Br. J. Pharmacol. (1988), **93**, 222–228

Gastric mucosal damage induced by local intra-arterial administration of Paf in the rat

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1 A technique for the close-arterial administration of substances to the rat stomach *in vivo* has been developed.

2 Intra-arterial infusion of platelet-activating factor (Paf, 10–50 ng kg^{-1} min^{-1} for 10 min) induced macroscopically assessed damage in the corpus mucosa, characterized as vasocongestion and necrosis.

3 The threshold intra-arterial doses of Paf that induced histologically assessed damage in the antrum and corpus of the stomach (10 and 5 ng kg^{-1} min^{-1}, respectively) produced minimal systemic hypotension (< 20 mmHg) suggesting a dissociation between these events.

4 Pretreatment with the Paf-antagonist, L-652,731 (2.5 mg kg^{-1} i.v.) prevented the gastric damage induced by local infusion of Paf.

5 Intravenous infusion of Paf (25 ng kg^{-1} min^{-1}) did not significantly damage the gastric mucosa, in contrast to the same dose infused locally, yet Paf administered by either route produced a comparable degree of hypotension. Such findings suggest minimal metabolism of Paf during its passage through the gastric circulation.

6 Local intra-arterial infusion of Paf in doses as low as 0.25 ng kg^{-1} min^{-1}, which had no systemic hypotensive actions, significantly induced gastric damage in the presence of intragastric 20% ethanol.

7 These observations support a local role for Paf in the pathogenesis of gastric irritation and ulceration, such as that observed during endotoxin shock or bacterial infection. The present technique is thus useful for the study of locally administered substances on gastric function and integrity.

Introduction

The endogenous pro-inflammatory phospholipid, Paf (Paf-acether; platelet-activating factor) induces a range of pathophysiological events that resemble shock states (Vargaftig *et al.*, 1981; Bessin *et al.*, 1983). These actions include increased vascular permeability, haemoconcentration, hypotension, neutrophil aggregation and lysosomal enzyme release (McManus *et al.*, 1980; O'Flaherty *et al.*, 1981; Sanchez-Crespo *et al.*, 1982; Bjork *et al.*, 1983; Braquet *et al.*, 1984, Doebber *et al.*, 1984). Following intravenous infusion in the rat, Paf also induces extensive damage to the gastric mucosa, (Rosam *et al.*, 1986). This mucosal damage, characterized macroscopically and histologically as diffuse hyperaemia, vasocongestion and necrosis is likely to result from the marked slowing and stasis of blood flow in the mucosal and submucosal microcirculation (Whittle *et al.*, 1986). Although the doses of Paf that induce these microcirculatory changes and tissue damage also reduce systemic arterial blood pressure (BP), such hypotensive effects alone are unlikely to induce mucosal disruption since other hypotensive agents do not cause similar damage (Rosam *et al.*, 1986). Furthermore, low doses of Paf, which did not lower BP, substantially augment the gastric mucosal damage that follows local mucosal application of acid-ethanol (Wallace & Whittle, 1986a).

In the present study, to clarify further the systemic and local actions of Paf on the rat stomach *in vivo*, a technique for the close-arterial administration of substances through the left gastric artery has been developed. The effect of the Paf-receptor antagonist, L-652,731 (Hwang *et al.*, 1985; Wu *et al.*, 1986) on these actions of Paf has also been investigated.

A preliminary account of part of this work has been communicated to the British Pharmacological Society (Esplugues & Whittle, 1987).

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Methods

Male, Wistar rats (230–255 g body weight) were deprived of food, but not water, for 18–20 h before the experiments. The rats were anaesthetized with sodium pentobarbitone (60 mg kg^{-1} i.p.) and the stomach exposed by a mid-line incision. The left gastric artery was carefully freed from connective tissue under a stereomicroscope, and cannulated with a modified short 23 g teflon cannula, fashioned from the outer sleeve of a 24 g i.v. cannula (H.G. Wallace Ltd, Colchester), as shown in Figure 1. Patency of the cannula was assured by observing the back flow of blood derived from the mucosal and submucosal vascular network and gastric muscle. Paf, or the vehicle (0.25% bovine serum albumen in saline) was infused into the left gastric artery for 10 min at a rate of 12.5 $\mu l\ min^{-1}$ by use of a Braun Perfuser pump.

In a further series of experiments, after ligation of the oesophagus and the pylorus, and immediately before the infusion of Paf, 2 ml of 20% ethanol in saline (v/v) were instilled into the gastric lumen via a 25 g needle inserted through the forestomach wall, with the ethanol being retained for the duration of the experimental period.

Mean systemic arterial blood pressure (BP) was measured from a cannula in a carotid artery connected to a pressure transducer (Elcomatic) and a chart recorder (Rikadenki, model R-50). In studies on the action of the Paf-antagonist, L-652,731, once a stable resting BP had been obtained and at least 20 min after exposing the left gastric artery, L-652,731 or its vehicle was injected into a tail vein. After 20 min, the local intra-arterial administration of Paf was commenced.

Assessment of damage

Twenty minutes after terminating the local intra-arterial infusion, the stomachs were removed and opened along the greater curvature. The stomachs

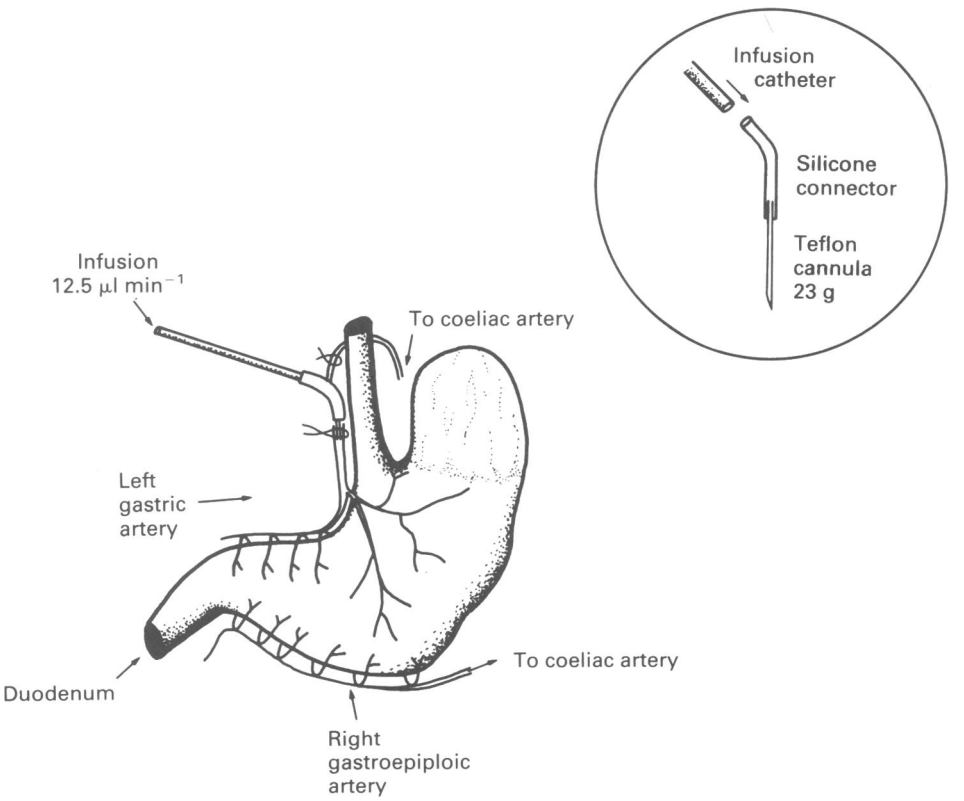

Figure 1 Technique for the local intra-arterial administration of substances such as Paf, to the rat stomach *in vivo*. The inset shows the 23 gauge teflon cannula that is ligated in place in the left gastric artery under a stereomicroscope.

were pinned out, mucosal side up, to a wax block and immersed in neutral buffered formalin and then photographed on colour transparency film. The extent of macroscopically-visible damage was determined from these projected transparencies in a randomized manner via computerized planimetry using an Apple IIe computer system. The mucosal damage was calculated as the % of glandular mucosa showing macroscopically-visible damage.

Two samples of the fundus (corpus) and one sample of the antrum were excised from standardized regions and were processed by routine techniques before embedding in paraffin. Sections (4 μm) were stained with haematoxylin and eosin and examined under a light microscope. The 1 cm length of each histological section was divided into 5–6 fields and each field further divided into 4 equal subsections. Each subsection was histologically assessed for epithelial cell damage (a score of 1 being assigned); glandular disruption, vasocongestion or oedema in the upper mucosa (a score of 2 being assigned); haemorrhagic damage in the mid to lower mucosa (a score of 3) and deep necrosis or ulceration (a score of 4). Each subsection was evaluated on a cumulative basis, the maximum score for each subsection thus being 10, and the total maximum score for each field being 40. The overall mean value of the scores for each of the 5–6 fields was taken as the histological index for that section. All determinations were performed in a randomized manner with both transparencies and histological sections coded to eliminate observer bias.

Drugs

Paf [1-O-alkyl-2-O-acetyl-*sn*-glyceryl-3-phosporylcholine, Sigma Chemical Co., St. Louis, MO] was stored at −20°C as a stock solution in chloroform (2 mg ml^{-1}). On the day of an experiment, an aliquot was dried under nitrogen and redissolved in 0.25% bovine serum albumen (BSA) in isotonic saline and kept on ice. L-652,731 (*trans*-2,5-(3,4,5-trimethoxyphenyl) tetrahydrofuran), a gift from Merck Sharp and Dohme Research Laboratories, Rahway, N.J., U.S.A., was dissolved in 100 μl of dimethyl sulphoxide (DMSO) immediately before i.v. injection. Evans Blue dye (Sigma) and ethanol (analar, B.D.H.) were dissolved in isotonic saline.

Statistical analysis

All data are expressed as mean ± s.e.mean. Comparisons between groups of parametric data were made by Student's *t*-test for unpaired data. Comparisons between groups of non parametric data (histological index) were made by the Mann-Whitney U-test. *P* values of less than 0.05 were taken as significant.

Results

Effect of left gastric artery ligation and perfusion

A series of control experiments were conducted to define the effects on mucosal integrity of the surgical procedures and local intra-arterial infusion under control conditions. Ligation of the left gastric artery for a period of 30 min, similar to that employed in the experimental protocols had no detectable effect on the macroscopic appearance of the mucosa, nor on the histological appearance of the tissue. Furthermore, local intra-arterial infusion (12.5 μl min^{-1}) of isotonic saline or the vehicle (0.25% BSA in saline) for periods of 30–80 min likewise caused no damage to the mucosal tissue, as assessed macroscopically or histologically (Figure 2). Thus, there is sufficient collateral blood flow in the mucosa, derived from the gastro-epiploic and hepatic branches of the coeliac artery to maintain mucosal integrity, as also demonstrated by the substantial back-flow of blood from the cannula in the left gastric artery. In 4 experiments to define the areas of distribution of the infusion from the left gastric artery, Evans Blue dye (5% in isotonic saline), infused over a 1–2 min period, was observed to distribute throughout the corpus and antral areas of the mucosa.

Paf-induced gastric mucosal damage

Local intra-arterial infusion of Paf for 10 min resulted in a dose-dependent increase in macroscopically-assessed damage to the gastric mucosa with Paf 10 ng kg^{-1} min^{-1} being the threshold dose for significant damage compared with control (Figure 2). With the highest dose of Paf studied (50 ng kg^{-1} min^{-1}) this damage affected 26.6 ± 5% of the surface of the mucosa. The nature of this damage was similar to that described following intravenous infusion (Rosam *et al.*, 1986), and was characterized by extensive hyperaemia and haemorrhage. This macroscopically-apparent damage was located in the acid-secreting corpus region of the stomach. Overall, no significant damage ($P > 0.05$) to the antral mucosa was observed macroscopically with any dose of Paf, although in two rats receiving Paf (25 ng kg^{-1} min^{-1}) minimal antral damage was detected (involving 2 and 4% of the total mucosal surface).

Histologically, Paf induced vascular congestion throughout the antral and corpus mucosal layer with extensive damage to surface epithelium, dilatation of the gastric glands and subepithelial oedema where the epithelium was intact. The threshold dose for a significant increase in the histological index in the corpus mucosa with Paf was 5 ng kg^{-1} min^{-1} (Figure 2). At this dose of Paf, there was a significant increase ($P < 0.05$) in the scores for glandular disruption,

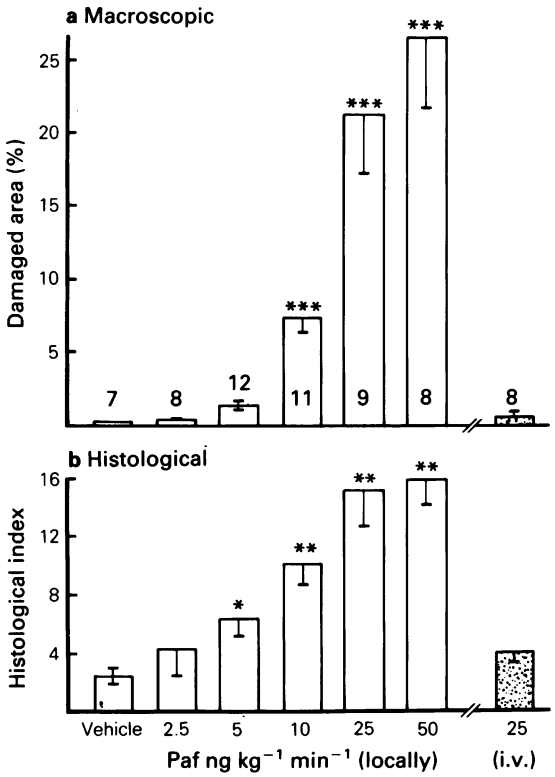

Figure 2 Effects of Paf following local intra-arterial (2.5–50 ng kg^{-1} min^{-1}) or intravenous (25 ng kg^{-1} min^{-1}) infusion for 10 min on the rat stomach *in vivo*. The degree of damage was assessed macroscopically as the % of the total mucosal area that exhibited damage (a) and histologically as a histological index (b). The macroscopically-apparent damage was located in the corpus mucosa, and the histological index for this region is shown for comparison. Results are expressed as mean of *n* (number above/in column) experiments; vertical lines show s.e.mean. Significant difference from the control (intra-arterial infusion of vehicle) is given as $*P<0.05$, $**P<0.01$, $***P<0.001$.

vasocongestion and haemorrhagic damage in the upper and mid regions of the mucosa. When the highest doses of Paf were infused, there were areas of necrosis in the mucosa, complete disruption of the glandular structure and, in some cases, ulceration and partial disappearance of the mucosa.

A significant ($P<0.05$) increase in the histological index for the antral area was observed with Paf (10 ng kg^{-1} min^{-1}). When assessed histologically, the antral damage induced by Paf was severe, particularly with the higher doses of 25 and 50 ng kg^{-1} min^{-1} (Table 1), and characterized by the same histological features as observed in the corpus.

Table 1 Effect of local intra-arterial or intravenous infusion of Paf (2.5–50 ng kg^{-1} min^{-1}) for 10 min in provoking damage to the gastric antral mucosa of the rat

Dose of Paf (ng kg^{-1} min^{-1})	*Route*	*Histological index*	(n)
Control	Local	3.6 ± 0.6	(5)
2.5	Local	3.1 ± 0.8	(10)
5	Local	5.3 ± 1.4	(10)
10	Local	6.8 ± 1.1*	(9)
25	Local	11.4 ± 1.8**	(9)
50	Local	10.8 ± 1.8**	(8)
25	i.v.	3.3 ± 0.6	(9)

Results, shown as the histological index, are the mean ± s.e.mean of *n* (number in parentheses) experiments. Statistical difference from the control (vehicle infused) is shown by $*P<0.05$, $**P<0.01$.

When infused intravenously for 10 min, Paf (25 ng kg^{-1} min^{-1}) did not induce significant mucosal damage, with both the macroscopical and histological scores being similar to those obtained in control (vehicle-perfused) rats. Previous studies have established that the threshold dose for gastric damage by this route was approximately 30 ng kg^{-1} min^{-1} of Paf infused for 20 min (Rosam *et al.*, 1986).

Effects on systemic blood pressure

Local intra-arterial or intravenous infusion of the vehicle had no significant action on resting BP, which remained between 100–120 mmHg. The local intra-arterial administration of Paf (5–50 ng kg^{-1} min^{-1}) induced a rapid hypotension which was maintained throughout the 10 min period of infusion (Figure 3). This effect on BP was dose-related with a maximum fall of −56 ± 4 mmHg with the highest dose of Paf

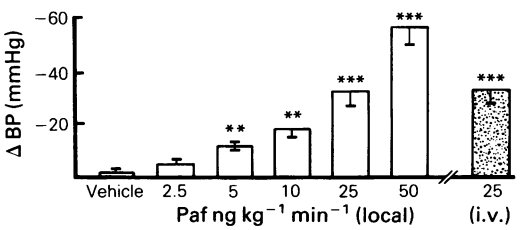

Figure 3 Effect of local intra-arterial (2.5–50 ng kg^{-1} min^{-1}) or intravenous (25 ng kg^{-1} min^{-1}) infusion of Paf for 10 min on mean systemic arterial blood pressure (BP). Results, shown as the fall in BP, are the mean of 5–12 experiments in each group; vertical lines show s.e.mean. Significant difference from control (vehicle-infused) group is given as $**P<0.01$, $***P<0.001$.

Table 2 Inhibition by the Paf receptor antagonist, L-652,731 (1–2.5 mg kg^{-1} min^{-1} i.v.) on the fall in mean systemic arterial blood pressure (BP) and area of macroscopically-assessed gastric mucosal damage induced by the local intra-arterial infusion of Paf (25 ng kg^{-1} min^{-1} for 10 min)

	Dose (mg kg^{-1})	*BP* (mmHg)	*Mucosal damage* (% area)	(n)
Paf alone	—	−33 ± 4	28 ± 4	(6)
+ L-652,731	1.0	−23 ± 8	26 ± 11	(3)
+ L-652,731	2.5	−9 ± 3**	2.1 ± 0.4**	(3)

Results, shown as the change in mean BP (mmHg) and gastric damage (% of total area of mucosa), are the mean ± s.e.mean of *n* (number in parentheses) experiments. Statistical difference from the Paf-alone group is shown by **$P < 0.01$.

(50 ng kg^{-1} min^{-1}). Following termination of the Paf infusion, BP gradually increased and by the end of the following 20 min period, BP values were very similar to basal levels, with all doses of Paf studied.

The route of administration did not influence this hypotensive action, since Paf (25 ng kg^{-1} min^{-1}) induced a comparable fall in BP when given intravenously or intra-arterially (−37 ± 4.9 and −32 ± 5.8 mmHg respectively, $n = 5$, $P < 0.05$).

Effects of the Paf-antagonist, L-652,731

Intravenous administration of the Paf-antagonist L-652,731 (1-2.5 mg kg^{-1}) had no effect on BP or on the macroscopic appearance of the mucosa. Pretreatment with L-652,731 (1-2.5 mg kg^{-1} i.v.), 20 min before Paf, caused a dose-dependent reduction in the degree of mucosal damage and the fall in BP induced by local intra-arterial infusion of Paf (25 ng kg^{-1} min^{-1} for 10 min) as shown in Table 2. With L-652,731 (2.5 mg kg^{-1}) the gastric damage induced by this dose of Paf was reduced by 93 ± 1.4% ($P < 0.01$) while BP was reduced by 73 ± 9 ($P < 0.01$).

The vehicle, DMSO (100 µl, i.v.) had no significant effect ($n = 6$, $P > 0.05$) on either the degree of gastric damage or fall in BP induced by intra-arterial infusion of Paf.

Effects of local intra-arterial Paf with intraluminal ethanol

In control rats receiving vehicle infused through the left gastric artery, the presence of 2 ml of 20% ethanol in the gastric lumen did not produce any macroscopically apparent damage to the gastric mucosa (Figure 4). However, when low doses of Paf (0.1–1 ng kg^{-1} min^{-1}) were infused locally for 10 min, areas of severe hyperaemia appeared, when observed 20 min following termination of the infusion. This hyperaemia was generally diffuse but in some cases there were local patches of more severe hyperaemia and haemorrhagic erosions. The degree of mucosal damage was dose-dependent and with the highest dose of Paf studied (1 ng kg^{-1} min^{-1}), 15.2 ± 3.1% of the total area of the mucosa was affected ($P < 0.01$ compared to control experiments with 20% ethanol alone). These lesions were limited to the corpus region, although the antrum was also affected in one out of five rats receiving Paf (1 ng kg^{-1} min^{-1}), with the damage to 2.6% of the mucosal area.

Discussion

In the present study, a technique for the local intra-arterial administration of substances to the rat

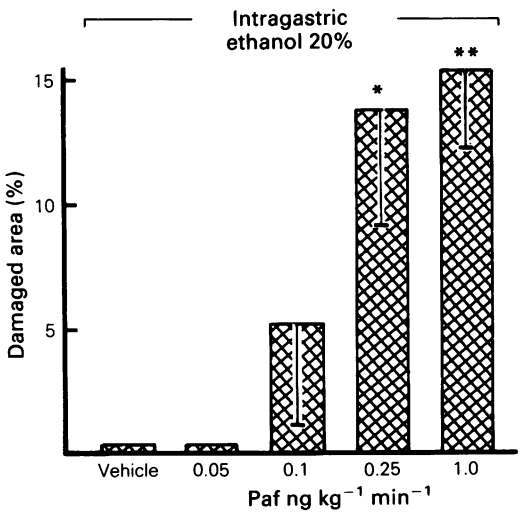

Figure 4 Potentiation of the gastric mucosal damage induced by intra-luminal instillation of 20% ethanol by local intra-arterial infusion of Paf for 10 min. Results, shown as the % area of total mucosal area that exhibited macroscopically assessed damage, are the mean of 5 experiments in each group; vertical lines show s.e.mean. Significant difference from control (vehicle-infused group) is given as *$P < 0.05$, **$P < 0.01$.

stomach *in vivo* has been developed. The left gastric artery, which supplies blood to the corpus and antral regions from the coeliac artery, was cannulated with a small flexible teflon cannula under a stereomicroscope (Figure 1). Despite interruption of the blood flow from this artery, the mucosa was still supplied with blood from the gastro-epiploic and hepatic arteries. Indeed, back flow of such blood from the left gastric artery, presumably derived predominantly from the extensive anastomosis between the submucosal vessels, was always assessed to confirm the patency of the cannula. Previous studies on blood flow in the rat gastric mucosa, using *in vivo* microscopy techniques 24 h following ligation of the left gastric artery, revealed that flow was still present in this area and was predominantly from the submucosal arterial plexus (Guth, 1972). In the current study, no macroscopic damage or histologically-visible disruption of the gastric mucosa was observed during the experimental period of ligation, or during an 80 min period of perfusion with vehicle through the left gastric artery. This technique thus allowed the study of the actions of the mediator, Paf, during close-arterial infusion. Studies with Evans Blue dye indicated that such an infusion was distributed throughout the gastric mucosa, in both antral and corpus regions.

Local intra-arterial infusion of Paf dose-dependently induced substantial gastric damage, located in the corpus mucosa as assessed macroscopically and histologically, which was characterized as vasocongestion and necrosis. Damage to the antral mucosa was also found on histological examination and resembled that found in the corpus. Since the threshold doses of Paf for the induction of histologically and macroscopically assessed corpus damage (5 and 10 ng kg^{-1} min^{-1}, respectively) only reduced BP by less than 20 mmHg, such gastric mucosal damage is unlikely to result from these minimal hypotensive actions. Furthermore, at intermediate doses, Paf (25 ng kg^{-1} min^{-1}) induced a comparable fall in BP following either intravenous or local intra-arterial infusion, yet only induced significant damage to the gastric mucosa following local intra-arterial administration, demonstrating a dissociation between these events. These findings would also indicate that at the higher doses of Paf, sufficient Paf reaches the systemic circulation to induce the hypotensive response, suggesting minimal metabolism of Paf during its passage through the gastric microcirculation. Previous studies have demonstrated that intravenous infusion of Paf substantially augmented the mucosal damage induced by topical application of acidified ethanol, using a rat *ex vivo* gastric chamber technique (Wallace & Whittle, 1986a). In the present study, local intra-arterial infusion of Paf in doses as low as 0.25 ng kg^{-1} min^{-1} significantly potentiated the macroscopic damage induced by intraluminal instillation of 20% ethanol. Since these doses of Paf induced no systemic hypotensive changes, these findings again demonstrate a direct action on the gastric mucosa, predisposing it to extensive damage by normally-weak irritants. A similar dissociation between hypotension and gastric ulceration induced by Paf has also been demonstrated in the dog. In that study, intra-arterial administration of Paf into the splenic artery supplying blood to the stomach, in doses having no effect on systemic cardiovascular parameters, induced local microcirculatory changes and extensive mucosal damage (Whittle *et al.*, 1987).

In previous studies, intravenous infusion of the structurally related precusor-breakdown product, lyso-Paf did not induce gastric damage, indicating that the effect of Paf was not simply a non-specific lytic action of a low-molecular weight phospholipid. This is further supported by the current finding that the Paf antagonist L-652,731 which has been shown to inhibit selectively the binding of radiolabelled Paf to specific binding sites *in vitro* (Hwang *et al.*, 1985), could substantially inhibit the degree of gastric damage induced by close-arterial infusion of Paf, as well as reduce the concurrent systemic hypotension. Earlier studies with L-652,731 have shown it to inhibit the effects of intravenously administered Paf on BP, neutrophil and leukocyte aggregation, increase in haematocrit and lysosomal enzyme release *in vivo* (Wu *et al.*, 1986). It has been proposed that the gastric damage evoked by Paf is a consequence of its effects on the mucosal microcirculation, inducing slowing and stasis of blood flow in the submucosal arteries and venules and the capillaries (Whittle *et al.*, 1986). Since Paf did not induce vascular constriction in these microvessels, it was considered that the extensive haemoconcentration and local white cell aggregation induced by Paf would prevent adequate blood flow and hence compromise the integrity of the mucosa (Wallace & Whittle, 1986b). The ability of L-652,731 to prevent such Paf-induced vascular and cellular events is thus likely to underlie its protective effects against the gastric mucosal damage.

The present finding that close-arterial administration of Paf can induce gastric mucosal damage gives support to the concept that its local release could play a role in the pathogenesis of gastric irritation and ulceration, including that observed during endotoxin shock (Rosam *et al.*, 1986; Wallace & Whittle, 1986c). Furthermore, the local release of Paf, along with other pro-inflammatory mediators such as leukotrienes induced by bacterial toxin, may account for the gastric and mucosal damage that is associated with colonization with *Campylobacter pyloridis* (Goodwin *et al.*, 1986). The present technique in the rat thus provides a useful model for the study of the local administration of substances on gastric mucosal integrity and can readily be adapted for the further exploration of the physiology and pathophysiology of gastric function.

J.V.E. is the recipient of a Fellowship from Conselleria de Cultura (Generalitat Valenciana, Spain). We thank Dr Chabala, Merck, Sharp and Dohme Research Laboratories, Rahway, for generously supplying L-652,731.

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(Received June 26, 1987.
Revised August 17, 1987.
Accepted September 7, 1987.)