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INDUCTION OF RAT LIVER MALIC ENZYME MESSENGER RNA ACTIVITY BY INSULIN AND BY FRUCTOSE

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The effects of insulin treatment and fructose feeding on malic enzyme [EC 1.1.1.40] mRNA in liver of diabetic rats were investigated. A high fructose diet, even without insulin treatment, increased the mRNA activity, much more than a high starch diet, but to a lesser extent than insulin treatment. The increase of malic enzyme activity was approximately comparable to the change in mRNA activity, but occurred 12 hr and 30 hr after the increase of mRNA in the fructose-fed rats with and without insulin treatment, respectively. Thus, insulin and fructose appear to induce malic enzyme primarily by increasing the activity of translatable mRNA.

It has been shown that dietary fructose has various effects on hepatic metabolism when compared with a glucose diet (1,2). These effects include alterations in activities of enzymes involved in glycolysis and lipogenesis (3). Although insulin is known to promote glycolysis and lipogenesis (4-9), a high fructose diet, even in diabetic rats, increased lipogenic enzymes 4-5-fold after 4-6 days (Fukuda, H. and Iritani, N., submitted). Very recently, it has been shown that regulation of rat liver L-type pyruvate kinase by insulin and by fructose is primarily via changes in the level of translatable mRNA (10). Mariash et al. have shown that induction of malic enzyme by both carbohydrate and thyroid hormone is mediated by an increase in malic enzyme mRNA (11). However, the molecular basis of the action of insulin and fructose on lipogenic enzymes has not been established. Therefore, in the present study, we have investigated the effects of insulin treatment and fructose feeding for diabetic rat on trans-

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latable malic enzyme mRNA activity by a cell-free translation assay of rat liver total RNA.

MATERIALS AND METHODS

Animals Male Wistar rats, 6-week-old, were made diabetic by intravenous injection of streptozotocin (5.5 mg/100 g, Sigma Chemicals Co.) after starvation for 20 hr. (12). Blood glucose was assayed (13) 2 days after streptozotocin treatment and rats with blood glucose levels of over 300 mg/dl were used for experiments. The diabetic rats adapted to a stock diet (Oriental Shiryo Co., Japan, No MF) were given a high fructose or a high starch diet and simultaneously insulin was injected in the insulin-treated group (at 7 p.m.). Lente insulin, slow acting insulin (Lilly Co.), was injected subcutaneously 3.5 U/rat at the beginning and then 2.5 U/rat every 12 hr. A high carbohydrate diet contained 67.4% fructose or corn starch, 18% casein, 9.5% cellulose, 5% salt mixture (14), 0.1% choline chloride and vitamins (14). The animals were allowed to take diet and water ad libitum and kept under an automatic lighting schedule from 7 a.m. to 7 p.m. at 24°C. The food intake was not significantly different from group to group.

Enzyme and antibody preparations Malic enzyme was purified to near homogenity as described previously (15). Antibody against malic enzyme was raised in rabbits and purified by affinity chromatography on a protein A-Sepharose Cl-4B column (16).

Translation of malic enzyme mRNA Total cellular RNA was isolated from frozen liver samples by extraction with 4 M guanidium thiocyanate (Fluka purum grade, Tridon Inc.) as described by Chirgwin et al. (17). Assays for malic enzyme mRNA activity were carried out with a nuclease-treated reticulocyte lysate system (18). The translation mixture contained 20 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (Hepes) (pH 7.4), 20 μM each of 19 amino acids (all except leucine), 60 μCi L-[4,5-^{3}H]leucine (130 Ci/mmol, Amersham), total RNA (750 μg/ml) and nuclease-treated rabbit reticulocyte lysate in total volume of 60 μl (19). After incubation at 30°C for 60 min, the reaction was stopped by the addition of 440 μl of 20 mM Tris/HCl, pH 7.4 containing 10^{-5}M $NADP^+$, 10^{-3}M EDTA, pH 7.4 and 10 mM mercaptoethanol and centrifuged at 105,000 x g for 60 min. Aliquots of 5 μl of the supernatant were taken for determination of radioactivity in total released proteins. (Trichloroacetic acid-insoluble materials were determined according to Pelham et al. (18)).

Malic enzyme was immunoprecipitated from 450 μl of the supernatant with the antibody and formalin-fixed Staphylococcus aureus (Zymed Laboratories) (20,21) as described previously (15). After 5 μg of purified malic enzyme was added to the immunoprecipitates, the immunoprecipitates were dissociated and subjected to electrophoresis on 10% sodium dodecyl sulfate/polyacrylamide slab gels by the method of Laemmli (22). Protein bands were located staining with Coomassie brilliant blue R 250. The malic enzyme radioactivity was eluted from the gel slices with NCS tissue solubilizer (Amersham) and determined in a liquid scintillation spectrometer.

RESULTS AND DISCUSSION

Malic enzyme mRNA was measured by using a cell-free translation system. Total RNA isolated from liver was translated in a nuclease-treated rabbit

reticulocyte system and the newly synthesized malic enzyme precipitated by indirect immunoprecipitation with the purified antibody and analyzed by sodium dodecyl sulfate/polyacrylamide slab gel electrophoresis and fluorography. The fluorograms were similar to those shown previously (15). The major radioactive band exactly coincided with the position stained with Coomassie blue. When normal immunoglobulin G was added to the system instead of the antibody, no radioactive band was observed. The addition of a large excess of unlabelled malic enzyme to the translation product prior to immunoprecipitation with antibody also resulted in the disappearance of the radioactive band corresponding to malic enzyme. These results indicated that the radioactive band represent malic enzyme synthesized in the reaction mixture. Since there was no visible band near that of the malic enzyme, quantification of newly synthesized malic enzyme was not effected by bands of contaminating protein. Furthermore, the amount of malic enzyme as well as of total protein synthesized in the translation system was proportional to the amount of total RNA added to the reaction mixture.

After the diabetic rats adapted to a stock diet were given a high fructose or a high starch diet and simultaneously injected with insulin, the time courses in activities of malic enzyme and its mRNA were followed. Malic enzyme activities in both dietary groups with insulin treatment were increased about 3-4-fold and 5-6-fold after 18 and 24 hr, respectively, and further increased thereafter (Fig. 1). The enzyme activities in the fructose-fed rats, even without insulin injection, were increased about 2-fold and 3.4-fold after 36 hr and 48 hr, respectively, whereas those of the starch-fed rats were still not increased after 48 hr. On the other hand, the mRNA activities in both dietary groups with insulin injection were increased 3-fold after 6 hr and up to about 15-fold after 24 hr, over those in rats adapted to the stock diet before the experiment (Fig. 2). Even without insulin treatment, the high fructose diet increased the mRNA activities 4-fold after 18 hr, whereas the high starch diet did not increase the mRNA activities until 48 hr. On the fructose-fed rats, the enzyme activities were induced 12 and 30 hr after

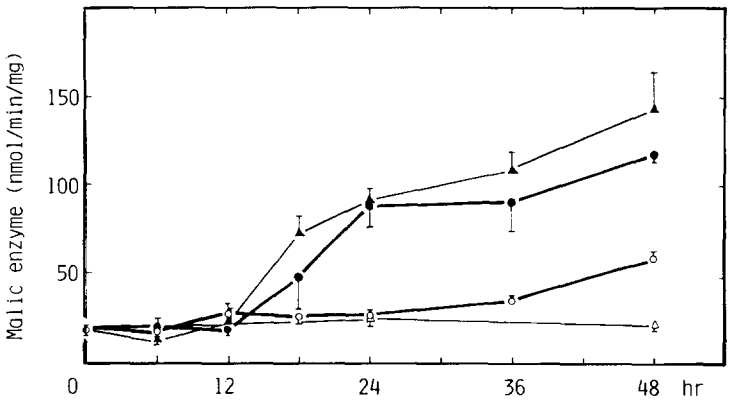

Fig. 1 The effects of fructose feeding and insulin treatment on changes in the activities of hepatic malic enzyme of diabetic rats. The diabetic rats adapted to a stock diet were given a high fructose diet (○,●) or a high starch diet (△,▲) at 7 p.m. (0 time) and simultaneously injected with Lente insulin (3.5 U/rat) in the insulin-treated groups (●,▲). Lente insulin (2.5 U/rat) was injected at 12, 24 and 36 hr after beginning the experimental diets. The animals were allowed to take diet and water ad libitum, and kept under an automatic lighting schedule from 7 a.m. to 7 p.m. Food intake was not significantly different from group to group. Each result represents mean ± S.E. of n=3 (nmol substrate utilized for malic enzyme per min at 37°C per mg protein in the 105,000 x g supernatant of liver homogenate).

the increase of mRNA activities with and without insulin treatment, respectively. In our previous experiment (Fukuda, N. and Iritani, N., submitted),

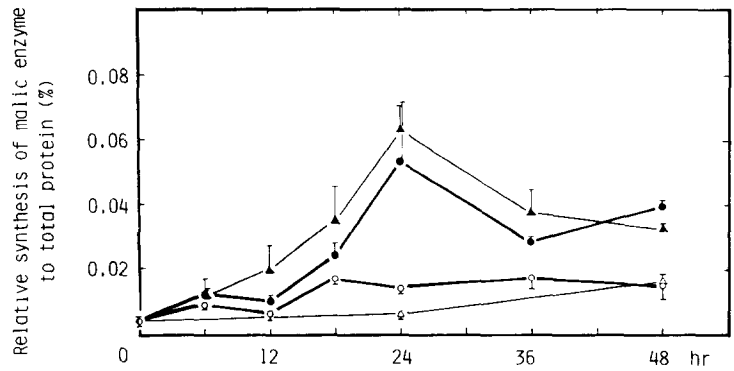

Fig. 2 The effects of fructose feeding and insulin treatment on changes in the activities of hepatic malic enzyme mRNA of diabetic rats. See the legend to Fig. 1 for animals. The diabetic rats were given a high fructose (○,●) or a high starch diet (△,▲) at 0 time. In the insulin-treated group (●,▲), Lente insulin was injected 3.5 U at 0 time, and 2.5 U at 12, 24 and 36 hr after giving the diet. Total RNA isolated from the rat livers was incubated with L-[4,5-^{3}H]leucine at 30°C for 60 min in a rabbit reticulocyte lysate system. The translation mixture contained 20 mM Hepes (pH 7.4), 20 μM each of 19 amino acids(all except leucine), 60 μCi of [^{3}H]leucine, 750 μg/ml of the total RNA and nuclease-treated reticulocyte lysate in total volume of 60 μl. The labelled translation products were subjected to indirect immunoprecipitation with antibody and separated by electrophoresis. Details are described in "Materials and Methods". Each results represents mean ± S.E. of n=3.

TABLE 1 THE EFFECT OF INSULIN INJECTION ON PLASMA INSULIN LEVELS OF DIABETIC RATS

Time after feeding the diets**	Fructose diet**		Starch diet**	
	Insulin-treated	Non-treated	Insulin-treated	Non-treated
hr				μU/ml
0*		(10.3 ± 3.57)***		
6	245 ± 65.9^{dh}	8.0 ± 1.87	192 ± 39.0^{d}	
12*	31.8 ± 13.0	17.0 ± 0.80	63.5 ± 2.50^{d}	
18	290 ± 63.6^{dh}	21.2 ± 5.91	200 ± 27.9^{d}	
24*	50.0 ± 9.17^{be}	18.3 ± 4.06	62.5 ± 11.9^{bf}	14.7 ± 3.21
36*	49.2 ± 19.2	18.2 ± 1.98	64.3 ± 14.2^{a}	
48	35.0 ± 3.47^{cei}	20.0 ± 3.56	64.5 ± 8.44^{cg}	10.8 ± 3.11

See the legend to Fig. 1 for details. *The diabetic rats were subcutaneously injected with insulin, 3.5 U/rat, at 0 time (7 p.m.), and 2.5 U/rat at 12, 24 and 36 hr after giving the experimental diets**. ***The diabetic rats were adapted to a stock diet before the experimental diets. Mean ± S.E. (n=3) Significantly different from 0 time, a: $P<0.05$, b: $P<0.02$, c: $P<0.01$, d: $P<0.001$; from non-treated, e: $P<0.05$, f: $P<0.02$, g: $P<0.01$, h: $P<0.001$; from starch diet, i: $P<0.05$

the enzyme activities show some increase 5 days after beginning the starch diet.

In the insulin-treated diabetic rats, the content of mRNA coding for malic enzyme was maximal 24 hr after insulin injection and reached the levels found for normal rats in our previous study (15). The plasma insulin levels were markedly increased 6 hr after the injection, and decreased after 12 hr (Table 1). However, the insulin levels of the diabetic animals injected with insulin every 12 hr were maintained at about the normal level (23) during the experiment. The half-life of malic enzyme is 2-4 days (24,25) and that of the mRNA is 10 hr (25) and it is possible that induction of malic enzyme cotinued after the decrease in the specific mRNA. The plasma insulin levels were higher in the fructose group than in the starch group 6 hr after the insulin injection, but this difference was reversed after 12 hr

Very recently, Spence and Pitot (26) showed that glucose addition to primary cultures of rat hepatocytes was without any effect on the level of malic enzyme; however, when insulin was present, glucose did increase the

levels of malic enzyme and other lipogenic enzymes. On the other hand, the addition of fructose or glycerol, even in the absence of insulin, increased the enzyme activities by 2-3-fold. The common metabolites of fructose and glycerol may induce some glycolytic and lipogenic enzymes by similar mechanisms in diabetic rats. Dietary fructose is known to have different metabolic effects from those of dietary glucose (27). Insulin has also been shown to induce glucokinase (28-30). In diabetic states, the level of fructokinase is not markedly reduced despite the low level of liver glucokinase, and fructose could be metabolized to yeild appreciable levels of glycolytic intermediates, which could be involved in the stimulation of the lipogenic enzymes. In any case, we have found that both fructose and insulin appear to induce malic enzyme primarily by increasing the activity of translatable mRNA. Similar results have been reported for L-type pyruvate kinase (10). It is reasonable to suppose that glycolytic and lipogenic pathways would be regulated coordinately in response to nutritional and hormonal stimuli.

REFERENCES

1. Volpe, J. J. and Vagelos, P. R. (1976) Physiol. Rev. 56, 333-417.
2. Winberry, L. and Holten, D. (1977) J. Biol. Chem. 252, 7796-7801.
3. Bailey, E., Taylor, C. B. and Bartley, W. (1968) Nature, 217, 471-472.
4. Lakshmanan, M. R., Nepokroeff, C. M. and porter, J. W. (1972) Proc. Natl. Acad. Sci., U. S. 69, 3516-3519.
5. Joshi, V. C. and Wakil, S. J. (1978) J. Biol. Chem. 253, 2120-2125.
6. Geelen, M. J. H., Harris, R. A., Beynen, A. C. and McCune, S. A. (1980) Diabetes 29, 1006-1022.
7. Pilkis, S. J. and Park, C. R. (1974) Ann. Rev. Pharmacol. 14, 365-388.
8. Tanaka, T., Harano, Y., Sue, F. and Morimura, H. (1967) J. Biochem. (Tokyo) 62, 71-91.
9. Nepokroeff, C. M., Lakshmanan, M. R., Ness, G. C., Muesing, R. A., Kleinsek, D. A. and Porter, J. W. (1974) Arch. Biochem. Biophys. 162, 340-344.
10. Noguchi, T., Inoue, H. and Tanaka, T. (1982) Eur. J. Biochem. 128, 583-588.
11. Mariash, C. N., Kaiser, F. E., Schwartz, H. L., Towle, H. C. and Oppenheimer, J. H. (1980) J. Clin. Invest. 65. 1126-1134.
12. Bar-On, H., Rohein, P. S. and Eder, H. A. (1976) Diabetes, 25, 509-515.
13. Miwa, I., Okuda, J., Maeda, K. and Okuda, G. (1972) Clin. Chim. Acta 37, 538-540.
14. Iritani, N., Fukuda, E. and Inoguchi, K. (1979) Atherosicerosis 34, 841-847.
15. Katsurada, A., Iritani, N., Fukuda, H., Noguchi, T. and Tanaka, T. (1982) Biochem. Biophys. Res. Commun. 109, 250-255.
16. Goding, J. W. (1978) J. Immunol. Methods 20, 241-253.
17. Chirgwin, J. M., Przybyla, A. E., McDonald, R. J. and Rutter, W. J. (1981) Biochem. 18, 5294-5299.
18. Pelham, H. R. B. and Hackson, R. L. (1976) Eur. J. Biochem. 67, 247-256.
19. Noguchi, T. and Tanaka, T. (1982) J. Biol. Chem. 257, 1110-1113.

20. Kessler, S. W. (1975) J. Immunol. 115, 1617-1624.
21. Olson, P. S., Thompson, E. B. and Granner, D. K. (1980) Biochem. 19, 1705-1711.
22. Laemmli, U. K. (1970) Nature, 227, 680-685.
23. Iritani, N., Fukuda, H., Tashiro, S. and Ikeda, Y. (1982) J. Biochem. (Tokyo) 92, 569-574.
24. Iritani, N., Fukuda, H. and Fukuda, E. (1981) Biochim. Biophys. Acta 665, 636-639.
25. Towle, H. C., Mariash, C. N., Schwartz, H. L. and Oppenheimer, J. H. (1981) Biochem. 20, 3486-3492.
26. Spence, J. T. and Pitot, H. C. (1982) Eur. J. Biochem. 128, 15-20.
27. Berghe, G. V. D. (1978) Curr. Top. Cell. Regul. 13, 97-135.
28. Sillero, A., Sillero, M. A. G. and Sols, A. (1969) Eur. J. Biochem. 10, 351-354.
29. Takeda, Y., Inoue, H., Honjo, K., Tanioke, H. and Daikuhara, Y. (1967) Biochim. Biophys. Acta 136, 214-222.
30. Gunn, J.M. and Taylor, C. B. (1973) Biochem. J. 136, 455-465.