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Involvement of hydroxyl radicals in neurogenic airway plasma exudation and bronchoconstriction in guinea-pigs *in vivo*

[2]Yu-Hong Lei, Peter J. Barnes & [1]Duncan F. Rogers

Department of Thoracic Medicine, National Heart & Lung Institute, Dovehouse Street, London SW3 6LY

1 Cigarette smoke induces plasma exudation in the airways of rodents by activation of capsaicin-sensitive 'sensory-efferent' nerves. The response is mediated predominantly by substance P (SP) and the magnitude of exudation is regulated by neutral endopeptidase (NEP). The component(s) of the smoke responsible for the activation of the nerves may be reactive oxygen radicals. We investigated the effect of the hydroxyl radical scavenger dimethylthiourea (DMTU), a regulator of superoxide anion, superoxide dismutase (SOD), and a regulator of hydrogen peroxide, catalase, on plasma exudation (measured using Evans blue dye) induced by cigarette smoke in guinea-pig main bronchi *in vivo*. The effect of DMTU on plasma exudation and non-cholinergic bronchoconstriction (measured as pulmonary insufflation pressure, PIP) induced by electrical stimulation of the vagus nerves was also assessed. Interaction between hydroxyl radicals and NEP was assessed with the NEP inhibitor phosphoramidon.

2 In each of the experiments, cigarette smoke increased plasma exudation by ~200% above air-exposed controls. Acute administration of DMTU (1.5 g kg^{-1}, i.v. for 20 min) significantly reduced cigarette smoke-induced plasma exudation by 69%. In contrast, neither SOD (240,000 u kg^{-1}, i.v.) nor catalase (400,000 u kg^{-1}, i.v.) significantly affected the exudative response.

3 Chronic pretreatment with DMTU (1.25 g kg^{-1} over 4 days) significantly reduced bronchial plasma exudation induced by cigarette smoke by 72%. Phosphoramidon (1.5 mg kg^{-1}, i.v.) completely reversed the inhibition by DMTU of cigarette smoke-induced plasma exudation.

4 Vagal stimulation increased plasma exudation by ~200% and PIP by ~250%. Acute treatment with DMTU had no significant inhibitory effect on these responses, whereas chronic pretreatment inhibited them by ~80%. Phosphoramidon reversed the inhibition by chronic DMTU.

5 SP (1 nmol kg^{-1}) increased plasma exudation by ~250%, a response which was not inhibited by either acute or chronic DMTU.

6 We conclude that hydroxyl radicals, rather than superoxide anion or hydrogen peroxide, are involved in the induction of neurogenic plasma exudation and bronchoconstriction induced by cigarette smoke or by electrical stimulation of the vagus nerves. These radicals also affect the activity of NEP. Acute DMTU may affect directly the neural actions of hydroxyl radicals contained in the cigarette smoke. Chronic pretreatment with DMTU may inhibit the neurogenic airway responses by effects on tachykinin biosynthesis and/or axonal transport.

Keywords: Oxygen radicals; hydroxyl radicals; neurogenic inflammation; dimethylthiourea; plasma exudation; bronchoconstriction; sensory nerve; cigarette smoke

Introduction

Inhalation of cigarette smoke provokes a number of airway defence mechanisms including cough, mucus secretion and changed pattern of breathing. In rats, passive inhalation of cigarette smoke induces plasma exudation into the airway tissue exclusively via activation of capsaicin-sensitive C-fibre afferents (Lundberg & Saria, 1983). These fibres may be considered 'sensory-efferent' nerves (Maggi & Meli, 1987) and their neurotransmitters are termed sensory neuropeptides and include substance P (SP) and neurokinin A (NKA) (Barnes *et al.*, 1991). In guinea-pigs, we have found that direct inhalation of cigarette smoke induces airway plasma exudation via activation of similar nerves (Lei *et al.*, 1995). Exudation is inhibited by tachykinin NK_1 (substance P) receptor antagonists (Delay-Goyet & Lundberg, 1991; Hirayama *et al.*, 1993; Lei *et al.*, 1995). A similar mechanism appears to operate for plasma exudation induced in rodent airways by capsaicin or by electrical stimulation of the vagus nerves (Lei *et al.*, 1992; Hirayama *et al.*, 1993). Phosphoramidon, an inhibitor of neutral endopeptidase (NEP), an endogenous enzyme which degrades SP (Borson, 1991), potentiates cigarette smoke-induced plasma exudation (Lei *et al.*, 1995). Thus, cigarette smoke causes airway plasma exudation via activation of sensory-efferent nerves and release of sensory neuropeptides which interact with tachykinin NK_1 receptors on the bronchial microvasculature. Substance P is the principal neurotransmitter involved. The magnitude of the response is regulated by endogenous NEP.

In both rats and guinea-pigs, it is the vapour phase of cigarette smoke which is responsible for the activation of the sensory nerves and the induction of plasma exudation (Lundberg *et al.*, 1983; Lei *et al.*, 1995). The particular phase, of which tar and nicotine are the principal components (Wartman *et al.*, 1959), does not appear to be involved in the neurogenic exudative response. The specific components of the vapour phase which are responsible for the nerve activation are unknown. Oxygen-derived radicals have been identified in both the particulate and vapour phases of cigarette smoke (Church & Pryor, 1985) and oxygen radicals will cause plasma exudation and lung oedema (Johnson *et al.*, 1981; Fox *et al.*, 1983). Dimethylthiourea (DMTU), a scavenger of the hydroxyl radical ($OH^{\cdot}$), inhibits cigarette smoke-induced bradypnoea, a response mediated via activation of C-fibres (Lee, 1990; Lee *et al.*, 1990) However, the role of oxygen radicals, either contained in the smoke or generated after inhalation, in

[1] Author for correspondence.
[2] Present address: Inflammatory Disease Research, G.D. Searle & Co., 4901 Searle Parkway, Skokie, IL 60077, U.S.A.

cigarette smoke-induced neurogenic plasma exudation is not clear. In addition, cigarette smoke degrades NEP (Dusser *et al.*, 1989), an effect possibly mediated via reactive oxygen species.

The present experiments assessed the role of oxygen radicals, in particular hydroxyl radical, in cigarette smoke-induced neurogenic airway plasma exudation in guinea-pig main bronchi *in vivo*. We used DMTU, given either acutely or chronically, to inhibit the effects of hydroxyl radicals (Fox *et al.*, 1983; Wasil *et al.*, 1987). The involvement of superoxide anion ($O_2^{\cdot}$) and hydrogen peroxide (H_2O_2) was assessed with superoxide dismutase (SOD) or catalase (Chance *et al.*, 1979) respectively. To investigate whether hydroxyl radicals had other effects on airway nerves, we determined the effect of DMTU on airway plasma exudation and bronchoconstriction induced by vagus nerve stimulation. The involvement of NEP in the neurogenic responses was assessed by use of phosphoramidon. We used Evans blue dye as a marker of plasma exudation (Rogers *et al.*, 1989) and pulmonary insufflation pressure (PIP) as an index of airway tone.

Methods

Animal preparation

Male Dunkin-Hartley outbred guinea-pigs (Charles River U.K. Ltd., Margate, Kent) weighing 260–440 g were anaesthetized with urethane (2 g kg^{-1}, i.p.). A tracheal cannula was inserted via a tracheostomy into the tracheal lumen and connected to a constant-volume mechanical ventilator (Harvard Apparatus Ltd., Edenbridge, Kent). A tidal volume of 10 ml kg^{-1} and a frequency of 60 breaths min^{-1} were used to ventilate the animals. Pulmonary insufflation pressure was measured by means of a pressure transducer (Sensortechnics, Rugby, Warwks.) connected to a side-arm in the expiratory limb of the ventilation tubing. Blood pressure was monitored throughout the experiments via a polyethylene catheter filled with heparin-saline (10 u ml^{-1}) inserted into the left carotid artery and linked to a pressure transducer (Bell and Howell, Basingstoke, Hants.) and two-channel recorder (Ormed Ltd., Welwyn Garden City, Herts.). Both jugular veins were exposed and covered with saline-dampened gauze to limit fluid loss. Apart from chronic administration of DMTU (see below) all drugs were administered via the jugular veins.

In experiments involving electrical stimulation of the vagus nerves, animals were pretreated with atropine, propranolol and phentolamine (1 mg kg^{-1} each, i.v.) 30 min before nerve stimulation to eliminate adrenergic and cholinergic influences. Bilateral cervical vagus nerves were exposed and cut 5 min before stimulation at 7 Hz, 5 V, 5 ms for 3 min. These parameters have been found to be optimal for increasing airway plasma exudation and to minimize blood pressure effects (Belvisi *et al.*, 1990). 'Sham-stimulated' animals were prepared similarly but were without electrical stimulation.

Quantification of plasma exudation

Evans blue dye (25 mg kg^{-1}) was used as a plasma marker (Rogers *et al.*, 1989) and was injected 1 min before stimulation (by cigarette smoke, vagal stimulation, SP or control stimulations). Five min after vagus nerve stimulation (Belvisi *et al.*, 1990), 15 min after cigarette smoke administration (Hirayama *et al.*, 1993), or 10 min after injection of SP (Rogers *et al.*, 1988), the systemic circulation of the animals was perfused with saline via the aorta to expel intravascular dye. The extrapulmonary (main) bronchi were removed and were 'blotted' dry by squeezing between filter papers and were placed in preweighed tubes. The Evans blue dye in the tissue was extracted in formamide at 37°C for 16–18 h. The concentration of extractable dye was quantified at 620 nm wavelength light absorbance with a spectrophotometer (PU8630 Kinetic Series, Phillips, Cambridge) and by interpolation on a standard curve of dye concentrations in the range 0.5–10 mg ml^{-1}. Tissue dye content was expressed as ng dye per mg wet weight tissue (ng dye mg^{-1} wet wt. tissue).

Preparation and administration of cigarette smoke

The cigarettes used were commercially-available, unfiltered, and in the U.K. government 'Middle Tar' category (nicotine content 1.2 mg per cigarette, carbon monoxide content 11 mg per cigarette). Cigarettes were lit in a fume cupboard where a laminar flow prevented smoke accumulation. Smoke was collected into a 60 ml polypropylene syringe. The first four syringe-fulls were discarded with the fifth syringe-full introduced into the tracheal cannula in a series of tidal volumes (10 mg ml^{-1}) via 3-way stopcock immediately rostral to the endotracheal tube. One tidal volume of cigarette smoke was given every five to six ventilated breaths of room air. Fifty tidal volumes of smoke were used in the present study because this number is submaximal for inducing plasma exudation in guinea-pig main bronchi (Lei *et al.*, 1995). Control animals had the same procedure, except 50 volumes of air drawn through an unlit cigarette were used.

Acute administration of DMTU, SOD or catalase

Guinea-pigs were injected with 1.5 g kg^{-1} DMTU (2 ml kg^{-1} of 750 mg ml DMTU, i.v. infusion over 5 min), or SOD (240,000 u kg^{-1}, i.v. bolus) or catalase (400,000 u kg^{-1}, i.v. bolus) respectively. Drug doses were selected according to Lai (1990; for DMTU) and Till *et al.* (1982: SOD and catalase). Control animals were injected with saline (1 ml kg^{-1}). Cigarette smoke was administered 5 min later. Separate animals were injected with DMTU (1.5 mg kg^{-1}, as above by i.v. infusion over 5 min) 5 min before vagal stimulation or injection of SP at a concentration submaximal for increasing plasma exudation (1 nmol kg^{-1}, i.v. bolus).

Chronic DMTU pretreatment and phosphoramidon

DMTU was administered chronically according to the protocol of Lai (1990) and was injected for 3 consecutive days at doses of 250, 125 and 125 mg kg^{-1} (intraperitoneal boluses). Saline vehicle (1 ml kg^{-1}) was administered similarly to control animals. On the day of experimentation (the fourth day), animals were given an infusion of DMTU (750 mg kg^{-1}, i.v. over 5 min) or saline 30 min prior to cigarette smoke administration, or SP or vagal stimulation. Some animals chronically pretreated with DMTU were given phosphoramidon (1.5 mg kg^{-1}, i.v. bolus) 25 min after the last infusion of DMTU and 5 min before cigarette smoke or vagal stimulation.

Drugs

The following were used: catalase, formamide, DMTU, phosphoramidon, SOD, SP and urethane (Sigma Chemical Co. Ltd., Poole, Dorset), atropine sulphate (Phoenix Pharmaceuticals Ltd., Gloucester), propranolol hydrochloride (Inderal: Imperial Chemical Industries plc, Macclesfield, Cheshire), phentolamine mesylate (Rogitine: Ciba Laboratories, Horsham, West Sussex), Evans blue dye (Aldrich Chemical Co. Ltd., Gillingham, Kent), saline (0.9% wv^{-1} sodium chloride BP for intravenous infusion: FL Manufacturing Ltd., Basingstoke, Hants.). Aliquots of 0.1 mmol ml^{-1} SP were stored at −20°C and diluted in saline on each day of experimentation.

Data analysis

Data in Results are expressed as mean and one s.e.mean. Tissue content of Evans blue dye was expressed as ng dye per mg wet tissue and changes in PIP were expressed as percentage increases for the peak of the response above pretreatment baseline values. Mean blood pressure was calculated from the

recorded traces as diastolic pressure + 0.33 (systolic pressure − diastolic pressure). Changes in mean blood pressure were expressed as a percentage of the mean blood pressure before nerve stimulation or drug administration. Data were analyzed by Student's t test for paired data (two-tailed) or the Mann-Whitney U-test (two-tailed). Probabilities less than 0.05 were considered statistically significant.

As an index of variability between different 'batches' of guinea-pigs, baseline blood pressure data for all animal treatment groups were analyzed by one-way analysis of variance (ANOVA). Mean basal carotid arterial blood pressure in the 120 guinea-pigs used was 35.8 ± 0.9 mmHg. There were no significant differences in baseline blood pressure among the groups ($F = 0.8$, $P = 0.65$). Thus, all animal groups are likely to have come from the same population. Variability in mean baseline blood pressure within each of the experimental groups was assessed by expressing the s.e.mean as a percentage of the mean. The mean of these percentage values was 18.3% ($\pm 2.0\%$) which is within acceptable limits of variability.

Results

Effect of acute DMTU, SOD or catalase on cigarette smoke-induced plasma exudation

The effect of the oxygen radical inhibitors is shown in Figure 1. Inhalation of cigarette smoke (50 puffs) induced a significant ($P < 0.01$) increase in Evans blue dye content in main bronchi of 251% above air-exposed controls, indicating an increase in plasma exudation. This increase is similar in magnitude to that found previously for a comparable administration of cigarette smoke (Lei *et al.*, 1995). DMTU alone had no significant effect on EB dye content in air-exposed controls (reduced by 17% compared to saline vehicle), but significantly ($P < 0.05$) reduced the cigarette smoke-induced increase in dye content by 69%. SOD had no significant effect on dye content in animals exposed either to air or cigarette smoke (19% and 13% reductions respectively). In contrast to both DMTU and SOD, catalase significantly ($P < 0.05$) increased the dye content of the air-exposed controls by 42%. However, similar to DMTU, catalase reduced the cigarette smoke-induced increase in dye content by 45%, although not significantly so. Thus, of the three drugs used, only DMTU inhibited cigarette smoke-induced increases in tissue Evans blue dye content without affecting the air-exposed controls and so was used throughout in the studies below.

Acute DMTU pretreatment alone decreased blood pressure by $25.9 \pm 4.4\%$ ($n = 16$, $P < 0.001$) and caused a transient, but non-significant, increase in PIP above baseline of $37.9 \pm 11.5\%$ ($n = 10$). Neither catalase nor SOD alone had any marked or significant effect on baseline blood pressure (decreased by $11.7 \pm 1.6\%$ and $0.9 \pm 0.6\%$ respectively) or PIP (0% change for both, $n = 8-9$).

Chronic DMTU pretreatment on cigarette smoke-induced plasma exudation and the effect of phosphoramidon

Data for these experiments are shown in Figure 2. In saline pretreated animals, inhalation of cigarette smoke significantly ($P < 0.01$) increased the Evans blue dye content of the main bronchi by 201% above air-exposed controls. Chronic DMTU had no significant effect on dye content in air-exposed animals

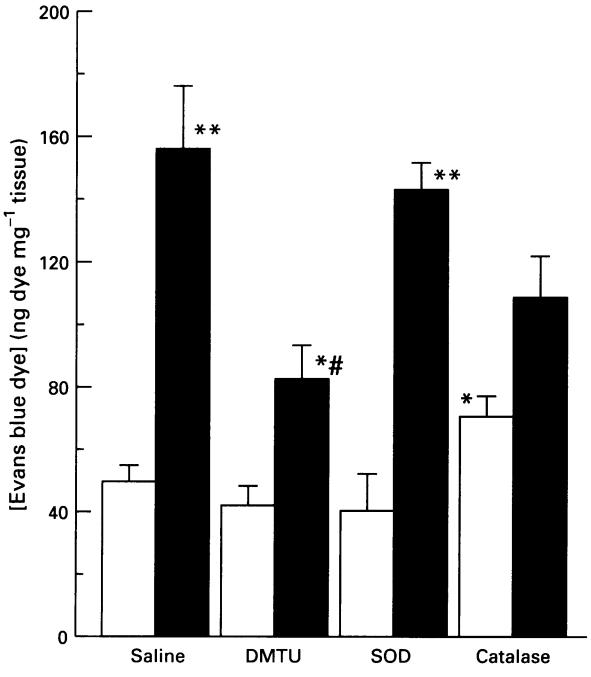

Figure 1 Involvement of oxygen-derived radicals in cigarette smoke-induced plasma exudation in guinea pig main bronchi *in vivo*. The hydroxyl radical scavenger dimethylthiourea (DMTU: 1.5 g kg^{-1}), the regulator of superoxide anion, superoxide dismutase (SOD: 240,000 u kg^{-1}), or the regulator of hydrogen peroxide, catalase (400,000 u kg^{-1}) were given acutely i.v. before cigarette smoke or sham-air. Control animals were injected with saline (1 ml kg^{-1}). Open columns, drugs + air; solid columns, drugs + cigarette smoke. Data are mean tissue content (with s.e.mean) of the plasma marker Evans blue dye for 4–6 animals per group. *$P < 0.05$, **$P < 0.01$ compared with corresponding air-exposed control; *$P < 0.05$ compared with saline + air; #$P < 0.05$ compared with saline + cigarette smoke.

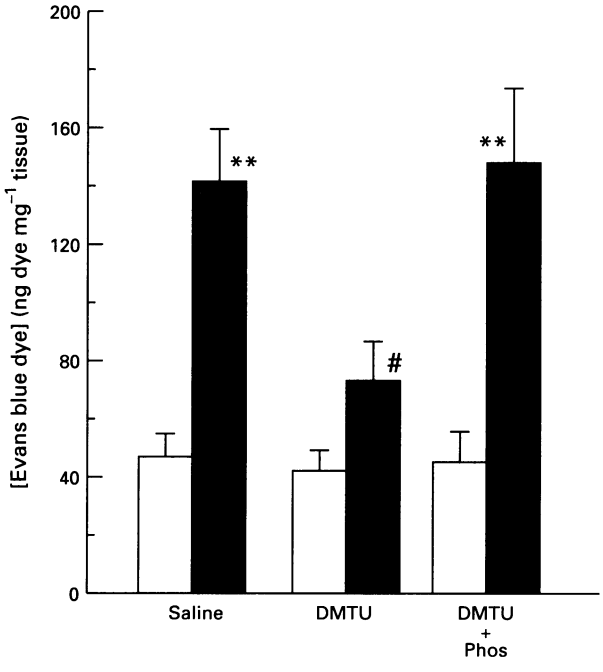

Figure 2 Effect of chronic pretreatment with dimethylthiouresa (DMTU) on cigarette smoke-induced plasma exudation in guinea-pig main bronchi *in vivo* and the effect of inhibition of neutral endopeptidase (NEP). Animals were given a total dose 500 mg kg^{-1} of DMTU over three days. Control animals were pretreated with saline (1 ml kg^{-1}). NEP was inhibited with phosphoramidon (Phos; 1.5 mg kg^{-1}). All drugs were given i.v. Open columns, drugs + air; solid columns, drugs + cigarette smoke. Data are mean tissue content (with s.e.mean) of the plasma marker Evans blue dye for 4–6 animals per group. **$P < 0.01$ compared with corresponding air-exposed control; #$P < 0.05$ compared with saline + cigarette smoke.

but significantly ($P<0.05$) reduced cigarette smoke-induced increase in dye content by 72% compared with saline controls. Phosphoramidon completely reversed the inhibition by DMTU of cigarette smoke-induced plasma exudation, without having any significant effect on air-exposed controls. Acute DMTU injection, after three day's chronic pretreatment, reduced blood pressure by $28.0\pm3.5\%$ ($n=16$, $P<0.01$).

Effect of acute or chronic DMTU on vagally-induced plasma exudation

Data for these experiments are shown in Figure 3. In animals in the 'acute' study, electrical stimulation of the vagus nerves significantly ($P<0.01$) increased the Evans blue dye content of the main bronchi by 167% above sham-stimulated values. Acute DMTU did not significantly reduce the vagally-induced increase in tissue dye content (Figure 3a). In animals in the 'chronic' study, electrical stimulation of the vagus nerves significantly ($P<0.01$) increased dye content by 250% above sham-stimulated values. Chronic pretreatment with DMTU significantly ($P<0.05$) reduced the vagally-induced increase in dye content by 82% (Figure 3b). Phosphoramidon reversed the inhibition by chronic DMTU of vagally-induced plasma exudation. In the presence of atropine, propranolol and phentolamine, vagus nerve stimulation decreased mean blood pressure by $43.7\pm2.6\%$ ($n=5$, $P<0.05$). In animals chronically pretreated with DMTU, vagal stimulation reduced blood pressure by $26.8\pm7.6\%$ ($n=5$, $P<0.05$).

Effect of acute or chronic DMTU on vagally-induced bronchoconstriction

Mean baseline PIP (measured just prior to any treatment) was 13.4 ± 0.2 cmH_2O ($n=120$). Data for these experiments are shown in Figure 4. In animals in the acute study, electrical stimulation of the vagus nerves caused a significant ($P<0.01$) increase in PIP of 200% above baseline, indicating bronchoconstriction. Acute DMTU did not significantly affect the vagally-induced increase in PIP (Figure 4a). In animals in the chronic study, electrical stimulation of the vagus nerves significantly increased ($P<0.01$) PIP by 295% above baseline. Chronic pretreatment with DMTU significantly ($P<0.05$) inhibited the increase in PIP by 84% (Figure 4b). Phosphoramidon reversed the inhibition by chronic DMTU of vagally-induced bronchoconstriction. In animals pretreated chronically with DMTU, the subsequent intravenous administration of DMTU caused a transient, but non-significant, increase in PIP above baseline of $32.0\pm8.4\%$ ($n=19$). The increases in PIP returned to baseline levels before vagal stimulation.

Effect of DMTU on SP-induced plasma exudation

In animals acutely or chronically pretreated with saline, SP significantly ($P<0.05$) increased Evans blue dye content in the main bronchi by 260% and 250% respectively ($n=5$ per group). These increases in plasma exudation were similar to those induced by either cigarette smoke or electrical stimula-

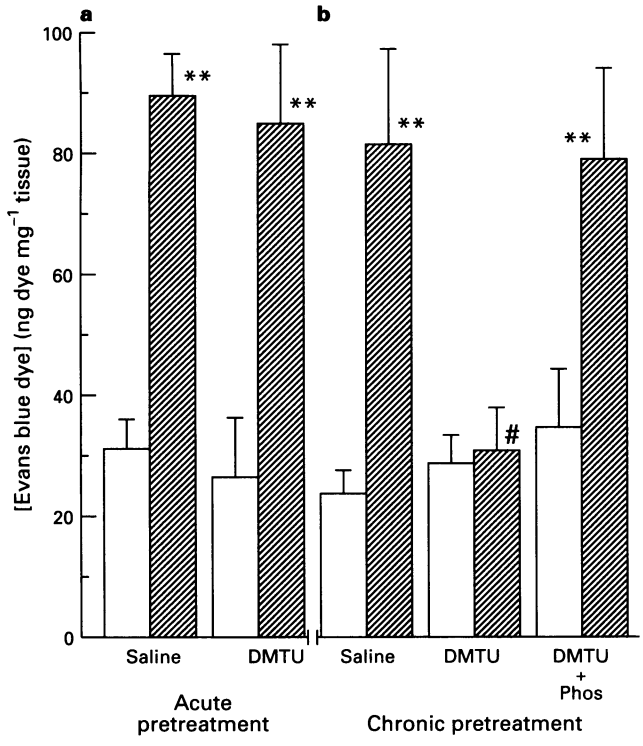

Figure 3 Involvement of hydroxyl radicals and neutral endopeptidase (NEP) in plasma exudation induced *in vivo* in guinea-pig main bronchi by electrical stimulation of the vagus nerves (7 Hz, 5 V, 5 ms for 3 min in the presence of atropine, propranolol and phentolamine, 1 mg kg^{-1} each, i.v.). The hydroxyl radical scavenger dimethylthiourea (DMTU) was given either acutely (1.5 g kg^{-1}) (a) or chronically (total dose 500 mg kg^{-1} of DMTU over three days) (b). NEP was inhibited with phosphoramidon (Phos; 1.5 mg kg^{-1}). Control animals were pretreated with saline (1 ml kg^{-1}, i.v.). Open columns, drugs + sham-stimulation; hatched columns, drugs + vagal stimulation. Data are mean tissue content (with s.e.mean) of the plasma marker Evans blue dye for 4–7 animals per group. $**P<0.01$ compared with corresponding sham-stimulated group; $\#P<0.05$ compared with chronic saline pretreatment + vagal stimulation.

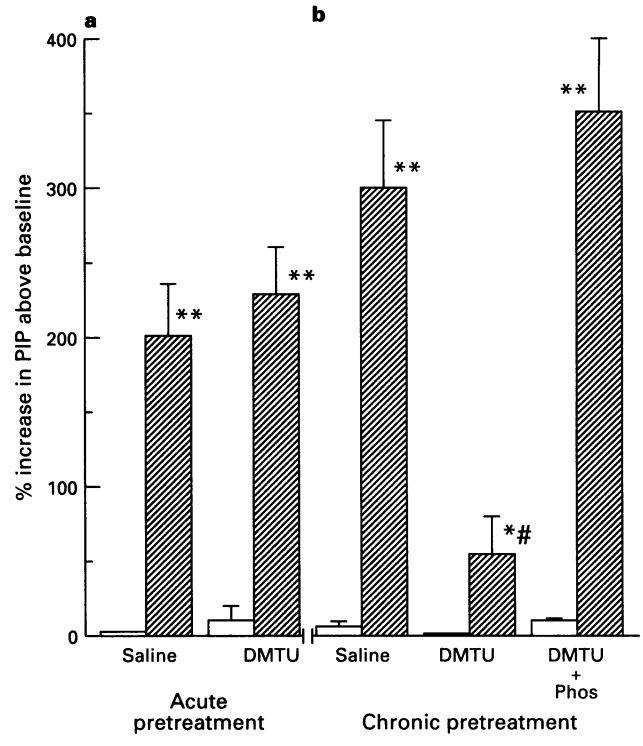

Figure 4 Involvement of hydroxyl radicals and neutral endopeptidase (NEP) in non-cholinergic bronchoconstriction induced *in vivo* in guinea-pigs by electrical stimulation of the vagus nerves (7 Hz, 5 V, 5 ms for 3 min in the presence of atropine, propranolol and phentolamine, 1 mg kg^{-1} each, i.v.). The hydroxyl radical scavenger dimethylthiourea (DMTU) was given either acutely (1.5 g kg^{-1}) (a) or chronically (total dose 500 mg kg^{-1} over three days) (b). NEP was inhibited with phosphoramidon (Phos; 1.5 mg kg^{-1}). Open columns, drugs + sham-stimulation; hatched columns, drugs + vagal stimulation. Data are mean % increase (with s.e.mean) in pulmonary insufflation pressure (PIP; an index of airway tone) above baseline for 4–7 animals per group. $*P<0.05$, $**P<0.01$ compared with corresponding sham-stimulated animals respectively. $\#P<0.05$ compared with chronic saline pretreatment + vagal stimulation.

tion of the vagus nerves. Neither acute nor chronic pretreatment with DMTU significantly affected the SP-induced increase in tissue dye content: dye content was in fact increased by a further 34±16% and 14±6%, respectively ($n=5$ per group), although not significantly so. SP reduced mean blood pressure by 44.0±2.0% ($n=4$), and the reduction was not significantly affected by either acute or chronic DMTU pretreatment ($n=8$, $P>0.05$ compared with SP alone).

Discussion

Neurogenic airway plasma exudation and non-cholinergic bronchoconstriction induced in rodents by cigarette smoke or by vagal stimulation are due to activation of capsaicin-sensitive 'sensory-efferent' nerves. The component(s) of the smoke responsible for activating the nerves are unknown. Cigarette smoke is a suspension of particulate matter in a gaseous or vapour phase. A number of reactive molecular species, in particular oxygen-derived radicals, are found in both the particulate and vapour phases of smoke (Church & Pryor, 1985). Tar in the particulate phase contains quinone-hydroquinone radicals which are capable of reducing molecular oxygen to superoxide leading to the generation of hydrogen peroxide and hydroxyl radicals. Removing the particulate phase from the smoke dose not diminish cigarette smoke-induced airway plasma exudation (Lundberg *et al.*, 1983; Lei *et al.*, 1995) which indicates that it is component(s) in the vapour phase which activate the nerves. The vapour phase of cigarette smoke contains oxygen-centred and carbon-centred radicals which are highly reactive and oxidize NO to NO_2. Displacement of nitrogen peroxide and hydrogen peroxide occurs in the vapour phase which generates hydroxyl radicals according to the reaction $NO_2+H_2O_2\rightarrow HONO_2+OH^{\cdot}$ (Church & Pryor, 1985). These data support previous experimental observations that the vapour phase is mainly responsible for cigarette smoke-induced airway plasma exudation (Lundberg & Saria 1983; Lei *et al.*, 1995) and bradypnoea (Lee, 1990).

In the present study, cigarette smoke-induced plasma exudation was inhibited by the scavenger of hydroxyl radicals, DMTU (Fox *et al.*, 1983; Fox, 1984; Wasil *et al.*, 1987), whether administered acutely or chronically. The inhibtion was unlikely to be related to non-specific effects of DMTU on blood pressure because, for example, calcitonin gene-related peptide, a sensory neuropeptide which markedly lowers blood pressure, does not induce airway plasma exudation nor does it potentiate SP-induced plasma exudation (Rogers *et al.*, 1988). Previous studies have shown that DMTU inhibits cigarette smoke-induced bradypnoea (Lee, 1990). In the present study, vagal stimulation induced neurogenic plasma exudation and, in the presence of atropine, also induced non-cholinergic bronchoconstriction. These responses were inhibited by chronic pretreatment with DMTU but not by acute treatment. These results are consistent with the finding that capsaicin-induced bronchoconstriction is inhibited by the same protocol of chronic DMTU pretreatment but not by acute treatment (Lai, 1990). We did not try an additional acute dose of DMTU in the present study because this has previously been found to be ineffective (Lai, 1990). The inhibition by DMTU in the studies above and in the present study imply that hydroxyl radicals are involved in neurogenic inflammation.

Substance P is the most effective of the tachykinins in inducing plasma exudation in guinea-pig airways (Rogers *et al.*, 1988), and presumably is the principal sensory neuropeptide involved in cigarette smoke-induced and vagally-induced plasma exudation. In the present study, DMTU did not affect SP-induced plasma exudation. The latter observation indicates that the inhibition by DMTU of neurogenic inflammation is via a neural (prejunctional) inhibitory effect rather than via a post-junctional effect, for example DMTU acting as a SP receptor antagonist or by directly affecting the bronchial microvascular cells to non-specifically inhibit leakage.

DMTU is an effective non-enzymatic scavenger of hydroxyl radicals *in vitro* and *in vivo* (Fox *et al.*, 1983; Fox, 1984; Wasil *et al.*, 1987). It has a comparatively long biological half-life (~36 h) and has very little effect on other oxygen-derived reactive species including superoxide anion or hydrogen peroxide (Fox *et al.*, 1983). A number of possible mechanisms underlying the inhibition of chronic pretreatment with DMTU on neurogenic airway responses have been proposed (Lai, 1990). For example, oxygen-derived free radicals may be involved in either the biosynthesis or axonal transport of tachykinins, or both. Thus, it is possible that chronic pretreatment with DMTU reduces tachykinin biosynthesis and/or their transportation to the C-fibre endings. Thus, the amount of available tachykinins which can be released in response to cigarette smoke or vagal stimulation is decreased which leads to a reduction in the functional response observed. Whether DMTU scavenges hydroxyl radicals contained in the smoke or generated neuronally by the actions of cigarette smoke is unknown. It is possible that there is no distinction between acute and chronic administration of DMTU. For chronic administration, the dose given on the day of experimentation, after three days' chronic treatment, was half that of acute administration (0.75 g kg^{-1} vs 1.5 g kg^{-1} respectively) (Lai *et al.*, 1990). Although reduced, the dose of 0.75 g kg^{-1} DMTU may still acutely scavenge sufficient hydroxyl radicals to reduce the exudative response to cigarette smoke, irrespective of the three day's pretreatment with DMTU. The latter suggestion is unlikely because in the present study vagus nerve stimulation increased both plasma exudation and airway tone and both of these responses were inhibited by chronic treatment but not by acute treatment with DMTU. In addition, doubling the acute dose of DMTU does not inhibit airway neurogenic bronchoconstriction (Lai *et al.*, 1990). Thus, it is the duration of treatment more than the dose of DMTU which differentiates between its acute and chronic effects.

In the present study, neither SOD nor catalase had any significant effect upon cigarette smoke-induced plasma exudation which indicates that neither superoxide anions nor hydrogen peroxide were involved in the activation of the nerves. *In vitro* studies have shown that SOD significantly reverses the reduction of NEP activity by cigarette smoke solution (Dusser *et al.*, 1989). However, the lack of effect of SOD on cigarette smoke-induced airway plasma exudation *in vivo* in the present study is consistent with previous results (Bjork *et al.*, 1980). The intravenous route of administration of SOD was used in the present study because it has been recommended as most effective (Bast *et al.*, 1991), although the short biological half-life of SOD may lead to reduced effective levels with time. Catalase inhibits production of hydrogen peroxide by transforming H_2O_2 into water and oxygen, and then reduces the concentration of hydrogen peroxide in the cells (Chance *et al.*, 1979; Till *et al.*, 1982). In the present study, catalase reduced cigarette smoke-induced plasma exudation in the main bronchi by 40% but increased plasma exudation in the air-exposed animals. Thus, it is difficult to interpret the effect of catalase on cigarette smoke-induced plasma exudation and thereby assess the contribution of hydrogen peroxide to the exudative response. However, no evidence for hydrogen peroxide decreasing NEP activity in the airway epithelium has been found (Murlas *et al.*, 1992), and neither SOD nor catalase affect capsaicin-induced bronchoconstriction (Lai, 1990). The involvement of these oxygen radicals in cigarette smoke-induced responses appears, therefore, to be minimal. However, it is possible that conversion of superoxide anion and hydrogen peroxide to hydroxyl radicals (Haber & Weiss, 1934) could contribute to the exudative response.

In vitro, the NEP activity of the tracheal epithelium has been found to be decreased after exposure to cigarette smoke (Dusser *et al.*, 1989). Similarly, exposure to hypochlorous acid (HOCl), a component of cigarette smoke, causes guinea-pig airway hyperresponsiveness and decreased NEP activity (Murlas *et al.*, 1992). In the present study, the inhibitory effect of chronic pretreatment with DMTU on plasma exudation induced by cigarette smoke or vagal stimulation and vagally-

induced bronchoconstriction were reversed by the neural endopeptidase inhibitor, phosphoramidon. These data indicate that it is hydroxyl radicals in the cigarette smoke which decrease NEP activity in the airways.

In summary, the results of the present work indicate that hydroxyl radicals, rather than superoxide anions or hydrogen peroxide, mediate the neurogenic airway plasma exudation and bronchoconstriction induced by cigarette smoke or electrical stimulation of vagal sensory nerves. Acute DMTU may affect directly the neural actions of hydroxyl radicals contained in the cigarette smoke. Chronic pretreatment with DMTU may inhibit the neurogenic airway responses, possibly due to its effects on tachykinin biosynthesis and/or axonal transport.

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