Chapter 9

*In vivo* effects of arginase inhibition and of L-arginine on allergen-induced airway hyperresponsiveness in a guinea pig model of allergic asthma

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Summary
In a guinea pig model of allergic asthma, using perfused tracheal preparations ex vivo, we have recently demonstrated that l-arginine limitation due to increased arginase activity underlies a deficiency of bronchodilating nitric oxide (NO) and airway hyperresponsiveness (AHR) after the allergen-induced early (EAR) and late (LAR) asthmatic reaction. In the present study, using the same animal model, we investigated the effects of the specific arginase inhibitor 2(S)-amino-6-boronohexanoic acid (ABH) and of l-arginine on the AHR after the EAR and LAR in vivo. Inhaled ABH (15 min, 25 mM nebulizer concentration) acutely reversed the AHR (defined as PC100 ratio pre/post challenge) after the EAR from 4.77 ± 0.56-fold to 2.04 ± 0.34-fold (P<0.001), while a tendency to reversal of the AHR after the LAR (from 1.95 ± 0.56-fold to 1.56 ± 0.47-fold, P<0.10) was observed. Quantitatively similar results were obtained by inhalation of l-arginine (15 min, 1 mM nebulizer concentration): from 4.09 ± 0.45-fold to 2.13 ± 0.43-fold (P<0.001) after the EAR and from 1.77 ± 0.17-fold to 1.30 ± 0.12-fold (P<0.05) after the LAR. No effects were observed with the inactive enantiomer d-arginine. ABH inhalation at 0.5 h before allergen challenge and at 8 h after the challenge caused significant protection against the AHR after both the EAR (from 6.33 ± 1.30-fold to 3.05 ± 0.51-fold; P<0.05) and LAR (from 2.08 ± 0.31-fold to 1.41 ± 0.25-fold; P<0.005). Interestingly, a 32.8-fold (P<0.01) higher concentration of ovalbumin was needed to induce airways obstruction after pretreatment with ABH. In conclusion, these data indicate that inhalation of ABH or l-arginine acutely reverses the allergen-induced AHR after the EAR and LAR, presumably by attenuating arginase-induced substrate deficiency to NO synthase in the airways. Moreover, ABH treatment before allergen exposure considerably reduces the sensitivity of the airways to inhaled allergen and protects against the development of AHR after both the EAR and the LAR. This is the first in vivo study indicating that arginase inhibitors may have therapeutic potential in allergic asthma.

Introduction
l-Arginine is a versatile semi-essential amino acid that acts as a substrate to various enzymes, including arginase and nitric oxide synthase (NOS) [1-3]. Arginase, which hydrolyzes l-arginine to l-ornithine and urea, is a key enzyme of the urea cycle in the liver, but also occurs in a variety of extrahepatic cells and tissues that do not express a complete urea cycle, including the airways [1-4]. Arginase exists in two isoforms. Type I arginase is a cytosolic enzyme primarily expressed in liver, whereas type II arginase is a mitochondrial enzyme that predominantly occurs in non-hepatic tissues. Arginase I and arginase II are both constitutively expressed in the airways, particularly in the bronchial epithelium and in fibroblasts [1-4].

The biological function of arginase in extrahepatic tissue is not entirely clear, but it has been implicated in the regulation of NO synthesis by controlling the
bioavailability of L-arginine to NOS [1,4-6]. Using intact guinea pig tracheal preparations in vitro, we have recently demonstrated that arginase activity in the airways is importantly involved in the regulation of airway responsiveness by attenuating the production of bronchodilating NO from nonneural (presumably epithelial) cells and from inhibitory nonadrenergic noncholinergic (iNANC) nerves, due to competition with constitutive NO-synthase (cNOS) isoforms for their common substrate [7,8][Chapter 6]. Remarkably, ex vivo experiments using airway preparations from a guinea pig model of allergic asthma have indicated that allergen challenge causes a considerable increase in arginase activity in the airways, which contributes substantially to airway hyperresponsiveness (AHR) and reduced iNANC activity after the early asthmatic reaction (EAR) by inducing a deficiency of both neuronal and nonneuronal cNOS-derived NO [9,10][Chapters 2&7]. Moreover, it was demonstrated that increased arginase activity also contributes to the AHR after the late asthmatic reaction (LAR), by causing a reduced bioavailability of L-arginine to iNOS [Chapter 4]. Reduced substrate availability to iNOS promotes the production of both NO and superoxide anion (O2-) by this enzyme, leading to the rapid formation of the highly reactive nitrogen species ONOO-, which has procontractile, cytotoxic and pro-inflammatory actions [11-15]. Increased arginase activity has also been observed in different mouse models of allergic asthma [16,17]. Very strikingly, in some of these models, it has been demonstrated that genes related to L-arginine metabolism, including arginase I and II, are among the most prominently overexpressed genes upon allergen exposure [16]. Accordingly, arginase activity and expression of both arginase I and arginase II in the lung may be induced by Th2 cytokines [16,19].

Increased arginase activity in the airways may not only compromise NO homeostasis, causing AHR by reduced bronchodilation and increased inflammation, it also generates L-ornithine, the precursor of L-proline and polyamines, which could be involved in cell proliferation and collagen synthesis associated with airway remodeling in chronic asthma [1-3,20].

The potential significance of arginase for the pathophysiology of human asthma has recently been indicated. Thus, increased expression of arginase I has been observed in epithelial and inflammatory cells in the airways of asthmatic patients [16]. Moreover, an increase of serum arginase activity has been noted in patients with severe asthma [21].

Collectively, these findings open a new horizon for the therapeutic potential of drugs targeting the arginase pathway in acute and chronic asthma. Recently, a number of novel, specific arginase inhibitors has been developed, the design of which being based on the interaction of L-arginine analogues with the binuclear manganese cluster in the arginase isoenzymes [22-24]. Of these, the specific, but subtype-nonselective, arginase inhibitor 2(S)-amino-6-borohexanoic acid (ABH) is the most potent one [23,24]. Recent in vivo studies indicated the effectiveness of
ABH in the potentiation of male and female sexual arousal [24,25] and in inhibiting experimental autoimmune encephalomyelitis [26].

Using a guinea pig model of allergic asthma, we now report the first in vivo data demonstrating that inhalation of ABH acutely reverses allergen-induced AHR after the EAR and LAR, which can be mimicked by L-arginine. Moreover, it is demonstrated that pretreatment with ABH considerably reduces the sensitivity of the airways to inhaled allergen and protects against the development of allergen-induced AHR after both reactions.

**Methods**

*Animals and sensitization procedure*

Outbred male specified pathogen-free Dunkin Hartley guinea pigs (Harlan Heathfield, UK) were used in this study. All animals, weighing approximately 250 g, were actively IgE-sensitized to ovalbumin as described by Van Amsterdam *et al.* [27]. The animals were operated on 2 weeks after sensitization and used experimentally in weeks 4 and 5 after sensitization. The animals were housed in individual cages in climate-controlled animal quarters and given water and food *ad libitum*, while a 12-h on/12-h off light cycle was maintained. All protocols described in this study were approved by the University of Groningen Committee for Animal Experimentation.

*Measurement of airway function*

Airway function was assessed by on-line measurement of pleural pressure (P$_{pl}$) under unrestrained conditions as described previously [28,29]. In short, a small fluid-filled latex balloon catheter was surgically implanted inside the thoracic cavity. The free end of the catheter was driven subcutaneously to the neck of the animal, where it was exposed and attached permanently. Via an external fluid-filled cannula, the pleural balloon catheter was connected to a pressure transducer (TXX-R, Viggo-Spectramed, Bilthoven, Netherlands). P$_{pl}$ (in cm H$_2$O) was measured continuously using an on-line computer system. Using a combination of flow measurement with a pneumotachograph, implanted in the trachea, and pressure measurement with the pleural balloon catheter, it was shown that changes in P$_{pl}$ are linearly related to changes in airway resistance and hence can be used as a sensitive index for allergen- and histamine-induced bronchoconstriction [28]. In this way, airway function can be monitored repeatedly and continuously for prolonged periods of time, while the animals are unaware of the measurements being taken.

During the experimental protocol (1-4 weeks after surgery) baseline P$_{pl}$-measurements remained stable and no signs of inflammation were observed at the sites of surgery.
Provocation procedures
Allergen and histamine provocations were performed by inhalation of aerosolized solutions. These provocations were carried out in a specially designed perspex cage of 9 l, in which the guinea pigs could move freely as previously described [28,29]. A DeVilbiss nebulizer (type 646) driven by an airflow of 8 l/min provided the aerosol with an output of 0.33 ml/min. The animals were habituated to the experimental conditions and the provocations procedure at least one week after surgery, when preoperative weight was restored, as described previously [29]. On the experimental days following the habitation procedure, allergen and/or histamine provocations were performed as described below. All provocations were preceded by an adaptation period of at least 30 min, followed by two consecutive control provocations with saline, each provocation lasting 3 min and separated by 7 min intervals. A baseline $P_{pl}$-value was calculated by averaging the $P_{pl}$-values from the last 20 min of the adaptation period.

In order to assess the airway reactivity for histamine, provocations with increasing concentration steps (6.25, 12.5, 25, 50, 75, 100 and 125 µg/ml) in saline were performed. Histamine provocations lasted 3 min and were separated by 7 min intervals. Animals were challenged until $P_{pl}$ was increased by more than 100% above baseline for at least 3 consecutive minutes. The concentration of histamine causing a 100% increase of $P_{pl}$ ($PC_{100}$) was derived by linear interpolation of the concentration-$P_{pl}$ curve and was used as an index for airway reactivity towards histamine. $P_{pl}$ returned to baseline within 15 min after the last histamine provocation.

Allergen provocations were performed by inhalation of increasing concentrations of 0.5, 1.0 or 3.0 mg/ml ovalbumin in saline and were discontinued when the first signs of respiratory distress were observed and an increase in $P_{pl}$ of more than 100% was reached.

Experimental protocols
Reversal of allergen-induced AHR by ABH and L-arginine
On two different occasions, separated by one week interval, guinea pigs were treated either with vehicle (saline) or drug (ABH, L-arginine or D-arginine), to establish the acute effects of these drugs on basal airway responsiveness to histamine as well as on allergen-induced airway hyperresponsiveness after the EAR and the LAR (Figure 1).

On the first experimental day, saline- or drug-induced effects on basal histamine reactivity were established. Thirty minutes after the assessment of the basal histamine $PC_{100}$, an aerosol of saline (control) or 25 mM ABH, 1.0 M L-arginine or 1.0 M D-arginine (nebulizer concentrations) was inhaled for 15 min. Following these inhalations, a second histamine $PC_{100}$-measurement was performed starting 15 min later. On the second day allergen provocations were performed. At 5 h and 23 h after ovalbumin provocation, histamine $PC_{100}$ values were measured to determine the allergen-induced AHR after the EAR and the LAR, respectively. Saline, ABH and
L- or D-arginine inhalations were performed at 5.5 h and 23.5 h after ovalbumin provocation, and subsequent histamine PC\textsubscript{100}-values were reassessed at 6 h and 24 h after the allergen provocation. Saline- and drug inhalations were alternated with one week interval in a random cross-over design (Figure 1).

**Prevention of allergen-induced airway hyperresponsiveness by ABH**

On the first experimental day, the basal histamine PC\textsubscript{100} was assessed. The next day, saline was inhaled for 15 min, 0.5 h prior to and at 8 h after allergen inhalation, and histamine PC\textsubscript{100} measurements were performed 6 and 24 h post allergen challenge. One week later, the same protocol was repeated with inhalations of either 25 mM ABH (nebulizer concentration) or saline at t = -0.5 and 8 h. All animals were challenged with increasing doses of allergen (0.5, 1 and 3 mg/ml) until obstruction.

**Figure 1:** Schematic illustration of the protocols used in this study. OA: ovalbumin challenge; PC\textsubscript{100}: histamine PC\textsubscript{100} measurement.
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**Data analysis**
The sensitivity to inhaled allergen was expressed as the total amount (mg) of allergen nebulized to obtain airway obstruction, which is the factor of the nebulized time (s), the allergen dose in the nebulizer (mg/ml) and the aerosol output (ml/min).

All data are expressed as means ± SEM. Statistical significance of differences was evaluated using a paired two-tailed Student’s *t*-test, and significance was accepted when *P*<0.05.

**Chemicals**
Histamine dihydrochloride, ovalbumin (grade III), aluminium hydroxide, L-arginine hydrochloride and D-arginine hydrochloride were obtained from Sigma Chemical Co. Saline was purchased from Braun (The Netherlands). 2(S)-amino-6-boronohexanoic acid was provided by Organon (Oss, The Netherlands).

**Results**

*Reversion protocol*
Figure 2 shows that ovalbumin induces a significant AHR after both the EAR and LAR, as indicated by significantly decreased PC100 values for histamine after these reactions. Inhalation of saline did not affect basal reactivity to histamine nor allergen-induced AHR after the EAR and LAR. Inhalation of the arginase inhibitor ABH was without effect on basal airway responsiveness, but reversed the allergen-induced AHR after the EAR, as indicated by the significantly increased PC100 value compared to the control measurement after this reaction. In addition, a trend towards a reduction in the AHR after the LAR was observed, while no significant AHR was present anymore after the ABH inhalation when compared to basal responsiveness (Figure 2).

Figure 3 demonstrates that ABH reduces the allergen-induced AHR, expressed as PC100 ratio pre/post challenge, from 4.77 ± 0.56-fold to 2.04 ± 0.34-fold (*P*<0.001) after the EAR and from 1.95 ± 0.23-fold to 1.56 ± 0.47-fold (*P*<0.10) after the LAR.

As ABH, inhalation of L-arginine did not affect basal airway reactivity to histamine (Figure 4). Remarkably, the AHR after the EAR and after the LAR were reversed to a similar extent as with ABH (Figures 4 and 5).
**Figure 2:** Effects of inhalation of saline (left panel) or the arginase inhibitor ABH (25 mM nebulizer concentration; right panel) on basal airway responsiveness toward inhaled histamine and on histamine hyperresponsiveness after the EAR and LAR. Two subsequent PC100-measurements were performed 30 min before (open bars) and 30 min after (filled bars) inhalation of saline or ABH. Data represent means ± SEM of 3-5 animals. *P<0.05, **P<0.01 and ***P<0.0001; n.s. = nonsignificant.

**Figure 3:** Effects of inhalation of ABH (25 mM nebulizer concentration) on the AHR after the EAR (panel A) and the LAR (panel B). AHR is defined as the ratio of the histamine PC100 values obtained before (basal) and after allergen challenge (after EAR or LAR, respectively). Aria represent means ± SEM of 3-5 animals. ***P<0.001 and *P=0.10 versus pretreatment.
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Figure 4. Effects of inhalation of saline (left panel) or L-arginine (1 M nebulizer concentration; right panel) on basal airway responsiveness toward inhaled histamine and on histamine AHR after the EAR and the LAR. Two subsequent PC_{100}-measurements were performed 30 min before (open bars) and 30 min after (filled bars) inhalation of saline or L-arginine. Data represent means ± SEM of 9 animals. *P<0.05, **P<0.01 and ***P<0.0001.

Figure 5: Effects of inhalation of L-arginine (1M nebulizer concentration) on the AHR after the EAR (panel A) and the LAR (panel B). AHR is defined as the ratio of the histamine PC_{100} values obtained before (basal) and after allergen challenge (after EAR or LAR, respectively). A value of 1 represents normoresponsiveness. Data represent means ± SEM of 9 animals. *P<0.05 and ***P<0.001 versus pretreatment.
As with saline, inhalation of the biologically inactive D-enantiomer of arginine did not affect basal airway responsiveness and allergen-induced AHR at all (Figure 6).

![Figure 6](image)

**Figure 6.** Effects of inhalation of saline (left panel) or D-arginine (1 M nebulizer concentration; right panel) on basal airway responsiveness toward inhaled histamine and on histamine AHR after the EAR and the LAR. Two subsequent PC_{100}-measurements were performed 30 min before (open bars) and 30 min after (filled bars) inhalation of saline or D-arginine. Data represent means ± SEM of 3 animals. *P<0.05 and **P<0.01.

**Protection protocol**

Interestingly, pretreatment with 25 mM ABH 0.5 h before allergen-challenge caused significant protection against the AHR after the EAR as compared to saline control treatment (Figure 7). Moreover, an additional inhalation of 25 mM ABH at 8 h after allergen challenge almost completely prevented the occurrence of AHR after the LAR. Pretreatment with saline did not affect the AHR after the EAR or LAR (Figure 7).

Figure 8 shows that ABH significantly reduced the allergen-induced AHR after the EAR from 6.33 ± 1.30-fold (saline control, week 1) to 3.05 ± 0.51-fold (P<0.05) and from 2.08 ± 0.31-fold to 1.41 ± 0.25-fold (P<0.005) after the LAR.
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Figure 7: Effects of inhalation of saline (grey bars, left panel) or ABH (25 mM; nebulizer concentration; black bars, right panel) at 0.5 h before and 8 h after allergen inhalation on airway responsiveness to histamine after the EAR (6 h) and LAR (24 h) in comparison with saline effects obtained in the same animals one week earlier (open bars). Data represent means ± SEM of 5 animals. *P<0.05 and ***P<0.001, n.s. = not significant.

Figure 8: Effects of inhalation of saline or ABH (25 mM nebulizer concentration) at 0.5 h before and 8 h after allergen inhalation on AHR to histamine after the EAR (black bars, panel A) and the LAR (black bars, panel B) in comparison with saline controls (open bars) obtained in the same animals one week earlier. AHR is defined as the ratio of the histamine PC100 values obtained before (basal) and after allergen challenge (after EAR and LAR, respectively). A value of 1 represents normoresponsiveness. Data represent means ± SEM of 5 animals. *P<0.05 and ***P<0.001 versus control.
Remarkably, after pretreatment with ABH in week 2 a 32.8-fold higher concentration of ovalbumin (1.31 ± 0.69 mg) was needed to induce airways obstruction compared to saline treatment of the same animals in week 1 (0.04 ± 0.01 mg; \(P<0.01\)), indicating that ABH considerably diminishes the sensitivity to the allergen. No significant increase in ovalbumin dose was observed for saline-treated animals (Figure 9).

**Figure 9:** Effects of pretreatment with saline or ABH (25 mM nebulizer concentration) on the ovalbumin dose required to induce airways obstruction (filled bars) compared to saline controls (open bars) obtained in the same animals one week earlier. Please note that ovalbumin dose is plotted logarithmically. Data represent means ± SEM of 5 animals. *\(P<0.05\) and **\(P<0.01\); n.s. = not significant.

**Discussion**

The present study for the first time demonstrates the importance of arginase in the pathophysiology of asthma *in vivo*. Using a guinea pig model of allergic asthma, we demonstrated that inhalation of specific, isoenzyme-nonselective arginase inhibitor ABH acutely reversed the allergen-induced AHR after the EAR and LAR, while pretreatment with the arginase inhibitor considerably reduced the sensitivity of the airways to inhaled allergen and protected against the development of allergen-induced AHR after both reactions. The allergen-induced AHR after the EAR and LAR was similarly reversed by L-arginine, indicating that arginase-induced deficiency of substrate to NOS isoenzymes in the airways may be involved.

Both *in vivo* and *ex vivo*, several studies in animals models [10,30-34][Chapter 7] and in asthmatic patients [35-37] have indicated that a deficiency of bronchodilating cNOS-derived NO is involved in the development of allergen-induced AHR. Recent studies have demonstrated that alterations in L-arginine
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homeostasis play a major role in allergen-induced NO deficiency and AHR. Thus, using perfused tracheal preparations from allergen-challenged guinea pigs, we have demonstrated that limitation of L-arginine availability to cNOS underlies the deficiency of contractile agonist-induced as well as iNANC nerve-derived NO after the allergen-induced EAR [10,38][Chapter 7]. A major mechanism causing attenuated L-arginine availability to cNOS is increased utilization of the substrate by arginase [9,10][Chapter 7]. Indeed, arginase activity is considerably increased in tracheal homogenates after the EAR [9][Chapter 2], while, just as exogenous L-arginine administration [10,38][Chapter 7], inhibition of arginase by the specific arginase inhibitor Nω-hydroxy-nor-L-arginine (nor-NOHA) normalized the AHR and the iNANC nerve-mediated airway smooth muscle relaxation, by restoring the production of cNOS-derived NO [9,10][Chapters 2&7].

Reduced L-arginine availability due to increased arginase activity also underlies the AHR after the LAR [Chapter 4]. Previously, we have shown that increased formation of the highly reactive procontractile and proinflammatory oxidant peroxynitrite is importantly involved in the AHR after the LAR [13]. This may be explained by observations that under conditions of low L-arginine concentration, iNOS, which is induced during the LAR [31], produces both NO and superoxide anion, leading to effective formation of peroxynitrite [11,39]. Increasing the concentration of L-arginine promotes NO production, while the generation of superoxide anion, and hence peroxynitrite, is reduced [11,39]. Fully in line with these observations, we recently demonstrated that administration of exogenous L-arginine or nor-NOHA to the hyperreactive airways, obtained after the LAR, reduced the AHR by increased production of bronchodilating NO [Chapter 4].

Taken together, the present in vivo data, demonstrating that inhalation of ABH as well as of L-arginine acutely reverses the allergen-induced AHR after the EAR and LAR, correspond closely to the ex vivo data described above, confirming the importance of arginase in the development of hyperreactive airways disease in the intact organism. We did not find an effect of the arginase inhibitor or L-arginine on basal airway responsiveness in vivo, which seems to be at variance with the previous ex vivo data [9,10,38][Chapters 2&7]. However, it is important to note that in the ex vivo studies tracheal preparations were used, which may not fully reflect the effects of constitutive arginase activity and endogenous L-arginine availability on NO metabolism and airway responsiveness of the entire respiratory tract.

As expected, inhalation of the biologically inactive enantiomer d-arginine did not affect the AHR after the EAR and LAR, supporting the hypothesis that L-arginine - and presumably the arginase inhibitor - reverses the AHR by stimulating NOS activity. Similar results were found in previous studies using perfused tracheal preparations [38].

Remarkably, inhalation of ABH 0.5 h prior to allergen provocation strongly reduced the sensitivity to the allergen as indicated by the higher dose of allergen required to obtain airway obstruction. Since ABH had no effect on basal lung
function and airway responsiveness to histamine, this may suggest that the arginase inhibitor is effective in inhibiting allergen-induced mediator release, which evokes the EAR. Although the role of arginase in allergic mediator release is presently unknown, it has been established that nitric oxide inhibits mast cell activation as well as a number of mast cell-mediated inflammatory processes [40], which could be compromised by endogenous arginase activity in the sensitized animals. It is important to note that challenge of the animals until airway obstruction in the presence of the arginase inhibitor still reduced the AHR to histamine after the EAR and the LAR, indicating that ABH does also protect against the development of AHR, irrespective of its acute anti-allergic effect. Obviously, this is also illustrated by the reversal of allergen-induced AHR when the arginase inhibitor is inhaled after the EAR or LAR.

In addition to attenuating NO synthesis, leading to allergen-induced AHR and enhanced inflammation in acute asthma, arginase may also be involved in airway remodeling in chronic asthma via the production of L-ornithine [1-3]. L-Ornithine is a precursor for the arginase downstream products L-proline and polyamines (putrescine, spermidine and spermine), which could promote collagen production and mesenchymal cell growth in the airway wall [1,20]. Interestingly, the Th2 cytokines IL-4 and IL-13 caused increased arginase activity and increased mRNA expression of arginase I and II in cultured rat fibroblasts, supporting a role for arginase in airway remodeling [19]. Moreover, elevated levels of putrescine were found in lung tissue of allergen-challenged mice [16], and increased levels of polyamines have been detected in serum of asthmatic subjects [41].

The significance of arginase in the pathophysiology of human asthma is just starting to emerge. It has recently been reported that the protein expression of arginase I is increased in bronchial lavage cells from asthmatic patients [42]. Moreover, enhanced mRNA expression of arginase I has been observed in bronchial biopsies of asthmatics, particularly in inflammatory cells and in the airway epithelium [42]. Remarkably, a striking reduction in plasma L-arginine levels was measured in patients with severe asthma experiencing an exacerbation, which was associated with a 3-fold increase of serum arginase activity [21]. Moreover, in some of these patients, arginase activity declined and L-arginine concentrations increased after improvement of symptoms [21]. Interestingly, single nucleotide polymorphisms (SNPs) in arginase I and arginase II have recently been found associated with atopy and risk of childhood asthma [43].

If the present study can be translated to human disease, arginase inhibitors may prove to be of great benefit in the treatment of inflammation-induced AHR, the most important hallmark of asthma determining disease severity. To prevent possible systemic adverse effects, development of these drugs should focus on the inhaled route. A role for L-arginine deficiency in AHR would predict a beneficial effect of L-arginine administration to patients with asthma, based on increased synthesis of
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bronchodilating NO and reduced production of peroxynitrite. Few studies have investigated the effects L-arginine administration to asthmatic patients thus far. Compared with a non-asthmatic control group, a pronounced dose-dependent effect of inhaled L-arginine on exhaled NO was observed in patients with mild asthma [44]. Although the effect of L-arginine on airway responsiveness was not measured, this would indicate that substrate limitation to NOS isozymes is present in these patients indeed. In another study, in asthmatic patients no effect of oral L-arginine was found on AHR to inhaled histamine as reflected by PC_{20}-values, although the dose-response slope was slightly reduced [45]. However, since there was neither an effect on exhaled NO [45], the administered dose could have been too low. Since orally administered L-arginine is effectively withdrawn from the portal blood by the liver and metabolized to urea [46], inhalation rather than oral administration seems to be the preferred route for L-arginine administration. It is important to note that chronic administration of L-arginine could also enhance synthesis of L-proline and polyamines, feeding airway remodeling. In this case, arginine supplementation could even be contraindicated.

Arginase has recently also been implicated in other respiratory diseases, such as cystic fibrosis. Thus, arginase activity is increased in sputum obtained from patients with cystic fibrosis as compared to healthy controls and a negative correlation between sputum arginase activity and levels of exhaled NO as well as FEV₁ was observed in these patients [47]. Interestingly, inhalation of L-arginine increased the levels of exhaled NO and improved pulmonary function in subjects with cystic fibrosis [48].

In conclusion, our data indicate that inhalation of ABH or L-arginine acutely reverses allergen-induced AHR after the EAR and LAR, presumably by attenuating arginase-induced substrate deficiency to NO synthase isozymes in the airways. Moreover, ABH treatment prior to allergen exposure considerably reduces the sensitivity of the airways to inhaled allergen and protects against the development of AHR after both the EAR and the LAR. Therefore, arginase inhibitors may have therapeutic potential in allergic asthma.

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References
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