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Review

Chemosensory ventilatory responses in the mutant mice with Presbyterian hemoglobinopathy☆

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ABSTRACT

The working hypothesis of this study was that chronically increased tissue oxygenation would facilitate respiratory endurance to chemical stimuli. We investigated the ventilatory responses to hypoxia and hypercapnia before and after carotid chemodenervation in the anesthetized, spontaneously breathing Presbyterian, which carry a low affinity variant of hemoglobin, and in wild-type mice. We found a dampening of all chemosensory responses in Presbyterian hemoglobinopathy. Particularly, the Presbyterian mouse with intact carotid body innervation was more vulnerable to hypoxia than the wild-type mouse, showing an accelerated decline in breathing frequency which was not counterbalanced by tidal respiration. We further found that chemodenervation in the Presbyterian mouse, performed in normoxia, led to respiratory arrest. The study shows enhanced susceptibility of respiration to hypoxia and indispensability of neural input from the carotid body for upholding the central respiratory controller's function in Presbyterian hemoglobinopathy. The study also suggests a relationship between hemoglobin-oxygen dissociation and respiration, which points to a metabolic, tissue oxygenation-linked component of respiratory regulation.

1. Introduction

The Presbyterian mouse have recently been embraced as a novel experimental model to study the posited influence on respiration of tissue oxygenation, as opposed to the widely recognized effects of arterial blood oxygen level (PaO_2). The mouse is a mutant that carries a low affinity for oxygen variant of the β-globin chain. In the variant, Asn108 is substituted for by Lys in the mouse genome by a targeted knock-in strategy (Shirasawa et al., 2003). Lower affinity for oxygen of the Presbyterian mouse's hemoglobin (HB Presbyterian), causing a rightward shift of the Hb-O_2 dissociation curve, results in a greater availability of oxygen to tissues.

The influence of tissue oxygenation on respiration dates back to the theories presuming the existence of chemoreceptors on the mixed venous side that would respond to the metabolic aspects of endogenous CO_2, as exemplified by exercise (Huszczuk et al., 1986). Such receptors, albeit present in other species such as birds (Boon et al., 1980), have not as yet been unequivocally proven in mammals. Pharmacologically-enhanced O_2 affinity by sodium cyanide failed to change the hypoxic ventilatory response in the rat (Birchard and Tenney, 1986). This result, however, does not invariably preclude the possible role of tissue oxygenation in breathing regulation in the condition of an innately decreased affinity for O_2 in the Presbyterian mouse, a model obviously superior to pharmacologic manipulations. Recently, Izumizaki et al. (2003) have shown decreased chemosensory responses in the awake Presbyterian mouse. The decrease in the hypoxic response was rather modest and although driven mostly by changes in breathing frequency, a pointy feature of possible carotid chemoreceptor involvement, the authors lean toward the central effect on the basis of unchanged magnitude of brief hyperoxic exposures during hypoxia of the Dejours type.

In awake species respiratory regulation is dependent, to a considerable extent, on consciousness-related effects mediated by higher brain (Gallego and Gaultier, 2000; Pokorski et al., 2003), which may hinder the interpretation of modest respiratory changes. Central effects are particularly evident when one uses noxious chemical stimuli, such as hypoxia, in usually freakish rodents; the ventilatory response being prolonged and the depressant phase delayed or virtually absent (Gozal, 1998). Anesthesia excludes the cortical regulatory components and also brings to

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sight independent, carotid body-mediated effects (Gautier et al., 1986). Therefore, in the present study we thought it worthwhile to reinvestigate the ventilatory responses to chemical stimuli in the anesthetized Presbyterian mouse, as compared with the wild type. Our working hypothesis was that the Presbyterian mutant mouse, due to genetically determined higher tissue oxygenation, would be able to better endure hypoxia and its depressant ventilatory aspects. Contrary to the presumption we report here a generally increased vulnerability to hypoxia and a strikingly determinative role of the carotid body neural input to the brain in upholding ventilation in normoxia in the Presbyterian mouse.

2. Material and methods

The study was approved by, and conducted in accord with the guiding principles of, the Animal Care and Use Committee of Showa University in Tokyo.

2.1. Creation of the Presbyterian mutant mouse

The Presbyterian mutant mice were raised at Tokyo Metropolitan Institute of Gerontology in Tokyo, Japan. The genomic procedures employed to create the Presbyterian mutant were described in detail elsewhere (Suzuki et al., 2002; Shirasawa et al., 2003). Briefly, the mouse was obtained by a targeted knock-in modification of the mouse genome in which Asn^{108} of the β-major globin chain was replaced by Lys residue. The mutant mice were cross-bred with the C57BL/6CrSLc mice for five successive generations. A PCR protocol was set up to confirm the genotype of the Presbyterian mouse by using genomic DNA isolated from tail snips. PCR amplification was carried out with the sense oligonucleotide 5′-ACC CAG CGG TAC TTT GAT AGC-3′ and antisense 5′-GCT ACT GAA GCT GCT TAA GGC AAC AGG-3′ primers. In addition, the mutant mouse's genotype was confirmed by the HPLC profile of its Hb. The profile showed that in the heterozygous mutant mice HB Presbyterian constituted about 30% fraction of the total Hb. This figure corresponds closely to the reported range of HB Presbyterian present in the human cases of the hemoglobinopathy (Harano et al., 1984).

2.2. Instrumentation

In total, 9 male heterozygous Presbyterian mutant mice of the mean age 24.4 ± 0.4 wk and weight 33.4 ± 1.2 g and 7 inbred wild-type male C57BL/6CrSlc mice 24.4 ± 0.6 wk old, weighing 31.9 ± 0.6 g were used for this study. All animals were housed in wire mesh cages, usually several mice per cage, in a local animal facility and were maintained under controlled temperature of 21 °C on a 12 h light-dark cycle until use. All mice were given a free access to standard chow and water. Experiments were conducted at room temperature of 22 ± 1 °C. Animals were anesthetized with urethane, 1.2 g/kg i.p. Supplementary injections of the anesthetic in the amount of 10% of the initial dose were given if any signs of too superficial anesthesia were noted, such as limb movement in response to surgical incision or on paw pinching. Skin incisions were preceded by local injection of lignocaine. The experiment started from taking the metabolic measurements (see details below) in the anesthetized but otherwise intact mouse. Then, the mouse was subjected to surgical procedures before taking the respiratory measurements. The mouse was surgically prepared for bilateral cutting of the sinus nerve. After inserting the tracheostomy tube through a mid-neck incision, the upper end of the trachea along with the esophagus were tied off, cut through, and retracted rostrally to make the carotid bifurcation region accessible. Following that, the superior cervical ganglion was removed on both sides and the carotid sinus nerve was visualized after removing the scraps of the surrounding connective tissue. The nerve was cut above the point of its branching off the glossopharyngeal nerve. Carotid body denervation was performed during room air breathing. The Presbyterian mice did not survive the denervation long enough to complete the protocol of chemosensory responses (see below). Both genotypes studied were instrumented in like manner. Rectal temperature was maintained at 36–37 °C throughout the tests with a servo-controlled heating lamp (Animal Controller ATB-1100, Nihon Koden, Tokyo, Japan) installed outside the body chamber. The temperature was measured during each recovery interval and adjusted, if required.

The number of animals was not even in the experimental groups studied (see details in the experimental protocol below). Some mice, particularly the Presbyterian mutant, poorly endured the first hypoxic exposure and were deemed unsuited for the continued experiment. Metabolic measurements, which also required hypoxic exposure, were taken in a setup disconnected from the plethysmographic chamber for ventilatory measurements. The number of dropouts after the measurement of metabolism was higher in the Presbyterian mutant group (3 cases) due to the after-hypoxia gasping or otherwise wobbly general condition after a prolonged or not full recovery from hypoxic depression.

2.3. Metabolic measurements

The measurement of aerobic metabolism was successfully performed in 9 Presbyterian mice during normoxia and 5 of the same lot during hypoxia (15% O_2 in N_2) and in 6 wild-type mice in either condition. Each mouse was placed in a chamber to which a steady flow of air was delivered by a vacuum pump. Fractions of O_2, CO_2, and N_2 were measured at the inflow and outflow of the chamber in an open-circuit routine with a mass spectrometer (ARCO-1000; ARCO System, Kashiwa, Japan). After achieving equilibration, the following metabolic indices were measured for 5 min: O_2 consumption VO_2, (ml/min STPD), CO_2 production (VCO_2, ml/min STPD) in the normoxic condition and then during the 15% hypoxia. All data were normalized to body weight in kg.

2.4. Ventilatory measurements

Ventilation and its responses to chemical stimuli were measured in 6 Presbyterian and 7 wild-type mice. The mouse was placed prone in the plethysmographic chamber and started breathing with room air. After 7–10 min period of stabilization in the chamber, 1 min recording of ventilation was taken as the basal normoxic level. The mouse was then subjected sequentially to two levels of hypoxia, 15% and 10% O_2 in N_2, hyperoxia consisting of 100% O_2, and next to two levels of hypercapnia of 5% and 9% CO_2 in O_2. CO_2 was allowed to run free during the hypoxic exposures. The gas trials were originally planned to last for 240 s. This time length turned out feasible for the hyperoxic and hypercapnic, but not hypoxic, trials. Particularly, the Presbyterian mice failed to endure hypoxic exposures, which especially concerned the lower 10% hypoxic level. To avoid irreversible hypoxic depression that would force to discard the animal from the experimental lot, hypoxic trials were discontinued at earlier, varied time points. The cutoff time point of hypoxic exposure, taken to include all hypoxic trials for statistical group comparisons, was set at 120 s. Consecutive trials were separated by room air breathing until full recovery was attained, which usually took 5–10 min. The gas mixtures were produced ad hoc from the gas tanks and the concentration of each gas was verified before and rechecked after the trial with a rapidly responding O_2 and CO_2 analyzer (Respina IH26, NEC, San-ei, Tokyo, Japan).

Ventilation was measured with a whole-body flow-through plethysmograph (PLY3211; Buxco Electronics, Sharon, CT). Individual mice were placed in the precalibrated plethysmographic