Effect of capsaicin on PAF-induced bronchial hyperresponsiveness and pulmonary cell accumulation in the rabbit

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1 Platelet activating factor (PAF), but not the carrier molecule bovine serum albumin (BSA) induced bronchoconstriction in the anaesthetized rabbit. This bronchoconstriction was not altered by prior treatment with capsaicin.

2 Rabbits demonstrated increased airways responsiveness to histamine 24 h after exposure to PAF but not to BSA. PAF failed to increase airways responsiveness to histamine in animals pretreated with capsaicin (80 mg kg⁻¹).

3 A significant increase in inflammatory cells was obtained in bronchoalveolar lavage (BAL) 24 h after PAF exposure in vehicle-treated rabbits. This was associated with an increase in the numbers of neutrophils and eosinophils. Capsaicin treatment inhibited the PAF-induced influx of inflammatory cells found in BAL, although this was not associated with an inhibition of PAF-induced pulmonary eosinophilia.

4 Capsaicin-induced motor effects were modest in epithelium-intact rabbit bronchial preparations, but were significantly enhanced in epithelium-denuded preparations in the presence of thiorphan. The contractile response to capsaicin was significantly inhibited in tissues exposed to a consecutive dose of capsaicin. Furthermore, ruthenium red abolished capsaicin-induced contraction in epithelium-denuded preparations.

5 Tissue content of calcitonin gene-related peptide-like immunoreactivity and substance P-like immunoreactivity was not reduced in bronchus and iris obtained from capsaicin-treated rabbits, although capsaicin-induced contractile responses in rabbit bronchus obtained from animals previously treated with capsaicin were significantly reduced. Furthermore, airway responses to histamine, methacholine and electrical field stimulation *in vitro*, were not altered by pretreatment of rabbits *in vivo* for 3 days with capsaicin.

6 In conclusion, PAF-induced airways responsiveness and pulmonary cell accumulation is inhibited by *in vivo* capsaicin pretreatment in the rabbit, via a mechanism that may not involve depletion of sensory neuropeptides.

Keywords PAF; bronchial hyperresponsiveness; capsaicin; sensory neuropeptides

Introduction

It is becoming increasingly apparent that platelet activating factor (PAF) may be an important mediator in the pathogenesis of asthma (Page, 1988) and it has been shown recently, that PAF induces an increase in airways responsiveness and recruitment of inflammatory cells in a number of species including the guinea-pig (Coyle *et al.*, 1988), rabbit (Coyle *et al.*, 1990a) and man (Cuss *et al.*, 1986). Neuropeptides including substance P, neurokinin A and calcitonin gene-related peptide (CGRP) may be localized to sensory nerves in the lung of a number of animal species including man (Lundberg *et al.*, 1984; Hua *et al.*, 1985; Palmer *et al.*, 1987; Hislop *et al.*, 1990) where they may play a physiological role in non-adrenergic non-cholinergic (NANC) transmission (Barnes, 1986). Furthermore, it has been suggested that stimulation of afferent sensory nerves by inflammatory mediators participate in an axon reflex involved in the pathogenesis of bronchial asthma (Barnes, 1986). Nonetheless, few studies have attempted to investigate formally the role of sensory neuropeptides in the pathogenesis of bronchial hyperresponsiveness and airways inflammation that characterize asthma.

Capsaicin, a chemical well recognized for its ability both to release acutely and deplete chronically the content of sensory

neuropeptides of a subset of primary afferent nerves in guinea-pigs and rats, has been widely used as a tool in these species to investigate the role of sensory neuropeptides in various biological processes (Maggi & Meli, 1988). Capsaicin has been shown to produce no protection (Ingenito *et al.*, 1989), partial protection (Saria *et al.*, 1983) or complete protection (Manzini *et al.*, 1987) against antigen-induced bronchospasm in guinea-pigs. Furthermore, antigen-induced bronchial hyperresponsiveness but not the pulmonary accumulation of eosinophils was inhibited in ovalbumin-sensitized guinea-pigs treated with capsaicin (Ladenius & Briggs, 1989). Similarly, toluene-diisocyanate (TDI)-induced bronchial hyperresponsiveness in guinea-pigs was inhibited by capsaicin pretreatment, although in this study no effect was seen on the pulmonary accumulation of polymorphonuclear leukocytes (Thompson *et al.*, 1987). Airways responsiveness to ovalbumin and 5-hydroxytryptamine was also reduced in ovalbumin-sensitized rats treated with capsaicin after but not before sensitization (Alving *et al.*, 1987).

Both rabbit and man are similar in the relatively sparse innervation by sensory nerves of their lungs (Laitinen *et al.*, 1983). Furthermore, capsaicin-induced contractile responses are modest in human bronchi *in vitro* (Lundberg *et al.*, 1983), a similar finding to that in the rabbit bronchus. Therefore, in the present study we have sought to investigate the contribution of sensory C-fibres in modulating PAF-induced bronchial

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hyperresponsiveness and cellular recruitment in the rabbit by investigating the effect of capsaicin pretreatment on these processes.

Methods

Capsaicin treatment

Rabbits were treated either with capsaicin (total dose of 80 mg kg⁻¹, s.c.) administered over a period of 3 days (5 mg kg⁻¹ on day 1, 50 mg kg⁻¹ on day 2, 25 mg kg⁻¹ on day 3) or with vehicle (ethanol 10%, Tween 10%, distilled water 80% s.c.). Both vehicle and capsaicin-treated rabbits received at intervals of 2 h, theophylline 2 mg kg⁻¹, atropine 1.2 mg kg⁻¹, diphenhydramine 2.5 mg kg⁻¹, and chlordiazepoxide 1.2 mg kg⁻¹ (i.p.). Vehicle or capsaicin was then injected 15 min after premedication. Lung function was assessed in rabbits 2 days after the last vehicle and capsaicin injection. In another series of experiments in which no lung function was performed in rabbits, *in vitro* experiments were performed 4 days after the last vehicle or capsaicin injection.

In vivo experiments

New Zealand White rabbits (3–3.5 kg) were anaesthetized with diazepam (5 mg ml⁻¹; 2.5 mg kg⁻¹, i.p.) and fentanyl citrate (0.315 mg ml⁻¹; 0.4 ml kg⁻¹, i.m.) and placed in a supine position. Rabbits were intubated with a 3.0 mm endotracheal tube which was connected to a pneumotachograph (Bore 00) and the flow determined by a Validyne pressure transducer. Pleural pressure was estimated by placing an oesophageal balloon in the lower third of the oesophagus to obtain the maximum end expiratory pressure. Thoracic pressure was measured by a sidehole catheter on the distal end of the tracheal tube. Transpulmonary pressure, the difference between thoracic and pleural pressure was measured using a differential pressure transducer (Validyne). The flow was integrated to obtain a continuous recording of tidal volume. Measurements of total lung resistance (RL) were calculated by a pulmonary mechanical analyser (Buxco, Model 6, Sharon, CT, U.S.A.) as described previously (Giles *et al.*, 1974). Anaesthesia was maintained throughout the course of the experiment by administration of 0.2–0.3 ml of fentanyl citrate intramuscularly every 30–40 min according to the protocol described by Fleck-nall (1987).

Assessment of airway responsiveness to histamine in vivo

Airway responsiveness to histamine was determined by exposing rabbits to increasing concentrations of histamine (1.25–80 mg ml⁻¹) administered to the lungs directly via an endotracheal tube. Aerosols were generated by a Devilbiss ultrasonic nebuliser which has previously been demonstrated to generate particles of which 80% are less than 0.5 µm in diameter.

On day 1, after measurement of baseline lung function, animals were exposed to an aerosol of sterile saline for 2 min immediately followed by lung function measurements. Increasing concentrations of histamine were administered, with each dose being given for 2 min until there was at least a doubling in RL. The concentration of histamine which produced a 50% increase in RL (PC₅₀) was determined for each animal and used as an index of airway responsiveness.

On day 2, vehicle and capsaicin-treated rabbits were re-anaesthetized and exposed to an aerosol of 0.25% bovine serum albumin (BSA) for 2 min, followed by aerosolised PAF (80 µg ml⁻¹) administered over a 1 h period as described by Coyle *et al.* (1990a). A group of vehicle-treated rabbits received an aerosol of BSA followed by exposure to an aerosol

of BSA containing the same concentration of ethanol as received by the PAF-treated animals (approx. 1%). In some animals, lung function was monitored for a 1 h period after exposure to BSA or PAF.

On day 3, increasing concentrations of histamine were administered to the anaesthetized rabbits and the PC₅₀ for histamine determined as on day 1.

Bronchoalveolar lavage

On completion of the concentration-effect curve to histamine on day 1 (pre) and day 3 (post), a bronchoalveolar lavage (BAL) was performed. Rabbits were lavaged by passing a cannula into the airways via the endotracheal tube until it was wedged at the first bifurcation. A volume of 3 ml of 10% normal rabbit serum in 0.9% sterile saline was injected and immediately aspirated from the lungs. Total cell counts were determined under light microscopy with an improved Neubauer haemocytometer. Cytospins were prepared, fixed and stained with Lendrum's stain. A total of 200 cells were counted differentially and classified as neutrophils, eosinophils or mononuclear cells based on standard morphological criteria.

In vitro experiments

Following the completion of a BAL on day 3 (described above), the rabbits were killed by exsanguination whilst under anaesthesia. The lungs were quickly removed and placed in cold oxygenated Krebs-Henseleit solution. Rabbit intrapulmonary bronchi (1–3 mm, i.d.) were dissected free of parenchymal tissue and visible blood vessels. Bronchial rings were suspended under 1 g tension in organ baths containing Krebs-Henseleit solution aerated with 95% O₂, 5% CO₂ and maintained at 37°C. Changes in isometric tension were measured with a Grass force-displacement transducer (FTO3C) and recorded on a Lectromed chart recorder. All bronchial preparations were allowed to equilibrate for 45 min and decreases in resting tension which may have occurred during this period were compensated for by readjustment of tension to 1 g. The bathing solution was changed every 15 min before the addition of pharmacological agonists.

Following equilibration in Krebs-Henseleit solution, bronchial preparations were chosen randomly and exposed to either methacholine (0.1–100 µM), histamine (1–100 µM), capsaicin (10–100 µM) or electrical field stimulation (EFS; 100 V, 0.5 ms, 0.1–30 Hz; 30 s duration). Furthermore, in all bronchial preparations exposed to capsaicin *in vitro*, a second application of capsaicin after 45 min was given to assess the effects of capsaicin-induced desensitization. In the case of the EFS studies, all bronchial preparations were preincubated for 30 min with indomethacin (5 µM) and propranolol (5 µM).

In other experiments, bronchi were obtained from animals in which no lung function was performed. New Zealand White rabbits (1.5–2.0 kg) were treated with vehicle or capsaicin as described above. Four days later, animals were killed with an overdose of ketamine (25 mg kg⁻¹, 0.5 ml kg⁻¹, i.v.). Main bronchi were dissected free from adhering lung tissue and bronchial rings were mounted on stainless steel hooks and suspended under 500 mg tension in a 5 ml organ bath containing Krebs-Henseleit solution as described above. In some preparations, the epithelium was removed by gentle rubbing of the luminal surface with a cotton wool applicator. The efficiency of this removal was confirmed histologically. Changes in isometric tension were measured with a Grass force-displacement transducer (FTO3C) and recorded on a Basile Unirecord 7050 polygraph. Each preparation was allowed to equilibrate for at least 30 min before the addition of pharmacological agonists. Indomethacin (5 µM) was present throughout the experiment. Thiorphan (10 µM) was added to the organ bath at least 10 min before administration of capsaicin

(100 μM), neurokinin A (NKA, 0.1 μM), substance P (1 μM) and calcitonin gene-related peptide (1 μM).

CGRP-LI and SP-LI radioimmunoassay

Calcitonin gene related peptide-like immunoreactivity (CGRP-LI) was assayed as previously described (Manzini *et al.*, 1989). Briefly, experimental samples or samples containing known concentrations of rat-CGRP standard were incubated for 48 h at 4°C with anti-human CGRP rabbit anti-serum (Peninsula CA, U.S.A.) that demonstrates 100% cross-reactivity toward both α and β rat-CGRP. Then [^{125}I]-human-CGRP (Amersham U.K.) was added and incubated for a further 48 h at 4°C. Separation of bound and free antigen was by double antibody precipitation. The lower detection limit was 2.5 fmol/tube.

Substance P-like immunoreactivity (SP-LI) was assayed as previously described (Geppetti *et al.*, 1987). Briefly, SP-standard or samples were incubated overnight at 4°C with rabbit anti-SP anti-serum and [^{125}I]-Bolton and Hunter conjugate SP (Amersham, U.K.). Bound and free antigen were separated by double antibody precipitation. The sensitivity of the assay was 1.1 fmol/tube. The anti-serum cross-reacts to 1% with NKA, 0.5% NKB and less than 0.1% with physalamin and eleodisin.

Analysis of results

Results from all studies were expressed as mean $\pm$ s.e.mean. Differences in the bronchoconstrictor responses to PAF were analysed with an unpaired Student's *t* test, and considered significant if $P < 0.05$. *In vivo* histamine potency derived from measurements of resistance (PC_{50}) was expressed as the derived PC_{50} (antilog PC_{50}) together with the lower and upper limits of the s.e.mean. The pre-post test differences for each group were analyzed with an analysis of variance (ANOVA) and the location of this difference was performed with an unpaired Student's *t* test (two-tailed) with a Bonferroni correction (Wallenstein *et al.*, 1980) and considered significant if $P < 0.05$.

The logarithm of the concentration of agonist or frequency of EFS which elicited a 50% maximal response was designated as $-\log_{10} \text{EC}_{50}$ (pD_2) or $-\log_{10} \text{EF}_{50}$ respectively. In the case of the EFS data, responses were expressed as a % of the maximum response to methacholine. Statistical analysis of agonist potency and EFS data was performed by ANOVA. In other experiments, motor responses were expressed as absolute increases in tension (mg) and as a % of the maximum response to methacholine or carbachol. The significance of differences between mean values was determined by the Wilcoxon test, Mann-Whitney U test or non-paired Student's *t* test, difference being considered significant if $P < 0.05$.

The variability of the results for the cell data was confirmed with a Bartlett's test for homogeneity of variance, and a logarithmic scale was found to be more appropriate. For the differential cells, analysis was performed on $\log_{10}(x + 0.005)$ because of recorded zero counts. Pre cell data were analysed by ANOVA to examine whether capsaicin treatment alone had an effect on cells in BAL. The pre-post test differences for each group were analyzed by ANOVA and the difference in means analyzed by a non-paired Student's *t* test with a Bonferroni correction and considered significant if $P < 0.05$.

Drugs

Calcitonin gene-related peptide, diphenhydramine hydrochloride, histamine dihydrochloride, histamine diphosphate, indomethacin, methacholine hydrochloride, neurokinin A, propranolol hydrochloride, ruthenium red, theophylline, thiorphan, Tween 80 (polyoxyethylenesorbitanmonooleate),

capsaicin (8-methyl-N-vanillyl-6-nonenamide), bovine serum albumin Grade V (endotoxin free) and normal rabbit serum were all obtained from Sigma (Poole, Dorset); PAF was purchased from Novabiochem (Nottingham, UK); chlordiazepoxide hydrochloride, diazepam (Roche, U.K.); fentanyl citrate (Janssen Pharmaceutical Ltd, U.K.). Composition of the Krebs-Henseleit solution was (mm): NaCl 117.6, NaHCO_3 25, D-glucose 11.1, KCl 5.4, MgSO_4 0.57, KH_2PO_4 1.03 and CaCl_2 2.5. Thiorphan was dissolved in 5% Na_2CO_3 , capsaicin in ethanol and all other drugs in Krebs-Henseleit solution.

Results

Effect of capsaicin treatment on PAF-induced bronchoconstriction

PAF (80 $\mu\text{g ml}^{-1}$) induced acute bronchoconstriction in vehicle-treated animals, with RL increasing by $100 \pm 15\%$ ($n = 4$). The PAF-induced bronchoconstriction was not altered in capsaicin-treated animals, as assessed by changes in RL ($135 \pm 25\%$, $n = 4$; $P > 0.05$). Vehicle-treated animals exposed to 0.25% BSA demonstrated a small but significant bronchoconstriction (RL $16 \pm 2\%$, $n = 3$; $P < 0.05$).

Baseline RL measurements in vehicle and capsaicin-treated animals were $15.2 \pm 1.7 \text{ cmH}_2\text{O l}^{-1} \text{ s}^{-1}$ ($n = 9$) and $14.7 \pm 2.2 \text{ cmH}_2\text{O l}^{-1} \text{ s}^{-1}$ ($n = 6$) respectively.

Effect of capsaicin treatment on PAF-induced bronchial hyperresponsiveness

Histamine induced a concentration-dependent increase in airways resistance in vehicle- and capsaicin-treated rabbits. Capsaicin treatment alone failed to alter significantly airway responsiveness to histamine (pre) ($P > 0.05$). A significant treatment effect was observed between post and prehistamine PC_{50} values for the 3 groups ($P < 0.005$). In vehicle-treated rabbits, the values of PC_{50} for histamine before and 24 h after exposure to aerosolized BSA were 20 mg ml^{-1} (12–32) and 17 mg ml^{-1} (13–23) respectively, ($n = 5$, Figure 1). However, there was a significant increase in airways responsiveness to histamine 24 h after PAF exposure compared with BSA exposure in vehicle-treated rabbits (pre PC_{50} 44.7 mg ml^{-1} (40–50); post PC_{50} 13.8 mg ml^{-1} (9–20), $n = 7$, Figure 1; $0.002 < P < 0.01$). However, in capsaicin-treated rabbits, PAF

Figure 1 Individual histamine lung resistance (RL) PC_{50} values before (pre) and 24 h after (post) aerosol exposure to bovine serum albumin (BSA) or platelet activating factor (PAF) in vehicle-treated (Veh) or capsaicin-treated (Caps) rabbits. Mean values are also shown and the vertical bars represent the lower and upper limits of the s.e.mean.

failed to increase significantly airways responsiveness to histamine compared with PAF exposure in vehicle-treated rabbits (pre PC₅₀ 35.1 mg ml⁻¹ (24–50); post PC₅₀ 39.4 mg ml⁻¹ (30–51), *n* = 6, Figure 1; 0.01 < *P* < 0.025).

Effect of capsaicin treatment on cell counts in bronchoalveolar lavage

Both total and differential cell counts were enumerated from bronchoalveolar lavage (BAL; Table 1). The percentage of neutrophils, eosinophils, and mononuclear cells recovered from BAL (pre) in vehicle-treated rabbits were 7 ± 4%, 1 ± 0.6% and 92 ± 10%, respectively (*n* = 11). Similarly, the percentages of neutrophils, eosinophils and monocytes recovered from BAL (pre) in capsaicin-treated rabbits were 21 ± 18%, 1.2 ± 0.9% and 78 ± 22%, respectively (*n* = 5). Capsaicin treatment alone failed to increase significantly the total number of cells recovered in BAL (*P* > 0.05), although a significant increase in the number of neutrophils was observed (0.02 < *P* < 0.05). A significant treatment effect was observed between post and pre total cell counts for the 3 groups (*P* < 0.005). Total cell counts were significantly elevated in BAL, 24 h after exposure to PAF compared with BSA in vehicle-treated rabbits (*P* < 0.005; Table 1). This was reflected by a significant increase in the number of neutrophils (0.02 < *P* < 0.05) and eosinophils (*P* < 0.005) but not monocytes (*P* > 0.05) in BAL.

The PAF-induced influx of total cells was significantly less in capsaicin-treated than in vehicle-treated rabbits (0.01 < *P* < 0.025), Table 1). Similarly, the PAF-induced influx of neutrophils was not observed in capsaicin-treated rabbits (*P* > 0.05; pre vs post). In contrast, there was no significant difference in the PAF-induced eosinophil influx in capsaicin-treated compared with vehicle-treated rabbits (*P* > 0.05).

Effect of capsaicin on in vitro smooth muscle function

In bronchi obtained from animals in which lung function was performed, methacholine, histamine and EFS produced a concentration/frequency-dependent contraction in rabbit intrapulmonary bronchi. The contractile potencies (pD₂) of histamine, methacholine and -log EF₅₀ values for electrical field stimulation (EFS) were not significantly different in preparations taken from vehicle or capsaicin-treated rabbits exposed to either BSA or PAF (*P* > 0.05, Table 2).

Table 2 Contractile potency (pD₂) of histamine, methacholine and -log EF₅₀ values for electrical field stimulation (EFS) in vehicle or capsaicin-treated rabbits exposed to either 0.25% bovine serum albumin (BSA) or platelet activating factor (PAF, 80 µg ml⁻¹)

	BSA/ Vehicle	PAF/ Vehicle	PAF/ Capsaicin
Methacholine	5.40 ± 0.09 (4, 17)	5.64 ± 0.18 (6, 23)	5.56 ± 0.08 (6, 29)
Histamine	4.67 ± 0.25 (3, 11)	4.73 ± 0.24 (6, 23)	4.70 ± 0.06 (5, 23)
EFS	0.484 ± 0.117 (4, 7)	0.514 ± 0.119 (7, 12)	0.774 ± 0.079 (7, 13)

Results expressed as mean ± s.e.mean. Numbers in parentheses indicate the number of rabbits followed by the total number of bronchial preparations used.

pD₂ values for histamine and methacholine and -log EF₅₀ values for EFS were not significantly different between groups (*P* > 0.05, ANOVA).

Capsaicin (10–100 µM) produced only modest contractions of bronchi from vehicle-treated rabbits (12 ± 3% methacholine *E*_{max}; *n* = 23 out of 41 bronchial preparations from 10 rabbits). In contrast, the response to capsaicin was significantly reduced when repeated a second time in these same preparations (3.5 ± 0.8%, *n* = 4 out of 33 preparations; *P* < 0.01, Wilcoxon rank test). Furthermore, capsaicin also produced contractions in bronchi from capsaicin-treated rabbits (3.7 ± 1.4%, *n* = 6 out of 21 bronchial preparations from 5 rabbits), although these were significantly smaller than in those from vehicle-treated rabbits (*P* = 0.025, Mann-Whitney U test).

In epithelium-intact bronchi obtained from animals in which no lung function had been performed, capsaicin (100 µM) produced a modest contractile response (13 ± 6% carbachol *E*_{max} in 4 out of 6 bronchial preparations) even in the presence of thiorphan (10 µM). In contrast, in epithelium-denuded thiorphan-pretreated preparations, capsaicin (100 µM) produced significantly greater contractions (29 ± 4%, *n* = 6, *P* < 0.05). Furthermore, capsaicin-induced contractile responses were significantly reduced in preparations from capsaicin-treated rabbits compared with those from vehicle-treated rabbits (14.0 ± 3.9%, *n* = 6 vs 30.5 ± 2.9%, *n* = 11;

Table 1 Number of cells recovered from bronchoalveolar lavage in vehicle-treated or capsaicin-treated rabbits before (pre) and 24 h after (post) aerosol exposure to bovine serum albumin (BSA, 0.25%) or platelet activating factor (PAF 80 µg ml⁻¹).

	× 10 ⁵ cells/ml			
	Total	Neutrophil	Eosinophil	Monocyte
BSA/Vehicle				
Pre	2.66 ± 0.40	0.30 ± 0.21	0.062 ± 0.028	2.30 ± 0.45
Post	2.54 ± 0.50	0.33 ± 0.26	0.006 ± 0.004	2.18 ± 0.60
PAF/Vehicle				
Pre	1.98 ± 0.28	0.055 ± 0.032	0.003 ± 0.003	1.94 ± 0.26
Post	11.8 ± 2.99*	6.71 ± 3.83~	0.565 ± 0.250*	4.56 ± 1.70
PAF/Capsaicin				
Pre	5.92 ± 2.31	1.25 ± 1.04 [#]	0.074 ± 0.056	4.62 ± 1.28
Post	5.64 ± 0.89†	1.43 ± 0.61 ^{NS}	0.502 ± 0.314 ^{NS}	3.76 ± 0.72

Results expressed as mean ± s.e.mean. Number of animals in each group are as follows: BSA/Vehicle, *n* = 5; PAF/Vehicle, *n* = 6; PAF/Capsaicin, *n* = 5. Capsaicin treatment significantly increased the number of neutrophils (pre) in BAL cf. vehicle treatment *0.02 < *P* < 0.05; non-paired *t* test). Analysis of the pre-post test differences revealed a significant difference in the 3 groups for total cells and eosinophils (*P* < 0.005).

PAF significantly increased the total number of cells, neutrophils and eosinophils recovered in BAL cf. BSA (**P* < 0.005, ~0.02 < *P* < 0.05 and **P* < 0.005 respectively, non-paired *t* test).

PAF-induced influx of total cells in BAL was significantly inhibited in capsaicin-treated cf vehicle-treated rabbits (†0.01 < *P* < 0.02, non-paired *t* test) but not eosinophils (^{NS}*P* > 0.05, non-paired *t* test). No significant increase in BAL neutrophils by PAF in capsaicin-treated rabbits (^{NS}*P* > 0.05, pre cf post; paired *t* test).

Figure 2 Tension generated in epithelium-denuded bronchi obtained from vehicle (a) or capsaicin-treated (b) rabbits to carbachol ($10\mu\text{M}$, open columns), capsaicin ($100\mu\text{M}$, cross-hatched column), neurokinin A ($0.1\mu\text{M}$, columns with crosses), substance P ($1\mu\text{M}$, stippled column) and calcitonin gene-related peptide ($1\mu\text{M}$, solid column) all in the presence of thiorphan ($10\mu\text{M}$) and indomethacin ($5\mu\text{M}$). *n* indicates the number of preparations tested. Response to capsaicin significantly reduced in bronchi from capsaicin-treated rabbits of vehicle-treated rabbits; * $P < 0.01$, non-paired *t* test

$P < 0.01$, Figure 2). Capsaicin pretreatment did not affect the responses to carbachol, substance P, CGRP or NKA (Figure 2).

The contractile responses of epithelium-denuded bronchial preparations to capsaicin were significantly inhibited by the presence of ruthenium red ($10\mu\text{M}$) ($30 \pm 6\%$ $n = 5$ vs $2 \pm 2\%$ $n = 6$, $P < 0.05$), whereas those to NKA ($0.1\mu\text{M}$) were not significantly affected by such treatment ($114 \pm 3\%$ vs $99 \pm 8\%$, $n = 6$, $P > 0.05$).

There were no significant differences in the tissue levels of CGRP-LI and SP-LI in bronchi and SP-LI in the iris from capsaicin-treated rabbits compared with the levels obtained in vehicle-treated rabbits ($P > 0.05$, non-paired *t* test, Table 3). In contrast, tissue levels of CGRP-LI in the iris from capsaicin-treated rabbits were significantly greater than in those from vehicle-treated rabbits ($P < 0.05$, Table 3).

Discussion

It has been demonstrated previously that in rabbit airways PAF can induce at least three effects: (a) platelet-dependent

(Coyle *et al.*, 1990a), histamine-mediated (Halonen *et al.*, 1985) bronchoconstriction; (b) non-specific increase in airways responsiveness to spasmogens (Nieminen *et al.*, 1989; Coyle *et al.*, 1990a) and (c) an influx of inflammatory cells in the lung as assessed by BAL (Coyle *et al.*, 1990a). Our present data confirm these results, and in addition indicate that *in vivo* capsaicin treatment significantly inhibits PAF-induced bronchial hyperresponsiveness and the recruitment of total inflammatory cells, while not significantly affecting PAF-induced bronchoconstriction.

Capsaicin has been used extensively in rats and guinea-pigs as a tool to investigate the function of primary afferent nerves and to reduce the levels of tissue sensory neuropeptides (Maggi & Meli, 1988). Its use has led to the suggestion that sensory neuropeptides may be important in a wide range of biological activities, including certain aspects of pulmonary pathophysiology. In the present study, we have demonstrated that bronchi removed from rabbits treated for 3 days with capsaicin, in contrast to those from vehicle-treated animals fail to contract *in vitro* to further stimulation by exogenously applied capsaicin. Furthermore, capsaicin-induced contractions were inhibited by pretreatment with ruthenium red, which has been proposed as a selective inhibitor of capsaicin-induced stimulation of sensory nerve endings (Maggi & Meli, 1988). Nevertheless, capsaicin pretreatment did not result in significant depletion of tissue neuropeptide levels in the rabbit (this study; personal communication J.-A. Karlsson).

A number of possibilities may explain the apparent dissociation between capsaicin-induced loss of function without loss in neuropeptide content. It is possible that part of the SP-LI and CGRP-LI detected in rabbit bronchi may be present in capsaicin-insensitive neuropeptide containing nerves, as has been demonstrated in the gut (Holzer *et al.*, 1980; Hua *et al.*, 1985), or that the majority of neuropeptides in sensory nerves exist in pools which are not sensitive to *in vivo* capsaicin treatment (Hakanson *et al.*, 1987), or that another neuropeptide may be involved. Alternatively, an impairment of sensory nerve function, without a reduction in neuropeptide content within the sensory nerve may occur (Lembeck & Donnerer, 1981; Maggi *et al.*, 1987; 1990a, b; Chahl, 1989). Intracameral injection of capsaicin into the eye of the rabbit induces miosis and hyperaemia which are not observed in rabbits treated neonatally or as adults with capsaicin (Butler & Hammond, 1980; Tervo, 1981). However, according to Tervo (1981), this loss of function in the eye is not associated with a loss in neuropeptide content, a finding confirmed in the present study. In contrast, capsaicin applied to rabbit saphenous nerve was accompanied by a reduction in neuropeptide content 9–12 days later, with a corresponding loss in function (Lynn & Shakhaneh, 1988). Whether this is a result of the much higher local concentrations of capsaicin around the nerve compared with that achieved within the eye or lungs following subcutaneous injection of capsaicin is not known. However, it does indicate that rabbit tissues have the potential to demonstrate a quantifiable loss in neuropeptide content when treated with capsaicin *in vitro* (Hakanson *et al.*, 1987; Lynn & Shakhaneh, 1988). However, a partial resistance of rabbit tissues to the excitatory actions of capsaicin has already been presented with regard to its effects on conduction in cutaneous nerves (Baranowski *et al.*, 1986), its inability to induce degeneration of primary afferents (Lynn & Shakhaneh, 1988) and its poor efficacy in producing desensitization (Amann & Lembeck, 1990). These studies indicate that, unlike rats and guinea-pigs, the rabbit is particularly resistant to the effects of capsaicin. However, whether this can account for the discrepancy between loss in function with no apparent loss in neuropeptide content is speculative. Lastly, capsaicin may produce an as yet unidentified non-specific action, although a non-specific effect of capsaicin on airway smooth muscle or nerves can be ruled out as contractile responses of bronchi induced by exogenously applied spasmogens or EFS were unchanged in tissues obtained from animals previously treated with capsaicin.

Table 3 Tissue levels of calcitonin gene-related peptide-like immunoreactivity (CGRP-LI) and substance P-like immunoreactivity (SP-LI) in bronchial and iridial tissue from rabbits pretreated with vehicle or capsaicin (80 mg kg^{-1})

	Bronchus (pmol g^{-1} wt weight)	Iris (pmol g^{-1} wt weight)	n
CGRP-LI			
Vehicle-treated	11.2 ± 1.5	35.8 ± 6.2	6
Capsaicin-treated	13.8 ± 2.1	$49.9 \pm 12.7^*$	6
SP-LI			
Vehicle-treated	0.96 ± 0.15	6.5 ± 1.1	6
Capsaicin-treated	1.01 ± 0.10	10.9 ± 1.3	6

Results expressed as mean $\pm$ s.e.mean. *n* represents the number of tissues tested.

Significant increase in CGRP-LI immunoreactivity in iridial tissue from capsaicin-treated compared with vehicle-treated rabbits: * $P < 0.05$, non-paired *t* test.

Capsaicin treatment alone did not alter airway responses to histamine *in vivo*. However, while it has been reported that responses to histamine are reduced in capsaicin-treated guinea-pigs (Biggs & Ladenius, 1990), it has also been reported that responses to muscarinic agonists are not affected (Thompson *et al.*, 1987; Biggs & Ladenius, 1990). Our data indicate that capsaicin does not mediate a non-specific effect on airway smooth muscle, nor does histamine induce bronchoconstriction by activation of sensory nerves. Against this background therefore, it is of interest that PAF-induced bronchial hyperresponsiveness to histamine was inhibited in rabbits treated for 3 days with capsaicin. Our results support previous observations that have demonstrated inhibition of TDI and antigen-induced bronchial hyperresponsiveness by capsaicin treatment of guinea-pigs (Thompson *et al.*, 1987; Ladenius & Briggs, 1989), although these authors concluded that sensory neuropeptides may be involved in the expression of bronchial hyperresponsiveness. The demonstration of a loss of function *in vitro*, as assessed by the lack of effect of capsaicin in treated animals, without a detectable loss in neuropeptide content, questions the involvement of the release of sensory neuropeptides in the PAF-induced increase in airways responsiveness observed *in vivo*.

We have also demonstrated in the present study that PAF induces an influx of inflammatory cells (both neutrophils and eosinophils) in the lung as assessed by BAL. We have also shown that this influx is inhibited in rabbits treated with capsaicin, an effect associated primarily with an inhibition of neutrophil accumulation. Whether this inhibition of neutrophil accumulation is responsible for the inhibition of the PAF-induced bronchial hyperresponsiveness by capsaicin remains to be established. Our previous work has suggested that both PAF (Coyle *et al.*, 1990a) and allergen-induced (Coyle *et al.*, 1990b) bronchial hyperresponsiveness in the rabbit are dependent upon platelet activation and associated with the eosinophil rather than neutrophil accumulation. Furthermore, PAF and allergen-induced eosinophil infiltration are platelet-

dependent, thus making it unlikely that the effects of capsaicin are mediated by alterations in platelet function, as this would have also resulted in a reduction in eosinophil infiltration, a finding not consistent with our present data with capsaicin-treated rabbits. Similarly, platelet depletion (Lellouch-Tubiana *et al.*, 1988), but not capsaicin-treatment (Ladenius & Biggs, 1989), inhibited antigen-induced pulmonary eosinophilia in allergic guinea-pigs.

The finding that capsaicin treatment failed to inhibit either allergen-induced influx of eosinophils in guinea-pig lung (Ladenius & Briggs, 1989), or PAF-induced pulmonary eosinophil accumulation in the rabbit (present study) suggest that eosinophil recruitment *per se* may not be responsible for the expression of bronchial hyperresponsiveness. This is supportive of data obtained in other species (Aoki *et al.*, 1988; Sanjar *et al.*, 1990). However, the activation status of the eosinophils in this study was not evaluated, and thus we cannot rule out the possibility that as we (Coyle *et al.*, 1990a, b) and others (Frigas & Gleich, 1986) have previously proposed, the eosinophil may still be important for the expression of PAF-induced bronchial hyperresponsiveness in the rabbit via the release of cytotoxic mediators. Nonetheless, the apparent dissociation between eosinophil accumulation and bronchial hyperresponsiveness suggested by our present data is also supported by other recent experimental results showing that in guinea-pigs, cytokines can cause pulmonary eosinophilia without bronchial hyperresponsiveness (Chapman *et al.*, 1990) and in man, chronic eosinophilic bronchitis is not associated with hyperresponsiveness (Gibson *et al.*, 1989).

In summary, the present results suggest that PAF-induced bronchial hyperresponsiveness and the infiltration of inflammatory cells (in particular neutrophils) may be inhibited by capsaicin pretreatment, but that these inhibitory effects may be independent of depletion of sensory neuropeptides.

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