Measurement of exhaled nitric oxide in man

Colin Borland, Yolande Cox, Tim Higenbottam

Abstract

Background—Nitric oxide is released from pulmonary endothelial cells and contributes to the low pulmonary vascular resistance. The resistance pulmonary arteries are in close anatomical proximity to membranous airways, so it is likely that some pulmonary endothelial nitric oxide will enter the airspace to allow its measurement in the exhaled breath.

Methods—Exhaled air was collected from a single full exhalation and during tidal breathing. This was analysed for concentrations of nitric oxide, nitrogen dioxide, and carbon dioxide to give alveolar (FA) and mixed expired (Fé) concentrations. Eight normal subjects were studied and laboratory air was similarly analysed using, respectively, chemiluminescent and infrared analysers.

Results—There was no relation between FA concentrations and the laboratory air concentrations. From the single breath, the ratio of (FAN0/FACO) × (FECO/FENO) had a mean value of 0.64 (95% confidence interval 0.7 to 1.14). As this does not differ from unity, nitric oxide is likely to be derived from the same regions of the lungs as carbon dioxide. During tidal breathing the FENO ranged from 8.3 to 20.3 parts per billion.

Conclusions—It is possible to measure endogenous pulmonary nitric oxide production in the exhaled air in man.

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Nitric oxide (NO) is a ubiquitous gaseous free radical which acts as an intracellular messenger with a range of regulatory roles in different tissues. In the endothelium, nitric oxide functions in vascular signalling and is responsible for the activity of endothelium derived relaxing factor. Released by pulmonary endothelium, nitric oxide contributes to the characteristically low pulmonary vascular resistance. Hypoxic lung disease may cause a reduction in pulmonary endothelium production. Inhaled concentrations of 40 parts per million (ppm), nitric oxide is a powerful selective pulmonary vasodilator. Implicit in this observation is the idea that gaseous nitric oxide can be exchanged between airspace and smooth muscle cells of resistance pulmonary arteries, which are in close proximity to membranous airways.

The single breath measurement involved a full breath to TLC, then a full exhalation to residual volume (RV). The last litre of air was collected into a PVC bag from which alveolar concentrations (FA) of nitric oxide, nitrogen dioxide, and carbon dioxide were measured. The mixed expired concentrations (Fé) were also measured from air collected through the full exhalation. All subjects undertook three measurements of each without holding breath.
Exhaled nitric oxide in man

Mean (SD) single breath and tidal breathing exhaled gas concentrations for nitric oxide (NO) and carbon dioxide (CO₂). Alveolar (Fₐ) and mixed expired (Fₑ) concentrations are shown for both NO and CO₂. The molar rate of output of the lungs is shown for tidal breathing (MNO).

<table>
<thead>
<tr>
<th>Subject</th>
<th>FANO (× 10⁻⁶)</th>
<th>FACO₂ (× 10⁻⁶)</th>
<th>FENO (× 10⁻⁶)</th>
<th>FECO₂ (× 10⁻⁶)</th>
<th>FENO/FACO₂</th>
<th>FENO/FANO</th>
<th>MNO (10⁻³ mol/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>4.7(0.6)</td>
<td>3.8(0.6)</td>
<td>4.7(0.6)</td>
<td>3.7(0.1)</td>
<td>12(1.1)</td>
<td>8.3(1.5)</td>
<td>3.6(0.9)</td>
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<tr>
<td>2</td>
<td>9.0(1.0)</td>
<td>5.1(0.3)</td>
<td>5.7(1.5)</td>
<td>3.3(0.2)</td>
<td>8.2(4.8)</td>
<td>17.3(4.6)</td>
<td>5.6(4.6)</td>
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<tr>
<td>3</td>
<td>14(0.2)</td>
<td>5.4(0.4)</td>
<td>12.3(2.5)</td>
<td>3.0(0.2)</td>
<td>8.7(2.6)</td>
<td>14.3(0.6)</td>
<td>4.0(1.0)</td>
</tr>
<tr>
<td>4</td>
<td>4.7(0.6)</td>
<td>3.8(0.6)</td>
<td>4.7(0.6)</td>
<td>3.7(0.1)</td>
<td>8.2(4.8)</td>
<td>17.3(4.6)</td>
<td>5.6(4.6)</td>
</tr>
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<td>5</td>
<td>11(0.5)</td>
<td>5.2(0.6)</td>
<td>7.3(1.5)</td>
<td>3.8(0.1)</td>
<td>9.3(0.8)</td>
<td>13.0(4.0)</td>
<td>5.0(1.3)</td>
</tr>
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<td>6</td>
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<td>6.0(1.7)</td>
<td>3.6(0.1)</td>
<td>8.3(0.1)</td>
<td>20.3(2.5)</td>
<td>6.5(0.3)</td>
</tr>
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<td>6.8(1.5)</td>
<td>3.5(0.1)</td>
<td>8.9(0.9)</td>
<td>15.9(2.9)</td>
<td>5.3(1.9)</td>
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<tr>
<td>8</td>
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<td>5.0(1.0)</td>
<td>3.5(0.1)</td>
<td>7.8(0.5)</td>
<td>17.7(1.2)</td>
<td>5.5(0.4)</td>
</tr>
<tr>
<td>Mean</td>
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<td>4.9</td>
<td>6.5</td>
<td>3.4</td>
<td>9.4</td>
<td>14.7</td>
<td>5.0</td>
</tr>
<tr>
<td>SD</td>
<td>3.3</td>
<td>0.6</td>
<td>2.8</td>
<td>0.3</td>
<td>1.6</td>
<td>3.8</td>
<td>0.9</td>
</tr>
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</table>
phric nitric oxide (FINO) by minute ventilation (obtained from Vr and f), and this product was then multiplied by 10^6/24.04 (10^-9 converts ppb to fractional concentration and 24.04 converts fractional concentration to molar concentrations at atmospheric temperature and pressure and water saturation). The MNO ranged from 3.6 to 6.5 × 10^-9 mol/min (table). The standard deviation of these mean values ranged from 0.3 to 4.6 mol/min.

Discussion

The concentration of nitric oxide in exhaled air varies in healthy subjects from 8.3 to 20.3 ppb during tidal breathing. From the single breath test, using the fractional alveolar and expired concentrations of nitric oxide and carbon dioxide, it can be predicted that the exhaled nitric oxide was derived from a similar region of the lung as carbon dioxide. An environmental source can be excluded, as the concentration of exhaled nitric oxide exceeded the concentration in laboratory air (fig). This contrasts with exhaled nitrogen dioxide, a gas known to interact with the lungs but which was shown not to be endogenously produced. The concentration of nitrogen dioxide in exhaled air was lower than environmental nitrogen dioxide. An endogenous source for exhaled nitric oxide was further supported by the experimental finding in guinea pigs and rabbits that inhibition of the nitric oxide synthase resulted in a fall in exhaled nitric oxide. Studies in isolated lungs perfused with colloid solutions further confirm this finding and suggest that pulmonary endothelium is the source of exhaled nitric oxide.

Cultured endothelial cells are capable of a substantially greater molar rate of production of nitric oxide than we observed during tidal breathing where the rates ranged from 3.6 to 6.5 × 10^-9 mol/min. However, exhaled nitric oxide will reflect only part of the nitric oxide production by pulmonary endothelial cells. Nitric oxide is the fastest known ligand of haemoglobin and is rapidly taken up by the lungs when inhaled in concentrations of parts per million, some 4-5 times faster than 0.2% of carbon monoxide. It can be assumed that much of the endothelial nitric oxide is taken up by circulating red cells. Only abuminally produced nitric oxide from the endothelial cells is likely to reach the airspace, and then only that not taken up by vascular smooth muscle cells. A complex dynamic exchange is likely to exist between the blood, the nitric oxide producing pulmonary endothelial cells, and the airspaces. Much remains unclear as to the nature of this dynamic process but breathhold at TLC for 60 s clearly alters the nitric oxide output in exhaled air. This, in part, may be explained by the increased diffusing capacity for nitric oxide (TLNO) which is seen at the increased lung volume at TLC. Increased TLNO at TLC may also explain the lowest value for FENO on maximal exhalation compared with spontaneous breathing (table).

It is therefore possible to measure endogenously produced nitric oxide in man from exhaled air. Strong circumstantial experimental evidence leads us to infer that it is derived from pulmonary endothelium at a similar site in the lungs to the evolution of carbon dioxide—that is, close to and including alveoli. The measurement from mixed expired air collected over five minutes of tidal breathing is more reproducible than from a single breath—dependent pulmonary blood flow is inactivated by enhanced TLNO at TLC. These measurements of exhaled nitric oxide may have particular importance in clinical practice in such disorders as hypoxic chronic obstructive lung disease where there is evidence of impaired pulmonary endothelial production of nitric oxide.4

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