Myocardial carnitine palmitoyltransferase I as a target for oxidative modification in inflammation and sepsis

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Abstract
CPT I (outer membrane carnitine palmitoyltransferase I) is a crucial enzyme in myocardial substrate selection. Two isoforms exist in the heart, the liver (L-) and muscle (M-) isoforms, which have different kinetic characteristics and alter in relative amounts during the neonatal/weaning/adult transition. CPT I is a point for control and regulation of fatty acid oxidation via modulation of its activity by malonyl-CoA, the concentration of which is set by acetyl-CoA carboxylase, AMP-activated protein kinase and malonyl-CoA decarboxylase in response to, for example, alterations in glucose supply. Systemic inflammatory responses and sepsis lead to myocardial dysfunction as part of multiple system organ failure. We have shown that: (i) myocardial CPT I activity is inhibited during neonatal sepsis; (ii) on the basis of inhibitor studies this inhibition appears to be of M-CPT I rather than L-CPT I; (iii) nitration of M-CPT I occurs, probably by peroxynitrite, and this may be responsible for the decrease in CPT I activity; (iv) myocardial CPT I activity is also inhibited in another model of systemic inflammatory response, namely intestinal ischaemia/reperfusion injury, but this can prevented by whole-body moderate hypothermia. Inhibition of M-CPT I would be predicted to alter myocardial substrate selection but there are several questions that remain to be answered.

Introduction
Sepsis, the systemic inflammatory response syndrome to an infectious cause, remains a major cause of morbidity and mortality particularly in infants and children [1]. A proportion of infants and children with sepsis progress to cardiac failure as part of multiple system organ failure (hepatic, renal, cardiac, pulmonary), the pathogenesis of which remains unclear [2–4]. The early events that follow the host response to sepsis or to systemic inflammation are represented by cytokine production. This process, essential for the antibiotic activity of phagocytes, produces significant quantities of reactive oxygen species including H2O2, superoxide (O2•−) and nitric oxide (NO•). However, in addition to an involvement in the bacterial killing process, reactive oxygen species can cause secondary damage to other organs, including the heart, which may influence the development of multiple organ failure syndrome [5–8].

CPT I (outer membrane carnitine palmitoyltransferase I) plays a pivotal role in fuel selection by the heart, being under the inhibitory control of malonyl-CoA [9–11]. Malonyl-CoA levels respond to glucose, hormonal and other regulatory signals via acetyl-CoA carboxylase, malonyl-CoA decarboxylase and 5’AMP-activated protein kinase [12]. However, direct modulation of CPT I activity would also influence substrate selection by the heart.

Effects of sepsis and systemic inflammation on CPT I activity
In an animal model of neonatal sepsis, we showed that heart CPT I activity was decreased by sepsis (Figure 1A), whereas CPT I activity in kidney and CPT II activity in both kidney and heart were unaffected [13]. Ischaemia and reperfusion of the intestine can be another cause of systemic inflammation, affecting liver [14], lungs [15], kidney [16] and heart [17,18]. We have recently shown that heart CPT I activity is decreased in an adult animal model of intestinal ischaemia/reperfusion injury [19] (Figure 1B), suggesting that inhibition of myocardial CPT I activity may be a common feature of systemic inflammation, or of inflammation localized to the heart. There are two isoforms of CPT I, the muscle (M-) isoform and liver (L-) isoform. The heart expresses both isoforms, whereas kidney only expresses the L- isoform. As heart was affected by sepsis, whereas kidney was not, we hypothesized that M-CPT I was specifically affected. Inhibitor studies with dinitrophenyl-etomoxir-CoA (which is more inhibitory towards L- than M-CPT I [20]) and malonyl-CoA (more inhibitory towards M- than L-CPT I) suggested that M-CPT I was indeed specifically inhibited during sepsis [21]. Western blotting...
Inhibition of CPT I activity by sepsis and inflammation

(A) Measurement of CPT I activity in heart mitochondria isolated from control and endotoxaemic suckling rats. Results are expressed as nmol/min per unit of citrate synthase (CS) activity; means ± S.E.M., n = 30, *P < 0.0007 versus control. (B) Measurement of CPT I activity in heart mitochondria isolated from adult control rats (C) and rats with intestinal ischaemia/reperfusion (I/R) injury. Data are expressed as in (A); means ± S.E.M., n = 8, *P = 0.01 versus control.

with specific antibodies against M- and L-CPT I, however, showed no differences in the amount of immunoreactive protein between control and septic hearts, indicating that the difference in CPT I activity was not due to changes in transcription or translation of CPT I [13].

Nitration of CPT I during sepsis

During endotoxaemia, significant quantities of superoxide, nitric oxide and peroxynitrite are produced in the heart, and this affects contractile function; peroxynitrite-mediated nitration of protein tyrosine residues has been suggested to be particularly important [22–28]. Superoxide, nitric oxide and peroxynitrite all inhibit cardiac CPT I activity in vitro [13], so we hypothesized that sepsis-induced inhibition of CPT I activity was due to nitration of M-CPT I. By immunoprecipitation of nitrated proteins from solublized cardiac mitochondria, followed by Western blotting against M- or L-CPT I, we showed that control mitochondria contain both nitrated M- and L-CPT I. During sepsis, nitration of M-, but not L-CPT I, was significantly increased, suggesting that peroxynitrite-mediated nitration of M-CPT I may be responsible for the observed inhibition of cardiac CPT I activity [29]. Whether some L- and M-CPT I is nitrated under normal conditions, or whether the baseline nitration that we detected is artefactual, is as yet unknown. In addition, it is of importance to determine the nitrated tyrosine residues within CPT I and to establish whether nitration affects activity.

Functional consequences of CPT I nitration

The heart normally relies on fatty acids for up to 75% of ATP requirements [30]; however, it additionally utilizes glucose, lactate or ketone bodies (Figure 2). If M-CPT I activity is impaired during sepsis, the balance of substrate utilization could be expected to be altered, such that the contribution of other substrates would be increased. As the Ki of malonyl-CoA on remaining CPT I activity is increased [29], the remaining CPT I activity would also be less easily suppressed by glucose/other regulators. However, succinyl-CoA:3-ketoacid-CoA transferase, a mitochondrial enzyme of ketone body utilization [31], is known to be nitrated and inhibited by endotoxaemia [27] and it is likely that aconitase of the Krebs cycle is also inhibited by peroxynitrite during sepsis [32,33]. Therefore, it is difficult to predict the overall effects of systemic inflammation and sepsis on substrate utilization. Although in our model of intestinal ischaemia/reperfusion injury CPT I activity was significantly decreased, phosphometabolites were unaltered, suggesting that a shift in substrate utilization takes place so that ATP/phosphocreatine supply is unaltered [19].

A role for CPT I nitration in preconditioning?

The heart can be preconditioned against ischaemic episodes by brief periods of ischaemia and reperfusion. This preconditioning can even take place between organs, e.g. a brief intestinal ischaemic insult can protect the heart against a subsequent ischaemic episode [34]. Peroxynitrite is thought to be involved in preconditioning [35] and it is thought that decreased fatty acid oxidation/increased glucose utilization protects the heart during ischaemia [36]. It is therefore possible that inhibition of CPT I activity by nitration could be involved in the preconditioning process, although further experiments would be necessary to investigate this hypothesis.

Therapeutic avenues to prevent myocardial dysfunction

A range of peroxynitrite scavengers/superoxide dismutase mimetics has been developed that are protective in a
variety of models of inflammation in which peroxynitrite is thought to be pathophysiologically involved [37]. These may additionally prevent myocardial CPT I nitration and myocardial dysfunction. Mild to moderate hypothermia has been shown to be protective in several different models of ischaemic or hypoxic injury, and we have recently shown that moderate hypothermia prevents liver bioenergetic failure [14], lung neutrophil infiltration [15] and the decrease in myocardial CPT I activity [19] subsequent to intestinal ischaemia/reperfusion injury.

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References

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