Malonyl-CoA inhibition of carnitine palmitoyltransferase I: response to Dr. Saggerson's Letter

Saggerson (1982) adds a further dimension to the controversy surrounding the characteristics of the malonyl-CoA inhibition of rat liver CPT I. While we are reluctant to belabour the issue, an attempt to clarify the picture in the interest of scientific accuracy appears to be in order.

The fact that in the rat starvation is associated with a reduction in the sensitivity of liver CPT I to the inhibitory effect of malonyl-CoA is not in dispute. What has been debated is the absolute I_{50} value for the interaction of malonyl-CoA with CPT I, the degree to which this increases with starvation, and the physiological significance of the change. Using three experimental approaches (method A, measurement of long-chain fatty acid oxidation; method B, measurement of CPT I using the isotope exchange assay; method C, measurement of CPT I by following the unidirectional conversion of palmitoyl-CoA into palmitoylcarnitine) we have routinely found the malonyl-CoA I_{50} to be about 1.5 and 3.0μM for 'fed' and 'fasted' liver mitochondria, respectively (McGarry *et al.*, 1978; McGarry & Foster, 1981; J. D. McGarry & D. W. Foster, unpublished work). By contrast, the equivalent values reported by Cook *et al.* (1980) and Ontko & Johns (1980) using method A were about 20 and 100μM, respectively. These were clearly shown to be artifactually elevated (McGarry & Foster, 1981).

Saggerson & Carpenter (1981), using method C, also reported surprisingly high I_{50} values for malonyl-CoA with 'fed' and 'fasted' mitochondria (5.4 and 32μM, respectively). In assessing these findings we stated (McGarry & Foster, 1981) that from the limited details provided it was not clear whether the authors were measuring only CPT I or a combination of CPT I and CPT II (the latter is not sensitive to malonyl-CoA). On the basis of Saggerson (1982) we now believe that they were in fact measuring primarily CPT I. How then can the difference between his results and ours be explained? We disagree with Saggerson's (1982) suggestion that the answer lies in the choice of assay for CPT I since, as noted above, we obtain similar results using all three procedures. Rather, we submit that the discrepancy stems from differences in the [palmitoyl-CoA]:[albumin] ratio employed. What are appropriate concentrations of palmitoyl-CoA and albumin for studies of this nature? Rat liver contains about 25 nmol per g wet wt. of long chain acyl-CoA (Cook *et al.*, 1977) and a cytosolic protein concentration of approx. 20%, of which some 5% is the fatty acid binding protein (Ockner *et al.*, 1982). Thus, for the assay of CPT I by method C we maintain that concentrations of palmitoyl-CoA and albumin of 40–50μM and 1–2%, respectively, (molar ratio of 0.3 or less) as used in this laboratory are probably not far removed from 'physiological'. Saggerson & Carpenter (1981) employed a similar concentration of palmitoyl-CoA but an albumin concentration of only 0.13% (molar ratio approx. 2). We consider this ratio to be excessive. As emphasized previously (McGarry & Foster, 1980, 1981) a [palmitoyl-CoA]:[albumin] ratio of this magnitude will yield spuriously high I_{50} values for malonyl-CoA. It will also tend to exaggerate the effect of starvation on this parameter. Both points are actually confirmed in Fig. 1 of Saggerson (1982).

Is the starvation-induced shift in malonyl-CoA sensitivity of CPT I physiologically important? Saggerson (1982) implies that we have rejected this possibility. This is not true. We do, however, feel that solid evidence in support of this concept is still lacking. By contrast, as amply documented (McGarry & Foster, 1981) there is good reason to believe that changes in the concentration of malonyl-CoA itself are of primary importance in the control of hepatic fatty acid metabolism *in vivo*. Saggerson (1982) has chosen to replot data from our previous papers (Fig. 2 in his Letter) in a manner which, at first glance, would support the notion that for any given malonyl-CoA concentration fatty acid oxidation is more rapid in 'fasted' than in 'fed' hepatocytes because of the higher I_{50} value for malonyl-CoA in the former. In doing so two important points (clearly spelled out in the original reports) have been overlooked. First, compared with 'fed' cells, 'fasted' hepatocytes are enriched in carnitine, a property that allows more efficient fatty acid oxidation for any given level of malonyl-CoA. Second, expression of the data on the basis of wet wt. of cells ignores the fact that 24 h of starvation results in a 35% increase in the number of hepatocytes per g wet wt. of liver. Correction for these two oversights would have yielded almost superimposable curves for 'fed' and 'fasted' cells.

In conclusion, it seems to us that the present controversy is more apparent than real and has arisen only because various groups have chosen subtly different methodologies to examine the same question. The interaction of malonyl-CoA with mitochondrial CPT I is a complex matter, the details of which remain to be delineated. We have tried in the past to emphasize the potential pitfalls inherent in such an endeavour. Hopefully, this communication will serve to reiterate the problems and

Abbreviations used: CPT I and CPT II, the overt and latent forms respectively of carnitine palmitoyltransferase (EC 2.3.1.21); I_{50}, the concentration of malonyl-CoA required to suppress CPT I activity by 50%.

thereby minimize the addition of further confusion to the field.

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(*Received 4 August 1982*)

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0306-3283/82/110527-02$01.50/1