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Ventilatory acclimatization to hypoxia in mice: Methodological considerations

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ABSTRACT

We examined ventilatory acclimatization to hypoxia (VAH) in CD1 mice, and contrasted results obtained using the barometric method on unrestrained mice with pneumotachography and pulse oximetry on restrained mice. Responses to progressive step reductions in O_2 fraction (21%–8%) were assessed in mice acclimated to normoxia and hypobaric hypoxia (barometric pressure of 60 kPa for 6–8 weeks). Hypoxia acclimation increased the hypoxic ventilatory response (primarily by increasing breathing frequency rather than tidal volume), arterial O_2 saturation (Sa_{O2}) and heart rate in deep hypoxia, hypoxic chemosensitivity (ventilatory O_2/CO_2 equivalents versus Sa_{O2}), and respiratory water loss, and it blunted the hypoxic depression of metabolism and body temperature. Although some effects of hypoxia acclimation were qualitatively similar between methods, the effects were often greater in magnitude when assessed using pneumotachography. Furthermore, whereas hypoxia acclimation reduced ventilatory O_2 equivalent and increased pulmonary O_2 extraction in barometric experiments, it had the opposite effects in pneumotachography experiments. Our findings highlight the importance of considering the impact of how breathing is measured on the apparent responses to hypoxia.

1. Introduction

Acclimation of lowland individuals to chronic hypoxia results in ventilatory acclimatization to hypoxia (VAH), characterized by increases in ventilation and ventilatory chemosensitivity that generally improve respiratory gas exchange and arterial oxygen saturation (Ivy and Scott, 2015; Powell et al., 1998, 2000). VAH is believed to arise from increased O_2 sensitivity of the carotid bodies along with changes in the processing of afferent inputs from the carotid body (central gain), which together increase respiratory motor output (Ivy and Scott, 2015; Kumar and Prabhakar, 2012; Powell et al., 1998). The increased O_2 sensitivity of the carotid bodies is associated with hypertrophy, hyperplasia, and/or neovascularization of the organ (Kusakabe et al., 1993; Pardal et al., 2007; Wang et al., 2008), as well as alterations in ion channel densities and/or neurotransmitter stores of the O_2-sensitive glomus cells (Hempleman, 1995, 1996; Prabhakar and Jacono, 2005). Central gain is believed to arise from increased afferent responsiveness in the nucleus tractus solitaries, due at least in part to enhanced glutamatergic signalling (Reid and Powell, 2005; Pamenter et al., 2014).

Many of the insights gained into the mechanisms of VAH have arisen from studies using a restricted number of laboratory strain rats and mice, which can differ appreciably in their tolerance of hypoxia. Adult rats suffer from high pulmonary artery pressures and growth of pulmonary vascular smooth muscle with chronic hypoxia, which likely restrains O_2 diffusion into the blood (Ge et al., 1998; Hoshikawa et al., 2003; Jochmans-Lemoine et al., 2015). In contrast to rats, mice increase ventilation by a greater magnitude after chronic hypoxia and they exhibit less vascular remodelling (Hoshikawa et al., 2003; Jochmans-Lemoine et al., 2015), each of which should facilitate gas exchange in hypoxic environments. These differences have been suggested to explain why mice have a wide altitudinal distribution while rats are generally absent from high-altitudes (Jochmans-Lemoine et al., 2015). Therefore, species differences in hypoxia tolerance are important to consider when generalizing the results of VAH studies across taxa.

Strain differences in hypoxia tolerance and in the ventilatory and cardiovascular responses to acute hypoxia can also be appreciable (Adachi et al., 2006; Lerman et al., 2002; Ward et al., 2007; Zwemer et al., 2007), and could foreseeably affect the outcome of hypoxia acclimation. Most studies investigating VAH in mice have focused on inbred strains from which genetically modified mice are

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derived, including C57BL/6 and 129/Sv strains (Bishop et al., 2013; Malik et al., 2005; Soliz et al., 2005, 2007; Villafuerte et al., 2007; Ward et al., 2007). Furthermore, changes in ventilatory sensitivity to hypoxia are often assessed during a single brief exposure to a single level of hypoxia. Very few studies have assessed VAH in outbred lab strains, such as CD1 mice, or have examined the shape of the ventilatory response to progressive increases in hypoxia severity.

There are various methods for measuring breathing, each of which has relative merits and drawbacks. Ventilatory measurements are most commonly made using the barometric technique, which determines tidal volume indirectly from changes in chamber pressure/flow that are caused by the changes in temperature and humidity of the air breathed during each breathing cycle (Drorbaug and Fenn, 1955; Jacky, 1980) and it allows for free movement of unrestrained animals. However, there are several potential limitations to the accuracy of this technique when body temperature, chamber temperature, and chamber humidity are not measured directly when breathing is being assessed (Mortola and Frappell, 2013,1998). Greater accuracy is afforded by direct measurements of breathing in restrained animals, in which the body is isolated in a closed chamber by creating a tight seal around the neck so that tidal volume can be measured directly using a pneumotachograph in the body chamber (Mortola and Frappell, 2013). Restraint also allows mice to be instrumented more easily with sensors to make additional measurements that are impractical in unrestrained mice. It is possible that disturbance associated with restraint and instrumentation could influence physiological measurements, as stress is known to alter hypoxic ventilatory responses in a sex-dependent manner (Fournier et al., 2007; Genest et al., 2004; Kinkead et al., 2005), but previous studies have reported variable differences in baseline breathing patterns between unrestrained and restrained mice (Dauger et al., 1998; DeLorme and Moss, 2002). It would therefore be valuable to consider whether the method for measuring breathing affects the apparent outcome of hypoxia acclimation.

Our objective here is to provide insight into the various issues mentioned above. We examined the effects of hypoxia acclimation on the respiratory, metabolic, and heart rate responses to acute progressive hypoxia in CD1 mice, a commonly used outbred lab strain. We also investigated if the apparent magnitude of VAH differed depending on whether mice were studied using the barometric (unrestrained) method or an alternative method whereby animals were restrained to measure breathing directly and were also instrumented with a pulse oximetry collar.

2. Materials and methods

2.1. Mice

25 CD1 mice (13 males, 12 females) were purchased from Charles River Laboratories and housed at McMaster University, Hamilton, ON, Canada. Mice were held in standard holding conditions at 24–25 °C and were provided with unlimited access to food and water on a 12:12-h light-dark photoperiod. All animal protocols followed guidelines established by the Canadian Council on Animal Care and were approved by the McMaster University Animal Research Ethics Board.

2.2. Acclimation treatments

Mice were acclimated to standard holding conditions in normobaric normoxia (n = 15, 8 males and 7 females), or to hypobaric hypoxia (n = 10, 5 males and 5 females) simulating the pressure at an elevation of roughly 4300 m (barometric pressure of 60 kPa, approximately 12.5 kPa O_2). Hypoxia acclimation was carried out in hypobaric chambers, by decreasing pressure to 84, then 67, and finally 60 kPa on each of the first three days. Mice were returned temporarily to normobaric conditions for <20 min for cage cleaning twice per week. Experiments to determine how hypoxia acclimation affected the responses to acute hypoxia were conducted after 6–8 weeks of acclimation.

2.3. Responses to acute hypoxia

We compared the ventilatory and metabolic responses to acute hypoxia between methods for measuring breathing: (i) the barometric technique on unrestrained and uninstrumented mice; and (ii) double-chamber pneumotachography on restrained mice that were instrumented with pulse oximetry collars. Each mouse was tested using both methods, with the exception of 7 normoxia and 2 hypoxia acclimated mice (greater than 45 g in mass), that could not be tested using the restrained method because they were too large to fit in our pneumotachography device (which is described below).

2.3.1. Hypoxia responses using the barometric technique

The first method employed a plethysmograph that allowed the mice unrestrained movement within the chamber. This cylindrical chamber (from Data Sciences International, St. Paul, MN, USA) was 670 ml in volume, with a small opening to the external environment to provide a natural leak and allow for changes in flow to be detected compared to a sealed reference chamber. The animal chamber contained a metal platform that elevated mice above any feces and urine that accumulated during the experiment. All mice were given 20–60 min to adjust to the chamber before experiments began, until they were noticeably relaxed with a stable breathing pattern, while breathing air (21% O_2, balance N_2) supplied to the chamber at 600 ml/min. Measurements were then made for 20 min at 21%, after which mice were exposed to 20 min step-wise decreases in inspired O_2 fraction (F_IO_2) of 16%, 12%, 10%, 9%, 8% at a constant incurrent flow rate to the chamber of 600 ml/min. Incurrent O_2 levels were mixed using precision flow meters (Sierra Instruments, Monterey, CA, USA) and a mass flow controller (MFC-4, Sable Systems, Las Vegas, NV, USA).

Breathing and gas composition (O_2, CO_2, H_2O) of chamber air flows were measured during the last 10 min at each F_IO_2. Changes in flow across the pneumotachograph were measured using a differential pressure transducer (Validyne DP45, Cancoppas, Mississauga, ON, Canada) and carrier demodulator (Validyne CD15, Cancoppas) to determine breathing frequency and tidal volume. Incurrent and excurrent air flows were subsampled at 200 ml/min; incurrent air was continuously analyzed for O_2 fraction (FC-10, Sable Systems), and excurrent air was analyzed for water vapour pressure (RH-300, Sable Systems) and was then dried with prebaked drierite and analyzed for O_2 and CO_2 fraction (FC-10 and CA-10, Sable Systems). Chamber temperature was continuously recorded with a thermocouple (PT-6, Physitemp). Rectal temperature was measured using a rectal probe (RET-3-ISO, Physitemp) at the end of the experiment and then 24 h later in normoxia (This was used as a proxy for rectal temperature at the start of the experiment, so we could avoid undue stress to the animals while also controlling for diurnal variation). All data was acquired using a PowerLab 16/32 and Labchart 8 Pro software (ADInstruments, Colorado Springs, CO, USA).

A subset of 5 mice from each treatment group had thermosensitive passive transponders (micro LifeChips with Bio-therm technology; Destron Fearing, Dallas, TX) implanted into the abdominal cavity ~2 weeks before acclimation. The transponders can detect body temperature and send the data passively to a hand-held scanner, allowing for body temperature to be measured whenever desired throughout experimental protocols. Mice were lightly anaesthetized using isofluorane and a microchip was injected