4 Carnitine palmitoyl transferase I and malonyl-CoA in the heart and skeletal muscle: does control analysis help to explain the paradox?

S. Eaton[1], N.P. Duran[1], K. Bartlett[2] and P.A. Quant[1]

[1]Unit of Paediatric Surgery, Institute of Child Health, 30 Guilford Street, London, WC1N 1EH, U.K.

[2]Sir James Spence Institute of Child Health, Royal Victoria Infirmary, Newcastle-upon-Tyne, NE1 4LP, U.K.

Introduction

Carnitine palmitoyl transferase I (CPT I), a transmembrane enzyme of the mitochondrial outer membrane, catalyses the transfer of an acyl moiety from a long-chain acyl-CoA ester to carnitine to form a long-chain acyl-carnitine ester, which can then enter the mitochondrion and undergo β-oxidation. The enzyme is a potential site for regulation of β-oxidation flux via its physiological inibitor, malonyl-CoA [1, 2] and is widely assumed to be the rate-limiting step in the β-oxidation of long-chain fatty acids in the heart [1] and other tissues. It has been found to have significant control over β-oxidation flux in hepatocytes [3–5], liver mitochondria [6] and astrocytes [7]. However, the concentration of malonyl-CoA in the heart is estimated to be in the range of 1-10 μM [2]. This greatly exceeds the IC_{50} of heart CPT I for malonyl-CoA [8] so it is difficult to see how β-oxidation proceeds in cardiac tissue if CPT I activity is rate-limiting for β-oxidation, unless most of the malonyl-CoA is intramitochondrial or bound and therefore not available to inhibit CPT I.

There are two isoforms (liver and muscle) of carnitine palmitoyl transferase I (CPT I) in the heart [9, 10]. Inborn errors of carnitine palmitoyl transferase II are well-known to present with myopathy and cardiomyopathy; defects in the liver isoform of CPT I have also been diagnosed but to date no patients defective in the muscle form of CPT I have been diagnosed. We wished to investigate the role of the two isoforms in control of β-oxidation in the heart and in skeletal muscle.

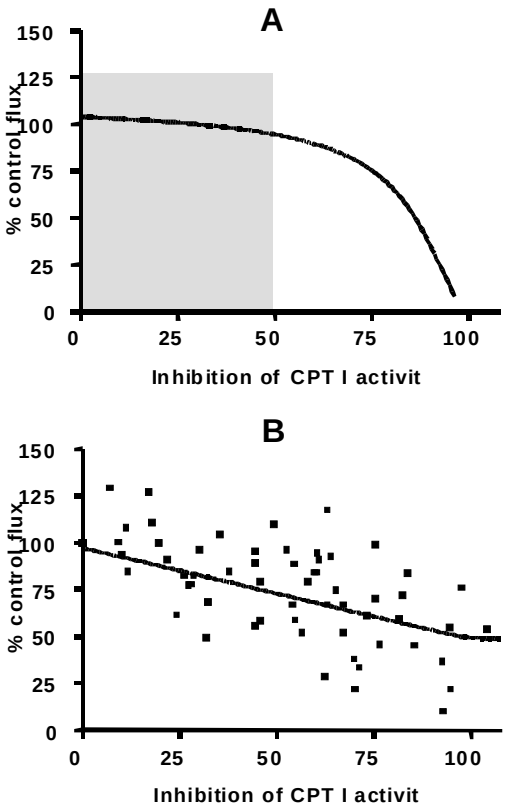

Fig. 4.1 Inhibition of CPT I activity

Methods

Mitochondria were isolated from the hearts and skeletal muscle of 11–15d old peak suckling Wistar rats as previously described [11]. Incubations were carried out at 37°C in a medium containing; 80 mM KCl, 10 mM Hepes, 5 mM $MgCl_2$, 2.5 mM KH_2PO_4, 1 mM EGTA, 1 mM ATP, 0.2 mg/ml cytochrome *c*, 30 mM creatine phosphate, 20 mM creatine, 0.1 mg/ml creatine phosphokinase (to maintain state 3.5), and 1.6 mg/ml bovine serum albumin, pH 7.2.

After 4 min preincubation with a range of concentrations of etomoxir-CoA and DNP-etomoxir-CoA, β-oxidation flux as total acid-soluble radioactivity (in the presence of 1 mM carnitine and 1mM malate [11] and CPT I activity (in the presence of 40 μg/ml myxothiazol [12]) were measured. Curve fitting and statistical analysis were carried out by means of linear and non-linear regression using the program Prism 2.01 (Graphpad Software, San Diego, California, USA) using the equation described by Gellerich et al. [13] for non-linear regression.

Results and discussion

Approximately 50% of CPT I activity in heart mitochondria could be inhibited by etomoxir-CoA before β-oxidation flux was affected (Fig. 4.1A). The full data and control analysis will be presented elsewhere, but the flux control coefficient of CPT I was 0.08 ± 0.05 (mean±SEM). This was contrary to the expectation that CPT I would be rate-limiting for β-oxidation in the heart. However, an explanation for these results could be that normally, the malonyl-CoA concentration in the heart is such that remaining CPT I activity is within the range where it can be considered to be rate-limiting (i.e., >50% inhibition of CPT I activity; the unshaded portion of the curve). An additional explanation for this phenomenon could be that the low flux control coefficient of CPT I over β-oxidation flux could be due to the presence of the liver isoform of CPT I at relatively high levels in the neonatal heart [9, 10]. To explore this question further, we attempted to repeat these incubations using DNP-etomoxir-CoA which, in contrast to etomoxir-CoA, is a specific inhibitor of the liver isoform at low concentrations [9, 10]. However, preliminary results indicated that DNP-etomoxir-CoA is not a suitable inhibitor: at low concentrations of DNP-etomoxir-CoA, β-oxidation flux was stimulated rather than inhibited. This appears to be due to the high instability of DNP-etomoxir-CoA; there is always some unesterified DNP-etomoxir present, which is a weak uncoupler of the respiratory chain (stimulated state IV oxygen consumption from glutamate plus malate by 30%). For these reasons, we decided to measure the flux control coefficient of CPT I over β-oxidation in skeletal muscle mitochondria from rats of the same age, as the liver isoform of CPT I is not present in this tissue. The results of this control analysis are presented in Fig. 4.1B. Although it appears that there may be a difference between heart and muscle in the control exerted by CPT I over β-oxidation flux (flux control coefficient = 0.49), the standard error of the flux control coefficient obtained for skeletal muscle is very large (0.41).

In both heart and skeletal muscle, malonyl-CoA, formed by the action of cytosolic acetyl-CoA carboxylase, is the physiological inhibitor of CPT I. These experiments used etomoxir-CoA rather than malonyl-CoA to titrate CPT I activity because etomoxir-CoA is a strong irreversible inhibitor of CPT I that forms a covalent adduct and is more potent than malonyl-CoA [14].

Conclusions

The apparent paradox of high rates of β-oxidation flux in the heart despite the presence of significant levels of malonyl-CoA may be explained by the low flux control coefficient of CPT I over β-oxidation flux. However, further experiments are necessary to define more precisely the role played by the two different isoforms of CPT I in the control of β-oxidation flux, particularly in relating these findings to the working heart.

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