Interaction of platelet-activating factor, spleen and atrial natriuretic peptide in plasma volume regulation during endotoxaemia in rats

Xiao-Wu Qu, Ranna A. Rozenfeld, Wei Huang, Susan E. Crawford, F. Gonzalez-Crussi and Wei Hsueh

Department of Pathology and Department of Pediatrics, Children's Memorial Medical Center, Northwestern University Medical School, Chicago, IL 60614, USA

(Received 28 January 1998; accepted after revision 17 June 1998)

1. We studied endotoxin (lipopolysaccharide, LPS)-induced platelet-activating factor (PAF) production in various visceral organs, and the effect of PAF antagonists or splenectomy on LPS-induced changes.

2. PAF production in response to LPS was highest in the spleen, followed by ileum, heart, lung and kidneys. None was found in the liver. The splenic response was rapid, reaching 10 times the basal level at 30 min. The increased PAF content in each organ was unrelated to the enzyme activity of either macrophages or neutrophils.

3. LPS-induced hypotension and haemoconcentration were largely prevented by PAF antagonists and splenectomy.

4. Plasma volume fell, and plasma atrial natriuretic peptide (ANP) rose, after LPS administration. Splenectomy or pretreatment with PAF antagonists almost completely prevented these LPS-induced changes at 30 min, but only partially reversed them at 90 min.

5. These results suggest that during endotoxaemia: (a) the spleen is the site of the highest endogenous PAF production; (b) the initial release of ANP is dependent on the production of endogenous PAF, and a PAF–ANP interaction mediates the early plasma volume reduction; (c) plasma volume reduction as well as ANP release depend on the spleen; (d) PAF mediated the hypotensive response and its action in the spleen; and (e) sequestered neutrophils are probably not the main source of PAF in the spleen.

PAF (platelet-activating factor, Paf-acether, 1-alkyl-2-acetyl-*sn*-glycero-3-phosphocholine), a phospholipid with potent biological effects (Benveniste, 1988; Snyder, 1990), is one of the main mediators in endotoxin shock, as supported by the following observations: (a) serum PAF level rises in sepsis (Heuer *et al.* 1991); (b) plasma PAF increases in animals following injection of lipopolysaccharide (LPS) (Chang *et al.* 1987; Dobrowsky *et al.* 1991), and the tissue PAF content is elevated in LPS-induced injury of the lung (Chang *et al.* 1987) and bowel (Hsueh *et al.* 1987); (c) PAF injection causes shock and tissue injury, mimicking the effect of LPS *in vivo* (Benveniste, 1988; Handley, 1990); and (d) furthermore, pretreatment with PAF antagonists prevents LPS-induced shock (Handley, 1990; Dobrowsky *et al.* 1991).

Since PAF is generally considered to be an autacoid, or local hormone, the content of PAF in the inflamed or injured tissues appears to be a more meaningful indicator than its serum level. However, tissue PAF production in inflammation has been studied to a limited extent, perhaps partly due to technical difficulties. Our previous animal studies have shown that the small intestine is the target of LPS-induced injury, and a high PAF level in the intestinal mucosa has been demonstrated following LPS administration (Hsueh *et al.* 1987). However, there has been no study comparing the endogenous PAF production of various organs.

In endotoxin shock plasma volume is reduced and haemoconcentration develops. Although the mechanism is unclear, it is generally assumed that blood volume changes are a consequence of the release of mediators such as tumour necrosis factor-α (TNF), PAF and peptide leukotrienes, with resulting vascular leakage or peripheral vasodilatation and venous blood pooling. An important physiological regulator of plasma volume is atrial natriuretic peptide (ANP), a cardiac hormone with profound effects on the cardiovascular system and kidneys (Espiner, 1994). Despite its potent vascular regulatory effect, and known changes in endotoxin shock (Gullichsen *et al.* 1989), its role in the pathophysiology of septic shock has not been studied. The goals of the present study are: (a) to compare the PAF level in various visceral organs in response to LPS; (b) to ascertain the possible cellular source of PAF in these organs; (c) to

define the role of the spleen and PAF in the regulation of blood volume in endotoxin shock; and (d) to study the interaction of PAF, spleen and ANP in endotoxin shock. We report here that splenic tissue is the site of the highest PAF biosynthesis during endotoxaemia, and that endogenous PAF induces ANP production, which may mediate plasma volume reduction.

METHODS

Materials

PAF (1-*O*-hexadecyl-2-acetyl-*sn*-glycero-3-phosphocholine) and lipopolysaccharide (LPS, from *Escherichia coli*) were purchased from Sigma. 1-*O*-[^{3}H]-hexadecyl-2-acetyl-3-phosphocholine (91 Ci mmol^{-1}) and [^{3}H]serotonin (11·1 Ci mmol^{-1}) were purchased from Amersham. Octadecyl (C18) columns were obtained from Varian (Harbor City, CA, USA). WEB 2170, a structurally unrelated PAF antagonist, was a generous gift from Dr H. Heuer, Boehringer Ingelheim, Mainz, Germany. The radioimmunoassay (RIA) kit for rat ANP was purchased from Peninsular Laboratories, Inc.

Animal experiments

Young male Sprague–Dawley rats (120–150 g) were anaesthetized with Nembutal (sodium pentobarbitone injection, 65 mg kg^{-1}, I.P.) and catheterized via the carotid artery and jugular vein for blood pressure recording and drug administration. The animals were divided into four groups to receive the following treatment regimes: (A) saline; (B) LPS (10 mg ml^{-1} saline, 10 mg kg^{-1}, I.V.); (C) PAF antagonist WEB 2170 (1·5 mg kg^{-1}, I.V.) and LPS (20 min after WEB); (D) splenectomy, followed by LPS. Blood samples were taken at time 0 (right before LPS injection), and at 15, 30, 60 and 90 min after LPS injection for the determination of haematocrit and WBC (white blood cell) count. At the end of the experiment (30 or 90 min after LPS injection), 3 ml of blood was collected from the carotid artery into a test tube containing 30 μl of 0·5 M EDTA. The blood sample was centrifuged at 500 *g* for 5 min, and the plasma was stored at −70 °C for later ANP extraction. The animal was then killed by an overdose of Nembutal (150 mg kg^{-1}, I.V.), and the heart, lungs, liver, kidneys, spleen and ileum were immediately removed. Only the ileum was used for intestinal PAF quantification because our previous study showed that the ileum is the major site of PAF production in the intestine (Qu *et al.* 1996). Portions of the tissue were homogenized in a chloroform–methanol (2 : 1, v/v) mixture for lipid extraction, and aliquots were stored at −70 °C for future enzyme studies. The animal protocol had been approved by the Institutional Animal Care and Use Committee.

Plasma volume determination

Plasma volume was measured by the Evans Blue dilution method as previously described (Wang *et al.* 1994). In brief, animals were prepared as described above, and 150 μl Evans Blue solution (5·0 mg ml^{-1} saline) was injected via the jugular vein catheter at time 0 (in sham-operated rats), or at 30 or 90 min after LPS administration. Two minutes after the Evans Blue injection, 200 μl blood was withdrawn from the carotid artery catheter into a test tube containing 3 μl of 0·5 M EDTA as anticoagulant. The blood was spun at 10 000 *g* for 5 min, and the Evans Blue concentration in the plasma was determined in a spectrophotometer at 605 nm. A standard curve was constructed with sera containing known concentrations of Evans Blue.

Tissue PAF extraction, purification and bioassay

Tissue PAF extraction and quantification were performed following our previously described procedure (Qu *et al.* 1996) with minor modifications. Briefly, total tissue lipid was extracted by Folch's method. Approximately 10^5 d.p.m. of [^{3}H]PAF was added into the tissue homogenate for the calculation of recovery. The organic phase was collected and dried with a vacuum evaporator or a stream of N_2. The lipid residue was reconstituted in 10% acetic acid solution. Tween-20 and ethanol (final concentrations $< 0{\cdot}15$ and $< 1{\cdot}5$%, respectively) were added to the residue, and thoroughly mixed, to facilitate solubilization.

PAF was partially purified by C18 column chromatography. The C18 column was activated by 6 ml of 100% methanol and acidified with 4 ml of 10% acetic acid. After loading the sample, the column was eluted sequentially with 1 ml 10% acetic acid (× 2), 2 ml ethyl acetate (× 6), and 2 ml of methanol (× 3). The collected methanol fraction (containing PAF) was mixed with chloroform and water to reach a chloroform : methanol : water ratio of 1 : 2 : 0·8, with DEAE-cellulose powder (0·1 g) added, and further addition of chloroform and water to make the chloroform : methanol : water equal to 1 : 1 : 0·9. The mixture was spun at 2000 r.p.m. for 5–10 min, and the lower phase was transferred to siliconized tubes and dried. Ethanol (50 μl) was added to the lipid residue to facilitate solubilization, and reconstituted in 450 μl saline containing 5 mg ml^{-1} albumin for subsequent bioassay. This method consistently yields an extraction efficiency of 65–75% or higher.

PAF was quantified by measuring [^{3}H]serotonin release from rabbit platelets as previously described (Hsueh *et al.* 1987). Briefly, [^{3}H]serotonin (1 μCi ml^{-1}) was added to heparinized (100 i.u. ml^{-1}), and diluted (1 : 4 with saline) platelet-rich plasma (PRP), prepared from rabbit blood (Qu *et al.* 1996), and incubated for 30 min. (Preliminary experiments indicate that 1 : 4 and 1 : 8 dilutions increased sensitivity of the assay.) After adding the samples and incubating for 2 min, the reaction was stopped with 20 μl of formaldehyde (1·5 M), and the samples centrifuged. WEB 2170 (10 μg ml^{-1}), a PAF antagonist, was used in duplicate to confirm that the active agonist in the sample was PAF. The concentration of ethanol in the PAF standard solution was identical to those of the test samples.

Enzyme assays

Myeloperoxidase (MPO, a marker enzyme for neutrophils) assay was performed according to published methods (Remick *et al.* 1990). Briefly, tissues were homogenized in 0·05 M potassium phosphate buffer containing 0·5% hexadecyltrimethyl-ammonium bromide (Sigma) and EDTA (5 mM), and sonicated. An aliquot was mixed with substrate (*O*-dianisidine HCl (Sigma) + H_2O_2 in potassium phosphate buffer) and its optical density read at 460 nm. A standard curve was constructed with serial dilutions of human MPO.

Acid phosphatase and β-glucuronidase (both are considered marker enzymes for macrophages) were assayed with a fluorometric method (Leaback, 1974). Tissue samples were homogenized in 0·5 M sodium acetate buffer (pH 5·0), and sonicated. An aliquot of the 1 : 100 diluted supernatant (diluted with acetate buffer) was mixed with the substrate (4-methylumbelliferyl phosphate for acid phosphatase and 4-methylumbelliferyl β-D-glucuronide trihydrate for β-glucuronidase), and incubated at 37 °C for 15 min. (Preliminary experiments were done to determine the time of incubation to attain zero kinetics.) The fluorescence was read with a spectrofluorometer (excitation, 375 nm; emission, 455 nm).

Extraction and quantification of ANP

ANP was extracted and partially purified from rat plasma according to a procedure manual of Peninsular Laboratories Inc. Briefly, 1 ml plasma sample was mixed with an equal volume of

buffer A (0·1 % trifluoroacetic acid) and centrifuged under 4 °C at 1500 *g* for 20 min. The mixture was loaded onto a C18 column which had been pre-washed with buffer B (60 % acetonitrile) then buffer A. The column was then washed with buffer A (3 ml × 3), and ANP was eluted with buffer B (3 ml × 3). The effluent of the final wash was collected, dried with a vacuum evaporator, and the debris was lyophilized. The final ANP content was determined by using a RIA kit from Peninsular Labs, following the instructions in the user's manual. The anti-rat α-ANP antibody used in the assay cross-reacts with ANP 8–33, atriopeptin III, urodilatin (all 100 %), ANP 18–28 (60 %), and does not (or minimally) cross-react with atriopeptin I and II, oxytocin, somatostatin or [Arg^8]-vasopressin.

Statistical analysis

All data are presented as the mean ± S.E.M. Two-way ANOVA (with Bonferroni method of pairwise comparison) was used for the analysis of all data with multiple groups and time points. By this method we analysed the data regarding the changes of blood pressure, WBC count, haematocrit, splenic PAF production, serum ANP level and the plasma volume of different groups. Student's unpaired two-tail *t* test was used for the comparison of data regarding tissue PAF content, and three marker enzyme contents, in each visceral organ, between LPS-treated and sham-operated animals. The significance level was set as 0·05 for all the tests.

RESULTS

The PAF content in the visceral organs

The basal level of PAF content in all the organs (heart, lungs, liver, spleen, kidneys and ileum) examined was low. The highest basal PAF concentration was in the spleen, followed by the ileum, liver and lungs. The heart and kidneys had the lowest baseline level (Fig. 1*A*). LPS (10 mg kg^{-1}) markedly increased PAF production in the ileum and spleen, and significantly raised the PAF content in the heart, lungs and kidneys, but not in the liver (Fig. 1*A*), 90 min after its administration. The increase was most pronounced in the spleen. The response was rapid, reaching almost 10-fold the resting level in 30 min (Fig. 2*A*). The splenic PAF concentration continued to rise, reaching more than 20-fold the baseline level at 90 min after LPS (Fig. 2*A*).

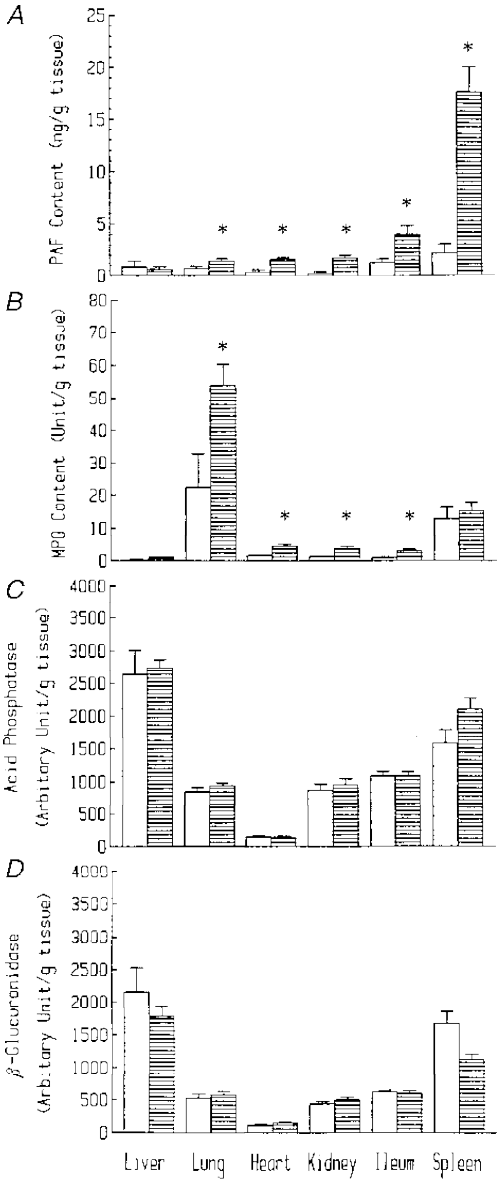

Figure 1. Changes in PAF content and enzyme activities in various organs after LPS injection

Tissue PAF content (*A*), myeloperoxidase (*B*), acid phosphatase (*C*) and β-glucuronidase (*D*) activities in various organs 90 min after LPS injection. □, sham-operated, $n = 7$; ▤, LPS (10 mg kg^{-1}), $n = 8$. * Significantly different between sham and LPS-treated groups.

The activity of the marker enzymes for macrophages and polymorphonuclear leucocytes (PMNs) in the organs

Since both macrophages and PMNs are known sources of PAF (Benveniste, 1988; Snyder, 1990), we investigated if the LPS-induced PAF production in different organs was parallel to the level of marker enzyme activity (an indicator of the numbers of cells) of neutrophils or macrophages in various organs. Lungs had the highest MPO activity in control rats, followed by the spleen (Fig. 1*B*). LPS administration resulted in PMN sequestration in the heart, lungs, small intestine and kidneys, reflected by a marked increase in MPO activity in these organs, but not in the liver and spleen (Fig. 1*B*), at 90 min. Liver, the organ containing the largest number of macrophages (Biozzi & Stiffel, 1965), showed the highest acid phosphatase and β-glucuronidase activities. LPS did not significantly change the activities of these enzymes (Fig. 1*C* and *D*). There was no consistent ratio between PAF content and any of the enzyme activities tested in various organs.

Changes of physiological parameters

Sham-operated animals showed no changes in blood pressure, WBC count, haematocrit (Fig. 3*A*–*C*) or plasma volume (Fig. 2*C*) during the entire experimental period. LPS (10 mg kg^{-1}) caused shock (Fig. 3*A*), leucopenia (Fig. 3*B*), and pronounced haemoconcentration which became apparent as early as 15 min after injection, and peaked at 30 min (Fig. 3*C*). LPS also induced a significant drop of plasma volume as early as 30 min (Fig. 2*C*). The splenic weight did not change significantly after LPS injection (3·4 ± 0·2 mg (g body weight)$^{-1}$, compared with 3·8 ± 0·2 mg (g body weight)$^{-1}$ of sham-operated rats).

Pretreatment with the PAF antagonist WEB 2170 (1·5 mg kg^{-1}) prevented the hypotension (Fig. 3*A*) and haemoconcentration induced by LPS (Fig. 3*C*). It also completely prevented the early phase (30 min) of plasma volume reduction induced by LPS. However, at a later phase (90 min), WEB 2170 pretreatment only partially reversed the plasma volume loss (Fig. 2*C*). WEB 2170 by itself had no effect on blood pressure, haematocrit, WBC count or plasma volume (Table 1).

The role of the spleen in endotoxin shock was examined by splenectomy before the LPS administration. We found that splenectomy, like PAF antagonists, markedly ameliorated the hypotension (Fig. 3*A*), and completely abolished the haemoconcentration induced by LPS (Fig. 3*C*). Splenectomy

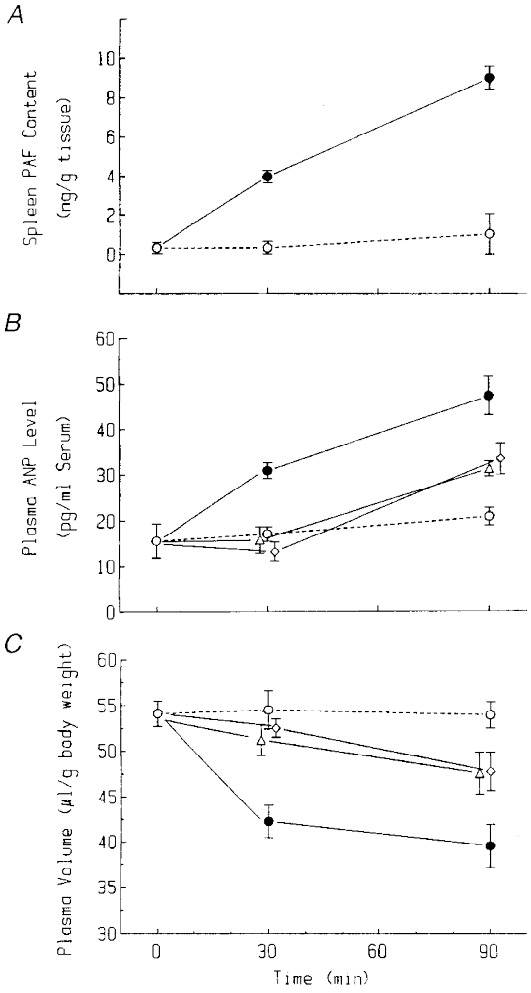

Figure 2. Changes in splenic PAF, plasma ANP and plasma volume after LPS injection

A, LPS-induced PAF production in spleen. $n = 4$ for both groups at each time point. LPS (10 mg ml^{-1}, 10 mg kg^{-1}, I.V.) was injected at time 0. A significant difference was found between sham-operated and LPS-treated groups at 30 and 90 min. *B*, LPS-induced elevation of plasma ANP and effects of splenectomy and WEB 2170. $n = 5$ for each group at all time points. A significant difference was found between LPS and the other 3 groups at 30 and 90 min. *C*, LPS-induced plasma volume reduction and effects of splenectomy and WEB 2170. $n = 7$ in each group at all time points. Sham-operated (○), LPS (●), WEB 2170 + LPS (△), splenectomy + LPS (◇). A significant difference was found between LPS and the other 3 groups at 30 and 90 min. A significant time trend was found in *A*, *B* and *C*.

Table 1. Lack of effect of WEB 2170 on systemic arterial pressure, peripheral WBC count, haematocrit and plasma volume

Time (min)	Blood pressure (mmHg)		Haematocrit (%)		WBC count (% time 0)		Plasma volume (μl g^{-1})	
	Sham	WEB	Sham	WEB	Sham	WEB	Sham	WEB
0	116 ± 3	118 ± 3	41·2 ± 0·4	42·0 ± 0·3	100	100	—	—
15	116 ± 3	111 ± 6	41·9 ± 0·5	42·0 ± 0·5	98·4 ± 4·8	101·3 ± 6·9	—	—
30	117 ± 4	116 ± 2	42·3 ± 0·6	42·0 ± 0·3	95·6 ± 5·0	90·7 ± 5·6	—	—
60	118 ± 3	111 ± 4	41·4 ± 0·7	42·3 ± 0·4	96·8 ± 8·8	101·8 ± 3·7	—	—
90	116 ± 3	106 ± 4	41·2 ± 0·7	42·0 ± 0·3	92·1 ± 4·7	99·9 ± 9·8	53·9 ± 1·5	54·3 ± 1·3

WEB 2170 (1·5 mg kg^{-1}, I.V.) was given at −20 min, then followed by saline at time 0. No significant difference was found between the sham-operated group and the group receiving WEB 2170 in any of the indices at all time points.

also abolished the initial (30 min) reduction of plasma volume induced by LPS, and significantly reversed it at 90 min (Fig. 2*C*).

Neither PAF antagonists nor splenectomy significantly affected LPS-induced leucopenia (Fig. 3*B*).

Plasma level of atrial natriuretic peptide (ANP)

Injection of LPS induced a marked elevation of plasma levels of ANP (Fig. 2*B*), a hormone involved in plasma volume regulation. It reached 1·5-fold basal level at 30 min, and attained 2·5-fold basal level at 90 min. The early increase (at 30 min) was completely abolished by pretreatment with

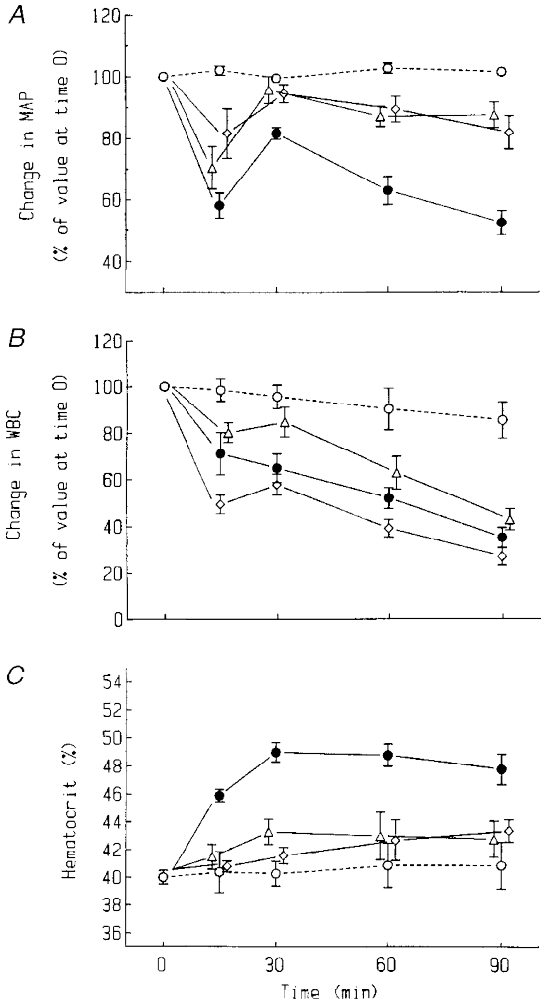

Figure 3. LPS-induced systemic hypotension, leucopenia and haemoconcentration; effects of splenectomy and pretreatment with PAF antagonist

LPS (10 mg ml^{-1}, 10 mg kg^{-1}, I.V.) was injected at time 0. Number of animals in each group at each time point: sham-operated, $n = 12$ (○); LPS, $n = 12$ (●); WEB 2170 + LPS, $n = 12$ (△); splenectomy + LPS, $n = 9$ (◇). *A*, mean arterial pressure (% of time 0 value). A significant difference was found between the LPS group and sham at 15 min, and between the LPS and the other 3 groups at 30, 60 and 90 min. *B*, white blood cell count (% of time 0 value). A significant difference was found between the LPS and sham groups at 15, 30 and 90 min. *C*, haematocrit (absolute value). A significant difference was found between the LPS group and the other 3 groups at 15, 30, 60 and 90 min. A significant time trend was found in *C*.

WEB 2170 or splenectomy (Fig. 2*B*). However, the late phase (90 min after LPS) of ANP elevation, like the changes of hypotension and plasma volume reduction, was only partially abrogated by PAF antagonists or splenectomy (Fig. 2*B*).

DISCUSSION

PAF, an important endogenous mediator in inflammation and septic shock (Benveniste, 1988; Snyder, 1990; Handley, 1990; Heuer *et al.* 1991), has many potent pro-inflammatory effects, such as promoting the expression of adhesion molecules (Lorant *et al.* 1991; Arnould *et al.* 1993), and the production of leukotrienes (Voelkel *et al.* 1982), TNF (Huang *et al.* 1994) and itself (Sun *et al.* 1994), as well as reactive oxygen species (Rouis *et al.* 1988). Plasma PAF increases following injection of endotoxin (Chang *et al.* 1987; Handley, 1990) or TNF (Sun *et al.* 1994), and during ischaemia/reperfusion (Filep *et al.* 1989). A rise in plasma PAF level occurs in sepsis and shock (Handley, 1990; Heuer *et al.* 1991). However, studies quantifying tissue PAF are few. Here we corroborate our previous findings that PAF production in the small bowel increases following LPS challenge (Qu *et al.* 1996), and that LPS-induced hypotension is mediated via endogenous PAF formation (Hsueh *et al.* 1987), since it is prevented by PAF antagonists.

We have compared the amounts of endogenous PAF produced in various organs, and found that PAF production rises in response to LPS in all the organs examined except the liver. The significant PAF production in the lung was concordant with previously published studies (Chang *et al.* 1987).

We found the spleen to be the main site of PAF during endotoxaemia. The implication of this organ selectivity is unclear. One of the main pathophysiological changes during septic shock is the reduction of blood volume, manifested by increased haematocrit. This process may be mediated via PAF, since previous studies (Handley, 1990), and the present one, show that pretreatment with PAF antagonists prevents the development of haemoconcentration. Our data suggest that both the spleen and endogenous PAF are important in the regulation of plasma volume during endotoxin shock, since splenectomy as well as PAF antagonists abrogated LPS-induced plasma volume reduction and haemoconcentration. Plasma volume reduction may result in hypotension, which was also prevented by these treatment regimes.

PAF is an autacoid with a short circulatory half-life. Thus, it is possible that a local effect of PAF mediates the plasma volume reduction during endotoxaemia. Splenic PAF might cause increased permeability of the sinusoids and loss of intravascular fluid into the splenic interstitium. However, this is unlikely, since the splenic weight did not change after LPS injection. A more attractive theory is that PAF induces the production of another hormone/mediator, which, in turn, causes blood volume reduction.

ANP is a physiological regulator of plasma volume. Injection of ANP results in an increase in haematocrit which cannot be accounted for by urinary losses (de Bold *et al.* 1981). ANP causes an increase in haematocrit without changes in the circulating red blood cell volume (Fluckiger *et al.* 1986; Trippodo *et al.* 1986; Kaufman, 1992). A recent study showed that the ANP-induced plasma volume reduction could be abrogated by splenectomy (Kaufman, 1992), presumably via ANP-induced increase in drainage of lymphatic fluid from the spleen. It has long been recognized that the spleen functions as a blood concentrator during splenic congestion by filtering off fluid into the lymphatic system (Kaufman & Deng, 1993). This physiological function may be mediated by ANP, since ANP receptors have been identified in the spleen (Farstad *et al.* 1991), and splenectomy abolished ANP-induced plasma volume changes. The present study further suggests that ANP may also play an important role in the pathophysiology of endotoxin shock, and that release of ANP is stimulated by endogenous PAF production.

The source of ANP in endotoxin shock is unclear. Although it is generally established that ANP is produced mainly by the cardiac atrium (Espiner, 1994), recent investigations demonstrated that this hormone is also produced by macrophages of the spleen (Throsby *et al.* 1991). Since PAF is a local hormone and often remains cell-associated after synthesis (Zimmerman *et al.* 1990), it is probable that the PAF synthesized locally in the spleen induces endogenous ANP release in this organ. However, PAF may also induce ANP release from the heart, as shown by previous investigators (Rayner *et al.* 1991). The present study shows that cardiac PAF rises significantly after LPS injection. Thus, PAF formed in the cardiac atrium might stimulate local release of ANP. However, the present study suggests that the early rise of plasma ANP after LPS injection may be dependent on the spleen and endogenous PAF, since it is abolished by splenectomy or PAF antagonists. In a later stage of endotoxaemia, part of the circulating ANP could originate from extra-splenic sites such as the heart. A PAF-independent pathway may also account for the release of ANP, since splenectomy and PAF antagonists abolished the rise of plasma ANP only partially.

The specific cellular source of PAF within splenic tissue remains unclear. The spleen has a large population of macrophages, a potential source. However, the liver, the organ with the largest number of macrophages (Biozzi & Stiffel, 1965) and the main distribution site of injected LPS (Ge *et al.* 1994), manifested only a weak PAF-synthesizing response. PMNs are another known cellular source of PAF, and PMNs respond to LPS with endothelial adhesion and transmigration into the tissue (Burns & Doerschuk, 1994), although this happens mainly in the lung (Farstad *et al.* 1991), not the spleen. Thus, LPS-induced PAF production is not parallel to the changes of PMN or macrophage numbers in the organ, suggesting that PAF is synthesized in cells other than these two. It is possible that PAF biosynthesis

takes place preferentially in the reticulo-endothelial system in the spleen.

Our data suggest a novel mechanism of haemoconcentration in endotoxaemia: LPS induces endogenous PAF biosynthesis in the spleen and in other organs. Local splenic PAF induces local release of ANP, which, together with ANP originating in the cardiac atrium and other sites, reduces plasma volume. The spleen may also play a direct role in plasma volume reduction by increasing drainage of lymphatic fluid from the spleen. The decreased blood volume reduces cardiac output resulting in poor tissue perfusion, which, if uncompensated, leads to irreversible shock.

Arnould, T., Michiels, C. & Remacle, J. (1993). Increased PMN adherence on endothelial cells after hypoxia: involvement of PAF, CD18/CD11b, and ICAM-1. *American Journal of Physiology* **264**, C1102–1110.

Benveniste, J. (1988). Paf-acether, an ether phospho-lipid with biological activity. *Progress in Clinical and Biological Research* **282**, 73–85.

Biozzi, G. & Stiffel, C. (1965). The pathophysiology of reticulo-endothelial cells of the liver and spleen. *Progress in Liver Diseases* **2**, 166–191.

Burns, A. R. & Doerschuk, C. M. (1994). Quantitation of L-selectin and CD18 expression on rabbit neutrophils during CD18-independent and CD18-dependent emigration in the lung. *Journal of Immunology* **153**, 3177–3188.

Chang, S. W., Feddersen, C. O., Henson, P. M. & Voelkel, N. F. (1987). Platelet-activating factor mediates hemodynamic changes and lung injury in endotoxin treated rat. *Journal of Clinical Investigation* **79**, 1498–1509.

de Bold, A. J., Borenstein, H. B., Veress, A. T. & Sonnenberg, H. (1981). A rapid and potent natriuretic response to intravenous injection of atrial myocardial extract in rats. *Life Sciences* **28**, 89–94.

Dobrowsky, R. T., Voyksner, R. D. & Olson, N. C. (1991). Effect of SRI 63-675 on hemodynamics and blood PAF levels during porcine endotoxemia. *American Journal of Physiology* **260**, H1455–1465.

Espiner, E. A. (1994). Physiology of natriuretic peptide. *Journal of Internal Medicine* **237**, 525–526.

Farstad, B. S., Sundrehagen, E., Opdahl, H. & Benstad, H. B. (1991). Pulmonary, hepatic and splenic sequestration of technetium-99m labelled autologous rabbit granulocytes: scintigraphic cell distribution after intravenous and intraarterial injection, exsanguination and intraarterial injection of cells passed through an intermediary host. *Acta Physiologica Scandinavica* **143**, 211–222.

Filep, J., Herman, F., Braquet, P. & Mozes, T. (1989). Increased levels of platelet-activating factor in blood following intestinal ischemia in the dog. *Biochemical and Biophysical Research Communications* **158**, 353–359.

Fluckiger, J. P., Waeber, B., Matsueda, G. M., Delaloye, B., Nussberger, J. & Brunner, H. R. (1986). Effect of atriopeptin III on hematocrit and volemia of nephrectomized rats. *American Journal of Physiology* **251**, H880–883.

Ge, Y., Ezzell, R. M., Tompkins, R. G. & Warren, H. S. (1994). Cellular distribution of endotoxin after injection of chemically purified lipopolysaccharide differs from that after injection of live bacteria. *Journal of Infectious Diseases* **169**, 95–104.

Gullichsen, E., Nelimarkka, O., Scheinin, M., Antila, K., Leppaluoto, J. & Nainikoski, J. (1989). Plasma levels of atrial natriuretic factor and catecholamines in endotoxin shock in dogs. *Acta Chirurgica Scandinavica* **155**, 567–571.

Handley, D. A. (1990). Platelet-activating factor as a mediator of endotoxin-related diseases. In *Platelet-Activating Factor in Endotoxin and Immune Diseases*, ed. Handley, D. A., Saunders, R. N., Houlihan, W. J. & Tomesch, J. C., pp. 451–495. Marcel Dekker, Inc., New York and Basel.

Heuer, H. O., Darius, H., Lohaann, H. F., Meyer, J., Schierenberg, M. & Treese, N. (1991). Platelet activating factor type in plasma from patients with septicemia and other diseases. *Lipids* **26**, 1381–1385.

Hsueh, W., Gonzalez Crussi, F. & Arroyave, J. L. (1987). Platelet-activating factor: an endogenous mediator for bowel necrosis in endotoxemia. *FASEB Journal* **1**, 403–405.

Huang, L., Tan, X., Crawford, S. E. & Hsueh, W. (1994). Platelet-activating factor and endotoxin induce tumour necrosis factor gene expression in rat intestine and liver. *Immunology* **83**, 65–69.

Kaufman, S. (1992). Role of spleen in ANF induced reduction in plasma volume. *Canadian Journal of Physiology and Pharmacology* **70**, 1104–1108.

Kaufman, S. & Deng, Y. (1993). Spleen control of intravascular volume in the rat. *Journal of Physiology* **468**, 557–565.

Leaback, D. H. (1974). *An Introduction to the Fluorometric Estimation of Enzyme Activities*, 2nd edn. Koch-Light Laboratories, Colnbrook, England.

Lorant, D. E., Patel, K. D., Mcintyre, T. M., Mcever, R. P., Prescott, S. M. & Zimmerman, G. A. (1991). Coexpression of GMP-140 and PAF by endothelium stimulated by histamine or thrombin: a juxtacrine system for adhesion and activation of neutrophils. *Journal of Cell Biology* **115**, 223–234.

Qu, X., Huang, L., Burthart, T., Crawford, S. E., Caplan, M. S. & Hsueh, W. (1996). Endotoxin induces PAF production in the rat ileum: quantitation of tissue PAF by an improved method. *Prostaglandins* **51**, 249–262.

Rayner, T. E., Menadue, M. F. & Oliver, J. R. (1991). Platelet-activating factor stimulates the release of atrial natriuretic factor from the rat heart. *Journal of Endocrinology* **130**, 281–288.

Remick, D. G., Strieter, R. M., Eskandari, M. K., Nguyen, D. T., Genord, M. A., Raiford, C. L. & Kunkel, S. L. (1990). Role of tumor necrosis factor-alpha in lipopolysaccharide-induced pathologic alterations. *American Journal of Pathology* **136**, 49–60.

Rouis, M., Nigon, F. & Chapman, M. J. (1988). Platelet activating factor is a potent stimulant of the production of active oxygen species by human monocyte-derived macrophages. *Biochemical and Biophysical Research Communications* **156**, 1293–1301.

Snyder, F. (1990). Platelet-activating factor and related acceptilated lipids as potent biologically active cellular mediators. *American Journal of Physiology* **259**, C697–708.

Sun, X., Caplan, M. S. & Hsueh, W. (1994). Tumour necrosis factor and endotoxin synergistically activate intestinal phospholipase A2 in mice. Role of endogenous platelet activating factor and effect of exogenous platelet activating factor. *Gut* **35**, 215–219.

Throsby, M., Lee, D., Huang, W. Q., Yang, Z. Y., Copplov, D. L. & Lim, A. T. (1991). Evidence for atrial natriuretic peptide-(5–28) production by macrophages of the rat spleen: an immunochemical and nonradioactive *in situ* hybridization approach. *Endocrinology* **129**, 991–1000.

Trippodo, N. C., Cole, F. E., Frohlich, E. D. & Macphee, A. A. (1986). Atrial natriuretic peptide decreases circulatory capacitance in areflexic rats. *Circulation Research* **59**, 291–296.

Voelkel, N. F., Reeves, J. T., Henson, P. M. & Murphy, R. C. (1982). Nonimmunological production of leukotrienes induced by platelet-activating factor. *Science* **218**, 286–288.

Wang, P., Ba, Z. F., Lu, M. C., Ayala, A., Harkema, J. M. & Chaudry, I. H. (1994). Measurement of circulating blood volume *in vivo* after trauma-hemorrhage and hemodilution. *American Journal of Physiology* **266**, R368–374.

Zimmerman, G. A., McIntyre, T. M., Mehra, M. & Prescott, S. M. (1990). Endothelial cell-associated platelet-activating factor: a novel mechanism for signaling intercellular adhesion. *Journal of Cell Biology* **110**, 529–540.

Acknowledgements

This work was supported by NIH grant DK34574.

Corresponding author

W. Hsueh: Department of Pathology, Children's Memorial Hospital, Chicago, IL 60614, USA.

Email: w-hsueh@nwu.edu