Inhibition by acetyl-CoA of hepatic carnitine acyltransferase and fatty acid oxidation

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At micromolar concentrations, acetyl-CoA inhibited hepatic carnitine acyltransferase activity and mitochondrial fatty acid oxidation. The inhibitory effects were not nearly as potent on a molar basis as those of malonyl-CoA; nevertheless, the cytosolic concentrations of acetyl-CoA, as yet unknown, may be sufficient ($>30\mu\text{M}$) to curtail appreciably the mitochondrial transfer of long-chain acyl-CoA units and fatty acid oxidation. Hence acetyl-CoA may also partially regulate hepatic ketogenesis.

By curbing the transfer of long-chain acyl-CoA units across the mitochondrial membrane, malonyl-CoA has emerged as the principal regulator of fatty acid oxidation (McGarry *et al.*, 1978*a*; Geelen *et al.*, 1980; McGarry & Foster, 1981; Saggerson & Carpenter, 1981*c*, 1982*a*; Robinson & Zammit, 1982). Numerous investigators have verified its intense inhibition of mitochondrial CAT, even at the minute cytosolic concentrations found in hepatocytes (Guynn *et al.*, 1972; Cook *et al.*, 1978; McGarry *et al.*, 1978*b*; McGarry & Foster, 1979; Zammit, 1981). Furthermore, the sensitivity of this membrane-bound enzyme to competitive inhibition by malonyl-CoA appears to be dependent on both the nutritional and the hormonal status of the animal (Ontko & Johns, 1980; Saggerson & Carpenter, 1981*b,c*, 1982*b*; Saggerson, 1982; Robinson & Zammit, 1982; Stakkestad & Bremer, 1983) and, perhaps directly, on the prevailing hepatic content of this metabolite *in vivo* (Robinson & Zammit, 1982; Zammit, 1981).

Recognizing the metabolic vantage point of CAT in controlling mitochondrial fatty acid oxidation, other compounds besides malonyl-CoA might affect this enzyme. Specifically, a cytosolic compound that may abound in far greater quantities than malonyl-CoA during lipogenesis, namely acetyl-CoA, was investigated. Although the metabolism of acetyl-CoA (via acetyl-CoA carboxylase) has been suggested as the rate-limiting step in triacylglycerol synthesis, acetyl-CoA might still assume importance as a regulator of CAT if the cytosolic concentrations

accumulate whenever hepatocellular conditions favour fatty acid esterification.

Materials and methods

Animals and preparation of liver mitochondria

Mitochondrial vesicles were prepared by osmotic freeze-thawing disruption of mitochondria as described by McGarry *et al.* (1978*a*). Except in experiments in which CAT was acquired from livers of pregnant rats, as mentioned in the text, mitochondria were isolated from 200–300 g male Sprague-Dawley rats, both fed (on standard laboratory chow) and 18–24 h starved.

Chemicals

All chemicals were purchased from Sigma Chemical Co., St. Louis, MO, U.S.A., except CoA, its esters and the radiolabelled compounds, which were obtained from P-L Biochemicals, Milwaukee, WI, U.S.A.

Assay of CAT activity

Assay II of McGarry *et al.* (1978*a*) was used, in which the 5 min reaction mixture (1 ml total volume) contained $50\mu\text{M}$ -palmitoyl-CoA and 2% (w/v) defatted bovine serum albumin (substrate/albumin molar ratio 0.18). Each tube contained 0.2 mg of mitochondrial protein. Our assay differed only in reaction temperature (37°C) and the use of $300\mu\text{M}$ -DL-carnitine instead of $200\mu\text{M}$ -L-carnitine. Protein was measured by the method of Lowry *et al.* (1951), with bovine albumin as standard. Since disrupted mitochondria were used in these experiments (see above), CAT activity refers to total CAT, i.e. the

Abbreviation used: CT, carnitine acyltransferase (carnitine palmitoyltransferase, EC 2.3.1.21).

sum of CAT I and CAT II, the enzymes located on the mitochondrial outer and inner membranes respectively.

Mitochondrial fatty acid oxidation

Since the fatty acid/albumin ratio, mitochondrial protein concentration and duration of incubation are important variables in ketogenesis experiments, the procedure as outlined by McGarry & Foster (1981) was adopted. Specifically, each tube contained, in a final volume of 2.5 ml: 2.2 ml of modified Krebs-Henseleit buffer (McGarry *et al.*, 1977), 10 μ mol of ATP, 2.2 μ mol of ADP, 125 nmol of CoA, 625 nmol of reduced glutathione, 20 mg of defatted albumin, 100 nmol of sodium palmitate, and 0.3 μ Ci of [1- ^{14} C]palmitate. Ketone bodies are defined as the acid-soluble products of palmitate oxidation. Our incubation time was 5 min, the mitochondrial protein content ranged from 0.2 to 0.5 mg, and our fatty acid (palmitate) concentration was 40 μ M, with a fatty acid/albumin molar ratio of 0.35. Total volume was 2.5 ml. Again, our reaction temperature was different (37°C) and 500 nmol of DL-carnitine was used in place of 250 nmol of L-carnitine.

Results and discussion

Although not as potent on a molar basis as malonyl-CoA, acetyl-CoA clearly inhibited CAT activity. At 10 μ M- and 40 μ M-acetyl-CoA concentrations in the reaction mixture, the inhibition of CAT activity from starved male rats was 14% and 24% of control values respectively (Fig. 1). The CAT inhibition with the same molar concentrations of malonyl-CoA was 29% and 41%, results similar to those reported by McGarry & Foster (1981). Admittedly, this represents a far greater percentage inhibition of CAT by equimolar concentrations of malonyl-CoA than was found by others (Saggerson & Carpenter, 1981a,c, 1982b, 1983), probably because, in these reports, the assay's substrate (50 μ M)/albumin molar ratio is appreciably greater (2.76 versus 0.18). This ratio and the effects of alterations in the albumin-substrate binding on the carnitine palmitoyltransferase (i.e. CAT) assay are the subjects of a related study (McCormick & Notar-Francesco, 1983). At higher molar ratios, the acetyl-CoA inhibition of CAT might be undemonstrable. As attested by others (Cook *et al.*, 1980; Ontko & Johns, 1980; Saggerson & Carpenter, 1981b, 1982b; Saggerson *et al.*, 1982; Robinson & Zammit, 1982), we found CAT prepared from livers of pregnant or fed rats, as compared with starved male rats, to be more sensitive to malonyl-CoA inhibition. For instance, with livers from pregnant rats, at 25 μ M-acetyl-CoA CAT activity was retarded by 21% of control, whereas malonyl-CoA at the same molar concentration produced nearly 50%

Fig. 1. Effects of acetyl-CoA (●) and malonyl-CoA (○) on CAT activity from starved male rats

CAT was isolated from 18–24 h-starved male rats, and 0.2 mg of mitochondrial protein was present in each assay. The duration of the reaction was 5 min, and the incubation mixture was as described in the Materials and methods section. The palmitoyl-CoA/albumin molar ratio was 0.18. Values are means $\pm$ S.E.M. (indicated by bars) for five experiments. Control CAT activity in the absence of any inhibitor was 1.1 ± 0.05 nmol/min per mg of protein at 37°C.

inhibition (Fig. 2); similar effector concentrations produced less inhibition on the enzyme isolated from starved male rats (Fig. 1). Again, acetyl-CoA inhibition was obvious, but not as impressive as with malonyl-CoA. When CoA was added to the mixture, CAT activity was attenuated to nearly the same degree as with acetyl-CoA; however, the addition of CoA, one of the products of the CAT reaction, may impede enzyme activity simply by creating assay conditions that are closer to equilibrium. With intact mitochondria from starved rats, the effects of acetyl-CoA on ketogenesis from palmitate were also examined (Fig. 3). Again ketogenesis was attenuated by acetyl-CoA, but appreciably less than by similar effector concentrations of malonyl-CoA. In contrast, a previous study of oleate oxidation in intact mitochondria failed to demonstrate any inhibition by acetyl-CoA, even at concentrations as high as 200 μ M (McGarry *et al.*, 1977). The reason for the dissimilarity in results is unclear.

The physiological role of acetyl-CoA inhibition of CAT activity is unknown, primarily because our knowledge of the cytosolic content of this compound

Fig. 2. Effects of acetyl-CoA (●), CoA (Δ) and malonyl-CoA (○) on CAT activity from pregnant fed rats. CAT was prepared from fed 19-day-gestation pregnant rats, and 0.2 mg of mitochondrial protein was added to each reaction. The assay was identical with that for the experiments illustrated in Fig. 1. Values are means $\pm$ S.E.M. (indicated by bars) from five experiments. Control CAT activity was 0.87 ± 0.06 nmol/min per mg of protein at 37°C .

Fig. 3. Effects of acetyl-CoA (●) and malonyl-CoA (○) on palmitate conversion into ketone bodies.

Intact mitochondria (0.2–0.5 mg of protein) from starved male rats were incubated with various concentrations of inhibitor. The reaction time was 5 min at 37°C with a fatty acid/albumin molar ratio of 0.35. Ketone bodies represent the acid-soluble radioactivity in the supernatant, as described in the Materials and methods section. Values represent means $\pm$ S.E.M. for six separate experiments. Control (no inhibitor present) rates of ketogenesis were 1.4 ± 0.2 nmol/min per mg of protein.

is vague. Indeed, the reported range of cytosolic acetyl-CoA concentrations is vast, anywhere from 5 to $86 \mu\text{M}$ (Siess *et al.*, 1978; Yeh *et al.*, 1981), depending, of course, on the nutritional status of the animals, rapidly with which the liver is frozen, and the technique by which the compound is measured. The total acetyl-CoA concentration in hepatocytes, ignoring intracellular compartments, is reportedly near or over $100 \mu\text{M}$ (Lumbers *et al.*, 1969; Guynn *et al.*, 1972; Kato, 1978). Complicating the issue is the relative cytosolic protein binding of acetyl-CoA and malonyl-CoA, assuming that only the unbound fraction would operate as a metabolic regulator. The cytosolic binding fractions for the short-chain CoA derivatives have yet to be determined, but, given the possible enormous differences in the total hepatic cytosolic concentrations of malonyl-CoA and acetyl-CoA, an enhanced cytosolic protein binding of the former would further accentuate the disparity in the free or unbound concentrations between these two compounds. In short, acetyl-CoA, more by quantity than quality, may also control fatty acid oxidation.

Acetyl-CoA can also regulate other enzymes, specifically malic enzyme (Zahltén & Nejtek, 1979) and pyruvate carboxylase. It is noteworthy that

CoA has emerged as a major regulator of fatty acid synthesis, principally by activating acetyl-CoA carboxylase (Yeh *et al.*, 1981).

Unarguably, malonyl-CoA is a more effective inhibitor of CAT than acetyl-CoA. In addition, indirect evidence supports the prominence of intracellular malonyl-CoA compared with acetyl-CoA as a metabolic regulator. In isolated hepatocytes, the inhibition of acetyl-CoA carboxylase by 5-tetradecyloxy-2-furoate, which may increase the cytosolic acetyl-CoA/malonyl-CoA ratio, produced an increase in oleate oxidation (Demaugre *et al.*, 1982). Nevertheless, should cytosolic acetyl-CoA concentrations increase appreciably during sustained stimulation of lipid synthesis, acetyl-CoA may assume physiological importance as a modifier of fatty acid metabolism, especially if both CoA

derivatives synergistically inhibit CAT activity. Indeed, Siess *et al.* (1976) have studied in detail the mitochondrial/cytosolic ratio of acetyl-CoA and other metabolites during states of fatty acid oxidation in isolated rat hepatocytes. They found that total ketone-body production from 0.9 mM-oleate was decreased by 30% by the addition of 10 mM-lactate and, noteworthy, this was paralleled by a rise in cytosolic acetyl-CoA content from an undetectable value to 46 μ M; simultaneous malonyl-CoA concentrations were, however, not measured. Furthermore, in the carbohydrate-fed state, the quantity of fatty acid synthetase in the liver may be rate-limiting, as evidenced by an intracellular malonyl-CoA content notably greater than this enzyme's K_m for this compound (Guynn *et al.*, 1972). Under these conditions both cytosolic malonyl-CoA and acetyl-CoA would be expected to accumulate.

The effect, if any, of acetyl-CoA on the reverse reaction, which generates palmitoyl-CoA and carnitine, is unknown. However, since glucagon increases mitochondrial acetyl-CoA (Siess *et al.*, 1977), this metabolite may temper or augment long-chain acyl-CoA trans-mitochondrial flux by affecting this reaction.

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