Vol. 83, No. 3, 1978 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
August 14, 1978 Pages 1191-1197

L-HISTIDINE INDUCED CHANGES IN ADENOSINE 3':5'-MONOPHOSPHATE LEVELS IN RAT BRAIN AND AMOUNTS OF APO-, HOLO-a AND HOLO-b FATTY ACID SYNTHETASES IN RAT LIVER*

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Received June 29,1978

SUMMARY: When fasted rats were fed a chow or fat-free diet supplemented 5% with L-histidine for three days, the brain adenosine 3':5'-monophosphate (cAMP) level increased. A 50% increase occurred in rats fed a chow diet and 20% increase in rats fed a fat-free diet. Purification of liver fatty acid synthetase and the isolation of liver apo-, holo-a and holo-b fatty acid synthetases demonstrated that L-histidine feeding caused changes in the relative amounts of these enzymes. Apo- and holo-b fatty acid synthetases increased while the holo-a form simultaneously decreased. This effect was observed in rats fed either chow or fat-free diets supplemented with L-histidine.

Dietary L-histidine supplementation induces hypercholesterolemia in the rat (1). This supplementation simultaneously increases cholesterol synthesis and decreases triglyceride synthesis, as measured by the conversion of labeled substrates into lipids in liver slices (1). Dietary histidine also depresses the activity of fatty acid synthetase in both liver and brain of rats fasted and then maintained on either a chow or fat-free diet (2). The increase in fatty acid synthetase activity, which is observed in the fasting/refeeding paradigm (3), is inhibited by adenosine 3':5'-monophosphate (cAMP).

Our investigation examined the relationship between cAMP levels and fatty acid synthetase activity in rats fasted and then refed L-histidine supplemented chow and fat-free diets. Previous work from our laboratory (2) has shown that L-histidine depresses fatty acid synthetase activity in rat brain as well as in liver. For technical reasons, we chose to measure cAMP levels in brain and fatty acid synthetase activity in liver of the same rats.

*This investigation was supported by grants from the Medical Research Service of the Veterans Administration Hospital and the University of Wisconsin.

0006-291X/78/0833-1191$01.00/0

Studies on the long-term control of fatty acid synthetase have suggested that changes in the rate of synthesis of this enzyme are responsible for increases and decreases in enzyme activity in the liver (4,5). Fasting decreases the rate of synthesis of fatty acid synthetase. When rats are refed a fat-free diet, the rate of synthesis increases. No change in the degradation rate of the enzyme was found to be responsible for the increase in enzyme activity (5). Studies on short-term control have suggested a second regulatory mechanism, i.e., the presence of apo- (devoid of 4'-phosphopantetheine) and holo- (containing 4'-phosphopantetheine) fatty acid synthetases (6,7), confirmed by the separation of apo- and holo- fatty acid synthetases with affinity chromatography (8). The holo- fatty acid synthetase has been further separated into two forms, holo-a (high specific activity, dephosphorylated) and holo-b (low specific activity, phosphorylated) by Qureshi et al. (9). Our investigation involved the use of this separation method to study the effect of L-histidine supplementation on fatty acid synthetase forms and activity. After separation, we isolated and measured the amounts of apo-, holo-a and holo-b fatty acid synthetases in the livers of rats fed a chow or fat-free diet following a two-day fast. Changes in the amount of these three enzymes were compared with the cAMP levels in the brain to suggest that L-histidine could affect fatty acid synthetase activity via changes in cAMP levels.

MATERIALS AND METHODS

The fat-free diet (Wooley & Sebrell), Mod. TD-71125 was from Teklad Test Diets, Madison, WI. The normal diet was ground Purina Formulab Chow. In the histidine-supplemented diets, L-histidine constituted 5% of the diets by weight. Experimental materials were obtained from the following sources: [^{3}H] cAMP (specific activity 36.6 Ci/μmole), [1-^{14}C] acetyl-CoA (specific activity 54 mCi/μmole and *Aquasol* from New England Nuclear, Boston, MA; cAMP, cAMP-dependent protein kinase (binding activity 0.1 pmole of cAMP per g protein), *Norit* charcoal, DEAE-cellulose, acetyl-CoA, malonyl-CoA, NADPH, crystalline pantetheine, caproic acid and dithiothreitol from Sigma Chemical Co., St. Louis, MO; Dowex AG 50W-X4 (H^+ form, 200-400 mesh), Dowex AG 50W-X2 (H^+ form, 50-100 mesh), Dowex AG 1-X2 (Cl^-form, 50-100 mesh) and Bio-Gel A-1.5 m (200-400 mesh) from Bio-Rad Laboratories, Richmond, CA; Sepharose 4B from Pharmacia Fine Chemicals, Uppsala, Sweden; bovine serum albumin from Nutritional Biochemicals, Cleveland, OH. All other chemicals used were of analytical grade.

A Gerling Moore microwave source (Model 4104) was used to sacrifice all rats (3.5 kW for 2.25 seconds). Spectrophotometric assays for fatty acid synthetase were carried out using a Zeiss M4Q II spectrophotometer. All radioactivity countings were done in a Nuclear Chicago scintillation counter Isocap/300. A *Polytron* (Brinkman Instruments, Des Plaines, IL) was used for homogenizing brain tissue.

Male albino rats weighing 180-200 g were obtained from the Holtzman Rat Co., Madison, WI. They were divided into four groups of five rats. At the beginning of the experiment, all rats were fasted for two days. Then they were refed the following control or experimental diets for three days: chow, 95% chow + 5% L-histidine, fat-free diet, and 95% fat-free + 5% L-histidine. Rats were housed singly in stainless steel cages. The light cycle was from 7:00 A.M. to 5:30 P.M. Sacrificing was done between 10:00 and 11:00 P.M. For cAMP assays, all five rat brains from each of the four groups were used. For the fatty acid synthetase studies, three randomly selected rat livers from each of the four groups were used.

DETERMINATION OF cAMP LEVELS IN RAT BRAIN: cAMP assays were carried out by the competitive protein binding method modified from Tovey et al. (10). Modifications included homogenizing brain tissue in 2 ml of 5% TCA for 10 sec and then centrifuging it at 15,000 x g for 10 min. The supernatant was passed through a 4 cm disposable pipette column packed with Dowex AG 50W-X4, 200-400 mesh, H^+ form. The column was washed with 1 ml ice-cold deionized water. The cAMP was eluted with another 3 ml of water. The cAMP was assayed using 50 µl of this eluate directly, bypassing ether extraction or lyophilization.

PREPARATION AND PURIFICATION OF FATTY ACID SYNTHETASE: Three rat livers from each group were pooled, weighed, minced, and then homogenized in a 0.1 M potassium phosphate buffer, pH 7.4, containing 4 mM $MgCl_2$, 1 mM EDTA and 2 mM dithiothreitol with 5 strokes of a Potter-Elvehjem homogenizer. Two ml of buffer were used per g of liver. The homogenate was centrifuged for 10 min at 20,000 x g. The supernatant solution was retained and recentrifuged at 100,000 x g for 60 min. The supernatant solution (100,000 x g fraction) was sealed in plastic tubes and stored at -20°C before purification. All the purification steps were carried out at room temperature according to the method of Nepokroeff et al. (11).

SEPARATION OF APO-, HOLO-a, AND HOLO-b FATTY ACID SYNTHETASES: Preparation of Sepharose ε-aminocaproylpantetheine (used for the affinity chromatographic separation of apo-, holo-a and holo-b fatty acid synthetase) was done according to the method of Lornitzo et al. (12). The successive elutions of different forms of the enzymes were carried out according to the procedure of Qureshi et al. (9) using the ε-aminocaproylpantetheine column. To determine the amount and specific activity of each form of the enzyme, fractions 2 to 14 (apo-), 21 to 30 (holo-a) and 36 to 45 (holo-b) were combined separately and dialyzed for three hr at room temperature with two changes of buffer. The dialysis buffer contained 0.5 M potassium phosphate, 1 mM EDTA and 10 mM dithiothreitol, pH 7.0. The assay for fatty acid synthetase activity in the holo-a and holo-b fractions was carried out according to the method of Neprokroeff et al. (11). The dialyzed apo- fatty acid synthetase was purified further by passing the enzyme through a Bio-Gel column (A-1.5 m, 200-400 mesh, in a 1.2 cm x 22 cm column) previously equilibrated with 0.4 M potassium phosphate buffer, pH 7.0, containing 1 mM EDTA and 2 mM dithiothreitol. The enzyme was eluted with the same buffer and 1 ml fractions were collected at 4°C. Fractions 12 to 17 were combined and dialyzed in the above buffer. The assay for acetyl-CoA-pantetheine transacylase of the apo- fraction was carried out by using the method of Lornitzo et al. (12).

Protein concentrations of liver and brain fractions were estimated by a modification of the biuret procedure (13) using bovine serum albumin as the standard. Statistical analysis was done with either a two-tailed Student's t-test or a two-way analysis of variance.

RESULTS

L-histidine supplementation caused an increase in the level of cAMP in rat brain (Fig. 1). This increase was 50% when L-histidine supplemented a chow diet and 20% when it supplemented a fat-free diet. Both increases were statistically significant ($p < 0.01$).

Significant changes in the distribution of the three different forms of fatty acid synthetase were observed in the livers of rats refed with L-histidine-supplemented diets (Table 1). They were determined by a two-way analysis of variance ($F_{(6,16)} = 76.7$; $p < 0.001$) which demonstrated a statistical interaction between diet and enzyme form distribution. These changes were characterized by a decrease in the ratio of holo-a to apo- and holo-b forms in liver. This decrease was observed when L-histidine was supplemented to either chow or fat-free diets. The identity of each apo- fatty acid synthetase fraction was confirmed by the presence of acetyl-CoA-pantetheine transacylase activity. Two elution profiles are presented in Fig. 2, characterizing the elution patterns of the three forms of fatty acid synthetase in chow and chow plus histidine conditions. When the three forms of fatty acid synthetase were separated on the Sepharose affinity columns, there was a large variation of protein recovery from column to column, even though the ratio of enzyme forms eluted remained constant (Table 1). Mean column recoveries ranged from 78% to 95%.

DISCUSSION

Nutritional and hormonal studies have suggested that cAMP may be involved in decreasing fatty acid synthetase in rat liver (3). cAMP and dibutyryl cAMP depress the incorporation of [^{14}C] acetate into liver lipids and fatty acids (14, 15). Thus, the depression of fatty acid synthetase due to L-histidine may be mediated by the elevation of histamine which then causes

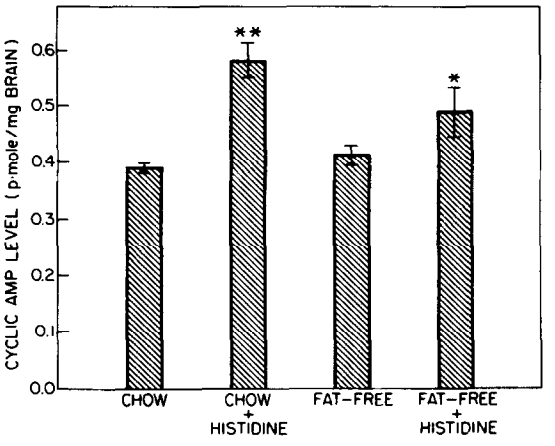

Fig. 1: Effects of L-histidine supplementation on cAMP levels in rat brain. The following diets: chow, 95% chow + 5% L-histidine, fat-free, and 95% fat-free + 5% L-histidine, were fed for three days to rats previously fasted for two days. Amount of cAMP is expressed as pmoles per mg wet weight of brain. Vertical bars represent standard deviations with five rats in each group. Asterisks indicate p values for comparison with the controls: $*p < 0.01$ (fat-free + histidine vs fat-free) and $**p < 0.001$ (chow + histidine vs chow).

TABLE I

EFFECT OF SHORT-TERM FEEDING OF L-HISTIDINE-SUPPLEMENTED DIET ON THE AMOUNT OF APO-, HOLO-a AND HOLO-b FATTY ACID SYNTHETASES IN RAT LIVER[a]

NUTRITIONAL STATE[b]	PROTEIN mg/liver				PERCENT RECOVERY
	DEAE	Apo-[d]	Holo-a	Holo-b	
Chow	12.7 ± 2.0[c]	1.9 ± 0.6	6.5 ± 2.0	1.7 ± 0.6	78 ± 15
95% Chow + 5% Histidine	12.9 ± 0.6	3.7 ± 0.4	3.2 ± 0.6	4.0 ± 0.6	85 ± 16
Fat-Free Diet	16.5 ± 2.0	1.9 ± 0.4	8.7 ± 1.5	2.6 ± 0.7	80 ± 8
95% Fat-free Diet + 5% Histidine	16.0 ± 1.5	5.4 ± 0.7	3.2 ± 0.5	6.6 ± 0.8	95 ± 8

a Time of killing is 10 P.M.
b Feeding period is 3 days following 2 days of fasting
c Mean ± SD; N=3 rats/group
d After purification by Bio-gel filtration

a rise in intracellular cAMP levels. Until recently, the in vivo assay for cAMP in brain or liver was hampered by the lack of a quick method to stop the

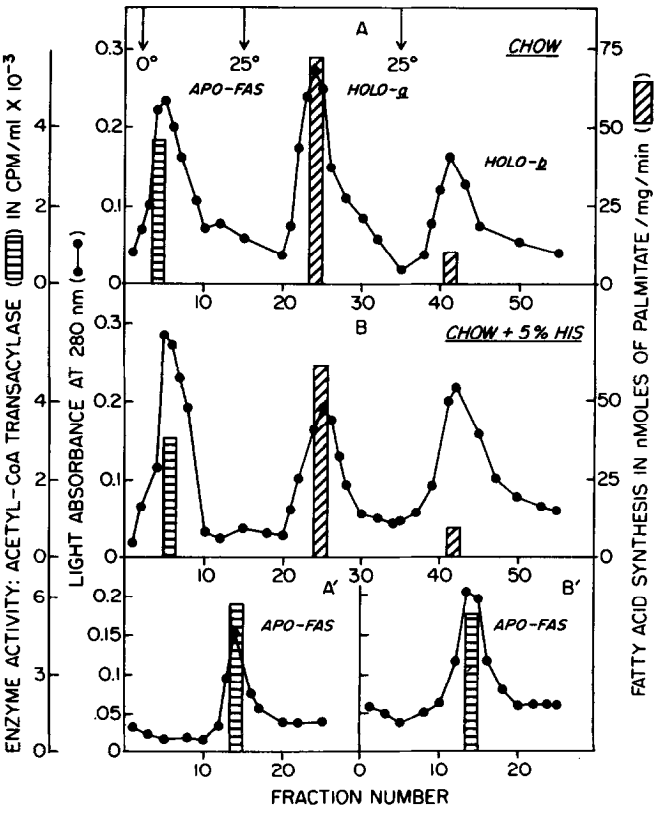

Fig. 2: Elution patterns of apo, holo-a and holo-b fatty acid synthetases by affinity chromatography. Each column contained 2 g of Sepharose-ε-amino-caproylpantetheine. Patterns are shown for rats fasted and refed with chow (A) and for rats fasted and refed with 95% chow + 5% L-histidine (B). For further purification of the apo- form in (A) and (B), combined fractions of each peak were passed through a Bio-Gel column. Elution patterns are shown for rats fasted and refed with chow (A') and for rats fasted and refed with 95% chow + 5% L-histidine (B'). Fatty acid synthetase activities ▨ for combined fractions of holo-a and holo-b fatty acid synthetase are presented as nmoles of palmitate formed per min per mg protein. Acetyl-CoA-pantetheine transacylase activities ▥ for combined fractions of apo- fatty acid synthetase are presented as cpm per ml x 10^{-3}. Protein concentration of each fraction was estimated by light absorption at 280 nm.

endogenous rise in cAMP levels which occurs with anoxia at the time of sacrifice. Microwave fixation appears to attenuate this problem (16) by rapidly denaturing the enzymes for the synthesis (adenylate cyclase) and degradation (phospho-diesterase) of cAMP. The results on form changes of fatty acid synthetase clearly demonstrate a reduction of holo-a with simultaneous increases of apo- and holo-b when cAMP levels rise. These effects are similar to those seen

in the case of glucagon (17).

Our results suggest that cAMP may be involved in the short-term regulation of rat liver fatty acid synthetase. It is uncertain whether cAMP regulates enzyme activity by indirectly interacting with a protein kinase [such as phosphorylase kinase (18)] which, in turn, suppresses fatty acid synthetase activity, or directly interacts with a fatty acid synthetase kinase. Additional studies will have to be pursued to determine the exact method of regulation.

Acknowledgements

We thank Cindy Birch for technical assistance.

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