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Metabolic effects of platelet-activating factor in rats *in vivo*

Stimulation of hepatic glycogenolysis and lipogenesis

Rhys D. EVANS,* Vera ILIC† and Dermot H. WILLIAMSON†

*Nuffield Department of Anaesthetics and †Metabolic Research Laboratory, Nuffield Department of Clinical Medicine, Radcliffe Infirmary, Woodstock Road, Oxford OX2 6HE, U.K.

1. The effects of platelet-activating factor (1-*O*-alkyl-2-acetyl-*sn*-glyceryl-3-phosphocholine; PAF) on hepatic metabolism *in vivo* in rats were studied. 2. PAF stimulated synthesis of hepatic lipid (saponified and non-saponified) in a dose-dependent fashion and caused hypertriglyceridaemia. There was no effect of PAF on lipogenesis in isolated hepatocytes. 3. High doses of PAF also decreased hepatic glycogen. 4. All doses of PAF decreased plasma insulin, and this was accompanied by hyperglycaemia, except at the lowest dose. 5. The selective PAF-receptor antagonist L659.989 prevented the stimulation of lipogenesis, but indomethacin did not.

INTRODUCTION

Platelet-activating factor (1-*O*-alkyl-2-acetyl-*sn*-glyceryl-3-phosphocholine; PAF) is a naturally occurring phospholipid with diverse and potent effects on several biological systems and is a putative mediator of endotoxic shock and asthma (for reviews see Hanahan, 1986; Braquet *et al.*, 1987; Snyder, 1989). The effects of PAF on some aspects of carbohydrate metabolism have been previously examined: PAF (at concentrations from 1 nM to 10 pM) stimulated glycogenolysis in perfused rat liver (Shukla *et al.*, 1983; Buxton *et al.*, 1984, 1986), but not in isolated hepatocytes (Fisher *et al.*, 1984). The absence of an effect of PAF in isolated hepatocytes may be due to the fact that its receptor is located on the Kupffer cells (Chao *et al.*, 1989). Glucose metabolism in hepatic parenchymal cells may be altered by products secreted from liver endothelial or Kupffer cells, e.g. prostaglandins (Casteleijn *et al.*, 1988; Kuiper *et al.*, 1989). PAF also induced glycogenolysis in foetal rabbit lung *in utero* (Hoffman *et al.*, 1988) and caused hyperglycaemia and increased glucose appearance in starved rats (Lang *et al.*, 1988). Other paracrine effectors of the immune system are also putative mediators of septic shock, e.g. tumour necrosis factor (TNF) and interleukin-1 (IL-1), and have a wide spectrum of metabolic effects (see Evans *et al.*, 1989); they may share common messenger pathways. Thus TNF-α stimulated glycogen breakdown in myotubes of the L6 muscle-cell line (Lee *et al.*, 1987), but its effect on hepatic glycogen is unknown. It has, however, been shown to stimulate hepatic lipogenesis in starved rats (Feingold & Grunfeld, 1987), an effect not shared with IL-1 in rats pretreated with glucose (Argilés *et al.*, 1989) and not seen in adipose tissue (Feingold & Grunfeld, 1987; Argilés *et al.*, 1989).

The possibility that PAF, like TNF, stimulates hepatic lipid metabolism has not been investigated; we have therefore examined its effects on hepatic lipogenesis and glycogen content *in vivo*, and their modulation by the cyclo-oxygenase inhibitor, indomethacin, and L659.989, a PAF-receptor antagonist (Hwang *et al.*, 1988; Ponpipom *et al.*, 1988).

EXPERIMENTAL

Rats

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 22±2 °C with a 12 h-light/12 h-dark cycle (light from 07:30 h).

Biochemicals

PAF and indomethacin were obtained from Sigma Chemical Co., Poole, Dorset, U.K. The synthetic PAF antagonist L659.989 (Hwang *et al.*, 1988; Ponpipom *et al.*, 1988) was generously given by Dr. William Parsons, Merck Sharp & Dohme Research Laboratories, Rahway, NJ, U.S.A. All enzymes and coenzymes were from Boehringer Corp. (London) Ltd., Lewes, Sussex, U.K.

Radioactive compounds

^3H_2O was obtained from Amersham International, Amersham, Bucks., U.K.

Effects of indomethacin and L659.989

Groups of rats were pretreated with either indomethacin (3.75 mg/kg body wt., in 0.5 ml of water; intraperitoneally) or L659.989 (5 mg/kg, in 0.25% methylcellulose; 2.0 ml by gastric intubation without anaesthetic) 20 min before PAF injection. A further group of animals was given 0.25% methylcellulose (2.0 ml) as controls.

Measurement of lipogenesis

Lipogenic rate *in vivo* was determined with ^3H_2O as previously described (Robinson *et al.*, 1978). Animals were injected subcutaneously with PAF or vehicle (0.25% BSA; 0.2 ml); after 1 h, 0.3 ml of ^3H_2O (3.0 mCi) was injected intraperitoneally, and after a further 55 min the rats were anaesthetized with pentobarbital (60 mg/kg body wt.). At 1 h after ^3H_2O injection, aortic blood was collected with a heparinized syringe for plasma and blood samples, and samples were taken of liver (triplicate), white adipose tissue (duplicate) and interscapular brown adipose tissue (single sample). Tissues were saponified, and non-saponified lipid and fatty acids were extracted by the method of Stansbie *et al.* (1976).

The non-saponified lipid was separated by t.l.c. with the solvent system hexane/diethyl ether/acetic acid (70:29:1, by vol.) on silica gel G plates (Gibbons *et al.*, 1983). Cholesterol standards were co-run, and the corresponding sample cholesterol bands removed and counted for radioactivity.

Lipogenesis in isolated hepatocytes was measured essentially

Abbreviations used: PAF, platelet-activating factor; TNF, tumour-necrosis factor; IL-1, interleukin-1.

Table 1. Effects of PAF on lipid synthesis, hepatic glycogen and blood metabolites *in vivo* in fed rats

PAF was injected subcutaneously at the stated dose 2 h before killing; control animals were injected with 0.25% BSA (vehicle). 'Combined' refers to saponified + non-saponified lipid. For further details see the text. Results are mean values ± S.E.M., with the numbers of observations in parentheses. Differences between PAF-treated and control groups by Student's *t* test are indicated: $*P < 0.05$, $**P < 0.01$, $***P < 0.001$.

Measurement	PAF treatment				
	Control (10)	10 μg/kg (8)	25 μg/kg (5)	50 μg/kg (3)	100 μg/kg (10)
^3H_2O incorporation into lipid (μmol of ^3H_2O/h per g wet wt. of tissue)					
Liver: combined	7.77 ± 0.974	11.6 ± 0.768**	27.4 ± 4.44***	20.3 ± 4.99**	18.1 ± 2.13***
saponified	5.07 ± 0.884	7.43 ± 0.719*	21.3 ± 4.27***	15.1 ± 3.25**	12.3 ± 1.57***
non-saponified	2.67 ± 0.162	4.13 ± 0.438**	6.20 ± 1.11***	5.20 ± 1.77*	5.80 ± 0.641***
cholesterol	2.09 ± 0.273 (5)	2.75 ± 0.671 (3)	3.76 ± 0.551*	4.10 ± 0.270**	3.21 ± 0.540 (5)
White adipose tissue: combined	3.96 ± 1.35 (6)	4.55 ± 1.00 (5)	–	–	10.7 ± 3.81 (6)
Brown adipose tissue: combined	44.5 ± 24.9 (7)	13.3 ± 4.31 (5)	–	–	41.5 ± 5.04 (6)
Hepatic glycogen (μmol of glucose/g wet wt. of tissue)	207.6 ± 16.4	182.3 ± 9.38	222.2 ± 31.3	36.4 ± 20.6***	72.5 ± 11.8***
Blood metabolites (μmol/ml)					
Glucose	6.61 ± 0.402	6.66 ± 0.315 (7)	10.3 ± 1.22**	10.2 ± 1.70**	11.8 ± 1.60**
Lactate	2.32 ± 0.145	1.98 ± 0.222 (7)	2.67 ± 0.196	3.53 ± 0.099**	2.21 ± 0.143
Plasma insulin (m-units/l)	82.9 ± 11.6	44.4 ± 8.72*	43.9 ± 6.02*	22.1 ± 2.08**	35.2 ± 5.14**
Plasma triacylglycerol (mg/100 ml)	51.9 ± 6.91	81.6 ± 5.64 (7)**	108.6 ± 13.1***	91.6 ± 15.2*	75.4 ± 5.73*

as described by Roberts *et al.* (1982), except that the exogenous substrate was L-lactate (2 mM) and pyruvate (0.2 mM).

Measurement of hepatic metabolites

A sample of liver was excised and rapidly freeze-clamped in light-alloy tongs cooled in liquid N_2 (Wollenberger *et al.*, 1960). The tissue was subsequently ground to powder, one portion being removed for determination of liver metabolites (glucose, lactate; Mercer & Williamson, 1987), and another portion used for determination of hepatic glycogen by the method of Keppler & Decker (1974) but with initial alkaline hydrolysis at 70 °C instead of deproteinization with $HClO_4$ as originally described.

Blood metabolites

Whole-blood glucose was determined by the method of Slein (1963) and lactate by the method of Hohorst (1963). Plasma triacylglycerol was measured by the method of Eggstein & Kreutz (1966).

Insulin

Plasma insulin was determined by radioimmunoassay with a rat insulin standard (Albano *et al.*, 1972).

RESULTS AND DISCUSSION

In liver, lipogenic rate was significantly increased at all doses of PAF; both saponified and non-saponified lipid contributed to this increase (Table 1). Within the non-saponified component, significantly increased cholesterol synthesis occurred at 25 and 50 μg/kg. Compared with the 'optimal' values of lipogenesis seen at 25 μg/kg, that at 100 μg/kg was significantly lower ($P < 0.05$; Table 1), and this may be explained by secondary, indirect, effects of PAF at the higher dose. These animals appeared unwell, with tachypnoea, lethargy and piloerection, and approx. 50% died within 30 min of injection of 100 μg of PAF/kg. Thus stress-hormone release (Lang *et al.*, 1988) and/or vasoconstriction in this group may have partly negated the effect of PAF to increase hepatic lipogenesis. Lipogenic rate in adipose tissue (brown and white) was unaffected by PAF treatment (Table 1).

A possible explanation for the stimulation of hepatic lipogenesis after PAF injection is that it is secondary to increased intrahepatic glycolysis as a result of glycogenolysis. PAF-induced increased glycogenolysis has been observed *in vitro* (Shukla *et al.*, 1983; Buxton *et al.*, 1984, 1986); in our experiments, hepatic glycogen content was significantly decreased in rats treated with 50–100 μg of PAF/kg compared with controls (Table 1), suggesting increased glycogenolysis *in vivo*. However, hepatic glycogen content was maintained at control values in rats treated with lower doses of PAF (up to 25 μg/kg), when increased hepatic lipogenesis was observed (Table 1), suggesting a lack of direct association between the two processes.

Measurement of blood metabolites demonstrated a dose-dependent hyperglycaemia; again, however, increased lipogenesis was noted at a PAF dose (10 μg/kg) at which blood glucose was unchanged (Table 1). Increased blood lactate occurred only at the 50 μg/kg dose of PAF (Table 1).

Surprisingly, the hyperglycaemia after PAF administration was accompanied by a decrease in plasma insulin (Table 1), suggesting that PAF can stimulate hepatic lipogenesis in the presence of lowered plasma insulin. The latter may be due to increased stress hormones (Lang *et al.*, 1988) or a direct effect of PAF. Decreased peripheral glucose utilization owing to the hypoinsulinaemia may explain the hyperglycaemia.

Plasma triacylglycerol concentration was increased after PAF administration at all doses; the changes paralleled those for hepatic lipogenesis (Table 1), i.e. maximum increase occurred at 25 μg of PAF/kg, with a relative decrease ($P < 0.05$) at higher PAF doses (Table 1). Again, stress effects at 'toxic' PAF doses may be responsible (see above). This hypertriglyceridaemia may be due to increased secretion of very-low-density lipoproteins resulting from the increased hepatic lipogenesis (Table 1) and/or decreased lipoprotein lipase activity (EC 3.1.1.34) in peripheral tissues, as occurs with TNF or IL-1 (see Evans *et al.*, 1989). Enhanced absorption of lipid as chylomicrons from the gut is an unlikely explanation for the hypertriglyceridaemia, as PAF is toxic to the gastrointestinal tract (Gonzalez-Crussi & Hsueh, 1983; Sun & Hsueh, 1988).

A possible direct effect of PAF on hepatic lipogenesis was

Table 2. Effects of indomethacin and PAF-antagonist L659.989 on lipid and carbohydrate metabolism after PAF treatment in fed rats *in vivo*

Animals were pretreated with indomethacin (3.75 mg/kg; intraperitoneally) or L659.989 (5 mg/kg in 0.25% methylcellulose; orally) or 0.25% methylcellulose (vehicle; orally) 20 min before injection of PAF (25 μg/kg) or 0.25% BSA (vehicle; subcutaneously). For further details see the text. Results are mean values ± S.E.M., with the numbers of observations in parentheses. Differences between PAF-treated and control (BSA-injected) animals by Student's *t* test are indicated: $*P < 0.05$, $**P < 0.01$, $***P < 0.001$. Differences between L659.989- and methylcellulose-pretreated groups given PAF, by Student's *t* test, are indicated: $\dagger P < 0.05$, $\dagger\dagger P < 0.01$.

Pretreatment...	Indomethacin		L659.989		Methylcellulose
Measurement	BSA (5)	PAF (4)	BSA (3)	PAF (7)	PAF (6)
^3H_2O incorporation into lipid (μmol of ^3H_2O/h per g wet wt. of tissue)					
Liver: combined	8.49 ± 0.991	21.7 ± 3.16**	7.97 ± 0.607	10.3 ± 0.899	17.7 ± 1.63**††
saponified	5.58 ± 0.969	16.3 ± 3.03**	5.67 ± 0.373	7.33 ± 0.982	13.1 ± 1.16**††
non-saponified	2.92 ± 0.308	5.40 ± 0.283***	2.30 ± 0.268	2.95 ± 0.305	4.58 ± 0.616*†
Hepatic glycogen (μmol of glucose/g wet wt. of tissue)	209.9 ± 13.1	181.6 ± 7.33	193.3 ± 21.4	194.5 ± 15.2	182.0 ± 19.4
Blood metabolites (μmol/ml)					
Glucose	6.78 ± 0.286	8.55 ± 0.448*	6.61 ± 0.103	6.55 ± 0.117	6.95 ± 0.259
Lactate	2.45 ± 0.154	2.92 ± 0.311	2.70 ± 0.097	2.37 ± 0.223	2.20 ± 0.108*
Plasma insulin (m-units/l)	52.8 ± 6.10	54.6 ± 3.56	54.6 ± 5.27	35.0 ± 7.81	33.7 ± 5.57*
Plasma triacylglycerol (mg/100 ml)	55.7 ± 8.38	99.0 ± 11.2*	55.2 ± 10.0	51.9 ± 8.26	78.9 ± 7.03†

examined *in vitro* by using isolated hepatocytes from fed rats and measuring ^3H_2O incorporation into lipid. Hepatocytes with lactate (2 mM) and pyruvate (0.2 mM) as substrates synthesized 1.65 ± 0.40 (5) μmol of non-saponified lipid/h per g fresh wet wt. of cells and 4.62 ± 0.66 (5) μmol of saponified lipid/h per g fresh wet wt. of cells, compared with 1.21 ± 0.05 (5) and 4.17 ± 0.52 (5) μmol/h per g fresh wet wt. of cells for non-saponified and saponified lipids respectively under identical incubation conditions but with the addition of 2 nM-PAF. These findings suggest that the action of PAF on hepatocytes *in vivo* is indirect. A possible mechanism is that PAF stimulates Kupffer cells, recently demonstrated to have PAF receptors (Chao *et al.*, 1989), which in turn signal to hepatocytes possibly via prostaglandin production; an example of the increasingly recognized paracrine intercellular communication (Casteleijn *et al.*, 1988; Kuiper *et al.*, 1989).

To test this hypothesis, the effects of the cyclo-oxygenase inhibitor indomethacin were investigated *in vivo* in rats given PAF (Table 2). In addition, the efficacy of the synthetic, selective and competitive PAF-receptor antagonist L659.989 (Hwang *et al.*, 1988; Ponpipom *et al.*, 1988) to reverse the effects of PAF was examined. A dose of 25 μg of PAF/kg was chosen, since this dose causes increased hepatic lipogenesis but did not decrease hepatic [glycogen] (Table 1). Pretreatment with indomethacin failed to prevent the PAF stimulation of hepatic lipogenesis; values for indomethacin-pretreated control and PAF (25 μg/kg)-injected groups (Table 2) were not significantly different from corresponding non-pretreated groups (Table 1). Furthermore, indomethacin pretreatment also failed to prevent PAF-induced hyperglycaemia and hypertriglyceridaemia (Table 2), suggesting any intermediate pathway of PAF action on hepatic metabolism does not involve eicosanoids. This view is in agreement with experiments by Lapointe & Olson (1989), where inhibition of the PAF-stimulated prostaglandin D_2 production in perfused livers by ibuprofen did not prevent the vasoconstriction or glycogenolytic responses to PAF. However, others have reported that infusion of indomethacin into perfused livers before PAF treatment results in a decrease in the effects of PAF (Altin *et al.*, 1987). Pretreatment with L659.989 prevented any significant increase in hepatic lipogenesis by PAF compared with controls (Table 2), an effect not due to the methylcellulose vehicle (Table 2). L659.989 also prevented PAF-induced hyperglycaemia and hypertriglyceridaemia. There was still a tendency for lower insulin with the antagonist, but indomethacin abolished the hypoinsulinaemic effect of PAF (Table 2).

To our knowledge, this is the first report of the effects of PAF on hepatic glycogen and lipid metabolism *in vivo*. The results confirm the findings of PAF-stimulated glycogenolysis in the perfused liver, but, in addition, demonstrate a potent stimulation of hepatic lipogenesis at PAF dosages less than those required to decrease hepatic glycogen. This, together with decreased plasma insulin, suggests that increased lipogenesis in liver after PAF treatment is not simply secondary to increased glycogenolysis and/or hyperglycaemia-induced insulin stimulation. The hepatocyte experiments *in vitro* indicate that the action of PAF on liver cells, at least regarding lipogenesis, is indirect, possibly via Kupffer cells and release of a mediator. The failure of indomethacin *in vivo* to prevent the PAF stimulation of hepatic lipogenesis is evidence against the involvement of eicosanoids. The similarity of the hepatic effects of PAF (Hanahan, 1986) and TNF (Evans *et al.*, 1989) suggests either that they share a common mediator and signalling system or that TNF treatment may increase the endothelial-cell production of PAF (Camussi *et al.*, 1987; Bussolino *et al.*, 1988).

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REFERENCES

Albano, J. D. M., Ekins, R. P., Maritz, G. & Turner, R. C. (1972) Acta Endocrinol. (Copenhagen) **70**, 487–509

Altin, J. G., Dieter, P. & Bygrave, F. L. (1987) Biochem. J. **245**, 145–150

Argilés, J. M., Lopez-Soriano, F. J., Evans, R. D. & Williamson, D. H. (1989) Biochem. J. **259**, 673–678

Braquet, P., Touqui, L., Shen, T. Y. & Vargaftig, B. B. (1987) Pharmacol. Rev. **39**, 97–145

Bussolino, F., Camussi, G. & Baglioni, C. (1988) J. Biol. Chem. **263**, 11856–11861
Buxton, D. B., Shukla, S. D., Hanahan, D. J. & Olson, M. S. (1984) J. Biol. Chem. **259**, 1468–1471
Buxton, D. B., Fisher, R. A., Hanahan, D. J. & Olson, M. S. (1986) J. Biol. Chem. **261**, 644–649
Camussi, G., Bussolino, F., Salvidio, G. & Baglioni, C. (1987) J. Exp. Med. **166**, 1390–1404
Casteleijn, E., Kuiper, J., Van Rooij, H. C. J., Kamps, J. A. A. M., Koster, J. F. & Van Berkel, T. J. C. (1988) J. Biol. Chem. **263**, 2699–2703
Chao, W., Liu, H., DeBuysere, M., Hanahan, D. J. & Olson, M. S. (1989) J. Biol. Chem. **264**, 13591–13598
Eggstein, M. & Kreutz, F. H. (1966) Klin. Wochenschr. **44**, 262–267
Evans, R. D., Argilés, J. M. & Williamson, D. H. (1989) Clin. Sci. **77**, 357–364
Feingold, K. R. & Grunfeld, C. (1987) J. Clin. Invest. **80**, 184–190
Fisher, R. A., Shukla, S. D., DeBuysere, M. S., Hanahan, D. J. & Olson, M. S. (1984) J. Biol. Chem. **259**, 8685–8688
Gibbons, G. F., Pullinger, C. R., Munday, M. R. & Williamson, D. H. (1983) Biochem. J. **212**, 843–848
Gonzalez-Crussi, F. & Hsueh, W. (1983) Am. J. Pathol. **112**, 127–135
Hanahan, D. J. (1986) Annu. Rev. Biochem. **55**, 483–509
Hoffman, D. R., White, R. G., Angle, M. J., Maki, N. & Johnston, J. M. (1988) J. Biol. Chem. **263**, 9316–9319
Hohorst, H. J. (1963) in Methods of Enzymatic Analysis (Bergmeyer, H. U., ed.), pp. 215–219, Academic Press, New York and London
Hwang, S.-B., Lam, M.-H., Alberts, A. W., Bugianesi, R. L., Chabala, J. C. & Ponpipom, M. M. (1988) J. Pharmacol. Exp. Ther. **246**, 534–541
Keppler, D. & Decker, K. (1974) in Methods of Enzymatic Analysis (Bergmeyer, H. U., ed.), vol. 3, pp. 1127–1131, Academic Press, New York and London
Kuiper, J., Zijlstra, F. J., Kamps, J. A. A. M. & Van Berkel, T. J. C. (1989) Biochem. J. **262**, 195–201
Lang, C. H., Dobrescu, C., Hargrove, D. M., Bagby, G. J. & Spitzer, J. J. (1988) Am. J. Physiol. **254**, E193–E200
Lapointe, D. S. & Olson, M. S. (1989) J. Biol. Chem. **264**, 12130–12133
Lee, M. D., Zentella, A., Pekala, P. H. & Cerami, A. (1987) Proc. Natl. Acad. Sci. U.S.A. **84**, 2590–2594
Mercer, S. W. & Williamson, D. H. (1987) Biochem. J. **242**, 235–243
Ponpipom, M. M., Hwang, S.-S., Doebber, T. W., Acton, J. J., Alberts, A. W., Biftu, T., Brooker, D. R., Bugianesi, R. L., Chabala, J. C., Gamble, N. L., Graham, D. W., Lam, M.-H. & Wu, M.-S. (1988) Biochem. Biophys. Res. Commun. **150**, 1213–1220
Roberts, A. F. C., Viña, J. R., Munday, M. R., Farrell, R. & Williamson, D. H. (1982) Biochem. J. **204**, 417–423
Robinson, A. M., Girard, J. R. & Williamson, D. H. (1978) Biochem. J. **176**, 343–346
Shukla, S. D., Buxton, D. B., Olson, M. S. & Hanahan, D. J. (1983) J. Biol. Chem. **258**, 10212–10214
Slein, M. W. (1963) in Methods of Enzymatic Analysis (Bergmeyer, H. U., ed.), pp. 117–123, Academic Press, New York and London
Snyder, F. (1989) Proc. Soc. Exp. Biol. Med. **190**, 125–135
Stansbie, D., Brownsey, R. W., Crettaz, M. & Denton, R. M. (1976) Biochem. J. **160**, 413–416
Sun, X.-M. & Hsueh, W. (1988) J. Clin. Invest. **81**, 1328–1331
Wollenberger, A., Ristau, O. & Schoffa, G. (1960) Pflügers Arch. Gesamte Physiol. Menschen Tiere **270**, 399–412

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