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The Role of Carnitine Acyltransferases in the Maintenance of Cell Function

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Summary. Carnitine acyltransferases catalyse equilibria between acyl-CoA esters and the respective acylcarnitines. Therefore, they act not only as pathway enzymes, but also as modulators of acyl-CoA concentrations within individual sub-cellular compartments. Because acyl-CoA esters are potent biologically active metabolites, carnitine acyltransferase activities are potentially able to affect a diverse range of physiological processes, ranging from insulin secretion, to appetite control, and insulin sensitivity of tissues. The distinctive subcellular distributions of the different types of carnitine acyltransferases also enables them to participate in the transfer of acyl moieties across intracellular membranes, and of particular acylcarnitine esters across the plasma membrane and into the plasma. Pharmacological strategies that make use of these properties to improve cell function are discussed.

Keywords. Carnitine; CPT; CAT; COT; Malonyl-CoA; Acetyl-CoA carboxylase; β-oxidation; Mitochondria; Peroxisomes.

Introduction

The coenzyme-A esters of fatty acids are highly active, both by giving rise to further pathway substrates that are potent modulators of cell function, and as effectors of many biological processes in their own right. Consequently, their concentrations within the different cell compartments are very closely regulated [1]. As with other metabolites, their effective concentration is dependent on their rate of synthesis and utilization through the activity of pathway enzymes. However, acyl-CoA esters share with each other (according to their chain length) their being substrates for a family of acyltransferases that equilibrate the individual molecular species with the corresponding acylcarnitines. The enzymes catalysing these equilibria belong to a family of carnitine acyltransferases. Therefore, these enzymes

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fulfil a very important role in the maintenance of cell function, in that they not only have specific functions (*e.g.* the transfer of acyl moieties across intracellular membranes – see below) but also have a strong influence on acute changes in concentration of pools of acyl-CoA esters in each individual cell compartment, which are kept separate owing to the impermeability of biological membranes to CoA and its esters. In this respect, carnitine acyltransferases occupy a unique niche in the maintenance of many cellular functions, ranging from fuel selection, to insulin secretion, and to appetite control [2].

The Carnitine Acyltransferases

There are three types of carnitine acyltransferase in mammalian cells, with a further subdivision in one of them. These are carnitine acetyltransferase (CAT), carnitine octyltransferase (COT), and the carnitine palmitoyltransferases (CPTs), with specificities for short-, medium-, and long-chain fatty acyl groups, respectively. The CPTs are further subdivided into those that are inhibited by malonyl-CoA (CPT 1), and one which is not (CPT 2). The inhibition of CPT 1 by malonyl-CoA is a major regulatory feature in metabolism as it provides a unique link between carbohydrate

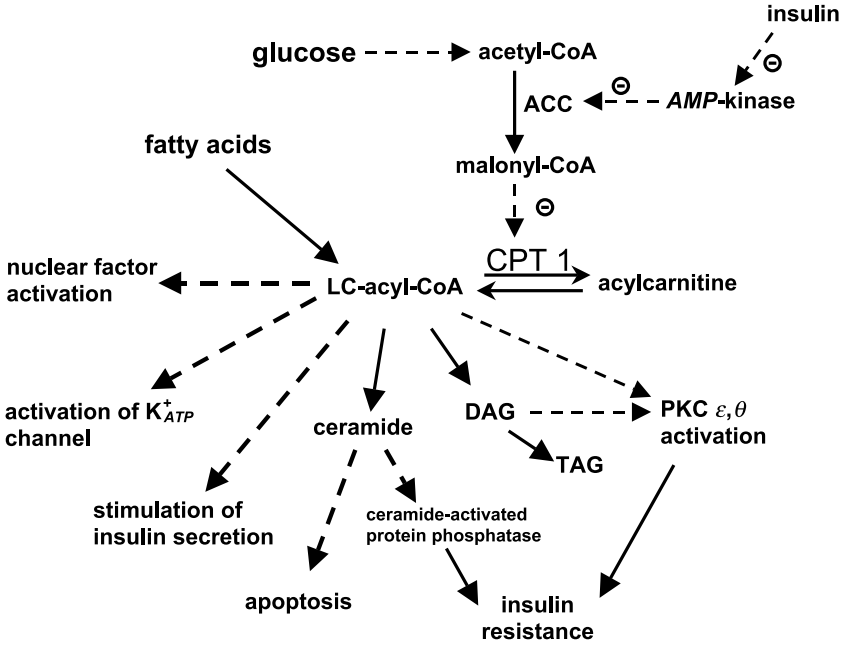

Fig. 1. The role of the inhibition of CPT 1 activity by malonyl-CoA in the integration of the effects of glucose and fatty acid metabolism on multiple functions in different cell types; common regulatory mechanisms affect cell function in a cell-type specific manner; for example, elevated long-chain acyl-CoA promote insulin secretion in the pancreatic β-cell, modulate the secretion of peptides involved in appetite control in the arcuate nucleus of the hypothalamus, and interfere with insulin signalling in many tissues; modulation of CPT 1 activity by malonyl-CoA and, in the case of CPT 1A, by changes in the sensitivity of the enzyme to its inhibitor, determine the degree to which these acyl-CoA effects occur

and fatty acid metabolism that recurs as a constant feature of mechanisms important in many cell functions (see Fig. 1).

Carnitine Palmitoyltransferases

In mammalian genomes there are three genes that code for CPT 1-type proteins, designated A, B, and C in the order in which their cDNA was cloned [3–5]. CPT 1A is also known as L-CPT 1 because it is the isoform first identified as the one that occurs in the liver. CPT 1B, also known as M-CPT 1 because of its preponderance in skeletal and cardiac muscle, is much more sensitive to inhibition by malonyl-CoA than CPT 1A, and has a much lower affinity for carnitine too. A third CPT 1-like protein, coded for by a third gene, and termed CPT 1C, is found expressed as the protein only in the brain of mammals and in transformed cells, although the mRNA for it is much more widely distributed [5]. The only biological function associated with CPT 1C (when expressed heterologously in the yeast *Pichia pastoris*) that has been described to date is its binding of malonyl-CoA with an affinity very similar to that shown by CPT 1A [5]. Both CPT 1A and 1B occur in the mitochondrial outer membrane of which they are integral proteins and have their acyl-CoA and malonyl-CoA-binding sites exposed on the cytosol aspect of the membrane [6].

By contrast, CPT 2 occurs as a protein that is loosely associated with the matrix aspect of the mitochondrial inner membrane. The distinguishing feature of CPT 2 kinetics from those of the CPT 1 proteins, is its insensitivity to malonyl-CoA. However, CPT 1 and CPT 2 work in tandem to effect transfer of long-chain acyl groups between the cytosolic compartment and the mitochondrial matrix, without the release of the free fatty acid, through the sequential formation of the carnitine ester and the reversal of this process through the action of CPT 2. Although both these enzymes catalyse reactions close to equilibrium, the flux is primarily towards the provision of long-chain acyl-CoA to the mitochondrial matrix for β-oxidation, owing to the high affinity and activity of a specific carnitine–acylcarnitine carrier resident within the mitochondrial inner membrane, and the rapid utilization of the acyl-CoA esters by the β-oxidation complex. Regulation of flux occurs primarily at the CPT 1-catalysed step, rather than CPT 2, through its inhibition by malonyl-CoA under normal conditions, although modest increases in protein expression of both CPT 1 [7] and CPT 2 [8] occur under conditions of increased fatty acid oxidation.

Physiological Modulation of Malonyl-CoA Sensitivity of CPT 1A

The regulation of CPT 1A activity by malonyl-CoA presents unique features. Although this isoform is much less sensitive to malonyl-CoA than CPT 1B, it has the ability to change its sensitivity to the inhibitor depending on physiological state, type of dietary fat intake, and hormonal status of the animal (for review, see Ref. [2]). This appears to occur due to the sensing by the protein of changes in the composition of the mitochondrial outer membrane in which it resides, as changes in sensitivity occur slowly over many hours [9–13], are stable in liver homogenates

even when no precautions are taken to prevent post-translational modifications [14], and can be mimicked by inducing changes in membrane fluidity *in vitro* [7]. Moreover, changes in outer membrane lipid order and composition correlate with the observed changes in malonyl-CoA sensitivity observed in mitochondria isolated from rats maintained under different physiological conditions [15]. Elucidation of the topology of the protein [6, 16, 17] within the membrane (polytopic, with two transmembrane segments with a 27-residue connecting loop that protrudes into the inter-membrane space) provides an explanation for this sensitivity of the protein to the physico-chemical state of the membrane, as well as for the ability of the protein to undergo major conformational changes in response to malonyl-CoA binding that enable it to fold tightly and make its core insensitive to proteases [18, 19]. These properties appear to derive from the fact that at least three types of intra-molecular interaction are made possible by this polytopic structure, namely, (i) between the (small) N- and (large) C-terminal (catalytic) domains that are both exposed on the outer surface of the membrane, (ii) between the two trans-membrane segments, and (iii) the TM segments and loop, as they affect inter-N- to C-domain interactions, although binding of malonyl-CoA and acyl-CoA both occur on the C-domain (for more detailed discussion, see Ref. [20]). That such N–C interactions actually occur in the native enzyme in its natural membrane environment has been demonstrated experimentally recently using a chemical cross-linking strategy [21]. In these experiments it was shown that a non-permeant, hydrophilic, bifunctional cross-linker (sulfo-KMUS) with a spacer arm of 15.7 Å is able to cross-link the N- and C-terminal domains both for the enzyme in intact rat liver mitochondria, and for that expressed in yeast. Moreover, it was shown that in liver mitochondria isolated from fasted or insulin-deficient rats, in which fatty acid oxidation is less sensitive to malonyl-CoA, cross-linking between the two domains is impaired, demonstrating a correlation between the intramolecular interaction between these two domains and the physiological function of this protein, as reflected in its kinetic characteristics. Indeed, the slow changes in malonyl-CoA sensitivity that CPT 1A undergoes during physiological perturbations such as the fed-to-fasted or diabetic-to insulin-replete transitions, are highly correlated with the ability of the liver to oxidise fatty acids (and thus form ketones, and support gluconeogenesis), indicating that these protein–membrane interactions are vital for the maintenance of these liver functions.

Recently, it has been found that at least two serine residues in rat liver CPT 1A are phosphorylated *in vivo* and that dephosphorylation brings about changes in catalytic activity and malonyl-CoA sensitivity, as well as changes in the mechanism of inhibition by malonyl-CoA with respect to acyl-CoA substrate [22]. However, the physiological relevance of these changes is difficult to ascertain, as dephosphorylation of these residues could not be achieved through the action of cytosolic phosphatases (to which the protein is physiologically exposed *in vivo*) but only after rupture of the mitochondrial inner membrane (by freezing and thawing mitochondria or liver extracts to expose the CPT 1 to a matrix protease). In addition, phosphorylation of these residues could only be achieved by casein kinase II rather than by serine protein kinases that are recognised as mediating cellular acute responses to changes in hormonal conditions (such as *cAMP*-dependent and *AMP*-stimulated kinases), suggesting that these residues may be constitutively

phosphorylated *in vivo* and may set the basal malonyl-CoA sensitivity of the enzyme, acute modulation of which is then mediated through the membrane-associated conformational changes mentioned above.

Indeed, both the extreme N- and C-termini of the protein have now been found to be important in determining malonyl-CoA sensitivity. Within the first two predicted α-helices of the N-terminal domain of rat liver CPT 1A, Glu3 is a positive determinant of sensitivity [23] whereas the combination of Ser24 and Gln30 is a potent negative determinant of this parameter [24, 25]. Therefore, membrane-induced changes in malonyl-CoA sensitivity are likely to involve intra-N-terminal conformational changes that alter the nature of the interaction between the regulatory N- and catalytic C-domains of the protein. Recently, we have found that, within the extreme C-terminus of rat CPT 1A, L764 is not only essential for correct folding of the protein (*cf.* CPT 1B [26]), but its exposure by sequential truncation of the short sequence C-terminal to it, also makes the enzyme more sensitive to malonyl-CoA (*Borthwick*, *Price*, *Jackson*, and *Zammit*, unpublished) indicating that this residue is crucial for the maintenance of native folding of the C-domain (to which both acyl-CoA and malonyl-CoA bind). Other work has also shown that larger truncations from the C-terminal end of the molecule prevent the correct folding of the catalytic domain [27].

Consequences of Inhibition of CPT 1 Activity

Besides the direct effect on the flux of fatty acids into the mitochondrial β-oxidative pathway, inhibition of CPT 1 activity (or over-supply of fatty acids in excess to the rate at which they can be oxidised) has other important consequences for cell function and, depending on the cell type, on whole-body metabolism. This is because it results in the elevation of the effective concentration of long-chain acyl-CoA esters (which are bound with very high affinity by acyl-CoA binding protein, ACBP [1]) and their diversion into the synthesis of complex lipids, including ceramides and diglycerides. Acyl-CoA esters themselves have important biological effects including: (i) Activation of the K^+_{ATP} channel which, in orexipeptide-secreting neurons in the arcuate nucleus of the brain, is known to link satiety signals, either directly *via* availability of fatty acids, or through *AMP*-kinase inhibition [28] and elevation of malonyl-CoA, to inhibition of CPT 1A. The consequent elevation of long-chain acyl-CoA levels leads to inhibition of food intake and inhibition of vagally mediated stimulation of hepatic glycogenolysis [29]; (ii) Amplification of insulin secretion through the provision of a coupling factor that enhances insulin secretion in response to secretagogues – an action that may account for the important role that long-chain fatty acids are now known to play in the compensatory hypersecretion of insulin characteristic of insulin-resistant conditions [30]; (iii) The development of insulin resistance in ectopic tissues, such as muscle, in which the rate of delivery of fatty acids or triglycerides exceeds the rate at which they can be oxidised (*e.g.* due to hyperlipidaemia or lack of exercise) owing to the effects of diglycerides and ceramide on the activity of protein kinases or phosphatases, respectively, which affect the phosphorylation state of proteins involved in the insulin-signalling cascade [31]; (iv) Modulation of apoptotic rate due to increased synthesis of ceramide, which is pro-apoptotic [32].

Therefore, it can be seen that control of CPT 1 activity has far-reaching consequences on cell function, and on the ability of tissues to respond to different physiological conditions. It is to be expected, therefore, that modulation of the activity of CPT 1A and 1B either directly or through altering their malonyl-CoA sensitivity continues to attract interest within the pharmaceutical industry as potential targets for the treatment of conditions associated with regulation of food intake, insulin secretion, and the metabolic syndrome.

The Medium- and Short-chain Carnitine Acyltransferases

Carnitine octanoyltransferase (COT) and carnitine acetyltransferase (CAT) are both found in the peroxisomal core; CAT is also found in the mitochondrial matrix [33]. But neither of them is present in the cytosolic compartment. Mature mitochondrial and peroxisomal CAT arise from transcription of the same gene as a result of differential splicing. This striking distribution may be related to the fact that, because fatty acid oxidation occurs both in peroxisomes (which have a preference for very-long chain fatty acid oxidation and perform only partial β-oxidation) and mitochondria, communication between the processes in the two sites needs to occur not only at the level of substrate flux, but also at the level of the complementary regulation of the two fluxes.

Role of CAT in Mitochondrial Function

The rationale for the localization of CAT within the mitochondrial matrix is that it acts as a means of regulating the intramitochondrial concentration of acetyl-CoA, which is an important metabolite that occurs at the intersection between entry of the products of metabolites derived from lipids, amino acids, and glucose into the tricarboxylic acid cycle on the one hand, and the synthesis of fatty acids and glucose (through its allosteric effects) on the other. Acetyl-CoA acts not only as a pathway substrate but also as an allosteric activator of PDH-kinase and pyruvate carboxylase. Therefore, CAT activity and the availability of carnitine assume an important role in determining intra-mitochondrial acetyl-CoA levels. While there are no reports that this activity is modulated acutely by metabolites, the availability of carnitine to the cell plays an important role in determining the position of the equilibrium between acetyl-CoA and CoA, which has consequences not only because of the actions of acetyl-CoA (above), but also because the intra-mitochondrial content of total CoA is limited, and in the absence sufficiently rapid regeneration of free CoA, further activation of substrates for oxidation is impaired. Importantly, the cellular pool of carnitine is much larger than that of CoA, and carnitine and its acylesters are permeable through membranes, because of the presence of specific carriers therein.

The role of peroxisomal CAT is more complicated, and will be discussed specifically below, but in terms of a role in the removal of products of fatty acid metabolism, especially in disease states characterised by enzyme deficiencies, the function is likely to be similar to that of mitochondrial CAT. This action of CAT has been suggested to underlie the beneficial effects of the supply of carnitine and its short-chain esters on the function of several tissues, especially under conditions

characterised by anoxia and reactive oxygen species formation. A comprehensive discussion of this aspect of the roles played by carnitine acyltransferases has been published recently [33, 34] and only a few salient examples will be discussed here.

Carnitine and, especially, propionyl-*L*-carnitine supplementation are beneficial for post-ischaemic recovery of cardiac function [34]. The effects of ischaemia are exacerbated by continued high rates of utilisation of fatty acids by the myocardium [35, 36]. High doses of carnitine inhibit fatty acid oxidation by the myocardium – an effect that is likely to be related to its ability to lower intramitochondrial acetyl-CoA levels and thus de-inhibit PDH activity, with consequent improvement in the rate of pyruvate oxidation. The much more potent action of propionyl-carnitine under the same conditions is probably due to the additional benefit of generating *ATP* through metabolism of the propionyl moiety to oxaloacetate [37, 38]. The much improved cardiac function that is observed upon treatment with propionyl-carnitine has been shown experimentally in hypertrophied hearts [39] and post-ischaemically [40, 41] although other studies have been less definitive [42]. Propionylcarnitine is also a good radical scavenger [43] a property that may contribute towards resistance against the effects of reactive oxygen species. Interestingly, acetylcarnitine, the direct product of CAT action on acetyl-CoA shows none of these effects, and this differential action of carnitine and its short-chain esters can be rationalised in terms of the metabolic interactions shown in Fig. 2. It can be seen that both carnitine and propionylcarnitine would be predicted to lower intramitochondrial acetyl-CoA levels, whereas acetylcarnitine, by virtue of a mass-action

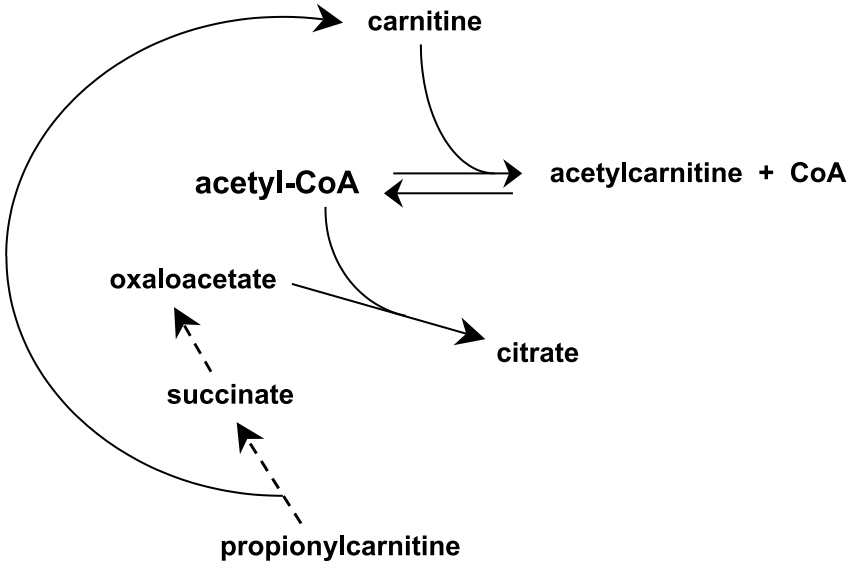

Fig. 2. Carnitine acetyltransferase-mediated equilibration between carnitine and its short-chain acyl esters modulates the intramitochondrial concentration of acetyl-CoA, with important consequences for tissue fuel-selection; carnitine and propionylcarnitine are anticipated to lower the concentration of intramitochondrial acetyl-CoA, thus de-inhibiting pyruvate dehydrogenase activity and favouring pyruvate oxidation over that of fatty acids in cardiac muscle; by contrast, acetylcarnitine, through its mass-action effect on the reaction catalysed by carnitine acetyltransferase (CAT) is expected to raise intramitochondrial acetyl-CoA

effect on CAT is anticipated to raise intramitochondrial acetyl-CoA, thus inhibiting pyruvate oxidation.

Role of COT and CAT in Mitochondrial-peroxisomal Acyl Trafficking

It has long been assumed that there is a route for partially oxidised products of peroxisomal fatty acid oxidation to be further processed by mitochondria. Peroxisomes partly oxidise very-long-chain fatty acids, generating shorter-chain acyl-CoA esters. The conversion of these products into the respective carnitine esters would be expected to provide a mechanism whereby the acyl moieties could be transported into mitochondria for complete oxidation. COT can only perform this function because of its wide chain-length specificity [44]. However, the experimental evidence in favour of this trafficking in the case of acetylcarnitine has been limited. In Ref. [45] it was shown that, in the liver, acetyl moieties are delivered to mitochondria from peroxisomes primarily as acetate, with small amounts of acetylcarnitine being detected when cells were incubated with carnitine, which may also have stimulated the oxidation of very-long-chain fatty acids by mitochondria [45]. In Ref. [46] it was shown that mitochondrial oxidation of propionyl moieties generated through peroxisomal β-oxidation of pristanic acid in human skin fibroblasts is dependent on the expression of the carnitine–acylcarnitine translocase (CACT), thus strongly suggesting that carnitine is involved in the transfer of the products of peroxisomal fatty acid oxidation to the mitochondria. Interestingly,

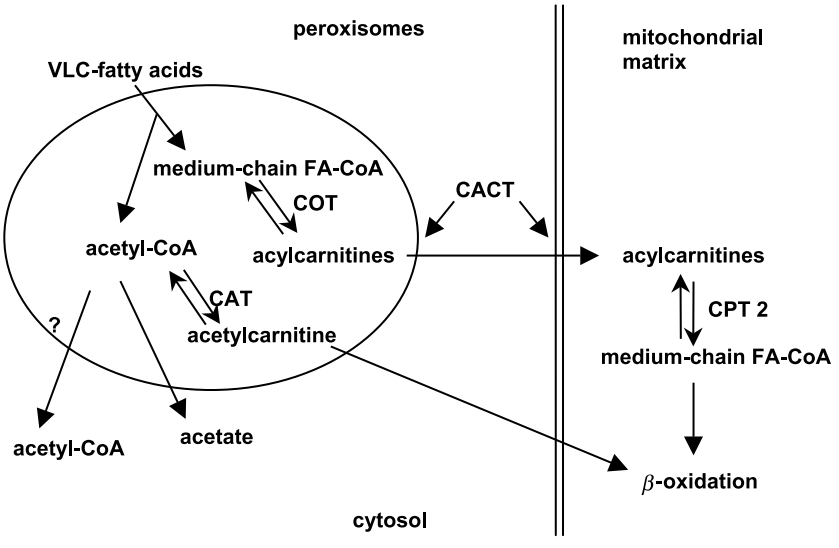

Fig. 3. The involvement of peroxisomal carnitine acetyltransferase (CAT) and carnitine octanoyltransferase (COT) in the provision of products of peroxisomal β-oxidation as substrates for mitochondrial oxidation; this widely-accepted scheme involves the export of acetylcarnitine and other (chain-shortened) acylcarnitines from the peroxisomes and transfer to the mitochondrial matrix through the action of the carnitine–acylcarnitine translocase (CACT); however, recent observations (see Fig. 4) suggest that the process is more complex, and may involve the release, from peroxisomes, of acetate and, possibly, acetyl-CoA

CACT has been found to be expressed both in peroxisomes and the mitochondrial inner membrane [47].

However, a recent series of observations on the fate of peroxisomally generated acetyl moieties in rat heart [48, 49] have raised the prospect that the acetylcarnitine resulting from peroxisomal β-oxidation is not transported to the mitochondria in this tissue, as ^{13}C-labelled acetyl moieties generated peroxisomally did not equilibrate with the C-atoms of citrate, suggesting that they do not give rise to intra-mitochondrial acetyl-CoA. By contrast, these authors showed that peroxisomal acetyl-CoA is a good precursor of (cytosolic) malonyl-CoA. Their data suggest that, whereas most of the malonyl-CoA derived from pyruvate originates from mitochondrial acetyl-CoA (*via* the sequential actions of citrate synthase and *ATP*-citrate lyase), that derived from fatty acid oxidation (not necessarily very-long-chain ones) originates primarily in the peroxisomes. Thus, a product of peroxisomal fatty acid oxidation may generate an inhibitor of mitochondrial fatty acid oxidation (Fig. 3). This is a highly interesting concept, especially as it can be

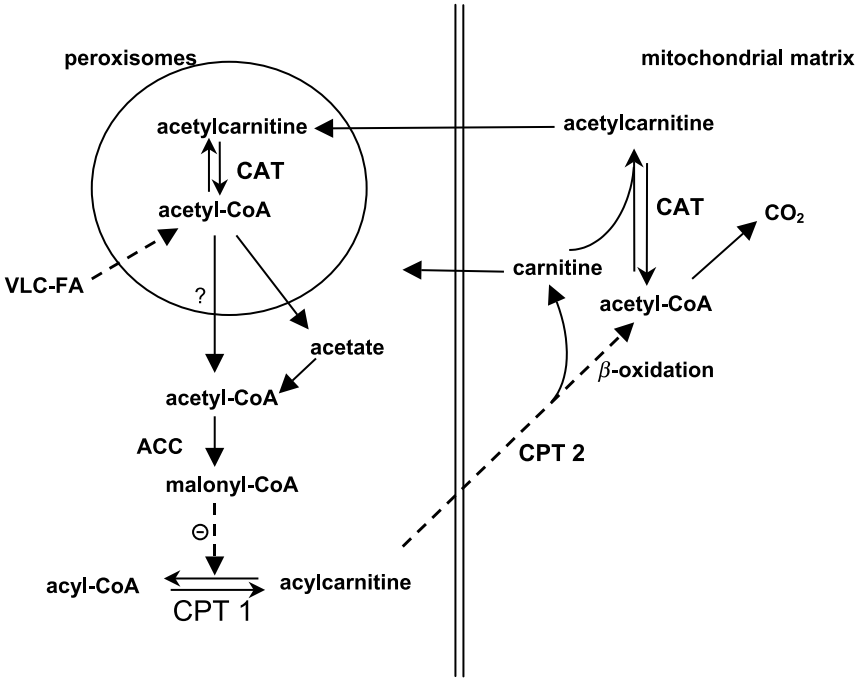

Fig. 4. Carnitine acetyltransferase in peroxisomes may have the function of providing acetyl moieties for the formation of cytosolic malonyl-CoA; acetyl moieties generated by peroxisomal fatty acid oxidation are a source of cytosolic malonyl-CoA, but not of C-atoms of mitochondrial citrate in rat heart (see Refs. [48, 49]); in combination with the absence of CAT from the cytosolic compartment, this necessitates the proposition that peroxisomes release acetate (as observed in hepatocytes [45]) and/or acetyl-CoA, for synthesis of malonyl-CoA through the action of acetyl-CoA carboxylase (the activity of which would be anticipated to be low under these conditions); conversely, acetylcarnitine produced through CAT activity within the mitochondrial matrix can also move into peroxisomes and generate cytosolic malonyl-CoA through this pathway, thus providing a feedback mechanism to limit excessive rates of fatty acid oxidation, though the malonyl-CoA-induced inhibition of CPT 1

extended (see Fig. 4) to the feed-back regulation of mitochondrial fatty acid oxidation through the reverse movement of acetylcarnitine from mitochondria to peroxisomes thus helping to moderate fatty acid utilisation (and possible generation of reactive oxygen species) under conditions of high rates of oxidation. However, it has to be appreciated that, under these conditions, the activity of acetyl-CoA carboxylase, required for the formation of malonyl-CoA from acetyl-CoA, is likely to be low although the data of Refs. [48, 49] suggest that a sufficient flux may still exist for peroxisomal acetylcarnitine to be a major source of cytosolic malonyl-CoA. That acetylcarnitine is released from the liver under these conditions is well established [50]. It is important to emphasise that such roles of acetylcarnitine are only possible because of the particular distribution of CAT (intra-peroxisomal and intra-mitochondrial) and its absence from the cytosolic compartment [51], implying that acetylcarnitine can act as a carrier of acetyl moieties (without being metabolised in the cytosol) between the two organelles and across the plasma membrane as a source of plasma acetylcarnitine, which increases markedly under conditions of increased fatty acid oxidation in the liver [50].

Conclusions

The importance of carnitine acyltransferases in the maintenance of cell function derives from their ability not only to act as pathway enzymes, but primarily from their role in controlling the pool-sizes of acyl-CoA esters (and free CoA itself). The high biological potency of these esters necessitates close metabolic control, and makes their metabolism, through the action of carnitine acyltransferases, a potential pharmacological target. This has the potential to affect such widely diverse processes as cardiac function, insulin secretion, insulin-sensitivity, and appetite control. Recent advances in the understanding of the molecular characteristics of the carnitine acyltransferases, as well as the tracing of the metabolic fluxes that occur between the different cellular compartments/micro-environments in which they occur offer a rationalisation for the use of such strategies.

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