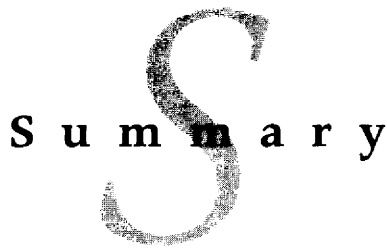
Molecular and biochemical aspects of carnitine biosynthesis

Vaz, F.M.

Publication date
2002



Summary

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Carnitine plays an important role in fatty acid metabolism where it is involved in the transport of activated fatty acids between cellular organelles. In mammals, carnitine is derived from dietary sources and *de novo* synthesis. The importance of carnitine in energy homeostasis is stressed by the occurrence of the disorder systemic carnitine deficiency, where the transporter in the plasma membrane responsible for the uptake of carnitine from the blood, the absorption of carnitine from dietary sources and the reabsorption of carnitine in the kidney, is defective. Carnitine is synthesised from protein-derived 6-N-trimethyllysine via 3-hydroxy-6-N-trimethyllysine, 4-N-trimethyl-aminobutyraldehyde and 4-N-trimethylaminobutyrate (4-N-butyrobetaine) (see chapter 2, FIG 3). The capacity of most mammals to synthesise carnitine endogenously has been known for over four decades. Until now, however, no disorders have been identified which were caused by a defective carnitine biosynthesis. As part of the diagnostic responsibilities of our laboratory, numerous patients have been identified suffering from various pathologies caused by unexplained disorders characterised by low carnitine levels. Although the sequence of metabolites leading to carnitine is known since the 1970's, at the start of our studies nothing was known about the enzymes of the carnitine biosynthesis at the molecular level. Furthermore, methods to determine the concentration of the metabolites preceding carnitine were either unavailable or too labour-intensive for routine application. Therefore, to investigate carnitine metabolism in full detail in these patients, we set out to identify the genes involved in carnitine biosynthesis and develop new methods to measure carnitine biosynthesis metabolites in body fluids. In Chapter 2 the current status of the field of carnitine biosynthesis and transport is reviewed including the results presented in the experimental chapters. Chapter 3 reports the identification of the cDNAs coding for rat, human and mouse 6-N-trimethyllysine, 2-oxoglutarate dioxygenase, which is the first enzyme of carnitine biosynthesis. The rat enzyme is localised exclusively in mitochondria and expression studies in yeast indicate that the enzyme is synthesised as a 47.5 kDa precursor, which is subsequently processed to a mature protein of 43 kDa, presumably upon import in mitochondria. Although we have attempted to measure the activity of the second enzyme, 3-hydroxy-6-N-trimethyllysine aldolase, we have thus far not succeeded in detecting this enzyme activity in rat liver homogenates. In chapter 4 we identified and characterised rat 4-N-trimethylaminobutyraldehyde dehydrogenase, which catalyses the penultimate step of carnitine biosynthesis. In addition, we found evidence which indicates that the known human aldehyde dehydrogenase 9 is the same enzyme as 4-N-trimethyl-aminobutyraldehyde dehydrogenase. Chapter 5 describes the purification and characterisation of rat 4-N-butyrobetaine, 2-oxoglutarate dioxygenase, the last enzyme of the carnitine biosynthesis. With sequence information of the purified rat protein, the human cDNA coding for this enzyme was identified and it was shown that 4-N-butyrobetaine, 2-oxoglutarate dioxygenase is primarily expressed in kidney and liver. Chapter 6 describes a novel method to quantify four of the five metabolites of carnitine biosynthesis in urine. With this method we obtained an indication of the control values for each of the metabolites in human urine and showed that full-term newborns are capable of carnitine biosynthesis from exogenous 6-N-trimethyllysine. In chapter 7 we resolved the molecular defect in three patients suffering from systemic carnitine deficiency and unambiguously identified *OCTN2* as the defective gene in this disorder. Summarising, we have succeeded in identifying three of the four genes coding for the enzymes of carnitine biosynthesis and we developed an assay to measure four of the five carnitine biosynthesis

metabolites in urine. With the knowledge of the genes involved in carnitine biosynthesis it should be possible to generate a knock-out mouse, where one of the genes of this pathway has been disrupted. This mouse model will give more insight in the consequences of a defective carnitine biosynthesis. Since we now have the ability to characterise the enzymatic, molecular and metabolic aspects of carnitine biosynthesis, a thorough investigation of the role of this pathway is possible and warranted, especially in patients with various clinical abnormalities and deficient carnitine levels. Such studies are now under way.