RAPID COMMUNICATION

Disodium Cromoglycate Attenuates Hypoxia Induced Enlargement of End-Expiratory Lung Volume in Rats

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Summary

Mechanism responsible for the enlargement of end-expiratory lung volume (EELV) induced by chronic hypoxia remains unclear. The fact that the increase in EELV persists after return to normoxia suggests involvement of morphological changes. Because hypoxia has been also shown to activate lung mast cells, we speculated that they could play in the mechanism increasing EELV similar role as in vessel remodeling in hypoxic pulmonary hypertension (HPH). We, therefore, tested an effect of mast cells degranulation blocker disodium cromoglycate (DSCG) on hypoxia induced EELV enlargement. Ventilatory parameters, EELV and right to left heart weight ratio (RV/LV+S) were measured in male Wistar rats. The experimental group (H+DSCG) was exposed to 3 weeks of normobaric hypoxia and treated with DSCG during the first four days of hypoxia, control group was exposed to hypoxia only (H), two others were kept in normoxia as non-treated (N) and treated (N+DSCG) groups. DSCG treatment significantly attenuated the EELV enlargement (H+DSCG=6.1±0.8; H=9.2±0.9; ml ± SE) together with the increase in minute ventilation (H+DSCG=190±8; H=273±10; ml/min ± SE) and RV/LV+S (H+DSCG=0.39±0.03; H=0.50±0.06).

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Chronic hypoxia and exposure to high altitude leads to the development of hypoxic pulmonary hypertension (HPH) characterized by increase of pulmonary arterial pressure, hypertrophy of the right heart and thickening of peripheral pulmonary vessels. Hypoxia concurrently increases the end-expiratory lung volume (EELV) (Barer *et al.* 1978). Although this increase has implications for mechanics of breathing (Barer *et al.* 1978, Vizek and Bonora 2001), its mechanism remains unclear. The fact that this enlargement persists after return to air breathing for weeks suggests that remodeling of the lung tissue rather than functional changes are involved. Hypoxia is known to induce increased activity of proteolytic enzymes as serine proteases and tissue metalloproteinases (Greenlee *et al.* 2007, Pejler *et al.* 2010), which has been shown to participate in remodeling of pulmonary vessels (Riley *et al.* 2000). It has been also shown that important source of proteolytic activity are pulmonary mast cells (Tozzi *et al.* 1998) and prevention of their degranulation attenuates vascular remodeling (Banasova *et al.* 2008). To test whether release of mast cells proteolytic enzymes is involved also in a mechanism responsible for EELV enlargement in chronic hypoxia, we decided to measure EELV changes in rats treated during hypoxic exposure by mast cell stabilizer DSCG. DSCG is compound which prevents mast cells degranulation and acts as anti-inflammatory agent (Theoharides *et al.* 2000).

Studies were performed in 20 adult male Wistar rats (Konárovice, Czech Republic) in accordance with the European Community and NIH guidelines for using experimental animals. All procedures were approved by

Table 1. Average body weight (BW), minute ventilation (V´$_E$) and right heart ventricle to left ventricle plus septum ratio (RV/LV+S) in normoxic (N) and hypoxic (H) nontreated groups and normoxic (N+DSCG) and hypoxic (H+DSCG) groups treated with DSCG.

Group	n	BW, g	V´$_E$, ml/min	RV/LV+S
N	5	352 ± 14	183 ± 7	0.231 ± 0.007
N+DSCG	5	379 ± 6	202 ± 6	0.253 ± 0.007
H	5	391 ± 10	273 ± 10 *	0.496 ± 0.057 **
H+DSCG	5	367 ± 7	190 ± 8	0.386 ± 0.026 ** †

* p<0.0001 H group versus all groups; ** p<0.01 H and H+DSCG groups versus N and N+DSCG groups; † p<0.05 H+DSCG group versus H group

our institution's Animal Studies Committee.

The experimental group (H+DSCG) was exposed to 3 weeks of normobaric hypoxia (F$_I$O$_2$ 0.1) in a normobaric hypoxic chamber (Hampl and Herget 1990) and treated with disodium cromoglycate DSCG (Sigma Aldrich, Prague, Czech Republic) applied intraperitoneally (40 mg/kg b.w.) during the first four days of hypoxia. One control group was exposed to hypoxia only (H), two others were kept in normoxia as non-treated (N) and treated (N+DSCG) groups. All groups were tested on the 21st day after the onset of experiment. PCO$_2$, temperature and humidity in the hypoxic chamber were controlled and kept the same as for normoxic controls.

Measurement of ventilation and EELV was performed in animals anesthetized with an intraperitoneal injection of Thiopental (ICN Czech Republic, Roztoky, Czech Republic; 40 mg/kg/ b.w.). They were intubated and placed in a whole body plethysmograph (Maxova and Vizek 2001). A tracheal cannula (ID 1.7 mm, OD 2.3 mm) was connected to an outer circuit ventilated with air. A specially designed computer program was used to calculate the rate of breathing, tidal volume, minute ventilation and EELV (Maxova and Vizek 2002). EELV was calculated from the changes in tracheal pressure and lung volume after the occlusion of the tracheal tube at the end of expiration. Rats were then overdosed with Thiopental and the hearts were removed from the chest. The right heart ventricle (RV) and the left ventricle plus septum (LV+S) were separated and weighed. RV/LV+S ratio was used as a marker of HPH.

Each ventilatory variable was averaged over six consecutive respiratory cycles. EELV values are the mean of three measurements. The results are presented as means ± SE. ANOVA with Fischer's PLSD test was used for statistical evaluation of the data, p<0.05 was considered as significant.

The average body weights (BW), minute ventilation (V´$_E$) and right heart ventricle to left ventricle plus septum ratio (RV/LV+S) are summarized in Table 1. Higher ventilation in group H was only due to increase in tidal volume, rate of breathing was similar in all groups (ANOVA, p=0.06).

Administration of DSCG did not affect EELV of animals kept in air. Exposure to chronic hypoxia increased EELV, but significantly less in the DSCG treated (H+DSCG group) than in the non-treated (H group) rats (Fig. 1).
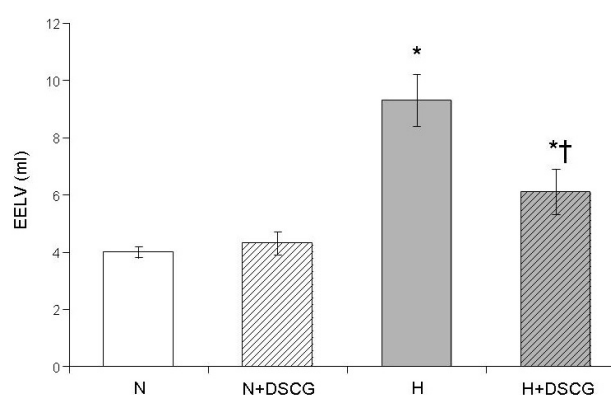

Fig. 1. End-expiratory lung volume (EELV, ml) in normoxic (N) and hypoxic (H) nontreated groups (unshaded) and DSCG treated (N+DSCG, H+DSCG) groups (shaded). * p<0.05 H and H+DSCG groups versus N group; † p<0.05 H+DSCG group versus H group

The main finding of our study is that prevention of mast cell degranulation at the onset of exposure to chronic hypoxia significantly attenuated EELV enlargement in chronic hypoxia.

To prevent release of substances from mast cells we used disodium cromoglycate (DSCG) in the same dose as in our previous experiments (Banasova *et al.* 2008, Maxova *et al.* 2010). We applied DSCG only

during the first four days of hypoxic exposure, because the initial phase of hypoxia is for the remodeling of the lung tissue crucial (Chovanec *et al.* 2009, Lachmanova *et al.* 2005). Administration of DSCG in early phase of hypoxia has effect on vascular remodeling. Histology revealed significantly decrease percentage of double laminated peripheral pulmonary vessels in DSCG treated group, gel electrophoresis of collagen showed less collagen fragments in extracts from peripheral pulmonary arteries (Banasova *et al.* 2008).

EELV increases could be classified as dynamic or static hyperinflation (Palecek 2001). Because components of dynamic hyperinflation – airway resistance, breathing frequency and postinspiratory activity of the diaphragm (DE) – in chronic normobaric hypoxia decrease (Habre *et al.* 2010) or do not differ from that of controls (breathing frequency – our results; DE – Vizek and Bonora (2001)) dynamic increase in EELV is not likely.

Hypoxia has been shown to induce the release of wide variety of proteolytic enzymes and growth factors in particular from lung mast cells (Maxova *et al.* 2008, Thakker-Varia *et al.* 1998). Enhanced collagenolytic activity in tissue (Novotná and Herget 1998, Riley *et al.* 2000) may result in an increase of the lung compliance (component of static hyperinflation). However, increased lung compliance was found only in newborn rats exposed to 6 days of hypoxia (Okubo and Mortola 1989), while others did not find in chronic hypoxia any increase in compliance (Barer *et al.* 1978) or decrease in elastance (Habre *et al.* 2010). Hypoxia may also trigger growth (Sekhon *et al.* 1995) and/or remodeling of the lungs similar to that seen during development of HPH. Estrada and Chesler (Estrada and Chesler 2009) have recently shown collagen-related gene and protein expression changes in lungs of chronically hypoxic mice.

We cannot outline the mechanism increasing EELV in chronic hypoxia, however the fact that it involves mast cells degranulation brings new aspect of the problem. Interestingly enough the similarity in effects of blocking of mast cells degranulation on increases in pulmonary blood pressure and EELV in chronic hypoxia suggests that mechanisms responsible for these changes are interconnected.

Conflict of Interest
There is no conflict of interest.

Acknowledgements

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