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Capsaicin pre-treatment prevents the development of antigen-induced airway hyperresponsiveness in neonatally immunised rabbits

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Abstract

The effect of a 3-day pre-treatment regime of capsaicin (8-methyl-*N*-vanillyl-6-nonenamide) (80 mg/kg s.c.) on airway changes induced by *Alternaria tenuis* aerosol challenge 3 days later was assessed in adult rabbits immunised from birth to the age of 3 months. Pre-treatment with capsaicin did not alter basal lung function or basal responsiveness to inhaled histamine. While capsaicin had no significant effect on the acute bronchoconstriction induced by antigen, this dose was sufficient to significantly inhibit the increase in airway responsiveness to inhaled histamine achieved 24 h following antigen challenge. The pulmonary recruitment of neutrophils and eosinophils induced by antigen was unaltered by prior treatment with capsaicin. In vitro contractile responsiveness to methacholine was not significantly different in bronchial tissues removed from capsaicin- and vehicle-pre-treated rabbits. In addition, there were no significant differences in responses to methacholine in preparations denuded of epithelium. Contraction of bronchial tissue induced by exogenously applied capsaicin in vitro, although modest, was significantly inhibited in capsaicin-pre-treated animals. In vehicle-pre-treated rabbits, contraction induced by a second challenge with capsaicin 45 min later was significantly reduced to a level that made responses not significantly different from those obtained in capsaicin-pre-treated tissues. The results of the present study demonstrate that antigen-induced airway hyperresponsiveness to inhaled histamine in immunised rabbits is inhibited by prior treatment with capsaicin. These findings suggest the involvement of capsaicin-sensitive nerves in antigen-induced airway hyperresponsiveness but not acute bronchospasm or cell infiltration induced by antigen.

Keywords: Airway hyperresponsiveness; Capsaicin; Sensory nerve; Inflammation; (Rabbit)

1. Introduction

Asthma is a disease characterised by a variety of features including reversible airway obstruction, increased airway responsiveness to various stimuli, and airway inflammation. The response of the neonatally immunised rabbit to antigen has been shown to mimic key aspects of the allergic asthmatic response, including the acute and late-onset airways obstruction (Larsen et al., 1987; Shampain et al., 1982), exacerbations of airway responsiveness (Bloom et al., 1988; Herd et al., 1994b) and pulmonary inflammatory cell infiltration (Marsh et al., 1985; Herd et al., 1994a). The allergic rabbit has therefore been used to investigate mechanisms contributing to such airway changes.

Sensory neuropeptides, including the tachykinins substance P and neurokinin A, and calcitonin gene-related peptide (CGRP) are stored and released from a subset of primary afferent neurones in the mammalian (including human) lung (Wharton et al., 1979; Lundberg et al., 1984; Hua et al., 1985; Palmer et al., 1987; Martling et al., 1988; Saria et al., 1988; Hislop et al., 1990). It has been proposed that these endogenous peptide mediators may play an important role in non-adrenergic, non-cholinergic (NANC) transmission in the lung (Barnes, 1986; Barnes et al., 1990). Furthermore, it has been suggested that stimulation of afferent sensory nerves by inflammatory mediators participates in an axon reflex involved in the pathogenesis of asthma (Barnes, 1986).

The tachykinins are potent bronchoconstrictor and vasodilator substances which have been shown to increase vascular permeability and mucus secretion in

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the airways, to stimulate cholinergic nerves, and to modulate the function of several inflammatory cells (see Barnes et al., 1991; Joos et al., 1994). Capsaicin (8-methyl-*N*-vanillyl-6-nonenamide) has been used extensively as a tool to investigate the function of sensory nerves in various experimental models (see Holzer, 1991) as it has been shown to release neuropeptides following acute administration and to deplete neuropeptides following chronic administration (see Maggi and Meli, 1988). Studies with capsaicin have demonstrated an involvement of sensory neuropeptides in pulmonary pathophysiology.

Rabbits treated with capsaicin at birth then immunised according to a previously described protocol (Minshall et al., 1993) have been shown to be less responsive to the bronchoconstrictor effects of inhaled histamine at 3 months of age (Riccio et al., 1993). This finding suggests that airway hyperresponsiveness in rabbits neonatally immunised may involve capsaicin-sensitive nerves. In guinea-pigs, capsaicin has been shown to inhibit (Saria et al., 1983; Manzini et al., 1987) or to have no effect (Ingenito et al., 1991) against antigen-induced bronchoconstriction and to have no effect against antigen-induced microvascular leakage (Lötvall et al., 1991). However, in some studies antigen-induced airway hyperresponsiveness was inhibited by capsaicin treatment (Alving et al., 1987; Ladenius and Biggs, 1989; Matsuse et al., 1991), although this has not been observed by all investigators (Ahlstedt et al., 1986). It appears from these findings that the influence of capsaicin depends on the immunization procedure, the species studied, and the capsaicin treatment protocol.

In the present study we have investigated the effects of in vivo capsaicin pre-treatment on antigen-induced bronchoconstriction, airway hyperresponsiveness and pulmonary cell infiltration in the neonatally immunised rabbit to ascertain the contribution of capsaicin-sensitive neurones in these events.

Preliminary findings of this work have been reported elsewhere (Herd and Page, 1994; Herd et al., 1994a).

2. Materials and methods

2.1. Animals

New Zealand White (NZW) rabbits (Froxfield Farms, Petersfield, Hampshire, UK) of either sex were used throughout the study. The immunisation protocol of neonatal rabbits has been previously described (Minshall et al., 1993). In brief, rabbits were injected intraperitoneally (0.5 ml) within 24 h of birth with *Alternaria tenuis* extract in aluminium hydroxide ($Al(OH)_3$) moist gel and saline in the ratio of 2:1:1. Antigen and adjuvant administration was repeated weekly for the first month and then biweekly for the following 2 months. The methods described in this study were subject to Home Office approval and performed under the Animals (Scientific Procedures) Act, 1986.

2.2. Capsaicin pre-treatment

Rabbits were treated with capsaicin (total dose of 80 mg/kg s.c.) or vehicle (10% ethanol, 10% Tween 80 and 80% 0.9% saline) administered over a 3-day period (5 mg/kg s.c. on day 1, 50 mg/kg s.c. on day 2 and 25 mg/kg s.c. on day 3). The volume of injection was 1 ml/kg.

2.3. Pulmonary function measurements

Immunised, adult rabbits (1.65–3.30 kg) were premedicated with ketamine hydrochloride (35 mg/kg i.m.) and xylazine (5 mg/kg i.m.). Neuroleptanalgesia was maintained throughout the course of the experiment by administration of ketamine hydrochloride (10 mg/kg i.m.) every 15–30 min (Flecknall, 1987). Animals were intubated with a cuffed endotracheal tube (3.0 mm internal diameter; Mallinckrodt Laboratories, Athlone, Ireland), which was connected to a heated (37°C) Fleisch pneumotachograph (size 00). Measurements of flow, pleural pressure, transpulmonary pressure (the difference between thoracic and pleural pressure) and tidal volume were made according to that previously described (Minshall et al., 1993). Total lung resistance (R_L) and dynamic compliance (C_{dyn}) values were calculated by an on-line respiratory analyser (Pulmonary Monitoring System (PMS) Version 5.1 Mumed, London, UK).

2.4. Experimental protocol

Lung function experiments were begun 3 days after the final capsaicin administration. On day 1, airway responsiveness to aerosolised histamine was determined as a measure of lung function. Following each 2 min aerosol of histamine, animals were disconnected from the ultrasonic nebulizer (Ultra-Neb 99; DeVilbiss Health Care, Heston, Middlesex, UK) and attached to the Fleisch tube. The following 10 breaths were recorded and the mean value calculated. Cumulative dose-response curves were established and the provocation concentration (PC) of histamine which produced 50% increase in R_L (PC_{50}) and 35% decrease in C_{dyn} (PC_{35}) was determined for each rabbit. In response to histamine, the maximum response for R_L and minimum response for C_{dyn}, the maximal percentage change in R_L and the minimal percentage change in C_{dyn}, together with the calculated parameters of R_L (PC_{50})

and C_{dyn} (PC_{35}) were used as indices of airway responsiveness. On day 2 antigen challenge was performed. Each antigen challenge consisted of a 4 min aerosol of saline followed by 5 consecutive aerosols of 4 min duration (total of 20 min) of *Alternaria tenuis* (20000 PNU/ml), after which time respiratory function was recorded as described above. On day 3, airway responsiveness to histamine was determined as on day 1. In separate control experiments, immunised rabbits were pre-treated with either vehicle or capsaicin and subjected to the same experimental protocol as described above, except that they were challenged with a comparable aerosol of 0.9% saline on day 2 instead of antigen.

2.5. In vitro experiments

Rabbits pre-treated with vehicle or capsaicin and subsequently challenged with antigen aerosol were further studied in vitro. One to 5 days following completion of the in vivo study rabbits were killed by an overdose of sodium pentobarbital (100 mg/kg i.v.), the lungs quickly excised and placed in cold, carbogenated Krebs-Henseleit solution. Intrapulmonary bronchi (2–5 mm internal diameter) were dissected free of parenchymal tissue and visible blood vessels. Four bronchial rings per rabbit were prepared, 2 of which had the epithelium removed by gentle rubbing of the luminal surface with stainless steel forceps. The bronchial rings were mounted under 1 g tension in 8 ml volume organ baths containing Krebs-Henseleit solution aerated with 95% O_2, 5% CO_2 and maintained at 37°C. Indomethacin (1 μM) was present in the Krebs-Henseleit solution throughout the experiment. 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 any decreases in resting tension 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 agents. Cumulative dose-response curves to methacholine (0.01–100 μM) were performed, followed by a single administration of capsaicin (100 μM). A second challenge of capsaicin (100 μM) was performed 45 min later to demonstrate capsaicin-induced desensitisation. The neutral endopeptidase inhibitor thiorphan (10 μM) was added to the organ bath at least 10 min prior to the administration of capsaicin.

2.6. Bronchoalveolar lavage

Bronchoalveolar lavage was performed immediately following histamine challenge on days 1 and 3. Saline (5 ml) was injected into the lungs through a polyethylene catheter (via the endotracheal tube) and then immediately aspirated and collected. Both total and differential cell counts were enumerated from bronchoalveolar lavage fluid as previously described (Minshall et al., 1993; Herd et al., 1994a).

2.7. Analysis of results

Bartlett's test for homogeneity of variances was used on all data to determine whether parametric or non-parametric statistics were to be applied. Statistical analysis was performed on $\log_{10}$ transformed PC_{50} and PC_{35} data (for lung function studies). Student's *t*-test for unpaired data was used to analyse the bronchoconstriction data (R_L and C_{dyn}) (expressed as percentage change from baseline response). Paired *t*-tests were used for the histamine lung function data before and after antigen challenge within vehicle and capsaicin-treated groups and unpaired *t*-tests were used for data between groups. Bronchoalveolar lavage cell data were analysed using the Kruskall-Wallis one-way analysis of variance and distribution-free multiple comparisons were then used to determine differences between groups. Unpaired *t*-tests were applied to the in vitro data. Results were considered significant if P values were < 0.05.

2.8. Drugs and chemicals

The drugs and chemicals used were: *Alternaria tenuis* extract (batch No. M1-152-7P16; 40000 PNU/ml, 1 mg/ml; Greer Laboratories, Lenoir, NC, USA); aluminium hydroxide moist gel (FSA Laboratory Supplies, Loughborough, Leicestershire, UK); capsaicin (8-methyl-*N*-vanillyl-6-nonenamide), chromotrope 2R, histamine diphosphate, indomethacin, methacholine hydrochloride, thiorphan, Tween 80 (polyoxyethylene-sorbitanmonooleate) (Sigma Chemical Co., Poole, Dorset, UK); haematoxylin (BDH Chemicals, Poole, Dorset, UK); Ketalar (ketamine hydrochloride, Parke-Davis, Pontypool, Gwent, UK), Rompun (23.32 mg/ml of 2-(2,6-xylidino)-5,6-dihydro-4*H*-1,3-thiazine-hydrochloride (equivalent to 20 mg xylazine)); sterile pyrogen-free 0.9% sodium chloride solution (saline; Baxter Healthcare, Thetford, Norfolk, UK). All reagents were of analytical grade.

3. Results

3.1. Baseline lung function

Baseline absolute values of airway resistance (R_L) or dynamic compliance (C_{dyn}) were not significantly different between vehicle and capsaicin-treated groups challenged with either saline (Table 1a) or antigen

Table 1
Baseline lung function prior to and 24 h following (a) control saline challenge, (b) antigen challenge in immunized rabbits pretreated with vehicle or capsaicin

		R_L (cm $H_2O/(l^{-1}\ s^{-1})$)		C_{dyn} (ml (cm $H_2O)^{-1}$)	
		Pre	24 h Post	Pre	24 h Post
(a)					
Vehicle	($n=6$)	29.2 ± 2.5	36.9 ± 2.8	3.7 ± 0.5	3.7 ± 0.6
Capsaicin	($n=6$)	34.0 ± 3.4	36.4 ± 4.4	4.0 ± 0.3	3.4 ± 0.4
(b)					
Vehicle	($n=10$)	30.4 ± 1.5	31.5 ± 1.4	4.3 ± 0.3	4.8 ± 0.3
Capsaicin	($n=10$)	29.0 ± 2.2	32.2 ± 1.4	4.0 ± 0.3	4.4 ± 0.3

Values represent mean ± S.E.M. for total lung resistance (R_L) and dynamic compliance (C_{dyn}) for the number of animals shown in parentheses.

(Table 1b) aerosol on either experimental day. The combined mean values are R_L: 30.4 ± 1.1 cm $H_2O/l/s$ ($n = 32$); C_{dyn}: 3.9 ± 0.2 ml/(cm H_2O) ($n = 32$).

3.2. Baseline airway responsiveness

No significant difference was observed between baseline airway responses to inhaled histamine, either maximum absolute R_L or C_{dyn} values (Table 2a), maximal percentage change in R_L or C_{dyn} (Table 2b) or PC_{50} R_L or PC_{35} C_{dyn} (Table 3a and Table 3b), at 3 months in rabbits pre-treated with vehicle or capsaicin. PC_{35} C_{dyn} values for the capsaicin-treated animals were lower than those for the vehicle-treated group; however, this difference was not statistically significant (Table 3b). The combined mean values are PC_{50} R_L: 9.75 ± 1.19 mg/ml ($n = 32$); PC_{35} C_{dyn}: 12.79 ± 1.23 mg/ml ($n = 32$).

3.3. Acute bronchoconstriction

The acute bronchoconstriction induced by inhaled saline was not significantly different in animals pretreated with capsaicin or vehicle for either the R_L or C_{dyn} component of the response (R_L: vehicle 18.6 ± 5.8%, capsaicin 16.0 ± 4.4%; C_{dyn}: vehicle −8.3 ± 1.6%, capsaicin −8.2 ± 2.4% ($n = 6$)). Similarly, the bronchoconstriction induced by antigen was not significantly different in groups of immunised rabbits pre-treated with vehicle or capsaicin (R_L: vehicle 29.8 ± 8.3%, capsaicin 18.3 ± 6.2%; C_{dyn}: vehicle −22.9 ± 3.5%, capsaicin −26.8 ± 4.1% ($n = 10$)). Only

Table 2
(a) Maximal recorded R_L (cm $H_2O/l^{-1}\ s^{-1}$) and minimum recorded C_{dyn} (ml cm H_2O^{-1}), and (b) maximal percentage increase in recorded R_L and maximal percentage decrease in recorded C_{dyn} obtained in response to histamine aerosol prior to and 24 h following control saline or antigen challenge in immunized rabbits pretreated with vehicle or capsaicin

			R_L		C_{dyn}	
			Pre	24 h Post	Pre	24 h Post
(a)						
Saline	Vehicle	($n=6$)	68.85 ± 4.65	71.42 ± 3.52	1.68 ± 0.25	1.82 ± 0.16
	Capsaicin	($n=6$)	65.42 ± 5.76	70.12 ± 2.38	1.72 ± 0.21	1.66 ± 0.11
Antigen	Vehicle	($n=10$)	67.25 ± 6.60	77.12 ± 5.66	2.16 ± 0.18	1.95 ± 0.22
	Capsaicin	($n=10$)	67.96 ± 6.63	71.95 ± 6.05	1.90 ± 0.16	1.78 ± 0.16
(b)						
Saline	Vehicle	($n=6$)	118.42 ± 13.35	98.20 ± 13.27	−51.25 ± 7.31	−43.99 ± 4.10
	Capsaicin	($n=6$)	76.99 ± 11.35	87.80 ± 13.01	−49.89 ± 4.73	−43.96 ± 2.33
Antigen	Vehicle	($n=10$)	126.63 ± 22.68	142.88 ± 17.78	−47.04 ± 4.75	−59.41 ± 4.90
	Capsaicin	($n=10$)	127.13 ± 21.02	114.45 ± 14.38	−51.94 ± 4.40	−54.51 ± 4.68

Values represent mean ± S.E.M. for the number of animals shown in parentheses.

Table 3
Airway responsiveness to inhaled histamine prior to and 24 h following (a) control saline challenge, (b) antigen challenge in immunized rabbits pretreated with vehicle or capsaicin

		Histamine R_L PC_{50} (mg ml^{-1})		Histamine C_{dyn} PC_{35} (mg ml^{-1})	
		Pre	24 h Post	Pre	24 h Post
(a)					
Vehicle	($n=6$)	8.65 ± 1.43	9.73 ± 1.47	11.59 ± 1.51	9.55 ± 1.36
Capsaicin	($n=6$)	9.18 ± 1.17	10.05 ± 1.59	9.23 ± 1.44	8.20 ± 1.34
(b)					
Vehicle	($n=10$)	12.70 ± 1.51	5.77 ± 1.24 *	22.75 ± 1.59	8.57 ± 1.39 *
Capsaicin	($n=10$)	8.31 ± 1.34	9.91 ± 1.40	9.31 ± 1.39	9.91 ± 1.50

Values represent geometric mean ± S.E.M. for total lung resistance (R_L PC_{50}) and dynamic compliance (C_{dyn} PC_{35}) for the number of animals shown in parentheses. * $P < 0.05$ compared with Pre control group.

the percentage change in C_{dyn} following saline aerosol was significantly different from that achieved following antigen ($P < 0.05$).

3.4. Airway responsiveness to histamine

Airway responses to inhaled histamine, either maximum R_L or minimum C_{dyn} values (Table 2a), maximal percentage change in R_L or C_{dyn} (Table 2b) or PC_{50} R_L or PC_{35} C_{dyn} (Table 3a) were not significantly different in animals pre-treated with capsaicin or vehicle 24 h following saline challenge compared with pre-saline values. Following antigen challenge to rabbits pre-treated with vehicle, maximum R_L (Table 2a) and maximal percentage change in R_L (Table 2b) were increased compared with capsaicin-treated groups; however, this difference did not reach statistical significance. Neither minimum C_{dyn} values (Table 2a) nor maximal percentage change in C_{dyn} (Table 2b) were significantly different from values obtained pre-challenge. Histamine PC_{50} (R_L) values and histamine PC_{35} (C_{dyn}) values were significantly decreased 24 h later ($P = 0.0169$ and $P = 0.0039$, respectively) (Table 3b; Fig. 1a and Fig. 1b). In rabbits pre-treated with capsaicin, histamine PC_{50} (R_L) and histamine PC_{35} (C_{dyn}) values were not significantly altered 24 h following antigen challenge compared with pre values ($P = 0.6031$ and $P = 0.8404$, respectively) (Table 3b; Fig. 1a and Fig. 1b). Capsaicin significantly inhibited antigen-induced airway hyperresponsiveness 24 h following antigen challenge (R_L and C_{dyn}) compared with the vehicle-treated group ($P < 0.05$) (Table 3b; Fig. 1a and Fig. 1b).

3.5. Bronchoalveolar lavage

The percentages of fluid recovered from bronchoalveolar lavage were not significantly different between vehicle- and capsaicin-treated animals before or after

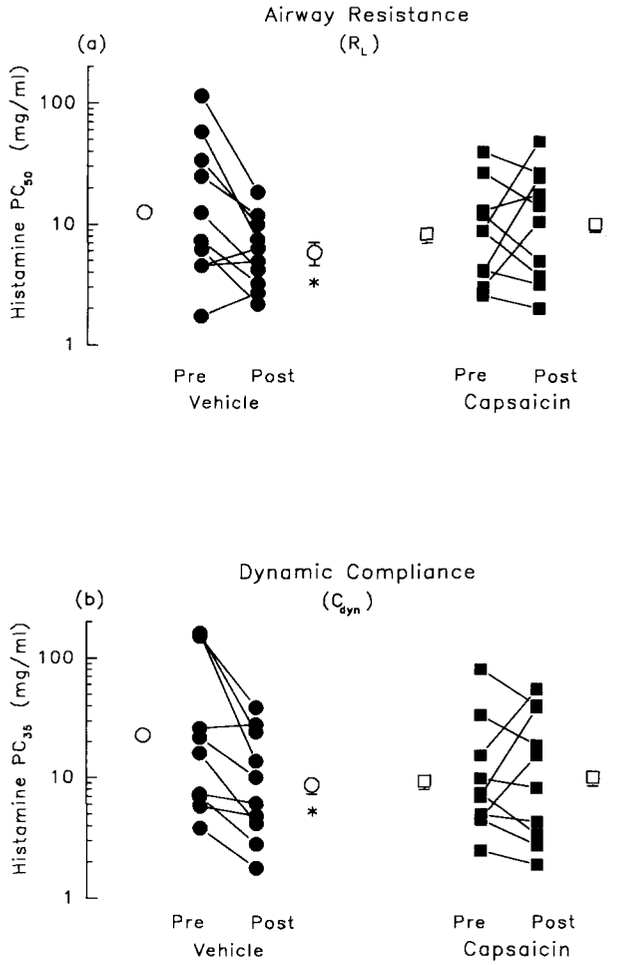

Fig. 1. Effect of vehicle (circles) ($n = 10$) and capsaicin (squares) ($n = 10$) pretreatment on antigen-induced airway hyperresponsiveness in immunized rabbits 24 h following antigen challenge. Closed symbols represent individual animal data and open symbols represent mean ± S.E.M. (a) Histamine PC_{50} is the concentration of histamine required to cause a 50% increase in airway resistance (R_L). (b) Histamine PC_{35} is the concentration of histamine required to cause a 35% decrease in dynamic compliance (C_{dyn}). * $P < 0.05$ compared with pre-antigen control.

Table 4
Total and differential cell numbers recovered from bronchoalveolar fluid prior to and 24 h following (a) control saline challenge, (b) antigen challenge in immunized rabbits pretreated with vehicle or capsaicin

			$\times 10^4$ cells ml^{-1}			
			Total	Neutrophils	Eosinophils	Mononuclear cells
(a)						
Vehicle	Pre	($n = 5$)	24.5 (4.0– 40.0)	0.32 (0.00– 0.735)	0.00 (0.00 – 0.00)	23.77 (4.00– 40.00)
	24 h Post	($n = 5$)	28.5 (10.0– 53.5)	7.40 (5.27– 22.47)*	0.00 (0.00 – 0.100)	17.53 (2.50– 31.03)
Capsaicin	Pre	($n = 5$)	38.0 (9.0– 77.5)	0.48 (0.00– 2.28)	0.00 (0.00 – 0.148)	35.72 (9.00– 77.50)
	24 h Post	($n = 5$)	34.5 (15.0–107.0)	20.36 (7.28– 34.78) *	0.00 (0.00 – 0.535)	14.45 (7.73– 71.69)
(b)						
Vehicle	Pre	($n = 9$)	30.5 (11.0– 57.0)	0.79 (0.00– 5.13)	0.00 (0.00 – 0.285)	29.89 (10.51– 51.59)
	24 h Post	($n = 8$)	38.8 (16.5–169.0)	14.92 (2.38–108.20) *	1.625 (0.340– 2.800) *	21.44 (12.95– 59.15)
Capsaicin	Pre	($n = 9$)	19.0 (10.5– 48.5)	0.47 (0.00– 3.88)	0.009 (0.00 – 0.440)	18.67 (10.50– 44.62)
	24 h Post	($n = 8$)	38.8 (8.5–229.5)	3.81 (0.62–126.20) *	1.097 (0.056–11.475) *	22.52 (3.05–103.28)

Values represent median values with the ranges in parentheses. * $P < 0.05$ compared with Pre challenge control.

Table 5
Percentages of individual cell types recovered in bronchoalveolar lavage fluid prior to and 24 h following (a) control saline challenge, (b) antigen challenge in immunized rabbits pretreated with vehicle or capsaicin

			Neutrophils	Eosinophils	Mononuclear cells
(a)					
Vehicle	Pre	($n = 5$)	1.0 (0.0– 3.0)	0.0 (0.0– 0.0)	99.0 (97.0–100.0)
	24 h Post	($n = 5$)	42.0 (17.0–74.0) *	0.0 (0.0– 1.0)	58.0 (25.0– 83.0) *
Capsaicin	Pre	($n = 5$)	1.0 (0.0– 6.0)	0.0 (0.0– 0.5)	99.0 (94.0–100.0)
	24 h Post	($n = 5$)	48.5 (32.5–71.0) *	0.0 (0.0– 0.5)	51.8 (28.5– 67.0) *
(b)					
Vehicle	Pre	($n = 9$)	4.5 (0.0–10.5)	0.0 (0.0– 0.5)	95.5 (89.5– 99.5)
	24 h Post	($n = 8$)	35.0 (14.0–64.0) *	3.75 (1.0– 5.0)*	61.0 (35.0– 84.0) *
Capsaicin	Pre	($n = 9$)	3.0 (0.0– 8.0)	0.0 (0.0– 1.0)	97.0 (92.0–100.0)
	24 h Post	($n = 8$)	19.25 (4.0–64.0) *	3.25 (0.5–19.5) *	67.5 (26.0– 95.0) *

Values represent median values with ranges in parentheses. * $P < 0.05$ compared with Pre challenge control.

challenge with either saline (Pre: vehicle $51.2 \pm 5.6\%$ ($n = 5$), capsaicin $55.2 \pm 5.2\%$ ($n = 5$); Post: vehicle $51.6 \pm 5.0\%$ ($n = 5$), capsaicin $57.8 \pm 5.0\%$ ($n = 5$)) or antigen (Pre: vehicle $61.8 \pm 3.6\%$ ($n = 9$), capsaicin $62.4 \pm 5.2\%$ ($n = 10$); Post: vehicle $60.6 \pm 3.5\%$ ($n = 9$), capsaicin $53.2 \pm 3.0\%$ ($n = 10$)).

Pre-treatment total cell counts were not significantly different in rabbits administered either vehicle or capsaicin (Table 4a and Table 4b), nor were they significantly different 24 h following saline challenge (Table 4a). Total leucocyte numbers were elevated in bronchoalveolar lavage fluid 24 h following antigen exposure in both treatment groups; however, this increase was not statistically significantly different ($P < 0.05$). The number of neutrophils in bronchoalveolar lavage fluid was significantly elevated 24 h following both saline and antigen exposure in vehicle- and capsaicin-treated rabbits with no significant differences between the groups (Table 4a and Table 4b). There was a significant increase in the number of eosinophils observed in bronchoalveolar lavage fluid in both vehicle- and capsaicin-treated groups 24 h following antigen ($P < 0.05$) (Table 4b). This eosinophil influx was not observed following saline challenge (Table 4a). There were no significant differences, however, in eosinophil numbers between the groups pre or post antigen (Table 4a and Table 4b). Mononuclear cell numbers were not altered following saline or antigen exposure in either treatment group (Table 4a and Table 4b).

Percentages of neutrophils, eosinophils and mononuclear cells recovered in bronchoalveolar lavage fluid before and after antigen and saline exposure are presented in Table 5. In all groups 24 h post challenge the percentage of neutrophils was significantly elevated ($P < 0.05$); however, only in bronchoalveolar lavage fluid recovered after antigen challenge was the percentage of eosinophils increased ($P < 0.05$). In all groups the percentage of mononuclear cells was significantly reduced 24 h following both saline and antigen challenge ($P < 0.05$).

3.6. In vitro smooth muscle function

Methacholine produced concentration-dependent contraction of rabbit intrapulmonary bronchi. Neither the contractile potency of methacholine (pD_2) or the maximum contractile response to methacholine (E_{max}) were significantly different in preparations taken from vehicle- or capsaicin-treated rabbits (Table 6). pD_2 values were greater in vehicle-treated ($P = 0.0517$) and capsaicin-treated ($P = 0.0905$) tissues denuded of epithelium; however, this difference was not statistically significant. There were no significant differences in E_{max} in epithelium-intact and epithelium-denuded preparations (Table 6). In both epithelium-intact and epithelium-denuded bronchi removed from vehicle-treated control rabbits, the in vitro contractile response to exogenously applied capsaicin (100 μM) was modest, yet significantly greater than that achieved in tissues removed from capsaicin-treated animals ($P < 0.05$) (Fig. 2). Responses were enhanced in epithelium-denuded tissues from both groups; however, this was not statistically significant (Fig. 2). The in vitro response to a second administration of capsaicin was not altered in epithelium-intact tissues from rabbits pre-treated with capsaicin but was significantly reduced in control tis-

Table 6
Contractile potency (pD_2) of methacholine and maximal response (g) to methacholine in epithelium-intact and epithelium-denuded bronchi removed from rabbits 24 h following antigen challenge in immunized rabbits pretreated with vehicle or capsaicin

	pD_2		E_{max} (g)	
	+ Epithelium	– Epithelium	+ Epithelium	– Epithelium
Vehicle	5.7 ± 0.1 ($n = 9$)	6.0 ± 0.1 ($n = 8$)	1.78 ± 0.36 ($n = 9$)	1.53 ± 0.5 ($n = 8$)
Capsaicin	5.7 ± 0.1 ($n = 10$)	6.1 ± 0.2 ($n = 10$)	1.83 ± 0.45 ($n = 10$)	1.53 ± 0.6 ($n = 10$)

Values represent mean ± S.E.M. for the number of animals shown in parentheses.

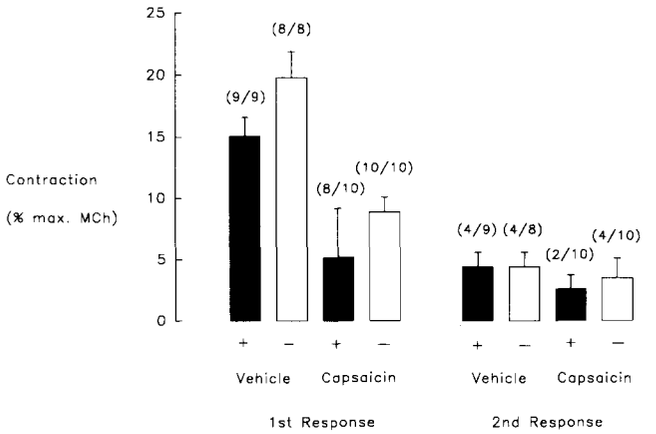

Fig. 2. Consecutive contractile responses to capsaicin (100 μM) (percentage of maximal methacholine response) in epithelium-intact (+) (solid bars) and epithelium-denuded (−) (open bars) bronchi removed from rabbits 24 h following antigen challenge in rabbits pretreated with vehicle or capsaicin. Values represent mean ± S.E.M. and in parentheses are shown the number of tissues that responded out of the total number of tissues studied.

sues ($P < 0.05$) (Fig. 2). In epithelium-denuded tissues the second capsaicin response was significantly less ($P < 0.05$) (Fig. 2). Contractions induced by the second administration of capsaicin were not significantly different in epithelium-intact or epithelium-denuded bronchi removed from vehicle- and capsaicin-treated rabbits (Fig. 2).

4. Discussion

Much of the current knowledge regarding the function of primary afferent neurones has been obtained in the last 20 years, not only as a result of the development of new experimental techniques, but from the availability of capsaicin, the pungent ingredient of hot red peppers (Holzer, 1991). Capsaicin is a substance considered selective for thin afferent neurones of mammalian species, nerves which are characterised by their dual sensory and efferent function (Maggi and Meli, 1988; Holzer, 1991). The efferent function is due to the release of sensory neuropeptides from the peripheral endings leading to a number of postjunctional actions in a variety of tissues. Both rabbit and human lung are similar in that they both receive a relatively sparse innervation by sensory nerves (Laitinen et al., 1983) and are poorly responsive to the contractile actions of capsaicin (Lundberg et al., 1983; Spina et al., 1991; present study).

In the present study, capsaicin pre-treatment alone had no effect on basal lung function, and basal responsiveness of the airways to inhaled histamine was unaltered by capsaicin, in contrast to the effect observed in the guinea-pig where capsaicin reduced the bronchoconstrictor response to histamine (Biggs and Ladenius, 1990). Furthermore, capsaicin pre-treatment did not alter the ability of bronchi to respond to methacholine ex vivo suggesting that chronic capsaicin does not have a non-specific effect on airway smooth muscle. This is in agreement with previous studies conducted in the rabbit (Spina et al., 1991; Riccio et al., 1993). However, a loss of the contractile response to exogenous capsaicin was demonstrated in vitro in bronchial tissues removed from capsaicin-treated animals, as previously reported (Spina et al., 1991; Riccio et al., 1993). The immediate bronchoconstriction induced by inhaled antigen in immunised rabbits was not affected by capsaicin pre-treatment prior to antigen challenge, suggesting a lack of involvement of sensory neurones in this response. In contrast, capsaicin pre-treatment did inhibit the enhanced responsiveness to inhaled histamine achieved 24 h following antigen exposure, a finding which supports previous studies in guinea-pigs (Ladenius and Biggs, 1989). These observations suggest a role for capsaicin-sensitive sensory nerves in the development of airway hyperresponsiveness following antigen exposure.

The capsaicin-induced contractile response is modest in human bronchi in vitro (Lundberg et al., 1983) as it is in rabbit bronchus (Spina et al., 1991). In the present study a loss of contractile function was demonstrated in vitro in bronchial tissues removed from capsaicin-treated animals. In previous studies from our group, a decreased neuropeptide content was not measured in bronchial tissues from rabbits subjected to a similar capsaicin pre-treatment regimen, a finding which perhaps questions the involvement of sensory neuropeptides in airway hyperresponsiveness observed in vivo in this species (Spina et al., 1991). However, we have shown desensitization of bronchi to capsaicin in vitro. Even though it has not been demonstrated in vivo in the rabbit (Tervo, 1991; Spina et al., 1991), loss of neuropeptide content has been demonstrated in rabbit tissues in vitro (Håkanson et al., 1987; Lynn and Shakhanbeh, 1988). It is possible therefore that the effect of capsaicin on antigen-induced hyperresponsiveness in the present study is via depletion of neuropeptides from capsaicin-sensitive nerves.

In bronchoalveolar lavage fluid recovered following antigen challenge, total inflammatory leukocytes were elevated in both capsaicin and control groups, although not significantly. Both neutrophils and eosinophils were significantly increased following antigen in both treatment groups. Neutrophils, however, were also elevated following challenge with saline, indicating the selective nature of the eosinophil recruitment induced by antigen exposure. It appears from these observations that pulmonary eosinophil infiltration induced by antigen does not depend on sensory nerve activation. This agrees with previous findings that eosinophil accumula-

tion into the lung induced by platelet-activating factor (Spina et al., 1991) or antigen (Matsuse et al., 1991) is unaffected by capsaicin pre-treatment.

It has been reported that antigen-induced airway hyperresponsiveness and eosinophil influx in the rabbit is secondary to platelet activation (Coyle et al., 1990). Therefore, it is of interest that capsaicin has been shown to inhibit platelet aggregation in vitro (Hogaboam and Wallace, 1991), suggesting that a mechanism unrelated to sensory nerves may exist via which this substance exerts its effect on airway responses. However, in the present study, airway hyperresponsiveness was inhibited by capsaicin in the absence of any effect on the eosinophil infiltration which would suggest that capsaicin is not working at the level of inhibiting platelet function in our experiments. In addition, platelet depletion (Lellouch-Tubiana et al., 1988), but not capsaicin treatment (Ladenius and Biggs, 1989), has been shown to inhibit antigen-induced eosinophil accumulation into the lungs of guinea-pigs.

Our results further support the idea that cell infiltration and airway hyperresponsiveness may be dissociated events as has been supported by others (Lundgren et al., 1988; Gibson et al., 1989; Ladenius and Biggs, 1989; Kings et al., 1990; Matsuse et al., 1991; Sanjar et al., 1990; Spina et al., 1991; Boulet et al., 1993; Vianna et al., 1993).

In conclusion, antigen-induced airway hyperresponsiveness in neonatally immunised rabbits can be inhibited by pre-treatment with capsaicin, an effect which appears not to be related to an action on the associated pulmonary infiltration of eosinophils.

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