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Effect of platelet agonists on airway reactivity and intrathoracic platelet accumulation

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1 Intravenous infusion of platelet activating factor (Paf), adenosine diphosphate (ADP), collagen and the thromboxane-mimetic U46619 induced a dose-related accumulation of ^{111}indium-labelled platelets into the thoracic region of anaesthetized guinea-pigs.

2 Intravenous infusion of Paf increased the reactivity of the airways to the spasmogen histamine. Such changes were not observed following treatment with ADP, collagen or U46619.

3 Paf-induced bronchial hyperreactivity is not secondary to pulmonary platelet recruitment, changes in basal lung function or cardiovascular changes.

4 Paf-induced bronchial hyperreactivity is not dependent upon the endogenous generation of ADP or thromboxane.

Introduction

Bronchial hyperreactivity is a characteristic feature of asthma (Hargreave, 1981), whereby asthmatics have an increased bronchoconstrictor response to a wide range of stimuli compared to normal healthy individuals. The pathological mechanisms underlying bronchial hyperreactivity are not fully understood, but may be related to inflammatory events occurring within the lung (Fabbri, 1985).

Platelet activating factor (Paf) is a product of a variety of inflammatory cell types, and has been proposed to be a mediator of inflammation and asthma (Morley *et al.*, 1984). In particular, Paf has been shown to induce a non-specific increase in bronchial reactivity to a wide range of spasmogens, in both experimental animals (Mazzoni *et al.*, 1985; Chung *et al.*, 1986; Barnes *et al.*, 1987) and man (Cuss *et al.*, 1986). In guinea-pigs, Paf-induced bronchial hyperreactivity has been shown to be dependent upon the presence of circulating platelets, and to be associated with a pulmonary recruitment of platelets (Mazzoni *et al.*, 1985; Deeming *et al.*, 1986).

The aim of the present study was to examine further the relationship between platelet activation and bronchial hyperreactivity. The ability of Paf to induce bronchial hyperreactivity and pulmonary platelet accumulation has been compared with other platelet agonists (ADP, collagen and the stable thromboxane-mimetic, U46619; Coleman *et al.*, 1981). Pulmonary recruitment of ^{111}indium labelled platelets has been continuously monitored *in vivo* by means of external scintillation detectors (Page *et al.*, 1982) concomitantly with monitoring changes in airways reactivity to the spasmogen histamine.

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Methods

Animals

Dunkin-Hartley guinea-pigs of either sex (350–600 g) were used throughout this study.

Airway responses in vivo

Guinea-pigs were anaesthetized with urethane (7 ml kg^{-1}, 25% w/v, i.p.) and the trachea, carotid artery and jugular vein cannulated for measurement of airway obstruction, systemic blood pressure and for the introduction of drugs respectively. Guinea-pigs were ventilated with room air at 70 strokes min^{-1} with a stroke volume of 1 ml 100 g^{-1}. Airways obstruction was measured by use of a differential pressure transducer connected to the side-arm of the tracheal cannula and expressed as intrathoracic pressure (ITP) in mmHg. Animals were administered bolus i.v. injections of histamine (10 μg kg^{-1}) at 10 min intervals. When consistent responses to this dose of histamine were obtained, animals were given a graded i.v.

infusion (5% for 10 min, 20% for 20 min, 75% for 30 min of the total dose received; total infusion time being 1 h) of Paf (600 ng $kg^{-1} h^{-1}$), ADP (1 or 10 mg $kg^{-1} h^{-1}$), collagen (0.5, 1 or 2 mg $kg^{-1} h^{-1}$), U46619 (5, 10 or 20 $\mu g\, kg^{-1} h^{-1}$) or 0.25% bovine serum albumin (BSA) saline (vehicle for Paf). Ten min after cessation of the infusion, guinea-pigs were rechallenged with i.v. histamine (10 $\mu g\, kg^{-1}$). Results are expressed as the change in airways response to histamine (10 $\mu g\, kg^{-1}$) in mmHg from the response obtained before infusion of the platelet agonist (Figure 1).

Preparation of labelled platelets

Blood was collected by cardiac puncture into 3.8% trisodium citrate (9:1v/v). After centrifugation at 200 *g* for 10 min, the platelet rich plasma (PRP) was transferred into round-bottomed polypropylene centrifuge tubes. The volume was made up to 10 ml with calcium-free Tyrode solution containing prostaglandin E_1 (PGE_1) 300 ng ml^{-1} (CFTPG). The platelets were then pelleted by centrifugation at 640 *g* for 10 min. The supernatant was discarded and the pellet washed free of plasma. The platelets were resuspended in 1.5 ml CFTPG. ^{111}Indium oxine (25–50 μCi) was added to the platelet suspension and incubated at 37°C for 1.5 min. The platelets were then pelleted again by centrifugation at 640 *g* for 10 min, and the free ^{111}indium oxine decanted. The platelet pellet was washed with CFTPG and the radiolabelled platelets finally resuspended in 1 ml CFTPG.

Administration and monitoring of labelled platelets

Radiolabelled platelets were administered into the jugular vein of guinea-pigs (anaesthetized with urethane) at least 30 min before studying the airway responses to histamine. The circulating platelets were continuously monitored in the thoracic region by use of 1 inch crystal scintillation probes located as close to the external surface of the thorax as possible so as not to interfere with basal respiration. The counts were estimated with a dual channel gamma-spectrometer (Nuclear Enterprises, NE4681) and logged with the aid of a special application interface within a microcomputer (IBM PC). This automated isotope monitoring system (AIMS 8000; Mumed Ltd.) permits sequential observations of predetermined duration to be recorded, and in this study, platelet counts were summated at 1 min intervals throughout the infusion period and for the following 30 min. The results are expressed as a % change in the counts in the thoracic region from those obtained immediately prior to infusion of the platelet agonist.

Materials

Histamine diphosphate salt, adenosine diphosphate (ADP) and bovine serum albumin (fraction V, essentially fatty acid-free) were purchased from Sigma and dissolved in physiological saline. PGE_1 (Sigma) was dissolved in ethanol. U46619 (11,9-epoxymethano-PGH_2) (Upjohn) was dissolved in saline, collagen (Horm Chemie) was diluted in isotonic glucose and Paf (Nova Biochem) was dissolved in 0.25% BSA saline buffer. ^{111}Indium oxine was purchased from Amersham International plc.

Statistics

Data are given as the mean ± s.e.mean of *n* observations. Significance was assessed by the non-parametric tests, the Mann-Whitney U test and the Wilcoxon signed-ranks matched-pairs test. A *P* value of less than 0.05 was considered significant.

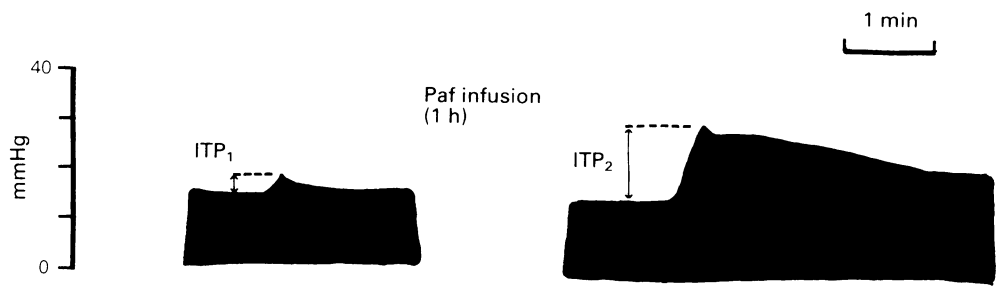

Figure 1 Representative trace showing the increase in airways obstruction (ITP) in response to histamine (10 $\mu g\, kg^{-1}$) before and after an infusion of Paf (600 ng $kg^{-1} h^{-1}$). The change in airway reactivity is expressed as $ITP_2 - ITP_1$ in mmHg.

Results

Intravenous infusion of Paf (600 ng $kg^{-1} h^{-1}$) induced a progressive accumulation of platelets into the thoracic region of guinea-pigs as shown in Figure 2. Such changes were not observed following infusion of the vehicle for Paf (BSA saline) (Figure 2). Minimal cardiovascular changes were observed in response to the infusion of Paf. A dose-related pulmonary platelet accumulation was observed following i.v. infusion of ADP or collagen (Figures 2 and 3). ADP (10 mg $kg^{-1} h^{-1}$) and collagen (0.5 and 1 mg $kg^{-1} h^{-1}$) gave a similar % increase (15%) in the thoracic platelet count during the infusion period, compared to that obtained with Paf (600 ng $kg^{-1} h^{-1}$). In contrast, the thromboxane-mimetic, U46619, was unable to induce an increase in thoracic platelet counts comparable to Paf (Figure 4) even at a high dose of 20 μg $kg^{-1} h^{-1}$, which produced substantial cardiovascular changes and increased basal ITP.

The thoracic accumulation of platelets was rapidly reversible following cessation of the infusion of ADP or U46619. However, following cessation of the infusion of Paf or collagen, platelets remained in the thoracic region for a longer period than following treatment with ADP. At the time of the histamine challenge post-infusion (70 min in Figure 3), the % increase in platelets in the thoracic region was similar in Paf- and collagen- (1 mg $kg^{-1} h^{-1}$) treated animals.

Associated with the pulmonary platelet recruitment following Paf infusion, there was an increase in the airway reactivity to histamine (10 μg kg^{-1}) as shown in Figure 5. Such an increase in airway responsiveness was not seen in vehicle-treated guinea-pigs (Figure 5). Infusion of ADP, collagen or U46619 (except at the high dose of 20 μg $kg^{-1} h^{-1}$) did not elicit increased

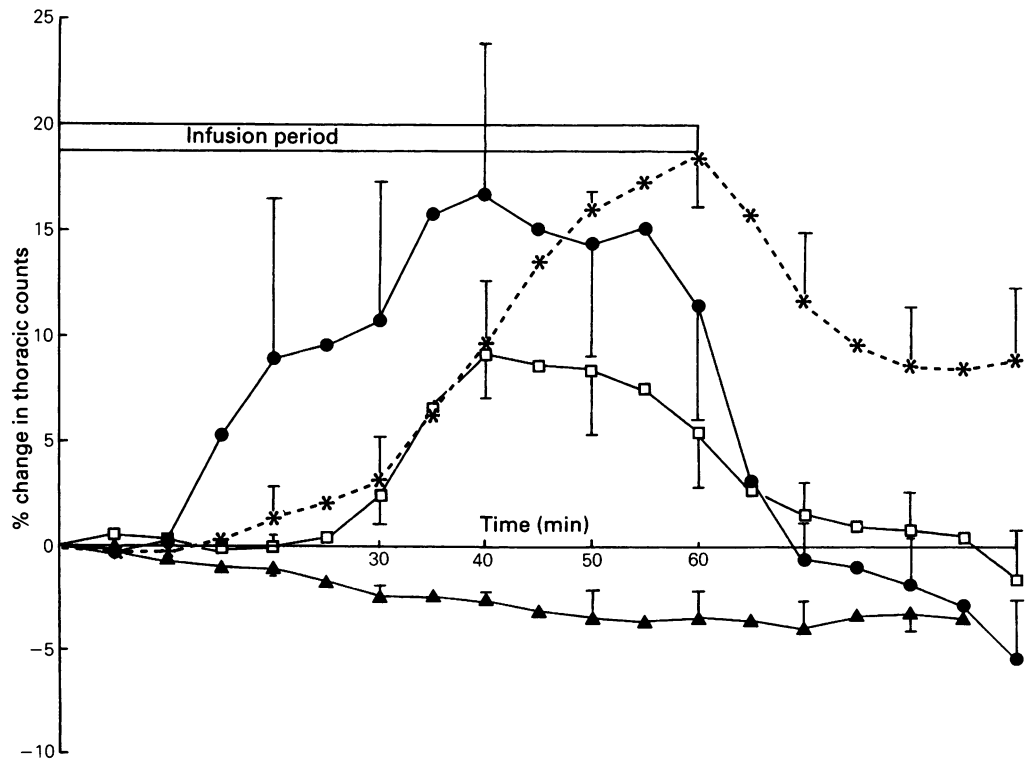

Figure 2 Percentage change in thoracic content of ^{111}indium oxine-labelled platelets following i.v. infusion of Paf (600 ng $kg^{-1} h^{-1}$) (★), ADP 1 mg $kg^{-1} h^{-1}$ (□) and 10 mg $kg^{-1} h^{-1}$ (●) or BSA saline buffer (▲). The infusion period was for 1 h and recordings made for a further 30 min in groups of $n = 5$ animals. Both Paf (600 ng $kg^{-1} h^{-1}$) and ADP (1 and 10 mg $kg^{-1} h^{-1}$) elicited significant increases at 60 min in comparison with BSA treatment ($P < 0.05$, Mann-Whitney U test). At 90 min, only Paf remained significantly different from BSA treatment ($P < 0.05$, Mann-Whitney U test). Furthermore, the response elicited at 60 min by ADP (10 mg $kg^{-1} h^{-1}$) is not significantly different from the response to Paf ($P < 0.05$, Mann-Whitney U test).

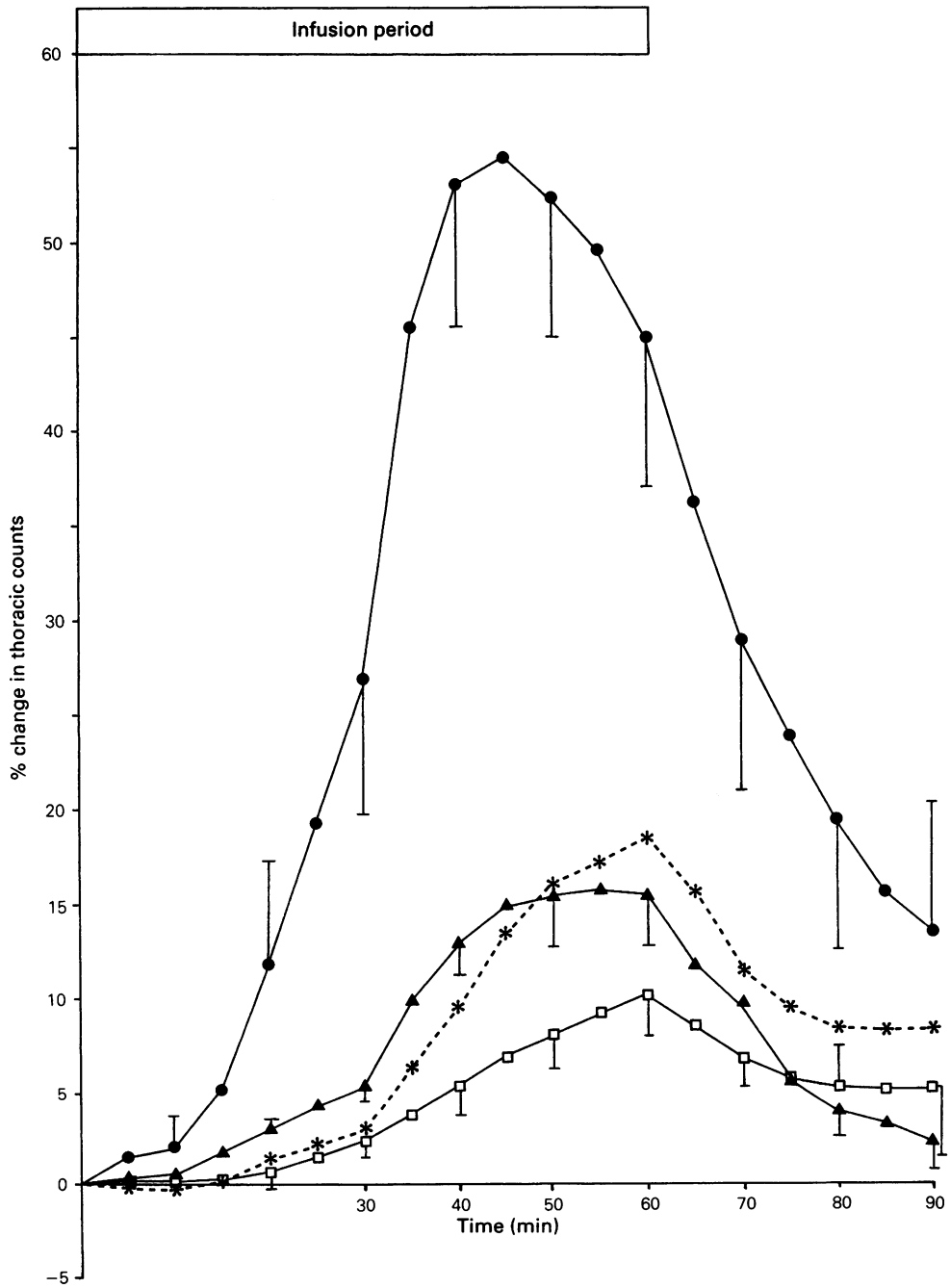

Figure 3 Percentage change in thoracic content of ^{111}indium oxine-labelled platelets following i.v. infusion of collagen 0.5 mg kg^{-1} h^{-1} (□) ($n = 5$), 1 mg kg^{-1} h^{-1} (▲) ($n = 7$) and 2 mg kg^{-1} h^{-1} (●) ($n = 3$), in comparison with Paf (600 ng kg^{-1} h^{-1}) (as in Figure 2 [★]). Both Paf (600 ng kg^{-1} h^{-1}) and collagen (0.5, 1 and 2 mg kg^{-1} h^{-1}) elicited significant increases at 60 and 90 min in comparison with BSA-treatment ($P < 0.05$, Mann-Whitney U test). Furthermore, the response elicited by collagen (0.5 and 1 mg kg^{-1} h^{-1}) is not significantly different from the response elicited by Paf (600 ng kg^{-1} h^{-1}) ($P < 0.05$. Mann-Whitney U test).

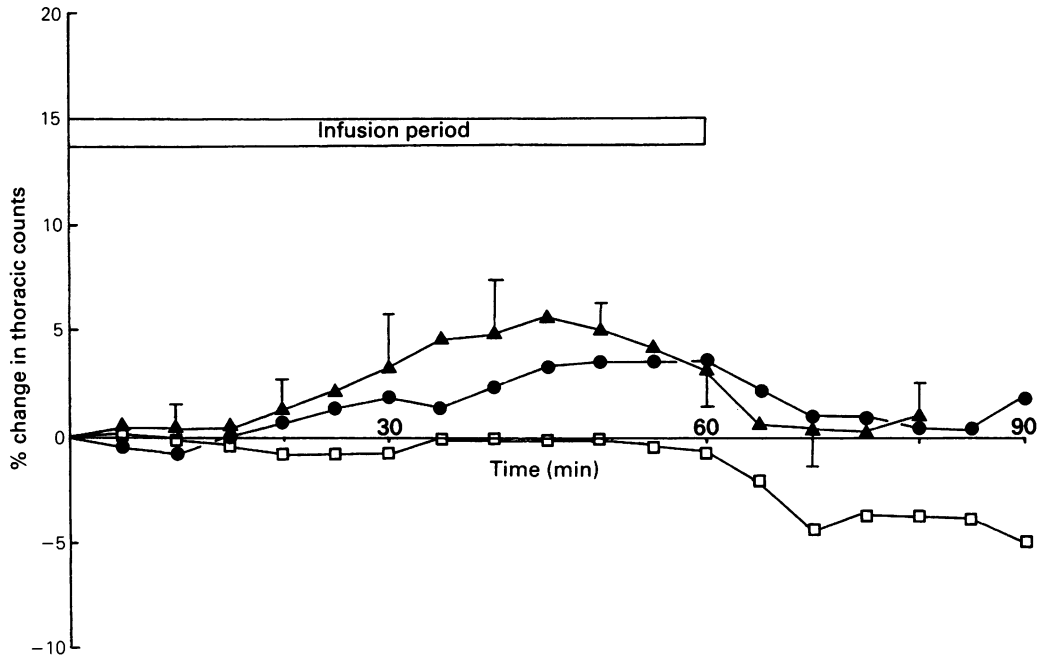

Figure 4 Percentage change in thoracic content of ^{111}indium oxine-labelled platelets following i.v. infusion of thromboxane-mimetic U46619 5 $\mu g\,kg^{-1}\,h^{-1}$ (□) ($n = 2$), 10 $\mu g\,kg^{-1}\,h^{-1}$ (●) ($n = 2$) and 20 $\mu g\,kg^{-1}\,h^{-1}$ (▲) ($n = 4$). U46619 (20 $\mu g\,kg^{-1}\,h^{-1}$) was significantly different from BSA at 60 min and this dose produced significantly less platelet accumulation than Paf (600 $ng\,kg^{-1}\,h^{-1}$) ($P < 0.05$, Mann-Whitney U test).

bronchial reactivity when compared to the BSA saline response, and ADP at 10 $mg\,kg^{-1}\,h^{-1}$ induced a decrease in the airways responsiveness to histamine (Figure 5).

Discussion

Paf has recently gained attention as a possible mediator of asthma as it can reproduce many of the characteristic features of this disease (Morley *et al.*, 1984; Cuss *et al.*, 1986) eliciting both bronchoconstriction (Vargaftig *et al.*, 1980; Cuss *et al.*, 1986) and long-lasting inflammatory changes in the lung (Camussi *et al.*, 1983). Exposure of both experimental animals and man to Paf induces a non-selective and long-lasting increase in bronchial reactivity to spasmogens (Mazzoni *et al.*, 1985; Chung *et al.*, 1986; Cuss *et al.*, 1986) and in guinea-pigs, Paf-induced bronchial hyperreactivity has been shown to be platelet-dependent (Mazzoni *et al.*, 1985). The present results confirm earlier observations that i.v. infusion of Paf to anaesthetized guinea-pigs induces an increase in the airway responsiveness to histamine (Mazzoni *et al.*, 1985; Barnes *et al.*, 1987). Further, they show that Paf at concentrations sufficient to induce an increased responsiveness of the airways to exogenous histamine will induce an associated recruitment of platelets into the thoracic region. This platelet recruitment is not readily reversible over the period of study and 30 min after the end of the infusion, there is still significant retention of platelets in the thoracic region.

The ability of the platelet agonist ADP, collagen and U46619 to induce comparable (and in the case of collagen greater) increases in pulmonary platelet recruitment, yet to have no effect on airway responsiveness suggests that platelet recruitment *per se* is not the sole determinant of increased airways responsiveness induced by Paf. These observations further suggest that endogenously generated ADP or thromboxane are unlikely to contribute to the increased airway responsiveness induced by Paf. Furthermore, as U46619 produced significant cardiac effects and increased basal ITP yet still had no effect on airways responsiveness, it is unlikely that Paf-induced bronchial hyperreactivity is secondary to changes in systemic blood pressure or changes in baseline lung function.

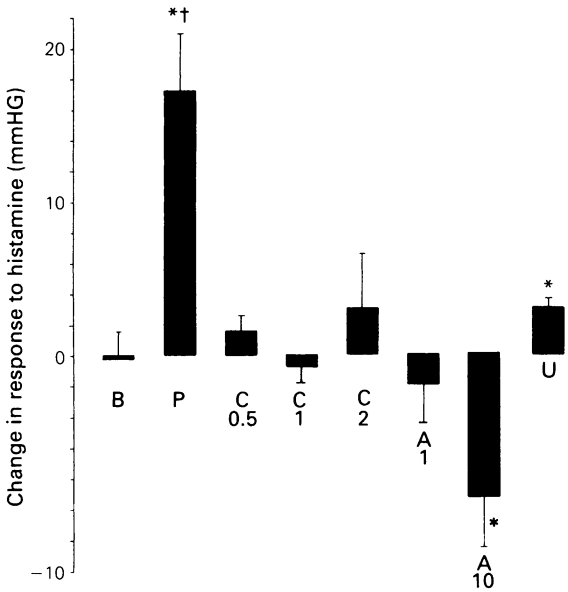

Figure 5 Change in airway reactivity to histamine after an infusion of BSA buffer (B), Paf (600 ng kg^{-1} h^{-1}; P), collagen (0.5–2 mg kg^{-1} h^{-1}; C), ADP (1 and 10 mg kg^{-1} h^{-1}; A) or U46619 (20 μg kg^{-1} h^{-1}; U). *$P < 0.01$ (significant difference in response to histamine comparing before and after infusion; Wilcoxan signed-ranks, matched-pairs test), †$P < 0.01$ (significant difference from BSA buffer response; Mann Whitney U test).

The pulmonary retention of platelets beyond the end of the Paf infusion may be related to the observation that Paf can induce extravascular diapedesis of platelets into pulmonary tissues (Lellouch-Tubiana *et al.*, 1985), such platelets being unlikely to return to the circulation. ADP is not able to induce such extravascular diapedesis (Lellouch-Tubiana *et al.*, 1985) and our observations are consistent with this finding as ADP-induced pulmonary platelet recruitment is rapidly reversible after cessation of the infusion. It is not known whether collagen induces extravascular platelet recruitment but in the present study, collagen also induces retention of platelets in the thoracic region beyond the infusion period. However, the inability of collagen to induce bronchial hyperreactivity would suggest that the prolonged retention of platelets within the thoracic region is also unlikely to be the central determinant of increased reactivity of the airways.

The precise activity of platelets contributing to platelet-dependent bronchial hyperreactivity induced by Paf in the guinea-pig remains unknown. However, Paf is known to affect cell types other than platelets and thus it is possible that airway hyperresponsiveness is secondary to activation of another cell type additional to the platelet. Paf has been shown to affect vascular endothelium (Morley *et al.*, 1983) and will elicit oedema formation in the airways (Evans *et al.*, 1987), a phenomenon recently suggested to be important for the induction of airway hyperresponsiveness (Persson, 1986). Furthermore, Paf is able to elicit both eosinophil (Arnoux *et al.*, 1985; Lellouch-Tubiana *et al.*, 1985) and neutrophil recruitment (Camussi *et al.*, 1983) into the lung. It has been suggested that both eosinophils (Frigas & Gleich, 1986) and neutrophils (Fabbri, 1985) may play a role in the induction of airway hyperresponsiveness in asthma and it is therefore possible that Paf-induced bronchial hyperreactivity involves activation of eosinophils or neutrophils in addition to platelets. These inflammatory properties of Paf have not been demonstrated with ADP, collagen or the thromboxane agonists *in vivo* which may explain why only Paf is able to induce airway hyperresponsiveness.

Platelets have classically been considered as blood elements involved in thrombosis and heamostasis, but there is now evidence to suggest that platelets may contribute to non-thrombotic conditions such as allergic inflammation (Capron *et al.*, 1985) and asthma (Morley *et al.*, 1984). Thus, the observation that Paf is the only platelet agonist able to induce bronchial hyperreactivity suggests that the relationship between Paf, platelets and other inflammatory cells in the context of bronchial hyperreactivity warrants further investigation.

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