Physiol. Res. 58: 917-920, 2009

RAPID COMMUNICATION

Hyperoxia Blunts Acute Hypoxia- and $PGF_{2\alpha}$-Induced Pulmonary Vasoconstriction in Chronically Hypoxic Rats

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Received August 4, 2009
Accepted September 2, 2009

Summary

We investigated the influence of oxygenation of *in vitro* lung preparation on the pulmonary vascular reactivity. Small pulmonary vessels isolated from adult male Wistar rats exposed for 4 days to hypoxia (F_{iO2} = 0.1, group CH) were compared with those of normoxic controls (group N). The bath in the chamber of small vessel myograph was saturated with gas mixture containing either 21 % or 95 % of O_2 with 5 % CO_2 and we measured the reactions of vessels to acute hypoxic challenge with 0 % O_2 or to $PGF_{2\alpha}$. We did not observe any difference of the contractile responses between both groups when the normoxic conditions were set in the bath. When the bath oxygenation was increased to 95 % O_2, the contractions induced by hypoxic challenge and $PGF_{2\alpha}$ decreased in chronically hypoxic rats and did not change in normoxic controls. We hypothesize that reduced reactivity of vessels from hypoxic rats in hyperoxia results from the effect of chronic hypoxia on Ca^{2+} signaling in the vascular smooth muscle, which is modulated by increased free radical production during the exposure to chronic hypoxia and further hyperoxia.

Key words

Hypoxia • Hyperoxia • Isolated pulmonary arteries • Hypoxic pulmonary vasoconstriction

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Several research groups demonstrated the decrease in reactivity of small pulmonary arteries (SPA) isolated from animals exposed to chronic hypoxia including attenuated response to acute hypoxia (McMurtry *et al.* 1978, Bee and Wach 1984, Reeve *et al.* 2001). In these measurements the gas mixture containing a high percentage of oxygen in organ bath was frequently used to ensure adequate oxygenation of SPA (Jones *et al.* 2000, Bonnet *et al.* 2001, Robertson *et al.* 2003, Snetkov *et al.* 2003). However, some other investigators used normoxic gas mixture for the bubbling of the bath (Dipp *et al*. 2001, Nagaoka *et al*. 2004). As early phases of the sojourn in chronic hypoxia are characterized by oxidant stress of pulmonary vascular tissue (Herget *et al.* 2000, Lachmanová *et al.* 2005) and hyperoxia is known to impair hypoxic vasoconstriction in pulmonary vasculature (Newman *et al.* 1981, Jones *et al.* 1983, Gurtner *et al*. 1985, Suzuki *et al*. 1998), we hypothesized that the reported attenuation of response to acute hypoxia may be at least partly due to hyperoxia in organ bath. Therefore we measured the reactivity to acute hypoxic challenge or to $PGF_{2\alpha}$ in SPA isolated from lungs of rats exposed to hypoxia for 4 days when incubated in saline saturated with normoxic or hyperoxic gas mixtures.

The experiments were done in adult male Wistar rats (200-250 g). Experiments were performed in accordance with the European Community and NIH guidelines for using experimental animals. All procedures were approved by the Animal Studies Committee of the Second Medical School, Charles University, Prague. Twelve animals were exposed for 4 days to isobaric hypoxia (FiO_2 = 0.1) in the hypoxic chamber (Herget *et al.* 1978), whereas 14 rats were kept in room air. Then the rats were euthanased by the intraperitoneal injection of

pentobarbital.

Heart and lungs were removed *en block* and placed into the cold physiological salt solution (PSS). The branches of the pulmonary arteries with external diameters of 300-400 μm were isolated and dissected under the microscopic control, cleared of surrounding tissue and rings (1.5-2.3 mm long) were mounted by wires to the jaws of small vessel myograph. The tissue chamber contained 37 °C warm PSS containing (in mM): NaCl 118, $NaHCO_3$ 24, KCl 4, $CaCl_2$ 1.8, $MgSO_4$ 1, NaH_2PO_4 0.434, glucose 5.56. Either 21 % or 95 % of oxygen in the gas was used for the saturation of vessel bath. 5 % CO_2 in the mixture maintained pH at ~7.4. The vessels were stabilized for 40 min and then normalized by the automatic stretching to the wall tension equivalent to the intravascular pressure 20 mm Hg.

At the start of each experiment, vessels were exposed to 80 mM K^+ until a maximal contractile response was observed. K^+-rich solution was obtained by replacing an equimolar amount of KCl for NaCl in PSS. This maximal contraction served as a reference response that was used to normalize subsequent contractile responses. Resting tension remained unchanged throughout the experimental period. The 80 mM K^+ contraction was repeated at the end of each experiment to prove the viability of pulmonary arterial rings during the whole experiment. The vasoreactivity was tested by recording the change in tension generated by small pulmonary arteries after the administration of 50 μM $PGF_{2\alpha}$ and to the acute hypoxic challenge. Acute hypoxic challenges were induced by 40 min of 0 % O_2 + 5 % CO_2 gas mixture. Precontraction required for hypoxic response was set by administration of $PGF_{2\alpha}$ in a dose contracting the vessel to 10 % of its maximal contraction (6-9 mmol/l). Results are expressed as means ± S.E.M. Significance was tested by ANOVA Fischer's-test at $P<0.05$ value.

The basic tested parameters (the mean diameter and the initial K^+-induced maximal responses) did not differ between the groups of normoxic and chronically hypoxic vessels. Hypoxia elicited a typical biphasic response (Leach *et al.* 1994). The first phase of hypoxic pulmonary vasoconstriction (HPV) consisted of a transient contraction followed by a partial relaxation. Then HPV continued by more slowly developing increase in tension – the second phase of HPV. Small pulmonary arteries from the chronically hypoxic rats incubated in the PSS bubbled with 95 % O_2 + 5 % CO_2 had a significantly smaller response to acute hypoxia, both in the first and the second phase of HPV in comparison to arteries isolated from normoxic rats (Fig. 1). In contrast, when the gas mixture used for the saturation of vessel bath contained 21 % O_2 balanced with 5 % CO_2, the responses of both groups to hypoxic challenge did not differ (Fig. 2).

Similarly, vasoconstriction induced by $PGF_{2\alpha}$ (50 μM) was attenuated in small pulmonary arteries from

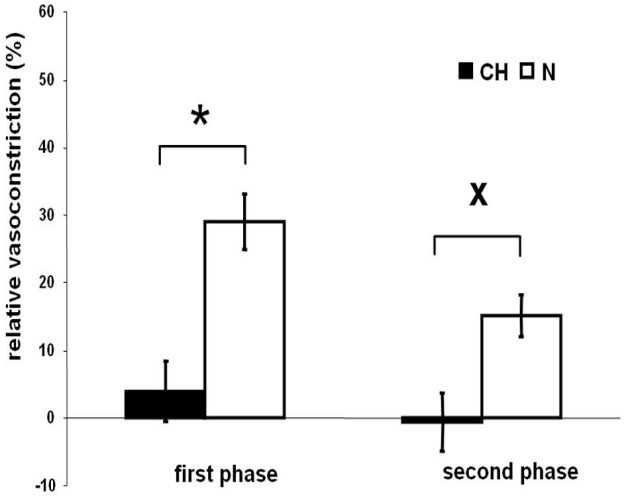

Fig. 1. Responses to acute hypoxic challenge (HPV) in pulmonary vessels isolated from the control rats (N) and from the animals exposed to hypoxia for 4 days (CH), when the bath of SPA was saturated with 95 % O_2. Relative vasoconstriction (%) = percent of the maximal contraction induced by 80 mM K^+ in PSS (means ± S.E.M.). * = vessels from chronically hypoxic rats contracted significantly less than those from normoxic rats in the first phase of HPV ($P<0.01$). x = response in the second phase of HPV was significantly lower in chronically hypoxic rats ($P<0.05$).

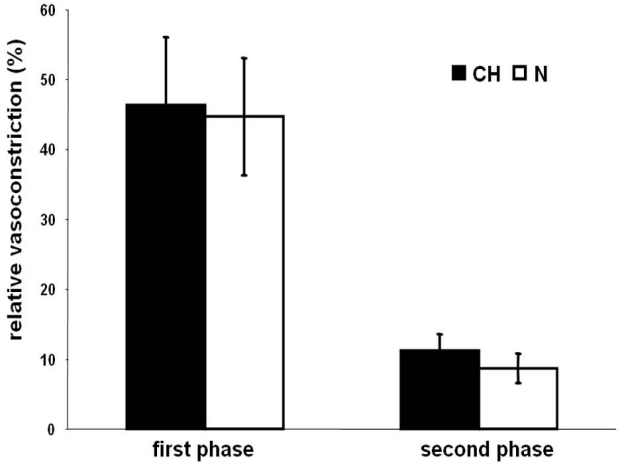

Fig. 2. HPV of SPA in groups CH (animals exposed 4 days to hypoxia) and N (control animals), when 21 % O_2 was used for the saturation of the chamber. Relative vasoconstriction (%) = percent of the maximal contraction induced by 80 mM K^+ in PSS (means ± S.E.M.). There were no significant differences between the groups.

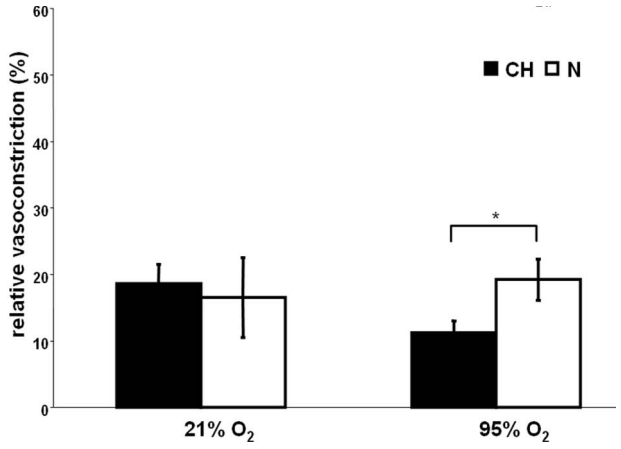

Fig. 3. Vasoconstriction induced by 50 μM $PGF_{2\alpha}$ in animals exposed for 4 days to hypoxia (CH) and control group (N), when 21 % or 95 % O_2 was used for the saturation of the bath. Relative vasoconstriction (%) = percent of the maximal contraction induced by 80 mM K^+ in PSS (means ± S.E.M.). * = vessels from chronically hypoxic rats contracted significantly less than vessels from normoxic controls ($P<0.05$), when the bath was bubbled with 95 % O_2.

chronically hypoxic animals, when the gas mixture used for the saturation of the myograph vessel bath contained 95 % O_2+ 5 % CO_2. The response to $PGF_{2\alpha}$ did not differ in groups of arteries from chronically hypoxic animals and controls, when the organ bath was bubbled with the normoxic gas mixture (Fig. 3).

The major finding of the present study is that small pulmonary arteries isolated from the lungs of rats exposed to 4 days of hypoxia are less reactive to hypoxic challenges and to $PGF_{2\alpha}$ if they are studied in hyperoxic bath solution. In normoxic bath solution they preserve the normal reactivity, comparable to the reactivity observed in pulmonary blood vessels isolated from rats not exposed to hypoxia. The oxygenation of incubation solution does not affect the reactivity to hypoxia and to $PGF_{2\alpha}$ in lung vessels obtained from control normoxic rats. This finding is important for the interpretation of results on pulmonary vascular reactivity in hypoxic pulmonary hypertension. The increase in pulmonary vascular resistance after the exposure to chronic hypoxia is the result of balance between the remodeling of the vascular wall of small pulmonary arteries (thickening the muscular media and adventitial fibrotization) and vasoconstriction. Observed decrease of vascular reactivity is probably resulting from chronic hypoxia-induced oxidant stress, which is enhanced by hyperoxia in vessel bath. Oxidant stress is involved in the regulation of pulmonary vascular tone in hypoxia (Hampl and Herget 1991). It is well known that chronic hypoxia alters pulmonary arterial reactivity as a consequence of an effect on both Ca^{2+} signaling and Ca^{2+} sensitivity of the contractile apparatus (Bonnet *et al.* 2001) by mechanism modulating RhoA/Rho-kinase activity (Broughton *et al.* 2008), which is regulated by reactive oxygen species production (Jernigan *et al.* 2008). In addition, hyperoxia causes vascular paralysis through oxidant-induced injury to the pulmonary vasculature (Gurtner *et al.* 1985). This can explain why the hyperoxic environment affects non-specifically the reactivity of small pulmonary arteries isolated from lungs of rats with hypoxic pulmonary hypertension and not from normoxic controls.

Conflict of Interest

There is no conflict of interest.

Acknowledgements

This study was partially supported by grants GAČR 305/06/P385, GAČR 305/05/08/108, Center 1M510.

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