The degradation of platelet-activating factor by high-density lipoprotein in rat plasma

Effect of ethynyloestradiol administration

P. Haydn PRITCHARD

Shaughnessy Hospital Lipid Research Group, Department of Pathology, University of British Columbia, Vancouver, B.C. V5Z 4H4, Canada

The degradation of platelet-activating factor (PAF; 1-*O*-alkyl-2-acetyl-*sn*-glycero-3-phosphocholine) in human plasma is brought about by a specific acetylhydrolase that is associated with low-density lipoprotein. In this study, the presence of a similar activity on high-density lipoprotein (HDL) of rat plasma has been demonstrated which is independent of lecithin-cholesterol acyltransferase (LCAT) activity. Furthermore, PAF acetylhydrolase activity may be decreased by 66% by treatment of rats with 17 α -ethynyloestradiol (1 mg/kg body wt.; 5 days). This treatment also decreased HDL cholesterol concentrations by 90%, but did not affect LCAT activity. Thus rat LCAT and PAF acetylhydrolase are associated with different subspecies of HDL.

INTRODUCTION

Exogenously administered PAF is rapidly degraded *in vivo*. This may be due to cellular catabolism [1], but a large proportion may be degraded in the plasma [2,3]. In fact, PAF activity has been reported to be decreased by 90% after incubation for 30 min with plasma [2]. Blank *et al.* [3] reported the presence in plasma of an acetylhydrolase activity that can catabolize PAF. This activity was shown to be increased in spontaneously hypertensive rats [3], suggesting that this enzyme may play an important physiological role in the regulation of circulating concentrations of PAF and, consequently, in the maintenance of blood pressure. Farr *et al.* [4] described a plasma acid-labile factor ('ALF') from human serum that is associated with the plasma lipoproteins. Subsequently, they suggested that the acid-labile factor is a specific phospholipase A activity associated with LDL [5]. During the preparation of the present paper, Stafforini *et al.* [6] have reported the complete purification of the human PAF acetylhydrolase. It appears to be specific for the *sn*-2 acetyl residue and to have an M_r of 43 000. We have confirmed the presence of such an enzyme activity in human LDL [7]. As rat plasma contains relatively small amounts of LDL [8], yet still retains a marked capacity for PAF degradation, we have carried out a series of experiments designed to characterize further the degradation of PAF in the rat.

In this paper, the activity that degrades PAF in rat plasma has been shown to be completely associated with HDL. In addition, as the major lipid-metabolizing enzyme associated with this lipoprotein is LCAT, the possibility that PAF degradation is brought about by the phospholipase A₂ component of this activity has been considered. It is concluded that the regulation of the plasma concentrations of this biologically active phospholipid may be a novel role for the rat HDL *in vivo*.

MATERIALS AND METHODS

Materials

Egg-yolk phosphatidylcholine, cholesterol and 17 α -ethynyloestradiol were purchased from Sigma. [7-³H]-Cholesterol was from ICN, and Bio-Gel A5.0 m was purchased from Bio-Rad.

Treatment of animals

17 α -Ethynyloestradiol (25 mg) was dissolved in ethanol and made up to 5 mg/ml with dimethyl sulphoxide. Samples (1 mg) were given to male Wistar rats (250 g) by daily intraperitoneal injections. Control rats received only 200 μ l of 20% (v/v) ethanol in dimethyl sulphoxide. After 5 days, the rats were anaesthetized with Nembutal, and 6–10 ml of blood was drawn from the abdominal inferior vena cava. EDTA was used as an anticoagulant. Plasma samples were separated from the blood cells by centrifugation for 15 min at 3000 *g* at 4 °C.

Lipid and lipoprotein analysis

Lipids in plasma and lipoprotein fractions were quantified by established enzymic methods for cholesterol, total and non-esterified cholesterol [9] and triacylglycerol [10]. HDL cholesterol was measured in plasma after manganese/heparin precipitation [11] of apoprotein-B-containing lipoproteins. Total lipid P was determined by a modification of the method of Bartlett [12]. HDL was isolated by preparative ultracentrifugation at a density of 1.063–1.21 g/ml [13].

Column chromatography

Samples (2 ml) of plasma from control or ethynyloestradiol-treated rats were incubated for 16 h at 4 °C in the presence of 10 μ Ci of [7-³H]cholesterol. In some experiments, 2 ml of normolipidaemic human plasma

Abbreviations used: PAF, platelet-activating factor (1-*O*-alkyl-2-acetyl-*sn*-glycero-3-phosphocholine); LDL, low-density lipoproteins; HDL, high-density lipoproteins; VLDL, very-low-density lipoproteins; LCAT, lecithin-cholesterol acyltransferase (EC 2.3.1.43).

was used. The samples were then applied to a Bio-Gel A5.0 m chromatographic column (90 cm $\times$ 1.5 cm) and eluted at 10 ml/h with 0.15 M-NaCl containing 10 mM-Tris/HCl, pH 7.4, 0.01 % EDTA and 0.3 % NaN_3 . Eluate from the column was collected in 2 ml fractions. As 90 % of the radiolabel was recovered in the lipoprotein classes, the assay of each column fraction for ^3H permitted us to establish the elution profile of the different plasma lipoproteins. The resulting peaks were identified by calibrating the column with authentic human ^{125}I -VLDL, -LDL and -HDL before each experiment [14].

Assay of LCAT

This activity was measured with an artificial substrate containing phosphatidylcholine (egg yolk)/[7- ^3H]-cholesterol (4:1 molar ratio) prepared essentially by the method of Batzri & Korn [15]. The incubation mixtures contained (final volume 0.2 ml): 250 nM-phosphatidylcholine; 62.5 nM-[7- ^3H]-cholesterol; 12 mg of bovine serum albumin/ml; 5 mM- β -mercaptoethanol; 50 μg of pure human apoprotein A-I/ml; and 15 μl of plasma or 50 μl of column fraction per assay. The mixtures were incubated for 30 min at 37 $^\circ\text{C}$ and stopped by the addition of 4 ml of chloroform/methanol (3:1, v/v). The lipids were extracted and the cholesteryl esters separated from the cholesterol substrate by t.l.c.

Assay of PAF acetylhydrolase

This activity was measured in the plasma and in column eluate fractions by a method similar to that described by Blank *et al.* [16], with 1-*O*-alkyl-2-[^3H]-acetyl-glycero-3-phosphocholine as a substrate. This was prepared in our laboratory as previously described [7]. The assay mixture contained: 50 μM -[acetyl- ^3H]PAF (25 nCi/nmol), 0.15 M-phosphate-buffered NaCl, pH 7.4, and 100 μl of column fraction or 5 μl of plasma (final volume 0.4 ml). The mixtures were incubated for 10 min at 37 $^\circ\text{C}$, and the lipids extracted by the method of Folch *et al.* [17]. The amount of ^3H recovered in the aqueous phase was determined by liquid-scintillation counting. The assays were linear with time for up to 15 min and with up to 10 μl of human or rat plasma.

RESULTS

Pre-calibration of the Bio-Gel A5.0 m column indicated that human VLDL was eluted at the void volume (fractions 28–31), LDL at fractions 31–48 and HDL in fractions 49–64. In rat plasma, PAF acetylhydrolase activity was also only detected in the HDL region, with no apparent activity in VLDL or LDL (Fig. 1). This is quite different from human plasma, in which PAF acetylhydrolase is found mainly in the LDL, with approx. 20 % in HDL (Fig. 1).

When rats were treated with 17 α -ethinyloestradiol, the plasma concentrations of triacylglycerol, total cholesterol and HDL cholesterol were dramatically decreased (Table 1). This is similar to observations by other workers, who produced lower rat plasma lipid concentrations by oestradiol treatment [18]. In the present study, it has been shown that 66 % of the PAF acetylhydrolase activity was removed from the plasma by ethinyloestradiol treatment, whereas no LCAT activity was removed (Table 1).

The marked changes in the HDL cholesterol from the

Fig. 1. Distribution of PAF acetylhydrolase in rat and human plasma

Samples (2 ml) of rat or human plasma were equilibrated for 16 h at 4 $^\circ\text{C}$ with 10 μCi of [^3H]cholesterol. The lipoprotein classes were then resolved on Bio-Gel A5.0 m. Column fractions were analysed for [^3H]cholesterol (—) and PAF acetylhydrolase activity (●). Normal human VLDL, LDL and HDL were eluted in fractions 28–31, 31–48 and 49–64 respectively.

oestradiol-treated rats (Table 1) demonstrate the profound effect of this treatment on the metabolism of this class of lipoprotein. The differences in response of the LCAT and PAF acetylhydrolase activity indicate that they are distinct enzymes that are associated with different subclasses of the HDL pool. This distinction was confirmed by their different responses to inhibition. LCAT activity was inhibited by 60–100 % by 0.01–1 mM-phenylmethanesulphonyl fluoride, -*p*-bromophenacyl bromide, -5,5'-dithiobis-(2-nitrobenzoic acid) and -diisopropyl phosphorofluoridate (Fig. 2), whereas only the last-named, and possibly the penultimate, could inhibit the PAF acetylhydrolase activity. However, concentrations of 1 mM and greater were required.

The fact that LCAT activity was not involved in the degradation of PAF in human plasma was confirmed by the high activity in the plasma of three patients with

Table 1. Effect of ethynylroestradiol on plasma lipid concentrations, LCAT and PAF acetylhydrolase in rats

Male Wistar rats (250 g) were given 1 mg of 17 α -ethynylroestradiol (EE) intraperitoneally daily for 5 days. After blood was drawn from the abdominal vena cava, lipid and protein concentrations and enzyme activities were determined as described in the Materials and methods section. The results are expressed as means $\pm$ s.d., and the numbers of animals used are indicated in parentheses: * indicates a significant difference ($P < 0.05$) from control values.

	Control	EE-Treated
Total cholesterol (mg/dl)	53 $\pm$ 5 (9)	14 $\pm$ 3* (9)
Non-esterified cholesterol (mg/dl)	14 $\pm$ 3 (9)	7 $\pm$ 3* (9)
Total triacylglycerol (mg/dl)	72 $\pm$ 32 (9)	10 $\pm$ 2* (9)
Total phospholipid (mg/dl)	9 $\pm$ 1 (5)	5 $\pm$ 1* (5)
HDL-cholesterol (mg/dl)	30 $\pm$ 3 (5)	5 $\pm$ 1* (5)
PAF acetylhydrolase (nmol/min per ml)	27 $\pm$ 8 (9)	9 $\pm$ 3* (9)
LCAT (nmol/h per ml)	29 $\pm$ 5 (9)	30 $\pm$ 5 (9)

Fig. 2. Inhibition of LCAT and PAF acetylhydrolase in rat HDL

Rat plasma HDL was isolated at 1.063–1.21 g/ml by differential ultracentrifugation. The activities of LCAT (●—●) and PAF acetylhydrolase (●---●) were measured at the indicated concentrations of phenylmethanesulphonyl fluoride (PMSF), *p*-bromophenacyl bromide (PBPB), 5,5'-dithiobis-(2-nitrobenzoic acid) (DTNB) and di-isopropyl phosphorofluoridate (DIFP). Results are means of duplicate incubations.

familial LCAT deficiency (192 $\pm$ 23 nmol/min per ml) compared with 11 controls (60 $\pm$ 11 nmol/min per ml).

DISCUSSION

The rat plasma PAF acetylhydrolase described in these experiments is associated with HDLs, and its activity is sufficiently high to play potentially a central role in regulating the concentrations of circulating PAF in the

vascular compartment. Moreover, the distinct substrate specificity of this enzyme may permit it to exist in this highly active state and yet be ineffective at degrading phospholipid with long-chain fatty acid groups esterified in the *sn*-2 position. In this study, the association of the acetylhydrolase in human plasma with LDL has been confirmed. A small amount (10–20%) is also recovered in the HDL when the lipoproteins are separated by gel filtration. This is less than the 30% amount recovered in

HDL after density-gradient ultracentrifugation [19]. As much as 30 % of the activity has also been recovered in HDL after differential centrifugation, which suggests that the enzyme may redistribute among the lipoprotein classes during isolation. The recovery of activity in the HDL from rat plasma may be due to its near-absence in the LDL [8] and the ability of the enzyme to partition between lipoprotein classes under defined conditions [19]. However, the factors that regulate the lipoprotein-binding site for the enzyme are not yet known.

The only other enzyme whose major protein pool is associated with the surface of lipoproteins is LCAT. This enzyme is located almost exclusively in the HDL of both rat and human plasma [20]. It uses phosphatidylcholine as a substrate for its acyltransferase activity, in which a fatty acid is removed from the *sn*-2 position of phosphatidylcholine and transferred to the 3-hydroxy group of free cholesterol [20]. It was initially postulated that PAF acetylhydrolase of rat plasma may be an expression of the phospholipase component of the LCAT enzyme reaction. Thus I attempted to see whether PAF acetylhydrolase activity in rat plasma was positively correlated with the LCAT activity, or whether it was merely a function of HDL concentrations.

In these experiments, rat plasma lipoprotein concentrations were modulated by a series of 17 α -ethynyl-oestradiol injections. This treatment greatly decreased the plasma VLDL and HDL concentrations, as indicated by the triacylglycerol and HDL-cholesterol concentrations. This lipid-lowering effect has been reported to be brought about by the expression of specific apolipoprotein B/E receptors in the liver of ethynyl-oestradiol-treated rats [18]. The decreased PAF acetylhydrolase activities may be explained by the removal of the circulating HDL particles by the liver [21]. However, LCAT activity was not affected by the oestradiol treatment. That this activity is associated with a class of HDL particles that does not contain apoprotein B or E supports the suggestion that LCAT is located within a unique subclass of HDL which is metabolized independently of the total HDL pool [22]. The difference in response of LCAT and the PAF acetylhydrolase to oestradiol treatment also indicates that they are distinct activities. This is supported by the differences in the ability of di-isopropyl phosphofluoridate, phenylmethanesulphonyl fluoride, *p*-bromophenacyl bromide and 5,5'-dithiobis-(2-nitrobenzoic acid) to inhibit these enzymes. Our observation that acetylhydrolase activity is not decreased in the plasma of patients with familial LCAT deficiency confirms this conclusion.

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REFERENCES

- Blank, M. L., Lee, T. C., Fitzgerald, V. & Snyder, F. (1981) *J. Biol. Chem.* **256**, 175–178
- Ostermann, G., Till, U. & Theilmann, K. (1984) *Thrombosis Res.* **30**, 409–418
- Blank, M. L., Hall, M. N., Cress, E. A. & Snyder, F. (1983) *Biochem. Biophys. Res. Commun.* **113**, 666–671
- Farr, R. S., Cox, C. P., Warlow, M. L. & Jorgensen, R. (1980) *Clin. Immunopathol.* **15**, 318–330
- Farr, R. S., Warlow, M. L., Cox, C. P., Meng, K. E. & Greene, D. E. (1984) *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **42**, 3120–3122
- Stafforini, D. M., Prescott, S. M. & McIntyre, T. M. (1987) *J. Biol. Chem.* **262**, 4223–4230
- Pritchard, P. H., Yeung, C. & Chonn, A. (1985) *Blood* **66**, 1476–1478
- Carlisle, S. I., Kudchodkar, B. J., Wang, C. S. & Lacko, A. G. (1986) *Mech. Ageing Dev.* **33**, 211–220
- Allain, C. C., Poon, L. S. & Chan, C. S. G. (1974) *Clin. Chem.* **20**, 470–475
- Bucolo, G. & David, H. (1973) *Clin. Chem.* **19**, 476–482
- Warnick, G. R. & Albers, J. J. (1978) *J. Lipid Res.* **19**, 65–69
- Bartlett, G. R. (1959) *J. Biol. Chem.* **234**, 466–472
- Wills, G. L., Lane, P. A. & Weech, P. K. (1984) *Lab. Techn. Biochem. Mol. Biol.* **14**, 18–116
- Rudel, L. L., Lee, J. A., Morrison, M. D. & Felts, J. M. (1974) *Biochem. J.* **139**, 89–95
- Batzri, S. & Korn, E. D. (1973) *Biochim. Biophys. Acta* **298**, 1015–1031
- Blank, M. L., Lee, T. C., Cress, E. A., Malone, B., Fitzgerald, V. & Snyder, F. (1984) *Biochim. Biophys. Acta* **124**, 156–163
- Folch, J., Lee, M. & Sloane-Stanley, G. H. (1957) *J. Biol. Chem.* **226**, 497–509
- Kovanen, P. T., Brown, M. S. & Goldstein, J. L. (1979) *J. Biol. Chem.* **254**, 11367–11373
- Stafforini, D. M., McIntyre, T. M., Carter, M. E. & Prescott, S. M. (1987) *J. Biol. Chem.* **262**, 4215–4222
- Dobiasova, M. (1983) *Adv. Lipid Res.* **20**, 107–194
- Windler, E. E. T., Kovanen, P. T., Chao, Y. S., Brown, M. S. & Goldstein, J. L. (1980) *J. Biol. Chem.* **255**, 10464–10471
- Chajek, T. & Feilding, C. J. (1978) *Proc. Natl. Acad. Sci. U.S.A.* **75**, 2445–2449