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The effect of ozone on reactivity of upper and lower airways in guinea-pigs

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1 The effect of ozone inhalation on the responsiveness of upper and lower airways to histamine was examined in guinea-pigs.

2 The exposure of conscious guinea-pigs to 3.5 p.p.m. ozone for 30 min rendered their lower airways 2–3 fold more sensitive to the bronchoconstrictor action of i.v. histamine, as assessed subsequently under anaesthesia.

3 The development of lower airway hyperreactivity was not modified by bilateral cervical vagotomy or pretreatment with BW 755C, FPL 55712 or SC-39070.

4 Under the conditions used, ozone exposure produced a mild inflammatory response as monitored by bronchoalveolar lavage, characterized by epithelial damage and prostaglandin E_2 generation, but no cellular infiltration or oedema.

5 In contrast to the lower airways, upper airway resistance was reduced by i.v. histamine. This response was not affected by ozone exposure.

6 Isolated tracheal preparations taken from ozone-exposed guinea-pigs were not significantly more sensitive to histamine than control tissues.

7 The mechanism of hyperreactivity in this model is unknown but does not depend on leukotriene generation or a vagal reflex.

Introduction

Airway hyperreactivity is a feature so characteristic of asthma that it has achieved diagnostic significance (Hargreave *et al.*, 1981). Although the mechanism of this hyperreactivity remains unknown, it is believed to be a manifestation of the inflammatory component of asthma which is receiving increasing attention (Chung, 1986). Indeed, stimuli which are known to cause inflammation, such as viral infections of the upper respiratory tract, have been shown to induce airway hyperreactivity in animals (Buckner *et al.*, 1985) and man (Empey *et al.*, 1976).

A particularly widely used stimulus has been the inhalation of ozone, which has been shown to induce airway hyperreactivity in rats (Evans *et al.*, 1986), dogs (Holtzman *et al.*, 1983), guinea-pigs (Murlas & Roum, 1985) and man (Holtzman *et al.*, 1979). The hyperreactivity produced in dogs is closely correlated with the influx of neutrophils into the airway epithelium (Holtzman *et al.*, 1983) and appears to be the direct result of the release of thromboxane A_2 from these cells (Aizawa *et al.*, 1985). In contrast, the hyperreactivity produced by ozone in guinea-pigs is established before the influx of any inflammatory cells (Murlas & Roum, 1985) and is reported to be enhanced by indomethacin (Murlas *et al.*, 1986) but inhibited by the leukotriene receptor antagonist FPL 55712 (7-[3-(4-acetyl-3-hydroxy-2-propylphenoxy)-2-hydroxypropoxy]-4-oxo-8-propyl-4H-1-benzopyran-2-carboxylic acid) and the leukotriene synthesis inhibitors BW 755C (3-amino-1-[*m*-(trifluoromethyl)-phenyl]-2-pyrazoline) and U-60,257 (6,9-deepoxy-6,9-(phenylimino)-$\Delta^{6,8}$-prostaglandin I_1) (Lee & Murlas, 1985; Murlas & Lee, 1985). These data suggest that ozone produces hyperreactivity in guinea-pigs by a leukotriene-dependent mechanism. This model was therefore investigated for its potential usefulness in the evaluation of novel inhibitors of leukotriene synthesis.

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Methods

Ozone exposure

Male Dunkin-Hartley guinea-pigs (500–800 g) were exposed to ozone while conscious and spontaneously breathing (except in experiments employing neuroleptanaesthesia) in a 0.12 m^3 perspex box. Ozone was generated by passing dry air through an ozone generator (Type BA.023012; Wallace and Tiernan, Tonbridge, Kent) and diluted with a flow of compressed air before being passed at a flow rate of 10 l min^{-1} into the base of the exposure chamber, directly beneath a floor consisting of a sheet of perforated perspex. After permeating the chamber the flow of ozone escaped from a port at the top of the chamber and was bubbled through a scrubbing solution of 5% KI in 50% acetic acid. Thorough mixing of the atmosphere within the chamber was ensured by a fan built into the roof of the chamber. The concentration of ozone at the centre of the chamber was continuously monitored by an ultraviolet ozone monitor (Dasibi Model 1008-AH; Glendale, California, U.S.A.). Unless otherwise stated, animals were exposed for 30 min to a concentration of 3.5 p.p.m. ozone (range 3.0–4.0). A constant humidity was ensured by placing water-saturated tissue paper in the subfloor space, immediately below the ozone entry port. Control animals were exposed to air in the same chamber. In some experiments 10 mg kg^{-1} FPL 55712 or 5% dextrose vehicle were administered subcutaneously (s.c.) 2 min before ozone exposure. In another series of experiments animals were dosed intravenously (i.v.) 5 min before ozone exposure with saline vehicle, BW 755C 10 mg kg^{-1} or SC-39070 (3-[7-(3-(2-n-propyl-3-hydroxy-4-acetyl-phenoxy)-propoxy)-2-methyl-3,4,dihydro-4-8-n-propyl-2H-1-benzopyran-2-yl]-propionic acid) 5 mg kg^{-1}.

Barbiturate anaesthesia

In the majority of experiments described, guinea-pigs were anaesthetized with sodium pentobarbitone (60 mg kg^{-1} i.p.) immediately after exposure to ozone. Cannulae were placed in the trachea, right jugular vein and right carotid artery (for measurement of arterial blood pressure and heart rate). The tracheal cannula was connected via a pneumotachograph (Fleisch type 4.0) to a respiratory pump, and the animal ventilated at 34 breaths min^{-1}, with a tidal volume of 10 ml kg^{-1}. An air-filled catheter was placed in the oesophagus to estimate intrapleural pressure, and transpulmonary pressure measured by a micromanometer (Furness Controls, Bexhill, East Sussex). A second micromanometer connected to the pneumotachograph recorded respiratory flow. Tidal volume was derived as the electrical integral of flow. From these variables, total lung resistance (R_L) was computed breath by breath and displayed on a Devices M19 recorder (Dixon *et al.*, 1979).

Thirty minutes after removing an animal from the exposure chamber, the reactivity of its lungs was assessed by administering increasing doses of histamine i.v. at 5 min intervals, and recording the resultant increase in R_L.

Neuroleptanaesthesia

A series of experiments was carried out in which airway reactivity was assessed in each animal before and after ozone exposure. This was not feasible in the animals anaesthetized with pentobarbitone due to the profound respiratory depression induced by this anaesthetic in guinea-pigs. Consequently, the technique of neuroleptanaesthesia described by Evans & Harrison (1980) was adopted, except that pethidine was used instead of phenoperidine.

Guinea-pigs were given sodium pentobarbitone 30 mg kg^{-1} i.p., followed after 15 min by i.p. injections of droperidol (5 mg kg^{-1}) and pethidine (20 mg kg^{-1}). When necessary, additional pethidine was administered i.v. before surgery. Further doses of 20 mg kg^{-1} pethidine were administered i.v. every 40 min throughout the experiment.

Guinea-pigs were prepared for recording R_L as described above, and a dose-response curve to histamine established. The animals were then disconnected from the apparatus, and upon resuming spontaneous respiration were exposed in the chamber to air or 3.5 p.p.m. ozone for 30 min. Some animals were exposed to 7.5 p.p.m. ozone (range 7.0–8.0). The animals were then removed from the chamber and reconnected to the recording apparatus. After 30 min a second dose-response curve to intravenous histamine was established.

At the end of the experiment the lungs were dissected out distal to the carina, blotted on tissue paper and weighed. After being dried out at 50°C for 24 h they were reweighed and the wet weight/dry weight ratio noted as a measure of oedema formation.

Lung lavage

The effect of ozone on lung cytology and mediator generation was studied in 20 guinea-pigs subdivided into 4 groups of 5. A control group was exposed to air for 30 min; the remaining 3 groups were exposed to 3.5 p.p.m. ozone for the same time. Immediately before exposure each animal received an i.v. injection of 1 ml kg^{-1} Evans Blue dye as a 1% solution in saline. Thirty minutes after the end of the exposure period the control group and one ozone-treated

group were killed with i.p. sodium pentobarbitone. The remaining two ozone-exposed groups were treated similarly at 90 and 150 min after the end of the exposure period.

In each animal the trachea was cannulated and the lungs lavaged with 2 × 5 ml aliquots of phosphate buffered saline. The chest was massaged for 15–20 s during each lavage. Lavage fluid was recovered with gentle suction, and the recovered volume noted. Each lavage sample was centrifuged at 700 *g* for 10 min at 4°C. Aliquots of the supernatant (1 ml) were taken for determinations of optical density at 620 nm using a Cecil spectrophotometer, as a measure of Evans Blue concentration. An additional 1 ml aliquot was stored at −20°C for subsequent measurement of prostaglandin E_2 (PGE_2) concentration using a radioimmunoassay kit (NEN Research Products, Stevenage, Herts.). The cell pellet was resuspended in 0.5 ml Hank's buffer containing 10 units ml^{-1} heparin, and a 50 μl aliquot diluted with 450 μl Kimura's stain (Kimura *et al.*, 1973). Cells were counted using a modified Fuchs-Rosenthal haemocytometer, counting at least 100 cells per sample.

Upper airway resistance

As guinea-pigs are obligatory nose breathers, the inhalation of ozone might be expected to affect the nasal passages as well as the lungs. This was investigated in a separate study. After exposure to ozone or air, guinea-pigs were anaesthetized with sodium pentobarbitone and prepared for the measurement of R_L as described above. In addition, a second cannula was placed in the pharyngeal end of the trachea just below the larynx and connected to a Fleisch 4.0 pneumotachograph. Air was humidified by being finely bubbled through a gas jar of water and then directed through the nasal passages via the heated pneumotachograph at a constant flow rate of 10 ml s^{-1}. The pneumotachograph was connected to a micromanometer to allow continuous monitoring of the flow rate. A side port of the pneumotachograph was used to measure pressure in the upper trachea with respect to atmosphere (i.e. the pressure gradient across the upper airways). To ensure that the air emerged through the nostrils, the mouth was tightly packed with plasticine to provide an airtight seal. The resistance of the upper airways was thus calculated at any time point as the pressure divided by the flow.

Increasing doses of histamine were administered i.v. at 3 min intervals to construct a dose-response curve. In a subgroup of animals this was later repeated after bilateral cervical vagotomy.

In vitro *preparations*

The reactivity of airway preparations *in vitro* can be enhanced considerably by removal of the epithelium (Holroyde, 1986). It is therefore conceivable that enhanced reactivity *in vivo* following exposure to ozone is merely the result of epithelial damage. To investigate this possibility, guinea-pigs were exposed to air or ozone, and after a further 30 min were killed by cervical dislocation. Paired tracheal preparations were set up as described previously (Holroyde, 1986), with one tissue in each pair being denuded of epithelium. Contractile dose-response curves to histamine were constructed on each tissue.

Drugs and solutions

Pentobarbitone sodium ('Sagatal', May and Baker, Dagenham, Essex), histamine dihydrochloride (Sigma, Poole, Dorset), pethidine hydrochloride (James Woolley and Sons Ltd., Manchester), droperidol ('Droleptan', Janssen, Grove, Oxford), Evans Blue (Searle Diagnostics, High Wycombe, Bucks), Hank's balanced salt solution (Gibco, Paisley, Scotland). BW 755C and the lysine salts of SC-39070 and FPL 55712 were synthesized in Fisons Medicinal Chemistry Laboratories.

FPL 55712 was dissolved in 5% dextrose; other compounds were dissolved in saline. BW 755C was prepared as the hydrochloride by dissolving in saline containing an equimolar concentration of hydrochloric acid. Doses of all compounds are expressed in terms of the active substance.

Analysis of results

All measurements of resistance include the resistance of the tracheal cannula. This was determined to be 25 $cmH_2O\,l^{-1}\,s$ for the lower airway cannula and 36 $cmH_2O\,l^{-1}\,s$ for the upper airway cannula.

Bronchoconstrictor responses to histamine were expressed as the percentage change in resistance compared with resting levels. From each dose-response curve the provocation dose of histamine producing a 100% increase in R_L (the PD_{100}) was calculated. In contrast to the lower airways, the resistance of the upper airways was reduced by histamine. This was quantified as the dose of histamine required to reduce resistance by 20% (i.e. the PD_{20}). In experiments using neuroleptanaesthesia, each animal served as its own control, allowing the PD_{100} values obtained before and after ozone treatment to be expressed as a ratio.

In experiments using tracheae *in vitro*, responses were calculated as the percentage of the maximal response and EC_{50} values calculated for each tissue.

The data were also pooled to allow the construction of composite dose-response curves.

Throughout, data were compared by use of a 2-tailed Student's t test for paired or unpaired data as appropriate. A probability value of 0.05 or less was considered significant.

Results

Barbiturate anaesthetized animals

Exposure to 3.5 p.p.m. ozone for 30 min produced no significant change in resting R_L ($71.8 \pm 7.5\,cmH_2O\,l^{-1}\,s$ after air exposure versus $73.3 \pm 6.4\,cmH_2O\,l^{-1}\,s$ after ozone; mean ± s.e. mean, $n = 15$), but significantly enhanced responsiveness to i.v. histamine. In the absence of drug pretreatment, ozone-exposed animals were 2.6 fold more responsive than air-exposed animals as determined by relative PD_{100} values (Table 1). Pretreatment with FPL 55712 $10\,mg\,kg^{-1}$ s.c. did not modify this hyperreactivity (Table 1). As shown in Table 2, development of hyperreactivity was also not prevented by pretreatment with BW 755C or SC-39070. Although statistically not significant, BW 755C tended to reduce the PD_{100} value, and it was noted empirically that animals treated with this compound displayed signs of laboured breathing after the first 15 min of ozone exposure.

Neuroleptanaesthetized animals

Before exposure the resting R_L in these animals was $67.0 \pm 2.5\,cmH_2O\,l^{-1}\,s$ (mean ± s.e. mean, $n = 15$), which was not different from that measured in barbiturate anaesthetized animals. However, this mode of anaesthesia considerably reduced sensitivity to histamine, with a mean PD_{100} of $11.8\,\mu g\,kg^{-1}$ compared with 5.1 under barbiturate anaesthesia. Nevertheless, sensitivity was approximately doubled after ozone exposure, with ozone concentrations of 3.5 and 7.5 p.p.m. being equieffective (Table 3). The development of hyperreactivity was not accompanied by oedema as determined by the lung wet/dry weight ratio. Indeed, there was no correlation between this ratio and the histamine dose-ratio (i.e. the ratio of PD_{100} values to histamine before and after exposure to air or ozone).

With this experimental protocol, ozone exposure often resulted in moderate bronchoconstriction which resolved over 10–15 min. Due to the experimental design, this was not noted in barbiturate anaesthetized animals as respiratory monitoring did not commence until 30 min after the end of exposure.

Lung lavage

After lavage of the lungs with 10 ml buffer, the volume recovered was 7.4 ± 0.4 ml (mean ± s.e.

Table 1 Effect of FPL 55712 on development of hyperreactivity to histamine after exposure to ozone in pentobarbitone-anaesthetized guinea-pigs

Pretreatment	PD_{100}	n	P
Air exposed			
None	5.36 ± 0.65	6	—
Ozone exposed			
None	2.05 ± 0.25	6	<0.001
Dextrose	3.08 ± 0.73	4	0.05
FPL 55712	2.38 ± 0.52	4	<0.02

Results are PD_{100} values (histamine $\mu g\,kg^{-1}$) expressed as mean ± s.e. mean. FPL 55712 ($10\,mg\,kg^{-1}$) or vehicle were given s.c. 2 min before ozone exposure. The P value refers to comparison with control group exposed to air.

Table 2 Effect of BW 755C and SC-39070 on ozone-induced hyperreactivity in pentobarbitone-anaesthetized guinea-pigs

Pretreatment	PD_{100}	n	P_1	P_2
Air exposed				
Saline	5.51 ± 0.67	9	—	<0.01
BW 755C	3.48 ± 0.55	5	NS	NS
Ozone exposed				
Saline	2.98 ± 0.48	9	<0.01	—
BW 755C	2.08 ± 0.18	9	<0.001	NS
SC-39070	3.06 ± 0.40	5	<0.05	NS

Results are PD_{100} values (histamine $\mu g\,kg^{-1}$) expressed as mean ± s.e. mean. Data compared with saline + air (P_1) or saline + ozone (P_2). NS = not significant.

Table 3 Ozone-induced hyperreactivity in neuroleptanaesthetized guinea-pigs

Exposure	*Dose-ratio*	*Lung weight (g)* *Wet*	*Dry*	*Wet/Dry*
Air	0.86 ±0.1	4.46 ±0.37	0.93 ±0.08	4.79 ±0.13
Ozone 3.5 p.p.m.	1.52* ±0.25	6.21 ±1.13	1.11 ±0.16	5.52 ±0.30
Ozone 7.5 p.p.m.	1.67* ±0.27	5.40 ±0.84	1.06 ±0.14	5.05 ±0.11

Dose-ratio is the ratio of the histamine PD_{100} before exposure to the PD_{100} after exposure in the same animal; numbers greater than 1 indicate hyperreactivity. Results are mean ± s.e. mean, $n = 5$–6.

* $P < 0.05$ compared with the air-exposed group.

Table 4 Analysis of guinea-pig lung lavage after ozone exposure

		Time after ozone exposure (min)		
	Control	*30*	*90*	*150*
Total cells	0.645 ±0.090	0.456 ±0.043	0.326* ±0.037	0.644 ±0.089
Macrophages	0.528 ±0.097	0.233* ±0.037	0.127** ±0.019	0.299 ±0.053
Eosinophils	0.065 ±0.018	0.044 ±0.006	0.036 ±0.017	0.074 ±0.006
Neutrophils	0.004 ±0.002	0.001 ±0.001	0.005 ±0.004	0.028** ±0.005
Epithelial cells	0.043 ±0.011	0.137* ±0.030	0.159* ±0.034	0.237** ±0.037
PGE_2	18.4 ±7.0	127.9*** ±20.0	586.0 ±287.0	46.4* ±7.0

Lungs were lavaged 30 min after exposure to air (control) or various times after exposure to ozone. Cell counts are expressed as millions per ml of recovered lavage fluid; prostaglandin E_2 (PGE_2) concentrations as pg ml^{-1}. All data are expressed as mean ± s.e. mean of 5 animals. *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$ for comparison with control group.

mean, $n = 20$). The recovery of lavage fluid was similar at all times after ozone exposure. The profile of the cells recovered is shown in Table 4. Ozone exposure resulted in an immediate increase in epithelial cells, indicative of damage sustained by the airways. Macrophages were reduced in number while eosinophils remained unaffected. Neutrophils were virtually absent until 150 min after ozone exposure, when the numbers were slightly elevated. Lymphocyte numbers were low and remained unchanged after ozone (data not shown).

Ozone exposure also resulted in mediator release as indicated by the generation of PGE_2, which reached a peak 90 min after ozone. The concentration of Evans Blue dye in the lavage fluid was low and remained unchanged after ozone, indicating no increase in pulmonary vascular permeability (data not shown).

Upper and lower airways

Simultaneous measurement of upper and lower airway resistance revealed that the resistance of the lower airways comprised approximately 8% of the total airway resistance. The resting resistance of the upper airways was found to be 1015.3 ± 144.1 $cmH_2O\,l^{-1}\,s$ (mean ± s.e. mean, $n = 8$) after exposure to air, and 992.1 ± 176.3 $cmH_2O\,l^{-1}\,s$ after exposure to ozone. The corresponding values for the lower airways in the same animals were 89.4 ± 8.5 and 88.9 ± 5.9 $cmH_2O\,l^{-1}\,s$.

Histamine produced a constriction of the lower airways but a dose-related reduction of upper airway resistance (Figure 1). Ozone enhanced the sensitivity of the lower airways but had no effect on the responses of the upper airways (Figure 2). Cervical vagotomy had no effect on responses of the upper airways to histamine, and did not affect the development of hyperreactivity in the lower airways. However, the sensitivity of the lower airways to histamine was significantly increased by vagotomy in ozone-exposed guinea-pigs, but not in control animals (Table 5).

Isolated trachea

Removal of epithelium enhanced sensitivity to histamine approximately six fold, regardless of whether animals had previously been exposed to air or ozone. Although the mean dose-response curves to histamine appeared to be shifted leftwards in ozone-

Table 5 Effect of bilateral cervical vagotomy on responses of upper and lower airways to histamine

Exposure	*Lower* (PD_{100}) ($\mu g\,kg^{-1}$)	*Upper* (PD_{20}) ($\mu g\,kg^{-1}$)
Air		
Pre vagotomy	6.20 ± 1.10	1.45 ± 0.16
Post vagotomy	5.95 ± 0.46	1.90 ± 0.50
Ozone		
Pre vagotomy	3.10 ± 0.06*‡	2.64 ± 1.20
Post vagotomy	1.98 ± 0.37†	1.60 ± 0.40

Results are expressed as mean ± s.e. mean of 5 animals. Result significantly different ($P < 0.05$) from: *air-exposed pre-vagotomy; †air-exposed post-vagotomy; ‡ozone-exposed post-vagotomy.

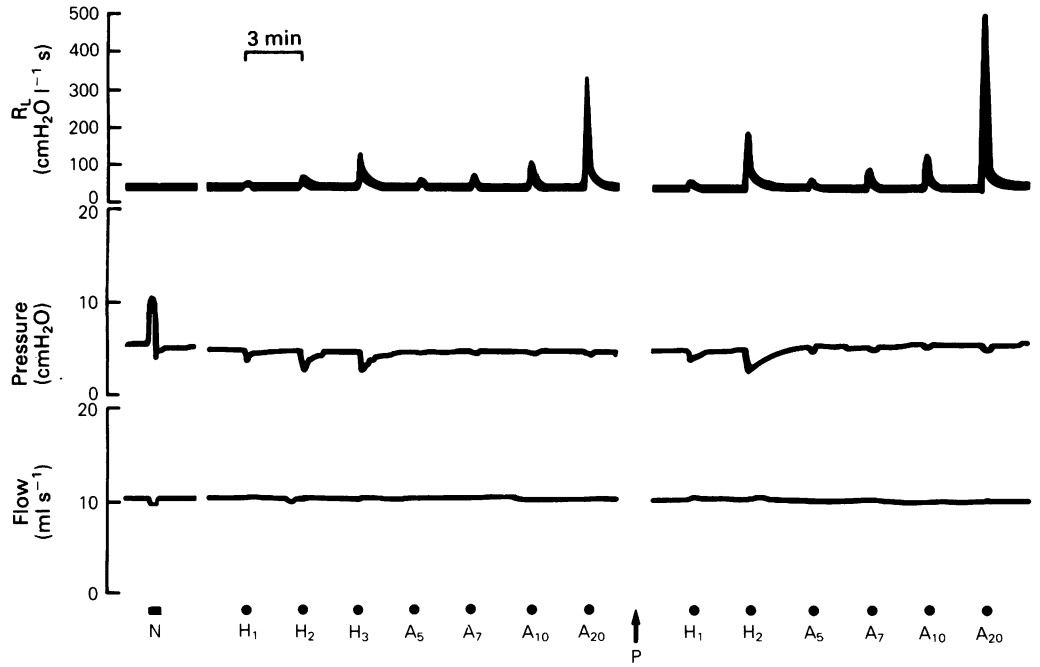

Figure 1 Responses of upper and lower airways of the anaesthetized guinea-pig to histamine (H) and acetylcholine (A) given intravenously before and after propranolol (1 mg kg^{-1} at P). All doses are expressed as μg kg^{-1}. From top to bottom the traces show: computed resistance of the lower airways (R_L); pressure gradient across the upper airways; flow rate of air passing through upper airways. At N, nasal resistance was increased by manually occluding the left nostril.

exposed tissues (Figure 3) this did not achieve statistical significance ($P = 0.06$). Prior exposure to ozone or removal of epithelium did not affect the maximal tension developed by these tissues in response to histamine.

Discussion

Exposure of conscious guinea-pigs to ozone resulted in airway hyperreactivity to histamine which was subsequently demonstrated under anaesthesia. The hyperreactivity was independent of the anaesthetic regime used (pentobarbitone or neuroleptanaesthesia) and was identical in magnitude to that obtained by Murlas & Roum (1985) who assessed airway reactivity in conscious animals. However, in contrast to the experiments of Lee & Murlas (1985), the present study provides no evidence of a role for leukotrienes in the development of this hyperreactivity. Hence pretreatment of animals with the leukotriene antagonists FPL 55712 and SC-39070, or the dual lipoxygenase/cyclo-oxygenase inhibitor BW 755C, failed to prevent the development of hyperreactivity after ozone exposure. In the

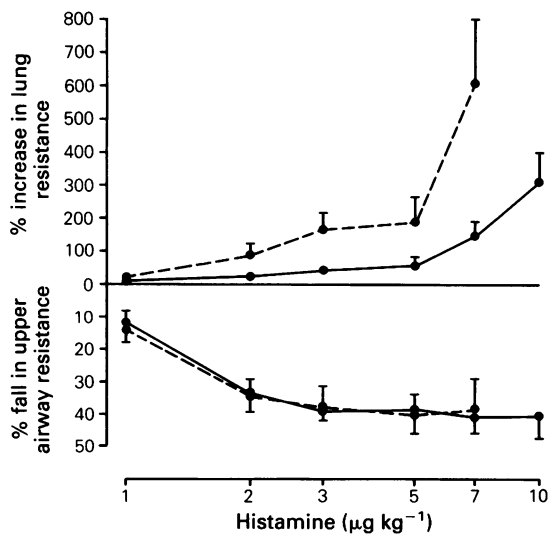

Figure 2 Effect of histamine on resistance of upper and lower airways in pentobarbitone-anaesthetized guinea-pigs with intact vagi after exposure to air (●—●) or ozone (●--●). Results are mean of 4–9 animals with s.e. mean shown by vertical lines.

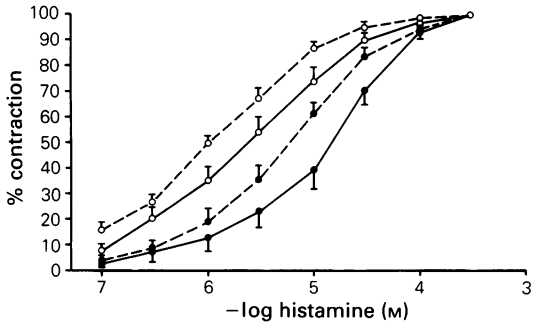

Figure 3 Effect of histamine on isolated tracheal preparations with intact (●) or denuded (○) epithelium taken from guinea pigs exposed to air (—) or ozone (---). Results are mean of 6 experiments with s.e. mean shown by vertical lines.

study described by Lee & Murlas (1985), FPL 55712 ($5\,mg\,kg^{-1}$) was administered i.v. 1 min before a 15 min period of ozone exposure, and airway reactivity was assessed after a further 30 min. As FPL 55712 is rapidly cleared from plasma (Sheard *et al.*, 1977) and has been found to have a biological half-life in guinea-pigs of only 8 min following i.v. administration (Carnathan *et al.*, 1987), it is difficult to understand how this compound could be effective under these conditions. In the present study FPL 55712 was administered s.c. at a dose of $10\,mg\,kg^{-1}$, which was calculated to maintain a plasma level of at least $1\,\mu g\,ml^{-1}$ for the duration of the experiment. Furthermore SC-39070, a leukotriene antagonist comparable in potency with FPL 55712 but with a longer duration of action (Carnathan *et al.*, 1987), was also tested. The ineffectiveness of both compounds cannot therefore be ascribed to pharmacokinetic problems.

It is interesting that several animals pretreated with BW 755C developed dyspnoea during exposure to ozone. Furthermore, this compound tended to enhance the sensitivity of control animals to histamine, although this was not statistically significant (Table 2). It is possible that both these observations reflect inhibition of cyclo-oxygenase by BW 755C, leading to a reduction in the levels of bronchodilator prostanoids. Non-steroidal anti-inflammatory agents such as indomethacin are known to enhance bronchoconstrictor responses of guinea-pigs to histamine (Mitchell & Adcock, 1987) and also to acetylcholine after ozone exposure (Murlas *et al.*, 1986).

As Murlas and co-workers monitored airway responses non-invasively using a plethysmographic technique (Murlas & Roum, 1985; Lee & Murlas, 1985), the possibility was considered that their data reflected changes in upper airways (particularly nasal passages) rather than the lung. Consequently, upper and lower airway resistances were measured separately but simultaneously in anaesthetized guinea-pigs. The data obtained show that the upper airways (larynx, pharynx, nasal passages) comprise the major portion, approximately 90%, of total airway resistance. This is a greater proportion than the 45% found in guinea-pig by Amdur & Mead (1958), but is similar to the 80% shown in the rat by DiMaria *et al.* (1987). Surprisingly, histamine consistently reduced the resistance of the upper airways while increasing that of the lower airways. This was unexpected as histamine has been demonstrated to increase nasal airflow resistance in dog (Lung *et al.*, 1984), rat (Misawa, 1987) and man (Bundgaard *et al.*, 1986). The studies of Murlas and co-workers employed acetylcholine as the bronchoconstrictor stimulus in animals pretreated with propranolol. These two agents were therefore investigated in a small number of experiments; acetylcholine was shown to have no effect on upper airway resistance while clearly producing bronchoconstriction. Pretreatment with propranolol enhanced bronchoconstrictor responses (particularly to histamine, Figure 1) but did not modify upper airway responses to histamine or acetylcholine. Almost certainly, upper airway responses made no contribution to the data obtained by Murlas and co-workers.

In the present study, upper airway reactivity was unaffected by ozone. Although the nasal airways were not examined histologically after ozone exposure it is likely that a degree of tissue damage had occurred, particularly as the upper airways of guinea-pigs remove approximately 50% of inhaled ozone (Miller *et al.*, 1979). Presumably, therefore, the mechanism of lower airway hyperreactivity, which is mediated ultimately by airway smooth muscle, does not apply to the nose, where airflow resistance is believed to be a reflection primarily of vascular smooth muscle tone.

The histological studies carried out by Murlas & Roum (1985) on tracheal tissue showed that ozone-induced hyperreactivity in guinea-pigs occurred before the infiltration of any inflammatory cells, and was accompanied only by signs of epithelial damage and an increase in the number of mast cells in the mucosa. However, the latter increase, did not correlate with the degree of hyperreactivity. These data are complemented by the results of the current lung lavage studies, which show that at the time hyperreactivity was demonstrable (30 min after ozone), the only significant changes were a reduction in numbers of macrophages and an increase in epithelial cells. Prostaglandin E_2 generation was markedly increased; it is likely that other mediators including leukotrienes were also elevated at this time, but no attempt was made to measure them. A reduction in

macrophage numbers after ozone was also noted by Coffin *et al.* (1968) in rabbits, and probably reflects killing of these cells, possibly followed by phagocytosis by other cells.

The absence of any change in Evans Blue content in these studies, or lung wet/dry weight ratio in earlier studies, demonstrates that hyperreactivity occurs in this model in the absence of any oedema. This is in contrast to the effect of cigarette smoke in guinea-pigs, which produces hyperreactivity of a similar magnitude at the same time point, together with oedema (Hulbert *et al.*, 1985).

The mechanism of ozone-induced hyperreactivity to histamine in this model is unknown. It appears to be restricted to the lower airways and is independent of cell infiltration, vagal reflexes, oedema and leukotriene generation. The enhancement of reactivity following vagotomy in ozone but not air-exposed animals is intriguing, and suggests that a vagally-mediated bronchodilator reflex may be activated by ozone exposure. The identity of this reflex and the mechanism of its activation deserve further investigation.

The possible central role of the epithelium remains unresolved. Clearly epithelial damage is produced by ozone, and it is known that total removal of epithelium enhances the sensitivity of airway muscle *in vitro* (Holroyde, 1986). Ozone exposure increased sensitivity to histamine to a similar extent *in vitro* and *in vivo*, although the statistical significance of the *in vitro* data must be considered borderline ($P = 0.06$). It is possible that ozone causes functional damage to the epithelium which is less severe than that produced by total epithelial removal. Consequently, to demonstrate statistical significance a considerably larger sample size would be required than that used in this study. Further studies to investigate this aspect are justified. If hyperreactivity in this model is solely the result of epithelial damage, then this model could prove particularly useful for the investigation *in vivo* of the purported epithelial-derived relaxant factor (Holroyde, 1986; Hay *et al.*, 1987).

Possible other mechanisms which also deserve investigation include the involvement of non-vagal reflexes, which could involve glossopharyngeal afferents (Biggs, 1984) or local axon reflexes (Barnes, 1986).

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