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Bronchoconstriction and endogenous nitric oxide in isolated lungs of spontaneously hypertensive rats

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Abstract

Bronchoconstrictor responses were measured in lungs isolated from spontaneously hypertensive (SHR) and normotensive rats, perfused via the airways. Lungs from SHRs were more responsive than lungs from normotensive rats to methacholine, 5-hydroxytryptamine (5-HT), arachidonic acid or prostaglandin H_2. The responses of SHR airways to methacholine or 5-HT were unaffected by pretreatment in vivo with an inhibitor of nitric oxide (NO) synthase, N^{ω}-nitro-L-arginine methyl ester hydrochloride (L-NAME, 30 mg kg^{-1}), although responses in normotensive airways to methacholine, but not to 5-HT, were enhanced. Antigen challenge of isolated lungs from actively sensitized rats elicited bronchoconstriction, not different between strains. Pretreatment with L-NAME increased the response to antigen challenge only in normotensive lungs. Compound 48/80 induced bronchoconstriction in lungs from either strain, equally. These responses to compound 48/80 were unaffected by L-NAME pretreatment. Thus, SHR airways lack relaxing factors and degranulation of mast cells in SHR lungs was not affected by endogenous NO.

Keywords: Bronchoconstriction; Lung; NO (Nitric oxide); Mast cell; Anaphylaxis; Spontaneously hypertensive, rat

1. Introduction

Bronchoconstriction is one of the acute responses to immunological challenge in the lung and has been long considered to reflect the release of a variety of smooth muscle spasmogens from mast cells in the process referred to as degranulation (Metcalfe et al., 1997; Black, 2002). In earlier work we had found that the mast cells in mesentery and skin of spontaneously hypertensive rats (the SHR strain) were resistant to degranulation following challenge with antigen in sensitised animals (Kwasniewski et al., 1998). It was therefore possible that the constrictor response to immunological challenge in the airways of lungs isolated from SHR, actively sensitised to ovalbumin, would be also decreased, relative to that in lungs from similarly treated normotensive rats. As we used the isolated lungs as both the generator of, and the assay for, the mixture of spasmogens released during antigen challenge, we also measured bronchoconstriction to known, standard agonists such as methacholine and 5-hydroxytryptamine (5-HT) to allow for any intrinsic difference in airway smooth muscle between strains. Such strain-related differences have already been shown for the vascular smooth muscle in the mesenteric bed (Chang et al., 2002; Liu et al., 2002; Touyz et al., 2002). Furthermore, since nitric oxide (NO) has been shown to be an inhibitor of smooth muscle contraction (see Nijkamp and Folkerts, 1995; Ignarro, 2002) and of mast cell degranulation (Forsythe et al., 2001; Coleman, 2002), any strain-related differences in bronchoconstriction following antigen challenge could also be a reflection of differences in endogenous NO generation in lung tissue. To assess this possibility in our model, synthesis of endogenous NO was inhibited with the nonselective NO synthase inhibitor, N^{ω}-nitro-L-arginine methyl ester hydrochloride (L-NAME; Hobbs et al., 1999).

Our experiments were therefore designed to test two main hypotheses. Firstly, that the SHR airways would exhibit less bronchoconstriction after antigen challenge, than airways from normotensive rats. The second hypothesis was that any decreased response in the SHR airways would reflect inhibition of the response by endogenous NO and thus would be reversible by treatment with L-NAME. Our results have been complicated by the observation that airways in lungs from SHR were more responsive to

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directly acting constrictor agonists than those in lungs from normotensive animals. However, disregarding this difference, the outcome, i.e., the bronchoconstriction following antigen challenge or compound 48/80, another stimulus for mast cell degranulation, was not different between strains. There was no support for our second hypothesis, as treatment with L-NAME did not alter the bronchoconstrictor response to mast cell degranulation in the SHR airways, although it did increase the response in airways from normotensive rats.

2. Materials and methods

2.1. Animals

Male Wistar normotensive rats and spontaneously hypertensive rats (SHR), 5–6 months old (body weight 280–350 g) obtained from Central Bioterium of Biomedical Sciences Institute, University of São Paulo, were used in this study. Mean arterial blood pressure, measured by the tail cuff method, was 114 ± 3 mm Hg in normotensive rats ($n = 12$) and 186 ± 6 mm Hg in SHR ($n = 12$) ($P < 0.001$). Animal care and research protocols were in accordance with the principles and guidelines adopted by the Brazilian College of Animal Experimentation (COBEA) and approved by the Biomedical Sciences Institute/USP—Ethical Committee for Animal Research (CEEA).

2.2. Sensitization protocol

Rats were sensitized with an intraperitoneal injection of 10 μg ovalbumin adsorbed to 10 mg of aluminum hydroxide ("Alumen", Aldrox®—Wyeth). Fourteen days after sensitization, the animals were killed and the lungs isolated, as described below.

2.3. Isolation of lungs and assessment of airway reactivity

Rats were anaesthetised with chloral hydrate (600 mg kg^{-1}; i.p.), the peritoneum was opened and animals exsanguinated by transection of the abdominal aorta. The thoracic cavity was then opened, the pulmonary artery cannulated and the pulmonary circulation perfused with 40 ml Krebs solution over 1 min, to wash out blood. A cannula was then inserted into the trachea and the lungs were removed carefully from the thorax and perfused through the tracheal cannula with warm and oxygenated (37 °C, 95% O_2 and 5% CO_2) Krebs solution at a constant flow rate of 5 ml min^{-1}. The Krebs solution was of the following composition (in mM): NaCl 115.0; KCl 4.7; $CaCl_2$ 2.5; KH_2PO_4 1.2; $MgSO_4$ 2.5; $NaHCO_3$ 25.0; glucose 11.0. A small incision was made in the lower end of each lobe to allow the outflow of the solution. The perfusion pressure was recorded on a Beckman R511A recorder, using Gould P23DB pressure transducers and was expressed as cm H_2O rather than mm Hg.

Airway reactivity was assessed, essentially as described earlier (Landgraf et al., 2003). After a short stabilization period (15 min), bronchoconstrictor responses were measured as increases over basal levels of perfusion pressure following bolus injections (150 μl) into the perfusate entering the lung. Increases in pressure due to injections of 150 μl of Krebs solution were not detectable.

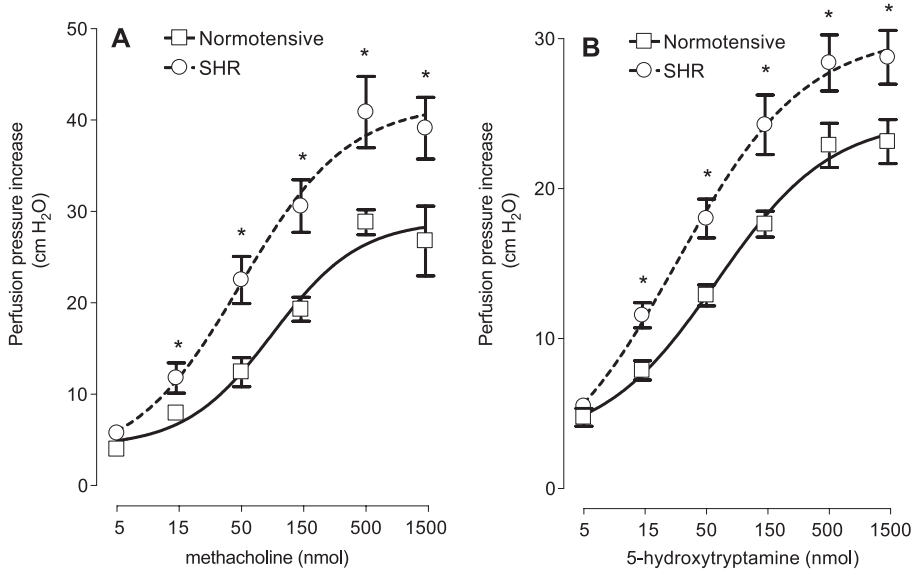

Fig. 1. Constrictor responses of perfused airways in isolated lungs from SHR or normotensive rats to methacholine (A) or 5-HT (B). Bronchoconstrictor effects were measured as increases in perfusion pressure following injection of bolus doses of agonist (150 μl) into the perfusate flow entering the lung. Values shown are the mean (± S.E.M.) pressure increase for each dose, given to five (methacholine) or eight (5-HT) lungs. For each agonist, the airways in the SHR lungs gave a greater response than those in lungs from normotensive rats. *Significantly different from corresponding value in normotensive lungs; $P < 0.05$.

Table 1
E_{max} and EC_{50} values for bronchoconstrictor effects of methacholine (1a) or 5-HT (1b) in isolated lungs from normotensive or hypertensive (SHR) rats

	E_{max} (cm H_2O)		EC_{50} (nmol)	
	Control	L-NAME	Control	L-NAME
a. Methacholine				
Normotensive	29.7 ± 1.1	39.4 ± 2.3	112 ± 30	130 ± 52
SHR	43.9 ± 3.0[a]	44.7 ± 1.4	73 ± 20	82 ± 20
b. 5-HT				
Normotensive	23.1 ± 1.5	21.0 ± 2.7	62 ± 12	80 ± 21
SHR	28.7 ± 1.8[a]	32.6 ± 1.7[a]	59 ± 18	45 ± 6

The values shown in this Table were obtained from the results presented in Figs. 1 and 3 and in Table 2. The effects of pre-treatment with L-NAME are most clearly shown in the E_{max} values where the strain related difference to methacholine was abolished but that to 5-HT was maintained.

[a] Significantly different from corresponding values for normotensive rats; $P<0.05$; $n=5-8$ lungs in each group.

2.4. Standard bronchoconstrictor agonists

Full dose–response curves were generated for methacholine and 5-hydroxytryptamine as standard constrictors of airway smooth muscle. Stock solutions of these agonists were prepared in isotonic saline and diluted immediately before use in Krebs solution to give dose ranges of 1–300 μg (5–1500 nmol) in 150 μl. In these experiments, each preparation of isolated lungs was exposed to a full sequence of doses, allowing pressure to fall to basal levels between doses. For exogenous arachidonic acid and prostaglandin H_2, the dose range was limited to three doses. Stock solutions of arachidonic acid or prostaglandin H_2 were prepared in absolute ethanol with final dilution as before in Krebs solution.

2.5. Responses to antigen challenge and compound 48/80

We used two other stimuli for bronchoconstriction, immunological challenge with antigen (ovalbumin) or a non-immunological stimulus, compound 48/80. Antigen or compound 48/80 were dissolved in saline and then diluted in Krebs solution and injected as described in a final volume of 150 μl. Antigen challenge was carried out with 150 μg of ovalbumin and three doses of compound 48/80 (100, 300 and 1000 μg) were used; each lung was given only one injection of a stimulus.

2.6. Inhibition of endogenous NO synthesis

Animals were treated with a nonselective inhibitor of NO synthesis, N^{ω}-nitro-L-arginine methyl ester hydrochloride (L-NAME), dissolved in isotonic saline and given i.v., 30 mg kg^{-1}, 30 min before killing the animals and isolation of the lungs.

2.7. Data analysis

Dose–response curves to methacholine or 5-HT are shown as the sigmoid curves generated from the results by the Graph Pad Prism 3.0 programme, using values from five lungs for each strain. Mean pressure responses to a single dose in the two strains were compared by Student's *t*-test for significant differences. Values of

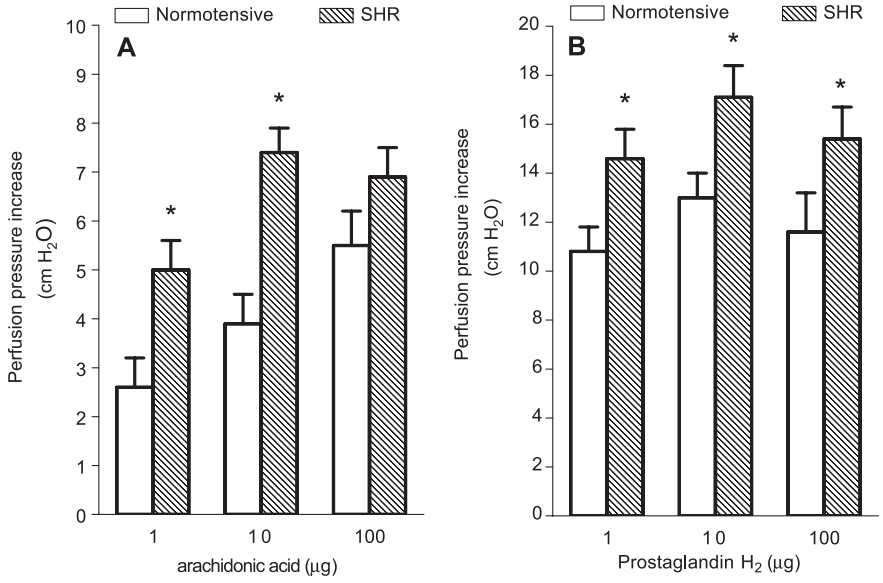

Fig. 2. Constrictor responses of perfused airways in isolated lungs from SHR or normotensive rats to arachidonic acid (A) or prostaglandin H_2 (B). Bronchoconstrictor effects were measured as described earlier. The heights of the bars represent the mean (± S.E.M.) pressure increase for each dose, given to $n=6$ (arachidonic acid) or 7 (prostaglandin H_2) lungs. For either agonist, the responses in the SHR lungs were greater than those in lungs from normotensive rats. *Significantly different from corresponding value in normotensive lungs; $P<0.05$.

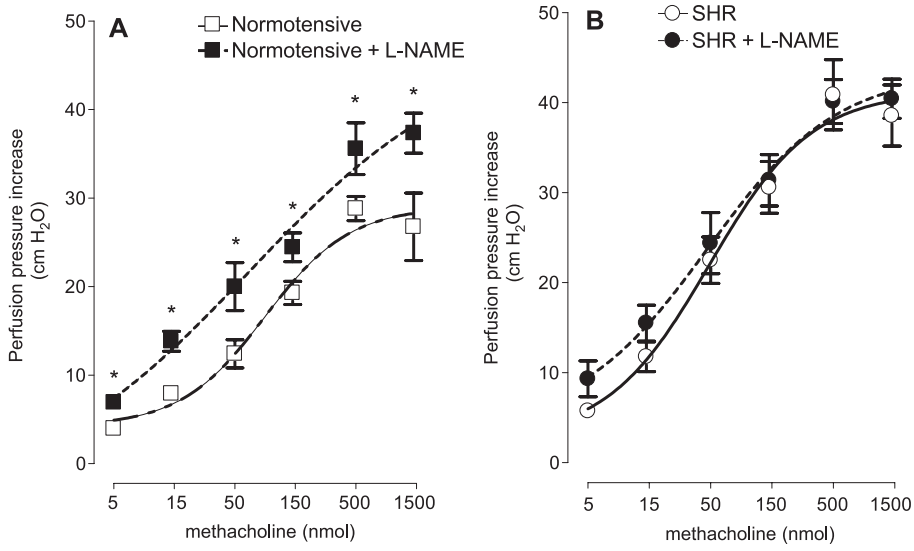

Fig. 3. Effect of pretreatment in vivo with L-NAME on the bronchoconstrictor response to methacholine in isolated lungs from normotensive (A) rats or SHR (B). The responses to methacholine in SHR lungs were not modified, whereas those in lungs from normotensive animals were increased, following pretreatment with L-NAME (30 mg kg^{-1}; 30 min). These potentiated responses in normotensive lungs were now identical to those in SHR lungs, with or without L-NAME pretreatment. Values shown are the mean (± S.E.M.) pressure increase for each dose, obtained from $n = 5–6$ lungs. *Significantly different from corresponding value without pretreatment; $P < 0.05$.

$P < 0.05$ were taken as showing a significant difference between means.

2.8. Drugs and reagents

The following compounds were purchased from Sigma, St. Louis, USA—ovalbumin (Grade III), compound 48/80, methacholine, 5-hydroxytryptamine, arachidonic acid, prostaglandin H_2 and N^{ω}-nitro-L-arginine methyl ester hydrochloride (L-NAME). Aluminium hydroxide (Aldrox®) was obtained from Wyeth, Whitehall, Brazil.

3. Results

3.1. Airway responses to standard bronchoconstrictors

During antigen challenge, mast cells release a wide range of potentially bronchoconstrictor substances (see Metcalfe et al., 1997). Since we used the airway smooth muscle in the isolated lungs as the detector system for antigen challenge, it was necessary to allow for variations in smooth muscle reactivity between strains by assessing responses to a standard agonist. The basal perfusion pressure with the fixed perfusate flow rate (5 ml min^{-1}) was not different between strains (normotensive 10.1 ± 0.7 vs. SHR, 11.5 ± 0.4: $n = 6$ for each group).

We chose as our standard airway constrictor, a cholinergic agonist, methacholine. Addition of methacholine (5–1500 nmol) to the perfusate entering the lungs induced constriction of the airways and a consequent increase in perfusion pressure. Dose–response curves for isolated lungs from either strain are given in Fig. 1A and show that, although methacholine induced perfusion pressure rises in both sets of airways, the airways from the SHR were approximately four times more responsive to methacholine than were airways from normotensive rats. For instance, a 15 cm H_2O increase in perfusion pressure was induced by 21 (± 3) nmol meth-

Table 2
Lack of effect of pretreatment with L-NAME on the airways perfusion pressure induced by 5-HT in normotensive or hypertensive (SHR) rats

Dose of 5-HT (nmol)	Perfusion pressure increase (cm H_2O)					
	5	15	50	150	500	1500
Normotensive control	2.8 ± 0.4	4.5 ± 0.2	9.0 ± 1.4	13.8 ± 1.7	18.9 ± 1.4	23.3 ± 1.5
Normotensive + L-NAME	2.4 ± 0.2	5.2 ± 1.0	9.2 ± 0.6	13.0 ± 1.1	16.6 ± 2.2	21.0 ± 2.7
SHR control	4.4 ± 0.4[a]	11.0 ± 2.0[a]	17.0 ± 1.8[a]	23.0 ± 2.5[a]	29.0 ± 3.1[a]	31.4 ± 2.3[a]
SHR + L-NAME	6.3 ± 1.3[a]	10.0 ± 1.3[a]	18.2 ± 2.4[a]	27.2 ± 2.5[a]	32.4 ± 2.0[a]	33.0 ± 1.9[a]

This Table shows the increased perfusion pressure induced by 5-HT in the isolated airways from normotensive and hypertensive rats (SHR). Groups of rats were injected (i.v.) with either isotonic saline (control) or L-NAME (30 mg kg^{-1}; + L-NAME) 30 minutes before isolating lungs. Values shown are the mean (± S.E.M.) results from 5 to 6 animals in each group. The treatment with L-NAME did not affect the responses of the airways from each strain when compared to the respective control groups. Note that responses in the SHR were always greater than in the normotensive lungs (see also Fig. 1B).

[a] Significantly different from normotensive control; ($P < 0.05$).

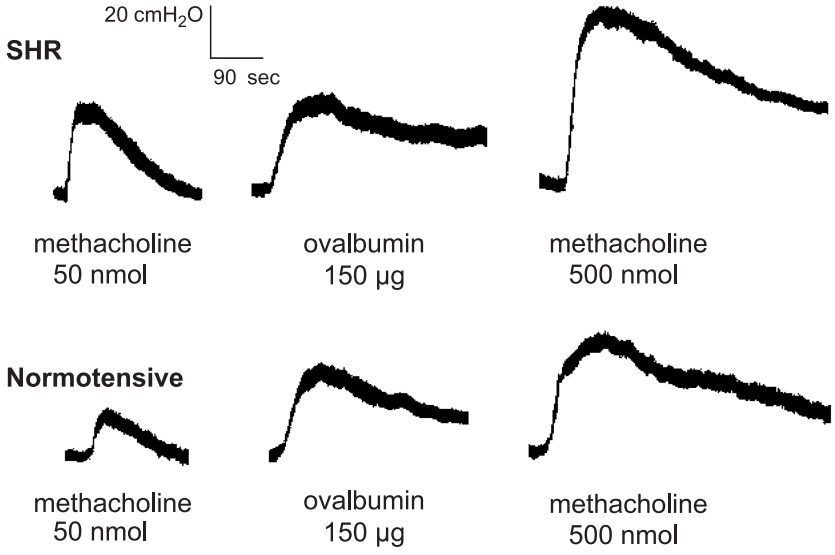

Fig. 4. Perfusion pressure recordings in lungs isolated from SHR or normotensive rats, sensitised to ovalbumin. Two responses to doses of methacholine (50 and 500 nmol) in a perfused lung are shown together with the response in the same lung to challenge with antigen (ovalbumin, 150 µg). Pressure increases to antigen challenge was very similar but responses to methacholine were quite different in lungs from the two strains.

acholine in SHR lungs but required 85 (± 20) nmol in lungs from normotensive rats (mean ± S.E.M.; $n = 5$ in both strains; $P < 0.05$). The maximal responses obtained in SHR airways were about 15 cm H_2O more than those in the lungs from normotensive rats.

Because of this initial difference in reactivity of airways between strains and because rat mast cells contain 5-HT as the major biogenic amine, we also measured airway responses to this agonist in lungs from either strain. As summarized in Fig. 1B, the airways in lungs from SHR were again more sensitive, by nearly threefold, than those from normotensive rats, over a range of doses (5–1500 nmol). The doses required for a 15 cm H_2O rise here were 36 (± 7) nmol in SHR lungs ($n = 9$) and 97 (± 16) nmol in the lungs from normotensive animals ($n = 8$; $P < 0.05$).

The values for E_{max} (as cm H_2O) and for EC_{50} (nmol) derived from these dose–response relationships are given for both methacholine and 5-HT in Table 1.

We further assessed airway responses to exogenous arachidonic acid, the precursor for all endogenous eicosanoids (prostaglandins and leukotrienes) and to prostaglandin H_2, the product of cyclooxygenase catalysis and precursor of prostaglandins and thromboxane A_2. Although the maximum of the constrictor response was less, in terms of cm H_2O, than those induced by the other two agonists, airways from SHR still gave a higher response at each dose level of either agonist than those in lungs from the normotensive rats (Fig. 2).

3.2. Effect of L-NAME on airway responses to standard bronchoconstrictors

Because we intended to assess bronchoconstriction in the presence of the NO synthase inhibitor L-NAME, we needed to estimate the effect of this inhibitor on the responses to the standard, directly acting agonists. We therefore repeated the assessment of airway constriction in lungs from animals pretreated in vivo with L-NAME (30 mg kg^{-1} i.v., 30 min before isolating lungs). This treatment raised systemic blood pressure (measured at 30 min) from 115 ± 5 to 140 ± 8 mm Hg in normotensive rats and from 175 ± 4 to 225 ± 14 mm Hg in SHR ($n = 5$). Although the constrictor responses of the normotensive airways to methacholine were enhanced (Fig. 3A), those of the SHR airways were not modified by pretreatment with L-NAME (Fig. 3B). The dose–response curve of the airways from normotensive rats pretreated with

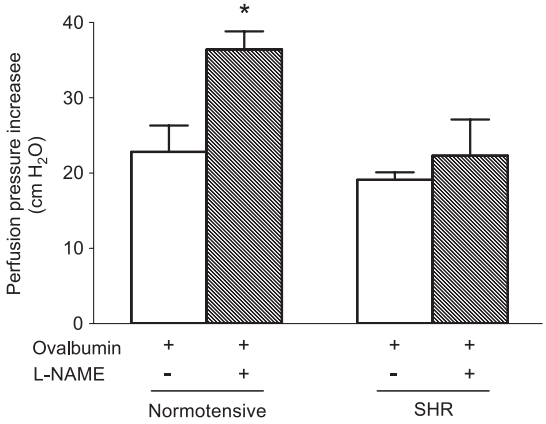

Fig. 5. Effects of pretreatment with L-NAME on bronchoconstriction induced by antigen challenge in perfused lungs. The heights of the bars represent the mean (± S.E.M.) of responses in lungs from sensitised rats of either strain challenged with ovalbumin. Antigen challenge induced the same extent of bronchoconstriction in lungs from SHR or normotensive rats. However, whereas the response in lungs from normotensive rats was increased, by about 60%, that in the SHR lungs was not modified after treatment with L-NAME. *Significantly different from value without L-NAME; $P < 0.05$; $n = 5–6$.

Table 3
Airway responses to antigen challenge expressed as equivalent doses of the standard agonist, methacholine

	Methacholine equivalents (nmol)	
	Control	L-NAME
Normotensive	402 ± 130	540 ± 40
SHR	41 ± 11[a]	23 ± 8[a]

Responses to antigen challenge, previously shown in Fig. 5 as increases in perfusion pressure (cm H_2O), have been converted to equivalent doses of the standard agonist by interpolation, using the responses obtained to methacholine in the same lungs. Expressed in this way, the responses to antigen challenge represented the release of markedly less spasmogens in lungs from SHR than were released in the lungs from normotensive rats. Pre treatment with L-NAME did not alter spasmogen release in either strain.

[a] Significantly different from corresponding values for lungs from normotensive rats; $P<0.05$; $n=5$-6 in each group.

L-NAME was now not different from that of airways from SHR rats, with or without L-NAME. However, pretreatment with L-NAME in vivo did not alter responses of SHR or normotensive airways to 5-HT (Table 2, lower two rows). The E_{max} and EC_{50} values from these experiments with L-NAME are also presented in Table 1.

3.3. Airway responses induced by antigen challenge

In lungs isolated from sensitized animals, challenge with antigen induced a bronchoconstrictor response in both SHR and normotensive strains. The increase in perfusion pressure following antigen was longer lasting than to methacholine (Fig. 4). As also illustrated in this figure, the bronchoconstrictor response to immunological challenge, in terms of increased perfusion pressure, was not markedly different in SHR and normotensive lungs. In Fig. 5, the mean values of these pressure increases are shown together with those in lungs from animals pretreated with L-NAME. It is clear that the pretreatment increased bronchoconstriction after antigen challenge only in lungs from normotensive animals with no

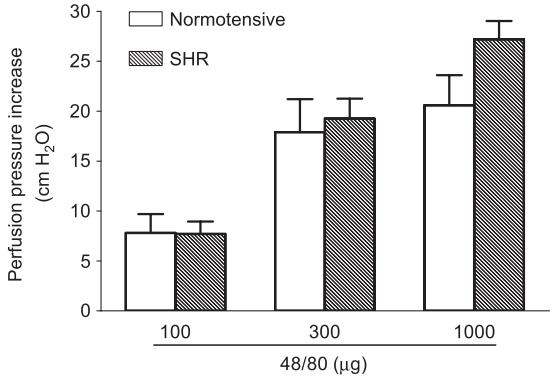

Fig. 6. Bronchoconstriction induced by compound 48/80 in perfused lungs. The heights of the bars represent the mean (± S.E.M.) of responses in lungs from sensitised rats of either strain challenged with compound 48/80, given at three dose levels. Note here that lungs from either strain were equally responsive to compound 48/80. Results shown are from five to seven lungs.

Table 4
Lack of effect of L-NAME on the bronchoconstrictor effects of a single dose of compound 48/80 in lungs from normotensive or hypertensive (SHR) rats

	Increased perfusion pressure (cm H_2O)	
	Control	L-NAME
Normotensive	7.6 ± 1.0	11.4 ± 1.0
SHR	11.8 ± 0.72	13.4 ± 1.6

Values shown are the mean (± S.E.M.) pressure increase for this dose (300 μg). Pre-treatment with L-NAME (30 mg kg^{-1}; 30 min) did not change the bronchoconstriction induced by compound 48/80 in either set of lungs ($n=5$ in each strain).

effects on SHR lungs. The airway response to antigen challenge can also be expressed in terms of the equivalent dose of a standard agonist, obtained by interpolation using the responses to methacholine in each lung (see Methods). Such methacholine equivalents are given in Table 3 and disclose a marked difference between strains, with or without L-NAME pretreatment. In lungs from unsensitised animals of either strain, challenge with ovalbumin produced no change in perfusion pressure (data not shown).

3.4. Airway responses induced by compound 48/80

In another set of experiments, each preparation of isolated airways was exposed to a single dose of compound 48/80, over a 10-fold range (100–1000 μg), eliciting a constrictor response. The perfusion pressure rise for a given dose of compound 48/80 did not differ between strains (Fig. 6). The contribution of endogenous NO to these responses was assessed by pretreatment of rats with L-NAME and stimulation with a single fixed dose of compound 48/80 (300 μg). As shown in Table 4, the perfusion pressure rise to the single dose of compound 48/80 was not changed in lungs from either strain.

4. Discussion

Our results have shown that constriction of the airways in lungs isolated from SHR was clearly different from that in lungs from normotensive rats. However, the nature of this difference depended on the mode of stimulation used to induce the bronchoconstriction. Furthermore, the involvement of endogenous NO in this response, as assessed after inhibition of NO synthases, also varied between the constrictor stimuli and between strains.

The increased sensitivity of the SHR airways to methacholine is compatible with reports of hyperresponsiveness of other smooth muscle, from blood vessels, in hypertensive rats (Kato et al., 1990; Chang et al., 2002; Liu et al., 2002; Touyz et al., 2002). For both types of smooth muscle this strain-related difference in response appeared to be due to a lack of endogenous NO, in the hypertensive strain relative to that in the normotensive rats. In our work, after inhibition

of endogenous NO synthase, the responses in airways from normotensive rats were increased to overlap with those of the SHR. Although there are reports of endogenous NO modulating airway responses in guinea-pig and human tissue (Belvisi et al., 1991; Ellis and Undem, 1992), in airways from mice, constrictor responses to carbachol were not affected by exposure to L-NAME in vitro (Kakuyama et al., 1999). These last authors concluded that effects of stimulating cholinergic neurones in small airways were modulated by both NO and cyclooxygenase products but that the effects of L-NAME were detectable only when cyclooxygenase had been already inhibited. The difference between our results and those of Kakuyama et al. (1999) may thus reflect the particular balance between NO and cyclooxygenase products in the two species. However, in our experiments, both the lack of modulation by L-NAME of responses in the SHR airway and the increased responses induced in normotensive airways to methacholine suggests that the strain-related differences in response to this agonist were completely accounted for by endogenous NO.

By contrast, the differential sensitivity of our preparations to another agonist, 5-HT was unaffected by pretreatment with L-NAME. We chose 5-HT as another agonist because it is the major biogenic amine in rat mast cells. This amine has also been shown in vascular smooth muscle to release a relaxing factor that was neither NO nor a cyclooxygenase product and has been characterised as an endothelium derived hyperpolarising factor (Yokota et al., 1994). More recently, contractions induced by 5-HT in bovine trachea were found to be resistant to modulation by L-NAME, whereas those to acetylcholine were potentiated (Spicuzza et al., 2002). In our airway preparations, we did not inhibit cyclooxygenase, so the criteria for defining an endothelium-derived hyperpolarizing factor (EDHF) were not met. Nevertheless, whatever the nature of the relaxing factor released by 5-HT in our preparations, it would appear that 5-HT generated more of it in airways from normotensive rats but less in those from the SHR.

The differing responses to exogenous arachidonic acid and to prostaglandin H_2 are less easy to analyse in terms of release of non-eicosanoid relaxing factors, as both compounds are metabolised further to a mixture of contractor and dilator eicosanoids (prostaglandins and leukotrienes). The net response of the airway smooth muscle would thus reflect the proportion of each eicosanoid synthesised in each tissue. However, this net response is relevant to our system as a mixture of eicosanoids is also released on degranulation of mast cells (Metcalfe et al., 1997) and responses to exogenous precursors of eicosanoids should give some indication of a differential responsiveness to eicosanoids released during degranulation, between strains.

Overall, our results with these direct stimulants of airway smooth muscle have shown a consistently greater response of SHR airways to three classes of agonist—methacholine, 5-HT and eicosanoids. For two of the agonists, the greater response is associated with a decrease in relaxing factors, probably NO with methacholine and possibly an EDHF with 5-HT. Our results suggest a general constrictor hyperreactivity, not related to any one particular type of agonist, in airway smooth muscle from the SHR. This hitherto unreported aspect of the SHR phenotype merits further and fuller analysis. Although we have related the increase in perfusion pressure to smooth muscle activity, there are several other factors which, in vivo, could also contribute to a greater narrowing of the airway lumen in the SHR. These include structural changes in the airway such as hypertrophy of the smooth muscle, changes in the proportions of smooth muscle and epithelium or subepithelial oedema. Such changes could also affect the access of agonist in the airway lumen to the receptors on the airway smooth muscle. Nevertheless, the advantage of a measure such as bronchoconstriction is that it is a functional measure and thus reflects the net overall response to the stimulus, allowing the integration of all factors. Assessment of the possible contributing factors is the next stage in the analysis of this unexpected phenomenon.

We planned the experiments on direct stimulation of airways as a background to our major purpose, the assessment of bronchoconstrictor responses to antigen challenge in the SHR airways. This assessment is indirect in that it represents both the response of the airway smooth muscle to the spasmogens released during the challenge and the immunological efficiency of that challenge. Compound 48/80 provides a stimulus similar to antigen challenge in that release of spasmogens from mast cells is involved in both. Both of these indirect modes of stimulation of airway smooth muscle gave essentially the same results, that there was no strain-related difference in the outcome of stimulation, i.e., bronchoconstriction. However, after inhibition of endogenous NO synthesis, only the bronchoconstriction in lungs from normotensive rats was potentiated, with no change in SHR lungs. Thus, in normotensive lungs the response was modulated by a relaxing factor, probably NO. This NO could be derived from the airway smooth muscle during its contractile response to the spasmogens generated by antigen challenge (see our results with methacholine), from the degranulation of the mast cells (Masini et al., 1991; Gilchrist et al., 2002) or from some other source. For the SHR lungs, the analysis is clearer; since the direct response of these airways was not affected by L-NAME, the lack of effect on antigen challenge strongly suggests that the lack of endogenous NO did not change the release of spasmogens during the challenge.

This lack of effect of NO synthase inhibition in the SHR lungs was unexpected as NO is well-established as an inhibitor of mast cell degranulation (Eastmond et al., 1997; Forsythe et al., 2001; Coleman, 2002). Exogenous NO is known to depress mast cell degranulation (Salvemini et al., 1991; Eastmond et al., 1997; Iikura et al., 1998) and in vivo there is evidence for tonic inhibition of mast cell function by endogenous NO (Kubes et al., 1993; Gaboury et al., 1996). Although isolated purified mast cells can

generate NO by themselves (Gilchrist et al., 2002) and peritoneal mast cells from SHR generate relatively less NO on activation (Masini et al., 1991), in our preparation, the mast cells are in situ and their behaviour could be greatly modified by NO derived from their cellular environment—smooth muscle, endothelium, epithelium. However, our experiments did not show any modulation of the release of spasmogens in response to degranulating stimuli (antigen challenge or compound 48/80) after inhibition of NO synthases in the SHR. This resistance to the effect of L-NAME could represent a generally depressed formation of endogenous NO in this strain. Thus the SHR airways in our preparation appeared not to produce any L-NAME-sensitive relaxing factor and a low output of endogenous NO has been reported in other tissues from SHR (Brovkovych et al., 1999; Sunano et al., 1999; Kumar et al., 2003). This lack of endogenous NO might reflect substrate depletion through increased arginase levels, recently proposed as a causative factor in other models of airway hyperresponsiveness (Meurs et al., 2003).

It is worth noting that although the bronchoconstriction induced by antigen or compound 48/80 was equal in the two strains, because the airways in SHR lungs were more responsive to the range of agonists we studied, that equal bronchoconstriction would represent a lesser amount of spasmogen released in the SHR. This is made clear by the values in Table 3. For instance, expressed as methacholine equivalents, spasmogens released in SHR lungs would be about 10-fold less than in lungs from normotensive rats. The corresponding analyses for equivalents of 5-HT or of arachidonic acid would give a different quantitative ratio but qualitatively there would still be less spasmogen release in SHR lungs than in normotensive lungs following antigen challenge. Because this stimulus releases a mixture of acutely acting bronchoconstrictor agents from mast cells, which we measure as increased perfusion pressure, it is difficult to estimate exactly how much less of this mixture the responses in the SHR lungs represent. Nevertheless, our observation that a wide range of agonist types (methacholine, 5-HT, arachidonic acid and prostaglandin H_2) all exhibited the same qualitative differential response in the SHR airways does strongly suggest that mast cells in SHR lungs release less spasmogens than those in lungs from normotensive rats. Thus the mast cells in SHR lungs appear to be resistant to antigen-induced degranulation, as we have already shown for two other sites, the mesentery and skin, in this hypertensive strain (Kwasniewski et al., 1998).

One possible consequence of a depressed mast cell response to antigen challenge in the SHR relates to the other non-spasmogenic components of mast cell degranulation. A variety of cytokines, chemokines and growth factors are released from mast cells during antigen challenge (Metcalfe et al., 1997; Page et al., 2001), which have important long-term effects upon the pathophysiology of asthma (Rossi and Olivieri, 1997; Williams and Galli, 2000; Page et al., 2001). Since many asthmatics exhibit an allergic component of their disease and the SHR are frequently used as models of essential hypertension in humans, our results suggest that subjects with essential hypertension may have lung mast cells less responsive to degranulation by antigens. As a consequence, there could be a lower incidence of asthma in the hypertensive patient population, a correlation open to relatively simple epidemiological assessment.

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