Effects of Development on Acetoacetyl-CoA Synthetase Biosynthesis in Rat Liver

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In order to investigate the physiological role of acetoacetyl-CoA synthetase (acetoacetate-CoA ligase, EC 6.2.1.16), a cytosolic acetoacetate-activating enzyme, the effects of animal development on the activity and content of the enzyme were examined in rat liver. In male rats, the enzyme specific activity increased 21-fold at 4 weeks of age from that at 2 weeks of age, and then gradually decreased, while in female rats, it increased similarly to that of male rats, but further increased, reaching a maximum about 3-fold higher than that of male rats, at 6 weeks of age. The developmental patterns of the enzyme content correlated with that of the enzyme specific activity. These results indicate that changes in this enzyme activity and content during the developmental process might influence the rate of ketone body utilization for the formation of physiologically important lipidic substances in rat liver.

Key words acetoacetyl-CoA synthetase; ketone body; development; hypercholesterolemia

Previously we showed that acetoacetyl-CoA synthetase (acetoacetate-CoA ligase, EC 6.2.1.16) is a discrete acetoacetate-specific ligase, and we purified for the first time from Zoogloea ramigera, a bacterium isolated from activated sludge, and rat liver. In Zoogloea ramigera, acetoacetyl-CoA synthetase is the enzyme essential for the metabolism of poly (3-hydroxybutyrate), a unique intracellular energy reserve of certain microorganisms. In mammals, acetoacetyl-CoA synthetase is present in the cytosolic fractions of various tissues, and acetoacetate is known to be effectively incorporated into cholesterol and fatty acids in rat liver, which contains virtually no activity of succinyl-CoA: 3-oxoacid-CoA transferase (EC 2.8.3.5), another acetoacetate-activating enzyme present in mitochondria. However, the physiological role of acetoacetyl-CoA synthetase has not yet been fully clarified.

We previously reported that ML-236B (compactin) and cholestyramine in the diet enhanced the acetoacetyl-CoA synthetase content in rat liver. A similar enhancing effect of a hypocholesterolemic compound is also known for 3-hydroxy-3-methylglutaryl-CoA reductase (HMG-CoA reductase), the rate-limiting enzyme in cholesterol biosynthesis. Since HMG-CoA reductase activity is known to be altered during animal development, in the present study we have examined whether the developmental process affects the activity and content of this enzyme in rat liver.

MATERIALS AND METHODS

Rats Young Sprague-Dawley rats (obtained from Tokyo Laboratory Animals Science Co., Tokyo, Japan) were separated from their mothers at day 21 after birth, and fed a standard diet ad libitum (Oriental Yeast Co., Ltd., Japan). On predetermined days, the animals were anesthetized with ether and killed by decapitation.

Preparation of Liver Supernatant All the following procedures were carried out at 4°C. The rat livers were excised and homogenized in 4 vol. of 10 mM Tris-HCl (pH 7.5) containing 10 mM 2-mercaptoethanol, 250 mM sucrose and 1 mM ethylenediamine tetraacetic acid (EDTA) by five strokes in a Teflon-glass homogenizer. The livers were then centrifuged at 10000×g for 20 min. The resultant supernatant was further centrifuged at 105000×g for 60 min. The supernatant fraction thus obtained was used for enzyme activity and immunological assays.

Enzyme Assay Acetoacetyl-CoA synthetase activity was assayed by following the formation of acetyl-CoA, which was produced from acetoacetyl-CoA and CoA with the reaction catalyzed by the addition of Z. ramigera thiolase.

Determination of Acetoacetyl-CoA Synthetase Protein Concentration by Enzyme Immunoassay (EIA) A protein sample was subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE; 10% acrylamide gel), and the separated proteins were then transferred electrophoretically (Semi-Dry Transfer System; Bio Craft, Tokyo, Japan) to a polyvinylidene difluoride (PVDF) membrane in 40 mM Tris–glycine containing 20% methanol and 0.1% SDS at 25°C for 1 h at 50 mA. The membrane was preincubated with Block Ace for 1 h at room temperature and then incubated overnight with anti-rat acetoacetyl-CoA antisera (1:1000) in Block Ace at 4°C. The membrane was washed three times with phosphate-buffered saline (PBS) containing 0.5% Tween 20 and reacted with peroxidase-conjugated goat antibody against rabbit IgG Fc at a 1/400 dilution in PBS containing 3% bovine serum albumin for 2.5 h at room temperature. Then, the membrane was washed twice with PBS containing 0.5% Tween 20 and twice with PBS, and the peroxidase activity was visualized with 3,3′-diaminobenzidine. The formed brown product was analyzed by the Discovery™ System (PD1, NY, U.S.A.) using purified rat acetoacetyl-CoA synthetase as a standard.

Materials Malate dehydrogenase of pig heart mitochondria and citrate synthase of pig heart were purchased from Boehringer Mannheim (Mannheim, F. R.G.); peroxidase-conjugated goat antibody to rabbit IgG Fc was from ICN Pharmaceuticals (Aurora, OH, U.S.A.); and Block Ace was from Dainippon Pharmaceutical Co., Ltd. (Osaka, Japan). Lithium acetoacetate was prepared as described previously. Other chemicals of reagent grade were purchased from commercial sources.

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RESULTS AND DISCUSSION

Figure 1A shows that at 2 weeks of age the acetoacetoy-CoA synthetase specific activity in the liver of both male and female rats was at about the same low level (0.015 ± 0.0015 ver. 0.0016 ± 0.0005 mU/mg protein). In the case of male rats, the enzyme specific activity displayed a 21-fold increase at 4 weeks of age (0.314 ± 0.0015 mU/mg protein) compared to that at 2 weeks of age, then gradually decreased. Although the enzyme specific activity of female rats increased similarly to that of male rats until 4 weeks of age, it continued to increase dramatically, reaching the maximum at 6 weeks of age (1.02 ± 0.05 mU/mg protein), a level about 3-fold higher than that in male rats. The developmental patterns of hepatic acetoacetoy-CoA synthetase content determined by EIA (µg immunoreactive protein/mg cytosolic protein) in male and female rats were almost parallel to those of the enzyme activity in male and female rats, respectively (Fig. 1A and B). The maximal values for male rats at 4 weeks of age and female rats at 6 weeks of age were 0.212 ± 0.004 µg/mg and 0.516 ± 0.051 µg/mg, respectively. These developmental patterns of acetoacetoy-CoA synthetase activity are quite similar to those published for lipogenesis and the male enzyme activity of rat liver, except that the maximum level for the female rat was much higher than that for the male rat in the case of acetoacetoy-CoA synthetase activity.

We previously reported that the feeding of two different hypocholesterolemic agents, cholestryamine (an anion exchanger and accelerator of bile acid excretion) and ML-236B (a competitive inhibitor of HMG-CoA reductase) increased the liver acetoacetoy-CoA synthetase content as in the case of HMG-CoA reductase. Such an effect on acetoacetoy-CoA synthetase has also been observed for pravastatin (data not shown), a new HMG-CoA reductase inhibitor recently frequently used for the treatment of hypercholesterolemia. These observations indicate that this enzyme plays a physiological role in cholesterol biosynthesis and regulated similarly to HMG-CoA reductase in rat liver. Weiss et al. reported a biphasic developmental pattern of alterations in hepatic HMG-CoA reductase activity, composed of alterations due mainly to the enzyme protein content and those due mainly to the catalytic efficiency of the enzyme. In our results on hepatic acetoacetoy-CoA synthetase, neither such biphasic alterations in enzyme activity nor significant differences between the profiles of the enzyme activity and the enzyme concentration were observed (Fig. 1). Therefore, acetoacetoy-CoA synthetase activity appears to be regulated mainly by gene expression during development after weaning. It is known that after weaning mammals undergo many changes in the activity of enzymes involved in hepatic lipogenesis and cholesterol biosynthesis, and the occurrence of such changes is thought to be mainly due to the transition of diet from high-fat milk to low-fat food. Thus, it is possible that the rapid increase in acetoacetoy-CoA synthetase level is also due to the transition of diet during the weaning. We reported that acetoacetoy-CoA synthetase is inhibited by fatty acyl-CoA, as in the case of acyl-CoA carboxylase, a rate-limiting enzyme for fatty acid biosynthesis. However, it is not clear whether this inhibition plays a significant role in the developmental alteration of acetoacetoy-CoA synthetase activity since the alteration seems to be derived substantially from the changes in enzyme content as described above.

It should be noted that after 6 weeks of age, both the enzyme activity and enzyme content of female rat liver are much higher than those of male rat liver (Fig. 1). The period during which this divergence arises coincides with the onset of sexual maturity in the rat. Since acetoacetate is known to be efficiently incorporated into cholesterol and fatty acids mediated by the action of acetoacetoy-CoA synthetase, it is likely that changes in this enzyme activity and content during the developmental process might influence the rate of ketone body utilization for the formation of biologically important lipidic substances in rat liver. Thus, it is possible that such divergence could be due to the influence of sex hormones and/or that acetoacetoy-CoA synthetase plays an important role in sexual maturity, mainly in the female through the ketone body utilization. Further investigation is required to clarify the influence of sex hormones on this enzyme activity.
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REFERENCES