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Hypercapnia Attenuates Hypoxic Pulmonary Hypertension by Inhibiting Lung Radical Injury

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Summary

Chronic lung hypoxia results in hypoxic pulmonary hypertension. Concomitant chronic hypercapnia partly inhibits the effect of hypoxia on pulmonary vasculature. Adult male rats exposed to 3 weeks hypoxia (Fi_{02}=0.1) combined with hypercapnia (Fi_{C02}=0.04-0.05) had lower pulmonary arterial blood pressure, increased weight of the right heart ventricle, and less pronounced structural remodeling of the peripheral pulmonary arteries compared with rats exposed only to chronic hypoxia (Fi_{02}=0.1). According to our hypothesis, hypoxic pulmonary hypertension is triggered by hypoxic injury to the walls of the peripheral pulmonary arteries. Hypercapnia inhibits release of both oxygen radicals and nitric oxide at the beginning of exposure to the hypoxic environment. The plasma concentration of nitrotyrosine, the marker of peroxynitrite activity, is lower in hypoxic rats exposed to hypercapnia than in those exposed to hypoxia alone. Hypercapnia blunts hypoxia-induced collagenolysis in the walls of prealveolar pulmonary arteries. We conclude that hypercapnia inhibits the development of hypoxic pulmonary hypertension by the inhibition of radical injury to the walls of peripheral pulmonary arteries.

Key words

Chronic hypoxia • Chronic hypercapnia • Pulmonary hypertension • Hypoxic lung injury

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Introduction

Chronic hypoxia causes hypoxic pulmonary hypertension (HPH) and structural remodeling of pulmonary blood vessels. Chronic lung hypoxia typically results from respiratory insufficiency or from breathing gas with a low oxygen concentration. The major difference between these two alternatives is in lung concentration of carbon dioxide. Whereas respiratory insufficiency in lung disease is most often characterized by hypercapnia and respiratory acidosis, oxygen deprivation in high mountains results in hypocapnia and alkalosis. In lung disease the onset of hypercapnia is an adverse prognostic event. Severity of cor pulmonale, however, was statistically linked with measurements of airway obstruction but not with hypercapnia (Wilkinson *et al.* 1988). In animal experiments, hypercapnia partially inhibited the development of HPH (Ooi *et al.* 2000, Herget *et al.* 2001, Herget *et al.* 2002, Howell *et al.* 2004, Kantores *et al.* 2006). In studies on high altitude dwellers (Canepa *et al.* 1956, Hultgren and Grover 1968) and patients with chronic obstructive pulmonary disease (COPD) (Lapp *et al.* 1971, Astin 1972), where both groups have similar levels of oxygen saturation of hemoglobin (~80 %), high altitude residents have P_{aCO2} ~31 torr and COPD patients ~46 torr. Hypercapnic sea level patients had less severe lung hypertension (mean pulmonary blood pressure 25 mm Hg) than mountain residents (35 mm Hg).

HPH results from hypoxia-induced radical tissue injury to the walls of peripheral pulmonary arterioles (Hampl and Herget 2000, Herget *et al.* 2000). The

Table 1. Body weight (BW), pulmonary artery pressure (PAP), cardiac output (CO), RV/LV+S ratio, DL ratio and plasma nitrotyrosine concentration in rats after 3 weeks exposure to ambient air (N), hypoxia (H) and hypoxia with hypercapnia (H + CO_2).

Group	BW (g)	PAP (mm Hg)	CO ($ml.min^{-1}$)	RV/LV+S	DL (%)	Nitrotyrosine (μM)
N	415±8*	15.6±0.9*	38.4±4.2	0.252±0.012*	18±2*	6.9±0.6*
H + CO_2	214±6	22.9±0.9^{+}	38.0±3.3	0.349±0.016^{+}	26±4^{+}	11.8±1.4^{+}
H	243±8	30.0±1.9	27.6±2.6	0.539±0.021	38±3	17.7±2.7

PAP – pulmonary arterial mean blood pressure, CO – cardiac output, RV/LV+S – ratio of right ventricular heart weight to the sum of weights of left heart ventricle and septum, DL – ratio of hypertrophied (double laminated) peripheral pulmonary arteries and total number of peripheral pulmonary arteries on section of both lungs. * $P<0.05$ between groups N and H + CO_2, $^{+}$ $P<0.05$ between groups H and H + CO_2.

initiation of HPH is characterized by the increase in production of NO and oxygen radicals (reactive oxygen species, ROS) (Lachmanová *et al.* 2005, Hampl *et al.* 2006). We hypothesize that hypercapnia restricts the HPH by inhibition of radical damage in the walls of the prealveolar pulmonary vessels. In this study on chronically hypoxic and chronically hypoxic and hypercapnic rats we characterized the developed pulmonary hypertension and analyzed the relation of hypercapnia to hypoxia-induced radical injury of peripheral pulmonary arteries.

Methods

Exposure to chronic hypoxia and hypercapnia

Experiments on three groups of adult male Wistar rats were performed in accordance with the European Community and NIH guidelines for using experimental animals. All procedures were approved by our institution's Animal Studies Committee.

The first group (group H) was exposed to chronic hypoxia in an isobaric hypoxic chamber (F_{iO2}=0.1). CO_2 was completely reabsorbed in closed circuit by KOH and soda lime (Hampl and Herget 1990). In the second group (H + CO_2), rats were exposed to hypoxia (F_{iO2}=0.1) and hypercapnia (F_{iCO2}=0.04-0.05). The increase in carbon dioxide concentration was achieved by bypassing the KOH absorber. The concentration of CO_2 was continuously monitored. The third group of rats (N) lived in atmospheric air. Rats were exposed to hypoxia or to hypoxia and hypercapnia for 3 weeks (study A) or 4 days (study B).

Study A was designed to investigate the effect of hypercapnia on the development of hypoxic pulmonary hypertension. Study B was focused on the effects of hypercapnia on ROS and NO production in early hypoxia-induced pulmonary vascular injury and on resulting metabolic changes in the walls of the peripheral pulmonary arteries.

After 3 weeks of exposure (study A) the rats were anesthetized with thiopental (30 mg/kg b.w. i.p.). In closed chest rats we measured the mean pulmonary arterial blood pressure (PAP) (Herget and Paleček 1972) and, after thoracotomy, the cardiac output (CO) by ultrasonic flow probe (Hampl *et al.* 2003). Hematocrit, plasma nitrotyrosine concentration (Herget *et al.* 2000) and concentration of lipofuscin-like pigments (lipid peroxidation endproducts) in erythrocyte membranes (Wilhelm and Herget 1999) were measured in samples of arterial blood. The heart was dissected and weighed in parts (Herget *et al.* 1978). The presence of chronic hypoxia-induced structural remodeling of peripheral pulmonary arteries was assessed by a quantitative histological method (Herget *et al.* 1978).

In rats exposed to 4 days hypoxia (study B) we measured the amount of exhaled NO in awake rats. After thiopental anesthesia, plasma concentration of NO plus its oxidation products (NOx) and nitrotyrosine concentration were measured by methods described elsewhere (Hampl *et al.* 2006). Following thoracotomy the lungs were exposed, cooled with ice and peripheral pulmonary arteries were dissected. From 20-25 vascular samples obtained from individual rats we isolated collagen proteins by limited pepsin digestion and individual collagen fractions were analyzed by gel SDS electrophoresis (Novotná and Herget 1998).

Statistical analysis

Results were evaluated by ANOVA with Fischer *post-hoc* test. Values of $p<0.05$ were considered

significant. The results are presented as means ± SEM.

Results

Study A – 3 weeks of exposure

Ten rats were included in each experimental group. All rats in groups N and H survived 3 weeks of experiment, one rat of the group H + CO_2 died after one week of exposure. The groups H and H + CO_2 had significantly lower body weight than normoxic controls N. The presence of high CO_2 concentration in inspired air significantly inhibited the development of HPH. Rats of the H + CO_2 group had significantly lower mean pulmonary arterial blood pressure, decreased right to left heart weight index and less muscularized peripheral pulmonary arteries than hypoxic rats with no hypercapnia (group H). Cardiac output did not differ between the groups. Hypercapnia, however, did not fully prevent the HPH and all indices of HPH were still significantly different from normoxic controls (Table 1). The values of hematocrit in groups exposed to hypoxia did not differ (H 74±2 %, H+CO_2 73±3 %) and were significantly higher than in normoxic controls (N 54±3 %, $P<0.001$). The concentration of lipofuscin-like fluorescent pigments was significantly lower in the group H + CO_2 than in the group H (Fig. 1) and did not differ from normoxic controls.

Study B – 4 days of exposure

Four days exposure to hypoxia increased significantly the amount of NO in exhaled air. This increase was significantly lower in the group exposed to hypoxia and hypercapnia Similarly the increase in plasma concentration of NOx and serum concentration of nitrotyrosine was significantly lower in group H + CO_2 than in group H (Fig. 2).

Exposure to hypoxia induced collagenolysis in the walls of peripheral pulmonary arteries which resulted in the presence of typical low molecular weight collagen fragments (Novotná and Herget 1998). This hypoxia-induced collagen cleavage was less prominent in the group H + CO_2 than in the group H (Figs 3 and 4). Analysis of collagenous proteins was made in 7 rats of groups H and H + CO_2 and in 6 rats of group N.

Discussion

The main finding of this study is that hypercapnia inhibits the development of HPH by interaction with radical tissue injury in the walls of prealveolar pulmonary vessels. The finding that HPH is attenuated by an increase in carbon dioxide concentration is in agreement with previously published studies (Ooi *et al.* 2000, Herget *et al.* 2001, Herget *et al.* 2002). The rats exposed to combined chronic hypoxia and hypercapnia had significantly lower pulmonary arterial blood pressure, significantly lower increase in right heart ventricle weight and less pronounced structural remodeling of the walls of peripheral pulmonary arteries. In acute experiments, hypercapnia added during hypoxic pulmonary vasoconstriction caused further constriction or dilatation of lung vessels, depending on the initial vascular tonus and species of experimental animal (Emery *et al.* 1977). Ventilatory consequences of hypercapnia may alter the PaO_2 for given FiO_2 in short-term exposures (Pepelko 1970). We were not able to measure PaO_2 in our rats acclimatized to chronic exposure. However, the absence of differences in hematocrit values found in H and H + CO_2 groups suggests that the presence of an important difference in the level of hypoxemia is unlikely.

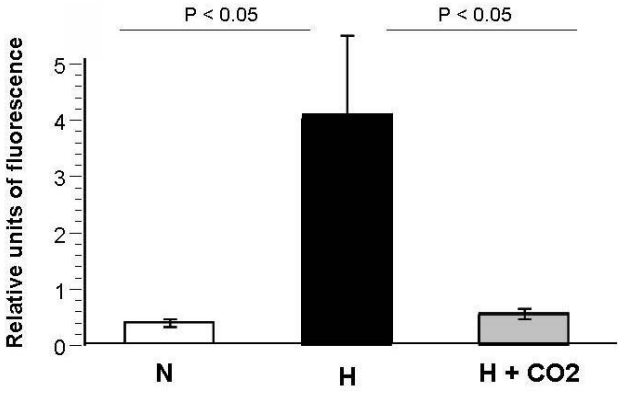

Fig. 1. Concentration of lipofuscin-like fluorescent pigments after 3 weeks exposure to ambient air, hypoxia and hypoxia with hypercapnia. H – group exposed to isobaric hypoxia (F_{iO2}=0.1), H + CO_2 – group exposed to isobaric hypoxia (F_{iO2}=0.1) and hypercapnia (F_{iCO2}=0.04-0.05), N – normoxic controls.

The present study indicates that the probable mechanism whereby hypercapnia inhibits HPH *via* the inhibitory effect of carbon dioxide on tissue hypoxia-induced radical injury to the walls of peripheral pulmonary arteries. In recent years, several studies have provided the solid evidence that radical tissue injury is a crucial mechanism in the development of HPH (for review see Hampl and Herget 2000). Release of both oxygen radicals (Hoshikawa *et al.* 1995, Nakanishi *et al.* 1995, Herget *et al.* 2000, Hoshikawa *et al.* 2001) and NO with its derivatives (Hampl and Herget 2000, Hampl *et al.* 2006) play a role. Production of NO and ROS peaks et the early phase of exposure to hypoxia (Hoshikawa *et al.*

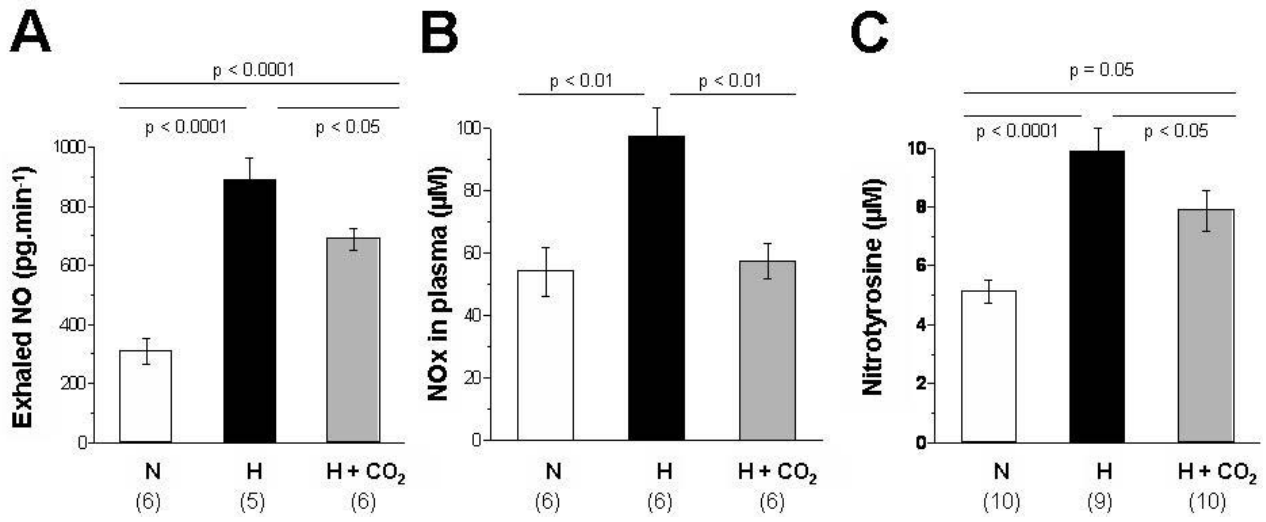

Fig. 2. Four days of exposure to ambient air (N), hypoxia (H) and hypoxia with hypercapnia (H + CO_2). Data are means ± SEM, numbers of rats are in parentheses. **A.** amount of expired of NO of awake rats, **B.** concentration of NOx in plasma, **C.** concentration of plasma nitrotyrosine (marker of superoxide and NO interaction).

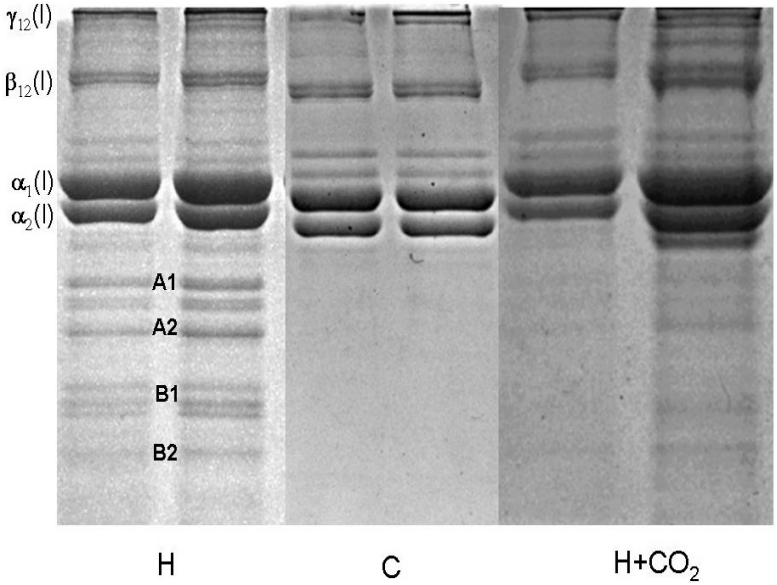

Fig. 3. Typical SDS-PAGE gel electrophoresis of collagen fraction isolated from peripheral pulmonary arteries of rats exposed to chronic hypoxia (H), normal control rat (C) and rats exposed to hypoxia and hypercapnia (H + CO_2). **γ** - gamma fraction, chain polymers of individual α chains of collagen type I; **β** - beta fraction, chain dimmers of individual α chains of collagen type I; **α_1** - individual α1 chains of collagen type I; **α_2** - α2 chains of collagen type I; **A1** – ¾ fragments of α1 chains; **A2** - ¾ fragments of α2 chains; **B1** - ¼ fragments of α1 chains; **B2 -** ¼ fragments of α2 chains.

2001, Lachmanová *et al.* 2005, Hampl *et al.* 2006). Several groups reported a protective role of hypercapnia against free radical injury (Shibata *et al.* 1998, Laffey *et al.* 2000, Lavani *et al.* 2007, Skoumalová *et al.* 2008). The mechanism of this action may be linked to the stabilization of iron-transferin binding due to synergistic action of bicarbonate (Edeker *et al.* 1995) or to the interaction of CO_2 with peroxynitrite and the formation of nitrosoperoxycarbonate with a very short lifetime and therefore restricted diffusion area (Lymar and Hurst 1996, Uppu *et al.* 1996). We assume that peroxynitrite, a highly reactive product of superoxide-nitric oxide interaction (Huie and Padmaja 1993, Beckman and Koppenol 1996), triggers the collagen breakdown in hypoxia-induced injury of prealveolar pulmonary blood vessels (Novotná and Herget 2002) and starts the process of remodeling. The finding that serum concentration of nitrotyrosine (marker of peroxynitrite formation (Beckman 1996) was significantly lower in the H + CO_2 group than in the hypoxic group without hypercapnia is, therefore, in agreement with our observation of less activated collagenolysis and less pronounced remodeling in vessels from rats exposed to hypoxia and hypercapnia.

The interpretation of our results is that hypercapnia inhibits hypoxia-induced tissue injury. Consequently, less NO and ROS is released and the vascular damage is less pronounced.

The alternative explanation is that HPH is decreased because of a vasorelaxant effect of CO_2. Belik *et al.* (2009) reported enhancement of endothelium-

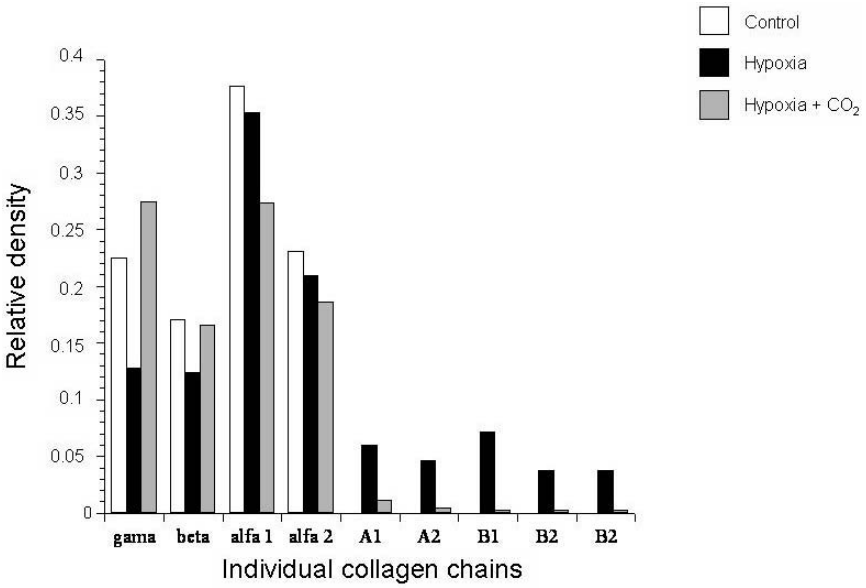

Fig. 4. Relative density of collagen γ, β and α fraction and ¾ and ¼ fragments A1, A2, B1, B2 isolated from peripheral pulmonary arteries of rats exposed to chronic hypoxia (H), normal control rat (C) and rats exposed to hypoxia and hypercapnia (H + CO_2), separated by SDS-PAGE gel electrophoresis.

dependent vasodilatation by severe chronic hypercapnia (10 %). An increase in NO production was ascribed to downregulation of arginase expression and a subsequent increase in endothelial NO production (Belik *et al.* 2009). This alternative is not supported by our finding of less exhaled NO in the group exposed to hypoxia and hypercapnia than in the group kept in hypoxia alone. It should be noted, however, that our rats were exposed to much less severe hypercapnia (4 %).

In summary, we report that concomitant hypercapnia inhibits the development of chronic hypoxic pulmonary hypertension. This is in agreement with the generally accepted permissive effect of hypercapnia (Laffey and Kavanagh 1999).

Conflict of Interest

There is no conflict of interest.

Acknowledgements

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