Effect of pH on malonyl-CoA inhibition of carnitine palmitoyltransferase I

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Malonyl-CoA inhibition of carnitine palmitoyltransferase I was found to be very pH-dependent. Malonyl-CoA concentrations causing 50% inhibition (I_{50}) at pH 6.0, 6.5, 7.0, 7.5 and 8.0 were 0.04, 1, 9, 40 and 200 μM respectively. It is suggested that a lowering of intracellular pH, such as might occur in ketoacidosis, may attenuate hepatic fatty acid oxidation by increasing malonyl-CoA sensitivity of carnitine palmitoyltransferase I.

Malonyl-CoA is a very important regulatory compound, since it serves as an intermediate for fatty acid synthesis and it inhibits carnitine palmitoyltransferase I, thereby inhibiting fatty acid oxidation (McGarry & Foster, 1980). [Carnitine palmitoyltransferase I refers to the enzyme (EC 2.3.1.21) located on the outside of the mitochondrial inner membrane, whereas carnitine palmitoyltransferase II refers to the enzyme bound to the matrix side of the membrane.] This dual role of intermediate and inhibitor provides a co-ordinated control mechanism for synthesis, esterification and oxidation of fatty acids. Support for this hypothesis is found in the inverse correlation between malonyl-CoA concentration and the rate of ketone-body synthesis when variations in malonyl-CoA content of isolated hepatocytes are induced by fatty acid-synthesis inhibitors such as glucagon and 5-(tetradecyloxy)-2-furoic acid (Cook et al., 1978). This correlation was not seen in hepatocytes isolated from 48 h-starved rats (Cook & Veech, 1978). Also consistent with this role for malonyl-CoA is the persistent propensity for fatty acid oxidation in perfused livers or isolated hepatocytes from starved rats (Rubenstein & Rubenstein, 1966; Ontko, 1972) that possess a malonyl-CoA-resistant carnitine palmitoyltransferase I (Cook et al., 1980). The occurrence of a carnitine palmitoyltransferase I that is less sensitive to malonyl-CoA (Cook et al., 1980), as well as the effects of protein and acyl-CoA concentrations (McGarry & Foster, 1981) and thiol-group activation (Saggerson & Carpenter, 1981), are all important factors in determining the amount of malonyl-CoA inhibition of carnitine palmitoyltransferase I. H^+ concentration is another factor that can greatly affect inhibition of enzymes by effectors, as with ATP: D-fructose 6-phosphate 1-phosphotransferase (Mansour, 1972). Most studies

to date on the inhibition of carnitine palmitoyltransferase I have been performed in the pH range 7.3–7.5 (McGarry et al., 1978; Ontko & Johns, 1980; Cook et al., 1980; Saggerson & Carpenter, 1981; Bremer, 1981), whereas cytosolic pH is thought to be approx. 7.0 (Hoek et al., 1980). Malonyl-CoA inhibition of carnitine palmitoyltransferase I was found in the present study to be greater at pH 7.0 and even greater at more acidic pH. This pH effect may provide a physiological mechanism for attenuating the production of ketone bodies during periods of acidosis.

Experimental

Materials

Essentially fatty acid-free bovine serum albumin, malonyl-CoA, oleoyl-CoA, L-carnitine hydrochloride, imidazole, Hepes [4-(2-hydroxyethyl)-1-piperazine-ethanesulphonic acid], EGTA, EDTA and reduced glutathione were obtained from Sigma Chemical Co. (St. Louis, MO, U.S.A.). DL-[*methyl*-^{3}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.).

Methods

Female Wistar rats weighing 200–250 g were fed *ad libitum* or starved for 48 h. Rat liver mitochondria were isolated by the method of Johnson & Lardy (1967), except that the final centrifugation step was performed at 8500 *g* for 5 min. The pellet from this step was resuspended to a protein concentration of 10 mg/ml in 0.25 M-sucrose (adjusted to pH 7.2 with Tris). Respiratory control ratios were consistently above 5 with 10 mM-glutamate and 0.5 mM-malate as substrates.

Carnitine palmitoyltransferase I activity was measured essentially as described by Bremer (1981), except that imidazole/Hepes was used as the buffer system. The ionic and osmotic strength of the buffer was determined essentially as described by Ellis & Morrison (1982). Sufficient KCl and sucrose were added to give an ionic strength of 0.1 M and an osmotic strength of 0.32 osM. The complete assay system (1 ml) contained 82–84 mM-sucrose, 60–70 mM-KCl, 35 mM-imidazole, 35 mM-Hepes, 1 mM-EGTA, 5 mM-reduced glutathione, 50 μM-oleoyl-CoA, 4 mg of bovine serum albumin/ml, 0.5 mM-L-carnitine (1 μCi of DL-[*methyl*-^{3}H]carnitine/μmol), 1 μg of antimycin A/ml, and malonyl-CoA at the indicated concentrations. The final pH was adjusted with the complete reaction mixture except for mitochondria, oleoyl-CoA, L-carnitine and malonyl-CoA (which were determined to have insignificant effects on pH). The pH was adjusted at 23 °C to 0.12 pH unit above the desired final pH to correct for the average change in pK_a for the buffering system when solutions were incubated at 30 °C. Oleoyl-CoA and mitochondria (0.2 mg of protein) were added to this solution in a polyethylene tube and preincubated at 30 °C for 5 min. Malonyl-CoA and L-carnitine were added to initiate the reaction, which was terminated by the addition of 0.5 ml of 6 M-HCl. Oleoyl[^{3}H]carnitine was extracted as described by Bremer (1963). The butanol extract was evaporated and counted for radioactivity in Scinti-Verse II (Fisher Scientific, Pittsburgh, PA, U.S.A.). Oleoyl-CoA recovered at the end of the assay averaged 85% and did not vary substantially with pH.

Incubations for the determination of malonyl-CoA were terminated with 0.2 ml of 60% (w/w) HClO$_4$. Malonyl-CoA assays were performed spectrophotometrically by the method of Guynn *et al.* (1972), with an Aminco DW-2 spectrophotometer. Protein was determined by the biuret method (Gornall *et al.*, 1949). Oleoyl-CoA was assayed as a HClO$_4$-insoluble CoA ester by the method of Michal & Bergmeyer (1974). Mitochondrial swelling was monitored by measuring the change in A_{540} under the above conditions (Packer, 1967).

Results and discussion

Intact mitochondria were used to determine the effect of pH on carnitine palmitoyltransferase I activity with oleoyl-CoA and DL-[*methyl*-^{3}H]-carnitine as substrates. Activity of the enzyme in the absence of malonyl-CoA was fairly constant over the pH range 6.5–7.5, but decreased at lower and higher pH (Fig. 1). This pattern, with the exception of decreased activity at pH 8.0, was similar to the pattern seen with purified carnitine palmitoyltransferase (Norum, 1964) and carnitine acetyl-
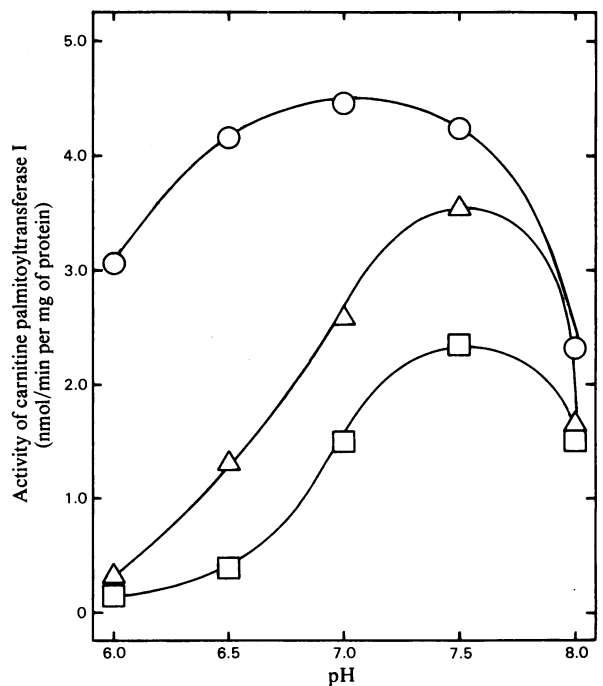

Fig. 1. *Inhibition of carnitine palmitoyltransferase I activity by malonyl-CoA as a function of pH*

Liver mitochondria (0.2 mg of protein) from fed rats were assayed for carnitine palmitoyltransferase I activity with no malonyl-CoA (○), 2 μM-malonyl-CoA (△) or 20 μM-malonyl-CoA (□) as described in the Experimental section. Values shown are the means of duplicate assays on mitochondria isolated from a single rat. Similar results were obtained in a separate experiment.

transferase (Markwell *et al.*, 1976). Adding malonyl-CoA changed the pH response, apparently shifting the pH optimum upward and increasing the rate of change in activity with respect to pH below the optimum. Mitochondria prepared from both starved and fed rats showed similar responses to malonyl-CoA as a function of pH, but mitochondria from fed animals exhibited greater sensitivity at all pH values (results not shown). I_{50} values (malonyl-CoA concentrations giving 50% inhibition) (±S.D.) determined on five fed and four starved rats in duplicate assays at pH 6, 7 and 8 were 0.04 ± 0.01, 9.0 ± 3.2 and 170 ± 49 μM for fed rats and 1.0 ± 0.6, 44 ± 11 and 249 ± 29 μM for starved rats.

Malonyl-CoA inhibition of carnitine palmitoyltransferase I was increased greatly at lower pH (Fig. 2). One major effect implied by the data is that the sensitivity of the enzyme to malonyl-CoA is increased at lower pH. Increased affinity of carnitine palmitoyltransferase I for malonyl-CoA caused by
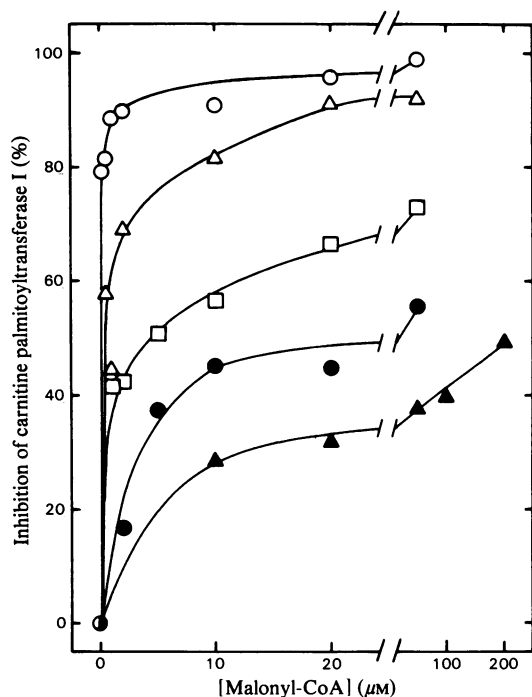
Fig. 2. *Effect of pH on malonyl-CoA inhibition of carnitine palmitoyltransferase I*
Liver mitochondria (0.2 mg of protein) from fed rats were assayed for carnitine palmitoyltransferase I activity with the indicated malonyl-CoA concentrations at pH 6.0 (O), 6.5 (△), 7.0 (□), 7.5 (●) and 8.0 (▲) as described in the Experimental section. Values shown are the means of duplicate assays on mitochondria isolated from a single rat. Similar results were obtained in a separate experiment. The pooled within-groups coefficient of variation was 7%, with an average control activity of 3.6 nmol/min per mg.

protonation of either malonyl-CoA or an amino acid residue of the enzyme could explain this effect. Histidine would titrate in this region (Segal, 1975). The variation in ATP inhibition of ATP: D-fructose 6-phosphate 1-phosphotransferase in the pH range 6–8 involves protonation of a histidine residue (Mansour, 1972). It is noteworthy that the inhibition by malonyl-CoA was much more responsive to changes in pH than was the velocity of the enzyme-catalysed reaction. This result suggests that the binding sites for malonyl-CoA and oleoyl-CoA are not identical. Another effect implied by the data is that the maximum inhibitable activity is increased at lower pH. One possible explanation is a change from complete inhibition at lower pH (enzyme–inhibitor complex totally inactive) to partial inhibition (enzyme–inhibitor complex partially active) at higher pH.

Precautions were taken because of potential swelling of mitochondria at alkaline pH and potential effects of ionic strength on carnitine palmitoyltransferase activity. The assay medium was maintained at a constant osmotic strength (0.32 osM) and ionic strength (0.1 M) by using a two-component buffer system and varying the KCl and sucrose concentrations. No mitochondrial swelling was detected when the A_{540} of suspensions was monitored under conditions identical with those in the enzyme assay.

The decreased sensitivity of carnitine palmitoyltransferase I to malonyl-CoA at higher pH cannot be accounted for by a decrease in malonyl-CoA concentration through higher rates of enzymic or non-enzymic hydrolysis. Direct determination of malonyl-CoA at the end of the incubations showed that the rate of hydrolysis was constant. Less than 10% of the malonyl-CoA was lost under the conditions of these experiments (results not shown).

The change in sensitivity of carnitine palmitoyltransferase I to malonyl-CoA as a function of pH may have several physiological implications. Studies *in vitro* performed at pH greater than 7 most probably underestimate the degree of inhibition *in vivo* by malonyl-CoA. It is proposed that a lowering of intracellular pH, such as might occur in keto-acidosis, may serve to dampen the rate of fatty acid oxidation by making malonyl-CoA a more effective inhibitor of carnitine palmitoyltransferase I. The pH of plasma falls in ketoacidosis, and evidence has been presented that the pH gradient across the plasma membrane is fairly constant over an external pH range of 6.85–7.35 (Deutsch *et al.*, 1979). The physiological purpose might be to establish feedback control over excessive production of acid by ketogenesis, much as lowering of pH is thought to limit lactic acid production by inhibition of 6-phosphofructo-1-kinase. Although convincing evidence has not been presented for changes in cytosolic pH in response to hormones, insulin has been shown to increase the plasma-membrane potential of adipocytes (Davis *et al.*, 1981) and muscle cells (Zierler, 1959). Since the protonmotive force across the liver plasma membrane appears to be zero (Hoek *et al.*, 1980), an increase in membrane potential produced by insulin may be balanced by an increase in pH gradient, resulting in a lower cytosolic pH. A decrease in pH under conditions where malonyl-CoA concentrations are expected to be high might result in increased inhibition of carnitine palmitoyltransferase I and lower rates of fatty acid oxidation.

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