Decreased vascular permeability response to substance P in airways of genetically hypertensive rats

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1 The inbred genetically hypertensive strain (GH) of the Otago Wistar rat possesses more sensory neurons containing the neuropeptide substance P (SP) than does its genetically related control normotensive strain.

2 As SP contributes to airway inflammation by increasing microvascular permeability, we assessed the extravasation of Evans Blue dye in trachea and main bronchus of anaesthetized GH and control rats, in the presence of endogenous (capsaicin-liberated) or exogenous SP.

3 Following intravenous administration of either capsaicin (75 µg kg⁻¹) or SP (3.3 nmol kg⁻¹), extravasation of Evans Blue in airways from GH rats was only about 60% of that in airways of control rats. This difference was not gender-specific and responses to capsaicin were abolished by pretreatment with a selective NK₁ receptor antagonist SR 140333 (360 nmol kg⁻¹).

4 By contrast, the extravasation of dye caused by intravenous 5-hydroxytryptamine (0.5 µmol kg⁻¹) was similar in magnitude in both GH and control strains.

5 Falls in systemic arterial blood pressure in response to exogenous SP (0.1–3 nmol kg⁻¹) or acetylcholine (0.2–2 nmol kg⁻¹) were also very similar between strains, but those in response to capsaicin (75 µg kg⁻¹) in the GH rats were about double those in control rats. The hypotensive response to SP was abolished by SR 140333, but that to capsaicin was unaffected.

6 Our results indicate that the increased peripheral innervation density by SP nerves in GH rats is accompanied by reduced inflammatory responses to SP. This does not involve decreased vasodilator potency of SP and is therefore probably related to altered endothelial responsiveness.

Keywords: Neuropeptides; trachea; sensory nerve; capsaicin; Evans Blue; hypotension; NK₁ receptor antagonist; substance P; hypertensive rat model

Abbreviations: ACh, Acetylcholine; CGRP, calcitonin gene-related peptide; GH, genetically hypertensive; 5-HT, 5-hydroxytryptamine; NGF, nerve growth factor; NO, nitric oxide; NOS, NO synthase; PACAP, pituitary adenylate cyclase activating peptide; SP, substance P

Introduction

Neuropeptides such as substance P (SP) are thought to be important inflammatory mediators in asthma (Barnes & Belvisi, 1997). Asthmatics have been reported as having increased numbers of intrapulmonary SP-containing axons (Ollerenshaw *et al.*, 1991) and as exhibiting increased levels of SP in bronchoalveolar lavage (Nieber *et al.*, 1992; Khwaja & Rogers, 1996). SP probably exerts its major effect in asthma by inducing airway oedema consequent to an increase in microvascular permeability (Joos *et al.*, 1994; Barnes & Belvisi, 1997). In rat trachea, microvascular permeability increase due to SP release can be induced by electrical stimulation of the vagal trunk (Lundberg & Saria, 1982) or by capsaicin (Germonpre *et al.*, 1995), making these manoeuvres an appropriate model for studies of inflammatory responses to SP.

The Otago strain of genetically hypertensive rat (GH) possesses selectively increased numbers of the polymodal C-fibre class of spinal sensory neurons. As a consequence, some peripheral tissues, including the trachea, are hyperinnervated by SP-containing sensory nerves and contain abnormally high levels of SP (Bakhle & Bell, 1994). We were therefore interested to determine whether the chronic presence of excessive amounts of neurogenic SP had an influence on the inflammatory responses of the airways in the GH strain. Some

preliminary results of this work have been communicated to the British Pharmacological Society (Bakhle *et al.*, 1998).

Methods

Animals

The rats used were bred in the Trinity College BioResources Unit from stock obtained in 1995 from the Wellcome Medical Research Institute, University of Otago (New Zealand) and consisting of GH animals and normal (N) Wistar animals from the colony that had produced the original GH strain. The integrity of each line was confirmed by establishing that the arterial blood pressures of each generation, as measured by tail-cuff plethysmography or by direct arterial cannulation, were consistently normotensive in N strain animals (<145 mmHg) and hypertensive in the GH animals (>190 mmHg).

Age-matched rats of each strain were anaesthetized with an intraperitoneal injection of sodium pentobarbitone (Sagatal; Servier Merieux, 60 mg kg⁻¹), placed on a stainless steel operating plate maintained at 35°C and allowed to breathe spontaneously. In all experiments, a polyethylene catheter (PE50) was passed down one external jugular vein to the region of the right atrium for injection of drugs. In experiments concerned with vascular responses, a second catheter was

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placed in the left common carotid artery for monitoring arterial blood pressure.

Chemicals

Acetylcholine (ACh), capsaicin, Evans Blue, formamide (SigmaUltra grade), heparin, 5-hydroxytryptamine creatinine phosphate (5-HT) and substance P (SP) were obtained from Sigma. The selective NK₁ antagonist, SR 140333, ((S)-1-{2-[3-(3,4-dichlorophenyl)-1-(3-isopropoxyphenylacetyl)piperidin-3-yl]ethyl}-4-phenyl-1-azoniabicyclo[2,2,2]octane chloride; Emonds *et al.*, 1993) was generously provided by Sanofi. Evans Blue was dissolved (30 mg ml⁻¹) in isotonic saline containing heparin (300 IU ml⁻¹) and the solution was filtered before use. Capsaicin was dissolved in 96% ethanol-Tween 80 (1:1, v/v) to give a stock solution of 10 mg ml⁻¹; SR 140333 was dissolved in ethanol to give a stock solution of 3.7 mM. All other solutions were made in saline.

Microvascular permeability measurements

Evans Blue (30 mg kg⁻¹) was given intravenously and was followed, 90 s later, with an injection of capsaicin (75 µg kg⁻¹), 5-HT (0.5 µmol kg⁻¹) or SP (3.3 nmol kg⁻¹), or an equal volume (0.2 ml) of saline. After a further 5 min, the chest was opened and the rat was perfused transcardially with 200 ml isotonic saline at a pressure of 100 cm water. The trachea and main bronchus were dissected free, blotted dry, weighed and extracted in 1.5 ml formamide at 37°C overnight. The optical density of the extract was measured at 620 nm and the Evans Blue concentration calculated from a standard curve of Evans Blue in formamide determined on the same day. Extravasated dye was expressed as ng dye (mg wet weight tissue)⁻¹.

In some animals given capsaicin or SP, the NK₁ antagonist SR 140333 (360 nmol kg⁻¹; Siney & Brain, 1996) was given as a single intravenous injection, 30 min before Evans Blue administration.

Blood pressure measurements

Arterial blood pressure was measured *via* a Statham pressure transducer and recorded using a MacLab data acquisition system. After allowing at least 10 min after arterial cannulation for stabilization of the preparation, blood pressure responses to intravenous injections of capsaicin (75 µg kg⁻¹), SP (0.1–3 nmol kg⁻¹) and ACh (0.2–2 nmol kg⁻¹) were obtained, before and after administration of SR 140333 (360 nmol kg⁻¹) intravenously.

Statistical analysis

Results are given as mean (±s.e.mean) values from the number of assays shown. Differences between means were assessed by Welch's test (no assumption of equal variances) and values of *P* < 0.05 were taken as significant.

Results

Changes in microvascular permeability

A small amount of extravasation of Evans Blue was detectable in tissues from animals that had received only saline challenge. This extravasation was similar in both trachea and bronchus and for both control (N) and GH strains (trachea; N, 33 ± 6 vs

GH, 33 ± 3; main bronchus; N, 38 ± 8 vs GH, 28 ± 3, all expressed as ng dye (mg wet weight)⁻¹; *n* = 8) and these results have been combined to give the baseline value shown as a dotted line in Figures 1 and 2.

Preliminary studies showed that 75 µg kg⁻¹ i.v. capsaicin produced a substantial and reproducible degree of extravasation without excessive mortality and this dose was used in all subsequent experiments. In both the trachea and the main bronchus, capsaicin caused a marked increase in extravasation above the baseline value, with a greater response in the bronchus. There was however a substantial strain difference in the magnitude of the effect of capsaicin, with the responses in both tissues of the GH animals being only about 60% of those seen in the comparable control (N) tissues (Figure 1). Capsaicin produced no visible blueing of the lung parenchyma, although blueing was clearly visible in the skin around the nostrils and in the ears.

Both SP (3.3 nmol kg⁻¹ i.v.) and 5-HT (0.5 µmol kg⁻¹) produced permeability increases similar in magnitude to those induced by capsaicin. As with capsaicin-induced extravasation, the responses to SP in both trachea and bronchus were substantially less in GH than in N animals (Figure 2a). By contrast, responses to 5-HT were of similar magnitude for both strains (Figure 2b).

The results described above were obtained using male rats. A limited series of experiments using female rats confirmed that the reduced extravasation in the trachea of GH animals to capsaicin (N, 72 ± 13; GH, 28 ± 5) or exogenous SP (N, 134 ± 6; GH, 105 ± 8; expressed as ng dye (mg wet weight)⁻¹; *P* < 0.05, *n* = 5 for each set) was independent of gender.

In another series of animals, the role of the NK₁ receptor in extravasation mediated by capsaicin was tested by pre-treatment with SR 140333 for 30 min before challenge. In these experiments, the responses to capsaicin were abolished in both N and GH strains (Figure 3).

Systemic blood pressure responses to capsaicin or to exogenous SP

Extravasation of plasma protein in areas of increased microvascular permeability may be modified by blood flow changes secondary to changes in systemic arterial pressure. We

Figure 1 Increased microvascular permeability in the upper airways of GH and control rats following capsaicin. Dye extravasation in trachea and main bronchus was markedly increased by i.v. injection of capsaicin (75 µg kg⁻¹) over that induced by injection of saline (dotted line). However the effect of capsaicin was consistently less in the GH rats than in the control (N) animals. The height of the bars represents the mean values (±s.e.mean; *n* = 8) of tissue content of extravasated dye. *Significantly different from value in control rats; *P* < 0.05.

Figure 2 Increased microvascular permeability following exogenous SP (3.3 nmol kg^{-1} i.v.) (a) or 5-HT ($0.5 \mu\text{mol kg}^{-1}$) (b). Both these exogenous pro-inflammatory agents increased dye extravasation, but only with SP was there a strain-related difference in response. Saline control values shown by the dotted line. The height of the bars represents the mean values ($\pm$ s.e. mean; $n=5$ for SP, $n=6$ for 5-HT) of tissue content of extravasated dye. *Significantly different from value in control rats; $P<0.05$.

therefore compared blood pressure responses to capsaicin and to SP between GH and control (N) strains and used ACh as an independent index of vascular reactivity. Comparison of vasodepressor responses to submaximal intravenous doses of either SP or ACh (Figure 4) showed no consistent difference between strains but, overall, there was a trend towards a greater response in the GH rats.

Intravenous injection of capsaicin ($75 \mu\text{g kg}^{-1}$) produced a depressor response which was more prolonged than that to SP (Figure 5). The magnitude of response to a second injection was frequently less than that to the first dose but responses to subsequent doses of capsaicin remained similar in size (Figure 5). Comparison of maximal hypotensive responses to the first dose of capsaicin showed a larger fall in GH animals ($111 \pm 11 \text{ mmHg}$, $n=5$) than in the N strain ($57 \pm 9 \text{ mmHg}$; $n=4$; $P<0.05$). In addition to the immediate depressor response, the first dose of capsaicin given invariably caused a maintained reduction in resting blood pressure. In control (N) animals, the resting arterial pressure fell after the first dose of capsaicin from a value of $129 \pm 5 \text{ mmHg}$ to a sustained value of $102 \pm 3 \text{ mmHg}$ ($n=5$). A closely similar magnitude of response was seen in GH rats, with the pre-capsaicin value of $186 \pm 3 \text{ mmHg}$ falling to a new baseline of $152 \pm 6 \text{ mmHg}$ ($n=6$).

Figure 3 Prevention of capsaicin-induced dye extravasation in rat upper airways by SR 140333. Pre-treatment with the selective NK₁ receptor antagonist SR 140333 (360 nmol kg^{-1}) completely prevented the effects of capsaicin ($75 \mu\text{g kg}^{-1}$) in either strain. The left hand bar of each pair represents dye extravasation induced by capsaicin alone (Cont) and the right hand bar extravasation induced by capsaicin after SR 140333 (SR). The height of the bars represents the mean values ($\pm$ s.e. mean; $n=8$) of tissue content of extravasated dye. *Significantly different from value in rats treated with capsaicin alone; $P<0.05$.

Involvement of the NK₁ receptor in the hypotensive responses to SP and capsaicin was tested in eight animals (4 N and 4 GH) by treatment with SR 140333 (360 nmol kg^{-1}). In all animals, SR 140333 abolished the depressor response to SP whereas the response to capsaicin was unaffected. The antagonist effect of SR 140333 on SP responses persisted for at least 30 min. Results from a typical experiment are shown in Figure 5. In an additional series of five animals (2 N and 3 GH), intravenous administration of the same dose of SR 140333, before capsaicin, was seen not to have any effect on resting blood pressure in either strain.

Discussion

Our experiments demonstrate that the upper airways of GH rats, which are hyperinnervated by sensory axons containing

SP (Bakhle & Bell, 1994), exhibit a markedly reduced capacity to increase microvascular permeability in response to either endogenous or exogenous SP. By contrast, the GH airways exhibited a normal capacity for microvascular permeability

increase in the presence of another pro-inflammatory mediator, 5-HT. Furthermore, the decreased inflammatory response was not likely to be due to altered circulatory dynamics in the GH, since both strains exhibited similar vasodepressor sensitivities to exogenous SP or ACh.

The inflammatory response to capsaicin may involve release not only of SP but also of other neuropeptides including calcitonin gene-related peptide (CGRP) and pituitary adenylyl cyclase-activating peptide (PACAP), which are powerful vasodilators (Brain *et al.*, 1985; Mulder *et al.*, 1994) and therefore potentiate extravasation of plasma proteins when microvascular permeability has been increased (Brain & Williams, 1989; Delay Goyet *et al.*, 1992). It does not seem likely, however, that the lesser inflammatory response to capsaicin in GH animals was due to an absence of contribution from these other peptides, since a similar strain difference was seen with the inflammatory responses to SP itself. Furthermore, involvement of receptors for peptides other than SP in the response to capsaicin was ruled out by the fact that this response was abolished by SR 140333, a selective NK₁ receptor antagonist (Emonds Alt *et al.*, 1993).

Another possible explanation for reduced inflammatory capacity in GH airways would be a reduced ability of the venular endothelium to change its structure and hence its permeability (Kenins *et al.*, 1984) in response to inflammatory mediators. However, the normal inflammatory response to 5-HT that we observed argues against a non-specific loss of the microvascular response in the GH strain.

A third possibility would be that the endothelium of the GH microvasculature is selectively insensitive to SP. The reduced permeability responses of GH animals to exogenous SP supported this explanation and it seems likely, therefore, that a subsensitivity to SP is responsible for the low level of permeability responses to capsaicin in GH airways.

Further possibilities include differences in receptor coupling to intracellular signalling pathways or in co-mediator biosynthesis, particularly nitric oxide (NO). Oedema, induced by exogenous SP or by stimulation of sensory nerves, has been shown to be decreased by inhibition of NO synthase (NOS) (Hughes *et al.*, 1990; Kajekar *et al.*, 1995). Indirect evidence of decreased NO synthesis has been described for blood vessels of the GH rat, *ex vivo* (Winquist *et al.*, 1984) and *in vivo* (Ledingham & Laverty, 1997) and similar changes in NOS in the vasculature of the airways could modulate permeability responses.

Figure 4 Vasodepressor responses to i.v. SP (a) or to ACh (b) in control (N) and GH rats. Over the range of doses shown, no consistent strain-related difference in hypotensive effect was evident. Where there was a difference, the responses of the GH rats were the greater. The values shown are the mean ($\pm$ s.e. mean) results from 3–4 animals at each dose. *Significantly different from value in control rats; $P < 0.05$.

Figure 5 Depressor effects of substance P (SP, 0.2 nmol kg^{-1}) and capsaicin (CAP, $75 \mu\text{g kg}^{-1}$) on arterial blood pressure in a GH rat. Note that resting blood pressure was maintained at a lower level following the first injection of capsaicin. Subsequent doses of capsaicin or SP caused reproducible blood pressure responses, with responses to capsaicin being longer lasting. Five minutes after i.v. injection of the NK₁ antagonist SR 140333 (360 nmol kg^{-1}), the response to SP was abolished whereas that to capsaicin was unaffected.

Another cause of subsensitivity to SP might be a decreased number of SP receptors in the GH. An acute loss of NK₁ receptors due to internalization occurs within minutes of agonist binding *in vivo* or *in vitro* (Garland *et al.*, 1994) and functional tachyphylaxis, compatible with receptor loss from the cell membrane, is a well established feature of SP-NK₁ receptor interactions (Quartara & Maggi, 1997). Since the GH is known to have elevated numbers of SP-containing sensory neurons and these provide an elevated innervation density in a number of peripheral tissues, including the airways (Bakhle & Bell, 1994), increased basal release of SP could result in a lower resting number of NK₁ receptors at the cell surface. Thus the response to the acute release of high levels of SP by capsaicin or to addition of exogenous SP could be attenuated by a pre-existing state of tachyphylaxis.

Alternatively, a chronic loss of NK₁ receptors could involve nerve growth factor (NGF). The overgrowth of SP-containing sensory neurons in the GH is coupled with reduced numbers of sympathetic neurons (Gurusinghe *et al.*, 1990) and both defects are normalized by administration of exogenous NGF in the neonatal period (Messina & Bell, 1991). Exogenous NGF increases the sensitivity of sensory neurons to capsaicin, probably by up-regulating capsaicin receptors (Bevan & Winter 1995; Hu-Tsai *et al.*, 1996). In view of the specificity of capsaicin for neurokinin-containing neurons, it is possible that neurokinin receptors may also be sensitive to modulation by NGF and that, therefore, reduced NGF levels may down-regulate receptor numbers.

SP is known to cause long-lasting excitation of sympathetic motoneurons (Holzer, 1988) and these neurons receive inputs from SP-containing sensory axons (Matthews & Cuello, 1984; Gurusinghe & Bell, 1989). In view of the increased density of SP-containing axons in sympathetic ganglia in GH animals, it has been suggested that enhancement of sympathetic drive by intra-ganglionic release of SP may contribute to the elevated blood pressure in this strain (Gurusinghe & Bell, 1989). However, this is not supported by our present observations. Thus, administration of SR 140333 in a dose that abolished

depressor responses to SP had no effect on resting blood pressure in the GH strain. Furthermore, the sustained reduction of resting blood pressure after an initial dose of capsaicin was similar in N and GH animals, although neurochemical data indicate that there is tonically elevated sympathetic drive in the GH rat (see Bell, 1996).

While the hypotensive response to exogenous SP was abolished by the NK₁ antagonist SR 140333, that due to capsaicin was hardly affected. One explanation for this difference could be that the depressor effect of capsaicin was mediated *via* a central, rather than a peripheral, mechanism. Centrally mediated cardiovascular effects of capsaicin have been demonstrated in rats (Donnerer & Lembeck, 1983), as have been centrally localized capsaicin-sensitive stores of SP (Virus *et al.*, 1982) and NK receptors (Otsuka & Yoshioka, 1993). However, intravenous SR 140333 is known to block the responses of thalamic neurons to mechanical nociception (Emonds Alt *et al.*, 1993). An equally viable explanation would be to attribute most of the systemic hypotensive effect of capsaicin to the release of CGRP. This neuropeptide is a potent vasodilator in rats (Claing *et al.*, 1992; Hall *et al.*, 1995) but as its effects are not mediated by the NK₁ receptor, they would not be susceptible to blockade by SR 140333.

Finally, the well documented ability of SP to degranulate mast cells (Metcalfe *et al.*, 1997) makes it of some interest to note that mast cells in the Kyoto strain of genetically hypertensive rat exhibit abnormal responses to a range of degranulating stimuli, such as deficient NO synthesis (Masini *et al.*, 1991) and lack of mediator release (Kwasniewski *et al.*, 1998). In view of these observations and our present results, it is possible that subsensitivity to the inflammatory actions of SP is a general characteristic of genetic hypertension.

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References

- BAKHLE, Y.S. & BELL, C. (1994). Increased numbers of Substance P-containing sensory neurones in a rat strain with a genetic neurotrophic defect. *Neuropeptides*, **27**, 169–174.
- BAKHLE, Y.S., BELL, C. & BROGAN, J.D. (1998). Paradoxical inflammatory response to capsaicin in a rat strain with increased tissue content of Substance P. *Br. J. Pharmacol.*, **123**, 75P.
- BARNES, P.J. & BELVISI, M.G. (1997). Sensory neuropeptides. In *Asthma*, eds. Barnes, P.J., Grunstein, M.M., Leff, M.R. & Woolcock, A.J. pp1051–1063. Philadelphia: Lippincott-Raven.
- BELL, C. (1996). Neurotrophic abnormalities and development of high blood pressure in genetically hypertensive rats. *Biomed. Rev.*, **6**, 43–55.
- BEVAN, S. & WINTER, J. (1995). Nerve growth factor (NGF) differentially regulates the chemosensitivity of adult rat cultured sensory neurons. *J. Neurosci.*, **15**, 4916–4918.
- BRAIN, S. & WILLIAMS, T.J. (1989). Interactions between the tachykinins and calcitonin gene-related peptide lead to the modulation of oedema formation and blood flow in rat skin. *Br. J. Pharmacol.*, **97**, 77–82.
- BRAIN, S., WILLIAMS, T.J., TIPPINS, J.R., MORRIS, H.R. & MACINTYRE, I. (1985). Calcitonin gene-related peptide is a potent vasodilator. *Nature*, **313**, 54–56.
- CLAING, A., TELEMAQUE, X., CADIEUX, A., FOURNIER, A., REGOLI, D. & D'ORLEANS-JUSTE, P. (1992). Nonadrenergic and noncholinergic arterial dilatation and vasoconstriction are mediated by calcitonin gene-related peptide and neurokinin-1 receptors, respectively, in the mesenteric vasculature of the rat after perivascular nerve stimulation. *J. Pharmacol. Exp. Ther.*, **263**, 1226–1232.
- DELAY GOYET, P., SATOH, H. & LUNDBERG, J.M. (1992). Relative involvement of Substance P and CGRP mechanisms in antidromic vasodilation in the rat skin. *Acta Physiol. Scand.*, **146**, 537–538.
- DONNERER, J. & LEMBECK, F. (1983). Capsaicin-induced reflex fall in blood pressure is mediated by afferent substance P-containing neurones via a reflex centre in the brain stem. *Naunyn-Schmied. Arch. Pharmacol.*, **324**, 293–295.
- EMONDS ALT, X., DOUTREMEPUICH, J.D., HEAULME, M., NELIAT, G., SANTUCCI, V., STEINBERG, R., VILAIN, P., BICHON, D., DUCOUX, J.P. & PROIETTO, V. (1993). In vitro and in vivo biological activities of SR 140333, a novel potent non-peptide tachykinin NK₁ receptor antagonist. *Eur. J. Pharmacol.*, **250**, 403–413.
- GARLAND, A.M., GRADY, E.F., PAYAN, D.G., VIGNA, S.R. & BUNNETT, N.W. (1994). Agonist-induced internalization of the substance P (NK₁) receptor expressed in epithelial cells. *Biochem. J.*, **303**, 177–186.
- GERMONPRE, P.R., JOOS, G.F., EVERAERT, E., KIPS, J.C. & PAUWELS, R.A. (1995). Characterization of neurogenic inflammation in the airways of two highly inbred rat strains. *Am. J. Respir. Crit. Care Med.*, **152**, 1796–1804.
- GURUSINGHE, C.J. & BELL, C. (1989). Substance P immunoreactivity in the superior cervical ganglia of normotensive and genetically hypertensive rats. *J. Auton. Nerv. Sys.*, **27**, 249–256.
- GURUSINGHE, C.J., HARRIS, P.J., ABBOTT, D. & BELL, C. (1990). Neuropeptide Y in rat sympathetic neurons is altered by genetic hypertension and by age. *Hypertension*, **16**, 63–71.

- HALL, J.M., SINEY, L., LIPPTON, H., HYMAN, A., KANG-CHANG, J. & BRAIN, S.D. (1995). Interaction of human adrenomedullin₁₃₋₅₂ with calcitonin gene-related peptide receptors in the microvasculature of the rat and hamster. *Br. J. Pharmacol.*, **114**, 592–597.
- HOLZER, P. (1988). Local effector mechanisms of capsaicin-sensitive sensory nerve endings: involvement of tachykinins, calcitonin gene-related peptide and other neuropeptides. *Neuroscience*, **24**, 739–768.
- HU-TSAI, M., WOOLF, C. & WINTER, J. (1996). Influence of inflammation or disconnection from peripheral target tissue on the capsaicin sensitivity of rat dorsal root ganglion sensory neurones. *Neurosci. Lett.*, **203**, 119–122.
- HUGHES, S.R., BRAIN, S. & WILLIAMS, T.J. (1990). Evidence that endogenous nitric oxide modulates oedema formation induced by substance P. *Eur. J. Pharmacol.*, **191**, 481–484.
- JOOS, G.F., GERMONPRE, P.R., KIPS, J.C., PELEMAN, R.A. & PAUWELS, R.A. (1994). Sensory neuropeptides and the human lower airways: present state and future directions. *Eur. Resp. J.*, **7**, 1161–1171.
- KAJEKAR, R., MOORE, P.K. & BRAIN, S.D. (1995). Essential role for nitric oxide in neurogenic inflammation in rat cutaneous microcirculation. Evidence for an endothelium-independent mechanism. *Circ. Res.*, **76**, 441–447.
- KENINS, P., HURLEY, J.V. & BELL, C. (1984). The role of substance P in the axon reflex in the rat. *Br. J. Dermatol.*, **111**, 551–559.
- KHWAJA, A.M. & ROGERS, D.F. (1996). Tachykinins: receptor to effector. *Int. J. Biochem. Cell Biol.*, **28**, 721–738.
- KWASNIEWSKI, F.H., TAVARES DE LIMA, W., BAKHLE, Y.S. & JANCAR, S. (1998). Impairment in connective tissue mast cells degranulation in spontaneously hypertensive rats: stimulus dependent resistance. *Br. J. Pharmacol.*, **124**, 772–778.
- LEDINGHAM, J.M. & LAVERTY, R. (1997). Nitric oxide synthase inhibition with N-nitro-L-arginine methyl ester affects blood pressure and cardiovascular structure in the genetically hypertensive rat strain. *Clin. Exp. Pharmacol. Physiol.*, **24**, 433–435.
- LUNDBERG, J.M. & SARIA, A. (1982). Capsaicin-sensitive vagal neurons involved in control of vascular permeability in rat trachea. *Acta Physiol. Scand.*, **115**, 521–523.
- MASINI, E., MANNAIONI, P.F., PISTELLI, A., SALVEMINI, D. & VANE, J.R. (1991). Impairment of the L-arginine-nitric oxide pathway in mast cells from spontaneously hypertensive rats. *Biochem. Biophys. Res. Commun.*, **177**, 1178–1182.
- MATTHEWS, M.R. & CUELLO, A.C. (1984). The origin and possible significance of substance P immunoreactive networks in the prevertebral ganglia and related structures in the guinea-pig. *Phil. Trans. Roy. Soc. Lond.*, **306**, 247–276.
- MESSINA, A. & BELL, C. (1991). Are genetically hypertensive rats deficient in nerve growth factor? *NeuroReport*, **2**, 45–48.
- METCALFE, D.D., BARAM, D. & MEKORI, Y.A. (1997). Mast cells. *Physiol. Rev.*, **77**, 1033–1079.
- MULDER, H., UDDMAN, R., MOLLER, K., ZHANG, Y.Z., EKBLAD, E., ALUMETS, J. & SUNDLER, F. (1994). Pituitary adenylate cyclase activating polypeptide expression in sensory neurons. *Neuroscience*, **63**, 307–312.
- NIEBER, K., BAUMGARTEN, C.R., RATHSACK, R., FURKERT, J., OEHME, P. & KUNKEL, G. (1992). Substance P and β -endorphin-like immunoreactivity in lavage fluid of subjects with and without allergic asthma. *J. Allergy Clin. Immunol.*, **90**, 646–652.
- OLLERENSHAW, S.L., JARVIS, D., SULLIVAN, C.E. & WOOLCOCK, A.J. (1991). Substance P immunoreactive nerves in airways from asthmatics and non-asthmatics. *Eur. Resp. J.*, **4**, 673–682.
- OTSUKA, M. & YOSHIOKA, K. (1993). Neurotransmitter functions of mammalian tachykinins. *Physiol. Rev.*, **73**, 229–308.
- QUARTARA, L. & MAGGI, C.A. (1997). The tachykinin NK₁ receptor. Part 1: ligands and mechanisms of cellular activation. *Neuropeptides*, **31**, 537–563.
- SINEY, L. & BRAIN, S. (1996). Involvement of sensory neuropeptides in the development of plasma extravasation in rat dorsal skin following thermal injury. *Br. J. Pharmacol.*, **117**, 1065–1070.
- VIRUS, R.M., MCMANUS, D.Q. & GEBHART, G.F. (1982). Capsaicin treatment in adult Wistar-Kyoto and spontaneously hypertensive rats: effects on Substance P contents of peripheral and central nervous system tissues. *Eur. J. Pharmacol.*, **81**, 67–73.
- WINQUIST, R.J., BUNTING, P.B., BASKIN, E.P. & WALLACE, A.A. (1984). Decreased endothelium-dependent relaxation in New Zealand genetically hypertensive rats. *J. Hypertens.*, **2**, 541–545.

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