STIMULATION OF RAT HEART LIPOPROTEIN LIPASE ACTIVITY BY 4-AMINOPYRAZOLO[3,4-d]PYRIMIDINE-INDUCED REDUCTION OF PLASMA TRIACYLGLYCEROL

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Received 19 February 1979

1. Introduction

Hormonal and nutritional regulation of lipoprotein lipase (LPL) activity of heart has been studied in the intact animal [1,2] and in isolated heart cells maintained in tissue culture [3]. Recently, cellular LPL activity was shown to decrease when the culture medium had been supplemented with 0.07 mg/ml of very low density lipoproteins (VLDL) triacylglycerol for 3 h [4]. Since the highest levels of enzyme activity were seen in cells grown in a medium with a very low triacylglycerol content, it seemed of interest to determine if elimination of plasma VLDL in the intact rat will result in an increase of heart LPL. This can be easily achieved by injection of 4-aminopyrazolo-[3,4-d]pyrimidine (4-APP), which has been shown to reduce markedly plasma lipoprotein levels [5]. The results of the present experiments were expected to elucidate the possible role of the substrate in the regulation of heart LPL activity in vivo.

2. Materials and methods

Male albino rats (200 g body wt) of the Hebrew University strain, kept in temperature- and light-controlled rooms were used. The rats had free access to food until 12:00 h, when they were injected intraperitoneally with 4-APP (50 mg/kg), in 10 mM phosphate buffer (pH 3.0) [6] or with buffer alone. Thereafter the rats were kept in wire-bottom cages and had access to water only. VLDL was isolated from plasma of sucrose-fed rats as in [7]. Chylomicrons were obtained from chylous pleural effusion and isolated and washed twice by centrifugation at 15°C for 30 min at 10 000 rev./min. Lipoprotein lipase activity of heart [8] and triglyceride hydrolase activity of liver [9] was determined on fresh tissue homogenates and enzyme activity of epididymal fat pads was carried out on acetone powders as in [10]. Serum triacylglycerol (TG) was determined as in [11]. In 4-APP injected rats 2.0 ml serum had been lyophilized and redissolved in 0.5 ml water prior to TG determination. Serum insulin was determined by a modified radioimmunoassay [12], and serum 11-hydroxycorticoids were determined by a fluorimetric method [13]. 4-APP was obtained from Aldrich Chem. Co., Milwaukee, WI.

3. Results

To study the relation between plasma TG level and LPL activity of heart, rats were treated with 4-APP and the enzyme activity was compared to that of control rats. As seen in table 1, 4-APP treatment for 22 h resulted in a marked fall in plasma TG level and a rise in the LPL activity of heart. A similar increase in heart LPL activity occurred also in non-fasted, 4-APP treated rats (data not shown). The 20–22 h interval after 4-APP treatment was used in all experiments, since no change in heart LPL activity was observed after 3 h and 6 h treatment. In order to determine whether the 4-APP effect on heart LPL

Table 1
Effect of VLDL and chylomicrons on lipoprotein lipase activity of heart in 4-APP-treated rats

Treatment	Lipoprotein lipase act.	Plasma	
	fatty acid released (μmol/g wet wt/h)	Glucose (mg/dl)	TG (mg/dl)
Control	98.2 ± 1.9 (18)	101	39
4-APP	170.8 ± 1.7 (20)	99	8
4-APP + VLDL (5 mg/h)	102.5 ± 1.8 (3)	–	35
4-APP + VLDL (15 mg)	103.3 ± 1.2 (12)	101	19
4-APP + chylomicrons (20 mg)	120.6 ± 1.3 (4)	97	8
4-APP + chylomicrons (2 × 20 mg)	119.3 ± 0.8 (3)	103	13

4-Aminopyrazolopyrimidine (4-APP), 50 mg/kg, was injected intraperitoneally. The rats were deprived of food and were kept individually in wire bottom cages. VLDL or chylomicrons were injected i.v. 19 h after 4-APP and all rats were killed 22 h after 4-APP. VLDL was either infused for 3 h at 5 mg triacylglycerol/h, or injected as a single dose. Chylomicrons were given as 1 or 2 injections, 90 min apart. Values are means ± SE or means. Numbers in parentheses represent no. rats

can be modulated by plasma VLDL or chylomicrons, various amounts of these lipoproteins were injected or infused and enzyme activity was determined 3 h thereafter. Injection of 15 mg VLDL-TG given as a single bolus or infused for 3 h at 5 mg/h resulted in a decrease in heart LPL activity to almost control values (table 1). Injection of chylomicrons resulted also in a decrease in heart LPL activity, but the effect was less pronounced. This could have been due to the more rapid clearance of chylomicrons from the circulation. An attempt was made to obtain a more sustained elevation in plasma TG level by intragastric administration of oil. However, only a small reduction in heart LPL activity occurred 3 h after oil feeding, probably due to impaired intestinal absorption of fat [14].

Plasma levels of glucose were determined in all groups of animals and ranged between 97–103 mg/dl. Serum insulin and 11-hydroxycorticoid levels after 4-APP treatment were not different from control values. The comparison of the effect of 4-APP treatment on LPL in the heart and adipose tissue and on hepatic TG hydrolase activities is presented in table 2. At the time when LPL activity in the heart increased by 70%, the enzyme activity in adipose tissue decreased by 60%, and the hepatic TG hydrolase by 40% (table 2).

4. Discussion

Lipoprotein lipase activity in the intact rat has been usually correlated with plasma levels of glucose, insulin or corticosteroid hormones [1,2]. In studies with cultured heart cells, addition of VLDL to the medium resulted in a fall in LPL activity which was very pronounced after 3 h incubation [4]. This fall suggested that a product of interaction between

Table 2
Effect of 4-APP on lipoprotein lipase of heart and adipose tissue and triacylglycerol hydrolase of liver

Enzyme source	Enzyme activity	
	Control	4-APP
Heart	97 ± 5.1 (n = 5)	167 ± 4.1 (n = 5)
Fat pad	165 ± 11.5 (n = 7)	63 ± 2.3 (n = 5)
Liver	80 ± 3.3 (n = 10)	50 ± 1.3 (n = 10)

Conditions as in table 1. All rats were deprived of food after the injection of 4-APP. Enzyme activity was determined on fresh homogenates of heart and liver and is expressed as fatty acid released, μmol/g wet wt/h. The enzyme activity of adipose tissue was determined on acetone powder and is expressed per g acetone powder. Values are means ± SE. Numbers in parentheses represent no. rats

VLDL and LPL depresses the synthesis of the enzyme. The present experiments were designed to test whether extreme reduction of plasma TG level will stimulate heart LPL activity. Such a reduction of plasma TG level in fasted rats was achieved by treatment with 4-APP which affects hepatic release of secretory proteins [5] and lecithin:cholesterol acyltransferase [15], without depressing total hepatic protein synthesis [5]. The increase of LPL activity 20–22 h after 4-APP was encountered only in the heart, while enzyme activity decreased in adipose tissue and liver. This fall in the LPL activity of adipose tissue and in TG hydrolase of liver could explain the reported reduction in lipolytic activity of postheparin plasma in 4-APP-treated rats [16]. In the 4-APP-treated rat when plasma TG was barely measurable, there was no change in plasma glucose, insulin and 11-hydrocorticoid levels and no change in plasma free fatty acid levels had been reported [16].

These findings of a rise in heart LPL activity concomitant with a marked reduction of plasma TG and the reversibility of this increase by injection of VLDL suggests that the heart enzyme might be regulated by its substrate, i.e., plasma triacylglycerol.

In the fasted rat of 200 g body wt, the rate of VLDL release by the liver has been estimated to range between 10–20 mg TG/h [17]. Thus the amount of VLDL injected presently into the 4-APP-treated rats was equivalent to ~50–25% of the hepatic release of VLDL in the non-treated rat, indicating that even the low values observed in the fasted state are sufficient to keep the heart LPL activity at the physiological low values.

The rate of clearance of plasma TG by the heart is determined largely by the concentration of the enzyme at the endothelial surface of heart capillaries and is maximal even at low triglyceride concentration, owing to its low Michaelis-Menton constant (K_m value) of 0.07 mM TG [18]. In the 4-APP treated rats, the plasma TG concentration was near the app. K_m value. Thus it seems that since the heart is largely dependent on availability of free fatty acids for energy production, the extreme fall in plasma TG provides a signal for new enzyme synthesis.

Acknowledgements

The excellent assistance of Mrs E. Bardach, Mr G. Hollander and Mrs Y. Dabach is gratefully acknowledged. Dr O. Stein and Dr Y. Stein are Established Investigators of the Ministry of Health. This investigation was supported in part by a grant from the United States-Israel Binational Science Foundation and from the Delegation Generale a la Recherche Scientifique et Technique of the French Government.

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