged 4 to 24 months of various experimental lots were killed by decapitation and all the subsequent procedures were performed at 0–4°C.

Hippocampus and striatum were isolated (<25 sec) in a refrigerated box at 0–4°C and immediately placed in an isolation medium (0.32 M sucrose, 1.0 mM EDTA-K^+, 10 mM Tris-HCl; pH 7.4). Briefly, the homogenate in the isolation medium was obtained (usually 5 ml final volume) by a Teflon-glass homogenizer (Braun S Homogenizer) and then centrifuged as previously described in details (20). The "crude" mitochondrial pellet containing synaptosomes was resuspended and placed on a discontinuous Ficoll-sucrose gradient and centrifuged as described previously (20,21) in a OTD-65B Sorvall Ultracentrifuge equipped with an AH-650 type rotor. The myelin fraction was sucked off by aspiration, and the purified mitochondrial pellet was resuspended in the isolation medium.

The synaptosomal pellet previously isolated from the Ficoll-sucrose gradient was lysed by resuspension in 5 ml of 6 mM Tris-HCl, pH 8.1 medium and the lysate was then centrifuged; the pellet obtained from lysed synaptosomes was resuspended in 0.6 ml (final volume) and was layered on a Ficoll discontinuous gradient consisting of two layers and centrifuged as described (20,21). At the end of this centrifugation, the upper phase contains the "light mitochondrial fraction" and the pellet the "heavy mitochondrial fraction". The washed pellets were finally resuspended in a small volume (usually 0.4 ml) of the isolation medium.

Enzyme Assays. On the different subcellular fractions, the maximum rates of the following enzyme activities were evaluated: citrate synthase (citrate oxaloacetate-lyase, EC 4.1.3.7) (22); malate dehydrogenase (L-malate:NAD^+ oxidoreductase, EC 1.1.1.37) (23); total NADH-cytochrome c reductase (NADH-cytochrome c oxidoreductase, EC 1.6.99.3)(24); cytochrome oxidase (ferrocytochrome c:oxygen oxidoreductase, EC 1.9.3.1) (25,26); glutamate dehydrogenase (L-glutamate: NAD^+ oxidoreductase deaminating, EC 1.4.1.3) (22). Protein concentration of samples was assayed according to Lowry et al. (27).

Enzyme Activity Calculation and Statistical Analysis. Enzyme activities were measured by graphic recordings for at least 3 min in a double beam recorder spectrophotometer (Perkin-Elmer, 544) and each value was calculated from two blind determination on the same sample. The enzyme specific activity was expressed as: μmoles of substrate transformed × min^{-1} × (mg of mitochondrial protein)$^{-1}$.

The ANOVA test was used to evaluate the interactions between vehicle- and L-acetylcarnitine- treated rats, at each individual treatment time, for each fraction tested and for each individual enzyme activity. Homogeneity of variance was checked by Bartlett's test.

RESULTS

Enzyme activities of mitochondrial fractions from control animals during aging are reported in Table I (hippocampus) and in Table II (striatum). Among the possible statistical comparisons indicated in these Tables, only statistical significance versus 4 month values will be considered here. The complete discussion about the relationships between catalytic enzyme activities up to 24 month of age and their biological significance are intriguing and reported elsewhere (28,29).

In general, these data indicate that in control animals of hippocampus: (a) at each age, the biochemical machinery is differently expressed in non-synaptic respect to synaptic light and heavy mitochondria; (b) the mitochondrial enzyme specific activities of Krebs' cycle (citrate synthase, malate dehydrogenase), related enzyme glutamate dehydrogenase and the electron transfer chain ones (NADH-cytochrome c reductase, cytochrome oxidase) progressively increase with advancing age up to 18 months; (c) the activities of the electron transfer chain enzymes decrease thereafter in non-synaptic and synaptic light mitochondria, showing a biphasic pattern of activity; (d) in the heavy mitochondria, the Krebs' cycle enzymes have lower activities that in other types of mitochondria at all the ages, being the lowest activity recorded at 12 months; (e) the synaptic light mitochondria possess the highest catalytic potentialities of citrate synthase, NADH-cytochrome c reductase and cytochrome oxidase.

Table I. Specific Enzyme Activities (μMoles Substrate·Min^{-1}·Mg of Protein^{-1}) of Indicated Enzymes Assayed on "Free", "Light" and "Heavy" Mitochondrial Fractions from Rat Hippocampus of Control Animals Aged 4, 8, 12, 16, 20, and 24 Months.

	"Free Mitochondrial Fraction"					
Enzymes|Age in Months	4 (a)	8 (b)	12 (c)	16 (d)	20 (e)	24 (f)
Citrate Synthase	0.060 ± 0.005	0.078* 0.006	0.081** 0.004	0.091** 0.008	0.091** 0.007	0.108*** 0.009
Malate Dehydrogenase	0.843 ± 0.044	1.010* 0.039	1.114*** 0.048	1.256*** 0.069	1.305*** 0.086	1.394** 0.160
NADH-cytochrome c Reductase (total)	0.122 ± 0.004	0.128 0.005	0.142** 0.004	0.136 0.005	0.148** 0.006	0.131 0.013
Cytochrome Oxidase	1.072 ± 0.127	1.044 0.079	1.358 0.136	2.304*** 0.232	2.387*** 0.184	1.982*** 0.082
Glutamate Dehydrogenase	0.085 ± 0.007	0.098 0.006	0.084 0.007	0.112* 0.007	0.119** 0.006	0.107 0.012
	"Light Mitochondrial Fraction"					
Citrate Synthase	0.094§§§ ± 0.005	0.109§§ 0.006	0.128*,§§ 0.013	0.118 0.012	0.156***§§§ 0.011	0.246***§§§ 0.032
Malate Dehydrogenase	0.517§§§ ± 0.029	0.601§§§ 0.041	0.528§§§ 0.053	0.665§§§ 0.065	0.818***§§§ 0.035	1.059*** 0.085
NADH-cytochrome c Reductase (total)	0.172§§ ± 0.008	0.162§§ 0.011	0.179§ 0.015	0.204§§§ 0.017	0.210*§§§ 0.010	0.178 0.012
Cytochrome Oxidase	3.678§ ± 0.447	5.291*§§§ 0.509	5.828**,§§§ 0.468	8.415***,§§§ 0.615	8.326***§§§ 0.319	7.313***§§§ 0.680
Glutamate Dehydrogenase	0.091 ± 0.006	0.096 0.007	0.095 0.008	0.093 0.011	0.134** 0.013	0.114 0.015
	"Heavy Mitochondrial Fraction"					
Citrate Synthase	0.083§ ± 0.004	0.057***,§§ 0.004 ○○○	0.046***§§§ 0.004 ○○○	0.069 0.008○○	0.062**§§ 0.004○○○	0.096○○ 0.007
Malate Dehydrogenase	0.430§§§ ± 0.021○	0.323*,§§§ 0.017 ○○○	0.244***§§§ 0.024○○○	0.382§§§ 0.037○○○	0.394§§§ 0.027○○○	0.468§§ 0.035○○○
NADH-cytochrome c Reductase (total)	0.093§§ ± 0.004○○○	0.058***,§§§ 0-.004 ○○○	0.046***§§§ 0.004 ○○○	0.062***§§§ 0.004 ○○○	0.062***§§§ 0.002 ○○○	0.067**§§ 0.007 ○○○
Cytochrome Oxidase	3.171§§ ± 0.472	1.791*,§ 0.237 ○○	1.752*○○○ 0.231	3.653§ 0.431○○○	3.105○○○ 0.381	2.268○○○ 0.173
Glutamate Dehydrogenase	0.087 ± 0.009	0.054**,§§§ 0.004 ○○○	0.039***§§§ 0.004 ○○○	0.067§§§ 0.007○○	0.054**§§§ 0.003 ○○○	0.060§ 0.008○

Statistical analysis: ANOVA test of data expressed as mean ± S.E.M.
3 significance levels: $P < 0.05$: 1 symbol; $P < 0.01$: 2 symbols; $P < 0.001$: 3 symbols.
Symbols of comparison: (a) versus (b)(c)(d)(e)(f):*
Enzyme activities are different from the same of free mitochondria: §; of light ones: ○.
Number of animals of each single fraction: 8–10. At 24 months n=5.

Thus these data are only in partial agreement with the observation that mitochondrial enzyme specific activities related to the Krebs' cycle and to the electron transfer chain are lower in synaptic mitochondria respect to the non-synaptic ones in cerebral cortex, striatum, and pons and medulla (30). In fact, these results confirm the existence of an intrinsic characterization between synaptic mitochondria themselves, *i.e.* "heavy" *versus* "light" ones (20,28,29).

Respect to hippocampus, specific enzyme activities of striatum are quantitatively different, while their patterns are qualitatively similar in the various types of mitochondria (Table II), with less striking differences of catalytic activities during aging (29).

Table II. Specific Enzyme Activities (μMoles Substrate·Min^{-1}·Mg of Protein^{-1}) of Indicated Enzymes Assayed on "Free", "Light" and "Heavy" Mitochondrial Fractions from Rat Striatum of Control Animals Aged 4, 8, 12, 16, 20 and 24 Months.

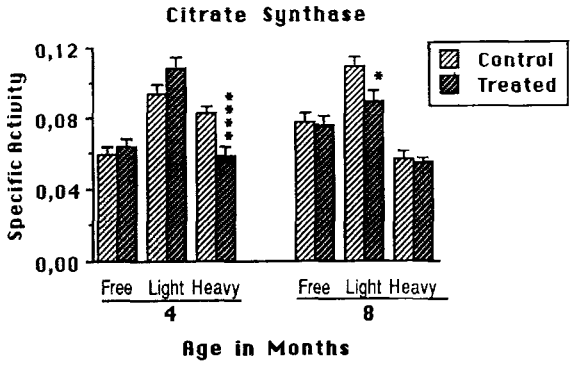
	"Free Mitochondrial Fraction"					
Enzyme|Age in Months	4 (g)	8 (h)	12 (i)	16 (j)	20 (k)	24 (l)
Citrate	0.078	0.073	0.081	0.104*	0.094*	0.117*
Synthase	0.005	0.005	0.007	0.009	0.004	0.016
Malate	1.016	1.013	1.179	1.259*	1.293*	1.409**
Dehydrogenase	0.062	0.045	0.048	0.058	0.067	0.112
NADH-cytochrome c	0.126	0.106	0.124	0.142	0.136	0.129
Reductase (total)	0.009	0.004	0.005	0.005	0.006	0.006
Cytochrome	1.537	1.261	1.455	1.578	2.888***	2.462**
Oxidase	0.187	0.103	0.134	0.133	0.279	0.141
Glutamate	0.107	0.088	0.080*	0.113	0.118	0.112
dehydrogenase	0.007	0.005	0.005	0.012	0.006	0.010
	"Light Mitochondrial Fraction"					
Citrate	0.080	0.107*,§	0.089	0.091	0.111*	0.177***
Synthase	0.007	0.008	0.008	0.008	0.009	0.023
Malate	0.449§§§	0.598**,§§§	0.437§§§	0.602*§§§	0.658**§§§	0.931***§
Dehydrogenase	0.029	0.022	0.041	0.065	0.058	0.027
NADH-cytochrome c	0.143	0.150§§§	0.150	0.156	0.179**§§	0.166§
Reductase (total)	0.007	0.010	0.016	0.021	0.008	0.009
Cytochrome	4.688§§§	5.293§§§	5.899§§§	9.663***§§§	10.085***§§§	8.479**§§§
Oxidase	0.435	0.489	0.649	0.658	0.689	0.667
Glutamate	0.084§	0.095	0.072	0.080	0.104*	0.099
Dehydrogenase	0.006	0.006	0.008	0.010	0.006	0.010
	"Heavy Mitochondrial Fraction"					
Citrate	0.059§	0.041*,§§§	0.031***§§§	0.047§§§	0.039*§§§	0.054§
Synthase	0.005o	0.003 ooo	0.002 ooo	0.006oo	0.003 ooo	0.003oo
Malate	0.309§§§	0.241*,§§§	0.187***§§§	0.304§§§	0.253§§§	0.307§§§
Dehydrogenase	0.022o	0.018 ooo	0.017 ooo	0.033oo	0.023 ooo	0.006ooo
NADH-cytochrome c	0.076§§§	0.051***,§§§	0.037***§§§	0.054**§§§	0.052***§§§	0.048**§§§
Reductase (total)	0.004ooo	0.003 ooo	0.002 ooo	0.004 ooo	0.003 ooo	0.002 ooo
Cytochrome	2.076ooo	1.369ooo	1.287ooo	1.653ooo	2.765ooo	1.622§§
Oxidase	0.385	0.114	0.138	0.264	0.288	0.097ooo
Glutamate	0.053§§§	0.033***,§§§	0.025***§§§	0.034**§§§	0.037**§§§	0.032**§§§
Dehydrogenase	0.004ooo	0.003 ooo	0.002 ooo	0.004 oo	0.003 ooo	0.004 ooo

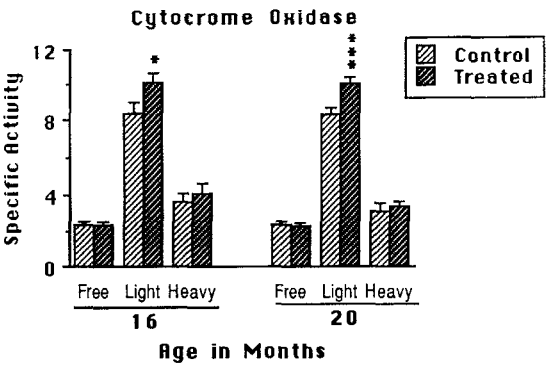
Statistical Analysis: ANOVA test of data expressed as mean ± S.E.M..
3 significance levels: P<0.05: 1 symbol; P<0.01: 2 symbols; P<0.001: 3 symbols.
Symbols of comparison: (g) versus (h)(i)(j)(k)(l):*
Enzyme activities are different from the same of free mitochondria: §; of light ones: o.
Number of animals of each single fraction: 8–10. At 24 months n=5.

The single i.p. administration of L-acetylcarnitine (100 mg·kg^{-1}) modified some enzyme activities only in the synaptic mitochondria from hippocampus and striatum.

In hippocampus the specific activities of citrate synthase and malate dehydrogenase of synaptic mitochondria were lowered by drug treatment at 4, 8, and 4, 24 months respectively (Figure 1). Cytochrome oxidase activity was increased by L-acetylcarnitine treatment at 16, 20 months on synaptic light mitochondria, while glutamate dehydrogenase activity was decreased by the drug on synaptic (4 months) and non-synaptic (20 months) mitochondria (Figure 2).

The enzyme activities of striatum of animals treated with L-acetylcarnitine are reported in Figure 3 and 4. Pharmacological treatment decreased the activity of citrate synthase and malate dehydrogenase mainly on synaptic mitochondria at 4, 8 and 8, 24 months of age, exactly as in hippocampus (Figure 3). Cytochrome ox-





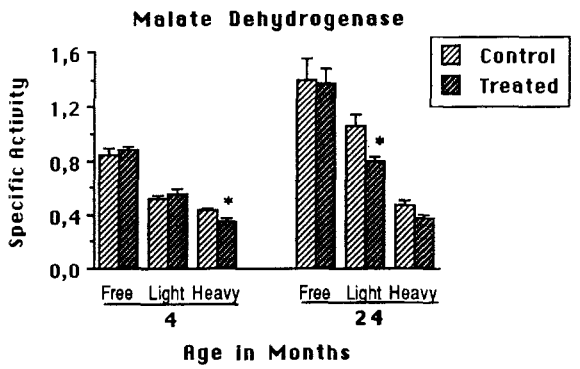


Fig. 1.

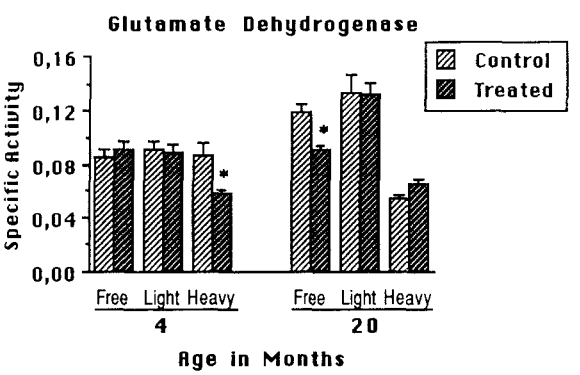


Fig. 2.

idase activity was increased by drug at 12 and 16 months of age on synaptic and non-synaptic mitochondria, while glutamate dehydrogenase activity was decreased at 8, 20 months (Figure 4).

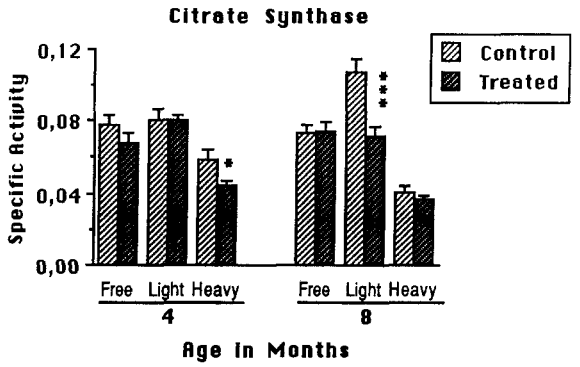
In both areas, the inhibition of citrate synthase activity was the main and constant effect of L-acetylcarnitine acute treatment, together with the inhibition of malate and glutamate dehydrogenase activities. Thus, L-acetylcarnitine exhibited an inhibitory trend of action on the enzyme activities of synaptic mitochondria related to the Krebs' cycle, while the drug was ineffective on the enzyme activities of non-synaptic mitochondria. The drug increased the activity of electron transfer chain cytochrome oxidase activity in synaptic and non-synaptic mitochondria of striatum at 12 and 16 months of age, while in hippocampus this effect was detected at 16 and 20 months in light mitochondria.

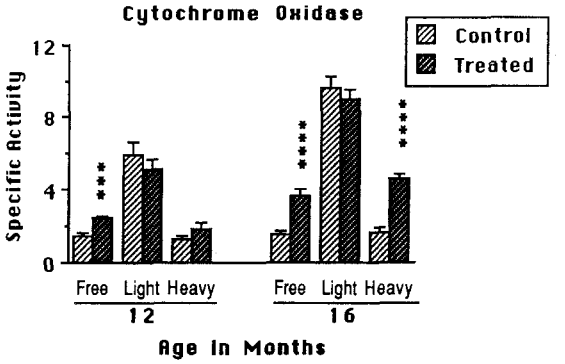
DISCUSSION

Systematic, diversified studies on mitochondrial enzyme systems related to energy transduction during aging confirm that the biochemical patterns of non-synaptic perikaryal and synaptic mitochondria are different and that enzyme activities undergo separate changes with age (29). The changes undergone by age of bioenergetic systems are very complex and have been extensively discussed previously by Hansford (see 31 for a review).

The distinct nature of these three populations of mitochondria, appears to be not only a subtle and unclear problem of microheterogeneity, but also a possible relevant factor with respect to aging processes and pharmacological treatment (20,29).

A lot of animals of different ages was subjected to





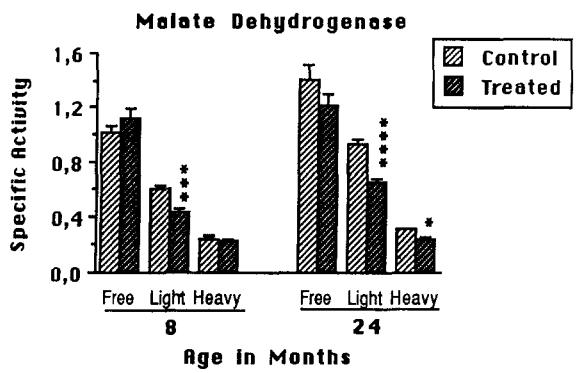


Fig. 3.

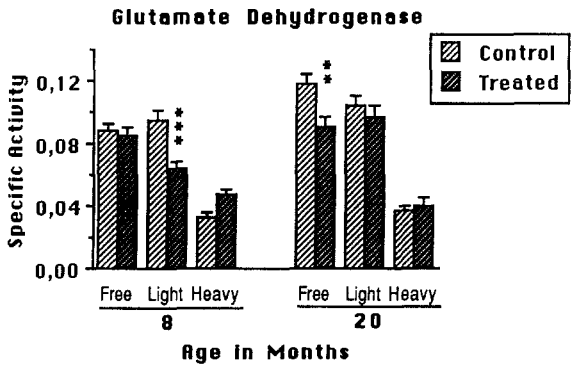


Fig. 4.

a single intraperitoneal injection of L-acetylcarnitine at the dose of 100 mg.kg^{-1} and sacrificed after 60 minutes, over the 4 to 24 months life-span, at 4 month time intervals.

The results can be summarized as follows. In "young" animals, acute treatment with L-acetylcarnitine decreased citrate synthase, malate deydrogenase and glutamate dehydrogenase activities in mitochondria obtained from synaptosomes of hippocampus and striatum. In "adult" animals, starting from 12 months of age, but noticeably at 16 and 20 months, cytochrome oxidase activity was increased by treatment either in non-synaptic (striatum) or in synaptic mitochondria (striatum and hippocampus). In "senescent" animals the acute treatment with L-acetylcarnitine decreased glutamate dehydrogenase activity in non-synaptic mitochondria of both areas and malate deydrogenase activity as evaluated on synaptic mitochondria.

These data indicate that the enzyme activities of synaptic mitchondria were affected by L-acetylcarnitine exogenous treatment, thus suggesting a specific neuronal trigger zone of action. Particularly, the activities of Krebs' cycle were inhibited by L-acetylcarnitine upon acute treatment.

The inhibition of citrate synthase, a key enzyme of the Krebs' cycle, confirms previous data on enzyme activity of hippocampus in young animals (20) and may be compared with previous observations (12,32) showing that a decrease in the flux of the citric acid cycle affects the mitochondrial formation of acetylcarnitine (12) or activate carnitine acetyl-transferase reactions (32).

In brain mitochondria, the inhibition of glutamate transamination by pyruvate metabolism may be induced by stimulating the flux through the pyruvate dehydrogenase complex (33). Hence, it seems likely that the inhibitory effect of a changed acetyl-CoA metabolism on glutamate transamination lies in competition of citrate synthase and aspartate aminotransferarase for common substrate, *i.e.* the oxaloacetate.

Even if this effect of the drug was observed in

"young" animal's hippocampus and striatum synaptic mitochondria, the interference on glutamate turnover by the acetyl-CoA metabolism related to the citrate synthase reaction is of interest, because an inverse relationship exists between the rate of carbon flux through the tricarboxylic acid cycle and the rate of cytosolic NADH reoxidation (33). However, with advancing age this effect of the drug disappears clearly, indicating that pharmacological action is superimposed to an intrinsic differential "maturation" of these enzyme systems of mitochondria (29).

The decreased glutamate dehydrogenase activity in synaptic mitochondria by acute exogenous treatment with L-acetylcarnitine corroborates the hypothesis of an interference with the glutamate metabolism at synaptic level. This is particularly important for hippocampus where the glutamate is a proper neurotransmitter and changes in its concentration may be of functional significance in this area (34). Microiontophoretic administration of L-acetylcarnitine affects electrical discharge of hippocampal neurons (35) and γ- aminobutyric acid induces a potent competitive inhibition of carnitine uptake in slices of cerebral cortex (36). The effect of the drug on glutamate dehydrogenase activity was observed in "young" as well as in "senescent" animals.

The effect of L-acetylcarnitine treatment on the activity of cytochrome oxidase is consistent with previous data (37,38) showing that this drug caused an high increase in the amount of a 16 kDa mitochondrial inner membrane protein (37) probably identified as the subunit IV of cytochrome oxidase, decreased by age in striatum (39). In addition, L-acetylcarnitine acts on mitochondrial DNA transcription processes erasing the reduction of subunit I of cytochrome oxidase (Col)mRNA in brain mitochondria (40), increasing the synthesis of all the mtRNA species of 28 months-old Fisher 344 rats, probably due to an improved mitochondrial metabolism (41).

In any case, **in vivo** administration of L-acetylcarnitine affects selectively some enzymes linked to energy metabolism of neurons, suggesting that the drug is not only implicated in the mitochondrial fatty acyl transport but involved in the brain energy metabolism.

ACKNOWLEDGMENTS

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REFERENCES

1. Fritz, I. B. 1963. Carnitine and its role in fatty acid metabolism. Adv. Lipid Res. 1:285–336.
2. Bremer, J. 1962. Carnitine in intermediary metabolism: reversible acetylation of carnitine by mitochondria. J. Biol. Chem. 237:3628–3632.
3. Clark, J. B., and Nicklas, W. J. 1970. The metabolism of rat brain mitochondria. J. Biol. Chem. 245:4724–4731.
4. McCamen, R., McCamen, M., and Stafford, M. 1966. Carnitine acetyltransferase in nervous tissue. J. Biol. Chem. 241:930–934.
5. Klingenberg, M., and Bode, C. 1965. Some aspects of the carnitine in fatty acid oxidation. Pages 87–95, *in* Wolf, G. (ed.), Recent Research in Carnitine, MIT Press, Cambridge.
6. Challoner, D. R., and Prols, H. G. 1972. Free fatty acid oxidation and carnitine levels in diphtheritic guinea pig myocardium. Scand. J. Clin. Lab. Invest. 51:2071–2076.
7. Engel, A. G., and Angelini, C. 1973. Carnitine deficiency of human muscle with associated lipid storage myopathy. A new syndrome. Science 179:899–902.
8. Markesberry, W. R., McQuillen, M. P., Procopis, P. G., Harrison, A. R., and Engel, A. G. 1974. Muscle carnitine deficiency. Association with lipid myopathy, vacuolar neuropathy, and vacuolated leukocytes. Arch. Neurol. 31:320–324.
9. Karpati, G., Carpenter, S., Engel, A. G., Walters, G., Allen, J., Rothman, S., Klassen, G., and Memer, O. A. 1975. The syndrome of systemic carnitine deficiency. Clinical, morphological, biochemical, and pathophysiological features. Neurology 25:16–24.
10. Van Tol, A. 1975. Aspects of long-chain acyl-CoA metabolism. Molec. Cell. Biochem. 7:19–31.
11. Vandyke, D., Markesberry, W. R., DiMauro, S., and Griggs, R. C. 1975. Hereditary carnitine deficiency of muscle. Neurology 25:154–159.
12. Pande, S. V., and Parvin, R. 1976. Characterization of carnitine translocase system of heart mitochondria. J. Biol. Chem. 251:6683–6691.
13. Boudin, B., Mikol, J., Guillard, A., and Engel, A. G. 1976. Fatal systemic carnitine deficiency with lipid storage in skeletal muscle, heart, liver, and kidney. J. Neurol. Sci. 30:313–325.
14. Borreback, B., Christiansen, R., Christophersen, B. O., and Bremer, J. 1976. The role of acyltransferase in fatty acid utilization. Circulat. Res. 38:16–20.
15. Parvin, R., and Pande, S. V. 1979. Enhancement of mitochondrial carnitine and carnitine acylcarnitine translocase-mediated transport of fatty acids into liver mitochondria under ketogenic conditions. J. Biol. Chem. 254:5423–5429.
16. Shug, A. L., Hayes, B., Huth, P. J., Thomsen, J. H., Bittar, N., Hall, P. V., and Dehling, R. M. 1980. Changes in carnitine-linked metabolism during ischemia, thermal injury and shock. Pages 321–340, *in* Frenkel, R. A. and McGarry, J. D. (eds.), Carnitine Biosynthesis, Metabolism and Function, Academic Press, New York.
17. Benzi, G., Gorini, A., Dossena, M., Taglietti, M., Fulle, D., and Pastoris, O. 1983. Recovery after hypoglycemic brain injury. Action of some biological substances on the cerebral metabolism. Biochem. Pharmacol. 32:1083–1091.
18. Benzi, G., Villa, R. F., Dossena, M., Vercesi, L., Gorini, A., and Pastoris, O. 1984. Cerebral endogenous substrate utilization during the recovery period after profound hypoglycemia. J. Neurosc. Res. 11:437–450.
19. Christiansen, R. Z. 1978. The effect of clofibrate-feeding on hepatic fatty acid metabolism. Biochim. Biophys. Acta 530:314–324.
20. Villa, R. F., Gorini, A., Zanada, F., and Benzi, G. 1986. Action of L-acetylcarnitine on different cerebral mitochondrial populations from hippocampus. Arch. Int. Pharmacodyn. 279:195–211.

21. Lai, J. C. K., Walsh, J. M., Dennis, S. C., and Clark, J. B. 1977. Synaptic and non-synaptic mitochondria from rat brain: isolation and characterization. J. Neurochem. 28:625–631.
22. Sugden, P. H., and Newsholme, E. A. 1975. Activities of citrate synthase, NAD^+-linked and $NADP^+$-linked isocitrate dehydrogenase, glutamate dehydrogenase, aspartate aminotransferase and alanine aminotransferase in nervous tissues from vertebrates and invertebrates. Biochem. J. 150:105–111.
23. Ochoa, S. 1955. Malic dehydrogenase from pig heart. Vol. 1, pages 735–739, *in* Colowick, S. P. and Kaplan, N. O. (eds.), Methods in Enzymology, Academic Press, New York and London.
24. Nason, A., and Vasington, F. D. 1963. Lipid dependent DPNH-cytochrome c reductase from mammalian skeletal and heart muscle. Vol. 6, pages 409–415, *in* Colowick, S. P. and Kaplan, N. O. (eds.), Methods in Enzymology, Academic Press, New York and London.
25. Smith, L. 1955. Spectrophotometric assay of cytochrome c oxidase. Vol. 2, pages 427–434, *in* Glick, D. (ed.), Methods of Biochemical Analysis, Wiley Interscience, New York.
26. Wharton, D. C., and Tzagoloff, A. 1967. Cytochrome oxidase from beef heart mitochondria. Vol. 10, pages 245–250, *in* Estabrook, R. W. and Pullman, M. E. (eds.), Methods in Enzymology, Academic Press, New York and London.
27. Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, J. 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. 193:265–275.
28. Villa, R. F., Gorini, A., Geroldi, D., Lo Faro, A., and Dell'Orbo, C. 1989. Enzyme activities of selected mitochondria from hippocampus and striatum in aging. Mech. Ageing Dev. 49:211–225.
29. Villa, R. F., and Gorini, A. 1991. Enzyme mitochondrial systems during aging: pharmacological implications. Life Sci. Adv., in press.
30. Leong, S. F., Lai, J. C. K., Lim, L., and Clark, J. B. 1984. The activities of some energy-metabolising enzymes in non-synaptic (free) and synaptic mitochondria derived from selected brain regions. J. Neurochem. 42:1306–1312.
31. Hansford, R. G. 1983. Bioenergetics in aging. Biochim. Biophys. Acta 726:41–80.
32. Hayes, B., and Shug, A. L. 1978. Factors affecting heart mitochondrial acetylcarnitine production. Fed. Proc. 37:1715.
33. Dennis, S. G. C., and Clark, J. B. 1978. The regulation of glutamate metabolism by tricarboxylic acid-cycle activity in rat brain mitochondria. Biochem. J. 172:155–162.
34. Cotman, C. W., and Nadler, J. V. 1981. Glutamate and aspartate as hippocampal transmitters: biochemical and pharmacological evidence. Pages 117–154, *in* Roberts, P. J., Storm-Mathisen, J., and Johnston, G. A. R. (eds.), Glutamate: Transmitter in the Central Nervous System, Wiley & Son, New York.
35. Tempesta, E., Janiri, L., Pirrongelli, C., and Ancona, L. 1982. The effect of microiontophoretically applied DL-, L- and D-acetylcarnitine on single central neurons. Neuropharmacology 21:1207–1210.
36. Huth, P. J., Schmidt, M. J., Hall, P. V., Fariello, R. G., and Shug, A. L. 1981. The uptake of carnitine by slices of rat cerebral cortex. J. Neurochem. 36:715–723.
37. Villa, R. F., Turpeenoja, L., Benzi, G., and Giuffrida-Stella, A. M. 1988. Action of L-acetylcarnitine on age-dependent modifications of mitochondrial membrane protein from rat cerebellum. Neurochem. Res. 13:909–916.
38. Turpeenoja, L., Villa, R. F., Magri, M. G., and Giuffrida-Stella, A. M. 1988. Changes of mitochondrial membrane proteins in rat cerebellum during aging. Neurochem. Res. 13:859–865.
39. Ragusa, N., Turpeenoja, L., Magri, G., Lahdesmaki, P., and Giuffrida-Stella, A. M. 1989. Age-dependent modifications of mitochondrial proteins in cerebral cortex and striatum of rat brain. Neurochem. Res. 14:415–418.
40. Gadaleta, M. N. 1990. Aging and mitochondrial DNA transcription process. It. Biochem. Soc. Transaction 1:64.
41. Fernandez Silva, P., Petruzzella, V., Fracasso, F., Gadaleta, M. N., and Cantatore, P. Age related changes of RNA synthesis in isolated mitochondria from rat brain. It. Biochem. Soc. Transaction 1:274.