

## Altered sensitivity of carnitine palmitoyltransferase to inhibition by malonyl-CoA in ketotic diabetic rats

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Carnitine palmitoyltransferase of liver mitochondria prepared from ketotic diabetic rats has a diminished sensitivity to inhibition by malonyl-CoA compared with carnitine palmitoyltransferase of mitochondria prepared from normal fed rats.

Malonyl-CoA is a potent inhibitor of fatty acid oxidation (McGarry *et al.*, 1977), especially in liver preparations from fed animals (Cook *et al.*, 1978), and the site of inhibition is the carnitine palmitoyltransferase step (McGarry *et al.*, 1977, 1978). In mitochondria from starved animals, however, fatty acid oxidation is resistant to inhibition by malonyl-CoA (Cook *et al.*, 1980; Ontko & Johns, 1980), and that resistance to inhibition is due to a difference in the sensitivity of carnitine palmitoyltransferase to inhibition by malonyl-CoA (Bremer, 1981; Saggerson & Carpenter, 1981). These studies suggest that carnitine palmitoyltransferase may be under some type of hormonal control, probably involving insulin and/or glucagon. More recently, some evidence has been presented that indicates such a mechanism exists (Harano *et al.*, 1982), but studies by Saggerson & Carpenter (1981) have indicated that, under certain conditions, mitochondria from fed rats possess a carnitine palmitoyltransferase that is not changed by treatment of the animals with streptozotocin. This phenomenon was re-investigated in the present study, where it was found that, when rats were injected with a dose of streptozotocin great enough to make them highly ketotic (see Schein *et al.*, 1971), the sensitivity of carnitine palmitoyltransferase to inhibition by malonyl-CoA was greatly diminished.

### Experimental

#### Methods

Female Wistar rats weighing 200–250 g were fed *ad libitum* (Wayne Lablox; composition 24% protein, 4% fat and 50% carbohydrate, by wt.) or starved for 48 h. Diabetic rats were produced as

described by Schein *et al.* (1971) by injecting rats with 150 mg of streptozotocin/kg body wt. Streptozotocin-injected animals were used for preparation of mitochondria on day 2 after injection; only those animals having urine ketone bodies  $\geq 80$  mg/dl (determined with Bili-Labstix; Miles Laboratories, Elkhart, IN, U.S.A.) were used for experiments. Animals were decapitated before isolation of mitochondria, and blood was collected for measurement of glucose, acetoacetate and 3-hydroxybutyrate by enzymic assays, by the methods of Lowry & Passonneau (1972), Williamson *et al.* (1962) and Brashear & Cook (1983) respectively. Stomachs of animals were checked to be certain that all fed animals, including diabetic animals, were well fed and that starved animals had no food in their gastrointestinal tract.

Liver mitochondria were isolated by the method of Johnson & Lardy (1967), and the final mitochondrial pellet was resuspended to a protein concentration of 10 mg/ml in 0.25 M-sucrose, adjusted to pH 7.2 by adding a few drops of 2 M-Tris base. Protein was determined by a biuret procedure (Gornall *et al.*, 1949). Respiratory control ratios (Estabrook, 1967) were 5 or greater with 10 mM-glutamate and 0.5 mM-malate as substrates.

Carnitine palmitoyltransferase activity was measured by a modification (Stephens *et al.*, 1983) of the method of Bremer (1981). Each assay contained, in a total volume of 1 ml: 82 mM-sucrose, 70 mM-KCl, 35 mM-imidazole, 35 mM-Hepes [4-(2-hydroxyethyl)-1-piperazine-ethanesulphonic acid], 1 mM-reduced glutathione, 50  $\mu$ M-oleoyl-CoA, 4 mg of bovine serum albumin, 0.5 mM-L-carnitine (1  $\mu$ Ci of DL-[methyl- $^3$ H]carnitine/ $\mu$ mol), 1  $\mu$ g of antimycin A and malonyl-CoA at the concentrations indicated. Assays were conducted at 30°C, pH 7.0.

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### Materials

Malonyl-CoA, oleoyl-CoA, L-carnitine hydrochloride, Hepes, EGTA, imidazole, 3-hydroxybutyrate dehydrogenase, reduced glutathione and essentially fatty acid-free bovine serum albumin were purchased from Sigma Chemical Co. (St. Louis, MO, U.S.A.). DL-[methyl- $^3\text{H}$ ]Carnitine hydrochloride was obtained from Amersham (Arlington Heights, IL, U.S.A.). Wistar rats were obtained from Harlan Industries (Indianapolis, IN, U.S.A.).

### Results and discussion

As intact mitochondria were used in these studies, only the carnitine palmitoyltransferase on the outer surface of the mitochondrial inner membrane was measured in our assay. The shape of the malonyl-CoA inhibition curve (Fig. 1) indicates that inhibition approached 100% in mitochondria from fed rats. As reported previously (Bremer, 1981; Saggerson & Carpenter, 1981), we also observed that the  $I_{50}$  (inhibitor concentration giving 50% inhibition) for malonyl-CoA inhibition of carnitine palmitoyltransferase was much greater in mitochondria from starved rats. Although inhibition of the enzyme in mitochondria from starved rats was only 70% at  $50\mu\text{M}$ , the highest concentration tested, the maximum inhibition approached 100%, and additional experiments with higher concentrations ( $100\text{--}300\mu\text{M}$ ) of malonyl-CoA have shown much greater inhibition. These results suggest that the mitochondria used in these experiments were intact and did not allow any of the inner carnitine palmitoyltransferase to be assayed. McGarry *et al.* (1978) have indicated that the inner enzyme is not inhibited by malonyl-CoA, so that inhibition of carnitine palmitoyltransferase by malonyl-CoA in broken mitochondria reaches a maximum of 50%.

At the time diabetic animals were used for these experiments, their stomachs were distended with food; the stomachs of the diabetic animals were much more distended than those of the normal fed animals, suggesting the typical hyperphagia of insulin-dependent diabetes. The diabetic animals were therefore well fed, but there were undoubtedly changes in metabolism because of their altered hormonal state.

In contrast with experiments reported by Saggerson & Carpenter (1981), our results indicated that mitochondria from streptozotocin-treated rats were almost identical with those from starved rats, i.e. carnitine palmitoyltransferase from both of these groups exhibited diminished sensitivity to malonyl-CoA inhibition. Our results would support the hypothesis that changes in sensitivity of carnitine palmitoyltransferase to malonyl-CoA

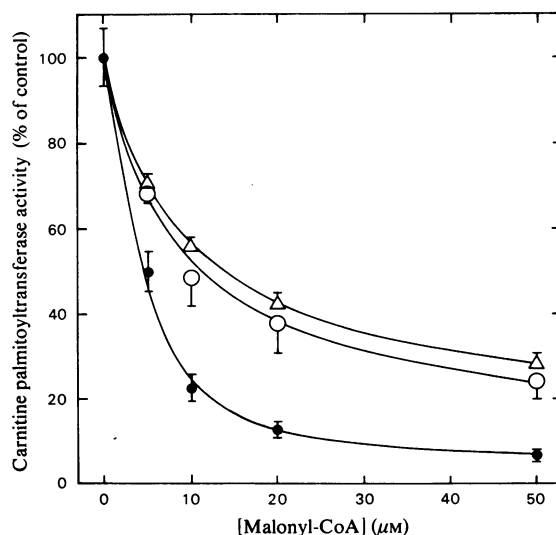


Fig. 1. Malonyl-CoA inhibition of carnitine palmitoyltransferase in intact mitochondria. Mitochondria were isolated from fed (●), starved (○) or streptozotocin-treated (△) rats. Each point represents the mean  $\pm$  S.E.M. for three animals.

inhibition are under hormonal control. Differences between our results and those of Saggerson & Carpenter (1981) can probably be explained by differences in the conditions under which the two studies were carried out. The primary difference was the dose of streptozotocin administered. The lower dose used by Saggerson & Carpenter (1981) would be expected to produce hyperglycaemic rats that were not highly ketotic and not dependent on insulin (see Mansford & Opie, 1968; Forster & Rudas, 1969; Junod *et al.*, 1969; Schein *et al.*, 1971). Although Saggerson & Carpenter (1981) reported that their animals were hyperglycaemic, no data were presented on ketone-body concentrations. In previous experiments (Gillim *et al.*, 1983) we found that the  $150\text{ mg/kg}$  dose of streptozotocin increased liver ketone bodies to 15 times that of the control animals, results similar to those reported by other laboratories (Schein *et al.*, 1971; Topping & Targ, 1975). Blood ketone bodies of the diabetic animals used in the experiments reported here were  $5.31 \pm 0.62\mu\text{mol/ml}$ ; blood glucose was  $25.8 \pm 0.2\mu\text{mol/ml}$  (mean  $\pm$  S.E.M.,  $n = 3$ ).

Another important difference between our experiments and those of Saggerson & Carpenter (1981) was the length of time between injection of streptozotocin and use of the diabetic animals. Wieland (1968) has cautioned against the use of chronically diabetic rats because of the depletion of fat stores, and he has pointed out that chronically diabetic rats do not represent an adequate model for the uncontrolled human

diabetic. Topping & Targ (1975), using rats that had been given a 100 mg/kg dose of streptozotocin, found that both plasma non-esterified fatty acids and ketone bodies were maximally increased at 48 h after injection and concluded that 48 h was the most advantageous time for studying experimental diabetes induced by streptozotocin.

The mechanism by which changes in the sensitivity of carnitine palmitoyltransferase occur is not known, but the finding that diabetic rats, like starved rats, have a diminished sensitivity to inhibition by malonyl-CoA strongly suggests that the mechanism operates under hormonal control.

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