Effects of platelet-activating factor on lipid metabolism in rats *in vivo*

Origin of the hypertriglyceridaemia

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Administration of platelet-activating factor (1-*O*-alkyl-2-acetyl-*sn*-glycero-3-phosphocholine; PAF) did not alter the rate of triacylglycerol entry into the plasma. The gastrointestinal absorption of [^{14}C]triolein was, however, inhibited by PAF, yet there was increased accumulation of [^{14}C]lipid in the plasma and hypertriglyceridaemia. The half-life of injected [9,10(n)- ^3H]triolein in the plasma increased by 47 %, and there was decreased accumulation of [^3H]lipid in brown adipose tissue. This was accompanied by a decrease in lipoprotein lipase activity. The hypertriglyceridaemia induced by PAF appears to be mainly due to decreased peripheral removal, one important site affected being brown adipose tissue.

INTRODUCTION

Acute changes in lipid metabolism, characterized by increased plasma triacylglycerols (hypertriglyceridaemia) and a stimulation of hepatic lipogenesis are commonly found during infective and other inflammatory states [1]. Recent evidence suggests that endogenous mediators released by cells of the immune system may be responsible for these effects [2]. Thus the peptide cytokines tumour necrosis factor- α and interleukin-1 have been implicated in the acute changes in lipid metabolism seen in sepsis [3]. Another effect of tumour necrosis factor- α administration is to decrease tissue lipoprotein lipase (EC 3.1.1.34) activity [4,5]; consequently there is some doubt as to whether the resultant hypertriglyceridaemia is due to increased hepatic very-low-density lipoprotein secretion and/or decreased peripheral utilization of triacylglycerols [6–8]. Recently, another putative mediator of endotoxic shock, platelet-activating factor (1-*O*-alkyl-2-acetyl-*sn*-glycero-3-phosphocholine; PAF) has also been shown to increase plasma triacylglycerols and hepatic lipogenesis in fed rats [9]. The objective of the present studies was to obtain information on the mechanism of the PAF-induced hypertriglyceridaemia.

EXPERIMENTAL

Materials

Animals. Female Wistar rats (160–200 g) were fed *ad libitum* on a chow diet consisting of 52 % carbohydrate, 21 % protein and 4 % fat (the residue was non-digestible material; Special Diet Services, Witham, Essex, U.K.) with free access to drinking water, and were maintained at an ambient temperature of $20 \pm 2^\circ\text{C}$ with a 12 h-light/12 h-dark cycle (light from 07:30 h). For experiments involving intravenous administration to conscious rats, indwelling polyethylene cannulae (internal diameter 0.58 mm; external diameter 0.96 mm) filled with 0.9 % NaCl were inserted into the left internal jugular vein and exteriorized between the scapulae under fentanyl/fluanisone anaesthesia 72 h before experimentation [10]. Food intake was measured in all rats in the 24 h period before experimentation.

Chemicals. [^{14}C]Triolein (glycerol tri[^{14}C]oleate) and glycerol tri[9,10(n)- ^3H]oleate were obtained from Amersham International, Amersham, Bucks., U.K., and [^{14}C]DDT [1,1,1-

trichloro-2,2-bis-(*p*-chlorophenyl)ethane], Triton WR 1339 (Tyl-oxapol), PAF and other biochemicals were from Sigma Chemical Co., Poole, Dorset, U.K.

Methods

Measurement of triacylglycerol entry rate into plasma. Plasma triacylglycerol entry rate was estimated essentially as previously described [11,12]. Triton WR 1339 (100 mg/ml in distilled water per 200 g body wt.) or 0.9 % NaCl was given intravenously to conscious rats, followed by PAF (25 $\mu\text{g/kg}$ body wt. subcutaneously) or vehicle (0.25 % BSA; 0.2 ml) 90 min before blood sampling. Plasma was centrifuged on a NaCl density gradient, and very-low-density-lipoprotein and chylomicron fractions were collected [13] and analysed for triacylglycerol content. The initial plasma triacylglycerol content in a separate group of animals not treated with Triton WR 1339 was used to calculate the increase in plasma triacylglycerol over 90 min.

Measurement of absorption, tissue accumulation and oxidation of enteral lipid. The metabolic fate of an orally administered [^{14}C]lipid load was examined as previously described [14]. [^{14}C]Triolein (0.7 g, 0.33 μCi) was given by oral gavage without anaesthetic after PAF injection (25 $\mu\text{g/kg}$ body wt. subcutaneously), and the animal was placed in a large desiccator to collect expired CO_2 hourly for 5 h by absorption in Lumasorb (May and Baker, Dagenham, Essex, U.K.). The control rats showed no signs of stress, whereas the PAF-treated rats exhibited decreased physical activity. PAF injection was repeated at 2.5 h, and 5 h after triolein administration the rat was anaesthetized with pentobarbital (60 mg/kg body wt.). Aortic blood was collected with a heparinized syringe and tissues were removed (liver, parametrial white and interscapular brown adipose tissue, lung, heart); the gastrointestinal tract was removed and homogenized in 150 ml of 3 % (w/v) HClO_4 . All tissues, the gastrointestinal tract and plasma, were saponified, and the lipid was extracted into light petroleum (b.p. 40–60 $^\circ\text{C}$) [15]. The extracted fatty acids were dissolved in 10 ml of scintillation fluid (Optiphase 'Safe'; LKB) for measurement of radioactivity and hence determination of [^{14}C]lipid accumulation. Triolein absorption was calculated by subtracting total gastrointestinal radioactivity from that administered; $^{14}\text{CO}_2$ production was estimated by counting radioactivity in a sample of the Lumasorb and was linear for the last 3 h [14].

Abbreviation used: PAF, platelet-activating factor.

Table 1. Effect of PAF on gastrointestinal absorption, $^{14}\text{CO}_2$ excretion and tissue [^{14}C]lipid accumulation after an oral [^{14}C]lipid load in fed rats

Rats were injected subcutaneously with PAF (25 $\mu\text{g/kg}$ body wt.) or normal saline, and [$1\text{-}^{14}\text{C}$]triolein was administered orally. $^{14}\text{CO}_2$ was collected hourly for 5 h, with repeat injections of PAF at 2.5 h; then blood and tissues were sampled. For further details see the text. Results are means $\pm$ S.E.M. for five observations; differences between PAF-treated and control animals by Student's *t* test are indicated: ***P* < 0.01, ****P* < 0.001.

Group	Absorption of ^{14}C lipid (% of administered dose/5 h)	$^{14}\text{CO}_2$ excretion (% of absorbed dose/h)	Tissue lipid accumulation (mg of absorbed lipid/5 h per g wet wt. of tissue)					Plasma triacylglycerol concn. (mg/100 ml)
			Liver	White adipose tissue	Brown adipose tissue	Lung	Heart	
Control	75.4 $\pm$ 2.05	7.37 $\pm$ 0.50	2.88 $\pm$ 0.34	3.83 $\pm$ 0.98	44.8 $\pm$ 3.85	1.01 $\pm$ 0.06	0.68 $\pm$ 0.04	31.3 $\pm$ 2.12
PAF	23.5 $\pm$ 3.11**	0.86 $\pm$ 0.46***	0.95 $\pm$ 0.35**	1.21 $\pm$ 0.63	5.35 $\pm$ 1.85***	0.28 $\pm$ 0.10***	0.38 $\pm$ 0.17	83.6 $\pm$ 14.8**

Measurement of plasma clearance and tissue accumulation of a triacylglycerol emulsion. An emulsion of triacylglycerols was freshly prepared by a modification of the method of Redgrave & Callow [16]. Briefly, triolein, glycerol tri[9,10(n)- ^3H]oleate and phosphatidylcholine were mixed and solvents evaporated under nitrogen; 0.2 M-Tris, pH 8.1, was added, and the mixture was emulsified at 4 $^\circ\text{C}$ by Ultra-Turrax disperser at 13 500 rev./min for 10 min. Defatted BSA was added to a final concentration of 0.32 % (w/v). At 90 min after injection (subcutaneous) of PAF or 0.9 % NaCl, rats were injected with 0.5 ml [$2\text{ }\mu\text{Ci}$; 10 mg (approx. 12 μmol) of triolein] of emulsion through internal jugular-vein cannulae, and blood was subsequently collected for 10 min from a tail-tip incision (about 50 $\mu\text{l/min}$). At 9 min after emulsion administration, the animals were injected with pentobarbital, and killed after the 10 min blood sample. Tissues (liver, parametrial white and interscapular brown adipose tissue and extensor digitorum longus muscle) were rapidly removed, weighed and saponified, and lipids were extracted as described above. Tissue [^3H]lipid accumulation was expressed as percentage of administered radioactivity per g wet wt. of tissue in 10 min; plasma ^3H clearance was calculated by counting radioactivity in whole plasma derived from tail-tip blood samples, and expressed as half-life (in min) for 50 % decrease in plasma radioactivity compared with the initial (1 min) sample.

Measurement of tissue blood flow. [^{14}C]DDT (10 $\mu\text{Ci/ml}$ per kg body wt.) in 0.9 % NaCl containing 2.5 % (v/v) Tween 20 (0.3 ml) was injected via indwelling internal jugular-vein cannula 60 min after PAF or control injection [17,18]. After 30 min, animals were anaesthetized, and tissues (liver, white and interscapular brown adipose tissue) removed and weighed. Tissue lipids were saponified and extracted as above, and counted for radioactivity.

Lipoprotein lipase. Lipoprotein lipase activities in parametrial and interscapular brown adipose tissue and heart were determined on acetone/diethyl ether-dried powders of the tissue, with [^3H]triolein as substrate [19,20].

Triacylglycerol concentration. Plasma triacylglycerol was measured by the method of Eggstein & Kreutz [21].

RESULTS AND DISCUSSION

PAF stimulates hepatic lipogenesis [9] and increases plasma non-esterified fatty acids [22], two factors which might lead to increased hepatic secretion of triacylglycerols in the form of very-low-density lipoproteins. To examine the contribution of increased entry of triacylglycerols into the plasma to the hypertriglyceridaemia observed with PAF [9], measurements were made of the increased plasma triacylglycerols after admin-

istration of the detergent Triton WR 1339, which decreases their removal by inhibition of lipoprotein lipase activity [23]. Plasma lipoproteins measured after Triton WR 1339 treatment arise from the liver (very-low-density lipoprotein) or the intestine (chylomicrons). Rats treated with PAF and Triton WR 1339 (90 min exposure; means $\pm$ S.E.M., *n* = 4–6) had a similar rate of entry of whole plasma triacylglycerols (24.7 $\pm$ 1.4 mg/100 g body wt. per 90 min), chylomicrons (5.1 $\pm$ 0.6 mg/100 g body wt. per 90 min) and very-low-density lipoprotein (17.5 $\pm$ 0.4 mg/100 g body wt. per 90 min) compared with saline- and Triton WR 1339-treated rats (31.8 $\pm$ 6.4, 9.4 $\pm$ 2.6 and 20.4 $\pm$ 3.1 mg/100 g body wt. per 90 min respectively). This suggests that the triacylglycerol entry rate into the plasma is not affected by PAF and that the hypertriglyceridaemia is due to some other mechanism. One possibility is that PAF inhibits assimilation of lipid by peripheral tissues and/or lipid oxidation.

To test this hypothesis, rats were given [$1\text{-}^{14}\text{C}$]triolein by oral gavage and the metabolic fate of the [^{14}C]lipid was examined (Table 1). PAF caused a large decrease (68 %) in lipid absorption, and at least part of this decrease was due to delayed gastric emptying (results not shown). Consequently, subsequent calculations were based on the absorbed dose, to allow for this difference. On this basis whole-body lipid oxidation (exhaled $^{14}\text{CO}_2$) was decreased by 88 % (Table 1). The combination of decreased lipid absorption and oxidation has previously been noted with the cytokines tumour necrosis factor- α [6] and interleukin-1- β [24]. This either suggests a specific effect of PAF to decrease lipid oxidation or may simply be a reflection of decreased availability of exogenous lipid to sites of lipid oxidation. Tissue lipid assimilation was calculated as mass of absorbed lipid (mg) accumulated per g wet wt. of tissue over 5 h without correction for the lower absorption with PAF, assuming uniform lipid- ^{14}C label distribution after gastrointestinal absorption. There was a significant decrease in PAF-treated rats of [^{14}C]lipid accumulation by liver, lung and brown adipose tissue (Table 1). When expressed as a percentage of the absorbed dose, there was no difference in liver or lung accumulation of lipid, but brown adipose tissue still showed a 3-fold decrease. Plasma [^{14}C]lipid was doubled after PAF injection, but this was not statistically significant [PAF-treated, 0.44 $\pm$ 0.20 (*n* = 5) mg of absorbed lipid/5 h per ml; controls, 0.24 $\pm$ 0.03 (*n* = 5) mg of absorbed lipid/5 h per ml]; however, when expressed as a percentage of the absorbed dose this difference was significant [PAF-treated, 0.30 $\pm$ 0.10 %/5 h per ml (*n* = 5); controls, 0.05 $\pm$ 0.01 %/5 h per ml (*n* = 5); *P* < 0.05]. As expected from previous work from this laboratory [9], the plasma triacylglycerol increased (170 %; Table 1).

PAF is known to have toxic effects on the gastrointestinal

Table 2. Effect of PAF on plasma clearance and tissue accumulation of an intravenous [³H]triacylglycerol emulsion and on tissue lipoprotein lipase activity in fed rats

PAF (25 µg/kg body wt.) was injected subcutaneously, followed 90 min later by a [³H]triolein-labelled emulsion through a jugular-venous cannula. Tail-vein blood was sampled for 10 min, then tissues were removed for lipid extraction. Plasma ³H clearance was calculated as the half-life in min of plasma radioactivity between 1 and 10 min after injection. Lipoprotein lipase was measured in separate experiments. Results are mean values ± S.E.M. for six to nine observations; significant differences between PAF-treated and control animals by Student's *t* test are indicated: **P* < 0.05, ***P* < 0.01.

Group	Plasma ³ H clearance (min)	Tissue [³ H]lipid accumulation (% of administered dose/g wet wt. of tissue per 10 min)				Lipoprotein lipase activity (nmol of fatty acid released/min per mg of acetone-dried tissue)		
		Liver	White adipose tissue	Brown adipose tissue	Muscle	White adipose tissue	Brown adipose tissue	Heart
Control	3.72 ± 0.15	2.12 ± 0.52	0.04 ± 0.01	0.94 ± 0.20	0.02 ± 0.01	0.91 ± 0.18	1.30 ± 0.09	0.54 ± 0.10
PAF	5.46 ± 0.39**	1.95 ± 0.47	0.06 ± 0.02	0.46 ± 0.07*	0.03 ± 0.01	0.86 ± 0.15	0.95 ± 0.05**	0.66 ± 0.09

tract, causing ischaemic necrosis at high doses [25]; the toxic effects of tumour necrosis factor on the intestine have also been ascribed to PAF [26]. That PAF severely inhibits gastrointestinal absorption of dietary lipid makes the hypertriglyceridaemia in the present experiments more striking. To confirm the apparent effects of PAF on peripheral triacylglycerol utilization, the clearance of an intravenously injected triacylglycerol emulsion [16] containing [9,10(n)-³H]triolein was measured (Table 2). The amount of triolein injected (10 mg) increased the plasma pool of triacylglycerols by 6-fold in control rats and 3-fold in PAF-treated rats, so that the initial concentrations were similar. The half-life of ³H radioactivity from the plasma was about 50% greater in PAF-treated rats, suggesting an inhibition of tissue assimilation of lipids. Of the tissues studied, brown adipose tissue showed a significant decrease in [³H]lipid accumulation (Table 2). The most likely explanation for this finding is a decreased rate of lipid removal from the plasma, although a theoretical possibility is increased tissue oxidation and/or re-export of labelled lipid. The former is unlikely, given the decreased ¹⁴CO₂ production rate (Table 1), and the short experimental period (10 min) would seem to exclude the latter.

Two mechanisms could explain the observed decreased [¹⁴C]lipid accumulation in brown adipose tissue: (a) depression of blood flow to tissue; (b) decreased tissue lipoprotein lipase activity. Tissue blood flow measured by the [¹⁴C]DDT method [17,18] showed no significant difference between controls and PAF-treated rats at the time of measurement (60–90 min; results not shown). Brown-adipose-tissue lipoprotein lipase activity was, however, decreased by 27% (Table 2).

At first sight it would seem unlikely that a tissue of small mass (1.5 g per 200 g body wt.) such as brown adipose tissue could make an appreciable contribution to plasma triacylglycerol removal in the rat. However, on a gram-weight basis this tissue is 7–14 times more active in the accumulation of dietary lipid than is liver (Table 1; [14]), and even in terms of total tissue masses it is more active. It should also be emphasized that the measured [¹⁴C]lipid accumulation in brown adipose tissue may be an underestimate because of the high rate of lipid oxidation in this tissue. Thus it is concluded that under the present experimental conditions the hypertriglyceridaemia on administration of PAF is mainly due to decreased removal of plasma triacylglycerols, and that an important contribution is made by decreased lipoprotein lipase activity in brown adipose tissue.

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