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The effect of platelet activating factor on pulmonary β-adrenoceptors

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1 An intravenous infusion of platelet activating factor (Paf) in the guinea-pig elicits an increase in bronchial responsiveness to the spasmogens, histamine and bombesin.

2 Airways obstruction induced by bombesin in Paf-treated animals is poorly reversed by isoprenaline compared to comparable airways obstruction induced by bombesin in vehicle-treated animals.

3 Isoprenaline induced a comparable dose-related relaxation *in vitro* of tracheal smooth muscle isolated from Paf- and vehicle-treated animals.

4 No change in β-adrenoceptor numbers or binding affinity was observed in lungs removed from Paf-treated animals in comparison with those from vehicle-treated animals, or after direct incubation with Paf *in vitro*.

5 The reduced bronchodilator responsiveness to isoprenaline in Paf-treated animals is not related to changes in pulmonary β-adrenoceptor function. These results suggest that non-spasmogenic elements may contribute to airways obstruction induced in hyper-responsive animals.

Introduction

Platelet activating factor (Paf, 1-*o*-alkyl-2-acetyl-sn-glyceryl-3-phosphorylcholine) is an ether-linked phospholipid with several biological properties which may be relevant to the pathogenesis of asthma (Morley *et al.*, 1984), including the induction of a long-lasting non-specific increase in bronchial responsiveness in both experimental animals (Mazzoni *et al.*, 1985a; Chung *et al.*, 1986) and man (Cuss *et al.*, 1986). In the guinea-pig, Paf-induced bronchial hyper-responsiveness has a pharmacological sensitivity reminiscent of antigen-induced bronchial hyper-reactivity in sensitized asthmatics, in that the development of hyper-responsiveness is prevented by four classes of prophylactic anti-asthma drugs (disodium cromoglycate, ketotifen, methylxanthines and glucocorticosteroids) but is not inhibited by β_2-adrenoceptor agonists (Mazzoni *et al.*, 1985b). Furthermore, the airways obstruction induced by spasmogens in Paf-treated animals is poorly reversed by β_2-adrenoceptor agonists, although the mechanism for this reduced sensitivity to β-adrenoceptor agonists has not been investigated. Preliminary evidence suggests that exposure of cerebellar tissue to Paf *in vitro* results in a loss of β_2-adrenoceptor binding sites, which can be prevented by prior incubation with a Paf antagonist (Braquet *et al.*, 1985). This suggests that Paf is able to down-regulate β_2-adrenoceptors and if such a phenomenon operates in the lung this could provide a plausible explanation for the loss of sensitivity to β_2-adrenoceptor agonists in Paf-treated animals. The present study has investigated the effect of Paf on β-adrenoceptor function in the lungs and airways of guinea-pigs both *in vitro* and *in vivo*. Preliminary results of this study have been presented to the British Pharmacological Society (Barnes *et al.*, 1986).

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Methods

Animals

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

Airway responses in vivo

Guinea-pigs were anaesthetized with urethane (25% w/v, 7 ml kg^{-1}, i.p.) and the trachea, carotid artery and jugular vein cannulated for measurement of airway obstruction, systemic blood pressure and the introduction of drugs, respectively. Airway obstruction was

measured via a pressure transducer connected to the side-arm of the tracheal cannula and intrathoracic pressure (ITP) expressed in mmHg. Animals were given i.v. bolus injections of histamine or bombesin which elicited threshold changes in ITP. When ITP had returned to baseline, animals were given a graded i.v. infusion of Paf in 0.25% bovine serum albumin (BSA) (3 ng kg^{-1} min^{-1} for 10 min, 6 ng kg^{-1} min^{-1} for 20 min and 15 ng kg^{-1} min^{-1} for 30 min via a constant infusion pump; total dose received 600 ng kg^{-1} over 1 h) or vehicle alone (0.25% BSA). Animals were then rechallenged 2 min after cessation of the Paf infusion with the dose of histamine or bombesin which had previously elicited a threshold effect on ITP to reassess airway responsiveness.

At the height of the airways obstruction induced by bombesin in Paf-treated animals, cumulative doses of isoprenaline (0.1, 1.0, 10 $\mu g\,kg^{-1}$) were administered intravenously at 1 min intervals and the change in ITP recorded. In vehicle-treated animals, larger doses of bombesin were administered until a level of airways obstruction comparable to that achieved in Paf-treated animals was obtained. Cumulative doses of isoprenaline (0.1, 1.0, 10 $\mu g\,kg^{-1}$) were then administered intravenously at 1 min intervals for determination of the change in ITP. At the end of some experiments lungs were immediately excised from both Paf- and vehicle-treated animals and immersed in Krebs-Henseleit buffer, oxygenated with 95% O_2 and 5% CO_2, for subsequent determination of airway responses *in vitro* and for β_2-adrenoceptor binding studies. Reversal of ITP by isoprenaline has been expressed as % change from the peak ITP induced by bombesin in vehicle and Paf-treated animals. Data were analysed by Student's unpaired *t* test for comparision of vehicle- versus Paf-treated animals and by paired *t* test with a Bonferroni correction for multiple comparisons (Wallenstein *et al.*, 1980) in the analysis of the effects of isoprenaline within the vehicle- and Paf-treated animals.

Airway responses in vitro

Tracheas from vehicle- and Paf-treated guinea-pigs were opened longitudinally by cutting through the cartilage and were sectioned into transverse segments 5 mm wide. Hooks were inserted into the cartilage and segments were mounted in 10 ml organ baths containing Krebs-Henseleit solution at 37°C and gassed with 95% O_2 and 5% CO_2. The Krebs-Henseleit solution had the following composition (mM): NaCl 118, KCl 5.9, $MgSO_4$ 1.2, $CaCl_2$ 2.5, NaH_2PO_4 1.2, $NaHCO_3$ 25.5, glucose 5.6 and contained 10 μM indomethacin. Changes in tension were measured isometrically with Grass FT.03 transducers and were recorded on a Grass 7D polygraph. An initial tension of 1.0 g was applied to the tracheal segments as this was found to be optimal for measuring changes in tension, and a period of 1 h was allowed for equilibration.

Cumulative dose-response curves were constructed to histamine in both BSA- and Paf-treated tissues. In separate segments, two doses of histamine which elicited similar levels of contraction in BSA- and Paf-treated tissues were used to construct relaxation dose-reponse curves to isoprenaline. Log EC_{50} values and maximal response to each agent were analysed by Student's unpaired *t* test.

β-Adrenoceptor binding studies

Tracheas and large bronchi were separated from peripheral parenchyma and placed separately in buffer (Tris-HCl 25 mM, 0.9% NaCl solution (w/v), 1.1 mM ascorbic acid; pH 7.4). Membrane preparations were made as previously described (Barnes *et al.*, 1979). Briefly, peripheral lung tissue or tracheas were homogenized in ice-cold Tris-HCl containing 0.32 M sucrose, then centrifuged at 1800 *g* for 10 min to remove unhomogenized debris; the supernatant was centrifuged at 40,000 *g* for 20 min, and the resulting pellet washed and centrifuged again. The final pellet was resuspended in incubation buffer at a concentration of approximately 1 mg protein ml^{-1}. Protein content was determined by the method of Lowry *et al.* (1951).

For saturation studies, fresh membranes were incubated with [^{125}I]-(-)-iodocyanopindolol ([^{125}I]-CYP) at 37°C for 90 min in a final volume of 250 μl (final concentration, 0.4 mg ml^{-1} for parenchyma membranes and 0.2 mg ml^{-1} for airways membranes), and the concentration of ICYP was varied between 12 and 400 pM. Incubations were done in duplicate; non-specific binding was determined by performing the same incubation in the presence and absence of 400 μM (±)-isoprenaline. Incubation was terminated by rapid filtration through glass fibre filters (Whatman GF/C); after filtration, filters were washed with 3 × 5 ml ice-cold incubation buffer and counted in a gamma counter at an efficiency of 67%.

In order to determine the direct effect of Paf on lung β_2-adrenoceptors, peripheral lung tissue from guinea-pigs was chopped with a MacIlwain tissue chopper (Gomshall, Surrey) into 200 μm pieces and incubated for 45 min in Tris-NaCl at 37°C with and without Paf (0.1 μM). Tissue was then washed twice with buffer, and membranes were prepared and binding experiments performed as described previously.

Drugs

Bovine serum albumin (BSA), indomethacin, urethane, isoprenaline, histamine and acetylcholine (ACh) were all purchased from Sigma. Paf and bombesin

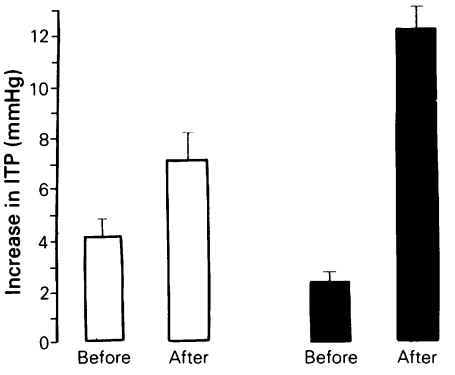

Figure 1 Peak increase in intrathoracic pressure (ITP) induced by histamine ($10\,\mu g\,kg^{-1}$) before and immediately following cessation of an infusion of Paf in 0.25% BSA ($600\,ng\,kg^{-1}$) (solid columns) or 0.25% BSA (open columns) over a period of 1 h. A significant increase in reactivity was observed following Paf infusion (paired t test, $P<0.001$). A small but significant increase in reactivity was observed following BSA infusion (paired t test, $P<0.005$). Increased reactivity induced by Paf was greater than that observed with BSA (Student's t test, $P<0.005$).

were obtained from Novabiochem AG, Switzerland and [^{125}I]-(−)-iodocyanopindolol ([^{125}I]-CYP: specific activity, 2000 Ci mmol^{-1}) was obtained from Amersham International plc.

Results

Effect of isoprenaline in vivo

The i.v. infusion of Paf ($600\,ng\,kg^{-1}\,h^{-1}$) elicited an increase in airway reactivity to threshold doses of histamine ($10\,\mu g\,kg^{-1}$) (Figure 1) or bombesin ($2\,\mu g\,kg^{-1}$) (Figure 2). An i.v. infusion of the phospholipid carrier molecule BSA also induced a small, but significant change in airway reactivity (Figure 1). In vehicle-treated animals, histamine induced an increase in ITP that reversed rapidly and spontaneously so that it was not possible to perform a relaxation dose-response study with isoprenaline. As it had been previously shown that ITP changes induced by the peptide bombesin were long lasting both in control and Paf-treated animals (Mazzoni *et al.*, 1985a), the effect of isoprenaline on reversal of increases in ITP was studied in animals where bombesin was utilised as a spasmogen. Bombesin ($5.7 \pm 0.7\,\mu g\,kg^{-1}$, $n = 7$) induced an increase in ITP in control animals comparable to bombesin ($3.0 \pm 0.6\,\mu g\,kg^{-1}$, $n = 6$) administered to Paf-treated animals (peak ITP response in control animals was 10.0 ± 1.6 mmHg, $n = 7$: 9.8 ± 2.1 mmHg in Paf-treated animals, mean ± s.e., $n = 6$, NS). Isoprenaline ($0.1-10\,\mu g\,kg^{-1}$) elicited a dose-related reduction in ITP induced by bombesin in vehicle-treated animals, whereas the same dose range of isoprenaline produced only minimal changes in ITP induced by bombesin in Paf-treated animals (Figure 3). Isoprenaline 0.1, 1.0 and $10.0\,\mu g\,kg^{-1}$ reversed the peak ITP to

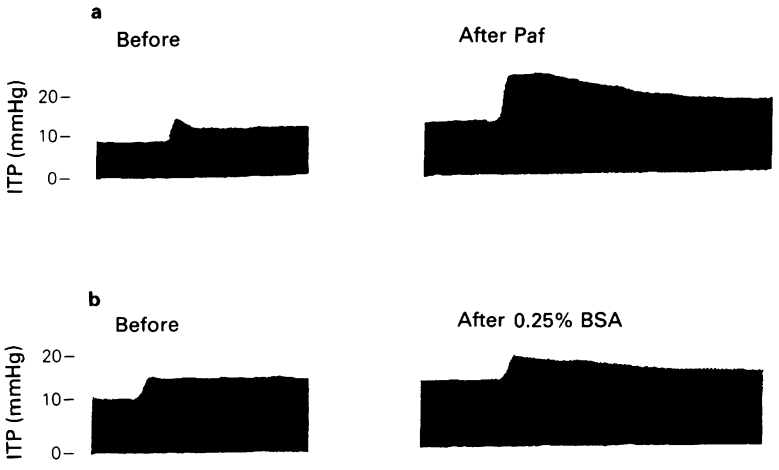

Figure 2 Representative traces of changes in intrathoracic pressure (ITP) following i.v. administration of bombesin ($2\,\mu g\,kg^{-1}$) before and after the infusion of Paf in 0.25% BSA ($600\,ng\,kg^{-1}$) (a) or 0.25% BSA (b) over a period of 1 h.

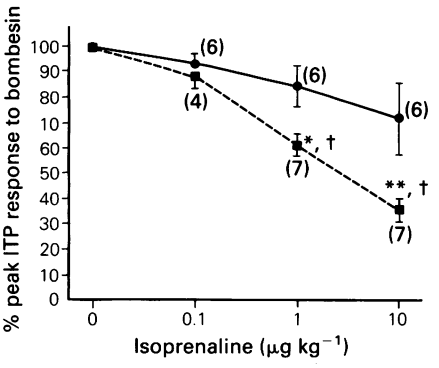

Figure 3 Dose-related reversal of bombesin-induced maximal change in intrathoracic pressure (ITP) by isoprenaline (0.1–10 $\mu g\,kg^{-1}$ i.v.) administered at 1 min intervals. Peak ITP change (100%) was not significantly different between Paf-treated (●) and vehicle-treated (■) animals. No significant reduction in the peak ITP change was observed in Paf-treated animals with any dose of isoprenaline utilized, whereas significant reductions in maximal ITP were observed in BSA-treated animals following 1 and 10 $\mu g\,kg^{-1}$ isoprenaline (*$P < 0.025$ and **$P < 0.005$, respectively; paired t test with a Bonferroni correction). Isoprenaline (1 and 10 $\mu g\,kg^{-1}$) gave a greater reduction in peak response induced by bombesin in vehicle-treated animals in comparision with Paf-treated animals (†$P < 0.025$, Student's t test). Mean results are shown of number of experiments in parentheses; vertical lines represent s.e.mean.

87.9 ± 5.1% ($n = 4$), 60.6 ± 4.5% ($n = 7$) and 35.2 ± 4.5% ($n = 7$) respectively in vehicle-treated animals and 93.3 ± 4.4% ($n = 6$), 84.4 ± 7.9% ($n = 6$) and 71.3 ± 13.6% ($n = 6$) in Paf-treated animals. At 0.1 $\mu g\,kg^{-1}$ there was no significant difference between vehicle- and Paf-treated animals, but significant differences were found at 1.0 $\mu g\,kg^{-1}$ ($P < 0.025$) and 10.0 $\mu g\,kg^{-1}$ ($P < 0.025$).

Airway reactivity in vitro

There was no difference in the ability of histamine to contract tracheal segments between BSA- and Paf-treated animals either in terms of maximal contraction elicited (expressed in g corrected for wet weight of tissue) or log EC_{50} (Table 1).

Two histamine concentrations were chosen for the subsequent evaluation of isoprenaline relaxation, 5×10^{-6} M which produced 61 ± 9% and 51 ± 9% of maximum histamine contraction in vehicle- and Paf-treated tissues, respectively, and 10^{-4} M which produced 100 ± 0% and 99 ± 1% of maximum histamine contraction in BSA- and Paf-treated tissues, respectively. At neither level of contraction was any difference in log EC_{50} or maximal relaxation to isoprenaline between vehicle- and Paf treated animals observed (Figure 4).

β-Adrenoceptor binding

In control animals, specific binding of [^{125}I]-CYP accounted for 75–90% of total binding at a concentration of 75 pM. Specific binding was saturable, reaching a maximum at 200 pM both in trachea and parenchyma. Scatchard analysis of the data showed a single population of receptors in parenchyma and trachea (Figure 5); the number of β_2-adrenoceptors was greater in parenchyma than trachea.

Neither the dissociation constants (K_D) nor maximal binding capacities (B_{max}) were significantly different in vehicle- or Paf-treated animals, either in parenchyma or trachea (Table 2). Similarly, in direct incubation experiments, no significant difference was found in K_D and B_{max} between control tissue and tissue incubated with Paf (Table 2 and Figure 5).

Discussion

The present study has confirmed the ability of Paf to induce a non-specific increase in airway responsiveness in the guinea-pig and has furthermore demonstrated that, in such hyper-responsive animals, isoprenaline is less effective as a bronchodilator. The ability of BSA to elicit a small, but significant increase in pulmonary responsiveness cannot be explained from the experiments performed: this may reflect a problem with prolonged experiments in anaesthetized

Table 1 Contractile responses of guinea-pig isolated trachea to histamine.

	Control ($n = 6$)	*Paf-treated* ($n = 7$)	*Statistical level*
Maximum contraction (g corrected for wet weight of tissue)	109 ± 14	85 ± 14	NS
− log EC_{50}	5.50 ± 0.16	5.37 ± 0.15	NS

Results are presented as mean ± s.e.mean.
NS: not significant.

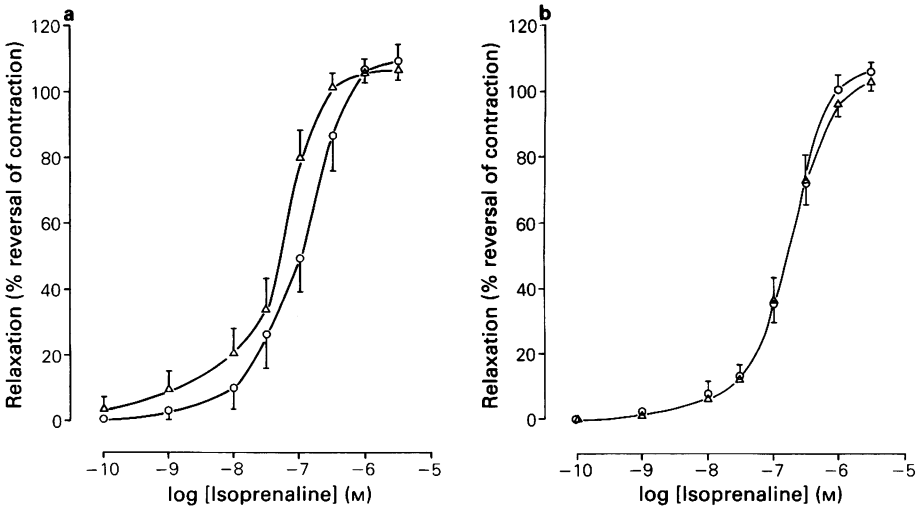

Figure 4 Relaxant effect of isoprenaline on tracheal segments obtained from BSA- (○) and Paf-treated (△) animals following precontraction with (a) 5×10^{-6} M histamine and (b) 10^{-4} M histamine. Points represent mean results of $n = 7$ for BSA- and $n = 6$ for Paf-treated animals. Vertical lines show s.e.mean.

animals ventilated with room air, since no such effect was observed in similar experiments using guinea-pigs ventilated with supplementary O_2 (Mazzoni *et al.*, 1985a,b). However, there was no reduced relaxant response to isoprenaline in tracheas taken from Paf-treated animals or vehicle-treated animals.

Pulmonary tissue from either control or Paf-treated animals has the same number of β-adrenoceptor binding sites, suggesting that the loss of sensitivity to isoprenaline is unlikely to be explained by a change in β-adrenoceptor binding sites. Our data also suggest that the previously described interaction between Paf and β-adrenoceptor binding sites may be tissue-specific since incubation conditions similar to those reported for cerebellar tissue (Braquet *et al.*, 1985) did not cause any change in pulmonary β-adrenoceptors. In this study we found that the β-adrenoceptor number was 3 fold greater in parenchyma than in trachea, which agrees with the previous results of Carswell & Nahorski (1983).

The present results indicate that the loss of sensitivity to isoprenaline in Paf-treated animals is unrelated to loss of β-adrenoceptor binding sites. The mechanism underlying the reduced sensitivity to isoprenaline in animals rendered hyper-responsive following exposure to Paf is unknown. However, in addition to being a potent spasmogen in experimental animals (Patterson & Harris, 1983) and man (Cuss *et*

Table 2 Binding characteristics of [^{125}I]-cyanopindolol to β_2-adrenoceptors in guinea-pig lung.

	Parenchyma		*Trachea*	
	B_{max} (fmol mg^{-1} protein)	K_D (pM)	B_{max} (fmol mg^{-1} protein)	K_D (pM)
Ex vivo				
Controls ($n = 6$)	233 ± 50	32 ± 40	112 ± 42	11 ± 2
Paf-treated ($n = 7$)	306 ± 50	46 ± 12	125 ± 30	13 ± 2
In vitro				
Controls ($n = 4$)	301 ± 31	30 ± 6		
Paf-treated ($n = 4$)	314 ± 32	31 ± 6		

Results are presented as mean ± s.e.mean.

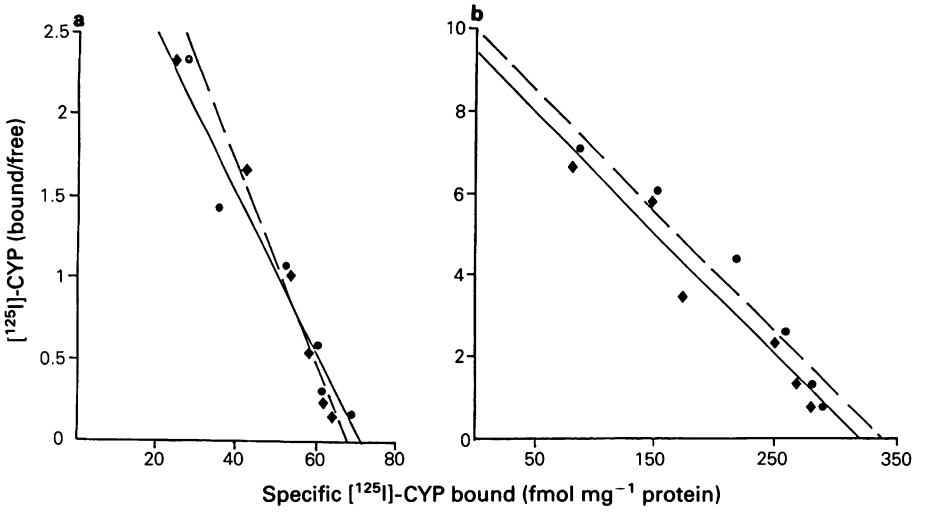

Figure 5 (a) Specific binding of [^{125}I]-cyanopindolol ([^{125}I]-CYP) to tracheal membranes obtained from control guinea-pigs (●) and Paf-treated animals (◆). (b) Specific binding of [^{125}I]-CYP to guinea-pig lung membranes. Chopped tissue was incubated for 45 min in the absence (●) and presence (◆) of 10^{-7} M Paf before the binding studies. In (a) and (b) data are expressed as a Scatchard plot. Data from a single experiment is shown which is representative of 7 experiments.

al., 1986), Paf is able to elicit increased vascular permeability in a variety of organs (Morley *et al.*, 1984) including the lung (Evans *et al.*, 1986) and to induce cellular infiltration in pulmonary tissue in which the predominant cell type is the eosinophil (Arnoux *et al.*, 1985; Lellouch-Tubiana *et al.*, 1985; McManus & Hass, 1986). As airway inflammation both accompanies and contributes to bronchial hyperresponsiveness in a variety of situations (Cockcroft, 1983; Metzger *et al.*, 1985; Fabbri, 1985), the reduced sensitivity to β-adrenoceptor agonists in hyper-responsive animals may be related to obstruction of the airways due to the infiltration of inflammatory cells and oedema which are not readily reversed by β-adrenoceptor agonists.

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