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Characterisation of the ventilatory response to hypoxia in a model of transgenic anemic mice

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Abstract

Both polycythemia and the increase in hypoxic ventilatory response (HVR) are considered as important factors of acclimatization to hypoxia. The objective of this study was to characterise the ventilation pattern at different inspired oxygen fraction in a model of chronic anemic mice. These mice have a targeted disruption in the 5′ untranslated region of the Epo gene that reduces Epo expression such that the homozygous animal is severely anemic. Ventilation in normoxia in Epo-TAg^{h} mice was significantly greater than in wild type, and the difference was mainly due to a higher tidal volume. HVR was higher in Epo-TAg^{h} mice at every FIO_2 suggesting a higher chemosensitivity. Resting oxygen consumption was maintained in anemic mice. Maximal oxygen consumption was 30% lower while hemoglobin was 60% lower in anemic mice compared to wild type. This small decrease in maximal oxygen consumption is probably due a greater cardiac output and/or a better tissue oxygen extraction and would allow these anemic mice to acclimatize to hypoxia in spite of low oxygen carrying capacity. In conclusion, Epo-TAg^{h} anemic mice showed increased ventilation and hypoxic ventilatory response. However, whether these adaptations will contribute to acclimatization in chronic hypoxia remains to be determined.

Keywords: Transgenic mice; Anemia; Hypoxic ventilatory response; Oxygen consumption

1. Introduction

The ventilatory response to hypoxia successively includes the acute ventilatory response to hypoxia, the relative decrease in ventilation (hypoxic ventilatory decline) after some minutes of exposure, and the time-dependent increase in ventilation that occurs with chronic hypoxic exposure of a few hours to several weeks. It is now well known that an increase in the acute hypoxic ventilatory response (HVR) contributes to the ventilatory acclimatization to hypoxia (Bisgard and Neubauer, 1995; Powell et al., 1998, 2000; Reeves et al., 1993).

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In anemia, a majority of reports indicates an absence of hyperventilation in normoxia (Woodson et al., 1978), or a small increase in ventilation related to the hypotension due to peripheral vasodilatation in hypoxia (Saiki et al., 1994; Sardella and Ou, 1993). The oxygen carrying capacity of the blood is reduced, resulting in tissue hypoxia. Nevertheless at rest in normoxia, oxygen delivery is maintained by an increase in cardiac output and tissue O_2 extraction (Gonzalez et al., 1994; Ickx et al., 2000; Kurdak et al., 1995) to preserve oxygen consumption (Ickx et al., 2000; Vaslef et al., 2001). However, it has been also shown a fall in oxygen consumption secondary to acute anemia induced by hemodilution (Kurdak et al., 1995).

In addition to the constraint of a decrease arterial O_2 content (CaO_2), exposure to hypoxia adds the constraint of a decrease arterial O_2 pressure (PaO_2) that triggers integrated responses at the respiratory, cardiovascular and hematological levels.

This study focuses on the time course and effect of acute hypoxic exposure on the ventilatory response in the erythropoietin SV-40 T antigen mouse (Epo-TAg^h). This mouse has a targeted disruption in the 5′ untranslated region of the Epo gene that reduces the Epo expression such that the homozygous animal is severely anemic (Binley et al., 2002). The use of the Epo-TAg^h allows us to determine the effect of anemia on HVR, without the adverse effects of bleeding or hemodilution.

Polycythemia is considered as an important factor of acclimatization to hypoxia, together with the increase of ventilation. Because, the beneficial role of polycythemia has been questioned, it was of interest to characterise the ventilation pattern and response to hypoxia in a model of chronic anemic mice.

2. Material and methods

2.1. Animals

Animal studies were performed in accordance with guidelines established by the French Ministère de l'Agriculture.

Six anemic SV-40 T antigen (Epo-Tag^h) and six healthy wild type (C57B16/CBA) mice, 6- to 8-week-old, were investigated. Animals were kept in a room with free access to food and water. Temperature ($21 \pm 2\,^\circ$C) and humidity ($49 \pm 3\%$) were checked daily. The animals were weighed before the experiments. At the end of the experiments a blood sample was taken from the tail vessel for hematocrit determination with a microcentrifuge (Microspin AMES, Germany) and hemoglobin measurement (OSM 3, Radiometer, Copenhagen, Denmark). After heart dissection, right ventricle (RV) free wall was completely separated from the left ventricle (LV) and septum (S). RV free wall and left ventricle with septum (LV + S) were weighed separately.

2.1.1. Ventilatory measurements

Ventilation was measured in conscious animals via a whole body single-chamber plethysmograph. The system consisted of a 312 ml plexiglas experimental chamber. The chamber provided a continuous airflow at 0.85 l/min via a manual flow meter system (Sho-Rate II 11355). Differential pressure between the experimental and reference chamber was measured with a differential pressure transducer (model DP45-18, Valydine). The pressure signal was sent to a demodulator (model CD1, Valydine) and data recorded by a Biopac system (BIOPAC System Inc. Santa Barbara, California, USA). Barometric pressure was measured routinely before experiments and temperature inside the chamber was kept between 21 and 23 °C and continuously monitored with a digital thermometer (Thermo Frigo OTAX). Ventilation ($\dot{V}_E$, ml/min BTPS/kg), respiratory frequency (f_R, breaths/min), and tidal volume (V_T, ml BTPS), were computed breath-by-breath throughout all baseline and experimental periods and were stored for offline analysis. Each animal was placed in the chamber and allowed at least 30 min to acclimatize before the assessment of ventilation. Baseline measurements were made when the animal was quiet but awake, and were averaged.

2.2. Hypoxic ventilatory response (HVR)

Measurements were performed in normoxia and after acute hypoxic exposure (FIO_2: 0.18, 0.15, 0.12, 0.10 and 0.08). At a given FIO_2 a measurement of ventilation was done every 5 min for a total of 20 min. Between each hypoxic exposure the mice was kept in normoxia for at least 2 h. Hypoxic gas mixture was obtained by mixing room air with N_2 in a 250 ml mixing-chamber connected to the plethysmographic chamber. Oxygen