LEVELS OF ACETYL COENZYME A CARBOXYLASE AND ITS EFFECTORS IN RAT LIVER AFTER SHORT-TERM FAT-FREE REFEEDING

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1. Introduction

Acetyl coenzyme A carboxylase (acetyl-CoA:CO_2 ligase (ADP), EC 6.4.1.2) plays a critical role in the regulation of fatty acid synthesis (see [1]). It was shown that in an earlier stage of starvation [2] or in acute decompensated alloxan-diabetes [3], the fatty acid-synthesizing capacity of rat liver slices is more depressed than can be accounted for by the level of the enzyme. Moreover, the content of acetyl-CoA carboxylase in rat liver can not change rapidly (see [4]), since the half-life for degradation of this enzyme was found to be 1–3 days in various metabolic states [1, 5, 6]. These findings suggested that changes in the catalytic efficiency of the enzyme, rather than changes in the enzyme quantity, may play an important role when the rate of fatty acid synthesis must be adjusted promptly. In fact, the catalytic activity of acetyl-CoA carboxylase of animal origin is known to be activated by tri- and dicarboxylic acids, most notably by citrate, and inhibited by long-chain acyl-CoA thioesters (see [1]).

In the present study, an attempt has been made to evaluate the relative importance of the control of the acetyl-CoA carboxylase content and that of its catalytic efficiency for the short-term regulation of fatty acid synthesis. For this purpose, we have studied the time course of changes in the levels of hepatic acetyl-CoA carboxylase, citrate and long-chain acyl-CoA thioesters as well as in the rate of fatty acid synthesis in liver slices, using rats which were fasted and subsequently refed a fat-free diet for varying periods of time. The results of this investigation support the view that the rapid rise in hepatic fatty acid synthesis observed after short-term refeeding is due principally to the modulation of the catalytic efficiency of acetyl-CoA carboxylase by changes in the activator and inhibitor concentrations.

2. Materials and methods

Male Wistar rats (150–200 g) were used in all experiments. Animals, which had been fasted for 48 hr, were refed a fat-free high-carbohydrate diet (Clea, Tokyo, Japan). At the indicated times, rats were anesthetized with ether and sacrificed by decapitation. After quick removal of the liver, a portion of it was frozen immediately with aluminium block cooled in dry ice–acetone for determinations of the citrate and long-chain acyl-CoA contents. The remainder of the liver was utilized without freezing for measurements of acetyl-CoA carboxylase activity in the soluble supernatant fraction and of fatty acid synthesis in tissue slices. Citrate was extracted by homogenizing the frozen liver with 3 vol of 6% (v/v) $HClO_4$ and determined by the fluorometric method with the use of citrate lyase and malate dehydrogenase (Boehringer, Mannheim, Germany) according to Williamson and Corkey [7]. Long-chain acyl-CoA thioesters were separated by acid precipitation and hydrolyzed to liberate free CoA as described by Saggerson and Greenbaum [8]; the CoA was then determined with the use of phosphotransacetylase (Boehringer, Mannheim, Germany) according to Abiko et al. [9]. Acetyl-CoA carboxylase activity was assayed at 37° by the $H^{14}CO_3^-$-fixation method as described previously [6]. One enzyme unit is defined as that amount

which catalyzes the carboxylation of 1 μmole of acetyl-CoA per min. For the determination of fatty acid synthesis, 0.5 g of liver slices was incubated with shaking for 120 min in 2.5 ml of Krebs–Ringer phosphate buffer pH 7.4 containing 5 μmoles of sodium [1-^{14}C]acetate (0.2 Ci/mole). Total lipids were extracted as described by Folch et al. [10]. After saponification with 10% ethanolic KOH at 60° for 1 hr, the nonsaponifiable lipid fraction was extracted three times with light petroleum (b.p., 40–45°). The aqueous phase was acidified with 6 N H_2SO_4, and the fatty acids were extracted three times with light petroleum. These extracts were combined, washed twice with water and evaporated for counting in the scintillator solution of Patterson and Greene [11].

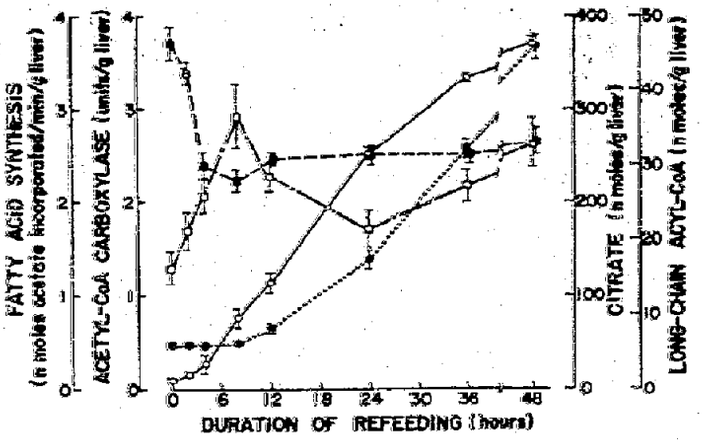

Fig. 1. Effects of fat-free refeeding on fatty acid synthesis in rat liver slices (o—o—o) and on the levels of hepatic acetyl-CoA carboxylase (■---■---■), citrate (□-·-□-·-□) and long-chain acyl-CoA thioesters (■——■——■). Results are given as means ± S.D. per gram of wet tissue. For each point, 6–8 rats were used.

3. Results

In fig. 1 are shown the changes in the fatty-acid synthesizing capacity of liver slices and in the levels of hepatic acetyl-CoA carboxylase, citrate and long-chain acyl-CoA thioesters observed upon fat-free refeeding of fasted rats. The rate of fatty acid synthesis from acetate began to increase within 2 hr of realimentation and kept rising during the whole period of 48 hr refeeding. The long-chain acyl-CoA content fell sharply within 4 hr and changed little thereafter. The citrate content increased during the first 8 hr, decreased thereafter to some extent and increased again after 24 hr. On the other hand, the level of acetyl-CoA carboxylase remained unchanged during the initial 8 hr. Only after this time, it began to increase and kept rising during the whole experimental period.

4. Discussion

Immunochemical studies carried out previously by our group [6, 12] and by Majerus and Kilburn [5] showed that the variations in the level of measured acetyl-CoA carboxylase activity in liver extracts derived from rats under different dietary and hormonal conditions, including fat-free refeeding, are actually determined by changes in the quantity of the enzyme protein. Hence the results of the present study indicate that the initial rise in the rate of fatty acid synthesis observed within 8 hr of realimentation is not due to an increased quantity of acetyl-CoA carboxylase but may rather be attributable to changes in the concentrations of allosteric effectors such as citrate and long-chain acyl-CoA thioesters. After the lapse of 8 hr, the content of acetyl-CoA carboxylase begins to increase, thus contributing also to the elevated rate of fatty acid synthesis. Previous studies showed that the contents of citrate and long-chain acyl-CoA thioesters in rat liver undergo diet-dependent changes [13–16], but no parallel determinations of the levels of hepatic acetyl-CoA carboxylase and its effectors were made for a period shortly after alteration of dietary conditions. Although the situation *in vivo* is hard to assess because of cell compartmentation and interaction between various cellular constituents, the present findings support the view that the modulation of the catalytic efficiency of acetyl-CoA carboxylase plays a principal role especially in the short-term regulation of fatty acid synthesis.

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References

[1] S. Numa, S. Nakanishi, T. Hashimoto, N. Iritani and T. Okazaki, Vitamins and Hormones 28 (1970) 213.
[2] H.M. Korchak and E.J. Masoro, Biochim. Biophys. Acta 58 (1962) 354.
[3] O. Wieland and I. Eger-Neufeldt, Biochem. Z. 337 (1963) 349.
[4] C.M. Berlin and R.T. Schimke, Mol. Pharmacol. 1 (1965) 149.
[5] P.W. Majerus and E. Kolburn, J. Biol. Chem. 244 (1969) 6254.
[6] S. Nakanishi and S. Numa, European J. Biochem. 16 (1970) 161.
[7] J.R. Williamson and B.E. Corkey, Methods in Enzymology 13 (1969) 434.
[8] E.D. Saggerson and A.L. Greenbaum, Biochem. J. 119 (1970) 193.
[9] Y. Abiko, T. Suzuki and M. Shimizu, J. Biochem. (Tokyo) 61 (1967) 10.
[10] J. Folch, M. Lees and G.H. Sloane-Stanley, J. Biol. Chem. 226 (1957) 497.
[11] M.S. Patterson and R.C. Greene, Anal. Chem. 37 (1965) 854.
[12] S. Numa, S. Nakanishi and N. Iritani, Proc. Jap. Conf. Biochem. Lipids 11 (1969) 235.
[13] W.M. Bortz and F. Lynen, Biochem. Z. 339 (1963) 77.
[14] P.K. Tubbs and P.B. Garland, Biochem. J. 93 (1964) 550.
[15] F. Lynen, Progr. Biochem. Pharmacol. 3 (1967) 1.
[16] C. Start and E.A. Newsholme, Biochem. J. 107 (1968) 411.