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Sensory neuropeptides and hypoxic pulmonary vasoconstriction in the rat

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Abstract

***Background*—Endogenous vasodilators such as endothelially derived relaxant factor have been shown to modulate hypoxic pulmonary vasoconstriction. Sensory peptides such as substance P (SP) and calcitonin gene related peptide (CGRP) are also potent pulmonary vasodilators in both animals and humans. Their possible role in the modulation of the normal hypoxic pressor response has been examined in an isolated, ventilated, and blood perfused rat lung preparation.**

***Methods*—Animals (n = 7) were pretreated with 50 mg/kg capsaicin administered subcutaneously to deplete nerve endings of sensory neuropeptides. A control group (n = 7) received a subcutaneous dose of capsaicin vehicle. One week later the rats were killed and the rise in pulmonary artery pressure was measured during four successive periods of hypoxic ventilation (FIO_2 0·03), and after four injections of angiotensin II (1·0 μg).**

***Results*—A 60% depletion of SP levels was measured in the sciatic nerves of animals treated with capsaicin. The hypoxic pressor response was not significantly altered in capsaicin treated animals compared with controls, except during the fourth hypoxic episode when it was augmented. The angiotensin II pressor response was the same in both groups during each of the injections.**

***Conclusion*—The sensory neuropeptide SP (and possibly CGRP) does not have a major role in modulating the pulmonary vascular response to hypoxia.**

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Although the phenomenon of hypoxic pulmonary vasoconstriction (HPV) was described over 40 years ago,[1] the underlying mechanism remains unknown. One possible explanation is the "indirect hypothesis" which suggests that mediator(s) released as a consequence of alveolar hypoxia lead to the contraction of pulmonary vascular smooth muscle.[2] Identifying such a substance has, however, proved difficult. A second explanation first proposed over 30 years ago suggested that HPV is a consequence of a local alveolar vascular reflex,[3] although this hypothesis has not been widely investigated. It is generally agreed that hypoxic vasoconstriction is not a centrally mediated phenomenon, being demonstrable in isolated, perfused lung preparations[4] and in transplanted human lungs.[5] Indeed, the possibility remains that HPV is mediated via an *intrapulmonary neural reflex*, perhaps through nerves located in pulmonary arteries.

Non-adrenergic, non-cholinergic (NANC) neural mechanisms have been described in many peripheral systems and NANC mediated vasodilation has been reported in both the pulmonary[6] and systemic[7] circulation of rodents. Neurotransmitters for NANC mediated vasodilation have not been identified positively, although neuropeptides such as substance P (SP) and calcitonin gene related peptide (CGRP) are likely candidates in some vessels.[8]

SP and CGRP are both known to be potent vasodilators of the pulmonary circulation in many species.[9–13] Available evidence suggests that the vasodilation effected by SP is endothelium dependent and that of CGRP is endothelium independent.[10 11] Endothelium derived relaxant factor appears to be released during hypoxia and modulates HPV.[14 15]

We tested the hypothesis that depletion of the vasodilator sensory neuropeptide SP (and CGRP) would augment HPV. Rats were treated with capsaicin which is known to cause the selective degeneration of unmyelinated chemosensitive primary afferent neurones.[16] The effects of this sensory neuropeptide depletion on hypoxic pulmonary vasoconstriction were investigated with an isolated, blood perfused, rat heart lung preparation. All procedures relating to animals were approved by the Home Office Licensing Authority, and in all cases protocols and procedures were endorsed on the relevant project and personal licences.

Methods

CAPSAICIN PRETREATMENT OF ANIMALS

To deplete sensory neuropeptides, male Wistar rats (300–350 g) were treated with capsaicin as described previously.[17] Briefly, capsaicin (Sigma, Poole, UK) was dissolved to a concentration of 50 mg/ml in 10% alcohol, 10% Tween, and 80% saline. Animals were anaesthetised with ketamine (50 mg/kg intramuscularly and xylazine (0·1 mg/kg

intramuscularly) and pretreated with terbutaline (0·1 mg/kg subcutaneously) and aminophylline (25 mg/kg intraperitoneally) 60 and 30 minutes before capsaicin (or vehicle) injection to prevent bronchospasm. A single dose of capsaicin (50 mg/kg subcutaneously) (group 1, n = 7) or vehicle (group 2, n = 7) in identical volumes was then administered after which the experimental animals were left in their cages for one week and allowed free access to food and water.

ISOLATED LUNG EXPERIMENTS

Following anaesthesia with diazepam (0·6 mg/kg) and 0·15 ml Hypnorm (fentanyl 0·315 mg/ml and fluanisone 10 mg/ml), a donor rat was exsanguinated and the blood placed into a reservoir (maintained at 40°C by a surrounding water bath) and used to "prime" the perfusion circuit which consisted, in order, of a left atrial cannula, reservoir, roller pump (Watson Marlow MHRE 200, Falmouth, UK), connecting tubing, bubble trap, side arm pressure transducer (Bio Medical Systems, Strathclyde, UK), and a pulmonary artery cannula.[4] The pressure transducer was connected to a recorder (Multitrace 2, Ormed, Hertfordshire, UK) to permit constant monitoring. Total blood volume within the circuit including the reservoir was 15–20 ml.

The experimental animals were anaesthetised (as above) and ventilated at a tidal volume of 4 ml and frequency of 15 breaths/min via a tracheostomy. The lungs were exposed through a median sternotomy and the animal heparinised and exsanguinated via the abdominal aorta. A purse string suture was placed around the left atrium to secure a catheter inserted through an atriotomy which was allowed to drain freely into a blood reservoir thereby ensuring a "left atrial pressure" of zero. A cannula was inserted into the main pulmonary artery via a right ventriculotomy and tied securely in place. A constant flow of 18 ml/min resulted in a mean (SE) initial pulmonary artery pressure (PPA) of 18 (1) mm Hg (n = 14). Blood gases were monitored during each experiment by collecting blood anaerobically from the left atrial line and analysing pH with a Corning blood gas analyser (178 pH/blood gas analyser, Corning, Essex, UK). Deviations in pH from normal (7·35–7·45) were corrected with small volumes of $NaHCO_3$ (1·0N) added to the reservoir.

The lungs were initially ventilated with a gas mixture of composition 21% O_2, 5% CO_2, and 84% N_2 (normoxia). After baseline PPA was stable the inspired gas mixture was changed to 3% O_2, 5% CO_2, and 92% N_2 (hypoxia) until the increase in PPA had reached a plateau. The ventilating gas was then changed back to normoxia and the pressure allowed to stabilise. After a stable baseline had been attained (6–7 minutes) angiotensin II (1·0 μg) was injected into the circuit just proximal to the pulmonary artery catheter and the rise in PPA recorded. This sequence was repeated so that each preparation had four episodes of hypoxic ventilation alternating with four injections of angiotensin II. The rise in PPA during hypoxic ventilation or following angiotensin II administration was determined as the difference between the peak PPA and the PPA measured in the immediately preceding normoxic period.

SUBSTANCE P ASSAY OF SCIATIC NERVES

After anaesthesia, but before thoracotomy, all experimental animals plus others pretreated with either capsaicin (n = 7) or vehicle (n = 7) had the right, left, or both sciatic nerves carefully dissected free of surrounding tissue. Care was taken not to touch or manipulate the nerve unduly. A segment of nerve (15–40 mg) was then excised and immediately placed into preweighed vials containing 0·1 mol acetic acid. The vials were then reweighed and maintained at −70°C until substance P assay was performed.

On the day of assay the vials of acetic acid were thawed, homogenised by sonication, and then boiled for 10 minutes. Samples were centrifuged at 3000 *g* for 10 minutes and the supernatants lyophilised and subsequently reconstituted in 0·05 mol phosphate buffer before radioimmunoassay. Samples were analysed in duplicate for SP like immunoreactivity in a competitive radioimmunoassay with the highly specific antibody α140 (kindly supplied by Dr M Hanley, MRC Neurobiology Unit, Cambridge) as previously described.[18] Standard curves were set up in triplicate with SP standards (Sigma, Poole, UK). Iodine-125 Bolton Hunter labelled SP was used as tracer (Amersham, Bucks, UK). After 48 hours incubation bound tracer was separated from free by precipitation with chilled ethanol and centrifugation at 4000 *g* for 15 minutes Supernatants were decanted and the radioactivity of the pellets counted and analysed on a crystal multigamma counter (Packard, Caversham, UK). The minimum detectable concentration of the assay is 0·1 pg SP/tube, with interassay variation of 9% and intra-assay variation of 4%. The cross reactivities of the antiserum with other tachykinins (neurokinin A, neurokinin B, SP 1–4 and SP 7–11) were all less than 0·05%.

STATISTICS

Normoxic and hypoxic PPA and percentage increase in PPA during hypoxia and angiotensin II injections in groups 1 and 2 were compared with one way analysis of variance. Post hoc analysis utilised the Fisher protected least significant difference test.[19] Substance P values were compared between groups 1 and 2 with an unpaired Student's *t* test. Results are expressed as means (SE) and p values less than 0·05 were considered significant.

Results

Capsaicin pretreatment (group 1) significantly decreased the SP content of the sciatic nerves from a mean (SE) level of

Mean (SE) room air (baseline) pulmonary artery pressure (mm Hg)

	Period of room air ventilation			
	1st	*2nd*	*3rd*	*4th*
Group 1 (capsaicin treated)	16 (1)	16 (1)	16 (1)	17 (1)
Group 2 (control)	19 (1)	19 (1)	19 (1)	20 (1)

12·1 (2·3) ng/g nerve tissue in controls to 4·7 (0·9) in those pretreated with capsaicin, representing a 62% decrease ($p < 0·01$).

There was no significant difference in baseline PPA between animals from group 1 (16 (1) mm Hg) and the controls (19 (1) mm Hg) (table). The hypoxic pressor responses in groups 1 and 2, expressed as percentage increase in PPA, are shown in fig 1. The HPV response was significantly greater in group 1 animals only during the fourth hypoxic episode ($p < 0·05$). There was no significant difference in the pressor responses to angiotensin II between groups 1 and 2 (fig 2).

Discussion

The main findings of this study were that sensory neuropeptide depletion by capsaicin caused only a small increase in HPV after successive exposures to hypoxia. The pressor response to angiotensin II was unaffected. There are several possible reasons that could explain the lack of a large effect of sensory neuropeptide depletion on the hypoxic pressor response in this study.

Firstly, both SP and CGRP are localised in afferent nerve fibres[20] and both are potent vasodilators in the bronchial and pulmonary circulations.[11–13] Although no potent antagonists of these peptides are available, pretreatment of adult rats with capsaicin is known to cause the selective degeneration of primary afferent neurones[16] which have been shown to contain both tachykinin—for example, substance P—and CGRP immunoreactive materials.[20 21] Studies have shown significant reduction in the SP and CGRP content of pulmonary and non-pulmonary tissue in several species following treatment with systemic capsaicin.[17 22] It is possible that capsaicin treatment in this study did not reduce the sensory neuropeptide content of intrapulmonary nerves. This is unlikely since, although we did not measure the amount of

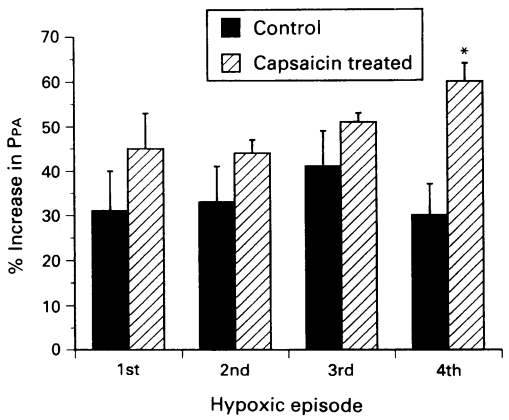

*Figure 1 Pressor response to each of four periods of hypoxic ventilation in control and capsaicin treated animals. *p < 0·05.*

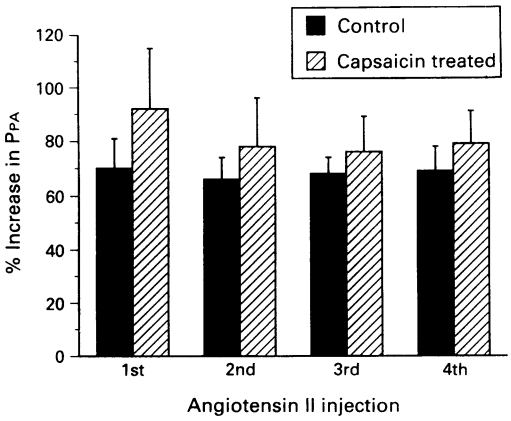

Figure 2 Pressor response to each of four angiotensin II injections (1·0 μg) in control and capsaicin treated animals.

CGRP present, the decrease in SP content of the sciatic nerve in animals treated with capsaicin in the current study was comparable to that reported in other studies where similar degrees of lung SP depletion has been noted after the same dose of capsaicin.[23] We were not able to assay the pulmonary vessels directly for SP content. In a pilot experiment there was insufficient SP for assay in pulmonary arteries from normal animals despite pooling of tissue. As the pulmonary artery did not have a large enough signal for detection with our assay, it was decided to assay sciatic nerve as this had previously been shown to contain large amounts of SP[24] and there is no reason to suspect that systemic capsaicin treatment would selectively diminish the SP content of sciatic and not pulmonary nerves.

A second explanation is that the residual SP remaining in the animals treated with capsaicin (38% of control) is enough to provide normal modulation of HPV through release of endothelium derived relaxant factor. This is also unlikely as a 60% reduction in SP content of pulmonary nerves has been shown to significantly reduce the non-cholinergic increase in insufflation pressure following vagal stimulation.[17]

The third and most likely explanation is that the sensory neuropeptide SP (and possibly CGRP) does not play an important part in modulating HPV in the rat. SP has been found in nerves adjacent to the rat pulmonary artery by immunohistochemistry[25–27] and SP receptors have been characterised in these vessels as being of the NK_2 subtype.[10 12] No physiological role for this peptide in the control of the pulmonary circulation has been described to date, however. It is of interest that a small, but nonetheless significant, augmentation in HPV was seen in lungs pretreated with capsaican after successive hypoxic challenges and that responses to the pressor angiotensin II were unaffected. This may indicate that sensory neuropeptides have a modulating effect on pulmonary vascular tone only in repeated or chronic hypoxaemia.

The baseline PPA was not significantly reduced in capsaicin treated animals compared with controls. This is in contrast to the report of Donnerer *et al*[28] where the resting

systemic arterial pressure from capsaicin treated rats was significantly less than that of controls. This suggests that the sensory neuropeptides SP (and probably CGRP) are not as important in the pulmonary circulation as the systemic circulation in maintaining normal vascular tone during normoxia. The reason for the difference between the pulmonary and systemic circulation is not clear, although it may relate to the higher resting tone of the systemic than the pulmonary circulation. Alternatively, the difference may be that our observations were made in vitro whereas Donnerer performed in vivo experiments.

In conclusion, our results do not suggest an important role for SP (and probably CGRP) in the modulation of normal pulmonary vascular tone or HPV in the normal rat. It remains to be determined whether these peptides play a part in the control of the pulmonary circulation in disease.

1 von Euler US, Lijestrand G. Observations on the pulmonary arterial blood pressure in the cat. *Acta Physiol Scand* 1946;**12**:301–20.
2 Voelkel NF. Mechanisms of hypoxic pulmonary vasoconstriction. *Am Rev Respir Dis* 1986;**133**:1186–95.
3 Rossier PH. The functional aspect of pulmonary hypertension. In: de Reuck AVS, O'Connor M, eds. *Problems of pulmonary circulation*. London: J and A Churchill, 1961:33–43.
4 McCormack DG, Barnes PJ, Evans TW. Evidence against a role for platelet-activating factor in hypoxic pulmonary vasoconstriction in the rat. *Clin Sci* 1989;77:439–43.
5 Robin ED, Theodore J, Burke CM, Oesterle SN, Fowler MB, Jamieson JC, *et al*. Hypoxic pulmonary vasoconstriction persists in the human transplanted lung. *Clin Sci* 1987;**72**:283–7.
6 Liu SF, Crawley DE, Evans TW, Barnes PJ. Endothelium-dependent nonadrenergic, noncholinergic neural relaxation in guinea pig pulmonary artery. *J Pharmacol Exp Ther* 1992;**260**:541–8.
7 Duckles SP. Neurogenic dilator and constrictor responses of pial arteries in vitro. *Circ Res* 1979;**44**:482–90.
8 Barnes PJ. Neuropeptides and airway smooth muscle. *Pharmacol Therap* 1988;**36**:119–29.
9 Archer AL, Kulik TJ, Chesler E, Weir EK. The effects of substance P on the preconstricted pulmonary vasculature of the anesthetised dog. *Proc Soc Exp Biol Med* 1986;**183**:19–27.
10 D'Orleans-Juste P, Dion S, Drapeau G, Regoli D. Different receptors are involved in the endothelium-mediated relaxation and the smooth muscle contraction of the rabbit pulmonary artery in response to substance P and related neurokinins. *Eur J Pharmacol* 1985; **125**:37–44.
11 McCormack DG, Mak JCW, Coupe MO, Barnes PJ. Calcitonin gene-related peptide vasodilation of human pulmonary vessels. *J Appl Physiol* 1989;**67**:1265–70.
12 McCormack DG, Salonen RO, Barnes PJ. Effect of sensory neuropeptides on canine bronchial and pulmonary vessels in vitro. *Life Sci* 1989;**45**:2405–12.
13 Adnot S, Cigarini I, Herigault R, Harf A. Effects of substance P and calcitonin gene-related peptide on the pulmonary circulation. *J Appl Physiol* 1991;**70**:1707–12.
14 Archer SL, Tolins JP, Raij L, Weir EK. Hypoxic pulmonary vasoconstriction is enhanced by inhibition of the synthesis of an endothelium derived relaxing factor. *Biochem Biophys Res Commun* 1989;**164**:1198–205.
15 Liu S, Crawley DE, Barnes PJ, Evans TW. Endothelium-derived relaxing factor inhibits hypoxic pulmonary vasoconstriction in rats. *Am Rev Respir Dis* 1991;**143**:32–7.
16 Jancso G, Kiraly E, Jancso-Gabor A. Pharmacologically induced selective degeneration of chemosensitive primary sensory neurones. *Nature* 1977;**270**:741–3.
17 Lundberg JM, Brodin E, Saria A. Effects and distribution of vagal capsaicin-sensitive substance P neurons with special reference to the trachea and lungs. *Acta Physiol Scand* 1983;**119**:243–52.
18 Carter DA, Lightman SL. Comparative distribution and cardiovascular actions of substance P and substance K within the nucleus tractus solitarius of rats. *Neuropeptides* 1986;**8**:295–304.
19 Winer BJ. *Statistical principles in experimental design*. New York: McGraw-Hill, 1971.
20 Lundberg JM, Anders FC, Hua X, Hokfelt T, Fischer JA. Co-existence of substance P and calcitonin gene-related peptide-like immunoreactivity in sensory nerves in relation to cardiovascular and bronchoconstrictor effects of capsaicin. *Eur J Pharmacol* 1985;**108**:315–9.
21 Franco-Cereceda A, Henke H, Lundberg JM, Petermann JB, Hokfelt T, Fischer JA. Calcitonin gene-related peptide (CGRP) in capsaicin-sensitive substance P-immunoreactive sensory neurons in animals and man: distribution and release by capsaicin. *Peptides* 1987; **8**:399–410.
22 Forsberg K, Karlsson JA, Theodorsson E, Lundberg JM, Persson CGA. Cough and bronchoconstriction mediated by capsaicin sensitive sensory neurons in the guinea-pig. *Pulmonol Pharmacol* 1988;**1**:33–9.
23 Shepherd MN, McGregor GP, Kurian SS, Haze AJ, Tyers MB, Morrison J, *et al*. Systemic administration of capsaicin induces a dramatic depletion of substance P containing nerve fibres from the rat lung. In: Skrabanck P, Powell D, eds. *Substance P*. Montreal: Eden Press,1983: vol 3, 221–2.
24 Colpaert FC, Donnerer J, Lembeck F. Effects of capsaicin on inflammation and on the substance P content of nervous tissues in rats with adjuvant arthritis. *Life Sci* 1983;**32**:1827–34.
25 Lundberg JM, Hokfelt T, Martling CR, Saria A, Cuello C. Substance P immunoreactive sensory nerves in the lower respiratory tract of various mammals including man. *Cell Tissue Res* 1984;**235**:251–61.
26 Wharton J, Polak JM, Bloom SR, Will JA, Brown MR, Pearse AGE. Substance P-like immunoreactive nerves in mammalian lung. *Invest Cell Pathol* 1979;**2**:3–10.
27 Polak JM, Bloom SR. Regulatory peptides and neuron-specific enolase in the respiratory tract of man and other mammals. *Exp Lung Res* 1982;**3**:313–28.
28 Donnerer J, Schuligoi R, Lembeck F. Influence of capsaicin-induced denervation on neurogenic and humoral control of arterial pressure. *Naunyn Schmiedebergs Arch Pharmacol* 1989;**340**:740–3.