

Minireview

Transport and metabolic effects of carnitine and acylcarnitines in brain*

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Many new experimental and clinical data concerning the physiological and pathophysiological role of carnitine have accumulated in recent years [1, 2]. It is now generally accepted that the major function of L-carnitine in the eukaryotic cell is to transfer acyl compounds (mainly long-chain fatty acids) from the cytosol to the mitochondrial matrix where these acids are further metabolized. L-Carnitine also facilitates removal from mitochondria of short-chain and medium-chain fatty acids that accumulate as a result of normal and abnormal metabolism [1 - 3]. The mechanism of carnitine-dependent transport of fatty acids through the inner mitochondrial membrane, the so called "carnitine shuttle", is schematically presented in Fig. 1. Besides a central translocation step, the pathway consists of several subsequent activation and deactivation reactions of a fatty acyl chain, catalysed by several enzymes of different specificities towards, e.g., degree of saturation and the chain length of the acyl moiety. A similar mechanism of translocation is also believed to operate in peroxisomes [1].

While the involvement of carnitine in fatty acid transport into mitochondria and peroxisomes appears the primary and very general physiological role of this compound, several other metabolic effects of carnitine and its acyl derivatives have recently been observed. Among them is the inhibition by palmitoylcar-

nitine of protein kinase C in various tissues like heart [4, 5], brain [6, 7] and epiderm [8]. It is supposed that this inhibition leads to other biological effects, e.g. inhibition of EGF association with cells [8], inhibition of melanoma cell proliferation [9] or blocking of intercellular communication in rat liver epithelium *via* gap junctions [10]. In heart, palmitoylcarnitine was also found to strongly inhibit sarcolemmal Na,K-ATPase [11]. Different acyl derivatives of carnitine were reported to reduce total brain RNA levels [12], whereas acetyl-L-carnitine was found to induce an increase of cytochrome oxidase subunit I mRNA in hypothyroid rat liver mitochondria [13].

CARNITINE AND BRAIN

Very little is known to date about the ways of carnitine accumulation in the brain and about further transport and metabolism of this compound within neural cells. More data is available as to the metabolic effects exerted by carnitine and its derivatives in cerebral tissue. For instance, it was found that in mouse brain synaptosomes L-carnitine binds efficiently to GABA receptors and competitively inhibits the uptake of GABA [14]. This effect could explain the observed elevation of GABA levels in mouse brain *substantia nigra* upon administra-

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¹Abbreviations: ASC, alanine, serine, cysteine amino acid transporting system; EGF, epidermal growth factor; GABA, γ -aminobutyric acid

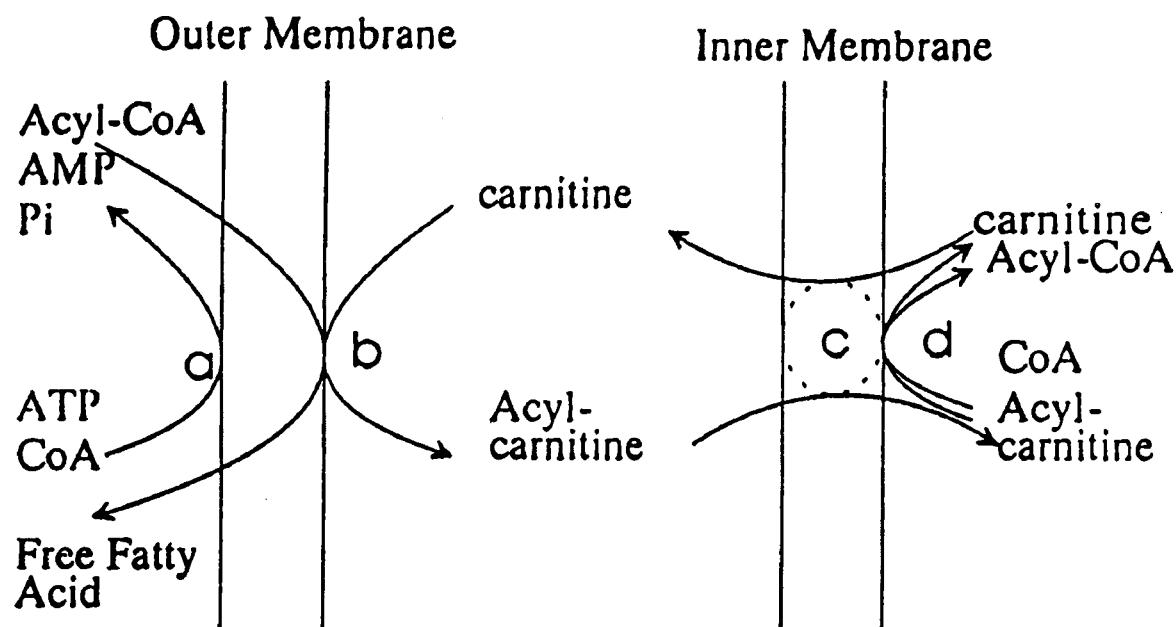


Fig. 1. Mitochondrial carnitine shuttle.

Single letters used in the scheme represent the following parts of the transport pathway: a, a cytoplasmic acylcoenzyme A synthetase; b, the outer mitochondrial membrane carnitine acyltransferase (acyltransferase I); c, inner mitochondrial membrane carnitine-acylcarnitine transferase (carnitine translocase, mitochondrial carnitine carrier); d, inner mitochondrial membrane carnitine acyltransferase (carnitine acyltransferase II).

tion of carnitine and acetylcarnitine to the animal [15]. Acetylcarnitine is also able to modulate neuronal response to acetylcholine, serotonin [16], and to noradrenaline [17]. All these observations bear some relation to the beneficial effects of carnitine administration in patients with brain diseases, although the mechanisms involved are unknown. In any case it is worth mentioning that L-acetylcarnitine was reported to have a strong therapeutic effect on senile brain [18] and, most strikingly, carnitine was found very potent in normalizing encephalopathic properties of Alzheimer cells in culture [19]. Thus, it is not surprising that, after liver and muscles, tissues already well characterized in terms of transport and metabolic role of carnitine, brain becomes now the subject of growing number of biochemical, physiological and medical studies concerning carnitine and its acyl derivatives.

Accumulation of carnitine in the whole brain

Heart and skeletal muscles have the highest content of carnitine and rat brain one tenth of that [20]. However, the activity of palmitoylcarnitine transferase in brain is comparable with that in kidney and skeletal muscles [20].

Mammals are able to synthesize carnitine [21]. Nevertheless, the enzyme catalysing the last step of its synthesis, hydroxylation of 4-butyrobetaine into carnitine (4-butyrobetaine hydroxylase), is present only in few tissues. In humans the enzyme is present in liver, kidney and brain [22, 23]. However, a very low activity of carnitine synthesis was found in the brain *in vivo* and thus it was postulated that carnitine must be transported through the blood brain barrier in order to reach levels normally found in the brain tissue [1, 3]. On the other hand, studies on the whole animal treated with carnitine intravenously revealed that the uptake of carnitine into brain is very slow [24]. Thus, it can not be excluded that carnitine synthesis is, at least to some extent, responsible for the observed accumulation of this compound in the brain, although no direct data are available.

Interestingly, accumulation of carnitine or its acyl derivatives was found very different in various regions of the central nervous system (a particularly high accumulation was found in *hypothalamus*) [20]. This may suggest a different distribution of the synthesizing machinery and/or of specific carnitine uptake system(s) in different regions of the brain.

Practically nothing is known about the possible transport of liver- or kidney synthesized carnitine through the blood-brain barrier, although it seems likely that such a transport occurs [1, 3], especially in the light of therapeutic effects of orally or intravenously administered carnitine and/or its esters on patients with brain diseases [2].

The blood-brain barrier, anatomically formed by cerebral endothelial cells [25], is known to be one of the most specialized and selective permeability barriers in vertebrates. Due to its existence, the composition of brain extracellular space drastically differs from that of blood and, even more important, it achieves a high degree of stability over a large range of variations in blood composition [25]. This is possibly due to highly specific transport regulatory mechanisms present in brain endothelium [26].

Since carnitine uptake, when measured in brain slices [27], is much faster than the accumulation in the whole brain measured *in vivo* [24], it seems clear that the blood-brain barrier limits the access of carnitine to the brain. It remains unknown, however, whether this process can be regulated and, especially, whether this access can be increased under some conditions.

Mechanisms of carnitine transport into cerebral cells

Again, as for the blood-brain barrier, virtually no information about carnitine uptake by neural cells can be found in the literature. A number of papers describing accumulation of carnitine in brain slices or in synaptosomes, as dealing with more artificial conditions, with no discrimination of different brain regions and types of cells, will be only briefly mentioned. For this reason we decided to use mainly our own observations on the subject, although most of them are very recent and have not yet been published.

Brain slices (e.g. [27]) and synaptosomal preparations (e.g. [14, 28]) show an efficient Na-dependent carnitine uptake. Experiments performed in our laboratory (unpublished observations) on carnitine uptake into endothelial, neuroblastoma and isolated rat *striatum* cells in culture showed that L-carnitine accumulation was much higher in neural cells (2.0 - 3.5 pmoles/mg protein) than in endothelial cells (0.8 pmoles/mg protein). Moreover, Na-de-

pendent uptake of carnitine in *striatum* cells contributed to 40 - 50% of the total accumulation of this compound, in contrast to the uptake measured in endothelial cells which was found fully Na-dependent. This suggested that more than one transport system may be responsible for carnitine uptake in nervous cells. In fact, an even more complex picture has been observed when comparing the transport of carnitine into cultured neuroblastoma cells with that into rat brain cortex cells isolated from either newborn or adult animals [29, 30].

The transport process in all cases was found fully inhibited by ouabain, i.e. Na-dependent. In addition, accumulation of carnitine by cortex cells from adult animals was found competitively inhibited by GABA, while no effect of this neurotransmitter was observed in neuroblastoma or in cortex cells isolated from 14-day old rats (suckling animals). Instead, in neuroblastoma the accumulation of carnitine was found inhibited by serine and cysteine, pointing to involvement of ASC-amino acid transport system. In these cells carnitine accumulation followed the first-order rate kinetics and, when analysed as a function of varying substrate concentration, turned out to be the sum of diffusion and saturable transport ($K_m = 170 \mu\text{M}$). Furthermore, L-carnitine transport in neuroblastoma was found to be inhibited also by choline at concentrations indicating an involvement of the low affinity choline translocating system [31]. In contrast, L-carnitine was found to pass through the plasma membrane of cortex cells isolated from suckling rats by diffusion only, indicating a lack of any barrier at the level of plasma membrane at this stage of development.

Some conclusions may already be drawn from these observations. First, brain appears to be very heterogenous in terms of carnitine uptake into cerebral cells. The most general mechanism seems to be Na-dependent, although the isolated *striatum* cells revealed also a part of uptake that was Na-independent. Second, transformed neural cells (here: neuroblastoma NB-2a) appear to use different translocating systems for carnitine uptake than that observed in cells isolated from normal brain (here: from *striatum* and cortex). It remains to be clarified whether the two systems identified in neuroblastoma to be engaged in carnitine uptake (namely, the ASC and the low affinity choline

translocators) may be similarly operational in some regions of normal brain, other than studied here. Third, the fact that the cortex cells isolated from newborn animals revealed carnitine uptake driven exclusively by diffusion may suggest a relatively late expression of carnitine translocating system(s) during brain development.

Carnitine shuttle in brain mitochondria

For a number of years it has been known that the carnitine shuttle exists in brain mitochondria, although its activity, as well as the β -oxidation of fatty acids taking place in the mitochondrial matrix, are much lower than in other tissues [1, 3]. Since glucose is the main energy source of adult brain, the relatively low activity of carnitine shuttle seemed fully justified. More recently, however, it has been observed that in the postnatal period the oxidation of brain fatty acids develops rapidly, to the level comparable to that usually found in liver, what is correlated with the increased activity of palmitoyl-CoA synthetase and carnitine palmitoyltransferase [32]. A more detailed characterization of the carnitine shuttle in brain mitochondria revealed a different substrate specificity than in other tissues; the system was found more specific towards short- than long-chain acyl derivatives of carnitine [33]. Interestingly, however, whereas a lot was known about the overall activity of the carnitine shuttle and some information was available on carnitine acyltransferases and acyl-CoA synthetases involved in activation reactions [1, 3], very few, if any, observations concerned the structure and function characteristics of the carnitine/acylcarnitine translocase, the central element of the scheme (see Fig. 1).

The carnitine carrier was isolated for the first time from liver, by chromatography on hydroxyapatite and celite [34, 35], and shown to consist of a polypeptide with the molecular mass of 32500 Da [35]. The purified protein catalyzed carnitine exchange and uniport after reconstitution into artificial lipid bilayers [36, 37] and revealed sensitivity to various SH group reagents [38].

Using a similar procedure, we have recently succeeded in purification of the carnitine/acylcarnitine translocase from rat brain mitochondria [33]. The carrier was found to consist of a polypeptide with the molecular

mass of 33000 Da, i.e. very similar to that described for liver [35]. The isolated protein seemed to be the same when isolated from mitochondria of very young or adult rat brains, although the activity of the carrier was dependent on animal age, being twice as high in suckling rats than in adults. It remains to be clarified whether this was due to a subsequent expression of two isoforms of the carrier, having similar molecular mass but differing in their activity, or to a posttranslational modification and/or regulation of the same translocating protein. Whatever is true, the present finding confirms earlier observations on high activity of the carnitine shuttle in mammalian brain of neonatals [32].

What is also interesting, is that the functional alteration of the carrier from more- to less-active form was found to occur after 2 - 3 weeks of postnatal brain development [33], approximately at the time of blood-brain barrier formation [25]. This would suggest that suckling animals are still able to fuel their high brain energy requirements by β -oxidation of fatty acids, this possible being due to the lack of blood-brain barrier and the high activity of carnitine shuttle. Later on in the development of brain the carnitine shuttle activity drops down [1, 32, 33] and its function possibly changes. As found in intact mitochondria and confirmed also on the isolated carrier from adult rat brain [33], the activity of the translocase and thus of the whole carnitine shuttle shows a relatively high specificity towards shorter-chain acyl carnitine derivatives. This, taken together with the fact that the association of carnitine acetyltransferase with mitochondria was observed in the nervous tissue [39], could suggest involvement of carnitine in transporting acetyl groups across the mitochondrial membrane for synthesis of acetylcholine [20]. Acetyl-CoA utilized for synthesis of acetylcholine in mammalian brain originates mainly from pyruvate and glucose [40], what would suggest the necessity of high activity of the pyruvate carrier in the nervous tissue. Indeed, this was observed in brain mitochondria [41]. Thus, it seems that the involvement in acetylcholine synthesis may be an important metabolic function of carnitine in adult brain, especially in the regions utilizing cholinergic transmission. Such a conclusion is further supported by the fact that a close relationship between carnitine and its derivatives accu-

modulating in neural cells and acetylcholine synthesis has recently been observed [42].

Metabolic effects of carnitine and acylcarnitines in brain

One of the most intriguing proposals was that carnitine and its acyl derivatives, chemically similar to choline and acetylcholine, may take part in neurotransmission [43]. In fact, it was shown that neurons of the cerebral cortex, i.e. cells specialized in cholinergic transmission, may be excited by acetylcarnitine [43]. This is not yet sufficient, however, to call this compound a neurotransmitter. Instead, it is now well documented that carnitine and acetylcarnitine are able to interact with GABA- [14], acetylcholine- [16], serotonin- and noradrenaline-receptors [17], thus modulating neurotransmission in different regions of the brain. It is unknown, however, whether these effects have any physiological significance *in vivo*. On the other hand, carnitine and its esters have been proven to be useful pharmacological agents for treatment of chronic degenerative diseases in aging human subjects [2]. For instance, acetylcarnitine has been shown in clinical trials to be effective in slowing down the progression of mental deterioration in Alzheimer's disease [44, 45]. The mechanism(s) of the observed effects are unknown but have recently been postulated to be associated with cholinergic nerve transmission [2].

Palmitoyl-CoA, whose level in the cell is in dynamic equilibrium with that of carnitine through activities of palmitoyl-CoA synthetase and palmitoylcarnitine transferase I, was reported to promote fusion of transport vesicles in Golgi cisternae [46]. This may be especially important in neural cells, since fusion phenomena in the Golgi apparatus were found to lead to an increased secretion of neurotransmitters in the presynaptic membrane [47 - 49]. Thus, carnitine appears to modulate neurotransmission in a still other way.

Palmitoylcarnitine has been reported to inhibit protein kinase C in melanoma cells [9] and the enzyme isolated from mouse brain [7]. Since protein kinase C plays a crucial role in intracellular regulation and has a direct influence on cell proliferation phenomena [50], such an effect of acylcarnitine derivatives may play a potentially important role in modulating brain metabolism [6, 7]. In fact, it has been

observed that palmitoylcarnitine is a potent inhibitor of melanoma proliferation, an effect ascribed to the interaction of the compound with protein kinase C [9]. In our hands, low concentrations (10 - 20 μM) of palmitoylcarnitine, but not carnitine, induced rapid differentiation of neuroblastoma NB-2a cells in culture. This effect was accompanied by a decrease in cell proliferation and a reduction in their viability (unpublished observations). Since palmitoylcarnitine appears especially potent in influencing brain tumor cells, possible clinical applications of this observation should be enquired in the future.

Finally, it has been shown that carnitine and its esters may protect cells from oxidative damage, both by inhibiting free-radical propagation and by contributing to repair of oxidized membrane phospholipids [51, 52]. Such processes can occur in many cell types, including brain, but are possibly less important in the central nervous system than they are for heart or epiderm, i.e. tissues more exposed to active forms of oxygen.

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