SOME MECHANISMS OF THE DIFFERENT EFFECTS OF SUCROSE ON THE BLOOD AND LIVER LIPID LEVELS IN EXPERIMENTAL ANIMALS

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Experiments on rats showed that inclusion of sucrose in the composition of diets with a high or normal carbohydrate content accelerates synthesis of apoproteins of pre-β-lipoproteins in the liver and their loading with endogenously formed lipids and facilitates the more rapid secretion of very-low-density lipoproteins (VLDLP) into the blood stream and a raised blood triglyceride level. However, the rate of complex formation between lipids and protein of the pre-β-lipoproteins in the liver also depends on the relative proportions of the carbohydrate and lipid components in the diet. On a diet with sucrose in which the ratio between carbohydrates and fats is physiological (2 : 1), a lower level of lipid loading of the apoproteins of pre-β-lipoproteins is found, with a consequent increase in the lipid content in the liver.

KEY WORDS: very-low-density lipoproteins; triglycerides; specific radioactivity

Investigations have shown that replacement of starch by sucrose in diets with a high or normal carbohydrate content leads to acceleration of lipogenetic reactions in the liver [2, 3, 10] and to an increase in the blood concentration of endogenous triglycerides [7]. Diets with sucrose in which the physiological ratio between calories derived from carbohydrates and saturated fat is unchanged also lead to an increase in the lipid content in the liver [4, 8].

These facts suggest that changes in the ratio between the calorific values of carbohydrates and fat have different effects on lipoprotein metabolism in the liver and blood.

The object of this investigation was to study the rate of biosynthesis of very-low-density lipoproteins (VLDLP) in the liver and the intensity of their secretion into the blood stream and also to study the composition of this lipoprotein fraction in animals on diets with sucrose and with a normal or high carbohydrate content.

EXPERIMENTAL METHOD

Experiments were carried out on 90 male Wistar rats with an initial weight of 120-140 g, receiving diets of equal calorific value (60 kcal/100 g body weight) for 30 days ad lib. The diet of the animals of the control group consisted of casein, lard, and starch (18, 26, and 56% respectively of the total calorific value). The diet of the animals of experimental group 1 was the same as the control except that part of the starch was replaced by sucrose (40% of the total calorific value). In the diet of the rats of experimental group 2 the carbohydrate (starch with sucrose) content was increased up to 71% of the total calorific value at the expense of a decrease in the proportion of fat to 11%. In this case the sucrose content was also 40% of the total calorific value. All diets contained the necessary amounts of salts and vitamins.

At the end of the period of feeding the animals received a subcutaneous injection of [^{3}H]methionine and sodium [2-^{14}C]acetate (100 μCi of each). The rats were killed in a fasting state 1 h after injection of the isotope.

The total lipid concentration in the blood and liver was determined by Folch's method. VLDLP were isolated from blood serum by ultracentrifugation in a density gradient of KBr solutions [6] on the VAC-60 ultracentrifuge at 100,000g for 2 h. The blood serum was first freed from chylomicrons by centrifugation (26,000g for 30 min), weighted with anhydrous KBr to d = 1.22 g/ml, and introduced beneath a two-step gradient of KBr solution with densities d = 1.006 and 1.063 g/ml.

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TABLE 1. Total Lipid Levels in Blood and Liver, Composition of VLDLP in Blood, and SR of Protein and Lipid Components of That Lipoprotein Fraction in Blood and Liver (in cpm/mg) 1 h after Injection of [^{3}H]Methionine and Sodium [^{14}C]Acetate

Test object	Index	Group of animals		
		control	1	2
Blood	Total lipids, mg%	404±21	475±26*	492±28*
	VLDLP:			
	Protein	3,5±0,42	4,2±0,38	3,7±0,45
	Triglycerides	32,8±4,8	48±5*	64±7,5*
	Phospholipids	14,4±2,0	12±2,1	13±2,8
	Cholesterol	4,1±0,6	4,6±0,7	3,8±0,5
	SR of apoproteins	761	1905	1759
	SR of lipids	1238	1920	3970
Liver	Total lipids, mg/g	62±3,4	86±1,4*	60±3,7
	VLDLP:			
	SR of apoproteins	830	1520	1426
	SR of lipids	1226	2027	3458

*P < 0.05.

Pre-β-lipoproteins were isolated from the liver by preparative disk electrophoresis in polyacrylamide gel on an apparatus the principle of which was taken from Gusev and Yazova [1]. The working chamber of the apparatus was filled with four layers of gel: 10% with pH 8.9, 5 and 3% with pH 8.9, and 3% with pH 6.7. The liver was homogenized in ice-cold physiological saline and centrifuged at 7000 rpm. The supernatant was weighted with glucose solution and bromphenol blue dye was added. The liver samples were introduced under a layer of electrode buffer in the upper chamber. Electrophoresis was carried out with a current of 250 mA until the front of the dye had reached the lower border of the barrier layer. After electrophoresis control strips were cut out of the gel for staining for lipids, after which the zone corresponding to pre-β-lipoproteins was identified. These were extracted by electrophoresis. The protein content in the lipoproteins thus obtained was determined by Lowry's method. The lipid extract was fractionated by thin-layer chromatography, after which the total cholesterol concentration in the eluates was determined by the method of Liebermann and Burchard and glycerides and phospholipids were determined as esterified fatty acids [11].

Radioactivity of tritium and carbon in the lipid and protein components of the pre-β-lipoproteins was recorded on the Isocap-300 counter (Nuclear Chicago), using PCS (Amersham) solubilizer.

EXPERIMENTAL RESULTS AND DISCUSSION

In the rats of both experimental groups receiving sucrose with the diet an increase in the total lipid level in the blood was found. An increase in the lipid content in the liver was found only in the animals of experimental group 1 (Table 1). The triglyceride content in the blood VLDLP of the animals of experimental group 1 exceeded the control by 45%, but in the animals of group 2 the triglyceride concentration in VLDLP was increased by almost 100%. An increase in the specific radioactivity (SR) of apoproteins and lipids in the pre-β-lipoproteins of the liver was observed in the animals of both experimental groups; the maximal value of SR of the lipids was found in the rats of experimental group 2. Although the increase in SR of apoproteins of VLDLP was virtually equal in degree in the blood of the rats receiving the diets with sucrose, SR of the lipids showed an unequal increase in the animals of the two experimental groups: In the animals of experimental group 2 it was almost twice as high as in those of group 1.

It can be concluded from these results that the synthesis of apoproteins of pre-β-lipoproteins is accelerated in the liver of rats receiving sucrose in diets with a normal or high carbohydrate content.

The results of these experiments also are evidence that newly formed lipids actively form complexes with protein in the liver of the animals of the experimental groups and are secreted into the blood stream in the composition of the pre-β-lipoproteins in amounts greater than in the control. This is confirmed by the increase in SR of the protein and lipid components of VLDLP in the liver and blood, and also by the increase in the triglyceride concentration in the blood VLDLP.

These results are in agreement with the limited information in the literature on the effect of readily assimilated carbohydrates of the diet on the rate of formation of VLDLP in the liver and of its secretion into the blood stream [5, 9]. However, when sucrose is used in diets with different relative proportions of carbohydrates and saturated fat the degree of loading of the protein component of VLDLP in the liver with lipids

varies. In rats receiving a diet with a high proportion of carbohydrates and a correspondingly low quantity of fat, the rate of complex formation of lipids with protein is higher than in animals receiving a balanced diet. This could be the reason for the difference in the change produced by them in the lipid content in the blood and liver.

Inclusion of sucrose in diets with a high or normal carbohydrate content thus accelerates the synthesis of apoproteins of pre-β-lipoproteins in the liver and their loading with endogenous lipids and it leads to a more intensive secretion of VLDLP into the blood stream and an increase in the blood triglyceride level. However, the rate of formation of lipid–protein complexes of pre-β-lipoproteins in the liver also depends on the relative proportions of the carbohydrate and fat components in the diet. If the diet contains sucrose and the ratio between carbohydrates and fats is physiological (2 : 1), a lower level of lipid loading of the apoproteins of pre-β-lipoproteins is found, with a consequent increase in the lipid content in the liver.

LITERATURE CITED

1. A. I. Gusev and A. K. Yazova, Biokhimiya, No. 1, 172 (1970).
2. B. G. Lyapkov, Yu. M. Zalesskaya, and V. F. Markelova, Vopr. Pitaniya, No. 2, 38 (1974).
3. V. F. Markelova, "Effects of qualitatively different diets on lipid metabolism," Doctoral Dissertation, Moscow (1974).
4. V. F. Markelova and Yu. M. Zalesskaya, Byull. Éksp. Biol. Med., No. 5, 41 (1974).
5. R. P. Eaton and D. D. Kipnis, Am. J. Physiol., 217, 1153 (1969).
6. R. H. Hinton, Y. al-Tamer, A. Mallinson, et al., Clin. Chim. Acta, 44, 267 (1973).
7. N. A. Kaufman and H. Stein, Atherosclerosis, 11, 365 (1970).
8. O. E. Michaelis and B. Szepesi, J. Nutr., 104, 1597 (1974).
9. G. Schonfeld, J. Lab. Clin. Med., 75, 206 (1970).
10. D. Zakim, R. S. Pardini, H. H. Herman, et al., Biochim. Biophys. Acta, 144, 242 (1967).
11. J. Verheyden and J. Nys, Clin. Chim. Acta, 7, 262 (1962).

ACTIVITY OF ENZYMES OF GLUCOSE-6-PHOSPHATE METABOLISM IN THE LIVER OF RATS WITH EXPERIMENTAL VALEXON POISONING

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Activity of hexokinase, glucose-6-phosphatase, and glucose-6-phosphate dehydrogenase in the liver of rats was studied after a single peroral dose of the organophosphorus insecticide Valexon. Administration of the compound caused increased activity in the homogenate and solubilization of glucose-6-phosphatase, activation of glucose-6-phosphate dehydrogenase, and inhibition of hexokinase. The changes were maximal 1 h after administration. It is postulated that the reduction in the intensity of formation and conversions of glucose-6-phosphate is a pathogenetic factor in the development of Valexon poisoning.

KEY WORDS: hexokinase; glucose-6-phosphatase; glucose-6-phosphate dehydrogenase; organophosphorus insecticide Valexon; rat liver

An important role in the formation and conversions of glucose-6-phosphate is played by the activity of hexokinase, glucose-6-phosphatase (G6Pase), and glucose-6-phosphate dehydrogenase (G6PD). Data are given in the literature on the effect of various chemicals containing chlorine, used as insecticides, on G6Pase and G6PD activity [1-3]. However, insecticides belonging to different classes of chemical compounds, notably the

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