Role of nitric oxide during hyperventilation-induced bronchoconstriction in the guinea pig

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Received 17 July 2000; accepted in final form 6 November 2000

Suman, Oscar E., and Kenneth C Beck. Role of nitric oxide during hyperventilation-induced bronchoconstriction in the guinea pig. J Appl Physiol 90: 1474–1480, 2001.—Airway function is largely preserved during exercise or isocapnic hyperventilation in humans and guinea pigs despite likely changes in airway milieu during hyperpnea. It is only on cessation of a hyperpneic challenge that airway function deteriorates significantly. We tested the hypothesis that nitric oxide, a known bronchodilator that is produced in the lungs and bronchi, might be responsible for the relative bronchodilation observed during hyperventilation (HV) in guinea pigs. Three groups of anesthetized guinea pigs were given saline and three groups given 50 mg/kg N\textsuperscript{G}-monomethyl-l-arginine (L-NMMA), a potent nitric oxide synthase inhibitor. Three isocapnic ventilation groups included normal ventilation [40 breaths/min, 6 ml/kg tidal volume (VT)], increased respiratory rate only (150 breaths/min, 6 ml/kg VT), and increased respiratory rate and increased volume (100 breaths/min, 8 ml/kg VT). L-NMMA reduced expired nitric oxide in all groups. Expired nitric oxide was slightly but significantly increased by HV in the saline groups. However, inhibition of nitric oxide production had no significant effect on rate of rise of respiratory system resistance (Rrs) during HV or on the larger rise in Rrs seen 6 min after HV. We conclude that nitric oxide synthase inhibition has no effect on changes in Rrs, either during or after HV in guinea pigs.

respiratory system resistance; asthma; exercise-induced asthma

AIRWAY FUNCTION DURING EXERCISE or isocapnic hyperventilation (HV) is relatively well preserved in asthmatic subjects (3, 5, 44) as well as in guinea pigs (35, 37), despite likely changes in airway fluid content that occur with hyperpnea. When airway function deteriorates during hyperpnea, it does so with a lesser magnitude compared with that seen in the posthyperpneic period. There are two possible explanations for this relative protection of airway function during hyperpnea. First, the bronchoconstrictor mediators might not be released during HV. Second, even when bronchoconstrictor mediators are released, dilator influences can develop during HV that prevent the full effects of the released mediators until HV stops (3, 45). Among the potential mediators responsible for the “bronchoprotection” of airway function during hyperpnea is nitric oxide (NO) (29, 48). In this paper, we investigate the possibility that NO protects airways from constriction until HV ends.

NO is a known bronchial and vasodilator produced by a variety of cells in the lungs and bronchi of humans (2, 4) and guinea pigs (29, 33, 48). Previous studies have yielded conflicting results as to the role of NO in modulating airway function. Mehta and colleagues (29) found an increase in bronchoconstriction during histamine-induced bronchoprovocation after inhibiting NO production with l-arginine methyl ester (L-NAME) in guinea pigs. In addition, Yoshihara and co-workers (48) found that L-NAME enhanced bronchoconstriction induced by cold air inhalation without HV in guinea pigs. Both studies thus suggest that endogenous NO exerted a protective effect. In contrast, Nogami and colleagues (33) found no effect of L-NAME on the increase in pulmonary resistance after a HV challenge in guinea pigs. However, their studies utilized L-NAME, which also has antimuscarinic effects (7) and might have altered the effects of NO by attenuating bronchoconstrictor responses to HV. Furthermore, these studies have detailed NO’s effect only in the post-HV period. The role of NO in controlling airway function during HV has not been studied. We therefore designed this study to test the hypothesis that NO production increases during HV challenge in guinea pigs, thereby protecting against bronchoconstriction until HV ceases. We tested this hypothesis by administering N\textsuperscript{G}-monomethyl-l-arginine (L-NMMA), a potent and specific NO synthase inhibitor, before HV challenge in guinea pigs.

METHODS

Male Hartley guinea pigs (n = 62; weight = 630.3 ± 74.8 g) were randomly assigned to one of three ventilation groups defined by respiratory frequency and tidal volume (ventilator settings) and one of three drug groups defined by the type of drug administered (Table 1).

Animal preparation. We anesthetized each animal using 1 ml/kg of ketamine-xylazine (100 mg/ml ketamine and 10 mg/ml xylazine) injected intramuscularly in the hind extremity. Once anesthetized, animals were intubated via a midline
tracheotomy and the lungs ventilated at a volume of 5.0–6.1 ml/kg using a small-animal ventilator (Harvard Apparatus, Millis, MA) attached via a 2-in. silicon rubber tubing (2.0 mm ID) with a Y piece at the end. A catheter was inserted into the jugular vein for administration of paralytic agent (vecuronium). The animal was placed inside a small-animal body plethysmograph, and 0.1 ml of vecuronium (1.0 mg/ml) was given intravenously to prevent breathing efforts from affecting subsequent respiratory system resistance (Rrs) measurements. Respiratory system mechanics and mixed expired NO (ExNO) were monitored for 25 min to establish pretreatment values. After 25 min, each guinea pig was given 50 mg/kg l-NMMA or saline intravenously to test the effect of saline or l-NMMA on pulmonary mechanics and ExNO production.

Pulmonary mechanics. Pressure at the mouth was measured with a calibrated pressure transducer (range ±200 cmH2O, Celesco Transducer Products, Canoga Park, CA) connected to a side tap of the endotracheal tube. Pressure in the small animal plethysmograph was measured using a ±2-cmH2O pressure transducer (Celesco Transducer Products). The latter pressure was proportional to flow across a screen pneumotachograph mounted in the rear wall of the plethysmograph. The flow signal was integrated digitally to produce a volume signal, which in turn was corrected for pressure in the plethysmograph by digitally subtracting a signal proportional to the pressure. The proportionality constant was adjusted to bring integrated box volume and integrated mouth volume signals into phase when the empty plethysmograph was pumped with a small-animal ventilator. The box flow signal was calibrated on each study day using a 5-ml gas-filled syringe to produce a known volume signal. The two pressure signals were fed into a personal computer-based data acquisition system at rate of 180 s⁻¹.

Rrs was measured on a breath-by-breath basis using techniques similar to those of Mead and Whittenberger (28) and Frank and colleagues (11). Respiratory system dynamic compliance (Crs, dyn) was obtained from ∆Vt/∆Pm, where ∆Vt indicates the change in volume from beginning of inspiration to end inspiration (at points of zero flow) and ∆Pm is the change in mouth pressure over the same interval. Rrs was then obtained from the slope by linear regression of the plot of flow vs. [Pm-(∆Vt/Crs,dyn)], which is equivalent to pressure change divided by change in flow (range of flow ± 20 ml/s) (3, 11). Data for at least five breaths were averaged at each time point of measurement.

Protocol. Animals inhaled dry air from a compressed gas tank throughout the experiment. Levels of ExNO were measured using a NO analyzer (model 88, Sievers, Boulder, CO). The analyzer itself was “zeroed” using the NO absorbent chamber supplied by Sievers and calibrated using a tank of 100 parts/billion NO in N2 (Scott-Marrin, Riverside, CA) at the beginning of each study. Before each measurement of expired NO, the inhaled gas was sampled and recorded. Anesthesia-type bag was connected to the inhaled port of the ventilator and fed by a mixture of compressed air and 5% CO2. Expired air emptied into a mixing chamber where ExNO was measured. Levels of ExNO were assessed every 2 min before and after HV.

Pre-HV baseline measurements were made ~30 min (28–31 min) after the induction of anesthesia. Tidal volume, mixed expired CO2, respiratory rate, and Rrs were measured every 2 min for 10 min. At each recording time, data for 10 breaths were recorded, followed by a full inspiration delivered via a syringe (20–25 cm of maximal airway pressure). Rrs measurements were taken by averaging data from breaths before the deep inspiration. At the end of 10 min, individual guinea pigs were randomly assigned to one of three ventilation strategies: one normal ventilation (NV) control group and two HV groups (Table 1). In the control group, the ventilator was set at 40 breaths/min and a constant tidal volume throughout the experiment. In the increased frequency group (HV150), the ventilator rate was increased to 150 breaths/min while tidal volume was maintained at resting levels during the 10 min of HV. In the second HV group (HV100), the respiratory rate was increased during the HV period to 100 breaths/min and the tidal volume was increased by 1.5–2.0 ml above pre-HV values for 10 min of HV. Animals were kept eucapnic by mixing 5% CO2 with compressed air in the inspired port of the ventilator. During the 10-min challenge period, Rrs was recorded every 2 min without the deep inspirations. After the hyperpneic challenge, the ventilator was returned to prechallenge settings (i.e., 40 breaths/min, 3.5 ml per breath) for 4 min. Measurements of Rrs were recorded every 2 min, again without deep inspirations.

Data analysis. The changes in pulmonary function during the early stages of HV were assessed by comparing Rrs at 2 min with the pre-HV value. The change in Rrs with time during HV was documented by the linear regression slope of Rrs against time from 2 to 10 min for each animal. The fractional bronchoconstrictor response after HV (PP) was determined by taking the largest Rrs measured post HV compared with the pre-HV value [largest post – pre]/pre]. The immediate bronchoconstrictor fractional response (IP) was similarly documented using the highest post-HV Rrs value compared with the last Rrs measurement made during HV (10 min) [largest post – 10 min]/10 min]. The IP response quantifies the degree of bronchoconstriction that occurs on cessation of HV that is beyond that which occurs during HV.

Statistical analysis. Means ± SE were calculated for ExNO levels and for Rrs. Both repeated-measures and non-repeated-measures ANOVA were used to test for the effects of ventilation strategy and drug treatment on Rrs across time. Tukey’s t-tests were conducted to compare ventilation and drug groups. To test for changes in Rrs during HV, the slope of Rrs vs. time was submitted to ANOVA and subsequent Tukey’s t-tests to test effects of drug and ventilation strategy. To be considered significant, the P value was set at 0.05.

RESULTS

Rrs measurement: effects of l-NMMA. There were no significant differences in Rrs among groups of animals
at baseline before administration of saline or L-NMMA and after drug treatment. The IP, PP, and the slope of Rrs were not statistically different between the saline and L-NMMA groups, indicating that L-NMMA had no effect on Rrs during any phase of the study (Figs. 1–3).

Rrs measurements: effects of ventilation. In both saline- and L-NMMA-treated groups, the mean post-HV Rrs responses (IP and PP) were largest in the HV100 group (larger tidal volume) compared with HV150 and NV. However, in the L-NMMA-treated animals, only the HV100 to normal ventilation comparison (IP and PP, Fig. 2) was significant. Similarly, the mean slope of Rrs was significantly steeper during HV100 compared with NV independent of drug treatment (Fig. 3). Mean slope of Rrs during HV was not significantly different between NV and HV150 groups, possibly because of two nonresponders in the L-NMMA treatment group. These results suggest that larger tidal volume ventilation produces a faster rise in Rrs during HV and a larger post-HV increase in Rrs compared with smaller volume but a higher respiratory rate ventilation.

NO measurements. All groups of guinea pigs had similar levels of ExNO before drug treatment (Table 2). Administration of saline did not affect ExNO levels (Fig. 4). However, L-NMMA-treated animals had decreased ExNO levels as expected (Fig. 4, bottom). There was a slight, but significant increase in ExNO levels measured 6 min after HV compared with before HV in the saline group (Fig. 4, top).

DISCUSSION

Inhibition of endogenous NO production with L-NMMA did not block the initial drop in Rrs observed at the onset of hyperpnea, and it had no effect on the...
L-NAME, but not L-NMMA, has been reported to have pulmonary resistance of L-NAME measured after a Nogami and collaborators (33), who found no effect on utilizing HV becomes difficult. Our results agree with mals, and, without HV, comparison with studies tion. However, they did not hyperventilate their ani-
hibited bronchoconstriction induced by cold-air inhala-
and co-workers (48) reported that endogenous NO in-
 pigs after histamine challenge. In addition, Yoshihara of NO in the bronchoprotection of airways in guinea 
colleagues’ previous study (29) that described the role (HV150).

Thus it is possible that airway NO production changed
increases in Rrs during or after hyperpnea in guinea 
pigs. Our results support our previous findings (45) that breathing with large tidal volume and increased frequency (HV100) produces a steeper rise in Rrs during HV than breathing with increased frequency alone (HV150).

Our findings are interesting in light of Mehta and colleagues’ previous study (29) that described the role of NO in the bronchoprotection of airways in guinea pigs after histamine challenge. In addition, Yoshihara and co-workers (48) reported that endogenous NO inhibited bronchoconstriction induced by cold-air inhalation. However, they did not hyperventilate their animals, and, without HV, comparison with studies utilizing HV becomes difficult. Our results agree with Nogami and collaborators (33), who found no effect on pulmonary resistance of L-NAME measured after a hyperpneic challenge in guinea pigs. However, the latter group did not report resistance changes during hyperpnea. Additionally, they, as well as others (29), have utilized L-NAME to inhibit NO synthesis. L-NAME, but not L-NMMA, has been reported to have muscarinic-receptor antagonistic action, which could have affected these results (7). Hogman and associates (18) found a weak bronchidilator effect of inhaled NO in human subjects with bronchial asthma and no effect in nonasthmatic individuals. Despite the finding by Hogman et al. of a bronchidilator effect of inhaled NO in humans, we found a lack of bronchidilator effect of endogenous NO during HV in guinea pigs.

The pattern of Rrs response during HV that we found in our present study is similar to that reported by other investigators (35, 45): i.e., an initial fall followed by a gradual increase (albeit small) in Rrs during HV. Ray and colleagues (35) interpreted the limited rise in Rrs during HV as a continued suppression of bronchoconstriction during hyperpnea. Although they listed factors that potentially could contribute to this bronchoprotection, their study was not designed to test the role of any specific dilator mechanism or mediator.

**Limitations of the model.** In the saline group, ExNO levels after HV were slightly but significantly different from ExNO levels before HV. Because of the known effects of increasing flow on ExNO measurement (39), we could not measure ExNO levels until after HV. Thus it is possible that airway NO production changed during HV. However, the response in Rrs both during and after HV was similar for both saline and L-NMMA groups, suggesting that the changes in Rrs were independent of ExNO levels. Potential systemic effects of L-NMMA administered intravenously include peripheral vasoconstriction, which could have caused a peripheral chemoreceptor-mediated reflex bronchoconstriction (46). However, no systemic effects of L-NMMA have been reported in studies in which L-NMMA was given via the intravenous or inhalation route in the dose that we utilized (33, 41, 47). In addition, Rrs measured 15 min after administration of L-NMMA but before HV had not changed from Rrs measured before administration of L-NMMA.

The reason for lack of effect of L-NMMA on Rrs includes the possibility that the available NO inhibitors might be poorly selective and lacking in potency. Inoue and associates (20) reported no significant inhibitory effect of L-NMMA alone on interleukin-8 (IL-8) production in the guinea pig after ozone-induced airway inflammation. Only when given in combination with L-NAME or with aminoguanadine (NO inhibitors) was IL-8 production significantly inhibited.

However, treatment with L-NMMA significantly re-
duced the ExNO levels found before HV. The levels of ExNO were not reduced to zero, but the measured inspired NO levels were near zero, suggesting that the ExNO originated from inside each guinea pig. In preliminary dose-response experiments, we found no further reduction in ExNO levels with doses of L-NMMA up to 100 mg/kg. In addition, we administered L-arginine via intravenous route in a separate group of ani-
mals (n = 35). L-Arginine caused an increase in ExNO levels, reversing the effects of L-NMMA as expected, but did not alter Rrs values.

**Table 2. Exhaled nitric oxide concentrations before hyperventilation in saline and L-NMMA groups**

<table>
<thead>
<tr>
<th>Drug and Ventilation</th>
<th>n</th>
<th>Baseline</th>
<th>Postdrug</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline</td>
<td>10</td>
<td>9.09 ± 0.13</td>
<td>9.22 ± 0.13</td>
</tr>
<tr>
<td>NV</td>
<td>10</td>
<td>11.4 ± 0.58</td>
<td>11.3 ± 0.58</td>
</tr>
<tr>
<td>HV150</td>
<td>10</td>
<td>10.0 ± 1.40</td>
<td>10.1 ± 0.46</td>
</tr>
<tr>
<td>L-NMMA (50 mg/kg)</td>
<td>10</td>
<td>11.9 ± 2.40</td>
<td>4.97 ± 0.26</td>
</tr>
<tr>
<td>NV</td>
<td>11</td>
<td>9.45 ± 1.70</td>
<td>4.51 ± 0.22</td>
</tr>
<tr>
<td>HV150</td>
<td>11</td>
<td>9.81 ± 1.66</td>
<td>4.29 ± 0.46</td>
</tr>
</tbody>
</table>

Values are means ± SE given in parts/billion; n, no. of guinea pigs.
Ketamine or vecuronium could have effects on the control of Rrs during these studies. Because all groups received ketamine and vecuronium, any effect specifically caused only by ketamine or by vecuronium would have been manifested in all groups. Although ketamine is an airway smooth muscle relaxant, it did not prevent the post-HV response or the progressive rise in Rrs during HV. We cannot rule out the possibility that ketamine or vecuronium blunted or enhanced the progressive increase in Rrs during HV or the post-HV bronchoconstrictor response. However, in both saline and L-NMMA unventilated groups, Rrs remained unchanged after administration of ketamine and vecuronium. In addition, administration of ketamine and vecuronium did not maximally relax airway smooth muscle because at the onset of HV all groups decreased Rrs initially.

NO has been postulated to be a marker of inflammation or a bronchodilator released in response to the inflammation (17). However, in our guinea pig model, endogenous NO does not seem to be involved in the control of airway tone during or after HV because both saline-treated and L-NMMA-treated groups had a similar degree of airway obstruction in response to HV. NO could promote an increase in Rrs by promoting airway blood flow and secretions. No differences in Rrs were found between saline and L-NMMA in Rrs after HV (when ventilated at 150 breaths/min or 100 breaths/min), suggesting that NO is not a “bronchopromoter” influence. A previous study by Sapienza and colleagues (41) suggests that NO might act as a bronchoconstrictor mediator. However, very high concentrations of endogenously released NO are needed to demonstrate this effect, beyond the concentrations of NO used in the present study.

Although NO does not seem to modulate the bronchoconstriction observed during or after hyperpnea, levels of NO might reflect vascular or inflammatory events in the airway (1, 9, 17).

Ray and colleagues (35–37) have shown that the time course and degree of bronchoconstrictor response are similar between humans and guinea pigs. In addition, the pulmonary response during isocapnic HV with dry gas is also similar between guinea pigs and humans. Although the guinea pig has been used as a model for HV-induced asthma and as a model of hyper-reactivity (8, 19, 31, 35, 36, 37, 38), caution is still warranted in extrapolating results from animal studies to humans. For example, the post-HV bronchoconstriction in guinea pigs is thought to be mediated primarily by the release of tachykinins and, to lesser extent, leukotrienes (36). In contrast to guinea pigs, in humans, the post-HV bronchoconstriction is thought to be mediated primarily by histamine, leukotrienes, and vagal nerve stimulation (10, 22, 24–26, 43).

Mechanisms. The mediators or mechanisms responsible for the bronchodilation or preservation of lung function observed during HV in both humans and guinea pigs remain unknown. A potential dilator mediator is prostaglandin E2 (16, 27, 30, 34, 38). However, we have reported that prostaglandin E2 is not likely the bronchodilator mediator operating during HV (45) and that the slow rise in Rrs during HV is dependent on a constrictor prostaglandin. Combined with the present results using L-NMMA, we can eliminate two potential dilator mediators.

Although other dilator mediators, such as calcitonin gene-related peptide (32) and nitrosothiol (15), might play a role in suppressing bronchospasm during hyperpnea, our study did not address the role of calcitonin gene-related peptide or nitrosothiol in hyperpnea-induced bronchospasm. Other naturally occurring bronchodilators include other prostaglandins (E2 and PGL2) (14), but their role is unlikely given our previous results (45). In addition to active mediators, either mechanical effect (12, 13, 40, 42) or an effect of the initial cooling of the airways in HV (15) could protect airways from hyperpnea-induced bronchospasm during HV.

Increased lung volume (stretching). Does HV have a mechanical dilatory effect? In a previous study (45), we reported that guinea pigs ventilated with an increase in tidal volume and respiratory rate (HV100) experienced larger changes in mean lung volume and a higher end-inspiratory volume than guinea pigs ventilated with an increase in respiratory rate only (HV150). Despite these differences in lung volume changes, the initial fall in Rrs at onset of HV was the same in both groups, suggesting little role for lung volume changes per se. Increased respiratory rate could also have a bronchodilator effect: in other species, pulmonary resistance drops with increasing respiratory frequency (6), probably because of a decreased effective tissue resistance (6, 21). Our results suggest that in guinea pigs there is a drop in Rrs with an increase in respiratory frequency between 40 and 100 breaths/min but little change between 100 and 150 breaths/min. However, our studies were not designed to examine the frequency dependence of Rrs in detail.

On the basis of our results, the following model for response to HV in guinea pigs emerges. During HV, there is an initial fall in Rrs caused predominantly by an increase in respiratory rate and tidal volume, although other active bronchodilator agents or mechanisms (e.g., airway cooling) cannot be ruled out. During continued HV, the rate of release of bronchconstrictor influences (prostaglandin, tachykinins) slowly increases, perhaps as a result of airway drying, cooling, or stretch, causing a slow and small rise in Rrs. Full bronchoconstriction does not develop during HV because of either the effects of airway cooling (14) or the mechanical effect of tidal stretches on cross-bridge function in airway smooth muscle (6, 12, 13, 40, 42). The present results indicate that NO is not a significant bronchoprotective mediator during HV in guinea pigs. When HV stops, full expression of the bronchoconstrictor effect by mediators such as prostaglandins and tachykinins is produced.

Although specific mediators can differ, changes in airway function during and after exercise in humans might exhibit similar mechanisms.

In conclusion, NO does not seem to be the mediator responsible for the suppression of HV-induced broncho-
constriction during hyperpnea in the guinea pig. In addition, No does not modulate the posthyperpnea bronchoconstriction in guinea pigs.

We appreciate the manuscript preparation work performed by L. L. Oeltenbruns and data analysis and presentation by Catherine M. Swee.

This work was supported by National Heart, Lung, and Blood Institute Grant HL-52230.

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