Effect of hyperbaric and normobaric oxygen on pulmonary endothelial cell function

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Allen MC, Watt SJ. Effect of hyperbaric and normobaric oxygen on pulmonary endothelial cell function. Undersea & Hyperbaric Med 1993; 20(1):39–48.—The effect of prior exposure to raised partial pressures of oxygen on pulmonary endothelial cell function was assessed in the isolated perfused rat lung preparation. Prolonged exposure to both 1 bar (48 h) and 2.5 bar (11 h) of oxygen caused pulmonary edema and dyspnea. Exposure to 1 bar oxygen for 48 h (approximately 0.66 lethal duration) caused a decrease in pulmonary 5-hydroxytryptamine (5-HT) clearance, suggesting compromised endothelial cell integrity. No change in 5-hydroxyindole acetic acid (5-HIAA) efflux was noted. However, exposure to 2.5 bar of oxygen for up to 11 h (approximately 0.85 lethal duration) did not decrease pulmonary 5-HT clearance, implying that endothelial cell integrity was not compromised after this oxygen exposure. Exposure to 2.5 bar oxygen resulted in a reduction in 5-HIAA efflux, possibly indicating a decrease in metabolism of cleared 5-HT. The absence of a demonstrable impairment of 5-HT clearance during the development of pulmonary toxicity on exposure to 2.5 bar of oxygen suggests that there may be important differences in pathologic mechanisms in response to oxygen exposure at partial pressures 1 and 2.5 bar.

5-hydroxytryptamine, normobaric, hyperbaric, oxygen

Breathing oxygen at elevated partial pressure is used therapeutically in many medical situations and by the diving industry during decompressions and in treatment of decompression sickness. However, breathing high partial pressures of oxygen (PO₂) may be associated with the development of pulmonary oxygen toxicity dependent on both the oxygen partial pressure and duration of exposure. Histologically, one of the earliest changes observed after exposure to a PO₂ of 1 bar is damage to the endothelial cells of the pulmonary circulation (1–3). These endothelial cells are responsible for the clearance of a number of circulating biologically active substances (4). It has been demonstrated by a number of workers that exposure to a PO₂ of 1 bar leads to a decrease in the clearances of noradrenaline, bradykinin, 5-hydroxytryptamine (5-HT), and angiotensin 1, all of which are normally endothelial cell functions (5–8).

To date, published histologic investigations have concentrated on the effect of exposure to a PO₂ of 1 bar or less, and most clearance studies have investigated the
effects of a PO$_2$ of 1 bar, although a few studies have used a PO$_2$ greater than normally encountered either in the diving industry or therapeutically (PO$_2$ = 4 bar) (5). Such a high PO$_2$ has the additional complication of concomitant CNS oxygen toxicity, which is recognized to potentiate pulmonary damage (9).

This study has investigated the effect of a range of PO$_2$'s encountered clinically or during diving (PO$_2$'s 0.21–2.5 bar) on pulmonary endothelial cell function assessed by monitoring pulmonary 5-HT clearance in the isolated perfused lung preparation, a technique that has been widely used in assessment of biochemical function of the pulmonary endothelium (6–8).

METHODS

Male Sprague–Dawley rats (230–280 g) were exposed to air or 100% oxygen at either 1 or 2.5 bar. Rats were placed in individual cages and allowed Oxoid breeding diet and water ad libitum. Caged rats were placed (maximum three at a time) in a Perspex pressure chamber. During the exposure the temperature was controlled within limits of 20° ± 2°C, humidity to 65 ± 15% and carbon dioxide to less than 0.006 bar. The chamber was flushed with the appropriate gas at a minimum of 1 liter · rat$^{-1}$ · min$^{-1}$.

After exposure, rats were anesthetized with urethane (1.5 g/kg i.p.). After tracheal cannulation, the chest cavity was opened and 500 IU of heparin injected into the right ventricular cavity. The heart, lungs, and trachea were dissected free, the apex of the heart cut away, and the pulmonary artery cannulated. The pulmonary circulation was flushed with 5 ml of Krebs solution containing heparin (100 IU/ml) before suspension in the perfusion apparatus (Fig. 1). The lungs were inflated with 10 ml of air, and the pulmonary circulation perfused with Krebs solution at 36°–37°C at a flow rate of 5 ml/min.

After an initial 30-min perfusion, a 1-min perfusate sample was collected (5 ml) and the preparation was perfused for 5 min with Krebs solution containing 5-HT (0.052–0.52 μM). Effluent perfusate samples (5 ml) were collected every minute. After a 5-min infusion of Krebs solution containing 5-HT, perfusion continued for

![FIG. 1—a schematic diagram of the isolated perfused lung.](image-url)
a further 10 min with normal 5-HT-free Krebs, and 1-min samples (5 ml) were collected 1, 2, 3, 4, and 10 min after the reversion to 5-HT-free Krebs.

Effluent perfusate samples were analyzed for 5-HT and its metabolite 5-hydroxyindole acetic acid (5-HIAA) by isocratic reverse-phase high pressure liquid chromatography using electrochemical detection. The buffer was a 0.2-M citrate-acetate buffer (pH 5.2) containing 500 mg/liter 1-octanesulfonic acid as an ion pairing agent, and 10% methanol. The buffer flow rate was 0.6 ml/min. The column used was a reverse phase Zorbax C8 column, run at room temperature with a 20-μl injection loop. Detection was with an electrochemical transducer using a glass carbon electrode (+ 0.6 V).

A complete calibration curve of peak height against concentration was constructed for 5-HT and 5-HIAA before every analysis of perfusate samples from two pairs of lungs. The concentrations of 5-HT and 5-HIAA in the perfusate were plotted against time. The clearance of 5-HT was estimated from the maximum 5-HT concentration in the effluent.

Clearance = 100–100 · maximum 5-HT concentration/ perfusate concentration

In addition, the areas under the 5-HT and 5-HIAA effluent concentration curves were estimated by the trapezium rule. Multiplying these areas by the Krebs flow rate (5 ml/min) gave the total amount of 5-HT surviving pulmonary transit and the total amount of 5-HIAA eluting from the lungs. Consequently, a second method of calculating 5-HT clearance is from the total amount of 5-HT surviving pulmonary transit.

Clearance = 100–100 · total amount of 5-HT surviving pulmonary transit/ total amount of 5-HT perfused into the lung

The changes in wet lung weight due to exposure were assessed in other animals. Rats were anesthetized as before, the trachea was cannulated, the chest cavity opened, and 500 IU of heparin was injected into the right ventricular cavity. The heart, lungs, and trachea were dissected free, the apex of the heart cut away, and a cannula tied into the pulmonary artery. The pulmonary circulation was gently flushed with 10 ml of normal saline containing 100 IU/ml of heparin. All non-pulmonary tissue including bronchi and trachea were removed, and the lungs were blotted and weighed.

Groups of rats were exposed to the following environmental conditions a) control (no chamber exposure), b) control chamber exposure to air at 1 bar for 48 h, c) 100% oxygen at 1 bar for 48 h, d) 100% oxygen at 2.5 bar for 9 h, and e) 100% oxygen at 2.5 bar for 11 h. The isolated lungs from subgroups were subsequently perfused with 5-HT at concentrations of 0.052, 0.13, and 0.52 μM. The mean peak 5-HT concentrations in the effluent or the total 5-HT–5-HIAA effluent contents were compared between groups using Student’s t test with a Bonferonni correction to account for multiple comparisons. Values presented are the mean ± SEM; a P value of <0.05 was considered significant.

RESULTS

The results (Tables 1 and 2) show 5-HT clearance based on both peak effluent concentration and total effluent content. These two methods gave identical results, so only those based on peak concentration will be discussed further.
### Table 1: Clearance and Metabolism of 5-HT by Pulmonary Endothelial Cells in the Isolated Rat Lung

<table>
<thead>
<tr>
<th>Oxygen Exposure (PO₂, bar)</th>
<th>Control</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>Control (air 0.21)</th>
<th>Control</th>
<th>Chamber 48 h</th>
<th>Chamber air 48 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>Exposure Duration, hours</td>
<td>20</td>
<td>6</td>
<td>13</td>
<td>48</td>
<td>5</td>
<td>6</td>
<td>13</td>
<td>48</td>
</tr>
<tr>
<td>Peak 5-HT Conc. µM</td>
<td>0.032</td>
<td>0.13</td>
<td>0.52</td>
<td>0.052</td>
<td>5.9</td>
<td>0.032</td>
<td>0.52</td>
<td>0.052</td>
</tr>
<tr>
<td>Total 5-HIAA Effluent Content, nmol</td>
<td>0.627 (0.021)</td>
<td>20.99 (3.4)</td>
<td>136.3 (13.3)</td>
<td>5.9 (1.57)</td>
<td>0.638 (0.031)</td>
<td>20.99 (3.4)</td>
<td>136.3 (13.3)</td>
<td>5.9 (1.57)</td>
</tr>
<tr>
<td>Percent Clearence</td>
<td>88</td>
<td>84</td>
<td>74</td>
<td>89</td>
<td>90</td>
<td>89</td>
<td>74</td>
<td>89</td>
</tr>
<tr>
<td>Percent Clearance Derived From Total 5-HT Efflux</td>
<td>89</td>
<td>84</td>
<td>74</td>
<td>89</td>
<td>90</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Represents total amount of 5-HIAA appearing in the effluent. The figures in brackets are the SEM.*
Table 2: Effect of Oxygen Exposure on the Clearance and Metabolism of 5-HT by Pulmonary Endothelial Cells in the Isolated Rat Lung

<table>
<thead>
<tr>
<th>Oxygen Exposure (P0₂), bar</th>
<th>Exposure Duration, hours</th>
<th>n</th>
<th>5-HT Perfusate Conc., μM</th>
<th>Peak 5-HT Conc in Effluent, nM</th>
<th>Percent Clearance Based On Peak 5-HT Conc in Effluent, %</th>
<th>Total 5-HIAA Effluent Content, nmol(^a)</th>
<th>Total 5-HT Effluent Content, nmol(^b)</th>
<th>Percent Clearance Derived From Total 5-HT Efflux</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0</td>
<td>20</td>
<td>0.052</td>
<td>6.5</td>
<td>88</td>
<td>0.627 (0.021)</td>
<td>0.138 (0.0129)</td>
<td>89</td>
</tr>
<tr>
<td>1</td>
<td>48</td>
<td>8</td>
<td>0.052</td>
<td>11.66(^d) (1.13)</td>
<td>77</td>
<td>0.528 (0.058)</td>
<td>0.259 (0.0205)</td>
<td>80</td>
</tr>
<tr>
<td>2.5</td>
<td>9</td>
<td>5</td>
<td>0.052</td>
<td>6.7</td>
<td>87</td>
<td>0.596 (0.047)</td>
<td>0.134 (0.0153)</td>
<td>90</td>
</tr>
<tr>
<td>2.5</td>
<td>11</td>
<td>6</td>
<td>0.052</td>
<td>5.68</td>
<td>89</td>
<td>0.471 (0.037)</td>
<td>0.135 (0.0231)</td>
<td>93</td>
</tr>
<tr>
<td>Control</td>
<td>0</td>
<td>13</td>
<td>0.52</td>
<td>13.63 (13.3)</td>
<td>74</td>
<td>3.896 (0.246)</td>
<td>3.33 (0.308)</td>
<td>74</td>
</tr>
<tr>
<td>1</td>
<td>48</td>
<td>6</td>
<td>0.52</td>
<td>123.4</td>
<td>76</td>
<td>3.457 (0.252)</td>
<td>2.731 (0.322)</td>
<td>79</td>
</tr>
<tr>
<td>2.5</td>
<td>9</td>
<td>7</td>
<td>0.52</td>
<td>175</td>
<td>67</td>
<td>2.662 (0.350)</td>
<td>4.237 (0.89)</td>
<td>67</td>
</tr>
<tr>
<td>2.5</td>
<td>11</td>
<td>8</td>
<td>0.52</td>
<td>138.1</td>
<td>73</td>
<td>2.645 (0.219)</td>
<td>3.09 (0.692)</td>
<td>76</td>
</tr>
</tbody>
</table>

\(^a\)Represents total amount of 5-HIAA appearing in the effluent from time 0 to 15 min.  \(^b\)Represents total amount of 5-HT surviving transit of the pulmonary vasculature.  \(^d\)Figures in brackets are the SEM.  \(^\ast\)Significantly different from control (P < 0.05).
Control animals demonstrated clearance of 5-HT from the perfusate which was concentration dependent, falling from 89% at a perfusate concentration of 0.052 μM to 74% at 0.52 μM (Table 1). 5-HIAA efflux was also dose dependent, rising with perfusate 5-HT concentration (Fig. 2). Mean lung wet weight was 1.19 ± 0.02 g (n = 13).

Rats exposed to air in the exposure chamber for 48 h had similar results. These animals were unaffected by chamber pressure and were not dyspneic. On dissection there was no evidence of pulmonary edema or pleural effusion. Rats exposed to 100% oxygen at 1 bar for 48 h were dyspneic and on dissection had evidence of pulmonary edema with pleural effusions. Lung wet weight was significantly increased to 2.25 ± 0.17 g (P < 0.05, n = 8). Clearance of 5-HT was significantly reduced at a perfusate concentration of 0.052 μM (77 vs. 88%) but not at a perfusate concentra-
PULMONARY ENDOTHELIAL CELL FUNCTION

tion of 0.52 μM (76 vs. 74%). 5-HIAA efflux was not significantly different from control results (Fig. 3, Tables 1 and 2). Rats exposed at 2.5 bar oxygen were also noted to be dyspneic. This became progressively more marked as exposure time increased. On dissection, the animals had evidence of pulmonary edema but pleural effusions were not found. Lung wet weight was increased compared to control at 2.0 ± 0.18 g (P < 0.05, n = 9, 11 h exposure). 5-HT clearance was unaffected at perfusate concentration 0.052 μM after either 9 or 11 h exposure (Fig. 3). A marginal reduction in 5-HIAA efflux occurred after 11 h but not after 9 h. Similarly, no change in 5-HT clearance was noted at a perfusion concentration of 0.52 μM after 9 or 11 h exposure, although a marginal reduction in 5-HIAA efflux was observed.

DISCUSSION

In control animals, clearance of 5-HT decreased as perfusate concentration of 5-HT increased, consistent with previous studies (10). This was anticipated for two reasons: a) 5-HT removal is a saturable carrier-mediated process (11), and b) it is a vasoconstrictor which may well shut down vascular beds and decrease the effective vascular bed area at high concentrations. However, Alabaster and Bakhle (12) demonstrated that only concentrations of 5-HT above 40 ng/m (0.104 μM) were associated with pulmonary vasoconstriction so that only the higher infused concentrations are

![Graphs showing concentration of 5-HT and 5-HIAA over time](image)

**FIG. 3**—Plot of 5-HT (solid circles) and 5-HIAA (open circles) concentration in the pulmonary effluent. Lungs were obtained from rats exposed to, a, control, n = 20; b, air (1 bar, 48 h, n = 5); c, oxygen (1 bar, 48 h, n = 8); d, oxygen (2.5 bar, 11 h, n = 6). Infused 5-HT concentration was 0.052 μM. Each point is the mean ± SEM.
likely to be associated with possible vasoconstriction (12). Control lungs demonstrated that perfusion with increasing amounts of 5-HT led to the appearance of increasing amounts of 5-HIAA in the effluent; the metabolite usually appearing in the effluent after 5-HT (Fig. 2b, c). However, infusion of low concentrations of 5-HT resulted in the earlier appearance of 5-HIAA. This may be due to the appearance of endogenous as well as exogenous 5-HIAA in the effluent or may represent differences in vascular perfusion while perfusing with differing 5-HT concentrations.

We also found the clearance of lower concentrations of 5-HT to be a more sensitive indicator of changes in endothelial cell function induced at 1 bar of oxygen. This is in agreement with previous published results. Ben-Harari et al. (13) infused a high concentration of 10 µM and were unable to detect a decrease in 5-HT clearance. Toivonen et al. (6) infused a concentration of 0.4 µM of 5-HT and were only able to detect changes in 5-HT metabolism after 60 h exposure to 1 bar of oxygen. Finally, Block and Fisher (14) infused a concentration of 0.25 µM and were able to detect a decrease in 5-HT clearance after 18 h exposure to 1 bar of oxygen. Our results after 48 h exposure at 1 bar of impaired clearance at perfusate concentration of 0.052 µM but not at 0.52 µM are therefore consistent. Low concentrations of 5-HT may be more sensitive to changes in 5-HT clearance for several reasons. First, low concentrations are sensitive to changes in km and Vmax of the uptake process, whereas high saturating concentrations will only be sensitive to changes in Vmax. However, all concentrations used here are well below the estimated km of 2.7 µM (15) so this explanation is unlikely. Second, at high concentrations of 5-HT, removal into the endothelial cell may be partially the result of diffusion, which will not be affected by changes in carrier transport. Finally, if the percentage clearance is high, as at low perfusate concentrations, then even a small change in the amount of 5-HT cleared will produce a relatively large change in the amount of 5-HT surviving pulmonary transit. This study along with others has demonstrated in vitro changes in pulmonary 5-HT clearance due to prior exposure to 1 bar oxygen. In addition, these changes have been confirmed in vivo, because Dobuler et al. (16) have shown changes in 5-HT clearance during a single pulmonary transit in conscious rabbits. This confirms that changes in clearance seen in vitro are representative of changes in vivo.

Exposure to 1 bar of oxygen led to a decrease in 5-HT (0.052 µM) clearance, but the reduction of 5-HIAA appearing in the perfusate did not achieve statistical significance. A decrease in 5-HT uptake (clearance) might be expected to result in both an increase in 5-HT surviving pulmonary transit and a decrease in 5-HIAA synthesis and efflux because uptake is the rate-limiting step in 5-HIAA formation (5). It must be remembered however, that although exposure to 1 bar of oxygen leads to an 80% increase in 5-HT surviving pulmonary transit, this represents only an 11% reduction in 5-HT uptake into endothelial cells. Consequently there is little change in substrate availability for monoamine oxidase (MAO). Exposure to 1 bar of oxygen also caused pulmonary edema resulting in dyspnea and an increase in lung wet weight. In addition, pleural effusions formed. These signs are well recognized (17).

Exposure to 2.5 bars of oxygen, however, produced some intriguing results. As expected it caused pulmonary edema with dyspnea and an increase in wet lung weight. However, unlike 1 bar exposures, no decrease in 5-HT clearance could be demonstrated. There was a decrease in 5-HIAA formation. Pleural effusions were not found, although the animals seemed equally dyspneic compared to those exposed at 1 bar.
PULMONARY ENDOTHELIAL CELL FUNCTION

The reasons for the difference between 1- and 2.5-bar exposures are unclear, although there are a number of possible explanations. The first is related to the duration of exposure. Endothelial cell damage may be a secondary effect that develops slowly, the animals dying of a primary pathology not involving endothelial cell function. It may be that at 2.5 bar the primary pathology occurs before endothelial damage develops. The duration of our studies at 2.5 bar was based on pilot experiments, which noted that the LD₅₀ at 2.5 bar is between 12 and 13 h; 9–11–h exposure therefore represents approximately 65–85% of the lethal exposure time, a similar proportion to 48 h at 1 bar (17). In addition, exposure times were long enough to cause dyspnea and pulmonary edema equally severe as that caused by 1-bar exposures. Longer exposures were therefore not possible. Another explanation is that an alternative pathology caused pulmonary edema but no endothelial cell damage at 2.5 bar. It may be that in the absence of this alternative pathology, exposures could be of longer duration and that eventually endothelial cell damage would result. Whatever the explanation, it suggests that more than one pathologic process is involved in pulmonary oxygen toxicity. Verification of this differential damage to endothelial cells might be obtained by a quantitative electronmicroscopic assessment of lungs from rats exposed to oxygen partial pressures greater than 1 bar. The 2.5-bar exposures demonstrated no change in 5-HT clearance (and hence 5-HT transport into the endothelial cell), but there was a significant fall in 5-HIAA efflux. The possible causes of this are as follows: a) Impairment of MAO A activity during exposure to 2.5 bars of oxygen. b) A decrease in aldehyde dehydrogenase activity. c) A decrease in the ability of the metabolite to diffuse from the endothelial cell.

Ben-Harari et al. (13) have shown that exposure of rats for 50 h to 1 bar of oxygen does not affect MAO A activity. However MAO A activity may be affected at higher partial pressures. A decrease in aldehyde dehydrogenase activity would decrease 5-HIAA production as it is the enzyme that generates 5-HIAA from 5-hydroxyindole-3-acetaldehyde. It has been shown that exposure of rats to HBO (60 psi) leads to a fall in dehydrogenase activity in rat lungs (18), although the dehydrogenase affected was not determined. Whatever the explanation for a decrease in 5-HIAA efflux, it was not seen after 1-bar exposures.

This study suggests that endothelial cell membranes may be an important site of damage after exposure to 1 bar of oxygen. This damage is associated with changes in membrane function such as 5-HT transport. Membrane damage is less likely to be a primary component to the pathology at oxygen partial pressures of 2.5 bar. However, these results would be consistent with cell membrane damage at 1 bar and intracellular biochemical changes at an oxygen partial pressure of 2.5 bar. Histologic and electronmicroscopic studies of lungs from rats exposed to 2.5 bars of oxygen would be valuable in further investigation of this phenomenon.

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