Ambient oxygen regulates epithelial metabolism and nitric oxide production in the human nose

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Nakano, Hitoshi, Hiroshi Ide, Toshiyuki Ogasa, Shinobu Osanai, Masanobu Imada, Satoshi Nonaka, Kenjiro Kikuchi, and Jun Iwamoto. Ambient oxygen regulates epithelial metabolism and nitric oxide production in the human nose. J Appl Physiol 93: 189–194, 2002.—The effects of ambient O2 tension on epithelial metabolism and nitric oxide (NO) production (VNO) in the nasal airway were examined in nine healthy volunteers. Nasal VNO, O2 consumption (VO2), and CO2 production (VCO2) were measured during normoxia followed by gradual hypoxia from 21 to 0% O2 concentration. Nasal VO2, VCO2, and respiratory quotient during normoxia were determined to be 1.19 ± 0.04 ml/min, 1.60 ± 0.04 ml/min, and 1.35 ± 0.04, respectively. Hypoxia exposure to the nasal cavity significantly decreased both VO2 and VNO (VO2: 1.60 ± 0.04 to 0.96 ± 0.08 ml/min (P < 0.01), VNO: 530 ± 15 to 336 ± 9 ml/min (P < 0.01)). VNO was reduced commensurately with gradual decline in O2 tension, and the apparent Km value for O2 was determined to be 23.0 μM. These results indicate that the nasal epithelial cells exchange O2 and CO2 with ambient air in the course of their metabolism and that nasal epithelial cells can synthesize NO by using ambient O2 as a substrate. We conclude that air-borne O2 diffuses into the epithelium where it may be utilized for either cell metabolism or NO synthesis.

The surface epithelium of airways has various physiological functions, including air conditioning, mucociliary clearance, and acting as a barrier against foreign bodies (28). In particular, ciliated cells, which have a high content of ATPase within cilia, provide these functions while consuming aerobic energy (27). Indeed, it has been demonstrated that O2 concentration in the sinus falls when the ostium is occluded in chronic sinusitis (2), suggesting that the sinus epithelium can continuously take up air-borne O2 and excrete CO2; i.e., gas exchange occurs. However, little is known of the metabolic properties and the source of O2 for epithelial metabolism.

Nitric oxide (NO), a highly diffusible gas, is synthesized enzymatically by NO synthase (NOS) from L-arginine and molecular O2, and it has various biological actions (20). In the nose, NOS is located in the superficial region of ciliated cells (7, 9, 25), and NO acts as an upregulator of mucociliary motion (13, 26) and as a host defense (3, 18). Extremely high concentrations of nasal NO have been found in exhaled air from the normal human nasal cavity (1, 17), but NO concentration is reduced in chronic sinusitis (16). On the other hand, exhaled NO output is reduced by hypoxia exposure in the human lower airways (6) and in isolated perfused rabbit lungs (11, 21, 29). In the human nasal cavity, NO output is depressed by hypoxia at O2 concentrations of <10% (8). However, the precise mechanisms responsible for the reduced nasal NO output by hypoxia remain unclear.

It has been demonstrated that cutaneous gas exchange of O2 and CO2 is a passive, diffusion-limited process in the amphibian skin (22). Furthermore, Km values for O2 have been determined in three isoforms of NOS from isolated cell preparations, suggesting that hypoxia limits the availability of substrate O2 for NOS (24). Thus we hypothesized that epithelial cell metabolism is associated with NO production and that both of these processes are regulated by ambient O2 tension. In the present study, we measured concentrations of NO, O2, and CO2 from the human nasal airway and examined the effect of varying O2 tension on the nasal NO and CO2 outputs.

METHODS

Subjects. Healthy adult volunteers with prior experience in respiratory physiology were recruited from hospital colleagues because the breathing maneuver required voluntary closure of the soft palate for several minutes. Subjects had no history of a recent upper airway infection or allergy. All subjects provided informed written consent. The study was approved by the Human Ethics Committee of Asahikawa Medical College.

Measurements of nasal NO, O2, and CO2. Nasal aspiration technique from nasal passages in series with closed soft palate was used to measure nasal NO, O2, and CO2 (4, 19). A schematic representation of the apparatus is shown in Fig. 1. In brief, a polystyrene tube was tightly attached into the vestibulum of one nostril to supply compressed NO-free air.

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containing 21% O₂-0.03% CO₂-78.97% N₂. The other nostril was connected to the same tube to draw gas inside the nose. To prevent outward leaks at the nostrils, the connected site was securely sealed with adhesive tape. Gas was aspirated from one side of the nostril to the other in series at a fixed flow of 300 ml/min. NO concentration was continuously measured by a chemiluminescence NO analyzer (model NOA 270B, Sievers, Boulder, CO) at the proximal site of the nasal orifice while O₂ and CO₂ concentrations were simultaneously monitored with a mass spectrometer (model Arco-1000, Arco System, Kashiwa, Japan). Each analog output of NO, O₂, and CO₂ has a signal-delay time that is caused by the response of machine and the dead space of sampling tube. Thus we adjusted the delay by using a computerized analog-to-digital and digital-to-analog converting device developed in our laboratory (12). The corrected signals were then transferred to a data acquisition system (MacLab, ADInstruments, Castle Hill, New South Wales, Australia) for real-time recordings and later analysis. Nasal NO production (VNO) was calculated by multiplying NO concentration [parts per billion (ppb)] by aspiration flow rate. Nasal O₂ consumption (VO₂) and CO₂ production (VCO₂) were calculated from the inflow-outflow O₂ and CO₂ differences multiplied by the aspiration flow rate. All values are presented at STPD conditions.

Subjects rested in a sitting position during the measurement. To isolate the nasal airway from the lower airway, the subject was instructed to take a shallow breath and to close the soft palate while watching a monitor displaying nasal CO₂ concentration. When the CO₂ concentration decreased to <1%, the nasal NO concentration reached a maximum steady plateau (Fig. 2). Inward leaks, produced by the opening of the soft palate, resulted in an abrupt rise in CO₂ concentration leaving the nose. When a leak was detected, the measurement was interrupted and repeated afterward. Subjects who could not close the soft palate were excluded from this experiment.

After the steady plateau level of nasal NO concentration was observed, 100% N₂ was slowly added to the inlet air while NO, O₂, and CO₂ concentrations were monitored. The O₂ concentration was gradually and progressively decreased from 21 to 0% for 5 min followed by a rapid increase to 21% (Fig. 3). VNO, VO₂, and VCO₂ were estimated during normoxic ventilation and at the end point of gradual hypoxia. The measurement was taken for one nostril and then for the contralateral nostril. Each measurement was repeated twice, and the average value of the individual measurements was used for further analysis.

Statistics. The differences in VNO or VCO₂ between normoxia and hypoxia were analyzed by ANOVA followed by a Scheffe’s post hoc t-test. The relationship between VNO and ambient O₂ level, which obeyed the Michaelis-Menten kinetics, was analyzed by the double-reciprocal method (Lineweaver-Burke plots) with linear least squares regression. In
RESULTS

Fourteen subjects (11 men and 3 women) attempted the experiment. However, five subjects failed to complete it because they were unable to maintain closure of their soft palate. Nine subjects (age range, 26–48 yr; all men) were successful.

When the nasal cavity was completely isolated from the lower airway, we found that O2 concentrations in nasal outflow were decreased compared with those in inflow, whereas CO2 concentrations in outflow were increased. From these results, we calculated nasal VO2 and VO2 during normoxia to be 1.19 ± 0.04 and 1.60 ± 0.04 ml/min, respectively (Table 1). In addition, the respiratory quotient (RQ) was >1 (1.35 ± 0.04).

Figure 3 illustrates representative recordings of the effects of gradual hypoxia on both NO and CO2 concentrations. Please note that the scale of CO2 concentration decreased commensurately with a gradual reduction in O2 concentration as well as CO2 concentration.

As shown in Fig. 4, VNO significantly decreased from 530 ± 15 nl/min during normoxia to 336 ± 9 nl/min (63% of control) at the end point of hypoxia (P < 0.01), and VCO2 significantly decreased from 1.60 ± 0.04 ml/min during normoxia to 0.96 ± 0.03 ml/min (60% of control) at the end point of hypoxia (P < 0.01).

In Fig. 5, the mean values of VNO are plotted against varying O2 concentrations, revealing a curvilinear relationship between VNO and O2 concentration. The shape of the curve resembles closely the plot for enzyme-substrate reaction that obeys Michaelis-Menten kinetics. By comparison, Kelley and DuBois (15) demonstrated that nasal superficial capillary blood flow was 13–16 ml/min out of a cardiac output of ~5,000 ml/min, or 0.3% of cardiac output. Although the tissue metabolism.

Table 1. Individual values of metabolic parameters during normoxia in the nasal cavity

<table>
<thead>
<tr>
<th>Subject No</th>
<th>VO2, ml/min</th>
<th>VCO2, ml/min</th>
<th>RQ</th>
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<tr>
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<td>1.30</td>
</tr>
<tr>
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</tr>
<tr>
<td>9</td>
<td>1.08</td>
<td>1.39</td>
<td>1.29</td>
</tr>
</tbody>
</table>

Mean ± SE 1.19 ± 0.04 1.60 ± 0.04 1.35 ± 0.04

VO2, O2 consumption; VCO2, CO2 production; RQ, respiratory quotient.

DISCUSSION

Nasal O2 and CO2 metabolism. We found that the nasal epithelium takes up ambient O2 and excretes CO2 under nornoxic conditions; i.e., epithelial gas exchange occurs. It has been reported that O2 is taken up by the sinus mucosa (2). However, little is known of the properties of cutaneous gas exchange in the nasal epithelium. In the present study, the nasal VO2 was determined to be 1.19 ml/min out of a body metabolic rate of ~250 ml/min, or 0.5% of total body metabolism. By comparison, Kelley and DuBois (15) demonstrated that nasal superficial capillary blood flow was 13–16 ml/min out of a cardiac output of ~5,000 ml/min, or 0.3% of cardiac output. Although the tissue metabolism.
is not necessarily proportional to the local blood flow, the higher percentage of the nasal metabolism than the blood flow indicates that, in addition to the O2 supplied from the blood, the nasal epithelium may consume much more O2 from the ambient air. With regard to the nasal CO2 production, 60% of control value was observed after 100% N2 exposure, suggesting that this CO2 may come from the O2 supplied from the blood in the course of ATP turn over rather than from the O2 from the ambient air.

On the other hand, the value of RQ we found in the nasal cavity (~1.35) is higher than that for the gas exchange of the mammalian lung; it is consistent with the value obtained for cutaneous respiration in amphibians (23). Hence, the human nasal epithelium may have metabolic functions similar to amphibian skin. It has been demonstrated that cutaneous gas exchange of O2 and CO2 is a passive, diffusion-limited process (24). Nevertheless, the Lineweaver-Burke plot between nasal VO2 and nasal VNO. Apparent Km value and Vmax are also shown. Error bars are omitted.

Nasal NO. Most of the nasal lumen is covered by densely ciliated columnar cells. This epithelium rests on a layer of collagen fibrils called the basement membrane. Beneath the basement membrane, there is the submucosa, which is rich in blood vessels. Because NO produced under the basement membrane would be trapped by hemoglobin circulating in blood vessels, it is likely that most of the NO excreted into the nasal cavity originates from the surface epithelium, which stains immunologically for NOS (7). In addition, NO concentration in the sinuses is extremely high and the sinus NO continues to diffuse into the nasal cavity (17). To estimate the net NO production in the nose, it is necessary to take into account the amount of NO removed by the processes of absorption and/or chemical reaction. As expected, according to the analysis by Dubois et al. (5), the amount of NO absorbed on the way through the nose was small in the present study because the gas flow rate we used was sufficiently high (300 ml/min).

In the present study, ventilation with 100% N2 gas in the nasal cavity elicited 37% suppression in NO output, with the remainder being 63%. In our previous study, however, inhalation of 100% N2 gas almost completely suppressed exhaled NO output in isolated perfused rabbit lungs (11). The most likely reason why 100% N2 did not fully eliminate nasal NO production could be that NO remained to diffuse into the nasal cavity from the sinuses, even during 100% N2 exposure, because O2 concentration in the sinuses was not decreased enough as a result of poor ventilation of the sinuses. Another possible explanation is that the blood-borne O2, which is required for tissue metabolism, may, in part, contribute to the remaining NO output. In support of this, both nasal vasoconstriction induced by a topical decongestant xylometazoline and reductions in the blood O2 content by maximal breath holding-elicited decreases in baseline levels of nasal NO output (8).

In the nasal airway, NOS has been identified in the epithelium close to the ciliated surface and within the lamina propria in nerves and vascular endothelium (7, 9, 25). Furthermore, NO is synthesized enzymatically by NOS from L-arginine and molecular O2 (20). It has been clearly demonstrated that NO production is regulated by ambient O2 tension through a mechanism that obeys Michaelis-Menten kinetics in the human lower airways (6) and in isolated perfused rabbit lungs (11, 29). In the human nasal cavity, NO output was depressed by hypoxia at O2 concentrations of <10% (8). In the present study, we found that nasal NO output was dependent on luminal O2 concentrations over the range from 0 to 21%. To estimate the NO kinetics, we simply analyzed the suppressed components of NO output by hypoxia because the remaining NO components seemed to account for NO from the sinuses or NO originated from the blood-borne O2 as mentioned previously. As a result, we could determine the apparent Km value for O2 to be 23.0 μM. These results indicate that the nasal epithelial cells take up ambient O2 and excrete NO into the nasal cavity in proportion to nasal O2 tension. It has been shown that there is an O2 gradient from the extracellular medium to the cytoplasm (14) and the plasma membrane acts as a minimal barrier to O2 diffusion (10). Nevertheless, the Lineweaver-Burke plot between nasal VO2 and O2 concentration displayed a linear relationship, indicating that nasal NO is synthesized by using the ambient O2 molecules as a substrate and that hypoxia limits the O2 available to NOS. The Km value obtained from the present study is close to the values (14.4 and 24.1 μM) obtained from isolated perfused rabbit lungs (11, 29) and the value (7.7 ± 1.6 μM) from cultured bovine aortic endothelial cells (24), suggesting that there are not huge species differences in the availability of O2 for NOS.

In contrast to these values, Dweik et al. (6) estimated the Km value to be 190 μM in the human lower airways. We, however, speculate that this higher Km value than what we reported in the present study may have resulted from these investigators failing to measure NO output.
(nl/min) and the fact that they obtained only a few data points below 21% O2.

**Significance of nasal NO and metabolism.** The apical region of the ciliated cell is filled with abundant mitochondria to supply energy for motion of cilia, and the dynein arm of cilia contains high content of ATPase to support its motility (27). NOS is located superficially within ciliated cells (25), and ciliary beat frequency is upregulated by NO (13, 26). Furthermore, NO exerts protective effects against viral and bacterial infections (3, 18). Thus it is likely that O2 demand and NO production are closely associated with both mucociliary function and host defense. In the present study, we found that hypoxia equally suppressed nasal VCO2 and VNO to 60% vs. 63% of control, respectively. With regard to structure and function considerations, both aerobic metabolism and NO production are limited by diffusion of O2, suggesting that mitochondrial respiration and NO synthesis in the epithelium require air-borne O2.

In chronic sinusitis, O2 tension in the sinus falls as a result of ostial dysfunction (2) while nasal NO levels are also markedly reduced (16). Therefore, it is likely that impaired ventilation through the ostium would lead to an O2 deficiency followed by a decline in NO production, resulting in disrupted mitochondrial respiration, mucociliary dysfunction, and bacterial infection, thereby bringing about epithelial damage. On the other hand, because not only the nasal cavity but also the lower respiratory tracts are covered with ciliated cells, epithelial metabolism and NO production in the entire airways are likely regulated as functions of ambient O2 levels. Indeed, it has been shown that exhaled NO from the lower airways is reduced by hypoxic gas inhalation in humans (6) and in isolated perfused rabbit lungs (11, 21, 29). Thus air-borne O2 may play an important role in both epithelial metabolism and airway function via NO.

In conclusion, we have demonstrated that nasal NO production is, in part, regulated by ambient O2 tension and that gaseous hypoxia in the nose diminishes epithelial cell metabolism. From these results, we conclude that air-borne O2 is taken up through the surface of nasal epithelia where it may be utilized for either cell metabolism or NO synthesis.

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