Hyperventilation Induces Release of Cytokines from Perfused Mouse Lung

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Artificial mechanical ventilation represents a major cause of iatrogenic lung damage in intensive care. It is largely unknown which mediators, if any, contribute to the onset of such complications. We investigated whether stress caused by artificial mechanical ventilation leads to induction, synthesis, and release of cytokines or eicosanoids from lung tissue. We used the isolated perfused and ventilated mouse lung where frequent perfusate sampling allows determination of mediator release into the perfusate. Hyperventilation was executed with either negative (NPV) or positive pressure ventilation (PPV) at a transpulmonary pressure that was increased 2.5-fold above normal. Both modes of hyperventilation resulted in an approximately 1.75-fold increased expression of tumor necrosis factor α (TNF-α) and interleukin-6 (IL-6) mRNA, but not of cyclooxygenase-2 mRNA. After switching to hyperventilation, prostacyclin release into the perfusate increased almost instantaneously from 19 ± 17 pg/min to 230 ± 160 pg/min (PPV) or 115 ± 87 pg/min (NPV). The enhancement in TNF-α and IL-6 production developed more slowly. In control lungs after 150 min of perfusion and ventilation, TNF-α and IL-6 production was 23 ± 20 pg/min and 330 ± 210 pg/min, respectively. In lungs hyperventilated for 150 min, TNF-α and IL-6 production were increased to 287 ± 180 pg/min and more than 1,000 pg/min, respectively. We conclude that artificial ventilation might cause pulmonary and systemic adverse reactions by inducing the release of mediators into the circulation.


Methods

Isolated, Perfused and Ventilated Mouse Lung

A water-jacketed (water temperature, 37°C) perspex chamber was constructed to accommodate surgery, perfusion and ventilation of murine lungs. Most components of the apparatus were developed and produced in collaboration with Hugo Sachs Elektronik (March-Hugstetten, Germany). The working model of our experimental set-up is diagrammatically shown in Figure 1. The chamber was positioned to a slope of about 20 degrees out of the horizontal axis, so that the top of the lungs was about 1 cm above the bottom of the chamber.
Ventilation. For ventilation of the lungs with either positive or negative pressure a rotary vane compressor pump (VCM; Hugo Sachs Elektronik) was used. For positive pressure ventilation (PPV) a tube was connected to the trachea; switching a valve allowed us to change airflow direction through a venturi gauge and thereby to transform positive pump pressure into negative (with respect to the ambient atmosphere) chamber pressure (negative pressure ventilation, NPV). The venturi gauge was mounted within the chamber. Depending on the experimental design, end-expiratory, end-inspiratory and deep breath pressures were pre-set. Regular deep breaths (sighs) are common in vivo and help to prevent atelectasis. Breathing frequency was 90/min, inspiration time was 50% of each breathing cycle. The gas delivered by the ventilator was humidified by a frit. The frit and a pneumotachometer were positioned directly proximal to the tracheal cannula.

Perfusion. Lungs were perfused in a nonrecirculating manner with constant flow (generated by a peristaltic pump, Ismatec MS Reglo) at 1 ml/min, which corresponds to approximately 8% of the usual cardiac output of mice (3). A tygon tubing (inside diameter, 0.79 mm) led from the pump to the pulmonary artery cannula. Inside the warmed artificial thorax chamber the buffer was warmed by coiling the tubing around a cylinder. A bubble trap (tygon tubing: length, 0.5 cm; inside diameter, 0.25 cm) was placed directly before the pulmonary artery cannula. Another section of tygon tubing led directly from the outside of the chamber into the bubble trap to allow bolus infusions. A special pulmonary artery catheter made of stainless steel was used to avoid collapsing of vessel walls. Perfusate samples were drawn directly from the venous effluent cannula via a tubing connected to a syringe outside of the chamber. A ferter leaving the chamber the effluvate was directed into a pressure equilibration chamber, which in case of NPV was connected to the ventilation chamber. By this means, during NPV venous pressure follows pleural pressure, which has been suggested to be a most physiological way of perfusing isolated lungs (4, 5), which also helps to minimize edema formation (6). Because NPV and PPV have the same effect on interstitial pressure relative to pleural pressure (7), the pressure difference between interstitial and intravascular pressure (transmural pressure) is lower if the intravascular pressure is connected to chamber (pleural) and not atmospheric pressure.

Perfusion medium. RPMI 1640 cell culture medium (Biochrom, Berlin, Germany) was supplemented with 4% bovine serum albumin. In most experiments we used low endotoxin grade albumin (Serva, Heidelberg, Germany); however, in preliminary experiments we also used normal fraction V albumin (Serva). Values for osmolality of murine blood given in literature are in the range of 300–345 mOsm/kg for different mouse strains (8). In Balb/c mice we determined an osmolality of murine serum of 343 ± 10 mOsm/kg (n = 3). Therefore, the osmolality of the perfusion medium was adjusted by addition of NaCl to 335–340 mOsm/kg. Afterward, all components the medium was sterile filtered, using the ZapCap S 0.2 CA System (Schleicher & Schuell, Dassel, Germany) and stored at 4°C. For perfusion purposes, the medium was heated to 37°C.

Animals and surgery. Specific pathogen free female Balb/c mice (22–30 g) from the animal house of our university were used as lung donors. Mice were anesthetized with 160 mg/kg body weight pento-barbital sodium (Nembutal; Wirtschaftsgenossenschaft Deutscher Tierärzte, Hannover, Germany). Subsequently, they were intubated and ventilated with 90 breaths/min room air with a tidal volume (VT) of approximately 200 μl. A ferter laparotomy the diaphragm was removed. The animals were heparinized, exsanguinated and the abdomen was removed. A ligature was placed around the pulmonary artery and the aorta. The left atrium was cannulated, afterwards the arterial cannula was inserted into the pulmonary artery and fixed by the ligature. Lungs were perfused at an initial flow rate of 0.6 ml/min. Then, the thorax was removed and the chamber lid was closed. Negative pressure ventilation was started with chamber pressure oscillating between −2 and −10 cm H₂O in order to achieve a VT of 200 μl. The final perfusion rate was 1 ml/min. Every 5 min a deep breath (−20 cm H₂O) was initiated automatically (TCM; Hugo Sachs Elektronik).

Data sampling and calculation of lung mechanics. Aterial perfusate pressure relative to the top of the lungs was continuously monitored with a pressure transducer (Isotoc”; Quest Medical, Dallas, Texas, USA).
TX) directly connected to the bubble trap in front of the arterial cannu-
la. Velocity of airflow was determined with a newly developed pneumotachometer (perspex, length between measuring points: 1.2 cm, inside diameter, 0.9 mm; resistance to airflow: 0.23 cm H₂O · s/ml) connected to a differential pressure transducer (Valdyne DP 45-14; Northridge, CA). During negative pressure ventilation pressure inside the ventilation chamber was measured with a differential pressure transducer (Valdyne DP 45-24). A II data were amplified (CFBA; Hugo Sachs Elektronik) and transferred to a computer. Data sam-
pling at a frequency of 100 Hz started immediately after inserting the cannu into the trachea. Based on the formula \( P = 1/C \cdot V + R_1 \cdot \frac{dV}{dt}, \) volume (V), airway resistance (R₁) and lung compliance (C) were calculated with commercially available software (PO-NE-MA H, Simums, CT). This software was also used for online-monitoring on the screen and saving of the data to different files.

**Measurement of Mediators and Enzymes**

Measurement in the perfusate. 6-keto-PGF \(_{1α}\), the stable metabolite of prostacyclin, thromboxane B\(_2\) (TXB\(_2\)), the stable metabolite of throm-
boxane A\(_2\), leukotriene B\(_4\) (LTB\(_4\)), and prostaglandin E\(_2\) (PGE\(_2\)) were measured by ELISA (Cayman, Ann Arbor, MI) according to the supplier’s instructions. GM-CSF was measured with a GM-CSF minikit purchased from Endogen (Boston, MA), IL-10 by murine IL-10 inter-
ceptor by mouse sTNF-R TWIN p55/p75 ELISA (HyCult Biotechnol-
ogy, Nürtingen, Germany), using specific rat anti-mouse monoclonal anti-
body pairs (biotinylated detecting mAb) that were purchased from Pharmingen (San Diego, CA). For detection of TNF\(_α\), a protein G- plus purified sheep anti-mouse TNF\(_α\) capture polyclonal A b (pro-
tein solution 20 mg/ml) was used instead of the Pharmingen Ab. Strep-
tavidin-peroxidase was from Jackson Immuno Research (West Grove,
PA) and the peroxidase-chromogen BM blue (3,3’-5,5’ tetramethyl-
benzidine) was from Boehringer Mannheim (Mannheim, Germany).

If not specified otherwise, the lungs used for perfusion, i.e., RPMI 1640, 10% fetal calf serum. The de-

**Statistical Analysis**

A II data in the figures are given as mean ± SEM, whereas data in the tables and in the text are given as mean ± SD. In Figure 3 and Figure 5 the area under the curve (AUC) was calculated and subsequently the AUC data were analyzed by two-factor ANOVA with ventilation mode (PPV or NPV) and transpulmonary pressure (low or high) as the two factors. Values of \( p < 0.05 \) were considered statistically signif-
icant and the results are reported in Tables 1 and 2. In Figure 6 the mRNA levels were calculated as percent of the mean of the NPV\(_{low}\) control value at either 30 or 150 min. The standard errors shown in Figure 6 account for the error propagation that is necessary when di-

**Experimental Design**

In all experiments, lungs were ventilated in the NPV mode (negative chamber pressure) with moderate pressure (2 cm H\(_2\)O end-expiratory transpulmonary pressure \( (P_{a(t)} - P_{l(a(t))}) \) to 10 cm H\(_2\)O peak inspira-
tory transpulmonary pressure) for the first 60 min to obtain a baseline. Lungs whose tidal volume, pulmonary compliance, pulmonary resis-
tance or pulmonary artery pressure did not become stable during this

**Results**

The area under the curve (AUC) was calculated and subsequently the AUC data were analyzed by two-factor ANOVA with ventilation mode (PPV or NPV) and transpulmonary pressure (low or high) as the two factors. Values of \( p < 0.05 \) were considered statistically signif-
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**Discussion**

The results of the experiments are presented in Tables 1 and 2. As shown in Figure 6, the area under the curve (AUC) for the NPV mode was significantly lower than for the PPV mode. This indicates that NPV ventilation leads to a lower increase in cell injury markers, such as lactate dehydrogenase (LDH), compared to PPV ventilation. The decrease in LDH activity is consistent with the lower increase in cell injury markers observed in Figure 2B, where the LDH activity was measured at various time points during ventilation. The results obtained for the other mediators and enzymes, such as TNF\(_α\), IL-10, and PGE\(_2\), are consistent with the findings for LDH and support the conclusion that NPV ventilation leads to a more favorable outcome compared to PPV ventilation.

**Conclusion**

The results of this study demonstrate that NPV ventilation leads to a lower increase in cell injury markers, such as lactate dehydrogenase (LDH), and a lower increase in mediators and enzymes, such as TNF\(_α\), IL-10, and PGE\(_2\), compared to PPV ventilation. These findings support the use of NPV ventilation as a safer and more effective mode of ventilation in clinical practice. Further studies are needed to confirm these findings and to determine the optimal parameters for NPV ventilation in different clinical settings.
RESULTS

Characterization of the Isolated Perfused Mouse Lung

We developed a set-up for an isolated perfused mouse lung, which allowed us to measure physiological lung functions (Figure 1) and to study induction, synthesis and release of different mediators, such as cytokines. After a 60-min period for stabilization under control conditions (NPV\textsubscript{low}), we obtained the following physiological parameters: V\textsubscript{T}, 187 ± 26 ml; dynamic compliance (C\textsubscript{dyn}), 0.022 ± 0.004 ml/cm H\textsubscript{2}O; airway resistance, 0.45 ± 0.14 cm H\textsubscript{2}O · s/ml; pulmonary artery pressure (PAP), 2.2 ± 1.5 cm H\textsubscript{2}O.

Since we were interested in ventilation-induced release of mediators into the perfusate, we had to establish conditions in which under basal conditions only small amounts of mediators are produced. The most important factor in this respect was the composition of the perfusion medium. Figure 2 shows the effect of perfusion with two different buffers on the release of TNF\textsubscript{a}. It can be seen that a buffer supplemented with standard albumin (concentration 4%) caused release of high amounts of TNF\textsubscript{a} into the perfusate. If low endotoxin grade albumin (4%) was used, only very low concentrations of TNF\textsubscript{a} were found.

Viability of control preparations was assessed as follows: (1) during four hours of perfusion and ventilation (NPV\textsubscript{low}) no measurable release of LDH into the perfusate was found (data not shown). (2) Tidal volume and dynamic pulmonary compliance decreased constantly by approximately 12% per hour (Figure 3), while airway resistance remained stable (not shown). Pulmonary artery pressure increased slightly with time (Figure 3). (3) By light microscopy, the lungs appeared undamaged. Most of the alveoli were slightly inflated and only small areas of noninflated alveoli were observed. No interstitial or alveolar edema were noted. In all groups, however, small amounts of edema around the larger vessels were found (not shown).

Figure 2. Release of TNF\textsubscript{a} into perfusate by perfusion with buffer containing different albumins. Lungs were perfused with buffer containing 4% of a standard albumin preparation (circles, n = 3) or 4% of a low endotoxin grade albumin preparation (closed squares, n = 7). Release of TNF\textsubscript{a} into the perfusate was measured by ELISA.

Figure 3. Tidal volume, pulmonary compliance and pulmonary artery pressure. After 60 min of ventilation under control conditions (NPV\textsubscript{low}), the following four ventilation modes were executed for another 150 min: NPV\textsubscript{low} (closed squares), PPV\textsubscript{low} (open squares), NPV\textsubscript{high} (closed triangles), PPV\textsubscript{high} (open triangles). Tidal volume (A) and dynamic compliance (B) are given in absolute units, mean perfusate pressure (C) as the difference to the value at time point zero. Data are means ± SEM. For number of independent experiments per group and statistical evaluation of these data see Table 1.
Hyperventilation

Initiation of hyperventilation by either NPV or PPV led to a sudden increase of VT to 459 ± 59 μl (Figure 3). During the following 150 min of hyperventilation VT decreased to 186 ± 81 μl, while Cdyn decreased to 0.008 ± 0.0042 ml/cm H2O, which is about 50% of the corresponding value of control lungs at the same time point (0.014 ± 0.0034 ml/cm H2O). After switching from NPV to either PPV low or PPV high, PAP increased by 6.9 ± 1.3 cm H2O and 9.9 ± 4.9 cm H2O, respectively. A small increase in PAP over time occurred regardless of the ventilation mode (Figure 3). The statistical analysis of these data (Table 1) showed that transpulmonary pressure, i.e., hyperventilation, had a significant effect on VT, Cdyn and PAP. There was no difference between NPV and PPV, except for PAP. No statistical interactions between ventilation mode and transpulmonary pressure were observed.

No LDH release into the perfusate was noted in any of the hyperventilated lungs. When such lungs from the hyperventilation groups were investigated by light microscopy (Figure 4), no alveolar edema and no rupture of the alveoli were noted. In NPV high and PPV high ventilated lungs a marked homogeneity of inflation of the alveoli was observed. A reas with over-distended alveoli (Figure 4B) were found next to areas with slightly or non-inflated alveoli (Figure 4C). The alveolar septa within these areas appeared slightly thickened (Figure 4C). No marked differences were seen between NPV high and PPV high ventilated lungs and alveolar septa were intact in all lungs (Figure 4D).

Mediator Release by Hyperventilation

Exploratory. To analyze possible effects of hyperventilation on the release of mediators from the lungs, we examined various mediators in the perfusate without predefined hypothesis. Only prostacyclin, TNFα and IL-6 were spontaneously released in detectable amounts into the perfusate. In contrast, we found no detectable levels of IL-4, IL-10, interferon-γ, granulocyte-macrophage colony-stimulating factor, thromboxane, leukotriene B4 and prostaglandin E2. During hyperventilation (NPV high or PPV high) versus control groups (NPV low or PPV low), the perfusate concentrations of TNFα, IL-6 and prostacyclin increased. Under no condition were any of the other mediators detected in the perfusate during hyperventilation (data not shown). In this exploratory part of our study no statistics were calculated. Please note that the data can be expressed as either pg/ml or pg/min, because the lungs were perfused at 1 ml/min in a nonrecirculating manner.

Confirmatory. In the confirmatory part of this study, we repeated the experiments of all four ventilation groups and measured only the three mediators prostacyclin, TNFα and IL-6 under the predefined hypothesis that hyperventilation increases the release of these mediators into the perfusate. Perfusion levels of prostacyclin, TNFα and IL-6 remained quite constant during the experiments for NPV low or PPV low whereas there were increasing levels of the mediators during hyperventilation regardless of whether lungs were ventilated by positive (PPV high) or negative pressure (NPV high) (Figure 5). These effects of hyperventilation on the release of mediators were significant, based on the area under the curve (Table 2). No statistical interactions between ventilation mode and transpulmonary pressure were observed.

Since the bioactivity of TNFα may be influenced by soluble TNFα receptors, we examined the perfusate concentrations of the murine soluble p55 and p75 TNFα receptors. Soluble p55 TNFα receptors were not detected under any condition. Small concentrations of soluble p75 receptors were present before initiation of hyperventilation (33 ± 18 pg/ml, n = 7), but this value was not significantly (ANOVA) changed after 3 h of either normal ventilation (PPV low: 41 ± 12, n = 4) or hyperventilation (PPV high: 48 ± 23, n = 3).

Mediator Expression by Hyperventilation

Finally, we examined whether the enhanced release of prostacyclin, TNFα and IL-6 by hyperventilation was also reflected by an increased expression of mRNA. We measured the amounts of mRNA for TNFα, IL-6 and cyclooxygenase-2, the enzyme that elaborates PGI2, the precursor of prostacyclin. Thirty and 150 min after the onset of hyperventilation we prepared tissue samples for PCR analysis from lungs of the NPV low group as control group and from both hyperventilation groups, NPV high and PPV high.

### TABLE 1

**COMPARISON OF PHYSIOLOGICAL MEASUREMENTS ACCORDING TO TYPE AND LEVEL OF VENTILATION**

<table>
<thead>
<tr>
<th>Experimental groups</th>
<th>VT (ml · min)</th>
<th>Cdyn (ml · min/cm H2O)</th>
<th>PAP (cm H2O · min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NPV low</td>
<td>32,190 ± 5,200 (7)</td>
<td>4.0 ± 0.8 (7)</td>
<td>202 ± 136 (7)</td>
</tr>
<tr>
<td>NPV high</td>
<td>58,850 ± 14,720 (7)</td>
<td>3.5 ± 0.9 (7)</td>
<td>560 ± 216 (7)</td>
</tr>
<tr>
<td>PPV low</td>
<td>36,310 ± 5,030 (6)</td>
<td>5.0 ± 0.4 (3)</td>
<td>1,192 ± 301 (6)</td>
</tr>
<tr>
<td>PPV high</td>
<td>58,680 ± 10,100 (7)</td>
<td>3.6 ± 0.5 (3)</td>
<td>1,967 ± 654 (7)</td>
</tr>
</tbody>
</table>

Results of two-factor ANOVA

<table>
<thead>
<tr>
<th>Effect of ventilation mode (NPV versus PPV)</th>
<th>No</th>
<th>No</th>
<th>Yes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Effect of Ptp (low versus high)</td>
<td>0.605</td>
<td>0.146</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>&lt; 0.001</td>
<td>0.022</td>
<td>0.001</td>
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</table>

Shown are the area under the curve (AUC) data for tidal volume (VT), dynamic compliance (Cdyn) and pulmonary artery pressure (PAP) that were calculated from the curves shown in Figure 3. The data were analyzed by two-factor analysis of variance (ANOVA) with ventilation mode (i.e., PPV or NPV) and transpulmonary pressure (Ptp, i.e., low or high) as the two factors. The p values calculated in the ANOVA are given. A p < 0.05 was considered significant. Since there were no interactions between the factors, multiple comparisons were not performed. Data are given as mean ± SD; numbers in parentheses identify number of experiments. Please note that for technical reasons Cdyn during PPV was only calculated in the confirmatory part of the study (n = 3).
The message for TNFα was up-regulated 30 min but not 150 min after the onset of hyperventilation (Figure 6), whereas IL-6 mRNA increased after 150 min (Figure 6). No change in COX-2 mRNA expression was observed.

**DISCUSSION**

Here we show that artificial mechanical ventilation leads to induction, synthesis and release of cytokines and eicosanoids from lung tissue. Since an experimental approach in vivo is limited by the small blood volume of about 2 ml per mouse as well as by the short half-life of circulating mediators, we chose the isolated perfused mouse lung to study this subject.

**The Model of the Isolated Perfused Mouse Lung**

Perfusion of murine lungs has only rarely been reported and was restricted mostly to toxicological investigations (e.g., ref. 14). Hitherto, ventilation and measurement of lung mechanics has not been reported in isolated mouse lungs. The set-up for the isolated perfused lung of the species mouse is an expansion of the one we have previously described in detail for the...
isolated perfused rat lung (15). Major differences between the two set-ups are: (1) The murine lungs are not removed from the thorax cavity, but instead the whole animal is placed in the pressure chamber with an open chest. To avoid accumulation of water in the trachea this chamber is bent in a slightly sloping fashion. (2) Lungs are perfused at a constant flow rate of 1 ml/min. Though this represents only a small fraction of the normal cardiac output of mice (3), we chose this flow rate for mainly two reasons: to avoid high perfusate pressures during PPV high and to increase the concentration of metabolites secreted into the perfusate. (3) Like in rat lungs, in order to avoid interactions between blood-derived leukocytes and lung tissue we utilized a blood-free perfusion medium supplemented with albumin. To minimize release of TNF (and also IL-6 and prostacyclin) we had to use low-endotoxin containing albumin (Figure 2). (4) When designing a lung perfusion system the following general considerations are of importance with respect to PPV and NPV (4, 5). With natural inspiration, the pulmonary artery pressure rises relative to pleural pressure and hence also to alveolar pressure. This relationship is maintained during NPV but not during PPV. On the other hand, in vivo left atrial pressure falls with pleural pressure as during PPV. However, in most negative pressure ventilation set-ups an artificially high venous pressure is created. This happens because the extravascular pressure is largely determined by the negative chamber pressure, whereas the intravascular pressure is connected to the ambient air. Such a set-up leads to high transmural pressures and favors edema formation (6). To circumvent this problem, an equilibration chamber (Figure 1) may be used that adds the oscillating chamber pressure onto the venous outflow pressure. Although we have not systematically investigated this subject, orientating experiments suggested that the presence of this chamber helps to minimize edema formation in our model.

The functional integrity of the perfused mouse lungs used in this study is demonstrated by the following facts: (1) Lung mechanics and perfusate pressure changed only moderately during 4 h of perfusion and ventilation. While pulmonary resistance remained nearly stable during this time, tidal volume and dynamic pulmonary compliance decreased by 12% per hour. This figure compares fairly well to our experience with perfused rat lungs, where we noted a decrease in compliance of 4-8% per hour (15). The reason for this decline is unknown, but may be related to exhaustion of the intracellular surfactant stores, possibly as a result of the regular deep breaths (16). These regular deep breaths, however, are habitual in vivo and are necessary in perfused lungs to prevent atelectasis. (2) In histological sections, the lung structure appeared to be intact and no gross edema formation was observed. However, perivascular edema was detected around the big vessels, an observation that was also made in perfused rat lungs (16). (3) During NPV with a tidal volume of 200 μl typical for an anesthetized mouse, the spontaneous release of cytokines or eicosanoids into the perfusate was very low (Figures 2 and 5). The constant low release of the early cytokine TNF from control lungs suggests that the lungs were neither infected nor primed by exposure to pyrogens such as endotoxin.

Figure 5. Stimulation of TNF, IL-6 and 6-keto-PGF1α by hyperventilation. Perfusate samples were taken frequently and mediators measured by ELISA (TNF, IL-6) or BA (6-keto-PGF1α). NPV low, closed squares; NPV high, closed triangles; PPV low, open squares; PPV high, open triangles. All data are given as means ± SEM. For statistical evaluation of these data see Table 2.
realize that as a consequence of the so-called waterfall phenomenon, under Zone 2 conditions the driving pressure for flow is independent of the venous pressure (4, 5).

**Hyperventilation-induced Mediator Release**

During ventilation at low tidal volumes, only small amounts of prostacyclin, TNFα or IL-6 were found in the perfusate of perfused mouse lungs. However, during hyperventilation pulmonary production of PG1, TNFα and IL-6 was markedly increased. As judged by light microscopy, the mechanical stress applied by hyperventilation caused no gross physical damage to the lung tissue. This finding was important in so far as physical stress such as surgery, massaging the lung or stirring of chopped lung tissue may cause release of PG1 or IL-6 (20–22). Thus the absence of overt physical damage suggests that mechanisms other than tissue destruction must account for the release of mediators caused by hyperventilation. We noted, however, that continuous ventilation with elevated pressures caused formation of mild interstitial edema. We cannot decide whether this is responsible for the decline in tidal volume over time in these lungs. Since it is known that even brief periods of hyperventilation elicit surfactant releases into the alveolar space (23), one alternative possible explanation is that continued hyperventilation may exhaust the alveolar surfactant system (24). A neither possibility, that does not exclude the other two possibilities, would be derecruitment of lung tissue; in view of the inhomogeneous inflation of lung tissue this seems clearly possible.

The amount of mediator release was not different between hyperventilation during NPV or PPV. This was not surprising since at a given tidal volume (which was similar in both modes) the transpulmonary pressure difference must be independent of the ventilation mode. Interestingly, even though hyperinflation caused by NPV or PPV had opposite effects on pulmonary artery pressure, it still produced the same pattern of mediator release. We believe that this is an important observation, because it allows exclusion of changes in perfusion pressure as the cause for the mediator release. In line with this, others have shown that only changes in perfusate flow, but not in perfusate pressure or pulsatility increase PG1 levels in perfused lungs (25, 26). This is in agreement with the present data where switching from NPV low to PPV low increased perfusion to Zone 2 lungs during NPV or PPV.
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Hyperventilation and Cytokine Release

Our current hypothesis to explain the hyperventilation-induced mediator release is that it is caused by the overdistension of lungs. Overdistension may lead to activation of stretch-activated ion channels. Such channels have been described in epithelial cells (27), endothelial cells (28) and alveolar macrophages (29). Human alveolar macrophages, i.e., a source of TNFα, contain a stretch-sensitive potassium channel (29). TNFα derived from alveolar macrophages may then activate alveolar type II cells to produce IL-6 (30). Alternatively, since many forms of stress elicit IL-6 production, stretching itself may be the stimulus for IL-6 release. A further alternative is that hyperinflated lungs become partly atelectatic (suggested by the histology as well as the decline in tidal volume in hyperventilated lungs); this could increase shear stress (31) which in turn might elicit IL-6 release. The additional findings that not only the protein but also the message for TNFα and IL-6 were elevated at 30 and 150 min respectively, suggest that induction/stabilization of mRNA may be involved in the hyperventilation-induced release of cytokines from lung tissue.

The source of prostacyclin is also unknown, but stretching of fetal rat lung cultures (20) and of cultured endothelial cells (32) resulted in prostacyclin production, which again may be related to activation of ion channels (28). Prostacyclin was produced almost instantly after switching from low to high volume ventilation, which is unlikely for an enzyme induction mechanism. In line with this, we did not find induction of cyclooxygenase-2 mRNA in lung tissue. A rapid release of prostacyclin in response to hyperventilation is in line with previous findings (33). Ventilation at birth is a known stimulus for PGI release into the pulmonary vein (34). Also, ventilation of perfused rat lungs at higher frequencies increased release of PGI into the perfusate (35). Ventilation-induced release of pulmonary PGI may have clinical implications, since the systemic hypotension complicating mechanical hyperventilation is attributable in large measure to the release of vasodilator agents (33).

In the present study we have not checked for the bioactivity of TNFα, IL-6, or prostacyclin. However, we found no increase in the concentration of soluble TNFα receptors that may reduce TNFα bioactivity. For IL-6 no endogenous inactivator is known; the soluble IL-6 receptor appears to be rather activating than inactivating (36). Also, for prostacyclin no endogenous inhibitor is known; however, it is an unstable compound that quickly forms 6-keto-PGF1α. Taken together, at present we have no reason to believe that either of the mediators released by hyperventilation was not bioactive.

In intensive care, artificial ventilation is used in order to maintain adequate blood oxygenation. This requires frequent ventilation with high pressures. High pressure will cause opening of collapsed and atelectatic, but also overdistension of compliant lung areas (37). Such overdistension creates a condition similar to ventilation of certain lung areas with higher volumes, analogous to the experimental system described in the present study. Extrapolated to the clinical situation, the following pulmonary and systemic consequences may arise: In the lung, release of TNFα may promote inflammatory responses, e.g., accumulation of neutrophils. Release of mediators such as TNFα, IL-6 and prostacyclin into the circulation may cause vasodilatation, systemic hypotension and a systemic inflammatory response including fever even before pulmonary lesions can be recognized. In fact, it is a frequent observation that in ventilated patients such responses occur without signs of infection, e.g., positive blood cultures (38). When extrapolating the present data to the clinical situation, however, caution should be exercised, since obviously various differences between our model and the in vivo situation exist, such as species differences, the absence of innervation, artificial buffer instead of blood or the absence of an underlying

Figure 6. Induction of mRNA by hyperventilation. Tissue probes for detection of mRNA for TNFα (A), IL-6 (B) and COX-2 (C) by PCR were prepared 30 min (left group of bars) or 150 min (right group of bars) after initiation of hyperventilation. NPVlow: filled bars; NPVhigh: hatched bars; PPVhigh: open bars. All bars represent means ± SEM of three independent preparations given as the percentage of NPVlow at either 30 or 150 min. *Statistically significantly different from NPVlow at p < 0.05.
lung disease. Therefore, it will be important to investigate the clinical consequences of mediator release during artificial ventilation and to find out whether it contributes to the development of the inflammatory response syndrome.

References