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Carnitine - a known compound, a novel function in neural cells

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Abstract. Carnitine (4-N-trimethylammonium-3-hydroxybutyric acid) seems to fulfill in the brain a different role than in peripheral tissues. Carnitine is accumulated by neural cells in a sodium-dependent way. The existence of a novel transporter in plasma membrane, specific to compounds with a polar group in the ß-position with respect to carboxyl group, has been postulated. The presence of a carnitine carrier in the inner mitochondrial membrane has been proven and the protein has been purified. It is postulated that its major role in adult brain would be translocation of acetyl moieties from mitochondria into the cytoplasm for acetylcholine synthesis. The latter process is stimulated by carnitine and choline in a synergistic way in cells utilizing glucose as the main energetic substrate. Carnitine metabolism in neural cells leads to accumulation of different acyl derivatives of carnitine. Palmitoylcarnitine can influence directly the activity of protein kinase C. An involvement of carnitine in a decrease of palmitate pool used for palmitoylation of regulatory proteins has been postulated.

Review

Key words: carnitine transport, acetylcholine synthesis, protein kinase C, palmitoylation (neuroblastoma, cerebral cortex cells)

INTRODUCTION

Many substances, for which the physiological role and pathways of metabolism have been established in peripheral tissues, play a special and/or different role in the central nervous system. For instance, certain amino acids which can enter the path of protein biosynthesis or, after transamination, supply energy through the tricarboxylic acid cycle, may fulfill in the central nervous system the role of neurotransmitters.

It is generally accepted that glutamate and GABA are the major amino acid neurotransmitters in the mammalian central nervous system, while other amino acids, as tyrosine, tryptamine are neurotransmitter precursors and others like glycine and proline, may modulate neurotransmitters' action. A high degree of integration of the central nervous system seems to take place due to communication and cooperation between very specialized cells. One of the important cases of such a cooperation between different cell types is a "glutamate-glutamine cycle" (Shank and Aprison 1977, Hertz 1979) - a process crucial for the synthesis and removal of glutamate. All brain glutamine synthetase activity has been found in glial cells (Martinez-Hernandez et al. 1977). Glutamine released to extracellular fluid does not cause depolarization of neurones, but, when taken up by these cells, is converted *via* neuronal glutaminase to glutamate. This cycle demands "trafficking" of nitrogen. For this purpose a special role was ascribed to leucine. As has been shown by Yudkoff et al. (1994), the nitrogen of leucine is efficiently metabolized to glutamine in astrocytes, which coincides with high activity of the branched--chain amino acids transferase reported for the brain (Hutson et al. 1992) and low oxidation rate of branched-chain α-ketoacids. The carbon backbone of leucine is released from astrocytes as α-ketoisocaproate (Bixel and Hamprecht 1995) and re-aminated at the expense of glutamate in nerve endings. This quickly formed leucine is released and most probably transported back to astrocytes from neurones (Yudkoff et al. 1994, Yudkoff et al. 1995), which results in a net return of nitrogen to glial cells.

Carnitine (4-N-trimethylammonium-3-hydroxybutyric acid) seems to play a different role in the central nervous system than in peripheral tissues. This compound is known to be involved in transfer of acyl compounds (mainly long-chain fatty acids) from the cytosol to the mitochondrial matrix where these acids are further metabolized. The pathway of carnitine-dependent transport of fatty acids through the inner mitochondrial membrane, the so-called "carnitine shuttle", consists of three steps (Fig. 1). (1) There are several enzymes synthesizing acylcarnitine derivatives from their acylCoA forms on the outer side of the outer mitochondrial membrane. These enzymes are characterized by different specificities towards degree of saturation and the chain length of the acyl moiety. (2) Acylcarnitine derivatives are translocated through the inner mitochondrial membrane by a carnitine carrier, a protein belonging to the family of mitochondrial carriers, capable of catalysing a uniport of carnitine or its exchange with acylcarnitines. (3) On the inner side of the inner mitochondrial membrane the acyl moieties are transferred to CoASH inside mitochondria by different carnitine acyl transferases. This involvement of carnitine in fatty acid transport to mitochondria has been well described for such tissues as muscles, kidney or liver. Carnitine *in vivo* is mainly synthesized in liver (Bøhmer 1974) and taken up by other tissues from circulation. Carnitine and its derivatives accumulate in nervous tissue (Bresolin et al. 1982, Shug et al. 1982), although the level of ß-oxidation of fatty acids in adult brain is relatively low (Warshaw and Terry 1976).

TRANSPORT OF CARNITINE IN THE BRAIN

Carnitine uptake by the brain

Carnitine is formed from 6-N-trimethyllysine after its liberation in protein breakdown (usually from myosin, actin and histones). The latter compound is subsequently cleaved to butyrobetaine aldehyde and glycine; the next steps are oxidation of butyrobetaine aldehyde to butyrobetaine and its hy-

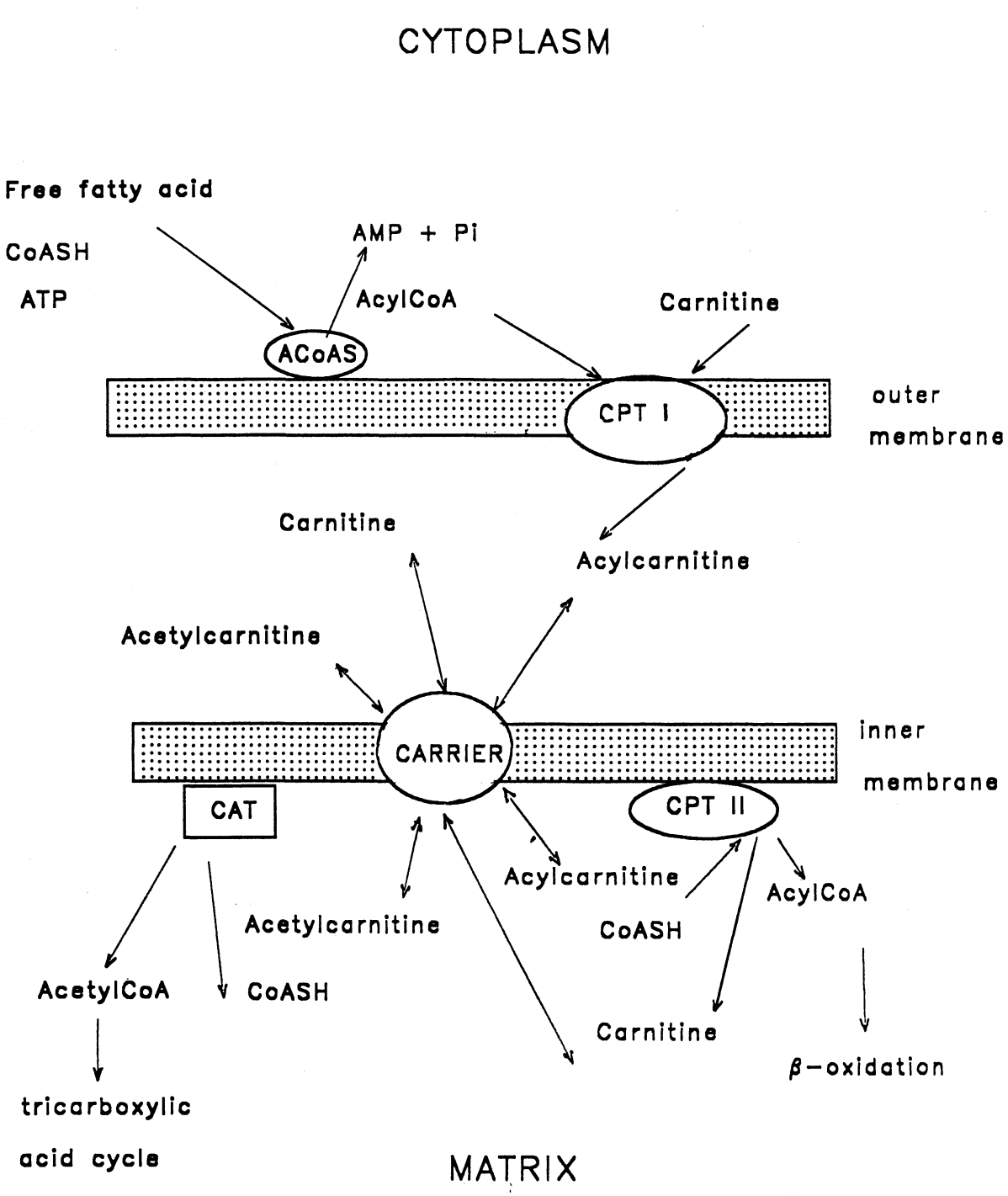

Fig. 1. Carnitine shuttle in mitochondria. The carnitine-dependent transport of activated fatty acids through the inner mitochondrial membrane. ACoAS, acylcoenzyme A synthetase (EC 6.2.1.3) and CPT I - carnitine palmitoyltransferase I (EC 2.3.1.21) in the outer mitochondrial membrane; CAT, carnitine acetyltransferase and CPT II - carnitine palmitoyltransferase II (EC 2.3.1.21) both in the inner mitochondrial membrane and reversible, which enables entry and exit of acyl groups into/from mitochondria; P_i, inorganic phosphate (Modified from Pande and Murthy 1994).

droxylation to carnitine (Lindstedt and Linstedt 1965, 1970, Hochalter and Henderson 1976, Hulse and Henderson 1980). The last reaction is catalysed by butyrobetaine hydroxylase, 4-trimethylaminobutyrate, 2-oxoglutarate: oxygen oxidoreductase (3-hydroxylating), EC 1.14.11.1, an enzyme belonging to

a group of oxidoreductases using 2-oxoglutarate as one donor, according to the following reaction:

4-trimethylaminobutyrate + 2-oxoglutarate + O_2 = = 3-hydroxy-4-trimethylaminobutyrate (carnitine) + + succinate + CO_2.

This enzyme was reported to reveal different tissue distribution in various species. In all species it is found in liver, and in the rat it is present in liver almost exclusively (Bøhmer 1974, Cox and Hoppel 1974, Haigler and Broquist 1974). Therefore carnitine has to be delivered to the brain from circulation, especially since plasma levels of butyrobetaine are hardly detectable even in humans (Lever et al. 1992).

Due to the fact that carnitine accumulates in the brain it has to have an ability to cross the blood--brain barrier. The content of carnitine was shown to be different in various regions of canine brain - the highest content being reported for cerebellar cortex (Shug et al. 1982), while in rat brain the hypothalamus turned out to have the highest content of free carnitine (above 2 nmoles/mg protein) and its acyl derivatives (Bresolin et al. 1982). Since hypothalamus has access to many substances circulating in blood through fenestrations, this observation may point to the existence of some limitations in carnitine crossing through the blood-brain barrier.

There are different active carnitine acyltransferases in brain. Although palmitoylcarnitine transferase seems to be about 100 times more active than carnitine acetyltransferase, the content of long-chain acylcarnitines does not exceed 15-20% of the total carnitine accumulation (Bresolin et al. 1982). This observation was also confirmed in the case of isolated rat cortical neurones (Wawrzeńczyk et al. 1995a, b).

Carnitine transport to neural cells

Several reports on carnitine accumulation by different tissues may be found in the literature. The possibility of exchange of carnitine and deoxycarnitine (butyrobetaine) was suggested from investigations performed *in vivo* after carnitine and deoxycarnitine administration (Sartorelli et al. 1989). Carnitine uptake by human fibroblasts was reported to be inhibited by preincubation with valproic acid (Tein et al. 1993). A saturable uptake of carnitine described for muscles was inhibited by butyrobetaine and choline chloride (Rebouche 1977).

The transport of carnitine into brain slices (Huth et al. 1981) and synaptosomal preparations (Zoccarato et al. 1983, Hannuniemi and Kontro 1988) was found to be inhibited by ouabain, i.e., to be sodium-dependent. Similar observations were reported for isolated rat cortical neurones (Nałęcz et al. 1995b) and also in the case of neuroblastoma NB-2a cells (Nałęcz et al. 1995a). These observations indicate that accumulation of carnitine is dependent on energy, but the involvement of ATP is indirect, by creating a Na^+ concentration gradient through Na^+, K^+- - ATPase.

It was also reported that accumulation of carnitine in rat brain slices (Fariello and Shug 1981, Huth et al. 1981) and synaptosomes (Zoccarato et al. 1983) was competitively inhibited by GABA. It has to be emphasized, however, that kinetic parameters of this uptake, being in mM range, exceeded the physiological concentrations of both GABA and carnitine. As shown by Nałęcz et al. (1995a) the accumulation of carnitine was found to be insensitive to GABA in neuroblastoma NB-2a cells. The measurements of carnitine accumulation in the isolated rat cortical neurones resulted in a decreased uptake of 50 μM carnitine in the presence of 1 mM GABA (Wawrzeńczyk et al. 1995b). The more detailed analysis revealed, however, that in the presence of GABA the level of acetylcarnitine decreased 1.6 fold. This observation was correlated to the measured inhibition of carnitine acetyltransferase (1.8 fold) (Wawrzeńczyk et al. 1995b), pointing to a possible effect of GABA on the further, intracellular metabolism of carnitine rather than on the transport phenomenon itself.

Although carnitine is structurally related to choline and betaines, these compounds did not affect its accumulation. Carnitine could be considered as N-methylated γ-amino acid, therefore, the influence of several amino acids or their analogs on carnitine accumulation was studied. Experiments

performed on NB-2a cells demonstrated that this process was inhibited by serine and cysteine (Fig. 2). Any involvement of amino acid transporting systems, ASC and asc (alanine, serine and cysteine specific) was, however, excluded due to the lack of any effect exerted by alanine. This observation seemed to be in agreement with the properties of ASC system as not tolerating N-methylation (Christensen 1984). The existence of a novel system transporting carnitine with a physiological K_m of 123 ± 13 μM was thus postulated (Nałęcz et al. 1995a). Such a system, although not necessarily exclusively specific toward this compound, can be characterized by its sodium-dependence and certain structural demands concerning substrate, namely the presence of a carboxylic group and a polar group in the ß-position (Fig. 2).

Carnitine transporter in brain mitochondria

Flux of metabolites into and out of mitochondria occurs due to the activity of many specialized carrier proteins, capable of exchanging substrates (e.g., adenine nucleotide translocator, α-ketoglutarate, glutamate/aspartate, monocarboxylate, dicarboxylate and tricarboxylate carriers) or catalysing their net uptake, in exchange of OH^- or co-transport with H^+ (e.g., phosphate, monocarboxylate, glutamate carriers). The carnitine transporter, a central part of the "carnitine shuttle" (Fig. 1), was reported to be able to perform both reactions in liver (i.e., an uniport of carnitine and its exchange with carnitine acyl derivatives), although the activity of the unidirectional transport of carnitine was shown to be lower than the process of exchange (Parvin and Pande 1979, Nol et al. 1985). Efflux of carnitine from preloaded mitochondria was much faster with longer carnitine acyl derivatives than with the shorter ones (Parvin and Pande 1979). The carnitine carrier from rat liver mitochondria has been purified (Indiveri et al. 1990) and the affinity of octanoylcarnitine for the transporter was shown to be much higher than that of either acetylcarnitine or carnitine itself (Indiveri et al. 1991).

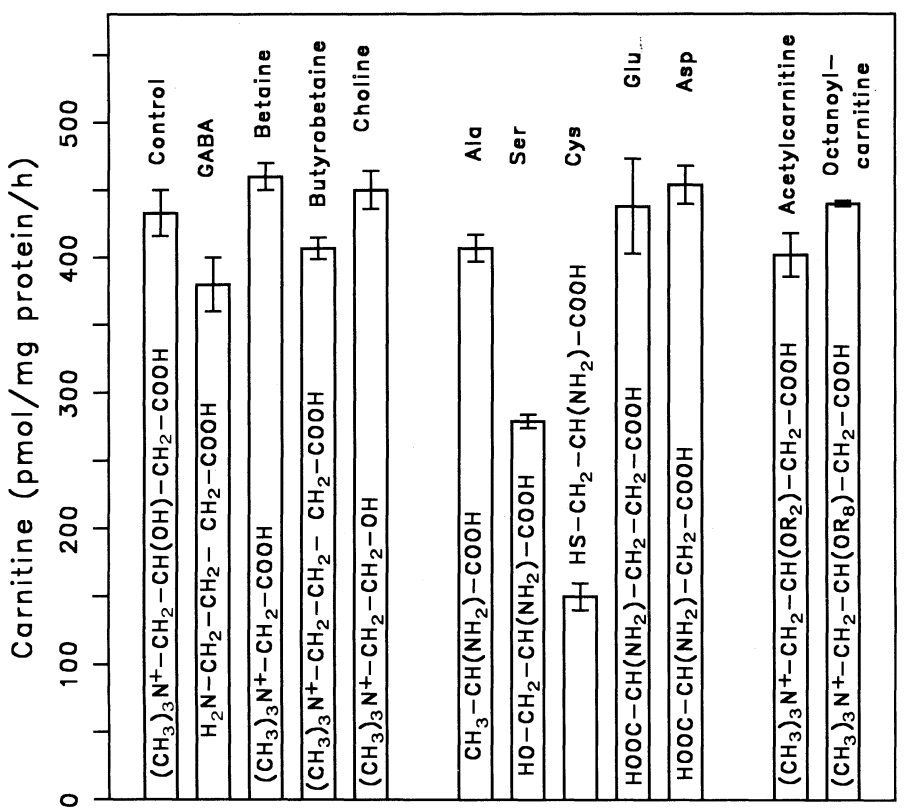

Fig. 2. Effect of different compounds on accumulation of carnitine in neuroblastoma NB-2a cells. Neuroblastoma NB-2a cells were incubated with 50 μM [^{3}H]carnitine in the presence of indicated compounds at 1 mM concentrations. The results present accumulation after 1 h. Values are means from 3 to 26 experiments ± SE. R_2 and R_8 represent acetyl and octanoyl moieties, respectively (Modified from Nałęcz et al. 1995a).

Proteins responsible for transport of certain metabolites through the inner mitochondrial membrane ar not expressed in all tissues to the same extent. It has been known that the activity of the tricarboxylate carrier is high in liver, being virtually absent in heart and brain (England and Robinson 1969, Sluse et al. 1971). Much higher activity of the monocarboxylate (pyruvate) carrier has been found in brain mitochondria in comparison with muscles and liver. Moreover, as demonstrated by Nałęcz et al. (1992) the carriers isolated from various tissues were characterized by different substrate specificity. Some of the inner mitochondrial membrane translocators, as proven for ADP/ATP translocator, were shown to be encoded by different nuclear genes, expressed differently in various tissues (Battini et al. 1987, Cozens et al. 1989, Powell et al. 1989).

The carnitine carrier activity was also reported for isolated rat brain free (nonsynaptosomal) mitochondria and the carnitine homoexchange was shown to be much higher in mitochondria from the suckling animals in comparison with adults (Kamińska et al. 1993). The protein responsible for carnitine transport was purified, reconstituted into phosphatidylcholine vesicles and the activity was correlated with the appearance of a polypeptide with M_r=33,000 (Kamińska et al. 1993). The activity of reconstituted carrier was higher when the protein was isolated from suckling animals and reached a constant, but much lower level after weaning. However, it would be too preliminary to speculate whether there are different isoforms of carnitine transporter expressed at different steps of brain development. Interestingly, the observed changes of carnitine transport activity may be correlated with the reported activation, at the same age, of palmitate oxidation and with a peak activity of carnitine palmitoyltransferase (Warshaw and Terry 1976). Thus a coordinated expression may exist of all the enzymes and transporters necessary for an effective ß-oxidation, a process preferential in young animals.

Further characterization of the brain mitochondrial carnitine carrier, on the basis of two-substrate kinetic analysis, implied other differences when compared with the transporter isolated from liver. A sequential mechanism and the formation of the ternary complex was postulated for the exchange reaction for brain mitochondrial carrier (Kamińska et al. 1995). This result resembled the two-reactant initial velocity studies of the other mitochondrial translocators, although a different mechanism (ping-pong) was postulated for the carnitine carrier in liver mitochondria (Indiveri et al. 1994).

It was also demonstrated that the reaction of carnitine/carnitine exchange catalysed by brain mitochondrial carnitine transporter was inhibited by carnitine acyl derivatives (Kamińska et al. 1995), however, in contrast to the results obtained with the carrier from liver, the inhibitory effect was less and less pronounced with the increase in the number of carbon atoms in the acyl chain of carnitine derivative (Fig. 3). This observation, showing high specificity of the inner mitochondrial membrane carnitine carrier towards acetylcarnitine, could suggest that the activity of this protein in the brain would rather deliver mitochondrial acetyl moiety to cytoplasm, than transport long-chain fatty acids for ß-oxidation, a process much less pronounced in the nervous system.

METABOLIC CONSEQUENCES OF THE CARNITINE SHUTTLE FUNCTIONING IN THE BRAIN

General bioenergetics of brain

Glucose is the main energetic fuel for the adult brain and, when metabolized to pyruvate by glycolysis, can enter the tricarboxylic acid cycle after transport by a monocarboxylate carrier and formation of acetylCoA due to the activity of pyruvate dehydrogenase. Although brain mitochondria contain considerable levels of palmitate activating enzymes (Vignais and Zabin 1958, Vignais et al. 1958, Beattie and Basford 1966, Bird et al. 1985), fatty acids in brain are preferentially incorporated into structural lipids rather than undergo ß-oxidation (Kawamura 1988). AcetylCoA can be also exported from mitochondria, either in the form of acetylcarnitine,

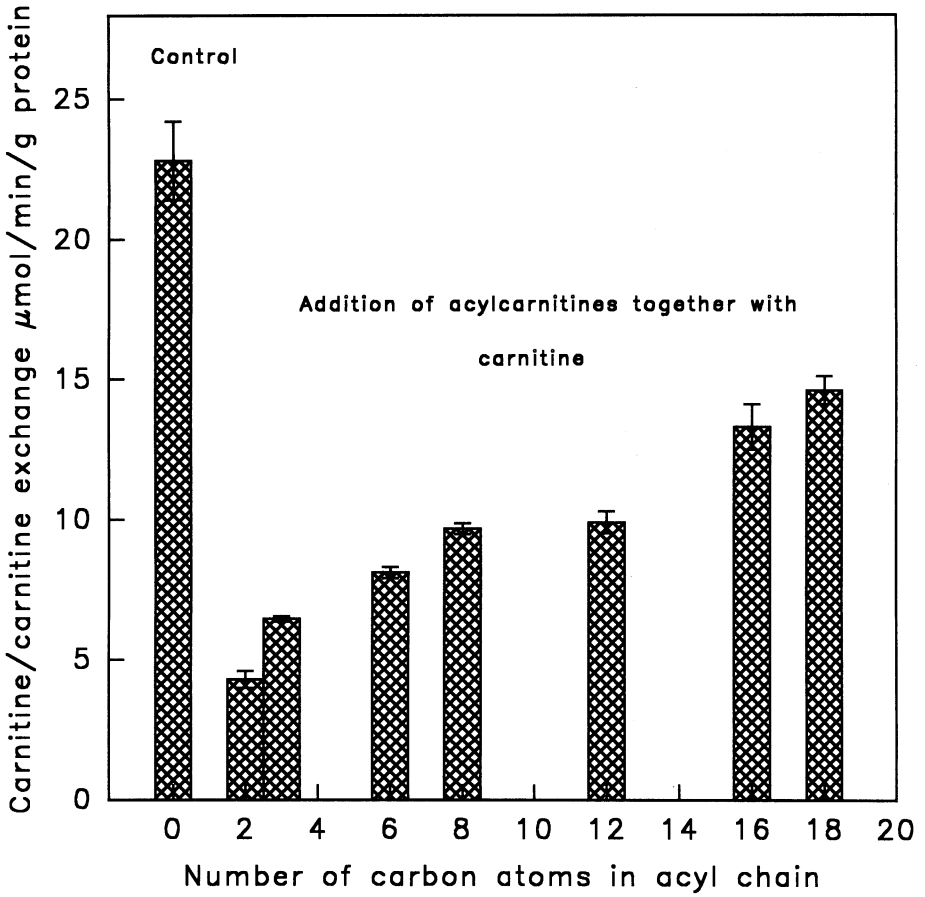

Fig. 3. Effect of acylcarnitine derivatives of different chain length on the carnitine transport. $Carnitine_{in}/carnitine_{out}$ exchange activity catalysed by the reconstituted carnitine carrier from rat brain mitochondria was measured for 7 min in the absence of (Control) or after simultaneous addition of the following acylcarnitine derivatives (3 mM): acetylcarnitine, propionylcarnitine, hexanoylcarnitine, octanoylcarnitine, layroylcarnitine, palmitoylcarnitine, stearoylcarnitine. Proteoliposomes were preloaded with 20 mM carnitine; the outside concentration of [^{3}H]carnitine was 0.5 mM. Data are the means ± SD from three experiments (Modified from Kamińska et al. 1995).

which on the cytoplasmic side can be transformed to acetylCoA by carnitine acetyltransferase (Fig. 4), or in the form of citrate, which results as well in an increase of acetylCoA pool in cytoplasm due to the action of citrate cleavage enzyme.

In the suckling mammal the main source of substrates for brain development is the mother's milk. It must be emphasized that the intensive myelinization process, demanding polar lipids and cholesterol, takes place during the suckling period (Nehlig and Pereira De Vasconcelos 1993). Moreover, it has been demonstrated that the capillary endothelium which forms the blood-brain barrier is incomplete in the rat until postnatal day 24 (Schulze and Firth 1992). In the milk of rats, lipids constitute almost 50% of weight of different components (protein, lactose) but in terms of calories they supply almost 70% of the total energy. These milk triacylglycerols must be processed to form ketone bodies (Williamson and Lund 1993) which are utilized by the brain of neonatal rats (Nehlig and Pereira De Vasconcelos 1993). The transport of ß-hydroxybutyrate through the blood-brain barrier is much higher during the suckling period than in the adults (Cremer 1981). The ketone bodies can enter mitochondria by a monocarboxylate carrier (as was demonstrated by the ability of catalysing an acetoacetate/α-ketoacid exchange by the carrier purified from brain mitochondria) (Nałęcz et al. 1992) and activation of acetoacetate to acetoacetylCoA leads to an increase of intramitochondrial acetylCoA pool (see Fig. 4).

AcetylCoA can be used in cytoplasm in one of three different pathways (1) synthesis of fatty acids, (2) synthesis of cholesterol, (3) formation of acetylcholine. In rat, between 6th postnatal day and the adult age, 77-86% of the total glucose used by the brain is directed for the production of CO_2 and only 14-23% for the synthesis of lipids (Yeh et al. 1977,

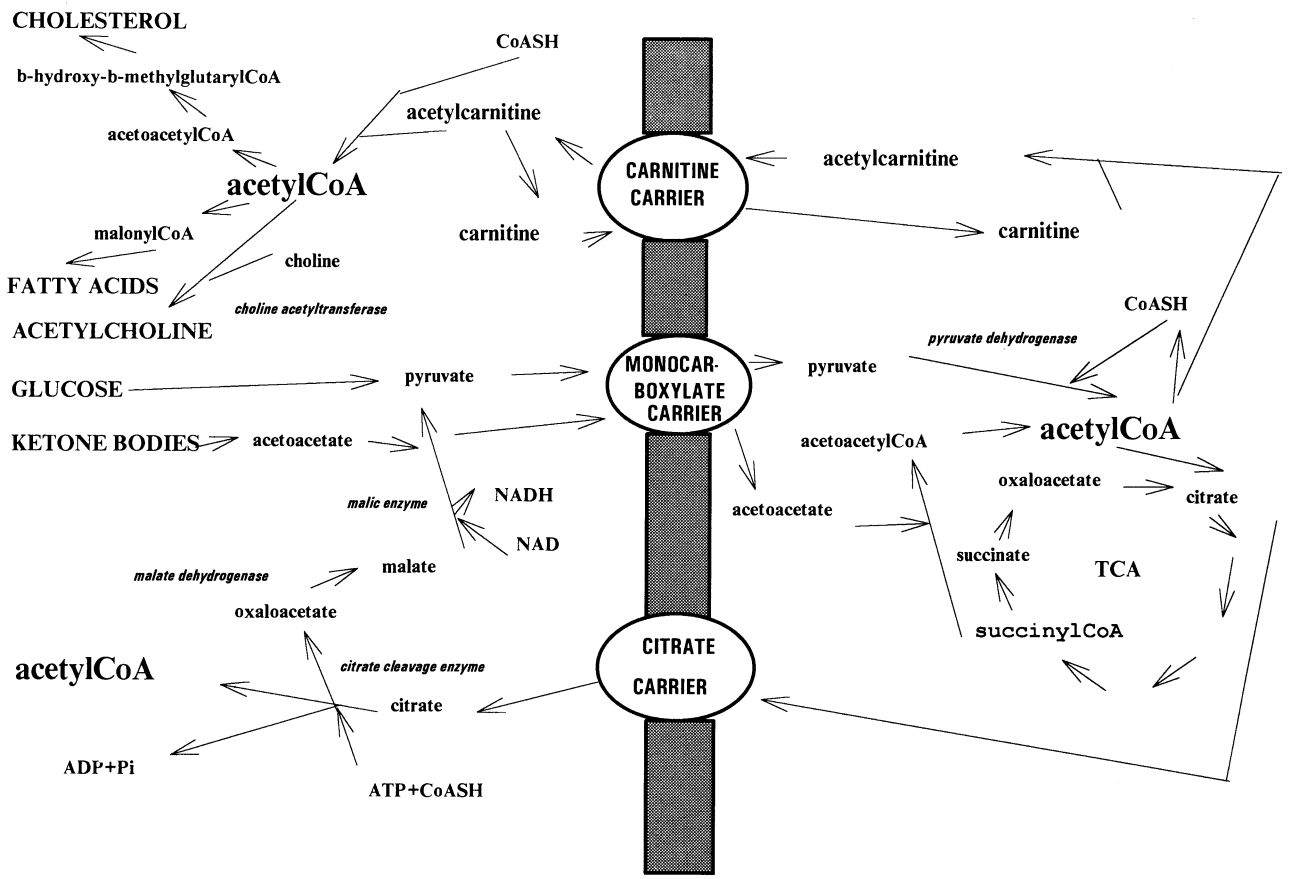

Fig. 4. General metabolic pathways involving acetylCoA. Left side corresponds to cytosolic compartment, the right one to mitochodrial matrix; TCA, tricarboxylic acid cycle.

Yeh and Sheenan 1985). The capacity to synthesize lipids from glucose was shown to peak at 7-15 days after birth (Maker and Hauser 1967, Patel and Tonkonow 1974, Cheseman and Hothersall 1988). In any case, it was demonstrated that ketone bodies were much more efficient than glucose as precursors of fatty acids and sterols in the brain of 18-days old rat.

Acetylcholine synthesis

Acetylcholine acts as the neurotransmitter on cholinergic synapses. Its synthesis takes place in cytoplasm and this process is catalysed by acetylcholine transferase (acetylCoA:choline O - acetyltransferase, EC 2.3.1.6) according to the equation: choline + acetylCoA = acetylcholine + CoASH.

The reaction is reversible, but the equilibrium is shifted to the right (Pieklik and Guynn 1975) which leads to the consumption of acetylCoA. Doležal and Tuček (1981), when checking different precursors which could supply acetyl moieties for the synthesis of acetylcholine, demonstrated for rat brain slices that utilization of citrate and acetate for the synthesis of acetylcholine was low, while pyruvate, glucose, and to a lower extent acetylcarnitine were much better substrates. The synthesis of lipids from pyruvate and citrate was comparable, when estimated from incorporation of ^{14}C from labelled precursors (Doležal and Tuček 1981). Citrate turned out to be a much better precursor for CO_2 production, consumed in the tricarboxylic acid cycle mainly by slices of rat caudate nuclei (Doležal and Tuček 1981). These results were in agreement with the previous observations that the citrate pathway, delivering acetyl CoA to cytoplasm, due to the action of citrate cleavage enzyme, supplied not more

than 30% of acetyl moieties for acetylcholine synthesis (Sterling and O'Neil 1978, Gibson and Shimada 1980, Tuček et al. 1981).

Due to the postulated role of carnitine in the export of acetyl moieties from brain mitochondria to cytoplasm, studies were undertaken in order to clarify the possible influence of carnitine on the synthesis of acetylcholine. This process was monitored in cortical neurones isolated from brains of suckling and adult rats (Wawrzeńczyk et al. 1995a) and also in neuroblastoma NB-2a cells (Wawrzeńczyk et al. 1994) with the use of radiolabeled glucose as a substrate. Acetylcholine synthesis was found to be much higher in suckling animals. Carnitine was shown to stimulate acetylcholine synthesis in a synergistic way, when added together with choline. This increase in acetylcholine synthesis reached 18% in cortical cells from adult rats (Wawrzeńczyk et al. 1995a) and even 36% in neuroblastoma cells (Wawrzeńczyk et al. 1994). The effect of added carnitine was, however, substantially different in the case of cortical cells isolated from suckling animals (Table I). Addition of carnitine decreased acetylcholine synthesis by 30%. This can be explained by a parallel increase in relative acetylcarnitine content in comparison with cells isolated from the adults (Wawrzeńczyk et al. 1995a). There was also observed a parallel high activity of carnitine acetyltransferase (Wawrzeńczyk et al. 1995a). Since the reaction catalysed by this enzyme is fully reversible (Bremer 1983, White and Scates 1990), a high accumulation of acetylcarnitine observed in suckling animals might be a consequence of a decresed amount of the other substrate of carnitine acetyltransferase, namely free CoASH used for the synthesis of lipids or cholesterol (Webber and Edmond 1979). It was, therefore, concluded that carnitine can stimulate acetylcholine synthesis by delivering acetyl moieties to cytoplasm in cells intensively utilizing glucose as the energetic substrate, for instance in adult brain or in transformed cells. This could, at least partially, explain the beneficial effects of carnitine administered to patients with neurodegenerative diseases (Spagnoli et al. 1991, Rebouche 1992, Forloni et al. 1994).

TABLE I

Effect of choline and carnitine on acetylcholine synthesis in cortical neurones

Added substance	Acetylcholine (pmol/mg protein)	
	adult rats	suckling rats
None (control)	256±24	363±10
L-carnitine (50 μM)	250±20	278±20
Choline (20 μM)	261±20	378±18
L-carnitine (50 μM) + choline (20 μM)	302±6	290±18

Synthesis of acetylcholine was estimated in the presence of 50 μM paraoxon (an inhibitor of acetylcholine esterase) with [^{14}C]glucose as the carbone source. The results represent mean values ± SEM from duplicates obtained in either 7 (adult rats) or 4 (suckling rats) experiments after 1 h of incubation (Modified from Wawrzeńczyk et al. 1995a).

Possible functions of long-chain acylcarnitines in brain

About 15-20% of carnitine accumulated in the brain is in the form of long-chain acyl derivatives (Bresolin et al. 1982). A similar value was obtained in experiments with isolated rat cortical neurones (Wawrzeńczyk et al. 1995b). Quite unexpectedly, however, a very high accumulation of palmitoylcarnitine was observed in neuroblastoma NB-2a cells (Nałęcz et al. 1995a). Addition of palmitoylcarnitine was reported to inhibit proliferation of melanoma (Vescovi et al. 1988) and neuroblastoma (Nałęcz et al. 1996) cells. Moreover, it was shown that different acyl derivatives of carnitine reduced the level of total RNA in brain (Albertini et al. 1989). Palmitoylcarnitine has been known to inhibit protein kinase C in various organs like heart (Katoh et al. 1981, 1983), brain and tissues like epiderm (Turner et al. 1982, Nakadate and Blumberg 1987). Our own observations indicated that the activity of protein kinase C measured with peptide {[ser^{25}] PKC-(19-31)} in permeabilized neuroblastoma NB-2a cells was inhibited by palmitoylcarnitine in a concentration-dependent way (Nałęcz et al. 1996).

Accumulation of palmitoylcarnitine in neuroblastoma NB-2a cells was found to be associated with diminished cell proliferation and promoted differentiation (Nałęcz et al. 1996). Although the mechanism of these effects has not been clarified yet, one explanation could point to the palmitoylation status of some protein(s).

It should be emphasized that, contrary to myristoylation, the posttranslational modification which occurs by attachment of palmitoyl moiety to cysteines in proteins is readily reversible (Milligan et al. 1995). The mechanism of this reaction has not been fully established yet, although some characterization of palmitoyl acyltransferase that palmitoylates myristoylated proteins has been reported (Berthiaume and Resh 1995). Palmitoylation is presumed to facilitate anchoring of proteins to membranes (Resh 1994) and to stabilize protein-protein interaction (Chow et al. 1987). What must be stressed is the fact that palmitoylated proteins belong to three classes: (1) receptors linked to guanine nucleotide binding proteins, (2) the α-subunits of heterotrimeric guanine nucleotide binding proteins, (3) nonreceptor tyrosine kinases of the Src family. All these types of proteins are involved in processes of cellular signalling, therefore it seems quite probable that a diminution of palmitate pool by accumulation of palmitoylcarnitine can affect cell cycle in proliferating cells and cause a dramatic change in gene expression turning on the mechanisms leading to differentiation, a phenomenon observed in transformed neural cells (for example neuroblastoma NB-2a). It is worth mentioning that the growth associated protein (GAP-43), the major protein of neurones growing during morphogenesis and regeneration, binds (in a reversible way) two palmitates to cysteines in positions 3 and 4 (Skene and Virag 1989). Moreover, it seems that only the non-palmitoylated form of this protein is able to interact and activate guanine nucleotide binding protein ($G_{o\alpha}$) (Sudo et al. 1992).

If carnitine forms a "sink" causing a decrease of palmitate pool, the administration of carnitine would change the acylation status of many important proteins, influencing various signal transduction pathways.

The knowledge about carnitine functions and transport in the nervous tissues seems also to have medical implications, since there are reported primary carnitine deficiencies based on defects in carnitine transport (Eriksson et al. 1989, Scholte et al. 1990), which may be correlated with acute encephalopathy (Kimura and Amemiya 1990).

In conclusion, there is more and more evidence that carnitine, apart from its main role in the transfer of acyl moieties to mitochondria, can play different roles in the brain, especially in neural cells. Still more information and data about the regulatory implications of carnitine in the brain are necessary.

ABBREVIATIONS

CoASH - Coenzyme A

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