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Hypoxia-induced ventilatory responses in conscious mice: Gender differences in ventilatory roll-off and facilitation

Lisa A. Palmer, Walter J. May, Kimberly deRonde, Kathleen Brown-Steinke, Benjamin Gaston, Stephen J. Lewis*

Pediatric Respiratory Medicine, University of Virginia School of Medicine, Charlottesville, VA 22908, USA

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ABSTRACT

The aim of this study was to compare the ventilatory responses of C57BL6 female and male mice during a 15 min exposure to a hypoxic–hypercapnic (H–H) or a hypoxic (10% O_2, 90% N_2) challenge and subsequent return to room air. The ventilatory responses to H–H were similar in males and females whereas there were pronounced gender differences in the ventilatory responses during and following hypoxic challenge. In males, the hypoxic response included initial increases in minute volume via increases in tidal volume and frequency of breathing. These responses declined substantially (roll-off) during hypoxic exposure. Upon return to room-air, relatively sustained increases in these ventilatory parameters (short-term potentiation) were observed. In females, the initial responses to hypoxia were similar to those in males whereas roll-off was greater and post-hypoxia facilitation was smaller than in males. The marked differences in ventilatory roll-off and post-hypoxia facilitation between female and male C57BL6 mice provide evidence that gender is of vital importance to ventilatory control.

1. Introduction

Exposure to an acute hypoxic challenge increases minute ventilation via activation of carotid body chemoafferents, which relay their information to the nuclei of the tractus solitarii (NTS) in the brainstem (Lahiri et al., 2006; Teppema and Dahan, 2010). NTS neurons relay this information to other brainstem sites, eliciting changes in peripheral motor output to elicit changes in ventilation (Lahiri et al., 2006; Teppema and Dahan, 2010). Although the brain also plays a dominant role in adjusting ventilation in response to changes in blood pCO_2 levels (Guyenet et al., 2010), the carotid bodies sense changes in blood proton concentrations (derived from changes in blood CO_2 levels via carbonic anhydrase) and play a role in the expression of the ventilatory responses upon exposure to hypercapnic challenges (Gonzalez et al., 1994; Fatemian et al., 2003; Forster and Smith, 2010). Unlike exposure to a hypoxic–hypercapnic (H–H) challenge, ventilatory drive can diminish during exposure to a hypoxic challenge. This ventilatory "roll-off" involves neurochemical processes in the NTS (Gozal et al., 2000) and the *direct* depressive effects of hypoxia on brain neurons regulating respiratory burst rhythm and amplitude (Martin-Body, 1988). Cessation of exposure to hypoxia often results in ventilatory parameters increasing or remaining above baseline for a substantial period of time. This "short-term facilitation (STF)" of ventilation, first detailed in anesthetized cats (Wagner and Eldridge, 1991), also occurs in awake or sleeping humans (Dahan et al., 1995) and rats (Moss et al., 2006), and is activated by a central neural mechanism with slow dynamics that drives ventilation independently of peripheral or central chemoreceptor inputs (Millhorn et al., 1980).

The roles of sex hormones and gender differences with respect to ventilatory control mechanisms, lung function, and ventilatory responses to hypoxic or hypercapnic challenges have received substantial attention (Saaresranta and Polo, 2002). In humans, hypoxia-induced ventilatory responses (HVR) have been reported to be equal in females and males (Marcus et al., 1994) or greater in females (Aitken et al., 1986). In contrast, it has been reported that women have a lower HVR (White et al., 1983) and hypercapnic drive (Patrick and Howard, 1972; White et al., 1983) than men. Disparate findings have also been generated in animals. For example, HVR has been reported to be greater in female than male rats via mechanisms independent of the activities of ovarian hormones (Mortola and Saiki, 1996), similar in female and male rats (Fournier et al., 2007) or smaller in female rats (Schlenker and Goldman, 1986). Studies in rats (Joseph et al., 2002) and mice (Gassmann et al., 2009) have ascribed disparities in HVR between males and females to gender-related differences in response/signaling processes in the carotid bodies.

* Corresponding author at: Pediatric Respiratory Medicine, University of Virginia School of Medicine, Room 2024, Building MR4, 409 Lane Road, Charlottesville, VA 22908, USA. Tel.: +1 434 243 6852.
E-mail address: sjl3s@virginia.edu (S.J. Lewis).

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Mice (Zwemer et al., 2007; Yamauchi et al., 2010) and carotid body preparations in or from these animals (He et al., 2000, 2002; Prieto-Lloret et al., 2007) are being used increasingly in studies of ventilatory control mechanisms. Hypoxia elicits ventilatory responses in mice that are reminiscent of those in other species including humans although there is substantial mouse-strain variability due to genetic discordance (Tankersley, 2001, 2003; Tankersley and Broman, 2004). Genetic studies have localized the variation in the acute HVR between mouse strains to chromosome 9 (Tankersley, 2001) whereas interactions between hypoxic and hypercapnic breathing are linked to chromosomes 1 and 5 (Tankersley and Broman, 2004). Importantly, hypoxia activates carotid body chemoafferent activity in anesthetized mice (Biscoe and Pallot, 1982) as well as an in vitro mouse carotid body/chemoafferent preparation (Donnelly and Rigual, 2000). Moreover, HVR is markedly attenuated after bilateral carotid sinus nerve (CSN) transection in anesthetized mice (Izumizaki et al., 2004). These findings are consistent with the vital role of carotid body/CSNs in the expression of HVR in humans (Timmers et al., 2003) and many animal species including rats (Roux et al., 2000). With respect to HVR, similar maximal increases in minute ventilation occur in female and male C57BL6 mice (Soliz et al., 2008, 2009), C57BL6/57 mice (Huey et al., 2000) and Swiss Webster mice (Schlenker and Goldman, 1986) although Gassmann et al. (2009) reported that HVR was greater in female than male Swiss Webster mice. In addition, roll-off in frequency of breathing was found to be more marked in female than male C57BL6 mice (Soliz et al., 2008, 2009) or similar between female and male C57BL6/65 mice (Huey et al., 2000). Post-hypoxia ventilatory responses in mice are also complex and marked differences between strains have been reported (Han et al., 2001; Yamauchi et al., 2010). For example, post-hypoxia depression is seen in C57BL/6J mice whereas facilitation is seen in C57BL6 and A/J mice (Han et al., 2001; Yamauchi et al., 2010).

To date, there are no published studies pertaining to gender differences in ventilatory responses to H–H challenges in mice. Moreover, few studies have examined gender differences in ventilatory roll-off in mice and these studies did not exhaustively analyze ventilatory parameters (Huey et al., 2000; Soliz et al., 2008, 2009). Finally, potential gender differences in the post-hypoxia and or post-H–H ventilatory responses in mice have not been reported. As a prelude to studies in genetically engineered mice (Palmer et al., 2012), this study compared the ventilatory responses of female and male C57BL6 mice during exposure to a H–H or a hypoxic challenge, and subsequent return to room air. The C57BL6 mouse is a common inbred strain that is widely used in ventilatory function studies (Soliz et al., 2008, 2009) and as common genetic background to transgenic mice (Liu et al., 2004; Palmer et al., 2012).

2. Methods

2.1. Mice

All studies were carried out in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH Publication No. 80-23) revised in 1996. The protocols were approved by the University of Virginia Animal Care and Use Committee. Adult female and male C57BL6 mice ($n = 6$ mice per gender per batch) were bought from Jackson Laboratories (Bar Harbor, MN, USA) to allow us to select females and males with similar body weights although this meant that the females were slightly older than the males (Table 2). A total of 52 C57BL6 mice (26 female and 26 male) were used in these studies. The status of the menstrual cycle was not taken into account because its effect on HVR is minor and ranges from an increased HVR in the luteal phase by 10–20% compared with the follicular phase (Behan and Wenninger, 2008; Dahan et al., 1998). In addition, Wenninger et al. (2009) found no correlation between the ratio of progesterone and estradiol and the HVR response.

Table 1
Definition of ventilatory parameters.

Parameter	Abbreviation, units	Definition
Frequency	f_R, breaths/min	Number of breaths per minute
Tidal volume	V_T, ml	Volume of air per breath
Ventilation	$\dot{V}$, ml/min	Frequency × tidal volume
Inspiratory time	T_I, s	Actual time of inspiration
Expiratory time	T_E, s	Actual time of expiration
End inspiratory pause	EIP, ms	Pause between the end of inspiration and the start of expiration
Tidal volume/inspiratory time	V_T/T_I, ml/s	Index of inspiratory drive[a]
Peak inspiratory flow	PIF, ml/s	Peak air-flow velocity during inspiration
Peak expiratory flow	PEF, ml/s	Peak air-flow velocity during expiration
Expiratory flow$_{50}$	EF_{50}, ml/s	Air-flow when 50% of tidal volume is expired
Relaxation time	T_R, s	Time for expiration to decrease to 36% maximum
Rpef	Rpef	Rate of achieving peak expiratory flow [Rpef = (time to PEF)/T_E]

[a] See Moss et al. (2006).

2.2. Ventilatory parameters

Ventilatory parameters (Table 1) were continuously recorded in four conscious unrestrained mice at a time using a whole-body chamber plethysmography system (PLY 3223; BUXCO Inc., Wilmington, NC, USA) described previously (Kanbar et al., 2010). Provided software constantly corrected digitized values for changes in chamber temperature and humidity and a rejection algorithm excluded motion-induced artifacts. Due to the minor differences in body weights between the males and females, parameters such as V_T, PIF and PEF, often corrected for body weight (Moss et al., 2006) are presented without corrections.

2.3. Experimental protocols

2.3.1. H–H challenge

A total of 14 female and 14 male mice were placed in the plethysmography chambers and allowed 45–60 min to acclimatize. The mice were then exposed to H–H via the re-breathing method used to study ventilatory responses in humans (e.g., Giannoni et al., 2009), and rats (e.g., Hayashi et al., 1982). Air-flow to the chambers housing the mice was stopped for 15 min allowing the mice to re-breathe their own air (inbuilt soft-ware adjusted flow-derived values for the concomitant increases in chamber temperature and chamber humidity). The major benefit of this model is that mice breathe air which becomes progressively more hypoxic and hypercapnic (these environmental changes drive the ventilatory responses), thereby mimicking many clinical scenarios (Dempsey et al., 2010). Moreover, hypercapnia is a potent arousal stimulus, especially if delivered rapidly, and as such, the gradual increase in environmental CO_2 limits the rate of arousal (Fewell and Konduri, 1998). After 15 min, the air-flow (room-air) was returned to the chambers and parameters recorded for a further 15 min.