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Role of L-arginine in the deficiency of nitric oxide and airway hyperreactivity after the allergen-induced early asthmatic reaction in guinea-pigs

*,[1]Jacob de Boer, [1]Michiel Duyvendak, [1]Fineke E. Schuurman, [1]F. May H. Pouw, [1]Johan Zaagsma & [1]Herman Meurs

[1]Department of Molecular Pharmacology, University Centre for Pharmacy, A. Deusinglaan 1, 9713 AV Groningen, The Netherlands

1 Using a guinea-pig model of allergic asthma, we investigated the role of L-arginine limitation in the allergen-induced deficiency of nitric oxide (NO) and airway hyperreactivity (AHR) after the early asthmatic reaction, by examining the effects of various concentrations of the NO synthase (NOS) substrate on the responsiveness to methacholine of isolated perfused tracheae from unchallenged (control) animals and from animals 6 h after ovalbumin challenge.

2 Preparations from ovalbumin-challenged guinea-pigs showed a 1.9 fold increase in the maximal response (E_{max}) to intraluminal (IL) administration of methacholine compared to controls ($P<0.001$). A similar 2.0 fold ($P<0.05$) increase in E_{max} to methacholine was observed in control airways incubated with the NOS inhibitor N^{ω}-nitro-L-arginine methyl ester (L-NAME; 0.1 mM, IL), while L-NAME had no further effect on the airways from ovalbumin-challenged animals.

3 In control airways, extraluminal (EL) administration of 0.3, 1.0 and 5.0 mM L-arginine all suppressed the E_{max} for methacholine by approximately 40% ($P<0.01$ all), whereas 5.0 mM D-arginine (EL) had no effect.

4 L-Arginine dose-dependently reduced the AHR to methacholine in tracheae from ovalbumin-challenged guinea-pigs, the responsiveness being normalized in the presence of 5.0 mM L-arginine. As in controls, 5.0 mM D-arginine was without effect.

5 The results demonstrate that deficiency of endogenous NO contributes to the allergen-induced AHR to methacholine after the early asthmatic reaction, which is reversed by exogenous administration of L-arginine. This indicates that limitation of substrate may underly the reduced cNOS activity and subsequent AHR after the acute asthmatic response.

Keywords: L-arginine; N^{ω}-nitro-L-arginine methyl ester; nitric oxide; nitric oxide synthase; methacholine; tracheal perfusion; airway hyperreactivity; asthma; allergic guinea-pigs

Abbreviations: AHR, airway hyperreactivity; cNOS, constitutive nitric oxide synthase; EL, extraluminal; E_{max}, maximal effect; IL, intraluminal; iNOS, inducible nitric oxide synthase; KH, Krebs-Henseleit; L-NAME, N^{ω}-nitro-L-arginine methyl ester; MBP, major basic protein; NO, nitric oxide; ΔP, differential (hydrostatic) pressure; pEC_{50}, $-\log_{10}$ of the concentration causing 50% of effect; P_{inlet}, (hydrostatic) pressure at the inlet; P_{outlet}, (hydrostatic) pressure at the outlet

Introduction

Allergen-induced early and late asthmatic reactions (Booij-Noord *et al*., 1971), infiltration and activation of inflammatory cells in the airways (De Monchy *et al*., 1985; Djukanovic *et al*., 1990) and an increase in airway reactivity (AHR) to non-specific stimuli, both after the early and after the late asthmatic reaction (Cockroft *et al*., 1977; Durham *et al*., 1988), are important features of allergic asthma.

Endogenous nitric oxide (NO) plays a central role in the regulation of airway tone as well as in asthmatic airway inflammation (Barnes & Belvisi, 1993; Gaston *et al*., 1994; Barnes & Liew, 1995), and may be importantly involved in the development of allergen-induced AHR (De Boer *et al*., 1996; Schuiling *et al*., 1998a,b). Thus, NO has a potent bronchodilator action by inducing relaxation of airway smooth muscle (Gruetter *et al*., 1989; Dupuy *et al*., 1992), is an important immunomodulator by promoting the proliferation of Th2 lymphocytes (Barnes & Liew, 1995), and, at high concentrations, may have deleterious effects in the airways by causing mucosal swelling (Kuo *et al*., 1992) and epithelial damage (Flak & Goldman, 1996).

NO is synthesized from the semi-essential amino acid L-arginine by the enzyme NO synthase (NOS), of which different isoforms have been identified (Forstermann *et al*., 1991). Constitutive NOS isoforms (collectively called cNOS) are mainly expressed in inhibitory nonadrenergic noncholinergic nerves, endothelial cells and airway epithelium (Fischer *et al*., 1993; Kobzik *et al*., 1993; Asano *et al*., 1994), and are thought to be primarily involved in the regulation of airway and vascular tone by the local production of small amounts of NO in response to physiological stimuli (Barnes & Belvisi, 1993). An inducible isoform of NOS (iNOS), producing large amounts of NO, is induced by proinflammatory cytokines during airway inflammation, particularly in inflammatory and epithelial cells (Barnes & Belvisi, 1993; Hamid *et al*., 1993; Asano *et al*, 1994), and may be involved in the detrimental effects described above.

Various observations have indicated the involvement of endogenous NO in the regulation of airway tone. Thus, *in vitro* studies have demonstrated that nonselective NOS inhibitors such as N^{ω}-nitro-L-arginine methyl ester (L-

*Author for correspondence.

NAME) and N^G-monomethyl-L-arginine (L-NMMA) cause enhanced muscarinic agonist-, histamine- and substance P-induced constriction of intact perfused guinea-pig tracheal tube preparations (Nijkamp *et al*., 1993; De Boer *et al*., 1996; Figini *et al*., 1997), while vasoactive intestinal peptide, endothelin-1 and bradykinin-induced relaxations were reduced in the presence of these inhibitors (Lilly *et al*., 1993; Filep *et al*., 1993; Figini *et al*., 1996). *In vivo*, inhaled NO reversed histamine and methacholine-induced bronchoconstriction in humans (Kacmarec *et al*., 1996), guinea-pigs (Dupuy *et al*., 1992) and dogs (Brown *et al*., 1994), while administration of L-NAME or L-NMMA caused enhanced bronchoconstriction in response to allergen (Persson *et al*., 1993; Mehta *et al*., 1997b), histamine (Nijkamp *et al*., 1993; Mehta *et al*., 1997a; Schuiling *et al*., 1998b) and bradykinin (Ricciardolo *et al*., 1994) in the guinea-pig and to bradykinin in mild asthmatics (Ricciardolo *et al*., 1996).

Using a guinea-pig model of acute allergic asthma, characterized by allergen-induced early and late asthmatic reactions, airway inflammation and AHR after both reactions (Santing *et al*., 1992; 1994), we have recently established that a deficiency of NO contributes to the increased *ex vivo* responsiveness of isolated perfused tracheae to methacholine and histamine after the early asthmatic reaction, at 6 h after inhalational challenge of the animals with ovalbumin aerosol (De Boer *et al*., 1996). Similarly, a deficiency of cNOS-derived NO was also found to contribute to the AHR to histamine *in vivo* after the early asthmatic reaction (Schuiling *et al*., 1998a,b), while restoration of NO activity, presumably by induction of iNOS during the late asthmatic reaction, contributed to the partial reversal of the AHR after this reaction (Schuiling *et al*., 1998a).

The cause of the allergen-induced NO deficiency after the early asthmatic reaction is presently unclear. Very recently, by investigating the effects of the O_2^- scavenger superoxide dismutase in perfused tracheal preparations from ovalbumin-challenged guinea-pigs, we demonstrated that this deficiency is not caused by depletion of NO due to its reaction with (inflammation-induced) superoxide anions (De Boer *et al*., 1998). In the present study, we examined the possibility of a reduced NO production due to limitation of substrate, by examining the effects of various concentrations of L-arginine on methacholine-induced constriction of perfused tracheal preparations from unchallenged control guinea-pigs and from ovalbumin-challenged animals obtained at 6 h after the challenge.

Methods

Animals

Outbred specified pathogen-free guinea-pigs (Charles River SAVO, Kiszlegg, Germany), weighing 500–800 g, were used in this study. All animals were actively IgE-sensitized to ovalbumin (OA) at 3 weeks of age as described by Van Amsterdam *et al*. (1989). In short, 0.5 ml of an allergen solution containing 100 μg ml^{-1} ovalbumin and 100 mg ml^{-1} $Al(OH)_3$ in saline was injected intraperitoneally, while another 0.5 ml was divided over seven intracutaneous injection sites in the proximity of lymphenodes in the paws, lumbar regions and the neck. The animals were used experimentally in weeks 4–8 after sensitization. The animals were group-housed in individual cages in climated animal quarters and given water and food *ad libitum*, while a 12-h on/off light cycle was maintained.

All protocols described in this study were approved by the University of Groningen Animal Health Committee.

Allergen provocation

Ovalbumin provocations were performed by inhalation of aerosolized solutions. The provocations were performed in a specially designed animal cage, in which the guinea-pigs could move freely (Santing *et al*., 1992). The volume of the cage was 9 l, which ensured fast replacement of the air inside the cage with aerosol and vice versa. A DeVilbiss nebulizer (type 646, DeVilbiss, Somerset, PA, U.S.A.) driven by an airflow of 8 l min^{-1} provided the aerosol required, with an output of 0.33 ml min^{-1}.

Allergen provocations were performed by inhalation of increasing aerosol concentrations of 0.5, 1.0, 3.0, 5.0 and 7.0 mg ml^{-1} ovalbumin in saline for 3 min, separated by 10-min intervals. Allergen inhalations were discontinued when the first signs of respiratory distress were observed. No anti-histaminic was needed to prevent the development of anaphylactic shock. Previous studies measuring pleural pressure changes in ovalbumin sensitized, permanently instrumented, unrestrained guinea-pigs have indicated that the allergen-induced early asthmatic reaction induced by this procedure is maximal within 20 min and lasts for up to 5 h (Santing *et al*., 1992; 1994).

Tracheal perfusion

Six hours after ovalbumin challenge, the guinea-pigs were sacrificed. Non challenged IgE-sensitized animals were used as controls. The animals were killed by a sharp blow on the head and exsanguinated. The tracheas were rapidly removed and placed in Krebs-Henseleit (KH) solution (37°C) of the following composition (mM): NaCl 117.50, KCl 5.60, $MgSO_4$ 1.18, $CaCl_2$ 2.50, NaH_2PO_4 1.28, $NaHCO_3$ 25.00, D-glucose 5.50; gassed with 5% CO_2 and 95% O_2; pH 7.4.

The tracheas were prepared free of serosal connective tissue and cut into two halves of approximately 17 mm before mounting in a perfusion setup, as described previously (De Boer *et al*., 1996). To this aim, the tracheal preparations were attached at each side to stainless steel perfusion tubes fixed in a Delrin perfusion holder. The holder with the trachea was then placed in a water-jacketed organ bath (37°C) containing 20 ml of gassed KH (the serosal or extraluminal (EL) compartment). The lumen was perfused with recirculating KH from a separate 20 ml bath (mucosal or intraluminal (IL) compartment) at a constant flow rate of 12 ml min^{-1}. Two axially centred side-hole catheters connected with pressure transducers (TC-XX,Viggo-Spectramed B.V., Bilthoven, The Netherlands) were situated at the distal and proximal ends of the trachealis to measure hydrostatic pressures (P_{outlet} and P_{inlet}, respectively). The signals were fed into a differential amplifier to obtain the difference between the two pressures ($\Delta P = P_{inlet} - P_{outlet}$), which was plotted on a flatbed chart recorder (BD 41, Kipp en Zonen, Delft, The Netherlands). ΔP reflects the resistance of the tracheal segment to perfusion and is a function of the mean diameter of the trachea between the pressure taps (Munakata *et al*., 1989). The transmural pressure in the trachea was set at 0 cm H_2O. At the perfusion flow rate used, a baseline ΔP of 0.1 to 1.0 cm H_2O was measured, depending of the diameter of the preparation.

After a 45 min equilibration period with three washes with fresh KH (both IL and EL), 1 μM isoprenaline was added to the EL compartment for maximal smooth muscle relaxation to assess basal tone. After three washes during at least 30 min, the

trachea was exposed to EL 40 mM KCl in KH to obtain a receptor-independent reference response. Subsequently, the preparation was washed four times with KH during 45 min until basal tone was reached and a cumulative concentration response curve (CCRC) was made with IL methacholine. When used, L-NAME (100 μM) was applied to the IL reservoir, 45 min prior to agonist-addition. L-arginine (0.3, 1.0 and 5.0 mM) and D-arginine (5.0 mM) were added to the EL reservoir, 30 min prior to agonist addition.

Data analysis

To compensate for differences in baseline ΔP and in ΔP changes in response to contractile stimuli due to variation in resting internal diameter of the preparations used, IL responses of the tracheal tube preparations to methacholine were expressed as a percentage of the response induced by EL administration of 40 mM KCl. The contractile effect of 10 mM methacholine (highest concentration) was defined as E_{max}. Using this E_{max}, the sensitivity to methacholine was evaluated as pEC_{50} ($-\log_{10}$ EC_{50}) value.

The results are expressed as means$\pm$s.e.mean. Statistical analysis was performed using the Student's *t*-test for unpaired observations. Differences were considered statistically significant at $P<0.05$.

Chemicals

Histamine hydrochloride, ovalbumin (grade III), aluminum hydroxide, (−)-isoprenaline hydrochloride, L-arginine hydrochloride, D-arginine hydrochloride and N^{ω}-nitro-L-arginine methyl ester (L-NAME) were obtained from Sigma Chemical Co. (St. Louis, MO, U.S.A.) and methacholine chloride from Aldrich (Milwaukee, WI, U.S.A.).

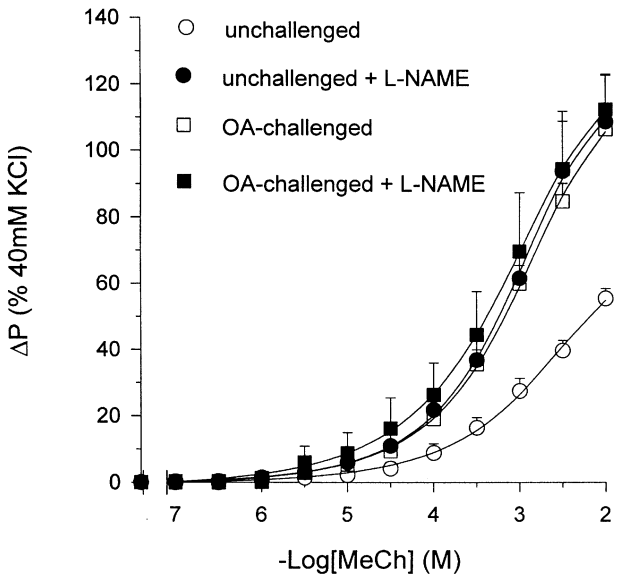

Figure 1 Methacholine (MeCh; IL)-induced constriction of intact perfused tracheae from unchallenged and ovalbumin (OA)-challenged guinea-pigs, in the absence and presence of 100 μM L-NAME (IL). Results are means$\pm$s.e.mean of 6–12 experiments.

Results

In perfused tracheal preparations of unchallenged guinea-pigs, the non-selective NOS inhibitor L-NAME (0.1 mM, IL) caused a significant 2.0 fold increase in the E_{max} of IL methacholine ($P<0.01$), without an effect on the sensitivity (pEC_{50}) to this agonist (Figure 1, Table 1).

In the ovalbumin-challenged group of animals, the ΔP response to KCl was unchanged compared to the unchallenged control group (not shown). However, a 1.9 fold increase in the E_{max} to IL methacholine was observed ($P<0.001$), without a change in pEC_{50} (Figure 1, Table 1). This increase was not further enhanced in the presence of L-NAME (Figure 1, Table 1).

In the control preparations, 0.3 mM L-arginine (EL) caused a significant decrease of the E_{max} to IL methacholine by approximately 40% ($P<0.01$), with no significant effect on the pEC_{50} for the agonist (Figure 2, Table 1). Higher concentrations of L-arginine (1.0 and 5.0 mM, EL) did not further decrease the responsiveness to methacholine, although there was a tendency to a gradually reduced sensitivity to the agonist (Figure 2, Table 1). In the presence of the inactive enantiomer D-arginine (5.0 mM, EL) the responsiveness to IL methacholine remained unaltered (Figure 2, Table 1).

In tracheae obtained from guinea-pigs at 6 h after allergen challenge, 1.0 and 5.0 mM L-arginine (EL) caused a significant, dose-dependent decrease of the E_{max} of IL methacholine, by 33% ($P<0.01$) and 65% ($P<0.001$), respectively (Figure 3, Table 1). In addition, there was a small but significant decrease in the pEC_{50} for methacholine in the presence of 1.0 mM L-arginine, which did not change further in the presence of 5.0 mM of the NOS substrate (Table 1). In the presence of 5.0 mM L-arginine, the responsiveness of the challenged tracheal preparations to methacholine was very similar to that of the unchallenged control preparations (Figures 2 and 3, Table 1). As in the control preparations, D-arginine had no effect on the responsiveness to

Table 1 Effects of L-NAME and L- and D-arginine on the responsiveness to methacholine of intact perfused tracheae from unchallenged and ovalbumin (OA)-challenged guinea-pigs

	Unchallenged		*OA-challenged*	
	pEC_{50} (−log M)	E_{max} (%KCl)	pEC_{50} (−log M)	E_{max} (%KCl)
Vehicle	3.01$\pm$0.11	55.5$\pm$3.1	3.14$\pm$0.07	106.4$\pm$3.8^{+++}
L-NAME				
0.1 mM	3.16$\pm$0.09	108.7$\pm$14.3**	3.15$\pm$0.24	112.4$\pm$10.4
L-arginine				
0.3 mM	3.33$\pm$0.40	33.0$\pm$4.7**	N.D.	N.D.
1.0 mM	2.84$\pm$0.10	32.9$\pm$3.4***	2.79$\pm$0.04*	71.8$\pm$6.6**/$^{+++}$
5.0 mM	2.68$\pm$0.13	31.1$\pm$7.5**	2.82$\pm$0.21	36.8$\pm$7.1***
D-arginine				
5.0 mM	3.03$\pm$0.16	57.5$\pm$10.6	3.28$\pm$0.35	99.9$\pm$5.9^{+}

Results are means$\pm$s.e.mean of 3–15 experiments. Statistical analysis: *$P<0.05$, **$P<0.01$ and ***$P<0.001$ compared to vehicle; $^{+}P<0.05$, $^{+++}P<0.001$ compared to unchallenged. N.D., not determined.

methacholine in the allergen-challenged preparations (Figure 3, Table 1).

Both in the control preparations and in the preparations of allergen-challenged animals, L-NAME and L- and D-arginine had no effect on basal airway tone (not shown).

Discussion

Using the nonselective NOS inhibitor L-NAME, we confirmed our previous finding (De Boer *et al*., 1996) that methacholine-induced constriction of guinea-pig tracheal tube preparations from unchallenged guinea-pigs is functionally antagonized by agonist-induced cNOS-derived NO. In the present study, it was also demonstrated that EL administration of the NO precursor arginine significantly reduced the responsiveness to methacholine in a stereoselective manner in these preparations, indicating that the production of agonist-induced endogenous NO may be enhanced by supplementation of substrate, and that the supply of L-arginine is a rate-limiting factor for cNOS activity in normal airways. This is in line with recent observations that oral administration (Kharitonov *et al*., 1995) as well as inhalation (Sapienza *et al*., 1998) of L-arginine cause an increase in exhaled NO in healthy human subjects.

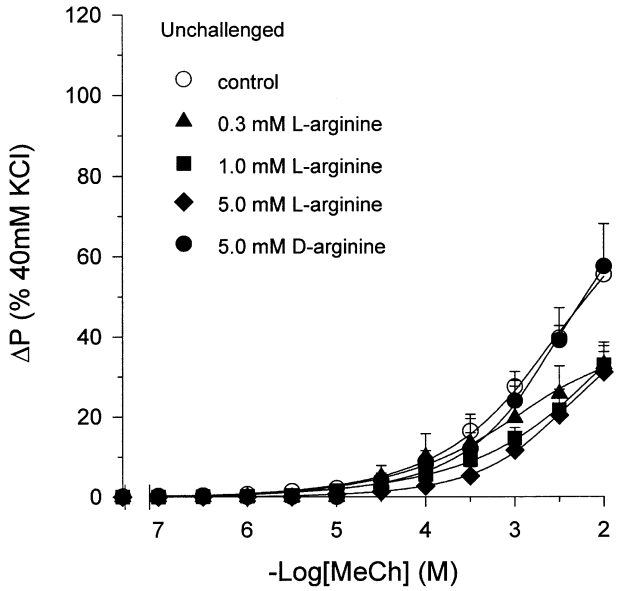

Figure 2 Methacholine (MeCh; IL)-induced constriction of intact perfused tracheae from unchallenged guinea-pigs in the absence (control) and presence of 0.3, 1.0 and 5.0 mM L-arginine (EL), and 5 mM D-arginine (EL). Results are means ± s.e.mean of 3–11 experiments.

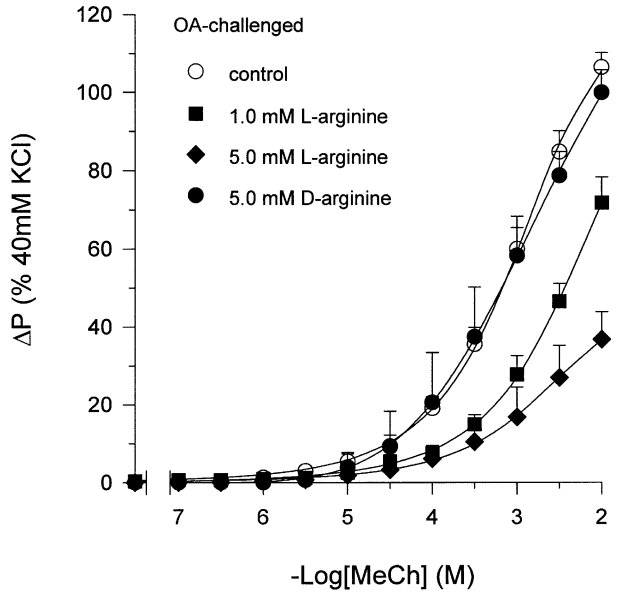

Figure 3 Methacholine (MeCh; IL)-induced constriction of intact perfused tracheae from ovalbumin (OA)-challenged guinea-pigs in the absence (control) and presence of 1.0 and 5.0 mM L-arginine (EL), and 5 mM D-arginine (EL). Results are means ± s.e.mean of 3–15 experiments.

The tracheal responsiveness to methacholine was markedly enhanced at 6 h after allergen challenge. As also found previously (De Boer *et al*., 1996), the observed airway hyperreactivity was closely mimicked by the administration of L-NAME to unchallenged control preparations, while, in addition, the hyperreactive preparations were unresponsive to the NOS inhibitor, indicating that a deficiency of agonist-induced NO is a major determinant of the observed hyperreactivity. The enhanced responsiveness to methacholine was completely reversed in the presence of 5 mM L-arginine, which indicates that limitation of substrate may underlie the deficiency of NO and subsequent AHR to methacholine after the early asthmatic reaction.

Different mechanisms could account for a limitation of L-arginine for cNOS activity after allergen challenge. First, recent observations in rat alveolar macrophages have indicated that polycationic proteins including eosinophil-derived major basic protein (MBP), which is released during the allergic asthmatic reaction and involved in the development of AHR (Gleich *et al*., 1988), may cause inhibition of cellular uptake of L-arginine by specific cationic amino acid transporters (Hirschmann *et al*., 1998). Using intact perfused guinea-pig tracheae, we recently demonstrated that the polycationic MBP analogue poly-L-arginine may indeed cause NO deficiency and AHR to methacholine in these preparations (Meurs *et al*., 1999).

Secondly, the bioavailability of L-arginine could be reduced by enhanced arginase activity in the airways. Arginase, which hydrolyzes L-arginine to L-ornithine and urea, is constitutively expressed in many cells throughout the body, including the lung (Aminlari & Vaseghi, 1992). Studies in murine bone marrow-derived macrophages have recently indicated that the expression of arginase may be selectively enhanced by T_H2 lymphocyte-derived cytokines (interleukin-4 and interleukin-10), which are involved in allergic inflammation, thus causing reduced NO production in these cells due to substrate depletion (Corraliza *et al*., 1995). Enhanced expression of inducible (type II) arginase by cytokines or lipopolysaccharide has also been observed in other cells, and has been proposed to be an important regulatory mechanism of NOS activity (Wang *et al*., 1995; Gotoh *et al*., 1996). Accordingly, in alveolar macrophages it has recently been shown that inhibition of arginase by N^{ω}-hydroxy-D,L-indospicine, a potent and specific arginase inhibitor (Vadon *et al*., 1996), causes a shift of L-arginine utilization to the NOS pathway (Hey *et al*., 1997).

Other hypothetical mechanisms that might be involved in L-arginine limitation for NOS activity are a reduced L-citrulline-L-arginine recycling (Chakder & Rattan, 1997), as well as a reduced affinity of NOS for the substrate. The latter idea would be in line with the reduced sensitivity to exogenous L-arginine in the challenged preparations, as indicated by the partial effect of 1.0 mM L-arginine in challenged airways compared to the full depression of the methacholine response in control preparations.

The observation that the enhanced methacholine reactivity in the NO-deficient tracheae at 6 h after allergen challenge can be fully reversed by L-arginine suggests that only the activity and not the protein expression of cNOS is reduced after allergen challenge. In addition, in a similar *in vitro* setting, we recently demonstrated that the deficiency of NO in hyperreactive tracheae is not caused by its possible reaction with superoxide anion in the airways (De Boer *et al*., 1998).

Different studies have indicated that the present *ex vivo* findings are relevant to the development of airway hyperreactivity *in vivo*. Thus, using the same conscious and unrestrained guinea-pig model of asthma, we have recently demonstrated that inhalation of the non-selective NOS inhibitor L-NAME, but not a selective dose of the specific iNOS inhibitor aminoguanidine, caused AHR to histamine before allergen challenge, indicating that cNOS-derived NO counteracts histamine-induced bronchoconstriction under basal conditions (Schuiling *et al*., 1998a,b). At 6 h after allergen challenge, both L-NAME and aminoguanidine had no effect on the allergen-induced AHR to histamine at this time point, indicating that a deficiency of (cNOS-derived) NO may contribute the AHR after the early asthmatic reaction (Schuiling *et al*., 1998a,b). In addition, by using aminoguanidine, it was pharmacologically established that bronchodilating iNOS-derived NO may contribute to the partial reversal of the AHR after the allergen-induced late asthmatic reaction (Schuiling *et al*., 1998a). In the same study, iNOS-derived NO also proved to have detrimental effects on airway reactivity by promoting airway inflammation and epithelial damage, indicating its dualistic role in the airways. In line with these observations, several studies have demonstrated the induction of iNOS mRNA, immunoreactivity and enzyme activity during (the time span of) the allergen-induced late asthmatic reaction (Yeadon & Price, 1995; Yan *et al*., 1995; Renzi *et al*., 1997).

Very interestingly, evidence for a deficiency of cNOS-derived NO modulating airway reactivity was recently also found in patients with severe asthma treated with corticosteroids, which suppresses the expression of iNOS (Ricciardolo *et al*., 1997). Thus, while the airway reactivity to bradykinin (and to methacholine) could be significantly increased by inhalation of L-NMMA in patients with mild asthma (Ricciardolo *et al*., 1996), a similarly enhanced AHR to bradykinin present in severe asthmatics was insensitive to the NOS inhibitor, indicating that a deficiency of NO contributed to the enhanced airway responsiveness in these patients. Preliminary evidence by the same authors suggests that this deficiency may indeed be induced by allergen challenge (Ricciardolo *et al*., 1999).

Also of interest is the recent finding by Gaston *et al*. (1998) that S-nitrosothiol concentrations in tracheal aspirates of children with near-fatal asthma were lower than those in non-asthmatic children. S-nitrosothiols are relatively stable NO adducts that may be formed by the reaction of NO with reduced thiols, such as reduced glutathion and cystein-containing peptides and proteins (Gaston *et al*., 1994). It has been reported that exogenously applied S-nitrosothiols may cause airway smooth muscle relaxation and bronchodilation in different species, including guinea-pigs (Jansen *et al*., 1992; Bannenberg *et al*., 1995). However, concentrations of endogenous S-nitrosothiols and their functional role in guinea-pig airways remain to be established.

A deficiency of cNOS-derived NO causing AHR to histamine was recently also demonstrated in guinea-pigs with a respiratory parainfluenza type 3 infection (Folkerts *et al*., 1995). As in the present study, the enhanced airway reactivity could be reversed by L-arginine, indicating that a deficiency of the NOS substrate may similarly be involved in virus-induced AHR. Moreover, in allergic asthmatics who were experimentally infected with rhinovirus-16, the increase in the airway reactivity to histamine was inversely correlated with the NO level in exhaled air, indicating the protective role of endogenous NO in virus-induced AHR (De Gouw *et al*., 1998).

Since L-arginine was shown to reverse the allergen-induced AHR after the early asthmatic reaction as well as virus-induced AHR, it could have therapeutic potential in patients with asthma. However, since L-arginine may also amplify the inflammatory effects of iNOS-derived NO present in most patients (Sapienza *et al*., 1998), its usefulness remains to be established.

In conclusion, our data demonstrate that a deficiency of endogenous NO contributes to the allergen-induced AHR to methacholine after the early asthmatic reaction, which can be reversed by exogenous L-arginine. This indicates that limitation of substrate may cause a reduced cNOS activity and subsequent AHR after the acute asthmatic response.

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