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STUDIES ON THE TRANSPORT OF CARNITINE IN THE BRAIN USING SYNAPTOSOMES ISOLATED FROM GUINEA-PIG CEREBRAL CORTEX

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Synaptosomes isolated from guinea pig cerebral cortex accumulate L-carnitine from the medium in an active process, dependent on the sodium gradient across the plasma membrane and on ($Na^+ + K^+$)-ATPase activity. L-Carnitine uptake is inhibited by oxidative phosphorylation uncouplers and by ouabain, a known inhibitor of ($Na^+ + K^+$)-ATPase. In addition, the omission of Na^+ or its replacement by Li^+ inhibited the transport, which was also competitively inhibited by γ-aminobutyrate. The kinetics of carnitine uptake show that the overall process would consist of two components: a passive diffusion and a carrier-mediated transport which is saturated at 1–2 mM carnitine concentration.

The main function of carnitine is the transport of activated long-chain acyls from the cytosolic to the intramitochondrial space [1]. The high concentration of carnitine in myocardial and skeletal muscles [2], which preferentially utilize long-chain fatty acids as an energy source [3], is in line with its function as acyl translocator. Less clear is the role of carnitine in tissues, such as nervous tissue, which utilize glucose as the major oxidizable substrate. The presence of appreciable amounts of carnitine in the nervous system and the capability of the brain to accumulate carnitine from the blood [2] implies that carnitine also plays a role in this tissue. Since carnitine concentration in the brain is higher than that normally present in blood plasma, an important problem is the mechanism by which carnitine is supplied to the brain. Recently Huth et al. [4] have presented evidence for an active transport system for carnitine uptake in rat brain slices. In the present paper, the problem has been re-examined using isolated synaptosomes from guinea-pig cerebral cortex, which represent a more specific system for the in vitro study of neuronal transport processes. The data here reported show the kinetics of L-carnitine uptake by synaptosomes and the dependence of the process on external Na^+.

Synaptosomes from cerebral cortices of Duncan-Hartley-strain guinea pigs (4–8 weeks old) were prepared by a modification of the method of Cotman and Matthews [5] employing a discontinuous Ficoll gradient, as previously described by Nicholls [6]. Protein was determined by the biuret method [7]. Synaptosomal pellets containing 6 mg of protein were resuspended in 4 ml of an air-saturated incubation medium at 30°C and pH 7.4, which contained 122 mM NaCl, 3.1 mM KCl, 1.2 mM $MgSO_4$, 0.4 mM KH_2PO_4, 5 mM $NaHCO_3$, 20 mM Na-Tes and L-[*methyl*-^{14}C]carnitine over the concentration range 0.1–3 mM (3.3 μCi/μmol). After 10 min preincubation, 10 mM D-glucose was added. Portions (0.5 ml) were withdrawn at de-

Abbreviations: FCCP, carbonyl cyanide *p*-trifluoromethoxyphenylhydrazone (proton translocator); Na-Tes, sodium 2-(2-hydroxy-1,1-bis(hydroxymethyl)ethylamino)ethane-sulphonate.

fined times and layered on to 250 μl of a mixture of 40% (v/v) Merck 550 silicone oil and 60% dinoylphthalate in an Eppendorf centrifuge tube and centrifuged for 1 min. The supernatant was carefully aspirated off and 1 ml distilled water was added onto the top of the oil. The water and oil phases were aspirated and 100 μl of 1 mM EDTA (pH 7.4)/0.1% NaCl/0.9% deoxycholate were added. The dissolved pellet was transferred into scintillation vials and the radioactivity was measured in a Beckman LS-100 C spectrophotometer. Tritiated water and [U-^{14}C]poly(ethylene glycol) were used to determine the total and the extrasynaptosomal volme. Enzymatic assay for L-carnitine was performed according to Marquis and Fritz [8]. 'Free' brain mitochondria were obtained as described by Nicholls [6].

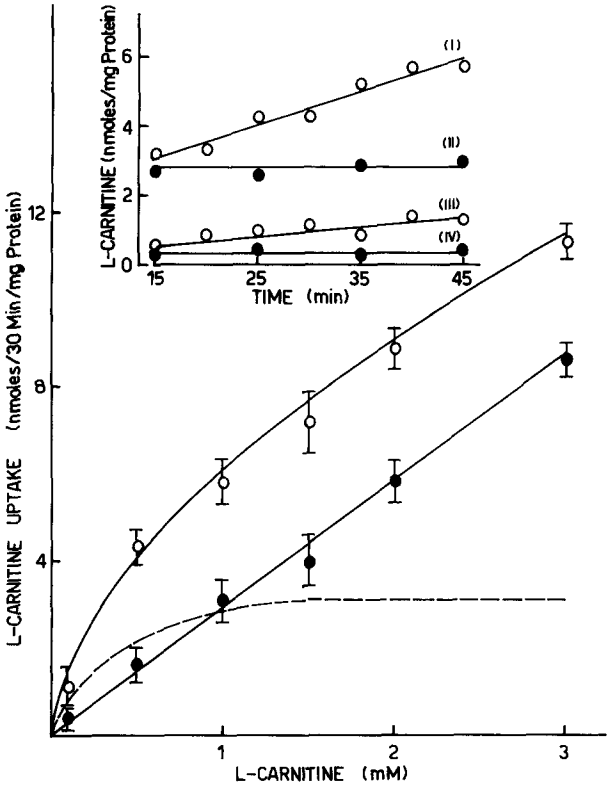

Fig. 1. The effect of L-carnitine concentration on its uptake by synaptosomes. Synaptosomes (1.5 mg protein/ml) were preincubated for 10 min at 30°C in the medium described in the text containing $^{3}H_2O$ (2 μCi/ml) and L-[*methyl*-^{14}C]carnitine at the indicated concentration. Subsequently, 10 mM glucose was added and samples were taken at defined timed as described in the text. (○) L-carnitine; (●) L-carnitine plus 0.7 mM γ-aminobutyric acid added 2 min before glucose addition. The average figure of 2.8 μl/mg protein was used for correction for carnitine contamination by the suspending medium. Points were means ± S.D. of at least five determinations. The dotted line was obtained by subtracting the diffusion component from total uptake. Insert: time-course of L-carnitine uptake by synaptosomes (I) 1 mM L-carnitine; (II) 1 mM carnitine plus 0.7 mM γ-aminobutyrate; (III) 0.1 mM carnitine; (IV) 0.1 mM carnitine plus 0.7 mM γ-aminobutyrate.

The time-course of L-[^{14}C]carnitine accumulation in synaptosomes is linear with time for at least 30 min (insert of Fig. 1). However in the presence of γ-aminobutyric acid, a competitive inhibitor [4], the amount of L-carnitine recovered in the pellet was independent of time. The same effect was obtained upon abolishing the energy source by omitting glucose in the medium or by preincubation with the uncoupler FCCP. The kinetics of L-[^{14}C]carnitine uptake by synaptosomes (Fig. 1) exhibit exponential behaviour similar to that observed in rat heart by Vary and Neely [9]. Fig. 1 also shows the clear-cut linear relationship obtained by plotting the amount of L-[^{14}C]carnitine taken up in the presence of γ-aminobutyrate against the external concentration of carnitine; this finding is indicative of a passive diffusion component. The dotted line obtained by subtracting the diffusion component from the total accumulation suggests the operation of a carrier-mediated transport component which is saturated at a carnitine concentration of 1–2 mM. Since the endogenous carnitine content of synaptosomal vesicles was negligible (results not shown), the

TABLE I

EFFECT OF MEDIUM IONIC COMPOSITION AND OUABAIN ON L-CARNITINE UPTAKE BY SYNAPTOSOMES

Synaptosomes were incubated as described in the text in the presence of 1 mM L-[^{14}C]carnitine (0.2 μCi/ml). Na^+ medium, 125 mM NaCl; sucrose medium, NaCl replaced by 250 mM sucrose; Li^+ medium, NaCl replaced by 125 mM LiCl. Ouabain, added 10 min before carnitine addition, was 0.3 mM. Data are expressed as mean ± S.D. of at least four determinations.

Conditions	nmol L-[^{14}C]carnitine/ 30 min per mg protein	% of control
Na^+ medium (control)	6.2 ± 0.45	100
Sucrose medium	0.9 ± 0.15	14
Li^- medium	1.0 ± 0.10	16
Na^+ medium plus ouabain	1.9 ± 0.40	30

possibility of an exchange can be excluded. Likewise, enzymatic determination of L-carnitine at the end of the incubtion indicated that the recovered radioactivity was accounted for by free carnitine. Moreover, since the ionic strength of the suspending medium is quite high, the possibility of carnitine binding to the synaptosomal plasma membrane should be irrelevant.

A significant contribution of contaminating mitochondria to carnitine uptake by synaptosomes can also be excluded by the finding that 'free' brain mitochondria [6] (1 mg protein/ml), incubated in the presence of 1 mM L-[^{14}C]carnitine accumulated only 1 ± 0.3 nmol/mg protein. This finding also suggests that the accumulated carnitine is localized in the cytoplasm and not sequestered inside the intrasynaptosomal mitochondria.

In agreement with the data reported by Huth et al. [4] in brain slices, a requisite for carnitine transport is the maintenance of the Na^+ gradient across plasma membrane by the energy-driven operation of $(Na^+ + K^+)$-ATPase. Indeed, Table I shows that carnitine uptake was inhibited by ouabain or by replacement of Na^+ in the medium with isoosmolar sucrose or Li^+. The Na^+ replacement by sucrose does not affect the membrane potential of synaptosomes, while the replacement of Na^+ by Li^+, as well as by ouabain, induces depolarisation of plasma membrane (data not shown). In both cases, however, L-carnitine uptake was inhibited, thus confirming further that the process is closely dependent on the inward-directed Na^+ gradient. The described mechanism of L-carnitine uptake by synaptosomes, similar to that found in brain slices by Huth et al. [4], is an important preliminary step in understanding the possible role played by carnitine in synaptosomes and in the nervous system in general.

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