BIOCHEMICAL JOURNAL LETTERS

Malonyl-CoA inhibition of carnitine palmitoyltransferase: interpretation of I_{50} and K_i values

Malonyl-CoA, an intermediate in the biosynthesis of fatty acids, is also a potent inhibitor of fatty acid oxidation (see review by McGarry & Foster, 1980). The discovery of McGarry *et al.* (1977) that malonyl-CoA inhibits long-chain fatty acid oxidation is a major contribution to our understanding of the co-ordinated control of the opposing pathways of fatty acid synthesis and degradation. Current evidence (McGarry *et al.*, 1977, 1978a,b; Saggerson & Carpenter, 1981; Bremer, 1981) indicates that carnitine palmitoyltransferase is the site of malonyl-CoA inhibition and that the inhibition is competitive with long-chain acyl-CoA (McGarry *et al.*, 1977, 1978a,b; Cook *et al.*, 1978; McGarry & Foster, 1979). Recently several groups of investigators have shown that the sensitivity to malonyl-CoA inhibition depends on the nutritional and/or hormonal state of the experimental animal (Cook *et al.*, 1980; Ontko & Johns, 1980; Saggerson & Carpenter, 1981; Bremer, 1981; Robinson & Zammit, 1982), e.g., the sensitivity to malonyl-CoA inhibition is much greater in the well-fed animal than in the starved animal. However, a controversy has arisen concerning the physiological importance and the magnitude of the variation in malonyl-CoA sensitivity (McGarry & Foster, 1981; Saggerson, 1982; Foster & McGarry, 1982; McGarry & Foster, 1982). In the discussion of those questions, the important distinction has not been made between the K_i , the dissociation constant for an enzyme-inhibitor complex, and the I_{50} value for inhibition in a defined reaction system. The purpose of this communication is to outline that distinction with regard to the inhibition by malonyl-CoA of carnitine palmitoyltransferase.

I_{50} may be defined as the inhibitor concentration that causes either 50% inhibition or half-maximal inhibition under a specified set of reaction conditions. For an inhibitor that is competitive with substrate, an increased substrate concentration requires an increased inhibitor concentration to maintain the same degree of inhibition; that is, the value of I_{50} will change with changes in the concentration of the competitive substrate.

K_i , in contrast, is the equilibrium constant for the

dissociation of the enzyme-inhibitor complex and is independent of the substrate concentration. As the reciprocals of association constants, K_i values indicate affinities of enzymes for inhibitors and are useful in comparing the effectiveness of several different inhibitors acting against the same enzyme. Although K_i is analogous to K_s , the dissociation constant for the enzyme-substrate complex (see discussion by Webb, 1963), K_i is often treated as an analogue of K_m , the substrate concentration that causes half-maximal reaction velocity and which permits calculations of relative velocities for any set of substrate concentrations [see, e.g., the abstract of McGarry & Foster (1981), where I_{50} values were referred to as K_i 's]. That, perhaps, is due to K_i having the units of concentration derived from the equilibrium ratio of $[E][I]/[EI]$. However, K_i gives no indication of the degree of inhibition unless K_m for substrate, concentration of substrate and concentration of inhibitor are all specified. While the numerical value of K_i does approach I_{50} at substrate concentrations that are very low relative to K_m , K_i should be viewed as an equilibrium constant rather than as an actual concentration of inhibitor. When seen as an equilibrium constant, it becomes clear why, in spite of suggestions to the contrary (McGarry & Foster, 1981), the K_i does not change when the concentration of enzyme or substrate changes. The K_i conceivably could change, however, if the enzyme were physically altered, e.g., by a different association with a membrane or a covalent modification.

The interpretation of I_{50} 's as K_i 's may be especially misleading in the case of malonyl-CoA inhibition of carnitine palmitoyltransferase, since binding to cellular protein has, so far, prevented measurement of the concentration of free acyl-CoA *in vivo* and also because the actual K_i of the enzyme for malonyl-CoA has never been determined. The value of $1.5 \mu\text{M}$ cited by McGarry & Foster (1981) is from a study in which palmitate oxidation rather than carnitine palmitoyltransferase activity was measured. Also, the values referred to as K_i 's by McGarry *et al.* (1978a,b), by Ontko & Johns (1980), by McGarry & Foster (1981) and by Hoppel (1982) should be called I_{50} 's, $I_{0.5}$'s (see Robinson & Zammit, 1982) or, simply, the malonyl-CoA concentrations that caused either 50% or half-maximal inhibition under the reaction conditions used in each instance. For a satisfactory

analysis of fatty acid oxidation *in vivo* or in whole cells, it would be useful to know the concentration of free acyl-CoA and the K_i of carnitine palmitoyltransferase for malonyl-CoA, which could be compared to K_s to indicate relative affinities of the enzyme for inhibitor and substrate. With those, it would become possible to make quantitative predictions about the action *in vivo* of malonyl-CoA as an inhibitor of fatty acid oxidation.

We may have contributed to this controversy by having mentioned K_i 's in two previous publications. The first of those (Cook *et al.*, 1978) described an attempt with isolated hepatocytes to determine the type of inhibition caused by malonyl-CoA. The cells were incubated with glucagon or 5-(tetradecyloxy)-2-furoic acid to vary the content of malonyl-CoA and with two different concentrations of oleate to vary the substrate, oleoyl-CoA, concentration. The ordinate intercepts of the resulting Dixon plot were proportional to the added oleate, suggesting that cellular oleoyl-CoA contents were also proportional. Consequently, the intersection of lines above the abscissa was taken to indicate a K_p , a value of 2 nmol/g wet wt. of cells. Conclusions about the accuracy of this value will have to await determination of K_i in a direct manner. K_i was also discussed in our paper (Cook *et al.*, 1980) on inhibition by malonyl-CoA of ketogenesis in isolated mitochondria. In that instance, slope and intercept terms in Dixon plots of data obtained with mitochondria from fed and starved rats were interpreted (see Rognstad, 1979) as being consistent with a larger K_i for malonyl-CoA in mitochondria from starved animals. It should be noted that K_i 's were not determined, nor were values for K_i 's suggested; the conclusion, instead, was that kinetic data were consistent with different K_i 's in the two nutritional states. Malonyl-CoA concentrations causing approx. 60% inhibition of ketogenesis in mitochondria from fed and starved rats were 20 μ M and 100 μ M, respectively. While we did not refer to those values as K_i 's, others (McGarry & Foster, 1981; Hoppel, 1982) have.

It has been suggested (McGarry & Foster, 1981) that the lower sensitivity of carnitine palmitoyltransferase to inhibition by malonyl-CoA observed with mitochondria from starved animals was due to problems in experimental design. As they found that the I_{50} value for malonyl-CoA varied with the concentration of fatty acid added, they (McGarry & Foster, 1981, 1982) concluded that our I_{50} (Cook *et al.*, 1980) was artifactually elevated because of the high concentration of fatty acid in our experiments. Variation of I_{50} with different concentrations of substrate and enzyme are to be expected on the basis of remarks above about the I_{50} for a competitive inhibitor. The question that most concerns us is whether *in vivo* the sensitivity of fatty acid oxidation to inhibition by malonyl-CoA varies with the

physiological state of the animal. Unfortunately, it is not possible to perform the tests *in vitro* at mitochondrial concentrations that are more than a few percent of those *in vivo*, and it is not known what concentrations of substrate, long-chain acyl-CoA, would approximate to those *in vivo*. We can, however, ask whether tests performed under constant reaction conditions show that sensitivity to malonyl-CoA is a function of the animal's physiological state. In our experiments, with 2.5 mg of mitochondrial protein per ml and 450 μ M-palmitate, there was a 5-fold difference in I_{50} 's for malonyl-CoA inhibition of fatty acid oxidation by mitochondria from fed and 48 h-starved rats (Cook *et al.*, 1980). The results of McGarry & Foster (1981) show a 3.3–4-fold difference in I_{50} for mitochondria from fed and 42 h-starved rats incubated at 0.4–0.6 mg of mitochondrial protein per ml and with 40 μ M-oleate. Although the observed I_{50} 's were different in the two studies, the agreement on the degree to which starvation lowers the sensitivity to malonyl-CoA is remarkably good in view of the large differences in experimental conditions.

The functional significance of the variation in carnitine palmitoyltransferase sensitivity to malonyl-CoA is best illustrated by the recent report of Robinson & Zammit (1982). Their results indicated that carnitine palmitoyltransferase is most sensitive to malonyl-CoA in animals with high tissue contents of malonyl-CoA and high rates of fatty acid synthesis. These authors proposed that this may be an amplification mechanism for the reciprocal regulation of fatty acid synthesis and oxidation. That is, in the physiological states with high rates of fatty acid synthesis, rates of oxidation are decreased because carnitine palmitoyltransferase is most sensitive to inhibition by malonyl-CoA, and the futile cycle of oxidation of newly synthesized fatty acids is prevented. In states such as starvation when amounts of free fatty acids are high, tissue contents of malonyl-CoA, rates of fatty acid synthesis and sensitivity of carnitine palmitoyltransferase are all low, and the rates of fatty acid oxidation are maximal.

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