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EFFECTS OF ETHYL-CPIB (CLOFIBRATE) ON TISSUE LIPOPROTEIN LIPASE AND PLASMA POST-HEPARIN LIPOLYTIC ACTIVITY IN RATS

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Abstract—The role of LPL in reducing the serum triacylglycerol concentration was investigated in rats fed a high sucrose diet containing 0.25% (w/w) ethyl-CPIB. Compared with sucrose-fed controls, drug treatment resulted in a fall in adipose tissue LPL activity and a rise in enzyme activity in thigh and heart muscle. Serum post-heparin lipoprotein lipase activity after a high dose of heparin was lower in ethyl-CPIB-treated rats than controls, but after a low dose of heparin the values were similar. The amount of LPL activator was decreased by the drug. Thus, the low serum triacylglycerol concentration observed in the ethyl-CPIB-treated rats cannot be explained by changes in functional LPL activity. The plasma triacylglycerol-lowering effect of the drug could be explained by the observed decrease in triacylglyerol output by the liver.

Treatment with ethyl-CPIB (clofibrate)† is known to lower an elevated serum triacylglycerol concentration [1] but the mechanism by which the drug acts is still under debate. Earlier workers have found that ethyl-CPIB decreases triacylglycerol synthesis in the liver [2, 3], decreases triacylglycerol secretion into the circulation in rats [4] but not in man [5], and improves the clearance of triacylglycerol from the blood as evidenced by increased removal rate of Intralipid [3, 5] and increased tissue LPL (EC 3.1.1. 34) activity [6, 7]. However, the results for the effect of ethyl-CPIB on tissue LPL activity have been variable [3] and there are few reports available on the effects of the drug on the activity of *functional* LPL [3].

It is possible that the drug could also alter the rate of removal of serum triacylglycerol as a result of changes in the serum concentration of the LPL activator which is required for the expression of enzyme activity [8]. This aspect is investigated here.

The present investigations were carried out to look at in more detail in rats the effect of ethyl-CPIB on the activity of LPL under the condition of elevated plasma triacylglycerol concentration produced by high sucrose feeding. Enzyme activity was measured in individual tissues and in post-heparin serum which provides a measure of total body functional LPL. The effect of ethyl-CPIB on the influx of triacylglycerol into the blood and the removal of triacylglycerol from the blood was also investigated.

MATERIALS AND METHODS

Experimental protocol

Male Porton Wistar rats, weighing 250–300g, were used throughout the study. Rats were caged in groups of four or six and given food and water *ad libitum*. The high sucrose diet, on a percentage (w/w) basis, consisted of 70% sucrose, 16% casein, 4% salt mix [9], 2% vitamin mix (BP R&M No. 1. Maintenance Pre-mix, BP Nutrition (U.K.) Ltd., Witham, Essex), 3% starch and 5% gelatine. Rats received the high sucrose diet for 4 days after which one group received the high sucrose diet containing 0.25% ethyl-CPIB (w/w) for another 4 days [1]. The control groups continued to receive the high sucrose diet without the drug for the same period. (The values are similar at 4 and 8 days of sucrose feeding for both the serum triacylglycerol concentration and adipose tissue LPL activity [10].) At the end of the feeding period the rats were anaesthetized lightly under diethyl ether before sacrifice. Starved rats were deprived of food for 24 hr before sacrifice.

Measurements

Lipoproteins were separated from serum samples by the method of Havel *et al.* [11] into VLDL ($d < 1.006$), LDL ($d = 1.006$–1.063) and HDL ($d = 1.063$–1.21). Epididymal and subcutaneous adipose tissue and thigh muscle were pooled for each group of rats and acetone–ether powders prepared according to the method of Ashby *et al.* [12]. The powders were stored, where necessary, at −20°C and assayed for LPL within 7 days. Adipose tissue powders were homogenized in 0.25 M NH_3/NH_4Cl buffer pH 8.1, and supernatants prepared by centrifugation (2500 *g* for 20 min at 4°). Where whole homogenates were assayed, heparin was added to the homogenizing buffer (final concentration 0.5 I.U./ml). Fresh hearts were homogenized in 0.25 M NH_3/NH_4Cl buffer containing 0.5 I.U./ml heparin (1g of heart to 20 ml of buffer) and aliquots of the homogenate immediately used for LPL assay according to the method of Riley and Robinson [13]. In this assay maximal enzyme

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† Abbreviations: ethyl-CPIB, ethyl-*P*-chlorophenoxyisobutyrate; HDL, high density lipoprotein; LDL, low density lipoprotein; LPL, lipoprotein lipase; PHLA, post-heparin lipolytic activity; VLDL, very low density lipoprotein.

activity is measured as activator is present in excess.

In the studies of serum PHLA rats were given an intravenous injection of heparin — either a high dose (500 I.U./kg) or a low dose 5 I.U./kg) — in the tail vein and then bled from the dorsal aorta after 10 or 2.5 min, respectively.

LPL activator was measured as the ability of the sample to activate an artificial triacylglycerol suspension (Intralipid Vitrum, Stockholm) as substrate, so that it could be hydrolysed by lipoprotein lipase. The assay was performed as described previously [14] except that the source of enzyme was rat adipose tissue. Measurement of influx of triacylglycerol into the blood from the liver was carried out using the procedure of injection of 10% Triton WR1339 [15]. Triton decreases the removal of serum triacylglycerol from the blood by inhibiting LPL activity or altering the surface properties of the lipoproteins (see [16]). The rate of triacylglycerol influx from the liver is calculated from the increase in plasma triacylglycerol concentration between zero time and 90 min after the injection of 10% Triton WR1339. The plasma volume was measured by injection of Evans Blue dye [16].

1-[^{14}C]palmitate–VLDL triacylglycerol was prepared by injecting 20μCi of the potassium salt of 1-[^{14}C]palmitic acid into each of six sucrose-fed rats. The rats were bled after 45 min and the serum samples obtained were pooled and the VLDL isolated as above. Only 1.7% of the total radioactivity in the VLDL prepared was found in free fatty acid, as measured according to Kelly [17], and not incorporated into triacylglycerol. The VLDL was used for injection, at a dose of 4 mg of triacylglycerol/kg, within 24 hr. Blood samples (0.4 ml) were taken from the tail vein at intervals over a period of 15 min and radioactivity was determined in 0.1 ml of serum samples added to 10 ml of scintillant (NE260, Beckman Inc.).

Serum triacylglycerol was determined by the method of Fletcher [18] and cholesterol according to Zlatkis *et al.* [19]. Protein was determined by the method of Lowry *et al.* [20] and insulin by that of Soeldner and Slone [21].

Ethyl-CPIB was a gift of I.C.I. Ltd., Macclesfield, U.K. Sources of all other materials were BDH Chemicals Ltd., Poole, U.K., or Sigma (London) Chemical Co., Dorset, U.K..

The significance of the differences between the results for the animal groups was estimated by using Behren's modification of Student's *t*-test [22].

RESULTS

LPL activity in tissues

The effect of ethyl-CPIB treatment on the activity of LPL in adipose tissue, thigh and heart muscle is shown in Table 1. Comparing the effect of the drug with values for fed animals, ethyl-CPIB caused a 50% drop in the total adipose tissue LPL activity measured in acetone–ether powder homogenates. Enzyme activity in supernatants from the homogenates prepared in the absence of heparin was also decreased by drug treatment. In contrast, LPL activity in heart and thigh muscle was higher in ethyl-CPIB-treated rats than control fed rats. The drug treatment resulted in a 70% increase in heart lipoprotein lipase activity.

Although rats fed a high sucrose diet containing 0.25% of ethyl-CPIB consumed as much food as control rats fed the high sucrose diet without the drug (28 ± 4 and 29 ± 2g/day respectively), animals treated with the drug gained less weight compared to the control fed rats. Mean weight gains for the 4 days of drug treatment were 14 ± 14 g for ethyl-CPIB-treated rats and 37 ± 10 g for control fed animals. For this reason 24 hr starved rats were also included in the study as a further control.

In agreement with other workers (see [23]) starvation produced lower adipose tissue LPL activity and higher heart and skeletal muscle enzyme activity when compared to control fed animals. In comparing enzyme activity in ethyl-CPIB-treated and starved

Table 1. Effects of ethyl-CPIB treatment on LPL activity of adipose tissue, heart and skeletal muscle and on serum insulin concentration

	Lipoprotein lipase activity (μmol free fatty acid/hr/g)		
	Control fed	Starved	Ethyl-CPIB-treated
Adipose tissue			
Supernatants from homogenates	14 ± 6 (6)	7 ± 4 (4)	6 ± 4 (6)
Homogenates	63	30	31
(+ heparin)	75	44	23
Heart	39 ± 15 (3)	89 ± 4 (3)	66 ± 12 (3)
Thigh muscle	15	34	38
Serum insulin (mU/l)	219 ± 34 (4)	11 ± 4 (3)	36 ± 24 (4)

LPL activity was determined in pooled samples from groups of 4–6 rats. Insulin was measured in pooled serum samples from the same groups. Mean values ± S.D. are given for the numbers of pooled samples shown in parentheses.

Table 2. Effect of ethyl-CPIB treatment on lipolytic activity in serum after injection of a high dose of heparin (500 I.U./kg) and a low dose of heparin (5 I.U./kg)

	Total post-heparin lipolytic activity (μmol free fatty acid/hr/ml)	Lipoprotein lipase activity (μmol free fatty acid/hr/ml)
After 500 I.U. of heparin/kg		
Experiment I		
Control fed	63 ± 8 (5)	
Ethyl-CPIB-treated	38 ± 4 (7)	
Experiment II		
Control fed	104 ± 4 (4)	88 ± 21 (4)
Starved	75 ± 14 (10)	60 ± 16 (10)
Ethyl-CPIB-treated	75 ± 12 (8)	55 ± 12 (8)
After 5 I.U. of heparin/kg		
Experiment I		
Control fed	2.08 ± 1.14 (4)	1.69 ± 0.93 (4)
Starved	9.43 ± 1.43 (4)	8.20 ± 1.27 (4)†
Ethyl-CPIB-treated	1.73 ± 1.35 (4)	1.49 ± 1.16 (4)†
Experiment II		
Control fed	5.50 ± 2.74 (4)	4.89 ± 2.44 (4)
Starved	9.45 ± 1.67 (4)	8.89 ± 1.57 (4)*
Ethyl-CPIB-treated	5.58 ± 1.28 (4)	4.59 ± 1.35 (4)*

* $P < 0.02$.
† $P < 0.001$.
Total serum lipolytic activity and lipolytic activity in the presence of sodium chloride (1M final concentration) were measured and LPL activity calculated. Values are given as means ± S.D. for the numbers of animals shown in parentheses.

rats, it is seen that the activity in the heart is greater in the starved rats while for thigh muscle the activities are the same.

It has been reported that in a variety of nutritional states muscle LPL activity is inversely correlated with the activity of the enzyme in adipose tissue and also with plasma insulin concentration [24]. Consequently, insulin concentration was measured in the present investigations. The results (Table 1) show a drop in serum insulin concentration in ethyl-CPIB-treated and starved rats compared with control fed animals.

LPL activity in post-heparin serum

The functional LPL activity is usually estimated in intact animals by the lipolytic activity in serum after injection of heparin (PHLA) [23]. However, the amount of enzyme released into the serum depends on the dose of heparin injected [25]. Therefore PHLA was measured at two different doses of

Table 3. Effects of ethyl-CPIB treatment on serum lipids and serum lipoprotein concentrations

	Control fed	Starved	Ethyl-CPIB-treated
Triacylglycerol (mM)			
Serum	4.14 ± 0.77 (12)†	1.05 ± 0.27 (8)	0.80 ± 0.20 (10)†
VLDL	3.49 ± 0.60 (4)	0.90 ± 0.30 (3)	0.58 ± 0.17 (4)
Cholesterol (mg/100ml)			
Serum	83 ± 11 (3)	71 ± 10 (3)	39 ± 7 (3)
VLDL	31 ± 4 (3)	18 ± 3 (3)	8 ± 5 (3)
LDL	11 ± 5 (3)	8 ± 2 (3)	9 ± 4 (3)
HDL	36 ± 10 (3)	31 ± 3 (3)	16 ± 10 (3)
Protein (mg/100ml)			
VLDL	0.56 ± 0.11 (4)	0.36 ± 0.09 (3)	0.35 ± 0.12 (4)
LDL	0.12 ± 0.15 (4)	0.11 ± 0.04 (3)	0.13 ± 0.08 (4)
HDL	1.09 ± 0.25 (4)*	1.23 ± 0.17 (4)	0.70 ± 0.17 (4)*

* $P < 0.02$.
† $P < 0.001$.
Cholesterol, protein and VLDL triacylglycerol values are for pooled serum samples from groups of four or six rats. The results are means ± S.D. for the numbers of groups shown in parentheses. Values for serum triacylglycerol are for individual samples and the results are means ± S.D. for the numbers of samples shown in parentheses.

Table 4. Effects of ethyl-CPIB treatment on serum lipoprotein lipase activator concentration

	Lipoprotein lipase activator (μl 2.5mM alkali/10μl serum sample)		
	Control fed	Starved	Ethyl-CPIB-treated
Serum	37 ± 5 (12)*	27 ± 5 (8)	16 ± 4 (12)*
VLDL	10 ± 3 (9)	5 ± 2 (8)	4 ± 2 (12)
Serum-VLDL	31 ± 6 (9)	22 ± 5 (8)	13 ± 3 (12)

* $P < 0.001$.

VLDL was isolated from serum obtained from groups of rats treated as described in the Materials and Methods section. Activator was measured in the VLDL, serum minus VLDL (serum-VLDL) fractions and serum samples. The results are means ± S.D. for the numbers of rats shown in parentheses.

heparin, a high dose and a low dose. As post-heparin serum also contains a hepatic lipase which contributes to the lipolytic activity of the serum, LPL contribution to total serum PHLA was calculated from measurements of the lipolytic rate observed in the absence and presence of 1M sodium chloride which inhibits LPL.

The results (Table 2) show that serum LPL activity measured after a high dose of heparin was lower by approximately 35% in ethyl-CPIB-treated and starved rats compared to control fed animals. However, after injection of a low dose of heparin the pattern was different. Under these conditions, the PHLA was now similar between ethyl-CPIB and normal fed animals but was lower than that found in starved animals.

Serum lipid and lipoproteins

Ethyl-CPIB treatment resulted in a fall in serum triacylglycerol and serum cholesterol concentrations (Table 3). There were decreases in both cholesterol and protein in serum VLDL and serum HDL fractions in drug-treated rats compared to control fed rats. Serum LDL protein and cholesterol concentrations were, however, unaffected by the drug treatment. HDL protein and cholesterol concentrations in the serum were lower in ethyl-CPIB-treated rats than starved animals.

Drug treatment resulted in marked alterations in the serum LPL activator concentration. Total serum activator concentration fell by 57% in ethyl-CPIB-treated rats compared to control fed animals (Table 4). This drop was accounted for by decreases in activator in both VLDL and serum-VLDL fractions. The serum LPL activator concentration was higher in starved rats than drug-treated animals. Ethyl-CPIB treatment, therefore, produced alterations in serum lipoprotein concentrations that were independent of starvation.

Triacylglycerol output by the liver

The rate of influx of triacylglycerol into the blood from the liver was determined by the increase in serum triacylglycerol concentration after injection of Triton WR 1339 (see Materials and Methods).

The plasma volume was similar for ethyl-CPIB-treated and control fed groups (11.2 ± 1.6 ml, 11.3 ± 1.1 ml respectively, six animals in each group). The rate of influx of triacylglycerol into the blood was reduced by 60% on drug treatment from 0.92 ± 0.22 μmol/min in the fed controls to 0.36 ± 0.09 μmol/min in the ethyl-CPIB-treated animals.

Triacylglycerol removal from the blood

In order to have a more complete knowledge of the effect of ethyl-CPIB on triacylglycerol metabolism, the clearance of VLDL triacylglycerol from the plasma was measured.

The removal of 1-[^{14}C]palmitate-labelled VLDL injected at a dose of 4 mg triacylglycerol/kg followed a first-order reaction as the logarithm of plasma radioactivity against time was linear. The fractional removal rate K_2 was calculated from the slope k according to the formula $K_2 = 2.3k \times 100$. The rate of removal was higher in the starved controls than in the fed groups (Table 5). K_2 for the ehtyl-CPIB-treated groups was similar to that for the starved animals.

DISCUSSION

In view of the lower rate of weight gains in the ethyl-CPIB-treated rats compared to control fed animals the question arises as to whether the effects of ethyl-CPIB-treatment are due to nutritional factors. The findings indicate that although ethyl-CPIB-treat-

Table 5. The effect of ethyl-CPIB on VLDL triacylglycerol removal from the blood

	Treatment	K_2 (%/min)
VLDL	Starved	9.5 ± 3.5 (8)
	Fed	4.7 ± 2.7 (7)*
	Ethyl-CPIB	10.0 ± 2.1 (6)*

* $P < 0.01$.

Rats were given an intravenous injection of 1-[^{14}C]palmitate-labelled VLDL. Values are given as means ± S.D. for the numbers of observations shown in parentheses.

ment and starvation produced lower serum triacylglycerol and lower serum VLDL protein concentrations than control fed animals, they differed greatly in other parameters as evidenced by the differences in the serum concentrations of HDL and the LPL activator. Therefore, the metabolic changes in the ethyl-CPIB-treated rats when compared to control fed animals are likely to be due to the effects of the drug rather than nutritional factors.

The findings in the present studies indicating a reduction in adipose tissue LPL activity by ethyl-CPIB treatment contrast with those of Tolman *et al.* [6] who reported a non-significant increase in adipose tissue LPL activity in high sucrose fed ethyl-CPIB-treated animals compared to control high sucrose fed rats.

Our findings of an increase in muscle LPL activity are in agreement with those of Lithell *et al.* in man [7] and the often observed inverse relationship between adipose tissue and muscle LPL activity [24]. The results also confirm the inverse relationship between serum insulin concentration and muscle LPL activity.

The present findings show no evidence of an increased functional lipoprotein lipase activity measured after either high or low doses of heparin in the intact ethyl-CPIB-treated rats compared to control fed animals. The results confirm the findings of Salaman and Robinson [25] that the response of serum PHLA to low and high doses of heparin are different. Thus, while serum PHLA measured at low doses of heparin was similar in ethyl-CPIB-treated and control fed animals (the values being lower than those in starved rats) the activity at high doses of heparin was higher in the control fed animals. It is likely that the two doses of heparin release dissimilar pools of LPL whose activity may be differentially affected by the various treatments. The results raise the question as to what dose of heparin releases the truly functional enzyme.

The question has been raised by Verschoor *et al.* [26] as to whether quantitative variations in adipose tissue LPL activity as directly measured give rise to useful insights into the kinetics of VLDL-triacylglycerol. Our results indicate that (i) as is already appreciated by many workers, the measurement of LPL activity in one kind of tissue alone is not sufficient because of the inverse alterations in adipose tissue and muscle; (ii) the measurement of PHLA activity may also not give a clear cut answer to the involvement of LPL as a determinant of the triacylglycerol concentration because of the different pattern of activities observed with different doses of heparin.

In the experiments involving VLDL removal, the fed rats, which would have the highest triacylglycerol concentrations of the three groups, showed the slowest rate of removal. On the other hand, the starved and ethyl-CPIB-treated groups, with similar low plasma triacylglycerol concentrations, gave higher K_2 values than the fed group. A number of studies in humans have shown an inverse relationship between K_2 and the serum triacylglycerol concentration [27, 28]. This could explain the above results and the apparent discrepancy between the results of the PHLA and Intralipid removal studies carried out by D'Costa *et al.* [3].

As regards other factors that might account for the observed lowering of the serum triacylglycerol concentration, since ethyl-CPIB treatment reduced rather than increased the serum concentration of the LPL activator in the present studies, the changes in the serum activator concentration cannot explain the lower plasma triacylglycerol concentration in ethyl-CPIB-treated rats. However, our results support the view that a decreased triacylglycerol output by the liver could account for the reduced serum triacylglycerol concentration in ethyl-CPIB-treated rats when compared to control fed animals.

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