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L-HISTIDINE-INDUCED SUPPRESSION OF LIPOGENIC ENZYMES*

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SUMMARY: Diets supplemented 5% with L-histidine produce hypercholesterolemia and increase cholesterol biosynthesis in rat liver. We now report an *inhibitory* effect of L-histidine on lipogenic enzymes in the liver. In this study, L-histidine was added to chow and fat-free diets and fed to rats for 18 days. After two days of fasting, the rats were refed the same diet for three days prior to sacrifice. L-histidine decreased fatty acid synthetase activity by 51% when it was added to the chow diet and by 26% when it was added to the fat-free diet. Isocitrate dehydrogenase activity was not altered significantly in rats fed diets supplemented with L-histidine.

Fasting decreases the activity of fatty acid synthetase (1-5) and acetyl-CoA carboxylase (2,3) in rat liver. When fasted animals are refed a fat-free diet, acetyl-CoA carboxylase and fatty acid synthetase activities increase to several times the normal levels (4-7). Feeding fasted rats a chow diet causes an overshoot of enzyme levels within two days, after which they return to normal levels (7). Using this paradigm, the study here described was designed to determine the effect of L-histidine diet supplements on the levels of acetyl CoA carboxylase, fatty acid synthetase, and isocitrate dehydrogenase in the 100,000 x g supernatant solution of rat liver homogenates.

MATERIALS AND METHODS

Experimental materials were obtained from the following sources: acetyl-CoA, malonyl-CoA, DL-isocitrate, glycylglycine, glutathione, NADP, NADPH, and ATP from Sigma Chemical Co., St. Louis, MO; EDTA from Fisher Scientific Co., Waltham, MA; bovine serum albumin from Nutritional Biochemicals Corporation, Cleveland, OH: [^{14}C] $NaHCO_3$ (specific activity 48 mCi/mmole) and *Aquasol* (scintillation solution) from New England Nuclear, Boston, MA. All other chemicals used were of analytical grade. The fat-free diet (Wooley &

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Sebrell), Mod. TD-71125 was from Teklad Test Diets, Madison, WI. The normal diet was ground Purina Formulab Chow. L-histidine constituted 5% of the diets by weight. Fatty acid synthetase and isocitrate dehydrogenase assays were made with a Carl/Zeiss M4QII spectrophotometer. An Isocap/300 Nuclear Chicago Scintillation Counter, Chicago, IL was used for radioactivity measurements.

TREATMENT OF ANIMALS: Twenty male albino rats from Holtzman Rat Co., Madison, WI, weighing 55 ± 5 g were obtained at 21 days of age. They were divided into five groups of four and fed normal or experimental diets ad lib. for 18 days. Following this period, one group was fed chow diet continuously (A). The remaining four groups were fasted for two days and then fed ad lib. for three days the following experimental diets: chow (B), 95% chow + 5% histidine (C), fat-free diet (D), and 95% fat-free diet + 5% histidine (E). This provided 21 days of experimental diet, as used in previous studies of amino acid feeding (8). Rats were housed singly in stainless steel cages. The light cycle was from 7:00 A.M. to 5:30 P.M.

PREPARATION OF RAT LIVER HOMOGENATE: Rats were killed by decapitation between 10:00 and 11:00 P.M. The livers were removed, placed on ice, weighed, minced, and homogenized in a 0.1 M potassium phosphate buffer, pH 7.4, containing 0.004 M $MgCl_2$, 0.001 M EDTA, and 0.002 M dithiothreitol with 5 strokes of a Potter-Elvehjem homogenizer. Two ml of buffer were used per gram of liver. The homogenate was centrifuged for 10 min at 20,000 x g and the supernatant solution was centrifuged at 100,000 x g for 60 min. The supernatant solution (cytosol fraction) was stored at -20°C until assayed; the enzymes were assayed without further purification. Protein concentrations were measured by a modification of the biuret procedure (9) using bovine serum albumin as a standard.

ENZYME ASSAYS: Acetyl-CoA carboxylase activity was assayed by a modified procedure described by Craig et al. (7). A 7 µl aliquot of the 100,000 x g supernatant cytosol solution of the rat liver (approx. 280 µg) was incubated at 37°C for 30 min in a 175 µl solution containing Tris-chloride (15 µMoles); glutathione (0.75 µMole); $MgCl_2$ (2 µMoles); EDTA (25 nmoles); bovine serum albumin (0.15 mg of proteins); and potassium citrate (5 µMoles). The pH of the final solution was 7.0. The carboxylation reaction was initiated by the addition of a 50 µl aliquot containing ATP (0.5 µmole), acetyl-CoA (50 nmoles), and 25 µl of [^{14}C] $NaHCO_3$ (2.5 µmoles and 1 x 10^6 dpm). A 250 µl volume of solution was incubated at 37°C for 10 min. The reaction was stopped by the addition of 50 µl of 6 N HCl. The samples were centrifuged for 10 min and 200 µl of the supernatant solution was placed in a counting vial and evaporated in a water bath at 80°C. Two hundred µl of distilled water were added to the vial to dissolve the precipitate; 10 ml of *Aquasol* were subsequently added. The samples were assayed for radioactivity. The total activity was expressed as nmoles of malonyl-CoA formed per min per g liver. This was equivalent to nmoles of HCO_3 fixed per min per g liver. Fatty acid synthetase was assayed according to the method of Nepokroeff et al.(10). The enzyme activity was expressed as µmoles of NADPH oxidized per min per g of liver. Isocitrate dehydrogenase activity was assayed by the method of Ochoa (11). The enzyme activity was expressed as µmoles of NADP reduced per min per g of liver. All statistical analysis was done with a two-tailed Student's *t*-test.

RESULTS

The fatty acid synthetase activity in liver homogenate from rats fed chow for three days after a two-day fast increased to 270% of the control

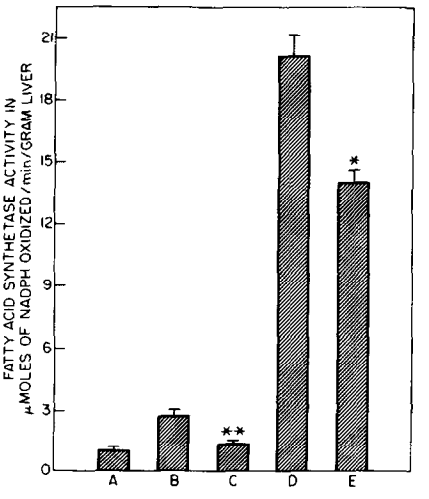

Figure 1: Effects of fasting and refeeding chow and fat-free diets with and without L-histidine supplementation on fatty acid synthetase activity of the 100,000 x g supernatant solution of liver homogenates of rats maintained in different nutritional states: continuously fed, chow (A); fasted-refed, chow (B); fasted-refed, 95% chow + 5% histidine (C); fasted-refed, fat-free (D); and fasted-refed, 95% fat-free + 5% histidine (E). The enzyme activity is expressed as μmoles NADPH oxidized per min. per g liver. The vertical bars represent the standard deviation with 4 rats in each group. Asterisks indicate p values for comparisons with the matched controls; *$p<0.01$ (E vs D) and **$p<0.001$ (C vs B).

level. The enzyme activity increased nineteenfold when a fat-free diet was fed after fasting. A 5% L-histidine supplement to the chow diet depressed the enzyme activity by 51%; activity decreased by 26% when 5% L-histidine was added to the fat-free diet. Fasted, refed rats given either chow or fat-free diets without histidine supplements were used as controls.

Fasting and refeeding chow diet also increased the activity of acetyl-CoA carboxylase ($p < 0.001$) to 195% of the control value (Table I). When rats were fasted and refed a fat-free diet, the enzyme activity increased to 356% of the control level. L-histidine supplementation suppressed these increases. Compared to fasted, refed controls, rats fed L-histidine had much lower levels of acetyl-CoA carboxylase on the chow diet ($p < 0.001$) and fat-free diet ($p < 0.05$).

TABLE 1

THE EFFECT OF FEEDING OF L-HISTIDINE SUPPLEMENTED DIET ON ACETYL-CoA CARBOXYLASE AND ISOCITRATE DEHYDROGENASE ACTIVITIES IN THE LIVER OF RATS MAINTAINED IN DIFFERENT NUTRITIONAL STATES [a]

	Nutritional State [b]	Acetyl-CoA Carboxylase		Isocitrate Dehydrogenase	
		Enzyme Activity [c]	Percent of Control Activity	Enzyme Activity [d]	Percent of Control Activity
A	Continuously Fed: Chow (ad lib.)	71.4 ± 6.4 [e]	-	6.63 ± 0.38	-
B	Fasted-Refed: Chow	139.4 ± 12.0	195	6.86 ± 1.13	103
C	Fasted-Refed: 95% Chow + 5% Histidine	65.5 ± 1.4 [f]	92	6.75 ± 1.27	101
D	Fasted-Refed: Fat-Free Diet	254.0 ± 22.0	356	6.89 ± 1.38	104
E	Fasted-Refed: 95% Fat-Free Diet + 5% Histidine	119.7 ± 4.3 [g]	167	6.50 ± 1.11	98

a. The methods of enzyme assay are described in the experimental section.
b. Feeding period was 21 days; N=4 rats/group
c. nmoles of product formed per minute per gram liver
d. μmoles of NADP reduced per minute per gram liver
e. Mean ± SD
f. Significantly different from matched control (B), $p<0.001$
g. Significantly different from matched control (D), $p<0.05$

The activity of isocitrate dehydrogenase did not change significantly (Table 1).

DISCUSSION

Our results show that L-histidine added to normal and fat-free diets depresses acetyl-CoA carboxylase and fatty acid synthetase activities in the livers of fasted, refed rats. L-histidine depressed to a similar degree (51-53%) the activities of both enzymes when it was added to chow diets, and that of acetyl-CoA carboxylase when it was added to fat-free diets. Fatty acid synthetase activity was depressed by 26% by the addition of L-histidine to fat-free diets.

Solomon et al. (12) showed that dietary histidine depresses the incorporation of [^{14}C] acetate into triglycerides in liver slices and increases the incorporation of the labeled substrate into cholesterol. Acetyl-CoA

is the common precursor for the biosynthesis of both cholesterol and fatty acids. The endogenous concentration of acetyl-CoA depends partly on the activities of enzymes which respond to histidine. The decrease in fatty acid synthesis in rat liver caused by L-histidine supplementation may be secondary to an increase in cholesterol biosynthesis or *vice versa*.

The fact that dietary L-histidine does not change isocitrate dehydrogenase activity in the cytosol suggests that this enzyme may not be controlled by the same mechanism that regulates fatty acid synthetase and acetyl-CoA carboxylase. It also suggests that isocitrate dehydrogenase may not be an important NADPH generating enzyme providing reducing equivalents for the enhanced lipogenesis that occurs during fat-free diet feeding.

Long-term changes in the activities of acetyl-CoA carboxylase and fatty acid synthetase have been attributed to changes in the rates of synthesis and degradation of these enzymes (13,14). However, it is also possible that short-term regulation by other control mechanisms might produce rapid changes in enzyme activities. The modification of fatty acid synthetase in rat and pigeon liver by a phosphorylation-dephosphorylation reaction, recently reported by Qureshi et al. (15), might be a short-term control mechanism of fatty acid synthetase activity. Preliminary data reported by Carlson and Kim (16-18) and Lee and Kim (19) suggest that rat liver acetyl-CoA carboxylase is regulated by a phosphorylation-dephosphorylation reaction similar to that of fatty acid synthetase. This mechanism of regulation may be of physiological importance in rats maintained on excess histidine diets.

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