Secondary carnitine insufficiency in disorders of organic acid metabolism: modulation of acyl-CoA/CoA ratios by L-carnitine in vivo

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L-Carnitine (3-hydroxybutyrobetaine) is biosynthesized in man by a series of steps from N-trimethyl-lysine to butyrobetaine, with a final hydroxylation step occurring specifically in liver, kidney and brain (Rebouche & Engel, 1980). The classical and best-known role of L-carnitine is in facilitating the transport of long-chain fatty acids across the mitochondrial inner membrane for β-oxidation, this occurring via the action of specific acyltransferases and translocases (Fig. 1a) (see, e.g., Hoppel, 1982). L-Carnitine may also mediate the removal of acetyl-CoA from the mitochondria as acetylcarnitine (Fig. 1b) and, as we and others propose, may have other important roles in the modulation of intramitochondrial acyl-CoA/CoA ratios (Bieber et al., 1982), of the availability of free CoA and in the detoxification and facilitated removal of excess and potentially toxic acyl groups from the mitochondrion and cell. Evidence for these proposed new roles for L-carnitine have now been provided by our studies on patients with disorders of organic acid metabolism.

We have observed increased acylcarnitine excretion in 26 children with inherited disorders of branched-chain amino acid metabolism characterized by accumulation of acyl-CoA intermediates and their abnormal organic acid metabolites (see, e.g., Chalmers & Lawson, 1982). Concentrations observed, expressed in nmol/mg of creatinine, were: methylmalonic aciduria, average 456 (range 74–1058; n = 10); propionic acidemia, average 382 (range 179–808; n = 7); isovaleric acidemia, 534, 217 (n = 2); multi-carboxylase deficiency, 940, 753 (n = 2); 3-hydroxy-3-methylglutaric aciduria, 649, 417, 92 (n = 3); and β-ketothiolase deficiency, 295, 309 (n = 2). Acylcarnitine excretion in normal subjects is 74 ± 40 nmol/mg of creatinine. Acylcarnitine/free-carnitine ratios in all patients studied were greatly increased above the normal value of 1.45, although urinary free carnitines were within the normal range in the majority of patients. These results suggest that acylcarnitine/carnitine and hence acyl-CoA/CoA ratios and homoeostasis in the mitochondrion are abnormal in these diseases and that, although carnitine biosynthesis and acylcarnitine formation are occurring, insufficient L-carnitine is available for the increased metabolic requirements secondary to the patients’ primary enzyme deficiencies. Further evidence for an increased require-
ment and thus secondary insufficiency of L-carnitine is provided by studies on a patient with methylmalonic aciduria. This disorder is characterized by accumulation of methylmalonyl-CoA and its metabolite methylmalonic acid and of propionyl-CoA and its abnormal metabolic products, for example methylcitric acid. Administration of an oral L-carnitine challenge resulted in a dramatic increase in acylcarnitine excretion, from 352 nmol/mg of creatinine to a peak value of more than 8000, representing a 24-fold increase in excretion. Plasma short-chain acylcarnitines increased from 17.1 µmol (normal range 5.7 ± 3.5 µmol) to 27.4 µmol, and free carnitine from 35.8 µmol (normal range 36.7 ± 7.6 µmol) to 66.1 µmol. The greatly increased acylcarnitine excretion was accompanied within 30 min of L-carnitine administration by a 6-fold increase in urinary hippurate excretion, from 800 nmol/mg of creatinine to a peak value of 5000 nmol/mg. The major acylcarnitine was identified as propionylcarnitine by use of fast atom bombardment mass spectrometry, showing that L-carnitine acts by detoxification and removal of propionyl groups, in accord with the decreased methylcitrate excretion.

The rapid and profound rise in hippurate formation and excretion provides further evidence for the underlying biochemical mechanisms involved. The rate-limiting step in hippurate biosynthesis is the ATP- and CoA-requiring benzoyl-CoA synthetase (Gatley & Sherratt, 1977), followed by glycine conjugation through the action of glycine N-acetyltransferase (EC 2.3.1.13) (Fig. 2). The removal of propionyl-CoA as propionylcarnitine releases free CoA and thereby stimulates the activity of key mitochondrial processes, for example pyruvate oxidation and tricarboxylic acid cycle activity (Fig. 1b). This in turn increases ATP synthesis via the respiratory chain and oxidative phosphorylation (Fig. 2), and the combined increased availability of free CoA and ATP is rapidly reflected in increased hippurate synthesis and excretion. The extremely rapid response in the absence of supplementary benzoyl indicates that benzoyl itself is already accumulating within the mitochondria. These results demonstrate the presence of secondary insufficiency of L-carnitine in these diseases and the role in vivo of L-carnitine in modulation of acyl-CoA/CoA ratios and of mitochondrial and cellular homeostasis.

This work was supported in part by grants from the National Reye's Syndrome Foundation, Ohio, the Reye's Syndrome Research Fund (Duke University Medical Center), the National Institutes of Health (grants AM15804 and the Muscular Dystrophy Association (U.S.A.). Studies on patients were approved by the Ethical Committee of Northwick Park Hospital and Clinical Research Centre, Harrow, U.K.


Energy provision in Chinese-hamster ovary cells

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We have shown that wild-type Chinese-hamster ovary cells utilize both D-glucose and L-glutamine as major energy sources, whereas a glycolytic mutant, R1.1.7 (Morgan et al., 1980), obtains essentially no energy from glycolysis and is absolutely dependent on respiration for its energy needs (Morgan et al., 1981). The energy yield from L-glutamine oxidation alone, however, was insufficient to explain the growth of the mutant, and we postulated that other substrates present in the medium must be oxidized (Morgan et al., 1981). We therefore decided to investigate which substrates could be oxidized in sufficient amounts to account for the apparent energy deficit in the glycolytic mutant.

The effect of substrates on oxygen uptake was measured in two ways, to determine which compounds might serve as energy sources. Firstly, washed cells were incubated with substrates for various times to ascertain which substrates were able to sustain high rates of oxygen uptake (equivalent to the initial endogenous rate). Alternatively, substrates were added directly to washed cells in the oxygen electrode chamber. L-Glutamine was the most effective substrate, and was able to sustain and stimulate oxygen uptake for up to 6 h. Sodium pyruvate and L-glutamate were partially effective. L-Alanine and malate were however, insufficient to explain the growth of the R1.1.7 cells: thus some glycolytic product inhibits pyruvate uptake and/or metabolism. The release of CO2 from [14C]pyruvate (but not that from [14C]acetate) depends on the operation of the tricarboxylic acid cycle and can therefore be used in an estimate of ATP yields. [2,4]C]Pyrurate utilization was measured only in R1.1.7 cells, where it is of major quantitative importance. The rate of CO2 release from [2,4]C]pyruvate was approximately one-third of that observed with [1,4]C]pyruvate and was essentially unaffected by the presence of glucose (Table 1). The rate of removal of pyruvate from the medium was essentially the same as in the experiment with [1,4]C]pyruvate, and thus the difference in rates of CO2 release reflect differences in the flux of pyruvate entering the tricarboxylic acid cycle. The acetyl-CoA that is not oxidized is probably converted into lipid. Similar observations and conclusions have been made for ascites cells (Lazo, 1981).

During growth on glucose, exogenous pyruvate metabolism results in the synthesis of 72.3 nmol of ATP/min per mg of protein in R1.1.7 cells and 21.8 nmol of ATP/min per mg of protein in CHO-K1 cells (Table 1). In the absence of glucose (and hence the absence of cell division) R1.1.7 cells obtain 60.3 nmol of ATP/min per mg of protein and CHO-K1 cells 57 nmol of ATP/min per mg of protein from pyruvate metabolism. We previously showed (Morgan et al., 1981) that...