Hypercapnia Attenuates the Hypoxia-induced Blunting of the Reactivity in Chronically Hypoxic Rats

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Running head: Hypercapnia in Pulmonary Arterial Reactivity

Summary

Chronic hypoxia causes oxidative injury of pulmonary vessels and attenuates their reactivity to different stimuli. When combined with hypercapnia, biochemical markers of this injury are reduced but the effect of concomitant hypoxia and hypercapnia on vascular reactivity is not fully understood. This study was therefore designed to test whether hypercapnia can prevent also the hypoxia-induced loss of reactivity of pulmonary vessels. The reactivity of vessels from rats exposed either to hypoxia or hypoxia combined with hypercapnia was tested using a small vessel myograph (M 500A, Linton, Norfolk, GB). The second and third intrapulmonary branches of pulmonary arteries were isolated under a dissecting microscope from lungs of 8 control rats (group N), 6 rats exposed to hypoxia for 5 days (isobaric, 10% O_2, group H) and 7 rats exposed to hypoxia combined with hypercapnia for 5 days (10% O_2, 5% CO_2, group H+CO_2). The transmural pressure was set by automatic normalization to 30 mmHg. The vessel size did not vary among the groups. After stabilization we challenged the vessels twice with KCl (80 mM) and once with $PGF_{2\alpha}$ (0.1 mM). There were no significant differences in KCl induced contractions among the groups. The responses to $PGF_{2\alpha}$ were expressed as a ratio to the maximal tension obtained by the exposure to 80 mM KCl. Contractions induced by $PGF_{2\alpha}$ were markedly reduced in group H (0.07±0.02) and in group H+CO_2 (0.26±0.03) in comparison with group N (0.83±0.07). The vessels of group H responded to $PGF_{2\alpha}$ less than those of group H+CO_2. However we observed the attenuated reactivity also in group H+CO_2 in comparison with N. Hypercapnia therefore partially blunted the hypoxia-induced loss of reactivity in pulmonary arteries. This finding supports the hypothesis that hypercapnia significantly alters the nature of lung injury induced by chronic hypoxia.

Key words

Isolated pulmonary arteries, hypoxia, hypercapnia.

Main body of the text

Sustained alveolar hypoxia increases the resistance of pulmonary vessels resulting in hypoxic pulmonary hypertension (HPH). The resistance increases because of remodelling of pulmonary vessels and their vasoconstriction (Reid 1986). In some experiments, hypercapnia partially inhibited the development of HPH (Herget *et al.* 2002; Herget *et al.* 2001; Howell *et al.* 2004; Kantores *et al.* 2006; Ooi *et al.* 2000). While it has been recently shown that the process of remodelling is less pronounced when hypoxia combines with hypercapnia (Chovanec *et al.* 2009; Veselá and Wilhelm 2002), the effect of combined hypoxia and hypercapnia on the reactivity of pulmonary vessels remains, as far as we know, not fully understood. Elucidation of this effect has clinical implications: constriction of pulmonary vessels diverts the blood flow from hypoventilated areas and the blunting of this response results in a ventilation/perfusion mismatch. In *in vitro* experiments, the initial phases of exposure to chronic hypoxia are characterised by the blunting of the isolated pulmonary vascular reactivity (McMurtry *et al.* 1978; Reeve *et al.* 2001). We hypothesize that exposure of animals to hypoxia with concomitant hypercapnia could protect also the pulmonary vascular responsiveness. Therefore we tested the response of isolated small pulmonary arteries from rats exposed to either chronic hypoxia or chronic hypoxia combined with hypercapnia.

Experiments on 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 mean body weight of animals was 279±41 g and didn't vary among the groups. The first group (group H, n=6) was exposed to chronic hypoxia for 5 days in an isobaric hypoxic chamber (FiO_2=0.1). CO_2 was completely reabsorbed in a closed circuit by KOH and soda lime (Hampl and Herget 1990). In the second group (H+CO_2, n=7), rats were exposed to hypoxia (FiO_2=0.1) and hypercapnia ($FiCO_2$=0.04-0.05) for 5 days. 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, n=8) lived in atmospheric air. After 5 days of exposure the rats were sacrificed using an intraperitoneal injection of thiopental.

Measurements of pulmonary arterial vasoconstriction were performed on stretched isolated vessel rings using a small vessel myograph (M 500A, Linton, Norfolk, GB). To obtain the rings, we excised the lungs *en bloc* and placed them in cold physiological salt solution (PSS, NaCl 6.954 g/l, KCl 0.35 g/l, $MgSO_4 . 7H_2O$ 0.289 g/l, $NaHCO_3$ 2.1 g/l, KH_2PO_4 0.161 g/l, glucose 1.091 g/l, $CaCl_2 . 2H_2O$ 0.368 g/l). Small pulmonary arteries (SPA, 250–550 μm i.d.) were dissected free of adventitia and mounted in the myograph. The temperature in the chamber was set at 37 °C and the solution was gassed continuously with 95% O_2+5% CO_2 (pH 7.4). After 30 min of stabilization the arteries were stretched to give an equivalent transmural pressure of 30 mmHg (Leach *et al.* 1991).

At the start of each experiment, vessels were exposed to 80 mM K^+ to reach a maximal contractile response. K^+-rich solution was obtained by replacing an equimolar amount of KCl for NaCl in PSS (KPSS). This maximal contraction served as a reference response and was used to normalize subsequent contractile responses. Resting tension remained unchanged throughout the experimental period. Then we stimulated vessel rings using 0.1 mM $PGF_{2\alpha}$. $PGF_{2\alpha}$ is the widely used vasoconstrictor affecting both the Ca^{2+} sensitivity and the Ca^{2+} influx (Snetkov *et al.* 2006).

At the maximum of the contractile response we induced the step-wise relaxation of SPA by the administration of acetylcholine (ACHe) in concentrations of 10^{-6}, 10^{-5}, 10^{-3} M. The concentration of acetylcholine in the bath was stepped up always after reaching the plateau of tension. The KPSS induced contraction was repeated at the end of each experiment to prove the viability of pulmonary arterial rings throughout the experiment. The statistical evaluation was performed using ANOVA and Fisher's *post-hoc* test with the significance $p \leq 0.05$.

The maximal tension induced by KPSS did not significantly differ among the groups of vessel rings. Thus neither hypoxia nor hypoxia combined with hypercapnia affected the maximal contractile response of pulmonary vessels. The contractile responses to the administration of 0.1 mM $PGF_{2\alpha}$ are shown in Fig.1. The results are expressed as the ratio of the $PGF_{2\alpha}$ stimulated contraction to the maximal response of each vessel induced by KPSS. Both groups exposed to hypoxia responded to $PGF_{2\alpha}$ less than controls but the tension developed by vessel rings from rats exposed to hypoxia and hypercapnia was significantly higher than that of rings from animals exposed to only hypoxia. Thus the hypercapnia partly prevented the inhibition of the pulmonary arterial reactivity induced by exposure to chronic hypoxia. The administration of acetylcholine in a concentration of 10^{-3} M returned the vascular tension to the baseline level in all three groups.

The main finding of this report is that hypercapnia attenuates the hypoxia-induced blunting of the reactivity of small pulmonary vessels. Chronic hypoxia has been shown to reduce or block the response of pulmonary vessels to the different stimuli including acute hypoxia or $PGF_{2\alpha}$ (McMurtry *et al.* 1978; Reeve *et al.* 2001). This decreased reactivity was attributed to radical injury of vascular tissue (Hampl and Herget 1991; Herget *et al.* 2000). Hypoxia induced formation of reactive oxygen species (Perez-Vizcaino *et al.* 2010) has been shown to increase Ca^{2+} influx into the vascular smooth muscle cells (Archer and Michelakis

2002) as well as the sensitivity of contractile apparatus to Ca^{2+} (Bonnet *et al.* 2001). Therefore the increased initial vascular tension (Broughton *et al.* 2008) seems acceptable explanation. The fact that we did not find any difference in responses to KCl stimulation suggested that chronic hypoxia or the hypoxia combined with hypercapnia did not change the ability of contractile elements to contract. Thus the hypoxia likely affected the mechanism controlling the contraction. The effect of hypercapnia could be explained by its scavenging activity (Chovanec *et al.* 2009; Laffey *et al.* 2000; Shibata *et al.* 1998; Skoumalová *et al.* 2008). However, although stepwise relaxation of all vessel rings by ACHe, which is used as a test of endothelial dysfunction (Furchgott and Zawadzki 1980), excludes serious damage of endothelial cells, the participation of endothelial cells in the changed vascular reactivity can not be ruled out. In conclusion, our data shows that relatively mild hypercapnia has a protective effect on hypoxia induced changes of vessel reactivity.

Conflict of Interest

There is no conflict of interest.

Acknowledgments

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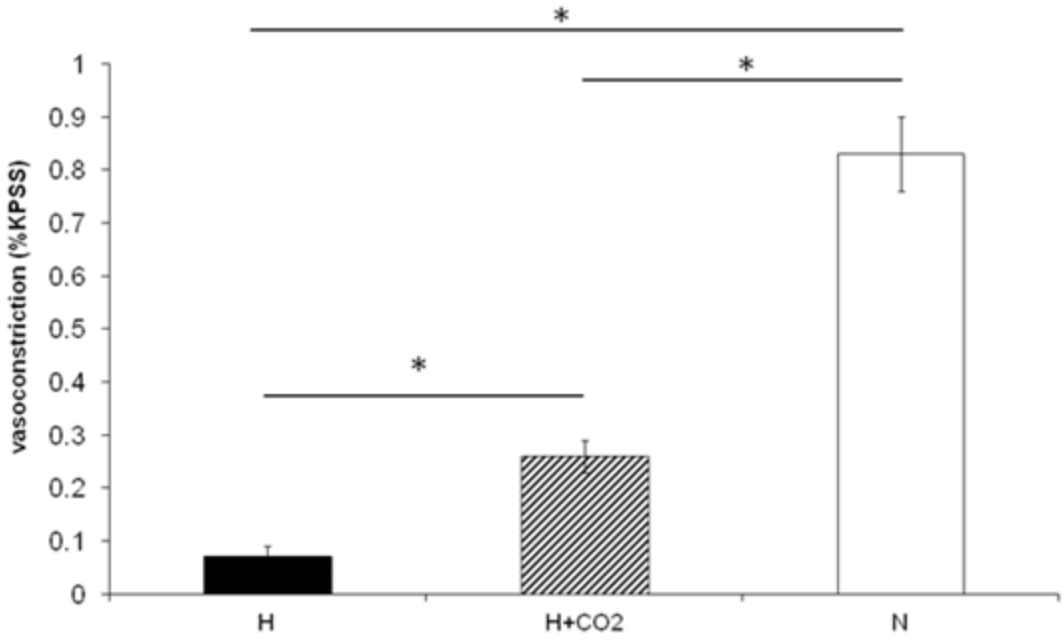

Fig. 1. The contractile responses of isolated pulmonary arteries (means ± S.E.M.) to the administration of 0.1 mM $PGF_{2\alpha}$ expressed as a ratio to the maximal tension obtained by the exposure to 80 mM KCl (KPSS) in each vessel ring. H - rats exposed to chronic hypoxia, $H+CO_2$ - rats exposed to hypoxia combined with hypercapnia, N - control normoxic rats, * - $P \leq 0.05$. Chronic hypoxia combined with hypercapnia attenuated the contractile responses of small pulmonary arteries less than hypoxia alone.

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