Regulation by oestrogen of carnitine palmitoyltransferase in hepatic mitochondria

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INTRODUCTION

Isolated perfused livers from female rats oxidize less fatty acid and esterify more fatty acid to triacylglycerol compared with livers from male rats (Soler-Argilaga & Heimberg, 1976), suggesting that oestrogen may play a role in the disposition of fatty acids by the liver. Indeed, livers from female rats that have been treated with ethynyloestradiol have an increased rate of esterification of fatty acids to triacylglycerol and a diminished capacity for fatty acid oxidation (Weinstein et al., 1979). Comparable results were reported in pregnant rats on day 19 of gestation, when oestrogen concentrations were greatly elevated (Wasfi et al., 1980). Understanding the mechanism of the anti-ketogenic effect of oestrogen may be important to our understanding of the physiological regulation of fatty acid oxidation and may have additional significance in therapy with oral contraceptives containing oestrogen.

Hepatic fatty acid oxidation appears to be regulated by modulation of the affinity of carnitine palmitoyltransferase for its physiological inhibitor, malonyl-CoA. Carnitine palmitoyltransferase from starved rats is much less sensitive, compared with the enzyme from fed rats, to inhibition by malonyl-CoA (Bremer, 1981; Saggerson & Carpenter, 1981). Starvation causes a mc.:test (60%) increase in activity of carnitine palmitoyltransferase, but decreases the affinity for malonyl-CoA by 10-fold, a change in affinity so great that physiological concentrations of malonyl-CoA may no longer have any inhibitory effect (Cook, 1984). Experimental insulin deficiency similarly diminishes the affinity of carnitine palmitoyltransferase for malonyl-CoA, but treatment of diabetic rats with insulin restores the enzyme to normal (Gamble & Cook, 1985). Data presented here indicate that the anti-ketogenic effect of oestrogen is exerted through a mechanism that is very similar to the mechanism responsible for the regulation of fatty acid oxidation in the starvation–feeding cycle and in diabetes. The primary effect of oestrogen in these experiments was to increase the affinity of carnitine palmitoyltransferase for malonyl-CoA.

EXPERIMENTAL

Treatment of animals

Female rats (175–225 g) of the Sprague–Dawley strain were obtained from Harlan Industries (Indianapolis, IN, U.S.A.). One group was allowed free access to food (Purina Lab Chow for Rats;Ralston Purina Co., Richmond, IN, U.S.A.) and was not treated with ethynyloestradiol. Another control group, that also received no oestrogen, was given the same quantity of food consumed by the group that received oestrogen. This group (pair-fed controls) was included because rats treated with a high dose of oestrogen consumed less food than did untreated rats (Weinstein et al., 1986). A third group of rats received 5 μg of ethynyloestradiol/kg body wt. per d for 5 days by subcutaneous injection. Ethynyloestradiol (100 mg) was dissolved in ethanol (0.5 ml) and diluted with propylene glycol (19.5 ml) for injection. All control animals received daily injections of the diluting solvents. In order to avoid problems arising from differences in food consumption in the high-dose oestrogen-treated group (see the Results section), additional experiments were carried out using rats that were treated and pair-fed as before but were starved for the 48 h immediately before enzyme assays were conducted. An additional group of rats received 15 μg of ethynyloestradiol/kg body wt. per d for 14 days and were also starved for 48 h before enzyme assays. Control rats for this group were also pair-fed along with the oestrogen-treated group before starvation. All animals were allowed free access to water.

Enzyme Assay

Rats were decapitated before livers were removed for the isolation of mitochondria (Johnson & Lardy, 1967). Mitochondrial pellets were suspended to a protein concentration of 20 mg/ml in 0.25 mM sucrose/1 mM EDTA/3 mM-Tris, pH 7.2. Protein was measured by a biuret procedure summarized by Layne (1975). Respiratory control ratios (Estabrook, 1967) were 5 or greater with 10 mM-glutamate and 0.5 mM-malate as substrates.
The outer carnitine palmitoyltransferase 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, 5 mm-GSH, 4 mg of bovine serum albumin, 0.5 mm-L-carnitine (0.4 μCi of L-[methyl-3H]carnitine/μmol) and 1 μg of antimycin A; oleoyl-CoA was present at 200 μM for measuring activity or at 75, 100 or 150 μM for measuring K_i values; malonyl-CoA was present at 0, 1, 2, 5, 15, 25 or 40 μM. All assays were conducted at 30°C and pH 7.0. K_i values were determined by the graphical method of Dixon (1953). Four experiments were averaged for the determination of each K_i value or estimate of activity.

**Fatty acid oxidation rates**

At the end of 5 days of treatment with the higher dose of ethynylestradiol, one group of rats was anaesthetized lightly with diethyl ether and livers were removed for perfusion in a recycling perfusion system (Heimberg et al., 1964, 1965; Weinstein et al., 1979). A complex of oleic acid and albumin was infused to give a steady-state concentration of approx. 0.4 mm-olate in the erythrocyte-free perfusates. Samples of perfusate were taken for the determination of ketone bodies (Michaels et al., 1951). Statistical evaluation of differences between means were determined by a two-tailed Student’s t test.

**Materials**

Ethynylestradiol, oleoyl-CoA, malonyl-CoA, L-carnitine hydrochloride, GSH, EDTA, imidazole, Hepes, and essentially fatty acid-free bovine serum albumin (used untreated for enzyme assays and treated to remove fatty acids for liver perfusions) were purchased from Sigma Chemical Co. (St. Louis, MO, U.S.A.). Oleic acid for liver perfusions was obtained from Nu Chek Prep (Elysian, MN, U.S.A.). L-[methyl-3H]Carnitine hydrochloride was obtained from Amersham Corp. (Arlington Heights, IL, U.S.A.).

**RESULTS**

The rate of fatty acid oxidation in perfused livers from control rats that were fed ad libitum was 2.5 ± 0.3 μmol of ketone bodies produced/min per g wet wt. of liver, but the livers from rats that were pair-fed along with the oestrogen-treated group oxidized olate at a rate of 5.7 ± 0.5 μmol of ketone bodies/min per g. The increase in ketogenic rate probably resulted from restricted food consumption by the pair-fed group. Treatment of rats with 5 mg of ethynylestradiol/day for 5 days decreased (compared with the pair-fed control group) the rate of olate oxidation to 3.0 ± 1.3 μmol of ketone bodies/min per g. These results with a high dose of oestrogen agree with previous data with a much lower (15 μg/kg per day for 14 days) dose of oestrogen (Weinstein et al., 1979) and indicate that oestrogen has an anti-ketogenic effect over a wide range of concentrations.

Inhibition of carnitine palmitoyltransferase by malonyl-CoA was somewhat greater in hepatic mitochondria from rats fed ad libitum compared with rats pair-fed along with the oestrogen-treated group (Fig. 1). Treatment with oestrogen caused greater sensitivity to inhibition by malonyl-CoA at all concentrations of inhibitor compared with the pair-fed control. The concentration of malonyl-CoA causing 50% inhibition in oestrogen-treated rats was only 4 μM, compared with 13 μM in the pair-fed group.

The activity of carnitine palmitoyltransferase in hepatic mitochondria from rats that were pair-fed along with the oestrogen-treated group was greater than the activity in hepatic mitochondria from rats that were fed ad libitum (Table 1). Oestrogen treatment resulted in activity that was identical with that in rats fed ad libitum, but less than in the appropriate pair-fed controls, suggesting that oestrogen treatment decreased the activity of carnitine palmitoyltransferase. The differences in enzyme parallelled differences in rates of ketogenesis in the perfused liver, but the differences in those rates of fatty acid oxidation were much greater than differences in activity. The K_i of carnitine palmitoyltransferase for malonyl-CoA (Table 1) changed in concert with differences in activity and with rates of fatty acid
Table 1. Activity of carnitine palmitoyltransferase and $K_i$ values for malonyl-CoA in intact mitochondria

Hepatic mitochondria were isolated from the five groups of rats indicated and assayed for carnitine palmitoyltransferase activity at the optimum concentrations of oleoyl-CoA (100 µM) and carnitine (0.5 mM). $K_i$ values were determined as described in the Experimental section. Results are presented as means ± S.E.M. for each group of rats ($n = 4$). There were statistical differences ($P < 0.05$) in activities between the pair-fed control group and the other two groups. There were statistical differences ($P < 0.001$) in $K_i$ values between the pair-fed control group and the other two groups and between the two starved groups.

<table>
<thead>
<tr>
<th>Treatment of rats</th>
<th>Carnitine palmitoyltransferase activity (nmol/min per mg of protein)</th>
<th>$K_i$ for malonyl-CoA (µM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fed (ad libitum)</td>
<td>7.2±2.1</td>
<td>1.5±0.3</td>
</tr>
<tr>
<td>Pair-fed</td>
<td>10.4±0.4</td>
<td>3.5±0.5</td>
</tr>
<tr>
<td>Fed + oestrogen</td>
<td>8.5±0.3</td>
<td>1.3±0.3</td>
</tr>
<tr>
<td>Starved</td>
<td>10.4±0.7</td>
<td>6.7±0.7</td>
</tr>
<tr>
<td>Starved + oestrogen</td>
<td>11.2±0.7</td>
<td>3.0±0.2</td>
</tr>
</tbody>
</table>

Oxidation, exactly as seen previously during starvation (Cook, 1984) and experimental diabetes (Gamble & Cook, 1985), i.e. modest increases in activity were associated with quantitatively more significant changes in the $K_i$ for malonyl-CoA.

The effects of oestrogen treatment seem quite clear when comparisons are made with the pair-fed control group, but the fact that the oestrogen-treated group was almost identical with the group fed ad libitum in all aspects stimulates questions regarding how rigorously one can control the nutritional status of animals in the various groups that were used in our experiments. To substantiate the effects of oestrogen on carnitine palmitoyltransferase, additional experiments were conducted using rats that were treated with ethynylestradiol or the dissolving vehicle exactly as before, except that the animals were starved for 48 h before they were used for enzyme measurements. Starvation caused a 30% increase in carnitine palmitoyltransferase activity, but the $K_i$ for malonyl-CoA was increased by 350% (Table 1) over the values observed in the group fed ad libitum. Oestrogen did not significantly lower enzyme activity in the starved rats, but the $K_i$ value for malonyl-CoA was decreased substantially by the oestrogen treatment.

We also confirmed the effect of oestrogen on $K_i$ values for malonyl-CoA, using another group of rats that were treated with a much lower dose of ethynylestradiol (15 µg/kg body wt. per day) and starved for 48 h before use. Results of that experiment showed that the $K_i$ for malonyl-CoA was again substantially decreased by oestrogen (7.7±1.1 µM in the starved control rats and 5.0±0.7 µM with low-dose oestrogen treatment; $P < 0.05$).

**DISCUSSION**

This study indicates that oestrogen is anti-ketogenic at both high and low doses and that it acts through changes in carnitine palmitoyltransferase, decreasing both activity of the enzyme and the $K_i$ value for malonyl-CoA. It is difficult to assess the relative importance of these two changes, since both effects would be expected to inhibit fatty acid oxidation. Oestrogen produced both changes in carnitine palmitoyltransferase of the fed rat, but in the starved rat the high dose of oestrogen substantially increased the affinity of carnitine palmitoyltransferase for malonyl-CoA without affecting activity, indicating that the two changes in enzyme properties may not always be inseparable.

The effects of oestrogen on carnitine palmitoyltransferase may explain the stimulatory actions of oestrogen on triacylglycerol formation by the liver (Watkins _et al._, 1972; Weinstein _et al._, 1979) and the hypertriglyceridaemia that is associated with oral contraceptives containing oestrogens (Aurell _et al._, 1966; Wynn _et al._, 1966). Inhibition of carnitine palmitoyltransferase has been shown by Ide & Ontko (1981) to stimulate hepatic triacylglycerol synthesis and lipoprotein secretion. Those authors stressed the importance of carnitine palmitoyltransferase in the reciprocal relationship that exists between fatty acid oxidation and very-low-density lipoprotein synthesis.

Oestrogen apparently had no effect on the positive co-operative inhibition characteristic of malonyl-CoA inhibition of hepatic carnitine palmitoyltransferase. Dixon plots confirmed those effects, i.e. upwardly curved lines were observed in plots of data from rats fed ad libitum but not in rats on food-restricted (pair-fed) diets or in rats that were starved for 48 h. The difference in curvature of lines of Dixon plots from pair-fed rats compared with rats fed ad libitum indicated that the two control groups were quite different. Those differences, and the fact that the pair-fed rats are deprived of energy, make them suspect controls for the oestrogen-treated fed rat, even though high-dose oestrogen treatment also induces decreased food consumption. It was because of those considerations that we repeated oestrogen treatment with a group of rats that were starved for 48 h before use. The data from that experiment confirmed the anti-ketogenic effect of oestrogen and suggested that oestrogen does have a regulatory effect on carnitine palmitoyltransferase that is not related to feeding behaviour. Furthermore, treatment of female rats with the low dose of estrogen substantially decreased the $K_i$ for malonyl-CoA, without having any significant effect on food consumption. Because we used normal female rats in these experiments, the effect of oestrogen may be underestimated, owing to the presence of endogenous oestrogen. Consideration of that fact supports the conclusion that the effect of oestrogen on carnitine palmitoyltransferase is indeed part of a physiological mechanism for regulating rates of hepatic fatty acid oxidation in the female rat.

The data presented here suggest that oestrogens should be considered with other hormones such as glucagon and insulin as being important in the regulation of fatty acid oxidation in the liver. Oestrogen apparently has its primary effect on the $K_i$ for malonyl-CoA, but the mechanism causing this change is still to be elucidated. It will be interesting to determine how these hormones interact to produce a co-ordinated control of hepatic fatty acid oxidation.

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REFERENCES


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