Br. J. Pharmacol. (1990), **100**, 69–72

Effects of aerosolised substance P on lung resistance in guinea-pigs: a comparison between inhibition of neutral endopeptidase and angiotensin-converting enzyme

[1]Jan O. Lötvall, Bengt-Eric Skoogh, Peter J. Barnes & K. Fan Chung

National Heart and Lung Institute, Department of Thoracic Medicine, Dovehouse Street, London SW3 6LY and Department of Pulmonary Medicine, Gothenburg University, Box 17301, S-402 64, Gothenburg, Sweden

1 We have examined in guinea-pigs, *in vivo*, the effects of inhibition of neutral endopeptidase (NEP) and angiotensin-converting enzyme (ACE) on the airway response to aerosolised substance P (SP). We aerosolised captopril (4.6 mM, 60 breaths; 210 nmol) to inhibit ACE and acetorphan (0.3, 1 and 3 mM, 60 breaths; 9 nmol, 33 nmol and 110 nmol respectively) to inhibit NEP. We also examined the effect of the highest dose of acetorphan (110 nmol) on the response to aerosolised acetylcholine (ACh).

2 Responsiveness to SP (or ACh) was measured as the change in lung resistance (R_L) induced by nebulisation of increasing concentrations of SP (or ACh) before and after treatment with the inhibitor. PC_{200}, defined as the provocative concentration inducing an increase in R_L of 200% above baseline, was calculated for each challenge.

3 Administration of acetorphan before the second SP-challenge induced a dose-dependent decrease in PC_{200} for SP amounting to 1.8 ($\pm$0.3) log units after treatment with 11 nmol acetorphan. Treatment with vehicle before the second SP-challenge or with 3 mM acetorphan before the second ACh-challenge had no significant effect on PC_{200}.

4 Treatment with captopril (21 nmol) induced only a small, nonsignificant leftward shift of PC_{200} to SP (0.3 $\pm$ 0.2 log units).

5 We conclude that a NEP-like enzyme, but not ACE, regulates the response to aerosolised SP. We suggest that the same is true for SP released endogenously from sensory nerve endings in the airway epithelial layer.

Introduction

Substance P (SP) is a potent neuropeptide present in airway sensory nerves of several species including guinea-pigs and man, and found in close relation to the airway smooth muscle, beneath and within the epithelium, around ganglion cells and around blood vessels in the airway interstitium (Lundberg *et al.*, 1984b). SP is a potent mediator of smooth muscle contraction, mucus secretion and microvascular leakage in airways of several species (Lundberg *et al.*, 1983; 1984a; Coles *et al.*, 1984; Lötvall *et al.*, 1989), and has been implicated in the pathogenesis of asthma (Barnes, 1986) and in bronchial hyperreactivity induced by respiratory viral infections (McDonald, 1988) or toluene diisocyanate (Sheppard & Scypinski, 1988).

Enzymes which degrade SP seem to be important regulators of its actions (Schwartz *et al.*, 1985; Borson *et al.*, 1987; Stimler-Gerard, 1987; Sekizawa *et al.*, 1987; Dusser *et al.*, 1988; Shore *et al.*, 1988; Sheppard & Scypinski, 1988; Umeno *et al.*, 1989). SP is degraded *in vitro* by several peptidases including neutral endopeptidase (NEP) and, to a lesser degree, angiotensin-converting enzyme (ACE) (Skidgel *et al.*, 1984). Although the lung contains both ACE and NEP activities, they are distributed differently between airway and vascular tissues, ACE being more active in vascular and NEP in airway tissues (Johnson *et al.*, 1985). By use of immunohistochemical staining, ACE has been shown to be localized at the luminal surface of the vascular endothelium, whereas NEP has been localized within epithelial cells of the alveolar septa and within tracheal smooth muscle and epithelium (Johnson *et al.*, 1985; Sekizawa *et al.*, 1987). Thus, the relative ability of ACE-inhibitors and NEP-inhibitors to facilitate the effects of exogenous SP *in vivo* may be influenced not only by the activity of these enzymes, but also by the route of administration for SP. In the guinea-pig, inhibition of ACE by captopril and of NEP by thiorphan, shifted the dose-response curve for the bronchoconstrictor effect of intravenously-administered SP to the same extent (Shore *et al.*, 1988) in spite of the *in vitro* observation that the SP-cleaving activity of ACE is less than that of NEP (Skidgel *et al.*, 1984).

We reasoned that if different distribution in the lung of ACE- and NEP-activity explained the results of Shore *et al.* (1988), then NEP-inhibition should be much more efficient than ACE-inhibition in shifting the dose-response curve to inhaled SP, because both the higher SP-cleaving activity of NEP and its localization in airway epithelial cells would work in that direction. We therefore compared in guinea-pigs the effects of NEP-inhibition and of ACE-inhibition on changes in lung resistance (R_L) induced by aerosolised SP. We administered SP in sufficient doses to provoke bronchoconstriction before and after inhibition of the degrading enzymes, thus enabling us to calculate the shifts of the concentration-response curve for SP within the same animal. We gave the inhibitors by aerosol and used captopril to inhibit ACE and acetorphan to inhibit NEP. Acetorphan is a prodrug becoming about 1000 times more active after hydrolysation into thiorphan (Lecomte *et al.*, 1986).

Methods

Preparation

We studied male Dunkin-Hartley guinea-pigs weighing 350–600 g. The animals were fed standardized guinea-pig chow and tap water freely. On the day of study they were weighed and anaesthetized with an initial dose of 6–8 ml kg^{-1} of urethane diluted to 25% w/v in 0.9% saline, injected intraperitoneally. Additional urethane was given as required to maintain appropriate anaesthesia level. A tracheal cannula (9–11 mm in length and 2.7 mm inner diameter) was inserted into the lumen of the cervical trachea through a tracheostomy, and tied snugly with suture material. A polyethylene catheter was inserted into the left carotid artery to monitor blood pressure and heart rate with a pressure transducer. The right external jugular vein was cannulated for the administration of i.v. drugs or fluids.

[1] Author for correspondence at London address.

The guinea-pigs were placed in a supine position with the tracheal cannula connected to a constant volume mechanical ventilator (Harvard model 50-1718, Edenbridge, U.K.). A tidal volume of 10 ml per kg and a frequency of 60 breaths per min was used. Transpulmonary pressure was measured with a pressure transducer (Furness, model FCO 40; $\pm 1000\,mmH_2O$, Glasgow), with one side attached to a catheter inserted into the right pleural cavity and the other side attached to a catheter connected to a side port of the intratracheal cannula. The ventilatory circuit had a total volume of 20 ml. Airflow was measured with a pneumotachygraph (Mercury, model F1, Glasgow) connected to a transducer (Model FCO 40; $\pm 1\,cmH_2O$: Furness Controls Ltd, Bexhill, Sussex). Tidal volume was obtained by electronic integration of the flow signal. The aerosols were generated with an ultrasonic nebuliser (PulmoSonic, Model 2511; DeVilbiss Co, PA, U.S.A.), and were administered to the airways through a separate ventilatory system, that bypassed the pneumotachygraph. The volume of this circuit was 50 ml. The output from this system, measured at the port of the tracheal cannula with an airflow equivalent to the ventilation rate, was 40 μl per 60 breaths.

We used a 6 channel recorder (model MX6, Devices Ltd, London) for flow, transpulmonary pressure and blood pressure measurements. Expiratory lung resistance (R_L) was calculated by the method of von Neergaard & Wirz (1927). Briefly, the pressure to overcome resistance (P_r) was divided by the airflow measured at a time point 0.1 s after beginning of expiration for each calculated breath.

Protocols

The animals were divided into six groups in order to study the effect of three different concentrations of acetorphan (0.3, 1 or 3 mM), captopril (4.6 mM) or vehicle on the response to SP, and the effect of acetorphan on the response to ACh. All these treatments were given for 60 breaths. The concentrations for acetorphan were selected according to preliminary experiments. The concentration for captopril was then chosen to give approximately the same relation to the highest concentration of acetorphan, as the relation between the dose for thiorphan and captopril in an earlier study using i.v. administration of the enzyme inhibitors (Shore *et al.*, 1988). ACh-challenged animals were treated with the highest concentration of acetorphan, in order to investigate the specificity of acetorphan for SP.

All animals were intially treated with propranolol ($1\,mg\,kg^{-1}$, i.v.), to counteract inhibitory effects on the airway smooth muscle induced by catecholamines released by urethane (Spriggs, 1965). Ten minutes later the guinea-pigs were exposed to an airway challenge with aerosolised saline (0.9%), given for 20 breaths and baseline R_L measurements were made. Five minutes later, a dose-response challenge to SP or ACh was started, by increasing the concentration in the nebuliser at half log molar concentrations, starting at 3 μM for SP, and at 30 μM for ACh. For each dose, the nebuliser was filled with 3 ml of the solution. All challenge inhalations were given for 20 breaths and at 5 min intervals. Two hyperinflations with twice the tidal volume were performed between each challenge, by blocking the outflow of the ventilator. The dose-response challenges were stopped when an increase of at least 200% in R_L was seen. Subsequently the animals were allowed to recover to stable R_L values before a single dose of enzyme inhibitor, or vehicle, was given by aerosol for 60 breaths from another nebuliser.

Fifteen minutes after treatment with enzyme inhibitor, or vehicle, a second dose-response challenge, similar to the previous one, was performed. A baseline R_L value was again obtained by exposing the animals to 20 breaths of saline (0.9%). The second SP dose-response was started at 0.03 mM for animals given 11 nmol of acetorphan, at 0.3 μM when given 3.3 nmol acetorphan, and 3 μM when given 0.9 nmol. The second ACh challenge, after acetorphan 11 nmol, was started at the same dose of ACh as the first challenge. For animals given captopril 21 nmol, or vehicle, the second challenges were started at 3 μM of SP. If not significant response was seen with SP at the maximum concentration of 1 mM, a second challenge, with the same concentration in the nebuliser, but given for twice the number of breaths (40), was performed. This dose was labelled 2 mM, for calculation of PC_{200} values.

The responses were evaluated as the maximum R_L values after each challenge, and were expressed as % increase above the R_L value obtained after aerosolised saline (0.9%). PC_{200} was defined as the provocative concentration for a 200% increase of R_L over baseline. PC_{200} was determined for each dose-response challenge, by log-linear interpolation between the concentrations connecting the points where R_L reached 200% above baseline (Figure 1).

Drugs

We used the following drugs: propranolol (Inderal, ICI); acetorphan (kindly donated by Bioprojet, Paris, France); captopril (Squibb); urethane diluted to 25% w/v in 0.9% saline; SP and ACh (Sigma Chemical Company, Poole, Dorset). SP and ACh were inhaled diluted in 0.9% saline. Acetorphan was stored at 4°C in a stock solution in 5% ethanol. Further dilution was made in saline to 3 mM, which was stored at −20°C. Equivalent concentrations of diluents were stored in a similar way and were used in control experiments. Captopril was dissolved in the same solvent as acetorphan.

Data analysis

Data are reported as mean ± s.e.mean. Statistical analyses were done on log-transformed PC_{200} values. For each experiment we calculated the $\log_{10}$ shift of PC_{200} between the first and second challenge. One way analysis of variance (ANOVA) and Dunnett's test (Zar, 1974) were used to determine significant differences among groups for the shift in PC_{200} and for changes in baseline R_L. Data were analysed with a Macintosh computer (Apple Computer Inc., Cupertino, Ca, U.S.A.) using a standard statistical package (StatView 512, Abacus Concept, Inc., CA, U.S.A.).

Results

Mean baseline values after aerosolised saline (20 breaths) for the different animal groups are given in Table 1. SP aerosol caused a dose-dependent increase in R_L at concentrations of 0.1 mM and higher (Figure 1). The cut-off level for definition of the provocative concentration, 200% increase of R_L above baseline, was found on the linear part of the concentration-response curve. Treatment with acetorphan caused a dose-dependent increase in airway responsiveness to aerosolised SP. The shift in PC_{200} was significant compared to that after vehicle for all three concentrations used. The highest dose of acetorphan (110 nmol) shifted PC_{200} for SP by 1.8 (±0.26) log units to the left (Figures 2 and 3). This shift was significantly larger than that induced by 0.9 nmol ($p < 0.05$) but not significantly different from that induced by 3.3 nmol acetorphan. In contrast, treatment with aerosolised captopril (4.6 mM;

Table 1 Baseline lung resistance values ($cmH_2O\,ml^{-1} \times s^{-1}$) in the various treatment groups prior to substance P (SP) or acetylcholine (ACh) challenge

Treatment challenge	n	*First challenge*	*Second*
Vehicle + SP	5	0.28 (0.03)	0.41 (0.06)
Acetorphan 3 mM + ACh	4	0.30 (0.04)	0.50 (0.17)
Acetorphan 0.3 mM + SP	6	0.27 (0.01)	0.49 (0.06)
Acetorphan 1 mM + SP	6	0.30 (0.02)	0.77 (0.16)
Acetorphan 3 mM + SP	7	0.34 (0.03)	0.79 (0.11)
Captopril 4.6 mM + SP	6	0.23 (0.01)	0.38 (0.04)

Values are mean ± s.e.mean

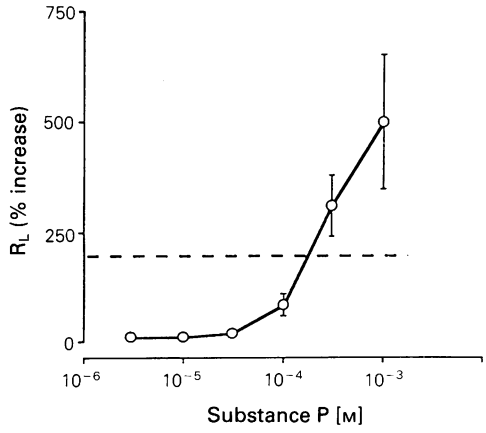

Figure 1 Mean increase in lung resistance (R_L), expressed as % above baseline, with increasing concentrations of substance P aerosol in 30 guinea-pigs ($n = 30$ up to 0.1 mM, $n = 27$ for 0.3 mM, and $n = 11$ for 1 mM), s.e.mean shown by vertical bars. We defined airway responsiveness to substance P as the provocative concentration needed to cause a 200% increase in R_L above baseline, as shown by the horizontal dotted line.

210 nmol) (Figure 2) or vehicle before the second SP challenge, or with 3 mM acetorphan before the second ACh-challenge, induced only small, nonsignificant shifts in PC_{200} (Figure 2).

The mean baseline R_L before the second dose-response challenge were in all groups, including the control group treated by vehicle, higher than before the first challenge

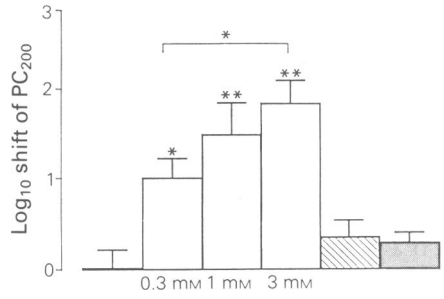

Figure 2 Leftward shift in PC_{200} (mean with s.e.mean shown by vertical bars) for substance P after treatment with vehicle (filled column), acetorphan (open columns at concentrations indicated) and 4.6 mM captopril (hatched column). The stippled column indicates the leftward shift in PC_{200} for acetylcholine after treatment with 3 mM acetorphan. Acetorphan induced a dose-dependent shift of PC_{200} for substance P, whereas captopril or vehicle had no significant effects. Acetorphan had no effect on the response to acetylcholine. (* $P < 0.05$; ** $P < 0.01$).

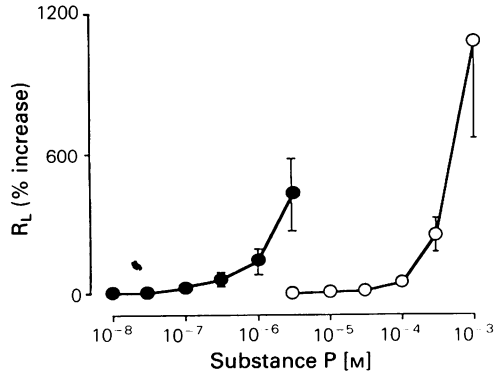

Figure 3 Mean concentration-response curves for substance P before (○) and after (●) aerosolisation of acetorphan (3 mM, 60 breaths; 110 nmol); s.e.mean shown by vertical bar. Acetorphan induced a significant leftward shift of the dose-response curve for substance P ($P < 0.01$).

(Table 1). However, this increase did not differ significantly between the groups. Baseline R_L did not correlate with the shifts in PC_{200} for SP-induced by acetorphan ($r = 0.09$).

Nebulisation of vehicle, acetorphan or captopril (60 breaths) induced a temporary increase in R_L in all groups. Compared to the increase in R_L induced by vehicle, only 2 nmol acetorphan induced significantly ($P < 0.01$) larger increases in R_L. There was a slight nonsignificant increase of the mean blood pressure in all groups 15 min after the nebulisation.

Discussion

This study shows that NEP-inhibition markedly potentiates the effect of aerosolised SP on lung resistance in guinea-pigs, whereas inhibition of ACE causes no significant potentiation. This is in contrast to studies where SP and the inhibitors were given intravenously in the same species. Inhibition of ACE was then as effective as inhibition of NEP in enhancing bronchoconstrictor responses (Shore *et al.*, 1988). Furthermore, the maximal shift of the dose-response curve for SP is clearly larger in this study using aerosol administration (1.8 log units) compared to the i.v. route (about 0.5 log units) (Shore *et al.*, 1988). The specificity of the effect of NEP-inhibition was demonstrated by its lack of effect on the response to aerosolised ACh.

Both NEP and ACE cleave SP (Skidgel *et al.*, 1984). The vascular endothelium is rich in ACE (Johnson *et al.*, 1985; Ryan *et al.*, 1985) implying SP administered i.v. is likely to be cleaved by ACE before reaching target tissues in the airways. However, the ACE-activity in airway tissue has been shown to be low compared to that of vascular tissue (Johnson *et al.*, 1985). Our data, showing no significant effect of ACE-inhibition on the response to aerosolised SP, indicates that the ACE-content in airway mucosa is low, which would explain the larger effect of NEP-inhibition on aerosolised SP compared to i.v. SP. Acetorphan and thiorphan also inhibit ACE (Schwartz *et al.*, 1981) which could contribute to the enhancement of the SP response (Skidgel *et al.*, 1984). However, this can be excluded as ACE-inhibition, since captopril had no significant effect. Furthermore, thiorphan is about 40 times less potent as an inhibitor of ACE than as an inhibitor of NEP (Schwartz *et al.*, 1981) and it does not affect ACE-activity in plasma when given by i.v. infusion (Spillantini *et al.*, 1986).

The maximal shift of PC_{200} for aerosolised SP induced by NEP-inhibition in our study agrees well with data from an earlier study by Dusser *et al.* (1988) using phosphoramidon for inhibition of NEP. They did not use sufficient SP-concentrations to elicit a bronchoconstrictor response before NEP inhibition, allowing calculation of the shift. However, if our concentration-response curve before NEP inhibition (Figure 1) is applied to their data, the calculated shift would be of the same magnitude as ours.

SP aerosol has given no bronchoconstrictor response in some earlier studies in guinea-pig (Dusser *et al.*, 1988) and in man (Joos *et al.*, 1987). However, if the SP aerosol is given in sufficiently high concentrations (about 1 mM), it induces airway narrowing in the absence of NEP inhibition (Lötvall *et al.*, 1989). The present and earlier (Dusser *et al.*, 1988) studies indicate that the probable explanation of the need for such high concentrations of SP is that NEP in airway epithelial cells constitutes a metabolic barrier to inhaled SP. When administered by the i.v. route, SP bypasses this barrier but may be metabolised by ACE in vascular tissues (Johnson *et al.*, 1985) and NEP in circulating blood (Spillantini *et al.*, 1986).

The mean baseline R_L before the second concentration-response curve had increased in all treatment groups including those treated with captopril and vehicle (Table 1). There was no significant difference between the groups in this respect, although the change in baseline R_L seemed to be more pronounced in acetorphan-treated animals, indicating a specific effect of acetorphan on R_L. This is further supported by

the observation that aerosolisation of 3 mM acetorphan, both in SP- and in ACh-challenged animals, induced a temporary increase in R_L that was significantly larger than that seen after treatment with vehicle. This indicates that aerosolisation of vehicle may induce a slight tachykinin release, the effect of which is then potentiated by acetorphan, but there is no direct evidence to support this hypothesis.

It should be pointed out that acetorphan is a prodrug that is about 1000 times more active after hydrolysation into thiorphan. Such hydrolysation has been demonstrated to take place in mouse brain membranes (Lecomte *et al.*, 1986) and in plasma (Spillantini *et al.*, 1986). Our data show that acetorphan also must be hydrolysed locally in the airways, presumably in the airway epithelium. Hydrolysation after systemic uptake would most probably provide too low concentrations to have any activity.

Our study confirms earlier studies demonstrating the key role for NEP-like enzymes in the lung for the control of the response to exogenous SP (Borson *et al.*, 1987; Stimler-Gerard, 1987; Sekizawa *et al.*, 1987; Dusser *et al.*, 1988; Shore *et al.*, 1988). It has extended these observations showing that the potentiation of the response to aerosolised SP by inhibition of NEP is indeed large, about two log units, whereas inhibition of ACE has no significant effect on the response to aerosolised SP.

This study also indicates the probable importance of NEP-like enzymes in airway epithelial damage. SP-reactive sensory nerves are present within and underneath the airway epithelium (Lundberg *et al.*, 1984b). Many airway irritants stimulate sensory nerves (Sellick & Widdicombe, 1971). It is then likely that airway epithelial damage can increase the responsiveness to various inhaled irritants via an impairment of the NEP-like activity. Support for this reasoning is given by studies showing that viral infections enhance the contractile responses to SP in guinea-pig bronchi (Saban *et al.*, 1987) and ferret trachea (Jacoby *et al.*, 1988). Furthermore, histories of infections in rats have been associated with a decreased activity of NEP and an enhanced SP-induced vascular leakage in the trachea (Borson *et al.*, 1989).

We thank Draco AB (subsidiary to Astra, Sweden) and the Swedish Heart-Lung Foundation for financial support. We thank Dr Claes-Göran Löfdahl and Dr Geoff Nichol for valuable discussion during the course of the study.

References

BARNES, P.J. (1986). Asthma as an axon reflex. *Lancet*, **i**, 242–245.

BORSON, D.B., BROKAW, J.J., SEKIZAWA, K., MCDONALD, D.M. & NADEL, J.A. (1989). Neutral endopeptidase and neurogenic inflammation in rats with respiratory infections. *J. Appl. Physiol.*, **66**, 2653–2658.

BORSON, D.B., CORRALES, R., VARSANO, S., GOLD, M., VIRO, N., CAUGHEY, G., RAMACHANDRAN, J. & NADEL, J.A. (1987). Enkephalinase inhibitors potentiate substance P-induced secretion of SO_4-macromolecules from ferret trachea. *Exp. Lung. Res.*, **12**, 21–36.

COLES, S.J., NEILL, K.H. & REID, L.M. (1984). Potent stimulation of glycoprotein secretion in canine trachea by substance P. *J. Appl. Physiol.*, **57**, 1323–1327.

DUSSER, D.J., UMENO, E., GRAF, T., DJOKIC, T., BORSON, D.B. & NADEL, J.A. (1988). Airway neutral endopeptidase-like enzyme modulates tachykinin-induced bronchoconstriction *in vivo*. *J. Appl. Physiol.*, **65**, 2585–2591.

JACOBY, D.B., TAMAOKI, J., BORSON, D.B. & NADEL, J.A. (1988). Influenza infection causes airway hyperresponsiveness by decreasing enkephalinase. *J. Appl. Physiol.*, **64**, 2653–2658.

JOHNSON, A.R., ASHTON, J., SCHULZ, W.W. & ERDÖS, E.G. (1985). Neutral metalloendopeptidase in human lung tissue and cultured cells. *Am. Rev. Respir. Dis.*, **132**, 564–568.

JOOS, G., PAUWELS, R. & VAN DER STRAETEN, M. (1987). Effect of inhaled substance P and neurokinin A on the airways of normal and asthmatic subjects. *Thorax*, **42**, 779–783.

LECOMTE, J.M., COSTENTIN, J., VLAICULESCU, A., CHAILLET, P., MARCAIS-COLLADO, H., LLORENS-CORTES, C., LEBOYER, M. & SCHWARTZ, J.C. (1986). Pharmacological properties of acetorphan, a parenterally active "enkephalinase" inhibitor. *J. Pharmacol. Exp. Ther.*, **237**, 937–944.

LUNDBERG, J.M., BRODIN, E., XIAOYING, H. & SARIA, A. (1984a). Vascular permeability changes and smooth muscle contraction in the relation to capsaicin-sensitive substance P afferents in the guinea-pig. *Acta Physiol. Scand.*, **120**, 217–227.

LUNDBERG, J.M., HÖKFELT, T., MARTLING, C.R., SARIA, A. & CUELLO, C. (1984b). Substance P-immunoreactive sensory nerves in the lower respiratory tract of various mammals including man. *Cell Tissue Res.*, **235**, 251–261.

LUNDBERG, J.M., MARTLING, C.R. & SARIA, A. (1983). Substance P and capsaicin-induced contraction of human bronchi. *Acta Physiol. Scand.*, **119**, 49–53.

LÖTVALL, J., LEMEN, R.J., BARNES, P.J. & CHUNG, K.F. (1989). Aerosolized substance P produces partially irreversible airway constriction in guinea pigs. *Am. Rev. Respir. Dis.*, **139**, (suppl), A236.

MCDONALD, D.M. (1988). Respiratory tract infections increase susceptibility to neurogenic inflammation in the rat trachea. *Am. Rev. Respir. Dis.*, **137**, 1432–1440.

RYAN, U.S., RYAN, W.J. & CRUTCHLEY, D.J. (1985). The pulmonary endothelial surface. *Fedn Proc.*, **44**, 2603–2609.

SABAN, R., DICK, E.C., FISHLEDER, R.I. & BUCKNER, C.K. (1987). Enhancement by parainfluenza 3 infection of contractile responses to substance P and capsaicin in airway smooth muscle from the guinea pig. *Am. Rev. Respir. Dis.*, **136**, 586-591.

SCHWARTZ, J.C., CONSTENTIN, J. & LECOMTE, J.M. (1985). Pharmacology of enkephalinase inhibitors. *TIPS*, **6**, 472–476.

SCHWARTZ, J.C., MALFROY, B. & DE LA BAUME, S. (1981). Biological inactivation of enkephalins and the role of enkephalin-dipeptidyl-carboxypeptidase ("enkephalinase") as neuropeptidase. *Life Sci.*, **29**, 1715–1740.

SEKIZAWA, K., TAMAOKI, J., GRAF, P.D., BASBAUM, C.B., BORSON, D.B. & NADEL, J.A. (1987). Enkephalinase inhibitor potentiates mammalian tachykinin-induced contraction in ferret trachea. *J. Pharmacol. Exp. Ther.*, **243**, 1211–1217.

SELLICK, H. & WIDDICOMBE, J.G. (1971). Stimulation of lung irritant receptors by cigarette smoke, carbon dust, and histamine aerosol. *J. Appl. Physiol.*, **31**, 15–19.

SHEPPARD, D. & SCYPINSKI, L. (1988). A tachykinin receptor antagonist inhibits and an inhibitor of tachykinin metabolism potentiates toluene diisocyanate-induced airway hyperresponsiveness in guinea pigs. *Am. Rev. Respir. Dis.*, **138**, 547–551.

SHORE, S.A., STIMLER-GERARD, N.P., COATS, S.R. & DRAZEN, J.M. (1988). Substance P-induced bronchoconstriction in the guinea pig, enhancement by inhibitors of neutral metalloendopeptidase and angiotensin-converting enzyme. *Am. Rev. Respir. Dis.*, **137**, 331–336.

SKIDGEL, R.A., ENGELBRECHT, S., JOHNSON, A.R. & ERDÖS, E.G. (1984). Hydrolysis of substance P and neurotensine by converting enzyme and neutral endopeptidase. *Peptides*, **5**, 769–776.

SPILLANTINI, M.G., GEPPETTI, P., FANCIULLACCI, M., MICHELACCI, S., LECOMTE, J.M. & SICUTERI, F. (1986). *In vivo* "enkephalinase" inhibition by acetorphan in human plasma and CSF. *Eur. J. Pharmacol.*, **125**, 147–150.

SPRIGGS, T.L.B. (1965). The effects of anesthesia induced by urethane or phenobarbitone upon the distribution of peripheral catecholamines in the rat. *Br. J. Pharmacol. Chemother.*, **24**, 752–758.

STIMLER-GERARD, N.P. (1987). Neutral endopeptidase-like enzyme controls the contractile activity of substance P in guinea pig lung. *J. Clin. Invest.*, **79**, 1819–1825.

UMENO, E., NADEL, J., HUANG, H.T. & MCDONALD, D.M. (1989). Inhibition of neutral endopeptidase potentiates neurogenic inflammation in the rat trachea. *J. Appl. Physiol.*, **66**, 2647–2652.

VON NEERGAARD, K. & WIRZ, K. (1927). Die messung der strömungswiderstände in den atemwegen des menschen, insbesondere bei asthma und emphysem. *Z. Klin. Med.*, **105**, 51–82.

ZAR, J.H. (1974). Multisample hypothesis. In *Biostatistical Analysis*. pp. 130–162. London: Prentice-Hall International, Inc.

(Received September 26, 1989
Revised January 12, 1990
Accepted January 19, 1990)