Nitric oxide derived from sympathetic nerves regulates airway responsiveness to histamine in guinea pigs

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Matsumoto, Koichiro, Hisamichi Aizawa, Shohei Takata, Hiromasa Inoue, Naotsugu Takahashi, and Nobuyuki Hara. Nitric oxide derived from sympathetic nerves regulates airway responsiveness to histamine in guinea pigs. J. Appl. Physiol. 83(5): 1432–1437, 1997.—Nitric oxide (NO), which can be derived from the nervous system or the epithelium of the airway, may modulate airway responsiveness. We investigated how NO derived from the airway nervous system would affect the airway responsiveness to histamine and acetylcholine in mechanically ventilated guinea pigs. An NO synthase inhibitor Nω-nitro-L-arginine methyl ester (L-NAME) (1 mmol/kg ip) significantly enhanced airway responsiveness to histamine but not to acetylcholine. Its enantiomer D-NAME (1 mmol/kg ip), in contrast, had no effect. The L-NAME-induced airway hyperresponsiveness was abolished by pretreatment with propranolol (1 mg/kg iv) and atropine (1 mg/kg iv). Stellatectomy at the caudal end of the tracheal midportion and mechanically ventilated with a respirator (model 683, Harvard Apparatus, South Natick, MA) at a constant tidal volume of 7 ml/kg and a rate of 60 breaths/min. To estimate pleural pressure, a fluid-filled catheter was introduced into the esophagus at a point at which the maximal amplitude of pressure was obtained. The animals were placed supine in a flow-type body plethysmograph made of Plexiglas with 2.8-liters dead space (customized, Chest Medical, Tokyo, Japan). The plethysmographic airflow was measured with a Fleisch pneumotachograph (model TV-132, Nihon Kohden, Tokyo, Japan) and a differential pressure transducer (model TP-60T2, Nihon Kohden). The transpulmonary pressure was estimated from the difference between the esophageal and airway opening pressures, measured by a differential pressure transducer (model TP-603T, Nihon Kohden). Total Rl was calculated from transpulmonary pressure and plethysmographic airflow.

Protocol 1. The effect of a NOS inhibitor was investigated as follows: a NOS inhibitor Nω-nitro-L-arginine methyl ester (L-NAME) (1 mmol/kg) or its inactive enantiomer D-NAME was administered by an ultrasonic nebulizer (model TUR-3200, Nihon Kohden) placed in line with the ventilator. Dose-response curves were constructed as follows: saline was given for 15 breaths, and the resulting Rl value was used as the baseline value. The aerosols were administered in sets of 15 breaths, separated by 5-min intervals. The bronchoconstrictor concentration was increased with each series of 15 breaths. The Rl was monitored for 5 min after each nebulization, and the maximum value was plotted against the agent concentration.

Airway Hyperresponsiveness to Various Bronchoconstrictors is Characteristic of Asthma. To treat this abnormal response, it is important to clarify the basic mechanism of airway responsiveness in the physiological state. Neural control is a key determinant of airway responsiveness in vivo.

Inhibitory nonadrenergic noncholinergic (iNANC) nerves constitute a major neural pathway inhibiting excessive bronchoconstriction in several mammalian species, including humans (6, 7, 9, 10, 16). We previously demonstrated that pharmacological blockade of the iNANC nerves causes airway hyperresponsiveness to inhaled 5-hydroxytryptamine in cats (1). This finding supports the contribution of iNANC nerves to the regulation of airway responsiveness. Recent investigations suggest that nitric oxide (NO) is one of the neurotransmitters released from iNANC nerves (3, 12, 22, 29, 30). However, the precise role of NO in airway responsiveness remains uncertain.

Although in vitro studies in guinea pigs indicate that NO is a principal neurotransmitter of the iNANC nerves (3, 22, 30), similar in vivo evidence is lacking. In addition, a recent neurohistological study has revealed that the airway ganglia of guinea pigs do not contain neuronal cell bodies positive for NO synthase (NOS) immunoreactivity. These NOS-positive cell bodies were identified in thoracic sympathetic ganglia, namely the stellate ganglia (15). This finding suggests the sympathetic nerves as a possible origin of NO and, thus, of the nitrinergic response in guinea pigs. However, no corresponding physiological evidence exists to support this hypothesis. To clarify the in vivo role of NO in the airways, we investigated its origins and effects on airway responsiveness in guinea pigs.

Methods

Measurements of total pulmonary resistance (Rl). A total of 75 Hartley-strain male guinea pigs weighing 500–600 g (Kyudo, Kumamoto, Japan) were anesthetized with 50 mg/kg ip of pentobarbital sodium. They were intubated via tracheotomy at the caudal end of the tracheal midportion and mechanically ventilated with a respirator (model 683, Harvard Apparatus, South Natick, MA) at a constant tidal volume of 7 ml/kg and a rate of 60 breaths/min. To estimate pleural pressure, a fluid-filled catheter was introduced into the esophagus at a point at which the maximal amplitude of pressure was obtained. The animals were placed supine in a flow-type body plethysmograph made of Plexiglas with 2.8-liters dead space (customized, Chest Medical, Tokyo, Japan). The plethysmographic airflow was measured with a Fleisch pneumotachograph (model TV-132, Nihon Kohden, Tokyo, Japan) and a differential pressure transducer (model TP-602T, Nihon Kohden). The transpulmonary pressure was estimated from the difference between the esophageal and airway opening pressures, measured by a differential pressure transducer (model TP-603T, Nihon Kohden). Total Rl was calculated from transpulmonary pressure and plethysmographic airflow.

Measurement of airway responsiveness. Airway responsiveness to histamine and acetylcholine (ACh) was determined by administering increasing concentrations of the agents via the endotracheal tube. Aerosols (output, 1.5 ml/min) were generated by an ultrasonic nebulizer (model TUR-3200, Nihon Kohden) placed in line with the ventilator. Dose-response curves were constructed as follows: saline was given for 15 breaths, and the resulting Rl value was used as the baseline value. The aerosols were administered in sets of 15 breaths, separated by 5-min intervals. The bronchoconstrictor concentration was increased with each series of 15 breaths. The Rl was monitored for 5 min after each nebulization, and the maximum value was plotted against the agent concentration to achieve a constant-volume history, hyperinflations (triplicate of tidal volume) were obtained between each agent challenge. The challenge was halted when Rl exceeded 200% of the baseline value. The provocative concentration, defined as the bronchoconstrictor concentration of agent that produced an Rl of 200% of the baseline (PC200), was calculated by log-linear interpolation from individual animals.
(1 mmol/kg) was administered intraperitoneally to the animals 30 min before the measurement of airway responsiveness to histamine or ACh. The effect of L-NAME was further examined in animals pretreated with propranolol (1 mg/kg) and atropine (1 mg/kg), both administered intravenously 10 min before the measurement of airway responsiveness.

Protocol 2. To determine whether the ganglionic blockade of nicotinic neural transmission would affect the modulatory role of L-NAME on airway responsiveness, the animals were treated with hexamethonium (2 mg/kg iv) 20 min after the administration of L-NAME. Airway responsiveness was determined 10 min later.

Protocol 3. To elucidate the role of the vagus nerve and the sympathetic nerves in L-NAME-dependent modulation of airway responsiveness, bilateral vagotomy and/or bilateral stellatectomy were performed 10 min before L-NAME administration. Airway responsiveness was determined 30 min later. Stellatectomy was performed as described previously (4, 27). In brief, the cervical sympathetic trunk was identified by using an operation microscope. The upper pole of the stellate ganglion was identified beneath the subclavian artery. The ganglion was dissected between the parietal pleura and the muscles of the chest wall. The combination of stellatectomy and vagotomy was chosen to interrupt sympathetic influence on airway function because a previous report had shown that the cervical sympathetic trunk could project to the airways through a vagal pathway via its communicating branch (4).

Drugs. Pentobarbital sodium was obtained from Abbott (North Chicago, IL). Histamine diphosphate, acetylcholine chloride, L-NAME, D-NAME, and hexamethonium chloride were obtained from Sigma Chemical (St. Louis, MO). Atropine sulfate was obtained from Tanabe Pharmaceutical (Osaka, Japan). Propranolol hydrochloride was obtained from Zeneka Pharmaceutical (Osaka, Japan). Histamine, ACh, L-NAME, and D-NAME were dissolved in saline at a concentration of 1 mmol/ml and hexamethonium at 2 mg/ml, respectively.

Data analysis. PC200 values were translated into log (PC200 × 100) and expressed as means ± SE. Baseline RL values were also expressed as means ± SE. Values between the two groups were compared by unpaired Student’s t-test. Values among more than two groups were compared by one-way analysis of variance followed by Bonferroni correction. A level of P < 0.05 was accepted as statistically significant.

RESULTS

Effect of L-NAME or D-NAME on baseline RL and airway responsiveness. To determine the influence of NO on airway responsiveness, we treated the guinea pigs with the NOS inhibitor L-NAME or with its inactive enantiomer D-NAME. Neither treatment significantly altered the baseline RL (Table 1). We then challenged the animals with increasing concentrations of histamine or ACh. Airway responsiveness was assessed by determining the PC200 of each agent. Treatment with L-NAME significantly enhanced the airway responsiveness to inhaled histamine, whereas D-NAME had no effect (Fig. 1). To determine whether this

Table 1. Baseline RL of guinea pigs in different experimental groups

<table>
<thead>
<tr>
<th>Response to Histamine</th>
<th>Response to Acetylcholine</th>
<th>Response to Histamine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>L-NAME</td>
<td>D-NAME</td>
</tr>
<tr>
<td>0.246 ± 0.007</td>
<td>0.244 ± 0.012</td>
<td>0.236 ± 0.012</td>
</tr>
<tr>
<td>Control</td>
<td>L-NAME</td>
<td>Hexamethonium</td>
</tr>
<tr>
<td>0.212 ± 0.007</td>
<td>0.230 ± 0.004</td>
<td>0.242 ± 0.013</td>
</tr>
</tbody>
</table>

Values are means ± SE. RL, pulmonary resistance; L-NAME, N(G)-nitro-L-arginine methyl ester; D-NAME, N(G)-nitro-D-arginine methyl ester. All response values were not significant.

Fig. 1. Effect of N(G)-nitro-L-arginine methyl ester (L-NAME) or its enantiomer D-NAME on airway responsiveness to histamine. Proactive concentrations (PC200) of histamine were determined in untreated, L-NAME-treated, and D-NAME-treated guinea pigs. Treatment with L-NAME, but not with D-NAME, significantly enhanced the airway responsiveness to inhaled histamine. Values are means ± SE of 5 animals; **P < 0.01; NS, not significant.
enhancement by L-NAME is derived from nonadrenergic noncholinergic mechanism, the effect of L-NAME was further examined in animals pretreated with propranolol and atropine. Treatment with L-NAME significantly enhanced airway responsiveness to histamine. Values are means ± SE of 5 animals; **P < 0.01.

Effect of hexamethonium on baseline RL and L-NAME-induced airway hyperresponsiveness to histamine. To elucidate the mechanism underlying the L-NAME-induced airway hyperresponsiveness to histamine, we pretreated some of the animals with the ganglionic blocker hexamethonium. This pretreatment, which did not affect the animal's baseline RL (Table 1), completely abolished the effect of L-NAME on enhancing the airway responsiveness to histamine (Fig. 4). These findings suggest the involvement of a neural component in the NO-mediated regulation of airway responsiveness to histamine.

Effect of vagotomy and/or stellatectomy on baseline RL and L-NAME-induced airway hyperresponsiveness to histamine. To determine which neural pathways could contribute to NO-mediated regulation of airway responsiveness, L-NAME treatment and histamine challenge were performed in animals that had undergone bilateral cervical vagotomy and/or stellatectomy. Neither of these procedures significantly altered the baseline RL value (Table 1). In animals that had undergone vagotomy, L-NAME significantly enhanced airway responsiveness (Fig. 5). However, L-NAME-induced airway hyperresponsiveness was not observed in animals...
pretreated with stellatectomy (Fig. 6) or vagotomy plus stellatectomy (Fig. 7).

Summary of log(PC200/100) of histamine in each group.
The values of log(PC200/100) of histamine in each group are summarized in Table 2. In vehicle-administered groups, the values in propranolol- and atropine-treated, hexamethonium-treated, vagotomized, and/or stellatectomized animals were significantly lower than those of sham-treated controls. In L-NAME-administered groups, there were no significant differences in the values among groups.

DISCUSSION

The present study demonstrated that the NOS inhibitor l-NAME, but not its inactive enantiomer d-NAME, enhanced airway responsiveness to inhaled histamine in guinea pigs, suggesting that NOS inhibition can induce airway hyperresponsiveness. The l-NAME-induced enhancement of airway responsiveness was still observed in animals pretreated with propranolol and atropine. By contrast, l-NAME had no effect on airway responsiveness to inhaled ACh. Pretreatment of the animals with the ganglionic blocker hexamethonium abolished the hyperresponsiveness to histamine produced by l-NAME. Stellatectomy, but not vagotomy, eliminated the l-NAME-dependent enhancement of airway responsiveness. The difference in the bronchoconstrictor effects of histamine and ACh is likely due to differences in the contribution of a neural reflex mechanism. Histamine causes bronchoconstriction not only directly by inducing the contraction of airway smooth muscle but also indirectly via the excitation of a cholinergic pathway by neural reflex (19, 25). ACh, in contrast, is less effective in eliciting bronchoconstriction by neural reflex (17, 18). Therefore, this difference in the bronchoconstrictor mechanisms of histamine and ACh suggests the involvement of a neural component in the l-NAME-induced airway hyperresponsiveness to histamine.

Several studies have indicated that epithelium-derived NO may affect airway responsiveness by inhibiting excessive bronchoconstriction (13, 26). If epithelium-derived NO played an essential role in modulating airway responsiveness, l-NAME would enhance airway responsiveness even in animals pretreated with hexamethonium. In the present study, however, pretreatment with hexamethonium abolished l-NAME-induced airway hyperresponsiveness. This discrepancy between our findings and the previous results may be explained partly by differences in the administration routes of l-NAME. In the previous study (26), l-NAME was administered by inhalation, which left the possibility that the agent selectively inhibited NOS in the epithelium but not in the nervous system. Conversely, the intraperitoneal administration of l-NAME used in this study may have affected NOS in the nerves but not in the epithelium.

Recent in vitro studies in several species have demonstrated that NO acts as neurotransmitter in iNANC nerves (3, 22, 30). It has been also reported that iNANC nerve-mediated bronchodilation was completely abolished by bilateral cervical vagotomy in cats (20). Moreover, we recently demonstrated that capsaicin-induced bronchodilation in cat airways precontracted by continuous 5-hydroxytryptamine infusion was abolished by treatment with hexamethonium or l-NAME (H. Aizawa, S. Takata, H. Inoue, K. Matsumoto, H. Nakano, and N. Hara, unpublished observations). These results indicated that in cat airways NO plays an essential role in the iNANC nervous system through a vagal reflex mechanism.

Interestingly, bilateral cervical vagotomy did not modulate the effect of l-NAME on airway responsiveness to histamine in guinea pigs in the present study. In contrast, previous studies found that electrical stimulation of the vagal nerve caused iNANC-mediated tracheal dilatation (6) and that vagotomy abolished the

![Fig. 6. Effect of stellatectomy on l-NAME-induced airway hyperresponsiveness to histamine. PC200 of histamine was determined in untreated and l-NAME-treated guinea pigs that had undergone a bilateral stellatectomy. No enhanced airway responsiveness in response to l-NAME was observed in these animals. Values are means ± SE of 5 animals.](image)

![Fig. 7. Effect of vagotomy plus stellatectomy on l-NAME-induced airway hyperresponsiveness to histamine. PC200 of histamine was determined in untreated and l-NAME-treated guinea pigs that had undergone bilateral cervical vagotomy plus stellatectomy. No enhanced airway responsiveness in response to l-NAME was observed in these animals. Values are means ± SE of 5 animals.](image)
30–60% of the afferent nerves in guinea pig intrapulmonary. Other investigators reported (8, 21, 23, 27) that neuronal cell bodies immunoreactive for NOS were present in guinea pigs. (3–5, 24) that vasoactive intestinal polypeptide is an important neurotransmitter. Indeed, several investigators have suggested that the contribution of nonnitrinergic iNANC nervous mechanisms may regulate airway responsiveness in guinea pigs (28). Thus our result seems inconsistent with those previous observations. Differences in the airway sites studied may account for this discrepancy. Both earlier studies had chosen the upper to midportion of the tracheal segment to obtain an iNANC response. In the present study, in contrast, the effect of L-NAME was evaluated in the airway distal to the tracheal midportion. Therefore, it is possible that in guinea pigs the vagal component of the L-NAME-sensitive response is of lesser importance in the airway distal than in those proximal to the midportion of the trachea. Vagotomy does not necessarily eliminate the contribution by postganglionic parasympathetic neurons. There may be a peripheral reflex activation of airway parasympathetic cholinergic and noncholinergic neurons. We previously demonstrated that atropine significantly inhibited the histamine-induced bronchoconstriction in animals with vagotomy (19). This suggests the existence of peripheral activation of parasympathetic cholinergic neurons. As far as the nitrinergic system is concerned, a possibility remains that vagotomy-resistant enhancement of airway responsiveness by L-NAME may be derived from the inhibition of peripheral activation of parasympathetic nitrinergic neurons. However, there has been no evidence to support that histamine could directly activate postganglionic nitrinergic neurons in vivo.

We previously showed that vagotomy significantly enhanced histamine-induced bronchoconstriction in guinea pigs (19). This was confirmed by the present finding that the vagal nerves have an inhibitory effect on airway responsiveness to histamine. It may reflect the nitrinergic iNANC nervous system in the tracheal segment, which attenuates histamine-induced airway response through a vagal reflex mechanism. However, this possibility is less likely because L-NAME did not enhance airway responsiveness in stellatectomized animals with the intact vagal nerves. Another explanation is the contribution of nonnitrinergic iNANC nervous system. Indeed, several investigators have suggested (3–5, 24) that vasoactive intestinal polypeptide is an alternative transmitter of airway iNANC nervous system in guinea pigs.

More importantly, Fischer et al. (15) reported that neuronal cell bodies immunoreactive for NOS were present in stellate ganglia but absent in airway ganglia. Other investigators reported (8, 21, 23, 27) that 30–60% of the afferent nerves in guinea pig intrapulmonary airways originate from dorsal root ganglia at the upper thoracic level of the spinal cord. These afferent nerves innervate the airways through a sympathetic pathway that includes the stellate ganglia. Our observations are consistent with the above reports, since L-NAME-induced airway hyperresponsiveness persisted in vagotomized animals but not in animals pretreated with vagotomy plus stellatectomy, which may interrupt both the afferent and the efferent postganglionic nitrinergic pathway in the thoracic sympathetic nervous system. Thus this study provides the first in vivo evidence that the release of NO from the sympathetic nervous system is important in regulating airway responsiveness. To confirm this, it is necessary to determine whether the direct stimulation of the sympathetic ganglia causes airway relaxation. In this regard, a very early study (11) reported that the electrical stimulation of the stellate ganglia caused marked bronchodilation in cats. However, it has been uncertain that the bronchodilation is observed even in animals pretreated with adrenergic blockers. Recently, Canning et al. (5) have demonstrated that tracheal NANC relaxations in guinea pigs are partly mediated by NO released from parasympathetic nerve endings derived from neurons intrinsic to the esophagus. The nitrinergic neural response in guinea pigs, therefore, may be unexceptionally derived from neurons located in the extra airway ganglia.

A recent study in humans identified NOS-immunoreactive neuronal cell bodies in the airway ganglia and in the vagal sensory ganglia (14). The presence or absence of NOS-containing neuronal cell bodies in the sympathetic ganglia, however, has not yet been investigated.

In summary, the NOS inhibitor L-NAME significantly enhanced the airway responsiveness to histamine, but not to ACh, in guinea pigs. The enhancing effect of L-NAME on airway responsiveness was not observed in animals pretreated with hexamethonium. Airway hyperresponsiveness to histamine was observed in animals with vagotomy but not in animals with stellatectomy. These findings suggest that NO derived from NOS in the sympathetic nervous system may regulate airway responsiveness.

Table 2. log(PC200 × 100) of histamine in different experimental groups

<table>
<thead>
<tr>
<th>Vehicle administration</th>
<th>Control or L-NAME</th>
<th>Propranolol + atropine</th>
<th>Hexamethonium</th>
<th>Vagotomy</th>
<th>Stellatectomy</th>
<th>Vagotomy + stellatectomy</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>log(PC200 × 100)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>l-NAME administration‡</td>
<td>2.104 ± 0.126</td>
<td>1.664 ± 0.055*</td>
<td>1.238 ± 0.165†</td>
<td>1.585 ± 0.203*</td>
<td>1.378 ± 0.135†</td>
<td>1.090 ± 0.169†</td>
</tr>
<tr>
<td>1.363 ± 0.214</td>
<td>1.326 ± 0.075</td>
<td>1.130 ± 0.148</td>
<td>0.988 ± 0.173</td>
<td>1.337 ± 0.085</td>
<td>1.185 ± 0.191</td>
<td></td>
</tr>
</tbody>
</table>

Values are means ± SE. *Significantly lower than control, P < 0.05; †significantly lower than control, P < 0.01; ‡all values not significant.
REFERENCES


