Arginase
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Chapter 5

L-Ornithine causes NO deficiency and airway hyperresponsiveness

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Summary
Using a guinea pig model of allergic asthma, we have recently demonstrated that arginase activity in the airways is increased after the allergen-induced early asthmatic reaction; this leads to airway hyperresponsiveness (AHR) by limiting the availability of L-arginine for constitutive nitric oxide synthase (cNOS) to produce bronchodilating nitric oxide (NO). Since arginase converts L-arginine into L-ornithine and urea, and L-ornithine may inhibit cellular uptake of L-arginine through γ+ carriers, we examined the effect of L-ornithine on the responsiveness to methacholine of intact perfused guinea pig airway preparations. Intraluminal (IL) incubation with 1.0 mM L-ornithine caused a 1.6-fold increase in airway responsiveness to IL methacholine compared to control (p<0.001). Incubation with a higher L-ornithine dose (5.0 mM) caused a similar (1.5-fold) increase in airway responsiveness (p<0.001). The NOS inhibitor L-NAME (0.1 mM; IL) also increased responsiveness to methacholine (1.8-fold; p<0.001). Coincubation with L-ornithine (1.0 mM) did not further enhance this increased airway responsiveness caused by L-NAME, indicating that L-ornithine induces AHR by decreasing NO production. Coincubation of 1.0 mM L-ornithine with the arginase inhibitor nor-NOHA (5.0 µM) strongly decreased airway responsiveness compared to L-ornithine alone, which could be prevented by coincubation with L-NAME. In addition, L-ornithine competitively inhibited arginase activity in lung, liver and kidney homogenates in a dose-dependent fashion. In conclusion, in addition to its direct competition with cNOS for the common substrate L-arginine, arginase may promote NO deficiency and AHR via the production of L-ornithine, presumably by inhibition of L-arginine uptake by γ+ carriers. Though L-ornithine is able to inhibit arginase activity as a feedback mechanism, inhibition of L-arginine uptake seems the dominant process regulating airway responsiveness.

Introduction
Arginase is an enzyme of the urea cycle, which metabolizes L-arginine to L-ornithine and urea [1]. Although abundantly expressed in the liver, arginase is also expressed in tissues that lack a complete urea cycle, including the airways [2-5]. Two distinct isoforms of arginase have been identified, which differ in cellular location and are encoded by different genes: the cytosolic isoform arginase I and the mitochondrial isoform arginase II. Arginase I is the predominant isoform and is mainly expressed in the liver, while arginase II is mainly expressed in extrahepatic tissues [1-3].

Extrahepatic arginases have been implicated in the regulation of nitric oxide (NO) production [6,7]. Since NO synthase (NOS) utilizes L-arginine to produce NO and L-citrulline, arginase may regulate NO synthesis via substrate competition with NOS. Three NOS isoforms have been identified: the constitutive NOS (cNOS) isoforms neuronal (nNOS or NOS I) and endothelial NOS (eNOS or NOS III), and inducible NOS (iNOS or NOS II) [8]. While nNOS and eNOS are constitutively expressed in the airways, mainly in inhibitory nonadrenergic noncholinergic (iNANC)
neurons (nNOS), endothelium (eNOS) and epithelium (nNOS and eNOS), iNOS is induced by proinflammatory cytokines during airway inflammation and is mainly expressed in macrophages and epithelial cells [8]. The relatively low concentrations of cNOS-derived NO are importantly involved in airway smooth muscle relaxation, while much higher concentrations of iNOS-derived NO also contribute to airway inflammation, by formation of significant amounts of the highly reactive nitrogen species peroxynitrite, the reaction product of NO and superoxide [2,8].

Both arginase I and II are constitutively expressed in the airways, particularly in the epithelium and in fibroblasts [4]. Recently, using guinea pig tracheal preparations we have demonstrated that arginase regulates airway smooth muscle tone by attenuation of the production of contractile agonist-induced non-neuronal [9] and iNANC nerve-derived neuronal [10][Chapter 6] NO, presumably by limiting the L-arginine availability to cNOS. Importantly, in a guinea pig model of allergic asthma we have demonstrated that a deficiency of both neuronal and nonneuronal cNOS-derived NO contributes to airway hyperresponsiveness (AHR) after the allergen-induced early asthmatic reaction (EAR) [11-14][Chapter 7]. A major mechanism underlying the allergen-induced NO-deficiency and airway hyperresponsiveness is reduced L-arginine availability to cNOS, caused by increased arginase activity after the challenge [13,15][Chapters 2&7]. A second mechanism contributing to the L-arginine limitation after the EAR is impaired cellular uptake of the amino acid via specific cationic amino acid transporters (CAT) of the y⁺ system, caused by increased secretion of polycations, presumably derived from activated eosinophils in the airways [16-18][Chapter 3]. L-Arginine limitation to iNOS due to increased arginase activity and polycation-induced inhibition of cellular uptake is also importantly involved in the AHR after the late asthmatic reaction, presumably by the concomitant synthesis of NO and superoxide by iNOS at low L-arginine concentrations, which results in effective formation of the proinflammatory and procontractile reaction product peroxynitrite [Chapter 4].

Although studies on the role of arginase in airway responsiveness and asthma have thus far mainly been focussed on substrate competition with NOS, a role for the arginase product L-ornithine and further downstream products like polyamines and L-proline can be envisaged. Polyamines and proline are known to be involved in cell proliferation and collagen production, respectively, which could play a role in airway remodelling in chronic asthma [1-3]. L-Ornithine by itself could have a more direct effect on airway responsiveness, as this cationic amino acid, being transported by the y⁺ system as well, competes with L-arginine for cellular uptake [19-21]. Another role for L-ornithine in L-arginine homeostasis and airway responsiveness could be feedback inhibition of arginase activity, since L-ornithine has shown to be a competitive inhibitor of arginase [22,23].

The role of L-ornithine in the regulation of airway responsiveness is presently unknown. Using intact perfused guinea pig tracheal preparations, we investigated the effect of L-ornithine on airway responsiveness to methacholine, specifically at the
level of NO homeostasis. Moreover, we studied the effects of L-ornithine on arginase activity in lung homogenates, as well as in liver (mainly arginase I) and kidney (mainly arginase II) homogenates to study arginase isoform-specific inhibitory activities of L-ornithine.

**Methods**

*Animals*

Outbred male specified pathogen-free Dunkin Hartley guinea pigs (Harlan Heathfield, UK), weighing 700 – 900 g, were used in this study. The animals were group-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.

*Tracheal perfusion*

The guinea pigs were sacrificed by a sharp blow on the head. After exsanguination, the trachea was rapidly removed and placed in a Krebs-Henseleit (KH) solution of 37°C (composition in mM: NaCl 117.50, KCl 5.60, MgSO4 1.18, CaCl2 2.50, NaH2PO4 1.28, NaHCO3 25.00, D-glucose 5.50), buffered at pH 7.4 and gassed with 95% O2 and 5% CO2. The trachea was prepared free of serosal connective tissue and fat and cut into two halves of approximately 16 mm each before mounting in a perfusion setup, as described by De Boer et al. [12]. In short, both sides of the tracheal preparation were fixed to stainless steel perfusion tubes mounted in a perfusion holder. The holder with the trachea was placed in a water-jacketed (37°C) organ bath containing 20 ml of gassed KH-solution (serosal or extraluminal (EL) compartment). The tracheal lumen was perfused with recirculating KH-solution from a separate 20 ml bath (intraluminal (IL) compartment) at constant flow rate of 12 ml min\(^{-1}\). Two axially centred side-hole catheters connected with pressure transducers (TXX-R, Viggo-Spectramed, Bilthoven, Netherlands) were situated at the proximal and the distal ends of the trachea to measure hydrostatic pressures (P\(_{\text{outlet}}\) and P\(_{\text{inlet}}\), respectively). The signals from the pressure transducers were fed into a differential amplifier to obtain the differential pressure (\(\Delta P = P_{\text{inlet}} - P_{\text{outlet}}\)). \(\Delta P\) was plotted on a flatbed chart recorder and reflects the resistance of the tracheal segment to perfusion, which is a function of the mean diameter of the trachea between the pressure taps [24].

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 40 mM KCl in KH (EL) to obtain a receptor-independent reference response. Subsequently, the preparation was washed four times with KH during 45 min until basal tone was reached again and a cumulative concentration
response curve was made with IL methacholine. When used, L-ornithine (1.0 or 5.0 mM), Nω-nitro-L-arginine methyl ester (L-NAME; 0.1 mM) and nor-NOHA (5.0 µM) were applied to the IL reservoir 40 or 45 (nor-NOHA) min prior to agonist-addition.

Arginase activity assay
Guinea pig lung, liver and kidney homogenates were prepared as previously described for tracheal preparations [15][Chapter 2]. Tissue was snap-frozen in liquid nitrogen and ground using a pestle and mortar in the presence of liquid nitrogen prior to homogenization in approximately 6-vol ice-cold homogenization buffer (20 mM Tris HCl; 2 µM phenylmethylsulphonyl fluoride, pH 7.4) using a Polytron homogenizer (Kinematica GmbH, Luzern, Switzerland). The homogenate was centrifuged at 20,000 g for 30 min at 4ºC and the supernatant was used for arginase assay. Arginase activity was determined by measuring the conversion of L-[guanidino-14C]arginine to [14C]urea, using a modified protocol as described by Custot et al. [25] In short, homogenate aliquots (50 µl) were incubated in a final volume of 150 µl, containing 25 mM Tris-HCl, 0.67 mM MnCl2, 1.66 mM L-arginine and 1 µl of L-[guanido-14C]arginine (51.5 mCi/mmol), pH 7.4 for 20 minutes at 37°C. Reactions were terminated by adding 450 µl of ice-cold stop buffer, containing 7 M urea, 0.25 M acetic acid, 10 mM L-arginine, pH 3.6. Samples were applied to vials containing 400 µl Dowex AG 50W-X8 (H+ form; 1 g/ml) and rotated for 2 minutes. Vials were centrifuged at 750 g for 1 minute and the resulting supernatants were centrifuged again at 750 g for 1 minute. Final supernatants (150 µl) were counted in triplicate in 4 ml Ultima Gold scintillation fluid using a Beckman LS 1701 liquid scintillation counter.

For Lineweaver-Burk analysis arginase activity was measured using various concentrations (1, 1.66, 4 and 10 mM) of L-arginine in the absence and presence of different concentrations of L-ornithine (1 and 10 mM). The specificity of the assay in measuring arginase activity was confirmed by the inhibitory effect of nor-NOHA [15][Chapter 2]. Protein concentrations were determined by the Bradford Coomassie brilliant blue method [26] using bovine serum albumin as a standard.

Data analysis
To correct for differences in baseline ∆P and in ∆P changes in response to contractile stimuli due to variation in 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 Emax [11,12]. Using this Emax, the sensitivity to methacholine was evaluated as pEC50.

Arginase activity was expressed as pmol urea produced per mg protein per minute. Kinetic parameters of arginase activity and its inhibition by L-ornithine were determined by Lineweaver-Burk analysis, by plotting 1/(arginase activity) versus
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1/(L-arginine concentration) for different L-arginine and L-ornithine concentrations. The results are expressed as means ± SEM. Statistical analysis was performed using the Student’s t-test for paired or unpaired observations as appropriate. Differences were considered statistically significant at \( P<0.05 \).

Chemicals

(-)-Isoprenaline hydrochloride, L-ornithine hydrochloride, L-arginine hydrochloride and \( N^\omega \)-nitro-L-arginine methyl ester were obtained from Sigma Chemical Co (St. Louis, MO, USA). and methacholine chloride from Aldrich (Milwaukee, WI, USA). \( L-[\text{guanido-}^{14}\text{C}] \)arginine (specific activity 51.5 mCi/mmol) was obtained from New England Nuclear Life Science Products, Inc (Boston, MA, U.S.A.). \( N^\omega \)-hydroxy-nor-L-arginine was kindly provided by Dr J.-L. Boucher (Université Paris V, Paris, France).

Results

In perfused guinea pig tracheal preparations, IL perfusion with 1.0 mM L-ornithine caused a 1.6-fold \(( P<0.001 \) ) increase in the Emax of methacholine, without an effect on the sensitivity (pEC\textsubscript{50}) to the agonist (Figure 1, Table 1). The airway responsiveness was not further increased after incubation with 5.0 mM L-ornithine (1.5-fold increase; \( P<0.001 \)) (Figure 1, Table 1).

In line with previous studies [15,17,27][Chapter 2], inhibition of cNOS with L-NAME (0.1 mM) increased the maximal airway response by 1.9-fold \(( P<0.001 \); Figure 2, Table 1), In the combined presence of L-NAME and L-ornithine the responsiveness to methacholine was not significantly different from that in the presence of L-NAME or L-ornithine alone (Figure 2, Table 1).

Figure 1: Methacholine-induced constriction of perfused guinea pig tracheal preparations in the absence and presence of 1.0 or 5.0 mM L-ornithine (IL). Results are means ± SEM of 5-14 experiments.
L-Ornithine and airway responsiveness

As previously demonstrated [9], inhibition of arginase with nor-NOHA (5.0 µM) resulted in a significant decrease in airway responsiveness (Figure 3, Table 1). Interestingly, nor-NOHA also reduced the increased airway responsiveness in the presence of 1.0 mM L-ornithine ($P<0.05$), which was completely prevented in the additional presence of L-NAME (Figure 3, Table 1). With all treatments, no effect on the sensitivity to methacholine was observed.

Figure 2: Methacholine-induced constriction of perfused guinea pig tracheal preparations in the absence and presence of 1.0 mM L-ornithine and/or 0.1 mM L-NAME. Results are means ± SEM of 4-14 experiments.

Figure 3: Methacholine-induced constriction of perfused guinea pig tracheal preparations in the absence and presence of 1.0 mM L-ornithine and/or 5.0 µM nor-NOHA with and without 0.1 mM L-NAME. Results are means ± SEM of 3-14 experiments.
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We also determined the effect of L-ornithine on arginase activity in guinea pig lung homogenates. Thus, at 1.66 mM L-arginine 1.0 mM and 10 mM L-ornithine significantly reduced lung arginase activity by 30.2% and 76.7%, respectively (Figure 4A). In comparison, incubation with 5.0 µM nor-NOHA inhibited lung arginase activity almost completely (94.7%; \( P < 0.005 \); Figure 4B).

### Table 1: Effects of IL L-ornithine, L-NAME and/or nor-NOHA on the responsiveness of perfused guinea pig tracheal preparations to IL methacholine.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>( E_{\text{max}} ) (% KCl) ± SEM</th>
<th>( pEC_{50} ) (-log M) ± SEM</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>51.4±1.6 ± 1.6</td>
<td>2.94±0.08 ± 0.08</td>
<td>14</td>
</tr>
<tr>
<td>1.0 mM L-ornithine</td>
<td>82.1±7.3 ± 3.02</td>
<td>3.02±0.12 ± 0.12</td>
<td>8</td>
</tr>
<tr>
<td>5.0 mM L-ornithine</td>
<td>75.4±7.9 ± 3.07</td>
<td>3.07±0.14 ± 0.14</td>
<td>5</td>
</tr>
<tr>
<td>0.1 mM L-NAME</td>
<td>95.6±7.3 ± 3.22</td>
<td>3.22±0.16 ± 0.16</td>
<td>5</td>
</tr>
<tr>
<td>(+ 1.0 mM L-ornithine)</td>
<td>103.9±5.4 ± 3.15</td>
<td>3.15±0.27 ± 0.14</td>
<td>5</td>
</tr>
<tr>
<td>5.0 µM nor-NOHA</td>
<td>32.0±1.4 ± 2.67</td>
<td>2.67±0.19 ± 0.19</td>
<td>5</td>
</tr>
<tr>
<td>(+ 1.0 mM L-ornithine)</td>
<td>58.0±3.3 ± 3.02</td>
<td>3.02±0.08 ± 0.14</td>
<td>4</td>
</tr>
<tr>
<td>(+ 0.1 mM L-NAME)</td>
<td>77.9±6.2 ± 2.87</td>
<td>2.87±0.13 ± 0.13</td>
<td>3</td>
</tr>
</tbody>
</table>

Results are means ± SEM of n experiments. ***P<0.001 compared with control; †P<0.05 compared with 1.0 mM L-ornithine; ‡P<0.001 compared with 5.0 µM nor-NOHA; #P<0.05 compared with nor-NOHA plus L-ornithine.

Figure 4: Inhibition of arginase activity in guinea pig lung homogenates by 1.0 or 10 mM L-ornithine (A) or 5.0 µM nor-NOHA (B). Arginase activity was measured in the presence of 1.66 mM L-arginine. Results are means ± SEM of 7 (A) and 5 (B) experiments. ***P<0.01 and ***P<0.005 as compared to control.
L-Ornithine and airway responsiveness

Lineweaver-Burk analysis of arginase activity in lung, liver and kidney homogenates revealed Michaelis-Menten kinetics (Figure 5). $V_{\text{max}}$ and $K_m$ values in guinea pig lung were determined to be $3.62 \pm 0.95 \text{ nmol/mg protein/min}$ and $2.35 \pm 0.46 \text{ mM}$, respectively (Table 2). $V_{\text{max}}$ and $K_m$ values for arginase in guinea pig liver were $654 \pm 150 \text{ nmol/mg protein/min}$ and $1.90 \pm 0.40 \text{ mM}$, respectively, and $19.5 \pm 4.50 \text{ nmol/mg protein/min}$ and $2.62 \pm 0.29 \text{ mM}$, respectively, for kidney (Table 2). Lineweaver-Burk plots of arginase inhibition experiments demonstrated that L-ornithine acts as a competitive inhibitor (Figure 5), with $K_i$-values for lung, liver and kidney of $1.38 \pm 0.30$, $1.37 \pm 0.35$ and $1.29 \pm 0.28 \text{ mM}$, respectively (Table 2).

Figure 5: Lineweaver-Burk plots of arginase activity in guinea pig lung (A), liver (B) and kidney (C) homogenates in the absence and presence of 1 or 10 mM L-ornithine. Results are expressed as means ± SEM of 5-7 experiments.
Table 2: Biochemical characteristics of arginase in guinea pig lung, liver and kidney.

<table>
<thead>
<tr>
<th>Tissue</th>
<th>( V_{\text{max}} ) (nmol/mg/min)</th>
<th>( K_m ) (mm)</th>
<th>( K_i ) (mm)</th>
<th>( n )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lung (arginase I &amp; II)</td>
<td>3.62 ± 0.95</td>
<td>2.35 ± 0.46</td>
<td>1.38 ± 0.30</td>
<td>6</td>
</tr>
<tr>
<td>Liver (arginase I)</td>
<td>654 ± 150</td>
<td>1.90 ± 0.40</td>
<td>1.37 ± 0.35</td>
<td>7</td>
</tr>
<tr>
<td>Kidney (arginase II)</td>
<td>19.5 ± 4.5</td>
<td>2.62 ± 0.29</td>
<td>1.29 ± 0.28</td>
<td>5</td>
</tr>
</tbody>
</table>

Discussion

Key observation of the present study is that the arginase product L-ornithine may cause a considerable increase in airway responsiveness to methacholine. As demonstrated in our previous studies [15,17,27][Chapter 2], NOS inhibition with L-NAME (0.1 mM) also increased airway responsiveness, to approximately the same degree. The observation that ornithine did not enhance airway responsiveness in the presence of L-NAME strongly suggests that a deficiency of cNOS-derived NO underlies the effect of L-ornithine.

The mechanism of L-ornithine-induced NO deficiency causing the increased agonist-induced airway responsiveness could well be related to inhibition of L-arginine transport into airway epithelial cells, as observed previously for (alveolar) macrophages [19,21]. Thus, L-ornithine as well as other cationic amino acids such as L-lysine compete with L-arginine for transport through the cationic amino acid transporter system \( y^+ \) [19,20,21,28]. A second mechanism by which cationic amino acids can decrease intracellular L-arginine availability to NOS is trans-stimulation of CAT transporters of the \( y^+ \) system by which extracellular cationic amino acids can stimulate efflux of intracellular L-arginine [29]. Accordingly, in cultured human endothelial cells it was shown that L-ornithine, and other cationic amino acids like L-lysine stimulate the efflux of L-arginine and decrease NO production by these cells [30]. Of note, in the present study the potentiation of airway responsiveness by L-ornithine was already maximal at a concentration of 1.0 mM, which is in line with the observation that 1 mM L-ornithine caused a (sub)maximal inhibition of cellular L-arginine uptake in rat alveolar macrophages [21]. It is also known that L-ornithine may inhibit cNOS activity as well; however, this requires about 10-fold higher concentrations [28].

Previous studies have indicated that L-arginine transport into alveolar macrophages and airway epithelium via \( y^+ \) transporters can also be inhibited by polycations such as poly-L-arginine and (eosinophil-derived) major basic protein [18]. Accordingly, in perfused guinea pig tracheal preparations, poly-L-arginine causes increased airway responsiveness to methacholine by inducing a deficiency of cNOS-derived NO [17]. Interestingly, the 1.6-fold increase in airway responsiveness caused by 1.0 mM L-ornithine was similar to the observed effect of poly-L-arginine [17].
The potential importance of reduced cellular uptake due to endogenous (poly)cations on the regulation of airway smooth muscle tone was illustrated by the observation in a guinea pig model of allergic asthma that heparin – acting as a (poly)cation scavenger [31] – is able to normalize the allergen-induced airway hyperresponsiveness *ex vivo*, after both the EAR [16][Chapter 3] and LAR [Chapter 4], by restoring the production of bronchodilating cNOS- and iNOS-derived NO, respectively. Thus, inhibition of cellular L-arginine uptake by endogenous cations - including L-ornithine and eosinophil-derived major basic protein - may cause substrate limitation to NOS isozymes and subsequent airway hyperresponsiveness after allergen challenge [16][Chapters 3&4]. Indeed, supplementation with exogenous L-arginine normalized allergen-induced airway hyperresponsiveness as well as impaired iNANC nerve-mediated airway smooth muscle relaxation after the EAR and/or LAR [11,13][Chapters 4&7]. Since the $y^+$ transporter system has a higher affinity for L-arginine than for L-ornithine [21], the ratio of both amino acids importantly regulates the L-arginine uptake and hence availability to NOS. Interestingly, although the local amino acid concentrations in the (diseased) airways are unknown, the plasma L-arginine/L-ornithine ratio was shown to be significantly decreased in patients with asthma, which was associated with increased arginase activity in the serum of these patients [32].

Decreased cellular L-arginine uptake due to inhibition of the $y^+$ transporters by endogenous (poly)cations including the arginase product L-ornithine is one mechanism that may be involved in L-arginine limitation and subsequent NO deficiency after allergen challenge. A second major mechanism contributing to attenuation of L-arginine may be increased L-arginine utilization by arginase. Using our guinea pig model of allergic asthma, we have demonstrated that arginase activity in the airways is strongly elevated both after the EAR and LAR, while the hyperresponsiveness of perfused tracheal preparations at these time points could be normalized after inhibition of arginase activity with nor-NOHA [15][Chapter 2]. Increased arginase activity also caused deficiency of iNANC nerve-mediated, nNOS-derived NO and impaired airway smooth muscle relaxation after the EAR, which was similarly due to limitation of L-arginine availability [13][Chapter 7].

Even in the presence of 1 mM exogenous L-ornithine, which causes maximal potentiation of airway responsiveness by this amino acid, inhibition of arginase by nor-NOHA was still effective in reducing this responsiveness. Since this effect was blocked with the NOS inhibitor L-NAME, this must indicate that arginase-induced attenuation of substrate availability to cNOS does still occur at maximally effective transport inhibition by the arginase product L-ornithine, implying competition between the two enzymes for their common substrate. The subtle balance between transport and substrate competition in regulating intracellular L-arginine levels is further indicated by the observation that L-ornithine completely reverses the reduction in airway responsiveness caused by nor-NOHA alone.
It has been reported previously that L-ornithine competitively inhibits arginase activity in rat liver and mammary gland with $K_i$ values of 1.0 mM and 1.1 mM, respectively [22,23,33]. In line with this finding, L-ornithine dose-dependently decreased arginase activity in guinea pig lung, liver and kidney homogenates. Lineweaver-Burk plots clearly showed that this inhibition is competitive, with $K_i$ values of 1.38 ± 0.30, 1.37 ± 0.35 and 1.29 ± 0.28 mM, respectively. All these values correspond closely to the reported values [22,23,33], and suggest no selectivity of L-ornithine with regard to arginase subtypes (mainly type I in liver and mainly type II in kidney). Furthermore, though 1.0 mM L-ornithine partially inhibits lung arginase activity, coincubation with 5.0 µM nor-NOHA, a concentration which almost abolishes tracheal [15] and lung arginase activities (Figure 4B), reverses the potentiating effect of L-ornithine on airway responsiveness in perfused tracheal preparations, suggesting that arginase is less dependent upon L-arginine uptake by cationic $\gamma^+$ transporters. Thus, though L-ornithine may act as a feedback regulator or arginase, its $\gamma^+$ carrier inhibitory properties are more important for regulating NOS activities and airway responsiveness.

Collectively, our data indicate that L-ornithine may regulate arginase activity and NO homeostasis in at least two distinct ways: first by decreasing the L-arginine availability via inhibition of cellular L-arginine uptake and secondly by direct competitive inhibition of arginase activity. Since in perfused guinea pig tracheal preparations the net effect of L-ornithine is increased airway responsiveness due to NO deficiency, it can be concluded that the impact of the inhibitory effect on L-arginine uptake is larger than that on arginase activity.

Acknowledgments
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References
L-Ornithine and airway responsiveness