Effects of hypercapnia with and without acidosis on hypoxic pulmonary vasoconstriction

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Ketabchi F, Egennazarov B, Schermuly RT, Ghofrani HA, Seeger W, Grimminger F, Shid-Moosavi M, Dehghani GA, Weissmann N, Sommer N. Effects of hypercapnia with and without acidosis on hypoxic pulmonary vasoconstriction. Am J Physiol Lung Cell Mol Physiol 297: L977–L983, 2009. First published August 28, 2009; doi:10.1152/ajplung.00074.2009.—Acute respiratory disorders such as acute obstructive pulmonary diseases and depression of the respiratory control center may induce alveolar hypoxia and hypercapnic acidosis, which can affect systemic regulatory processes and pulmonary function. However, the use of sodium bicarbonate, one of the most essential extracellular buffers for the normalization of pH in different pathological conditions of respiratory acidosis, is controversial. During resuscitation, application of sodium bicarbonate is generally not recommended for just correction of pH due to severe side effects (40). Furthermore, in permissive hypercapnic strategy, beneficial effects of acidosis have been discussed in the literature (5). In animal studies, a decreased development of pulmonary edema and reduced hypoxemia during hypercapnic acidosis suggests valuable effects of acidosis (13, 14, 25–27), whereas from other studies it could not be distinguished whether the effects were related to hypercapnia or acidosis (12, 43). In addition, the mechanisms of improvement of oxygenation and reduced lung edema formation are not known. Improvement in ventilation-perfusion matching and/or of gas diffusion across the alveolocapillary membrane may be related to confounding factors in this regard.

Ventilation-perfusion matching in the lung is achieved by the mechanism of hypoxic pulmonary vasoconstriction (HPV). HPV is the result of constriction of precapillary vessels supplying blood to areas of the lung with poor alveolar ventilation or hypoxia. In critically ill patients, for example with alveolar edema, a failure of HPV may result in life-threatening hypoxemia. However, the effect of hypercapnic acidosis on HPV is not known. Moreover, it still remains elusive whether hypercapnia or acidosis affects pulmonary artery vascular tone. Whereas evidence exists that metabolic acidosis increases pulmonary artery pressure, there are inconsistent findings as to whether hypercapnic acidosis has any effect on pulmonary artery pressure (2–4). Some reports have indicated that in the isolated lung preparations, hypercapnia with normal pH increases or does not change pulmonary artery vascular tone (16, 30, 31, 33). On the other hand, in vivo studies have indicated that sodium bicarbonate administration in metabolic acidosis increases or does not change pulmonary artery pressure during recovery from cardiopulmonary resuscitation in anesthetized animals (18, 22).

Few studies have addressed the role of nitric oxide (NO), which is known as a highly important factor in hypoxic pulmonary vascular reactivity, for hypercapnia and/or acidosis on HPV and pulmonary vascular resistance (28, 37). Nevertheless, some investigators found a decrease in NO production caused by hypercapnia (1, 29, 42), and others describe an increase (15, 41, 44). These studies were challenged by other investigations postulating that NO does not contribute to the regulation of pulmonary vascular tone in under hypercapnia (17, 33).

Against this background, we investigated 1) the effects of hypercapnic acidosis and hypercapnia with normal pH on pulmonary vascular tone in normoxia and hypoxia, 2) the effects of hypercapnic acidosis and hypercapnia with normal pH on pulmonary edema development and capillary permeability, and 3) the role of NO in hypercapnia-dependent effects on...
the pulmonary circulation. Our investigations were performed in intact rabbit lung, a model that allows detailed quantification of pulmonary vascular resistance, HPV, and capillary permeability as well as analysis of the role of NO in the effects of hypercapnic acidosis and hypercapnia with normal pH without having the problems of interventions of systemic regulatory mechanisms.

MATERIALS AND METHODS

Lung isolation, perfusion, and ventilation. Animal experiments were approved by the Animal Ethics Authority (Regierungspräsidium) of Giessen, Germany.

The model of isolated perfused rabbit lungs has been described previously (39). Briefly, pathogen-free male rabbits (2.8–3.8 kg body wt) were deeply anesthetized with an intravenous application of ketamine (30–50 mg/kg) and xylazine (6–10 mg/kg) and treated with the anticoagulant heparin (1,500 U Ag body wt). The trachea was cannulated, and animals were ventilated with room air (30 ml tidal volume, frequency 30 strokes per minute). The lungs were perfused with Krebs-Henseleit solution (perfusate) through the pulmonary artery. After rinsing the lungs with 1 l of perfusate, for washout of the blood, the perfusion circuit was closed for circulation and the start of the experiments. Meanwhile, the flow rate was slowly increased from 20 to 150 ml/min, and concomitantly the left atrial pressure was set at 2 mmHg. A positive end-expiratory pressure of 2 cmH2O was also chosen for prevention of regional alveolar collapse.

The isolated perfused lung was placed in a temperature-equilibrated housing chamber and freely suspended from a force transducer (Hottinger Baldwin) for continuous monitoring of organ weight. The whole system (perfusate, reservoirs, tubing, and housing chamber) was heated to 38.5°C. Pressures in the pulmonary artery, left atrium, and trachea were continuously registered. All lungs included in the study 1) exhibited a homogeneous white appearance with no signs of hemostasis, edema, or atelectasis, and 2) revealed constant mean pulmonary artery and peak ventilation pressures in the normal range and were also isogravimetric during the first 50 min of steady-state period.

Composition of ventilatory gas and perfusate. Four different gas mixtures were employed for lung ventilation (gas mixing chamber, KM 60-3/6MESO; Witt, Witten, Germany). 1) Normoxia plus normocapnia: 21% O2-5.3% CO2 balanced with N2. 2) Normoxia plus hypercapnia: 21% O2-11% CO2 balanced with N2. 3) Hypoxia plus normocapnia: 3% O2-5.3% CO2 balanced with N2. 4) Hypoxia plus hypercapnia: 3% O2-11% CO2 balanced with N2. The perfusate used for this study contained 120 mM NaCl, 1.1 mM K2HPO4, 1.3 mM MgPO4, 4.3 mM KCl, 2.4 mM CaCl2, 13.32 mM glucose, and 50 g of hydroxyethylamyllopectin (200-kDa mol mass), and pH was adjusted with NaHCO3 [8.4% sodium bicarbonate (Serag-Wiessner)] to normal physiological range of 7.35–7.40. In hypercapnic acidosis conditions, pH was allowed to decrease, whereas in hypercapnia with normal pH, extra NaHCO3 was added to adjust perfusate pH at range 7.35–7.40. A membrane oxygenator in the afferent branch of the circulation assured physiological O2 and CO2 partial pressures of the perfusate, for the second time 30 min after the last hypoxic ventilation maneuver of 10-min duration each and 15-min normoxic ventilation interval were performed. Then, Kn was measured for the second time 30 min after the last hypoxic ventilation challenge. In nitro-L-arginine (L-NNA)-treated groups, L-NNA was added to the perfusate at 10 min before the onset of the first hydrostatic challenge.

In separate experiments, Mn[III]tetraakis(4-Benzoic acid)porphyrin chloride (MnTBAP; Calbiochem, San Diego, CA) was added to the perfusate after the first hypoxic challenge. Data are given as percentage of the strength of the first hypoxic challenge.

For provocation of ventilation-perfusion mismatch polyoxyethylene sorbitan monolaurate (5% solution of Tween 20; Sigma, Deisenhofen, Germany) was nebulized in the inspired gas using the Aeroneb Nebulizer System (Respiromics, Herringsch, Germany) as described before (23). These experiments were performed in a time course corresponding to the hypoxia experiments with the Tween 20 application before the corresponding onset of the first hypoxic challenge. Samples for PO2 measurements and the shunt fraction were taken in parallel every hour. The trachea was opened in the middle and the ventilation was increased to V/Q determination in isolated lungs by MIGET. The ventilation-perfusion (V/Q) distributions were determined by the multiple inert gas elimination technique (MIGET) as described by Wagner et al. (36), which has been adapted to blood-free perfused rabbit lungs (23).

Monitoring of exhaled NO. The technique of exhaled and perfusate NO levels has been previously described (28). In brief, an aliquot of the mixed expired gas was continuously forwarded to a chemiluminescence NO analyzer (28 NOA; Sieves Instruments, Boulder, CO) to measure exhaled NO concentration. For determination of NO concentrations released into the buffer fluid, samples were taken every 25 min and analyzed as described before (28).

Statistics. Data are the means ± SE. Analysis of variance with the Student-Newman-Keuls post hoc test was used for comparison of more than two groups. For the comparison of two groups, Student’s t-test was applied. Significance was assumed for P < 0.05.

RESULTS

HPV exhibited good reproducibility over the observation period when lungs were challenged with repetitive hypoxic ventilation maneuvers (3% O2) of 10-min duration alternating with 15-min periods of normoxic ventilation under normocapnia and physiological pH (Fig. 1A; Table 1).

Under condition of hypercapnic acidosis (Table 1), no alteration in HPV was observed at the 1st repetitive maneuvers compared with normocapnia. However, the strength of HPV was increased during the course of repetitive hypoxic ventilation maneuvers, being significantly higher at the 7th and 8th maneuver of hypercapnic acidosis compared with normocapnia (Fig. 1A). Normoxic vascular tone assessed before the 1st hypoxic maneuver in the normocapnic experiments was 9.1 ± 1.6 mmHg and did not change during the course of the experiment. Also, no difference was found in the normoxic vascular tone assessed before each hypoxic maneuver when comparing normocapnia with hypercapnic acidosis. In contrast to hypercapnic acidosis, the performance of the experiments under hypercapnia with normal pH (Table 1) resulted in a transiently increased strength of HPV during the 1st to 6th hypoxic ventilation maneuver (Fig. 1A). As for hypercapnic acidosis, no effect on normoxic vascular tone was perceived under hypercapnia with normal pH.

Nebulization of Tween 20 increased shunt flow determined by MIGET and decreased oxygenation over time in normocapnia (Fig. 1B). The modulation of HPV during
hypercapnic acidosis and hypercapnia with normal pH was reflected by a time-matched decrease of shunt flow and improvement of oxygenation in Tween-induced lung injury (Fig. 1B).

Capillary permeability, assessed by the $K_f$ at the beginning of the experiments and 30 min after the 8th hypoxic ventilation maneuver, revealed no significant alterations under hypercapnic acidosis but an increase during hypercapnia with normal pH (Fig. 2), the condition that increased osmolarity of the perfusate due to addition of sodium bicarbonate (Table 1).

Repetitive hypoxic ventilation resulted in a cyclic and reversible decrease in the exhaled NO levels under normocapnia condition (Fig. 3A). Whereas baseline NO exhalation was not significantly different in hypercapnic acidosis and hypercapnia with normal pH (Fig. 3B), the decrease in exhaled NO on hypoxic ventilation challenge was more pronounced in hypercapnic acidosis compared with hypercapnia with normal pH (Fig. 3C). In contrast, no alterations could be detected in intravascular NO release (Fig. 3D).

Inhibition of lung NO production, by L-NNA (100 μM), amplified the strength of HPV in normocapnia as well as in hypercapnic acidosis and hypercapnia with normal pH compared with the experiments with the presence of NO activity, with exception of the last two maneuvers during hypercapnic acidosis (Fig. 4). The increased strength of HPV during hypercapnia with normal pH observed during intact NO synthesis (Fig. 1) compared with normocapnia was even more intensified during inhibition of NO activity (Fig. 4). In contrast, the

### Table 1. pH, $P_{CO_2}$, $P_{O_2}$, and $HCO_3^-$ in the perfusate during the different experimental conditions

<table>
<thead>
<tr>
<th>Condition</th>
<th>pH</th>
<th>$P_{CO_2}$, Torr</th>
<th>$P_{O_2}$, Torr</th>
<th>$HCO_3^-$, mmol/l</th>
<th>Osmolarity, mosM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normocapnia</td>
<td>7.36±0.00</td>
<td>37±0</td>
<td>51±6</td>
<td>20±0</td>
<td>297±1</td>
</tr>
<tr>
<td>Hypercapnia</td>
<td>7.09±0.00*</td>
<td>72±1*</td>
<td>62±1</td>
<td>21±0</td>
<td>298±1</td>
</tr>
<tr>
<td>Hypercapnia with Normal pH</td>
<td>7.38±0.00</td>
<td>72±1*</td>
<td>64±1</td>
<td>42±0*</td>
<td>332±1*</td>
</tr>
</tbody>
</table>

Values are means ± SE given for $n=5$ experiments each, derived before the 2nd measurement of the capillary filtration coefficient ($K_f$). No difference of the respective parameters could be detected during the course of the experiment (data not shown). *Significant difference compared with normocapnia. In hypercapnia with normal pH, pH was corrected to the depicted values by the addition of sodium bicarbonate.

Fig. 1. The strength of hypoxic pulmonary vasoconstriction (HPV) in repetitive hypoxic ventilation maneuvers during normocapnia, hypercapnic acidosis, and hypercapnia with normal pH. A: strength of HPV, given as the maximum increase in pulmonary artery pressure ($\Delta PAP$) during 10-min hypoxic ventilation maneuvers (3% $O_2$). Hypoxic challenges were performed either under normocapnia, hypercapnic acidosis, or hypercapnia with normal pH. Data are from $n=5$ experiments each. B: shunt flow in percent measured by the multiple inert gas elimination technique (MIGET). Local hypoxia was induced by alveolar damage by aerosolization of Tween 20 into the inspired gas. Shunt flow was determined during normocapnia, hypercapnia with acidosis, and hypercapnia with corrected pH every 60 min. Data are from $n=3$ experiments each. C: $P_{O_2}$ in the perfusate after oxygenation by the lung. Data are from $n=5$ individual lungs each. *Significant differences in hypercapnia with normal pH compared with normocapnia. #Significant differences in hypercapnic acidosis compared with normocapnia.

Fig. 2. The effect of repetitive hypoxic ventilation maneuvers during normocapnia, hypercapnic acidosis, and hypercapnia with normal pH on capillary permeability. Capillary permeability was quantified as the capillary filtration coefficient ($K_f$) before (pre) the 1st hypoxic ventilation maneuver and 30 min after (post) cessation of the 8th maneuver. Data are from $n=5$ experiments each. *Significant differences compared with normocapnia.
difference in HPV between normocapnia and hypercapnic acidosis observed during intact NO synthesis in the 7th and 8th hypoxic maneuver was lost in the experiments with inhibition of NO generation as HPV was not further augmented by L-NNA at these time points in hypercapnic acidosis (Fig. 4).

No significant increase was detected for normoxic vascular tone during inhibition of NO synthesis under all three conditions (data not shown).

Interestingly, despite amplification of HPV, the increase in the $K_{fc}$ value induced by hypoxic hypercapnia with normal pH (Fig. 2) was obviated when lung NO synthesis was inhibited by L-NNA (Fig. 5).

Application of the SOD mimetic MnTBAP did not affect the course of HPV alteration, neither in hypercapnia with acidosis nor during normal pH (Fig. 6).

Fig. 3. Nitric oxide (NO) release in repetitive hypoxic ventilation maneuvers during normocapnia, hypercapnic acidosis, and hypercapnia with normal pH. A: original recording of exhaled NO changes for repetitive hypoxic ventilation under normocapnic conditions. Bars indicate hypoxic ventilation maneuvers (3% O₂). B: baseline normoxic NO exhalation assessed directly before each hypoxic ventilation maneuver. C: decrease of exhaled NO (ΔNO) during repetitive hypoxic ventilation. The maximum decrease within each 10-min period of hypoxic ventilation is given. Data are from $n = 4$ experiments each.

Fig. 4. Strength of HPV in repetitive hypoxic ventilation maneuvers during normocapnia, hypercapnic acidosis, and hypercapnia with normal pH in the presence of nitro-L-arginine (L-NNA). A: the strength of HPV is given as ΔPAP during 10-min hypoxic ventilation maneuvers (3% O₂). *Significant differences compared with normocapnia. B: percent changes in the strength of HPV induced by the application of L-NNA. Data are calculated in percent of values in the absence of L-NNA (100%) given in Fig. 1. Data are from $n = 4$ experiments each.
Hypercapnic acidosis is a common condition in acute respiratory disorders and in the therapeutic approach to permissive hypercapnic ventilation. However, it is not clear whether acidosis should be compensated under these conditions (7, 13). Both protective (32) and deleterious (40) effects of acidosis have been described. Moreover, it is not clear whether hypercapnia or acidosis promote the observed beneficial effect of permissive hypercapnic ventilation (12). Protective effects of hypercapnic acidosis might suggest a better ventilation-perfusion matching and improvement of diffusion capacity. Nonetheless, until now, the effects of acidosis and hypercapnia on pulmonary arterial pressure, and in particular under conditions of alveolar hypoxia, are debatable. We therefore addressed the effects of hypercapnia with normal pH and hypercapnic acidosis on HPV, pulmonary gas exchange, and capillary permeability. Moreover, we addressed the role of NO in the changes of HPV and Ktc in hypercapnic acidosis and hypercapnia with normal pH. We found an increased HPV under conditions of hypercapnic acidosis compared with normocapnia ventilation. This suggests an improvement of ventilation-perfusion matching in hypercapnic acidosis in injured lungs under clinical conditions, where an irregular distribution pattern of alveolar ventilation can lead to a high shunt fraction and thus severe hypoxemia (23). Indeed, an increase in HPV improved ventilation-perfusion matching demonstrated by an increased arterial PO2 as well as a decreased shunt when gas exchange was impaired by Tween nebulization. Increased intensity of HPV thus may explain the observations of improved oxygenation of the arterial blood in permissive hypercapnia stated by Laffey and colleagues (14).

During hypercapnia with normal pH, HPV was only transiently enhanced, which suggests an advantage of hypercapnic acidosis over hypercapnia with normal pH in terms of increased HPV over time.

Previous reports have described both vasodilative and vasoconstrictive responses of lung vasculature to hypercapnia and acidosis (2, 4, 6, 21, 34, 35). Although the interaction of increased osmolarity, due to addition of sodium bicarbonate, with vascular tone should be considered seriously, our data demonstrate that increased perfusate osmolarity did not alter normoxic vascular tone during hypercapnia with normal pH. However, increased osmolarity may have detrimental effects on the endothelial barrier function as Ktc values were increased in our study in hypercapnia with normal pH. Along these lines, it has been suggested that such bicarbonate-mediated effects may be caused by a sodium bicarbonate-induced increase in superoxide levels (19, 20).

Sodium bicarbonate is the most important extracellular buffer that neutralizes acidosis induced by hypercapnia. Interaction of excess H+ during hypercapnia with added sodium bicarbonate during hypercapnia with normal pH produces more CO2 that easily diffuses into the cell and quickly lowers intracellular pH (8, 11, 22, 38). Thus the intensification of HPV seen during hypercapnia with normal pH is possibly the result of decreased intracellular pH with hypoxia. With regard to the finding of a sodium bicarbonate-induced increase in superoxide levels, this interpretation is also in line with the absence of an effect of the SOD mimetic MnTBAP on the course of HPV in hypercapnia with normal pH (19). However, the quantitative differences found in our study between HPV during hypercapnic acidosis, hypercapnia with normal pH, and normocapnia could not be related to edema formation because amplification of HPV did not correlate with increased lung permeability (increased Ktc and lung weight gain), and edema was only found in hypercapnia with normal pH.

In addition, our findings showed the beneficial effects of hypercapnic acidosis with regard to capillary permeability because quantification of Ktc indicated an increase in capillary permeability only in hypercapnia with normal pH, not in hypercapnic acidosis. This finding may well explain the decreased edema formation in hypercapnia with permissive acidosis as reported by Laffey et al. (13).

It has been previously shown that hypoxic ventilation induces a drop of exhaled NO, and it has been suggested that this decrease contributes to the onset of HPV, whereas a hypoxia-independent portion of lung NO synthesis, detectable in the exhaled gas as well as in the intravascular space, antagonizes excessive vasoconstrictions (9). Against this background, we
examined the effect of hypercapnia and acidosis on lung NO release and its possible interaction with the strength of HPV. Intravascularly released NO levels were not altered, neither during hypoxic ventilation compared with normoxia nor by hypercapnic acidosis or by hypercapnia with normal pH compared with normocapnia. In contrast, exhaled NO was consistently more decreased during hypoxic ventilation periods in hypercapnic acidosis compared with hypercapnia with normal pH or normocapnia. Speculatively, this higher decrease of NO during hypoxic ventilation in hypercapnic acidosis is caused by alteration of pH, although in another study exhaled NO was not altered during extracellular acidosis (1).

Although the decrease of exhaled NO during hypoxia has been suggested to trigger HPV (9), the observed differences of exhaled NO in both of the hypercapnic groups cannot fully explain the amplification of HPV as they do not entirely match the amplification kinetics of HPV. Whereas exhaled NO was decreased in hypercapnic acidosis throughout and in hypercapnia with normal pH during the first hypoxic challenge, HPV was amplified only during the last two hypoxic maneuvers in hypercapnic acidosis and the first six maneuvers in hypercapnia with normal pH. This discrepancy may be explained by the fact that exhaled NO only partially reflects physiological activity of NO. To further elucidate this point, we inhibited lung cellular NO production by adding l-NNa to the perfusate before exposing the lungs to experimental conditions. l-NNa increased HPV in all groups with exception of the 7th and 8th hypoxic maneuver in hypercapnic acidosis. l-NNa administration resulted in an equalization of the strength of HPV in normocapnia compared with hypercapnic acidosis but not in normocapnia compared with hypercapnia with normal pH. The fact that the differences in the strength of HPV between normocapnia and hypercapnic acidosis disappeared during NO inhibition suggests that NO plays a key role for the gradual increase in HPV in hypercapnic acidosis. The discrepancy that a decrease in exhaled NO levels was induced by hypercapnic acidosis throughout the experiments whereas an amplification of HPV occurred only during the 7th and 8th hypoxic maneuver may be explained by additional transiently active vasodilatory mechanisms (e.g., prostacyclin) that prevent and thus mask the amplification of HPV at maneuvers 1-6. In contrast to hypercapnic acidosis, a key role of NO could not be found for the hypercapnic group with normal pH as the amplification kinetics of HPV during preblocked NO synthesis was similar to that during intact NO synthesis in these experiments.

Furthermore, the absence of any effect of hypercapnic acidosis on capillary permeability may also be related to the decreased level of NO production because inhibition of NO lessened the augmented $K_{ic}$ seen in hypercapnia with normal pH.

As alterations in superoxide levels during hypoxia have been suggested to mediate HPV, and 2) shown to interfere with NO levels (10), we used the SOD mimetic MnTBAP to address a specific role of superoxide-mediated NO alteration for the amplification of HPV in hypercapnic acidosis, the group that, according to our data, is suggested to be dependent on NO alterations. Comparing the amplification profile of HPV during the course of the experiments for both hypercapnic acidosis and hypercapnia with normal pH in the presence and absence of MnTBAP, no effect of superoxide scavenging on the strength of HPV could be observed. These experiments most likely exclude a possible effect of superoxide on NO levels as the underlying mechanism of the hypercapnic effects on HPV.

In summary, we showed that, in isolated lung preparation, hypercapnia with or without acidosis has beneficial effects on lung function in terms of HPV and ventilation-perfusion matching. Together with the finding that hypercapnic acidosis also prevents edema formation, hypercapnic acidosis may be advantageous over hypercapnia with normal pH in terms of gas exchange.

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