

- Klebanoff, S. J., Olszowski, S., Van Voorthuis, W. C., Ledbetter, J. A., Waltersdorff, A. M., & Schlechte, K. G. (1992). Effects of gamma-interferon on human neutrophils: Protection from deterioration on storage. *Blood*, 80(1), 225–234.
- Leatherman, B. D., Owen, S., Parker, M., Chadwick, S., Fornadley, J. A., Colson, D., & Fass, P. (2007). Sublingual immunotherapy: Past, present, paradigm for the future? A review of the literature. *Otolaryngology – Head and Neck Surgery*, 136(Suppl 3), S1–20.
- MacDowell, A. L., & Peters, S. P. (2007). Neutrophils in asthma. *Current Allergy and Asthma Reports*, 7(6), 464–468.
- Maestrelli, P., De Fina, O., Bertin, T., Papiris, S., Ruggieri, M. P., Saetta, M., Mapp, C. E., & Fabbri, L. M. (1999). Integrin expression on neutrophils and mononuclear cells in blood and induced sputum in stable asthma. *Allergy*, 54(12), 1303–1308.
- Mann, B. S., & Chung, K. F. (2006). Blood neutrophil activation markers in severe asthma: Lack of inhibition by prednisolone therapy. *Respiratory Research*, 7, 59–69.
- Monteseirin, J. (2009). Neutrophils and asthma. *Journal of Investigational Allergology and Clinical Immunology*, 19(5), 340–354.
- Potapinska, O., Gorska, E., Denkow, U., Zawadzka-Krajewska, A., Kulus, M., & Wasik, M. (2009). The usefulness of CD203c expression measurement on basophils after activation with grass pollen and *Dermatophagoides pteromyssinus* antigens. Preliminary study. *Pneumonologia i Alergologia Polska*, 77(2), 138–144.
- Romagnani, S. (2000). The role of lymphocytes in allergic response. *The Journal of Allergy and Clinical Immunology*, 105, 399–408.
- Sale, R., Sabatini, F., Silvestri, M., Serpero, L., Petecchia, L., & Rossi, G. A. (2004). Concentration-dependent activity of mometasone furoate and dexamethasone on blood eosinophils isolated from atopic children: Modulation of Mac-1 expression and chemotaxis. *International Immunopharmacology*, 4(13), 1687–1696.
- Silvestri, M., Spallarossa, D., Battistini, E., Sabatini, F., Pecora, S., Parmiani, S., & Rossi, G. A. (2002). Changes in inflammatory and clinical parameters and in bronchial hyperreactivity asthmatic children sensitized to house dust mites following sublingual immunotherapy. *Journal of Investigational Allergology and Clinical Immunology*, 12(1), 52–59.
- Spoelstra, F. M., Berends, C., Dijkhuizen, B., de Monchy, J. G., & Kauffman, H. F. (1998). Effect of theophylline on CD11b and L-selectin expression and density of eosinophils and neutrophils in vitro. *European Respiratory Journal*, 12(3), 585–591.

Chapter 11

L-Arginine Supplementation and Experimental Airway Hyperreactivity

M. Antosova and A. Strapkova

Abstract The interest in L-arginine metabolism was triggered primarily by the discovery of nitric oxide (NO) synthesis in mammals and its remarkable biological roles. The real role of L-arginine in the airway hyperreactivity (AHR) has not been established yet. Therefore, we studied whether supplementation of L-arginine can influence the experimental AHR evoked by two different triggers – allergen and exogenous irritant (toluene vapours). Male TRIK strain guinea pigs were used in the study. We used two patterns of pretreatment with L-arginine *in vivo*, short- and long-term, in a dose of 300 mg/kg administered i.p., after which we studied reactivity of airway smooth muscles *in vitro*. Pretreatment with L-arginine for 3 days decreased the airway smooth muscle reactivity induced by toluene vapour, whereas pretreatment for 17 days was without any additional effect on smooth muscle reactivity. The short-term pretreatment in ovalbumin-induced hyperreactivity caused an increase in airway smooth muscle reactivity to lower concentrations of both bronchoconstrictors. On the other side, this pretreatment significantly decreased smooth muscle reactivity to high concentrations of both bronchoconstrictors. Supplementation of L-arginine resulted in a modification of the airway smooth muscle response. The effect of supplementation was different depending on the AHR trigger, airway region and pretreatment duration. The results also underscore the importance of an optimal L-arginine level for the control of bronchial tone.

Keywords Airway hyperreactivity • Arginase • L-arginine • Nitric oxide • NO synthase

11.1 Introduction

L-arginine is the basic substrate for nitric oxide biosynthesis. This semi-essential amino acid is also a precursor for the synthesis of various proteins, urea, polyamines, proline, glutamate, creatine and agmatine. The synthesis and catabolism of L-arginine is influenced by several enzymes such as arginine succinate synthase, two isoenzymes of arginase, four NO synthases and arginine decarboxylase. Changes in the activity of these enzymes play an important role in the determination of L-arginine metabolic pathway under physiological and pathological conditions (Wu and Morris 1998).

M. Antosova (✉) • A. Strapkova
Department of Pharmacology, Jessenius Faculty of Medicine, Comenius University,
Sklabinska 26 St., 036 01 Martin, Slovakia
e-mail: antosova@fjmed.uniba.sk

M. Pokorski (ed.), *Respiratory Regulation - The Molecular Approach*,
Advances in Experimental Medicine and Biology 756, DOI 10.1007/978-94-007-4549-0_11,
© Springer Science+Business Media Dordrecht 2013

Intracellular concentration of L-arginine is higher than 100 $\mu\text{mol/l}$. Its utilization depends not only on the presence of enzymes, but also on their catalytic effect. The optimal level of L-arginine in humans is sufficient for continuous production of NO (Chandran et al. 1998). Although the catalytic activity of NO synthase is relatively low ($K_m(\text{L-Arg})$ 1.4–32.2 $\mu\text{mol/l}$ – depending on the isoform type), exogenous L-arginine can increase the bioavailability of nitric oxide (Su et al. 1997). Therapy with L-arginine is associated with increased concentrations of NO in exhaled air and with increased concentration of L-arginine and nitrate in plasma. This confirms that an increased amount of the substrate can increase endogenous NO production (Kharitonov et al. 1995). NO synthesis is dependent not only on the bioavailability of L-arginine, but also on different cofactors.

Another important enzyme using L-arginine is arginase. In humans it is constitutively expressed in two forms: arginase I and arginase II. Both enzymes have different properties, they are unevenly distributed in tissues and cells and have also different regulation of gene expression (Jenkinson et al. 1996; Li et al. 2001). Induction of arginase of any type may reduce the bioavailability of L-arginine for NO synthesis and subsequently attenuate the effects mediated by NO (Gobert et al. 2000). It is assumed that arginase II is involved in the regulation of bioavailability of L-arginine for NO synthesis, although it is not confirmed which type of arginase competes for substrate (Boucher et al. 1999). The imbalance between the activity of NO synthase and arginase is induced, e.g., by the action of inflammatory mediators (Cook et al. 1994). The maximal activity of arginase at physiological conditions significantly exceeds that activity of NO synthase. While arginase activity in rat liver is about 1,400 $\mu\text{mol/l/min/mg}$, NO synthase activity is approximately 1 $\mu\text{mol/l/min/mg}$ (Reczkowski and Ash 1994; Griffith and Stuehr 1995). In terms of inflammation, this rate may even increase, therefore L-arginine can be considered as a major limiting factor for NO production, the lack of which may contribute to the development of airway hyperreactivity (AHR).

The metabolic pathway of L-arginine and balance between arginase and NO synthase are important factors preventing the onset and development of AHR (North et al. 2009). Endogenous NO is involved in the regulation of airway responsiveness to bronchoconstrictive stimuli, including the muscarinic receptor agonists, histamine and bradykinin (Meurs et al. 2003). A number of studies have shown that deficiency of endogenous NO promotes AHR in response to various stimuli (de Boer et al. 2001).

Deficiency of NO is the main stimulus for the development of AHR after the late asthmatic response. This deficiency is due to reduced availability of L-arginine in the airways, which may be caused by two different mechanisms: increased activity of arginase competing with iNOS for the common substrate, or increased release of eosinophilic substances, which inhibits the transport of L-arginine to NO-producing cells (Maarsingh et al. 2009).

A decrease in bioavailability of the basic substrate for NO synthesis – L-arginine can influence bronchial tone and can be one of the factors contributing to AHR. Therefore, we studied whether supplementation of L-arginine can influence the experimental AHR or whether it has beneficial effects on airway reactivity evoked by two different triggers – allergen or exogenous irritant.

11.2 Methods

Experimental protocols had been approved by a local Ethics Committee of the Jessenius Faculty of Medicine in Martin, Slovakia, in accordance with internationally accepted recommendations regarding the experimental animal care and use. Outbred male TRIK strain guinea pigs (250–300 g) were used in the experiments. The animals were housed individually in climate-controlled commercial animal cages. The guinea pigs were weighed before and during the study and had *ad libitum* access to water and food.

11.2.1 Study Design

We recorded changes in airway smooth muscle reactivity after L-arginine pretreatment in response to toluene or allergen exposure. We divided the animals into seven groups, each made up of eight animals: four experimental and three control ones. All animals from the experimental groups received L-arginine (Sigma Aldrich, St. Louis, MA), dissolved in water, in a dose of 300 mg/kg daily, i.p. as short-, for 3 days, and long-, for 17 days, pretreatment. The animals in the control groups received 'water for injection' alone – 1 ml/kg.

The first experimental group received L-arginine 30 min before toluene exposure during 3 consecutive days (short-term pretreatment).

The second experimental group received L-arginine for 17 days (long-term pretreatment); during the last 3 days 30 min before toluene exposure.

The third experimental group received L-arginine 30 min before each allergen exposure (short-term pretreatment: three times).

The fourth experimental group received L-arginine during over the whole period of sensitization – 14 days – once a day (long-term pretreatment).

All animals in the control groups received 'water for injection' as above outlined under otherwise the same experimental and hyperreactivity paradigms.

11.2.2 Airway Hyperreactivity Induction

Toluene exposure. The method of *in vivo* exposure to toluene vapours described by Strapkova et al. (1996) was used in this study. The guinea pigs were spontaneously breathing toluene vapors in a Plexiglas exposure chamber which consisted of the compressor, flow-meter, vaporizer and exposure cage. The device was situated in the fume-cupboard at 22°C. Toluene vapours were delivered into the cage at a constant flow of 4 l/min. The average concentration of toluene was 6 mg/l (1,600 ppm). The duration of each exposure was 2 h for 3 consecutive days.

Allergen sensitization. Guinea pigs were sensitized with ovalbumin (OVA, Sigma Aldrich). The animals received OVA during 14 days: the 1st day – 100 μg OVA dissolved in 1 ml saline (0.5 ml – subcutaneously in the neck and 0.5 ml i.p.), the 3rd day – OVA in the same dose i.p. only. The guinea pigs inhaled 0.1% OVA solution for 3 min on the 14th day.

11.2.3 Airway Smooth Muscle Reactivity

Airway smooth muscle reactivity was recorded in response to cumulative doses (10^{-8} – 10^{-3} mol/l) of histamine and acetylcholine (Sigma Aldrich, St. Louis, MA) after 1 h of tissue incubation *in vitro*. The animals were killed 24 h after last toluene or allergen exposure. The trachea and lungs were removed and thin organ strips were prepared and placed into a bath with Krebs-Henseleit solution containing 110.0 mmol/l NaCl, 4.8 mmol/l KCl, 2.35 mmol/l CaCl_2 , 1.20 mmol/l MgSO_4 , 1.20 mmol/l KH_2PO_4 , 25.0 mmol/l NaHCO_3 and 4 g glucose in glass-distilled water. The solution was continuously aerated with a mixture of 95% O_2 and 5% CO_2 at pH 7.5 ± 0.1 and temperature $36.0^\circ\text{C} \pm 0.5^\circ\text{C}$. The strip endings were connected to a force transducer and an amplifier (RES s.r.o., Martin, Slovakia). Changes in tension were recorded on PC with specific software (RES s.r.o., Martin, Slovakia). The tissue strips were exposed initially to the tension of 4 g (30 min – loading phase). Thereafter, the tension was reduced to the baseline of 2 g (30 min – adaptation phase). The Krebs-Henseleit solution was exchanged every

10 min. A cumulative concentration-response curve to 10^{-8} – 10^{-3} mol/l histamine or acetylcholine was determined for every strip.

All results were expressed as means $\pm$ SE. Statistical analysis was performed using one-way analysis of variance ANOVA. Comparisons of baseline values between groups were analyzed with a two-sided *t*-test. All statistical analyses were done with Microsoft Excel and Microcal Origin 7.0 (OriginLab, Data analysis and Graphing Software). Differences were considered statistically significant with $p < 0.05$.

11.3 Results

11.3.1 Pretreatment with L-Arginine in Toluene-Induced Airway Hyperreactivity

Short-term pretreatment with L-arginine caused significant changes in *in vitro* reactivity of trachea and lung tissues obtained from the animals exposed to toluene *in vivo*. There were increases in tracheal smooth muscle reactivity in response to the lowest concentrations of both mediators (10^{-8} – 10^{-7} mol/l of histamine and 10^{-8} mol/l of acetylcholine) in comparison with the control group. However, we further recorded a significant decrease in tracheal smooth muscle reactivity with gradually increasing concentrations of the mediators (10^{-4} – 10^{-3} mol/l of histamine (Fig. 11.1a) and 10^{-5} – 10^{-3} mol/l of acetylcholine) (Fig. 11.1b). In contrast, lung smooth muscles responded to histamine with an insignificant decrease in reactivity (Fig. 11.1c), but with a dedicated decrease in reactivity in response to acetylcholine at all concentrations used after short-term administration of L-arginine (Fig. 11.1d).

Long-term pretreatment with L-arginine resulted in insignificant decreases in both tracheal and lung smooth muscle reactivity (data not shown).

11.3.2 Pretreatment with L-Arginine in Allergen-Induced Airway Hyperreactivity

The effects of short-term pretreatment with L-arginine in the ovalbumin sensitized guinea pigs followed by the allergen exposure *in vivo* on the *in vitro* reactivity of tracheal smooth muscles to histamine and acetylcholine were in general similar to those observed for the toluene-exposed animals above outlined. The tracheal smooth muscles responded with increases in reactivity at the lowest concentrations of both mediators, followed by decreases at higher concentrations after short-term pretreatment with L-arginine (Fig. 11.2a, b).

With regard to the long-term pretreatment with L-arginine, the response of the tracheal smooth muscle, taken from the allergen-sensitized guinea pigs, to histamine was similar to that observed after the short-term pretreatment; increases in reactivity at low concentrations and decreases at higher concentrations of histamine (Fig. 11.2a). Concerning acetylcholine, the effects were more variable. Compared with control reactivity, there was first an increase at the lowest 10^{-8} mol/l acetylcholine concentration, but at higher acetylcholine concentrations no appreciable changes in the amplitude of muscle contraction were observed (Fig. 11.2b).

Lung smooth muscle, taken from the allergen-sensitized guinea pigs, responded with marked increases in reactivity due both to histamine (10^{-8} – 10^{-7} mol/l) (Fig. 11.2c) and acetylcholine (10^{-8} – 10^{-7} mol/l) (Fig. 11.2d) after both short- and long-term L-arginine pretreatment. The increases were however apparent only at low concentrations of the mediators and were stronger after the short-term pretreatment with L-arginine compared with the long-term pretreatment (Fig. 11.2c, d).

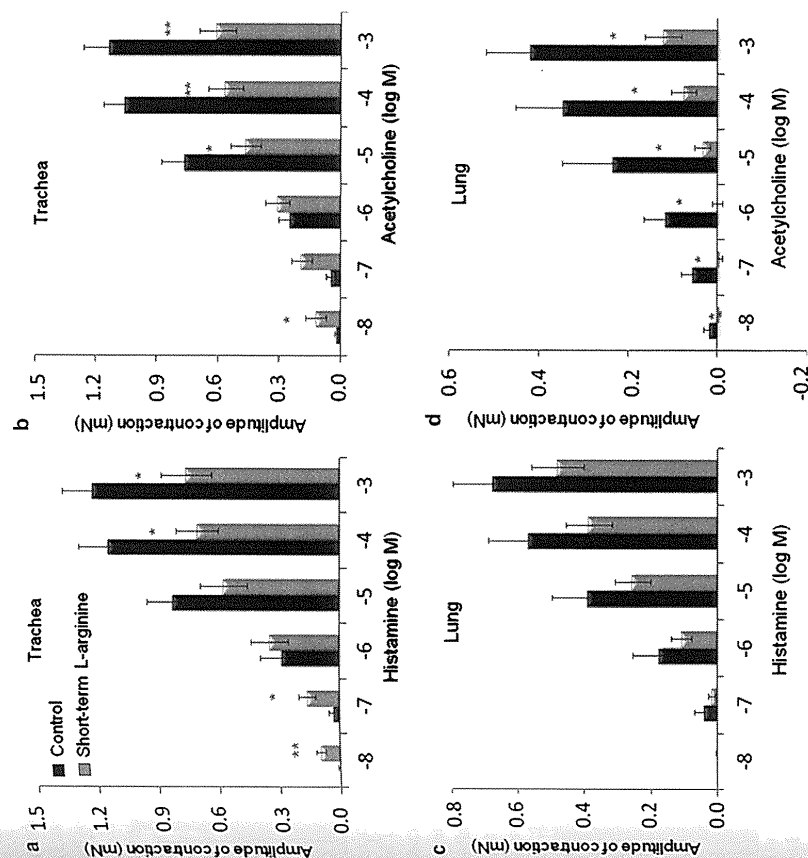


Fig. 11.1 Tracheal (Panels a–b) and lung (Panels c–d) tissues reactivity to histamine and acetylcholine *in vitro* after short-term pretreatment with L-arginine in guinea pigs exposed to toluene *in vivo*; * $p < 0.05$; ** $p < 0.01$

11.4 Discussion

We focused in our work on changes in airway hyperreactivity induced by toluene and ovalbumin after application of L-arginine, the main precursor for NO synthesis. Many experimental studies have demonstrated that a deficit rather than an increase of NO (mainly iNOS-derived) is a major cause of airway hyperreactivity after the late asthmatic response. NO deficiency caused by reduced availability of L-arginine in the airways may be a consequence of two different mechanisms. First, increased activity of arginase that competes with NOS for a common substrate, and second, increased release of eosinophil-derived substances (e.g., peroxidase, polycation proteins) that inhibit the transport of L-arginine to NO-producing cells (Maarsingh et al. 2009).

We found that short-term application of L-arginine caused substantial reductions in tracheal smooth muscle reactivity to both mediators and a reduction in lung tissue reactivity to acetylcholine in toluene-induced hyperreactivity. We can suppose that the ultimate effect of L-arginine depends

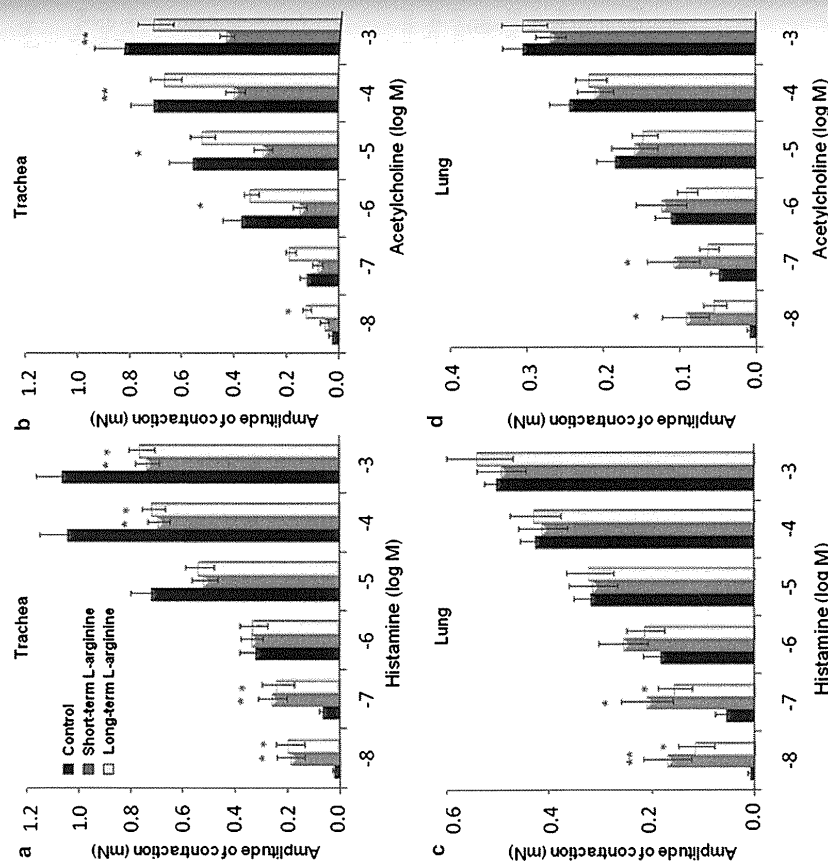


Fig. 11.2 Tracheal (Panels a-b) and lung (Panels c-d) tissue reactivity to histamine and acetylcholine *in vitro* after short- and long-term pretreatment with L-arginine in guinea pigs sensitized with ovalbumin and subsequently exposed to the allergen *in vivo*; * $p < 0.05$; ** $p < 0.01$

on the type of enzyme which is dominant in its synthesis and on the way of its administration. The question therefore arises which enzymes are important in the pathogenesis of airway hyperreactivity. Substitution of L-arginine and its utilization with constitutive NO synthase isoforms have predominantly beneficial effects. Increased of iNOS activity or activity of other enzymes (especially arginase) may induce the opposite situation. The literature shows an effort to clarify these hypotheses. Inhalation of L-arginine does not worsen symptoms or lung function in patients with mild asthma (Sapienza et al. 1998). Oral administration (Kharitonov et al. 1995) or inhalation of L-arginine in patients with primary ciliary dyskinesia improves cilia dysfunction and respiratory antibacterial protection (Loukides et al. 1998). L-arginine modifies the endothelial dysfunction in patients with COPD (Hutchinson et al. 2001). L-arginine pretreatment also improves airway hyperreactivity induced by viruses, which also correlates with increased NO in exhaled air (de Gouw et al. 1999). In reducing symptoms of asthma or COPD, L-arginine could also work, in addition to NO

modulation, by its antioxidant properties due to the presence of guanidine group in its molecule (Lass et al. 2002). It is possible that some of these mechanisms could be involved in the protective effects of L-arginine in toluene-induced hyperreactivity in the present study.

In the experiments concerning allergen-induced hyperreactivity of airway smooth muscles, short-term pretreatment with L-arginine decreased the tracheal reactivity to both histamine and acetylcholine, with the exception of the lowest histamine concentrations. In the case of lung tissue, short-term application of L-arginine caused an increase in reactivity in response to the lowest concentrations of both mediators.

In vivo studies in experimental models of guinea pigs with allergic asthma, iNOS-derived NO has a beneficial effect on allergen-induced airway hyperreactivity after the late asthmatic response, and a partial weakening of bronchial hyperreactivity in relation to its bronchodilator effect (Maarsingh et al. 2009). On the other side, disruption of the delicate balance between NO synthase and arginase may lead to reduced availability of L-arginine for constitutive isoforms of NOS and thus increased oxidative stress and airway hyperreactivity. Mahalirajan et al. (2009) observed the effect of high doses of L-arginine metabolizing enzymes and the subsequent biological response, such as cGMP production, lipid peroxidation, peroxynitrite formation, hyperreactivity and airway inflammation in ovalbumin-sensitized mice models of asthma. L-arginine significantly reduced AHR and airway inflammation. In addition, L-arginine increased NO levels in exhaled air and nitro-markers of oxidative stress, e.g., nitrotyrosine. This was associated with reduced activity and expression of arginase-1, increased expression of eNOS and reduction of iNOS in bronchial epithelium.

The reason why we did not observe a beneficial effect of long-term L-arginine pretreatment in the airway hyperreactivity conditions is not readily explicable, but that phenomenon has been observed by others as well. De Gouw et al. (1999) described that orally administered L-arginine had no effect on airway responsiveness to histamine in patients with asthma. Takano et al. (1998) found that oral administration of L-arginine increased airway inflammation. Therefore, we can assume that long-term application of L-arginine inhibit its short-term beneficial effects on airway hyperreactivity. An increase in the substrate for NO production probably inhibits eNOS, resulting in amplification of bronchoconstriction. L-arginine may increase NO production by iNOS or, conversely, may increase production of free radicals, which is associated with increased readiness of airway smooth muscle to bronchoconstriction. The differences in the action of L-arginine in different regions of the respiratory system may be caused by different localization of enzymes that utilize L-arginine and by diverse locations of antioxidant mechanisms in the upper and lower airways (Strapkova et al. 2008). The proximal airways also have a higher number of L-arginine utilizing NOS neurons than the distal parts. Therefore, NO works as a bronchodilator mainly in the proximal part of the airways (Prado et al. 2005). Another explanation is that the respiratory system responds to ovalbumin with adaptive responses in larger airways in which it regulates the concentration of L-arginine in the epithelial cell layer (Kenyon et al. 2008).

Some authors report a different respiratory response to histamine and acetylcholine, while reactivity to histamine is more pronounced (Matsumoto et al. 1997; Strapkova et al. 2008). This can be caused by differences in the proportion of neuronal reflex mechanisms. Histamine induces bronchoconstriction directly and indirectly. Direct contraction of airway smooth muscle is mediated through a receptor and indirect one is through neuronal reflexes and the excitation of cholinergic neural pathways. Acetylcholine is less effective for neural reflex mediated bronchoconstriction (Matsumoto et al. 1997). In our experiments, the response to both mediators did not differ significantly. The disagreement with other authors may be caused by using a different stimulus to induce airway hyperreactivity. In our case, the stimuli were an exogenous irritant and ovalbumin sensitization.

In summary, pretreatment with L-arginine resulted in a modification of the response of airway smooth muscles. The effects of supplementation depended on the airway hyperreactivity trigger, airway region and pretreatment duration. The results show a protective effect of short-term pretreatment with L-arginine mainly in irritant-induced experimental airway hyperreactivity. The study underscores the importance of L-arginine for the control of bronchomotoric tone.

Acknowledgments This work was supported by Grant MZ SR 2007/46-UK-11, VEGA and by the Center of Experimental and Clinical Respiratory.

Conflicts of interest: The authors declare no conflicts of interest in relation to this article.

References

- Boucher, J. L., Moali, C., & Tenu, J. P. (1999). Nitric oxide biosynthesis, nitric oxide synthase inhibitors and arginine competition for L-arginine utilization. *Cellular and Molecular Life Sciences*, 55, 1015–1028.
- Chandran, S., Sridhar, N., & Veeranjayulu, A. (1998). Nitric oxide: Concepts, current perspectives and future therapeutic implications. *Indian Journal of Pharmacology*, 30, 351–366.
- Cook, H. T., Jansen, A., Lewis, S., Largen, P., O'Donnell, M., Reaveley, D., & Cattell, V. (1994). Arginine metabolism in experimental glomerulonephritis: Interaction between nitric oxide synthase and arginase. *American Journal of Physiology. Renal Physiology*, 267, 646–653.
- de Boer, J., Meurs, H., Flendrig, L., Koopal, M., & Zaagsma, J. (2001). Role of nitric oxide and superoxide in allergen-induced airway hyperreactivity after the late asthmatic reaction in guinea-pigs. *British Journal of Pharmacology*, 133, 1235–1241.
- de Gouw, H. W. F. M., Verbruggen, M. B., Twiss, I. M., & Sterk, P. J. (1999). Effect of oral L-arginine on airway hyperresponsiveness to histamine in asthma. *Thorax*, 54, 1033–1035.
- Gobert, A. P., Daulouede, S., Lepoivre, M., Boucher, J. L., Boulet, B., Bugnet, A., Cesuglio, B., Veyret, B., & Vincendau, P. (2000). L-arginine availability modulates local nitric oxide production and parasite killing in experimental trypanosomiasis. *Infection and Immunity*, 68, 4653–4657.
- Griffith, O. W., & Stuehr, D. J. (1995). Nitric oxide synthases: Properties and catalytic mechanism. *Annual Review of Physiology*, 57, 707–736.
- Hutchinson, S. J., Sievers, R. E., Zhu, B. Q., Sun, Y. P., Stewart, D. J., Parnley, W. W., & Chatterjee, K. (2001). Secondhand tobacco smoke impairs rabbit pulmonary artery endothelium-dependent relaxation. *Chest*, 120, 2004–2012.
- Jenkinson, C. P., Grody, W. W., & Cederbaum, S. D. (1996). Comparative properties of arginases. *Comparative Biochemistry and Physiology. 114B*, 107–132.
- Kenyon, N. J., Bratt, J. M., Linderholm, A. L., Last, M. S., & Last, J. A. (2008). Arginase I and II in lungs of ovalbumin-sensitized mice exposed to ovalbumin: Sources and consequences. *Toxicology and Applied Pharmacology*, 230, 269–275.
- Kharitonov, S. A., Lubec, G., Lubec, B., Hjeltn, M., & Barnes, P. J. (1995). L-arginine increases exhaled nitric oxide in normal human subject. *Clinical Science*, 88, 135–139.
- Lass, A., Suessenbacher, A., Wolkart, G., Mayer, B., & Brunner, F. (2002). Functional and analytical evidence for scavenging of oxygen radicals by L-arginine. *Molecular Pharmacology*, 61, 1081–1088.
- Li, H., Meininger, C. J., Hawker, J. R., Jr., Haynes, T. E., Kepka-Lenhart, D., Mistry, S. K., Morris, S. M., Jr., & Wu, G. (2001). Regulatory role of arginase I and II in nitric oxide, polyamine and proline syntheses in endothelial cells. *American Journal of Physiology. Endocrinology and Metabolism*, 280, E75–E82.
- Loukides, S., Kharitonov, S. A., Wodenhouse, T., Cole, P. J., & Barnes, P. J. (1998). Effect of L-arginine on mucociliary function in primary ciliary dyskinesia. *Lancet*, 352, 371–372.
- Maarsingh, H., Bossenga, B. E., Bos, I. S. T., Volders, H. H., Zaagsma, J., & Meurs, H. (2009). L-Arginine deficiency causes airway hyperresponsiveness after the late asthmatic reaction. *European Respiratory Journal*, 34, 191–199.
- Maballirajan, U., Ahmad, T., Leishangtem, G., Joseph, D. A., Dinda, A. K., Agrawal, A., & Ghosh, B. (2009). Beneficial effects of high dose of L-arginine on airway hyperresponsiveness and airway inflammation in a murine model of asthma. *The Journal of Allergy and Clinical Immunology*, 125, 626–635.
- Matsumoto, K., Aizawa, H., Takata, S., Inoue, H., Takahashi, N., & Hara, N. (1997). Nitric oxide derived from sympathetic nerves regulates airway responsiveness to histamine in guinea pigs. *Journal of Applied Physiology*, 83, 1432–1437.
- Meurs, H., Maarsingh, H., & Zaagsma, J. (2003). Arginase and asthma: Novel insights into nitric oxide homeostasis and airway hyperresponsiveness. *Trends in Pharmacological Sciences*, 24, 450–455.
- North, M. L., Khanna, N., Marsden, P. A., Grasmann, H., & Scott, J. A. (2009). Functionally important role for arginase-I in the airways hyperresponsiveness of asthma. *American Journal of Physiology. Lung Cellular and Molecular Physiology*, 289, L677–L683.
- Prado, C. M., Leite-Maldonado, E. A., Kasahara, D. I., Capelozzi, V. L., Martins, M. A., & Tibério, I. F. (2005). Effects of acute and chronic nitric oxide inhibition in an experimental model of chronic pulmonary allergic inflammation in guinea pigs. *American Journal of Physiology. Lung Cellular and Molecular Physiology*, 289, L677–L683.

Reczkowski, R. S., & Ash, D. E. (1994). Rat liver arginase: Kinetic mechanism, alternate substrates, and inhibitors. *Archives of Biochemistry and Biophysics*, 312, 31–37.

Sapiezna, M. A., Kharitonov, S. A., Horvath, I., Chung, K. F., & Barnes, P. J. (1998). Effect of inhaled L-arginine on exhaled nitric oxide in normal and asthmatic subjects. *Thorax*, 53, 172–175.

Strapkova, A., Nosolova, G., & Hanacek, J. (1996). Effect of irritants on airways reactivity. *Central European Journal of Public Health*, 4, 54–56.

Strapkova, A., Antosova, M., & Nosolova, G. (2008). Relation of L-arginine to airway hyperreactivity. *General Physiology and Biophysics*, 27, 85–91.

Su, Y., Couch, M., & Block, E. R. (1997). Substrate inhibition of nitric oxide synthase in pulmonary artery endothelial cells in culture. *Nitric Oxide*, 1, 469–475.

Takano, H., Lim, H. B., Miyabara, Y., Ichinose, T., Yoshikawa, T., & Sagai, M. (1998). Oral administration of L-arginine potentiates allergen-induced airway inflammation and expression of interleukin-5 in mice. *Journal of Pharmacology and Experimental Therapeutics*, 286, 767–771.

Wu, G., & Morris, S. M. (1998). Arginine metabolism: Nitric oxide and beyond. *Biochemical Journal*, 336, 1–17.