

# Prevention of Mast Cell Degranulation by Disodium Cromoglycate Delayed the Regression of Hypoxic Pulmonary Hypertension in Rats

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## Key Words

Chronic hypoxia • Disodium cromoglycate • Hypoxic pulmonary hypertension • Mast cells • Rat • Recovery

## Abstract

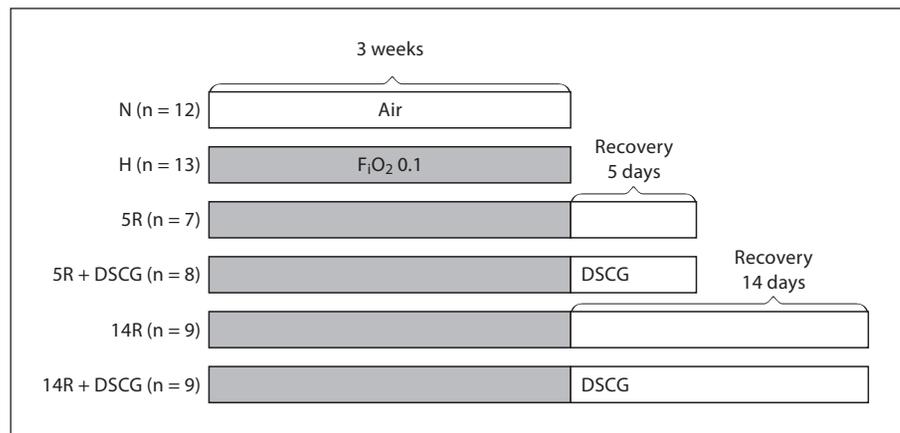
**Background:** Pulmonary vascular remodeling induced by chronic hypoxia regresses after return to normoxia. This regression is associated with an increased amount of collagenase in pulmonary mast cells and increased collagenolytic and elastolytic activity in the lung tissue. **Objective:** The role of lung mast cells during recovery from chronic hypoxia was tested by the inhibition of their degranulation by disodium cromoglycate (DSCG). **Methods:** Male Wistar rats (n = 46) were exposed to isobaric hypoxia (3 weeks, F<sub>i</sub>O<sub>2</sub> 0.1). Thirteen of them were tested immediately at the end of exposure, 17 were treated with DSCG during the first 4 days of recovery and tested on the 5th or 14th day of recovery, 16 untreated animals were measured at the same time intervals. These groups were compared with 12 animals kept in normoxia. The rats were anesthetized (Thiopental) and their pulmonary arterial blood pressure (PAP), cardiac output and heart weight were tested, as well as the collagen composition of the walls of the peripheral pulmonary arteries. **Results:**

DSCG applied during the first 4 days of recovery from chronic hypoxia blocked the decrease in PAP during the early phase of recovery and had no influence on PAP at a later phase. DSCG administration prevents collagen splitting in peripheral pulmonary vessels at the early phase of recovery. PAP and right ventricle hypertrophy were normalized after 14 days of return to normoxia. **Conclusions:** Mast cell degranulation plays a role in the regression of pulmonary hypertension during the early phase of recovery from chronic hypoxia.

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## Introduction

Chronic hypoxia results in hypoxic pulmonary hypertension (HPH) characterized by fibrotization and muscularization of the walls of the peripheral pulmonary arteries, reversible after return to normoxia [1]. The time frame of these changes depends on the magnitude and/or duration of hypoxia, and occurs over a number of weeks. Restoration of the hemodynamic parameters is usually slower than biochemical changes in the wall of pulmonary arteries. Tozzi et al. [2] describe normalization of the right



**Fig. 1.** Study design.

ventricular pressure after 14 days of recovery, and Fried and Reid [3] show significantly increased pulmonary arterial pressure (PAP) even after 1 month of exposure to room air. The lowering of PAP is accompanied by restoration of normal vascular architecture. This process is associated with a decrease in collagen and elastin content and an increase in collagenolytic and elastolytic activity. Normalization of proteolytic, collagenolytic and gelatinolytic activities in pulmonary arteries occurs within 14 days of recovery [4], hydroxyproline content and the ratio of elastin to protein do not differ from the control vessels within 7 days after return to air [5]. The increase in immunoreactive collagenase in pulmonary mast cells in the adventitia and media of hypertrophied pulmonary vessels [2], suggests that pulmonary mast cell collagenase plays a specific role. While the development of hypoxic pulmonary hypertension has been studied in depth, including in our previous paper, which shows that mast cell degranulation plays a role in the initiation of hypoxic pulmonary vascular remodeling [6], comparatively less is known about the mechanisms involved in recovery. The involvement of mast cells in the recovery phase is mostly based on indirect evidence [2, 7]. The present study was therefore designed to elucidate the role of mast cell degranulation in recovery from chronic hypoxia.

## Material and Methods

### Study Design

Six groups of adult male Wistar rats (Anlab, Prague, Czech Republic) were used. Experiments 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 normoxic group that served as the control was kept in air. Five groups of rats were exposed to permanent hypoxia (FiO<sub>2</sub> 0.1) using a normobaric chamber [8] for 3 weeks [9, 10]. Two groups of the animals were treated with disodium cromoglycate [6, 11, 12] (Cromolyn sodium salt, Sigma Aldrich, Prague, Czech Republic; 40 mg/kg body weight (BW), i.p. once a day) for the first 4 days of recovery and they were measured after 5 or 14 days of recovery in air. The study design is shown in figure 1.

### Hemodynamic Measurements

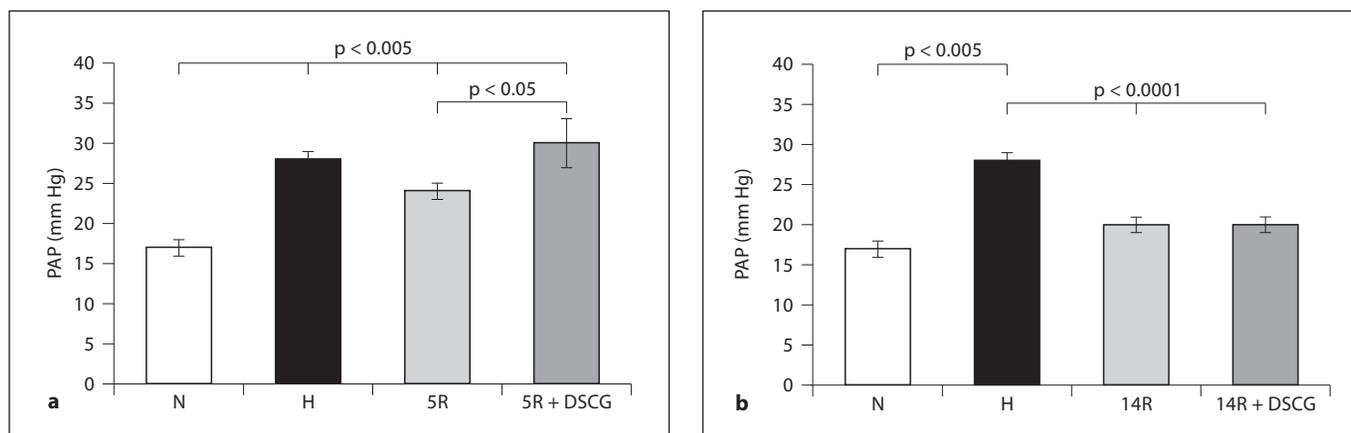
Mean PAP was recorded in rats anesthetized with Thiopental (ICN, Roztoky, Czech Republic; 40 mg/kg BW, i.p.). In rats spontaneously breathing room air, the pulmonary artery was catheterized via the right jugular vein without opening the chest [10, 13]. Final data were computed from a 2-minute recording of mean PAP. Blood samples for hematocrit were obtained from this vein. Cardiac output was estimated by an ultrasonic flow probe placed at the ascending aorta after opening the chest under mechanical ventilation with room air (50 breaths/min by positive pressure; Rodent ventilator model 683, Harvard Apparatus, Holliston, Mass., USA) [14]. After taking hemodynamic measurements, the heart was removed from the chest. The right heart ventricle and the left ventricle plus septum were separated and weighed.

### Collagen Composition of the Walls of Peripheral Pulmonary Arteries

The lungs of animals from group N, H, 5R and 5R + DSCG (fig. 1) were used to assess the collagen composition of the peripheral pulmonary vessels. The 3rd and 4th branches of the peripheral pulmonary arteries were dissected, digested by pepsin, and the supernatant with collagen proteins was analyzed by SDS-PAGE electrophoresis [15].

### Analysis

The statistical analyses were performed using StatView 5.0 (SAS Institute, Cary, N.C., USA). Comparison procedures were made by ANOVA with Fischer's post-hoc test. Values of  $p < 0.05$  were considered significant. The results are presented as means  $\pm$  SE.



**Fig. 2.** Pulmonary arterial pressure in early phase of recovery (a) and late phase (b).

**Table 1.** Body weight, hematocrit and hemodynamic parameters

Group	n	BW g	HTC %	CO ml/min/ 100 g BW	(LV + S)/BW mg/100 g BW
N	12	351 ± 9	48 ± 1*	8.5 ± 0.3	189 ± 6
H	13	316 ± 9 <sup>††</sup>	56 ± 1 <sup>†</sup>	8.0 ± 0.4	183 ± 5
5R	7	375 ± 12	51 ± 1* <sup>†</sup>	9.1 ± 0.6	199 ± 4
5R + DSCG	8	394 ± 17	52 ± 1* <sup>†</sup>	8.4 ± 0.7	195 ± 4
14R	9	350 ± 4	47 ± 1*	8.1 ± 0.6	171 ± 4
14R + DSCG	9	341 ± 5	47 ± 1*	7.0 ± 0.5	177 ± 3

CO = Cardiac output; HTC = hematocrit; LV + S = left ventricle plus septum.

\*  $p < 0.05$ , normoxic and recovery groups versus the H group.

<sup>†</sup>  $p < 0.05$ , H and 5R groups versus the N group.

<sup>††</sup>  $p < 0.05$ , hypoxic group versus all groups.

## Results

Exposure to hypoxia had little effect on the body weight of the experimental rats. The body weight of the rats recovering from hypoxia did not differ from the normoxic controls (table 1).

The only difference in PAP between those groups treated with DSCG and the untreated group occurred at the early phase of recovery, when it was higher in the treated rats (fig. 2a). DSCG had no effect on PAP at the late phase of recovery, when it returned to control (normoxic) values (fig. 2b). Values of cardiac output and the relative weights of the left ventricle plus septum are summarized on table 1. The relative weights of the right ven-

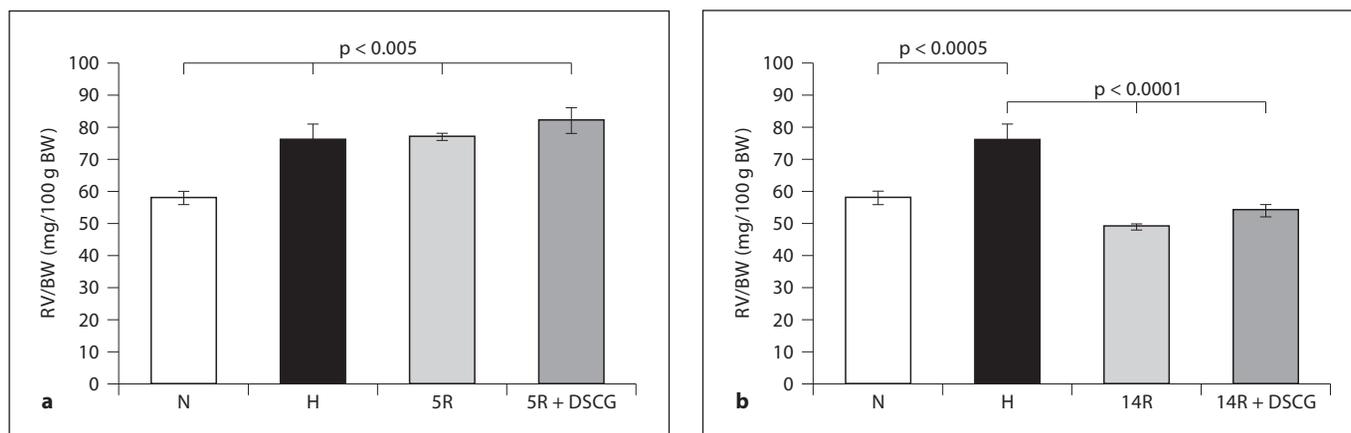
tricle differed between groups but they were not affected by DSCG (fig. 3).

Analysis of collagen extracts isolated from the peripheral pulmonary arteries of rats exposed for 21 days to hypoxia, and of DSCG-untreated rats after 5 days of recovery from 21 days of hypoxia, showed the presence of the 3/4 and 1/4 cleavage fragments of  $\alpha 1$  and  $\alpha 2$  collagen type I chains. Moreover, collagen extracts from the peripheral pulmonary arteries of rats recovering for 5 days from chronic hypoxia showed a peptide doublet (fig. 4) of collagen cleavages with a molecular mass close to  $\alpha 1$  (I) and  $\alpha 2$  (I). These collagen fragments are probably the products of telopeptidase collagenolytic activity of MMP-13 [16]. The doublet was not present in the hypoxic, normoxic and DSCG-treated groups (fig. 4).

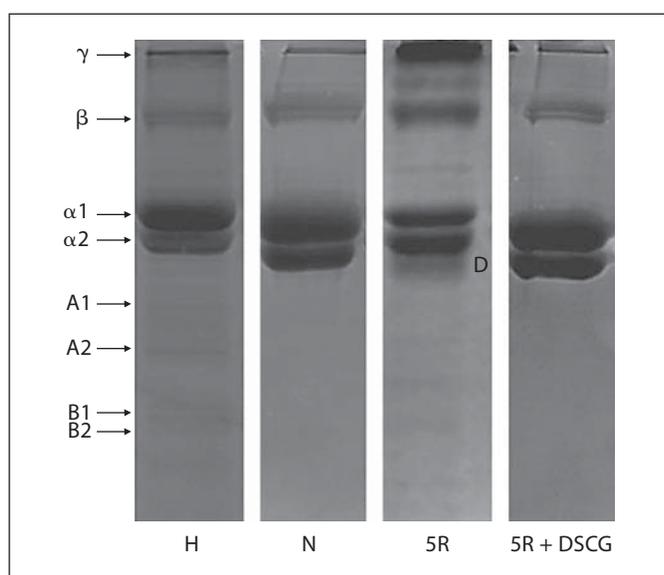
## Discussion

The present study was designed to extend our previous report [6] by determining the role of the mast cells in the process of pulmonary vascular remodeling. The main finding is that prevention of mast cell degranulation by DSCG after the return from hypoxia to normoxia delayed the lowering of PAP. As far as we know, this is the first study showing the effect of DSCG administration during recovery from chronic hypoxia. It clearly supports our hypothesis that mast cell activity plays a role in the recovery of lung vascular structure after the brief period in chronic hypoxia.

The pattern and timing of pulmonary vascular convalescence, among other factors, influences the clinical out-



**Fig. 3.** Relative weights of the right ventricle measured at 5 days (a) and 14 days (b) of recovery in air.



**Fig. 4.** SDS-PAGE electrophoresis of collagen extracts isolated from the peripheral pulmonary arteries shows presence of 3/4 and 1/4 cleavage fragments of  $\alpha 1$  and  $\alpha 2$  collagen type I chains. Extracts from rats recovering for 5 days from chronic hypoxia showed a peptide doublet (D) of collagen cleavages with molecular mass close to  $\alpha 1$  (I) and  $\alpha 2$  (I).

come of patients recovering from left heart failure. The search for factors which determine this process is therefore clinically relevant.

We used the proven dose and method of DCSG administration, as described in our previous study [6]. DCSG treatment did not affect other changes typical af-

ter return to normoxia (i.e. a decrease in hematocrit and the relative weight of the right heart).

In our experiment, the time course of the heart weight and hematocrit restoration was rapid. Fourteen days after returning to breathing room air, there were no differences in any of the measured parameters between the treated groups and the normoxic controls. On the 14th day of recovery, Tozzi et al. [2] observed normalization of the right ventricular pressure only, while the weight of the right ventricle and hematocrit were still increased.

Exposure to chronic hypoxia leads to the development of hypoxic pulmonary hypertension characterized by increased PAP, right heart ventricular hypertrophy and remodeling of the peripheral pulmonary vessel walls [17]. The onset of vascular remodeling is accompanied by an increase in collagenolytic activity of extracellular matrix and the presence of specific collagen cleavage products [15] which may induce the proliferation of mesenchymal cells [18]. These changes are at their maximum during the first week of hypoxic exposure and then do not progress [10]. Prevention of mast cell degranulation attenuates the development of hypoxic pulmonary hypertension in rats exposed to chronic hypoxia [6].

The collagenolytic activity again increases after the return to normoxia (recovery) [2] as well as the number of MMP-13 positive mast cells at the prealveolar arterial level [2, 19]; but in this case it leads to the regression of pulmonary vascular remodeling and return to normal PAP values. Interestingly, the collagen cleavage products found in the walls of the peripheral pulmonary arteries during recovery differ from those found at the onset of hypoxia. After 5 days of recovery, we detected a peptide

doublet of collagen cleavages with a molecular mass close to  $\alpha 1$  (I) and  $\alpha 2$  (I), which is not present in walls of similar arteries during the onset of hypoxia. This finding suggests that the nature of collagenolytic activity during recovery may differ from that described in the onset of hypoxic exposure. It is worth noting that a similar type of collagenolytic activity was also found in rats exposed to hyperoxia [16].

In conclusion, we have shown that in rats degranulation of mast cells plays an important role in the early phase of recovery from hypoxic pulmonary hyperten-

sion, and that the collagenolytic activity in the lung tissue during recovery from hypoxia differs from that seen during the onset of hypoxia.

### Acknowledgments

The study was supported by the Grant Agency of Charles University 7228/2007, the Grant Agency of the Czech Republic 305/08/0108, and by the Cardiovascular Research Centre MSMT 1M0510.

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