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

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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% O₂, 90% N₂) 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.

Keywords

Hypoxia; hypercapnia; minute ventilation; male and female mice

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 solitarius (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 pCO₂ levels (Guyenet et al., 2010), the carotid bodies sense changes in blood proton concentrations (derived from changes in blood CO₂ 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

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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 (Shlenker 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.

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 et al., 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.

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.

2.3.2. Hypoxia challenge—A total of 12 female and 12 male mice were placed in the plethysmography chambers and allowed 45–60 min to acclimatize. Data was continuously recorded (i.e., breath by breath) throughout acclimatization (the last 15 min was used for analysis and graphing) and subsequent experimentation. After acclimatization, the mice

were exposed to a standard hypoxic challenge of 10% O₂, 90% N₂ (Gassmann et al., 2009; Gozal et al., 2000; Teppema and Dahan, 2010) for 15 min after which time they were re-exposed to room air and data recorded for 15 min.

2.4. Statistics

The recorded data (1 min bins) and derived parameters, V_T/T_I and Response Area (cumulative percent changes from pre-challenge values) were taken for analyses. Pre-challenge bins excluded occasional marked deviations from resting due to movements or scratching by the rats. The data are presented as mean $\pm$ SEM. All data were analyzed by one-way or two-way analysis of variance followed by Student's modified *t* test with Bonferroni corrections for multiple comparisons between means using the error mean square terms from the analyses of variance. A value of $P < 0.05$ denoted statistical significance.

3. Results

3.1. Body weights, ages and baseline ventilatory parameters of males and females

As summarized in Table 2, the body weights of the female mice were slightly less than those of males for the H-H study (-4.8% , $P < 0.05$) and for the hypoxia study (-6.1% , $P < 0.05$). The females were older than the males in the H-H (+14 days, $P < 0.05$) and hypoxia (+18 days, $P < 0.05$) studies (Table 2). Resting ventilatory parameters in male and female mice used in the H-H the hypoxia studies are summarized in Tables 3 and 5, respectively. There were no gender differences in most ventilatory parameters although resting V_T/T_I , PIF and PEF were lower in females than males.

3.2. Ventilatory responses elicited by exposure to hypoxia-hypercapnia

The ventilatory responses elicited by H-H are summarized in Fig. 1 and Table 4. H-H elicited (a) increases in f_R and V_T and therefore, $\dot{V}$, (b) decreases in T_I and T_E and an increase in EIP, (c) an increase in V_T/T_I , and (d) increases in PIF and PEF. Return to room-air elicited (a) a brief increase in f_R accompanied by a steady fall in V_T and therefore a brief increase in $\dot{V}$, (b) a brief decrease in T_I (co-incident with the increase in f_R), a gradual increase in T_E , and a rapid recovery of EIP, (c) a brief increase in V_T/T_I (due principally to the decrease in T_I), and (d) a brief increase in PIF and a gradual decline in PEF. All responses gradually returned to pre-H-H levels within 15 min. The responses during H-H and upon return to room-air were similar in males and females.

3.3. Ventilatory responses elicited by exposure to hypoxia

f_R , V_T and $\dot{V}$ —Exposure to hypoxia elicited similar immediate increases in f_R in males and females (Fig. 1, Table 5). These responses were not sustained such that f_R values were similar to initial resting values within 9–15 min. This roll-off in f_R was similar in males and females and the cumulative response (R_C) was similar in both genders (Table 6). Return to room-air elicited a greater initial increase in f_R in males than in females (Table 5). R_C was greater in males (Table 6). Hypoxia elicited a greater immediate increase in V_T in males than females (Fig. 1, Table 5). The increases in V_T subsided to a greater extent in females such that R_C was smaller in females (Table 6). Upon return to room air, V_T remained higher in males than females such that the R_C was greater in males (Table 6). Hypoxia elicited similar immediate increases in $\dot{V}$ in male and female mice (Fig. 1, Table 5). These responses were not sustained in females such that $\dot{V}$ returned to initial normoxia values within 9–15 min whereas $\dot{V}$ did not return to pre-hypoxia values in males. Thus roll-off in $\dot{V}$ was greater in females and R_C was smaller (Table 5). Return to room-air elicited an initial increase in $\dot{V}$ that was greater in males than in females (Table 5) and the R_C was greater in males (Table 6).

T_I, T_e and EIP—Hypoxia elicited similar immediate decreases in T_I in males and females (Fig. 2, Table 5). The responses were not sustained such that T_I was similar to resting values between 5–15 min. Roll-off and R_C were similar in both genders (Table 6). Return to room-air elicited a similar initial decrease in T_I in males and females (Table 5) and the R_C values were similar in each gender (Table 6). Hypoxia elicited similar immediate decreases in T_e in male and female mice (Fig. 2, Table 5). These responses were sustained in male mice such that T_e remained below pre-hypoxia levels throughout the hypoxic exposure. In contrast, the responses were not sustained in females such that T_e was similar to initial normoxia values within 4–5 min. The R_C during exposure to hypoxia was significant in males but not in females (Table 6). Return to room-air elicited minimal changes in T_e such that values remained below pre-hypoxia levels in males and at pre-hypoxia levels in females (Tables 5 and 6). Hypoxia elicited an immediate decrease in EIP in females but not males (Fig. 2, Table 5). The responses in females recovered within 11–15 min. The R_C was significant in females but not males (Table 6). Upon return to room-air, the changes in EIP were negligible in males and females (Tables 5 and 6) except for a decrease at the 1 min time-point in females (-0.32 ± 0.06 sec, $P < 0.05$).

Respiratory Drive—Hypoxia elicited similar immediate increases in V_T/T_I in males and females (Fig. 3, Table 5). These responses were not sustained such that V_T/T_I was similar to initial normoxia values within 9–15 min. However the degree of roll-off in V_T/T_I was greater in females than males such that the R_C was smaller in females (Table 6). Return to room-air elicited an initial increase in V_T/T_I that was greater in males than in females (Table 5). The R_C during re-exposure to room air was also greater in males than in females (Table 6).

PIF and PEF—Hypoxia elicited similar immediate increases in PIF in males and females (Fig. 3, Table 5). These responses were not sustained such that PIF was similar to initial normoxia values within 11–15 min. Roll-off in PIF was similar in males and females, thus R_C was similar in both genders (Table 6). Return to room-air elicited a greater initial increase in PIF in males than in females (Table 5). As such, R_C was greater in males (Table 6). Hypoxia elicited similar immediate increases in PEF in males and females (Fig. 3, Table 5). These responses were subject to roll-off but remained above resting levels throughout exposure to hypoxia. R_C was similar in males and females (Table 6). Return to room-air elicited greater initial increases in PEF in males than females (Table 5). The R_C was greater in males than in females (Table 6).

EF₅₀, T_R and R_{pef}—Hypoxia elicited similar immediate increases in EF₅₀ in males and females (Fig. 3, Table 5). These responses were subject to roll-off such that EF₅₀ values returned to pre-hypoxia levels within 5–10 min. R_C during exposure to hypoxia was similar in males and females (Table 6). Return to room-air elicited an initial increase in EF₅₀ in males but not females (Fig. 3, Table 5). R_C was also greater in males than in females (Table 6). Hypoxia elicited minimal initial T_R responses in male or females (Fig. 3, Table 5). However, T_R gradually decreased in the males whereas it did not change in females. Accordingly, R_C was significant in males only (Table 6). Return to room-air elicited minimal changes in T_R in males (i.e., values remained below pre-hypoxia levels) or in females (no change from pre-hypoxia levels). A significant R_C was noted for males but not females (Table 6). Hypoxia elicited immediate increases in R_{pef} (i.e., PEF was attained more quickly) of similar magnitude in males and females (Fig. 3, Table 5). These responses subsided within 5 min such that roll-off was similar in males and females. The R_C was similar in males and females (Table 6). Re-exposure to normoxia elicited minimal R_{pef} responses in males and females (Tables 5 and 6).

4. Discussion

4.1. Summary of Findings

The major findings of this study were that (a) ventilatory responses to H-H were similar in male and female C57BL6 mice, (b) the initial ventilatory responses to hypoxic challenge were similar in males and females whereas roll-off was greater in females, and (c) STF was greater in males. It therefore appears that gender differences in hypoxic signaling pathways exist in these mice whereas no such differences exist in hypercapnic signaling pathways.

4.1. Baseline ventilatory parameters

Wenninger et al (2009) reported that normalization of ventilatory volumes to body weight provided erroneous indices of ventilatory sensitivities to hypercapnia and suggested that expressing HVR as a change from pre-values without body weight corrections is a better index of ventilatory responses (*e.g.*, our use of %cumulative changes from pre-values). We selected females and males of similar body weights, although this resulted in the use of females that were older than males (*e.g.*, 102 ± 3 vs 88 ± 3 days). Others have reported minimal differences in baseline ventilatory parameters or HVR in 8 strains of female or male mice including C57BL6 mice between the ages of 90–120 days (Soliz et al., 2008, 2009; Wenninger et al., 2009). Most resting parameters (*i.e.*, f_R , V_T , $\dot{V}$, T_I , T_E , EF_{50} , TR and R_{pF}) were similar in our female and male C57BL6 mice (Table 7). These results agree with evidence that f_R , V_T and $\dot{V}$ were equal in females and males (Card et al., 2006) but contrast with evidence that V_T was lower in females (Carey et al., 2007), and that $\dot{V}$ was higher in females (Soliz et al., 2008, 2009; Gassmann et al., 2009). These disparities may be due to the time given the mice to acclimatize to the ventilatory chambers. Major differences in f_R ranging from 150 to 500 breaths/min in females and 200–500 breaths/min in males have been reported (Card et al., 2006; Carey et al., 2007; Gassmann et al., 2009; Soliz et al., 2008, 2009). The f_R values in our female and male C57BL6 mice (*e.g.*, 229 ± 3 and 222 ± 4 breaths/min, respectively) fall in the middle of the reported values.

The finding that PIF and PEF were less in females than males (Table 7) are consistent with those of Carey et al (2007) but contrast to evidence that resting flows are equal in female and male C57BL6 mice (Card et al., 2006). Gender differences in 7 mouse strains (not including C57BL6) exist in most lung function parameters although normalization to body weight or lung size usually compensates for gender differences (Reinhard et al., 2002). However, total lung capacity is higher in female than male mice even when corrected for body weights. Moreover, specific lung volumes are higher in female than male C57BL6 mice (Schulz et al., 2002) and gas-exchange surface area is higher in female than male CD-1 mice (Massaro et al., 1995). As such, females may not need to generate the same maximal flows at rest and diminished HVR or post-hypoxia ventilatory responses in female C57BL6 mice may be due to differences in ventilatory signaling, and/or hypoxic pulmonary vasoconstriction (HPV), which would affect gas exchange, blood gas chemistry and therefore ventilatory drive (Fuchs et al., 2010). The finding that V_T/T_I was lower in female than in male mice suggests that females have lower respiratory drive at rest. Whether females have lower input from carotid body chemoafferents or lower activity of brainstem circuitry controlling timing between breaths (Mellen and Mishra, 2010) is not known.

4.2. Responses during and after hypoxia-hypercapnia challenge in male and female mice

H-H elicited similar ventilatory responses in male and female C57BL6 mice that were equivalent to those elicited by gas-mixtures such as 5% CO₂, 10% CO₂, 75% N₂ (Xu et al., 2007). The lack of ventilatory roll-off during H-H is consistent with roll-off being due to alterations in hypoxic-signaling pathways (Tankersley 2003). Genetic aspects of the interaction between hypoxic and hypercapnic signaling pathways are known (Tankersley

2003, Tankersley and Broman, 2004). Multiple central/peripheral neural mechanisms contribute to this interaction (Dempsey et al., 2010). The gradual and gender equivalent increases in EIP during H-H are likely due to hypercapnia-induced changes in central neural networks driving respiration since hypoxia decreased EIP (in males at least, see below). Return to room air following H-H caused an initial increase in f_R (with a decrease in T_I but not T_E), $\dot{V}$, V_T/T_I and PIF of 2–3 min in duration, and then a gradual decline to pre-HH levels. Gender may not be important in these responses since they were similar in males and females. The possibility that hypercapnia masks post-hypoxia responses could not be ascertained since the lack of roll-off during H-H meant that ventilatory parameters were at maximum, thereby precluding direct comparison to hypoxia challenge.

4.3. Responses to hypoxic challenge in male and female C57BL6 mice

The changes in f_R , V_T , $\dot{V}$, PIF and PEF elicited by hypoxia in the male mice were similar to those reported by others (Carey et al., 2007; Gassmann et al., 2009; Huey et al., 2000) whereas the changes in other variables (see below) have not been reported. In our male mice, hypoxia elicited (a) increases in f_R , V_T and $\dot{V}$, (b) decreases in T_I and T_E (but not EIP), (c) increases in V_T/T_I , PIF, PEF, EF_{50} and R_{pef} (i.e., PEF was achieved faster), and (d) a gradual decline in T_R (Table 7). These findings demonstrate that hypoxia stimulates the rate and depth of breathing, and peak efficiencies of respiration in male C57BL6 mice (Carey et al., 2007). Hypoxia-induced increases in f_R in females (peak responses and roll-off) were similar to those of males, whereas the peak increases in V_T and $\dot{V}$ were less in females and roll-off was greater (Table 7). Although the f_R responses and decreases in T_I were similar in males and females, the decrease in T_E , which was sustained during exposure to hypoxia in males, was transient in females. As such, gender has subtle influences on the mechanisms regulating the f_R component of HVR. This is supported by our findings that hypoxia had less influence on respiratory drive (V_T/T_I) in females than males. Since the f_R responses were similar in females and males, it appears that input from the carotid sinus chemoafferents and the central processing of this input with respect to f_R is not gender-dependent. Our findings that gender influences hypoxia-induced changes in respiratory timing is supported by the novel finding that EIP decreased during hypoxia in females (and displayed roll-off) but not EIP in males. It appears that the processes driving the respiratory cycle respond differently in male and female C57BL6 mice and that these processes are sensitive to hypoxia in females only. Moreover, since the cumulative increases in V_T and respiratory drive during hypoxia were less in females than in males (i.e., greater roll-off in females), it is evident that mechanisms underlying the adaptive responses to hypoxia are also under gender control. It is likely that gender differences exist in peripheral effector pathways controlling respiration. Although hypoxia elicited similar initial increases in PIF, PEF, EF_{50} and R_{pef} in males and females, hypoxia elicited a gradual decrease in T_R in males but not in females. Gender differences in HVR in our C57BL6 mice are unlikely to be due to differences in hypoxia-induced decreases in body temperature, blood pressure, or metabolic rate since no-gender differences in these responses have been reported in mice (Huey et al., 2000; Soliz et al., 2008, 2009; Gassmann et al., 2009,) or rats (Wenninger et al., 2009).

Female mice may be more susceptible to the direct effects of hypoxia on central pathways controlling ventilation (Martin-Body, 1988), which would manifest as greater roll-off. As mentioned, total lung capacity, specific lung volumes and gas-exchange surface area are higher in female than male mice. Accordingly, diminished HVR (and post-hypoxia responses) in our female mice may be due to increased pulmonary efficiency (i.e., greater roll-off due to more effective oxygen exchange in the lungs) and gender differences in ventilatory signaling. Hypoxic challenges decrease arterial blood pO_2 and pCO_2 and end-tidal O_2 and CO_2 (Mortola and Saiki, 1996; Soliz et al., 2008, 2009). A fall in pO_2 is a

bronchodilator stimulus (Wetzel et al., 1992) whereas a fall in $p\text{CO}_2$ is a bronchoconstrictor stimulus (Lindeman and Freed, 1993). In addition, the hypoxic gas mixture delivered to our mice was “dry air”, a bronchoconstrictor stimulus in canine airways (Lindeman and Freed, 1993). It is likely that changes in airway resistance during hypoxic challenge will involve the interplay between these bronchodilator and bronchoconstrictor stimuli. Gender differences in airway responses to hypoxia/hypocapnia/dry air have not been found in mice although Mortola and Saiki (1996) found that arterial $p\text{CO}_2$ levels during hypoxia fell more in female than male rats due to a greater increase in f_R . Possible gender differences in HPV, which would affect gas exchange, blood gas chemistry and therefore ventilatory drive (Fuchs et al., 2010), are also possible. Since HPV is diminished in female as compared to male mice (Lahm et al., 2008), diminished HPV in our female mice would limit the rate of blood deoxygenation and diminish the initial magnitude of the increase in V_T . It is also possible that gender differences in hypoxia-induced cerebral vasodilation, which blunt the ventilatory responses, or hypocapnia-induced cerebral vasoconstriction, which would promote ventilatory responses (Ainslie and Ogoh, 2010), may underlie differences in the ventilatory responses of female and male mice during hypoxic challenge. Ventilatory roll-off during hypoxia has been ascribed to mechanisms including changes in brainstem neurochemistry, cerebral vasodilation, a decrease in metabolic rate, and the depressant effects of hypoxia on brainstem neurons driving ventilation. In contrast, roll-off has not been ascribed to a temporal decrease in carotid sinus chemoafferent activity because nerve activity remained elevated during the roll-off phase of hypoxic exposure in anesthetized cats (Vizek et al., 1987). However, there are clear examples in which CSN activity in mice shows roll-off during brief exposures to hypoxia (Kline et al., 2002; Prieto-Lloret et al., 2007). The temporal decrease in CSN activity may be due to decreased release of excitatory “neurotransmitters” from carotid body primary glomus cells and/or diminished responsiveness of chemoafferent nerve terminals to the neurotransmitters. Accordingly, gender differences in roll-off of carotid body/chemoafferent activity may underlie the temporal differences in HVR of the female and male mice used in the present study.

4.4. Post-Hypoxia ventilatory responses in male and female C57BL6 mice

Return to room air elicited a series of responses in male mice, including (a) increases in f_R , V_T and $\dot{V}$, (b) decreases in T_I and T_E , (c) an increase in respiratory drive (V_T/T_I) but no change in EIP, (d) increases (i.e., above pre-hypoxia and hypoxia values) in PIF and PEF and EF_{50} , and (e) a diminished T_R (i.e., equivalent to hypoxia values) but no change in R_{pef} from pre-hypoxia or hypoxia values (Table 7). These findings are consistent with evidence that STF occurs in animals and humans (Powell et al., 1998; Dahan et al., 1995), and is activated by central mechanisms that drive ventilation independently of peripheral/central chemoreceptor inputs (Millhorn et al., 1980). STF is most evident with V_T or phrenic neural output (Powell et al., 1998). However, STF in our male C57BL6 mice involved sustained increases in f_R and (less pronounced) increases in V_T . Differences in species probably determine the relative contributions of f_R and V_T to STF. For example, the magnitude but not the time-course of changes in STF is often determined by the prevailing level of arterial $p\text{CO}_2$, which can vary between species (Powell et al., 1998). STF is evident in the output of many motor nerves following cessation of electrical stimulation of the CSN including phrenic and inspiratory intercostal nerves (see Powell et al., 1998). Perhaps the most striking feature of the present study was the dramatic difference in STF between female and male C57BL6 mice (Table 7). The females displayed (a) smaller increases in f_R , V_T , $\dot{V}$, PIF and PEF, EF_{50} and respiratory drive (V_T/T_I), (b) similar decreases in T_I and EIP but substantially smaller decreases in T_E and T_R , and (c) similar to male mice, minimal changes in R_{pef} . These findings suggest that the mechanisms responsible for the expression of STF are not as evident in female as in male C57BL6 mice. Whether the gender differences in expression of STF in C57BL6 mice reside within the carotid body complex or brainstem

sites controlling ventilation remain to be established. Nonetheless the mechanisms warrant careful investigation considering the potential importance of STF to ventilatory control in humans and animals (Powell et al., 1998).

4.5. Summary and Perspectives

This study demonstrates that marked gender differences in HVR and STF exist between male and female C57BL6 mice. The use of genetically-engineered mice allow for the assessment of the roles of genes and their products in ventilatory processes (Kline et al., 2002; Lahiri et al., 2006; Soliz et al., 2008, 2009; Palmer et al., 2012). These mice also promote our understanding of the processes underlying gender differences in ventilatory processes (Palmer et al., 2012).

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***Highlights**

- Marked gender differences in ventilatory responses during hypoxic challenge in C57BL6 mice.
- Marked gender differences in ventilatory responses following hypoxic challenge in C57BL6 mice.
- No gender differences during or after hypoxic-hypercapnic challenge in C57BL6 mice.

Fig. 1. Changes in ventilatory parameters during hypoxic-hypercapnic challenge (AIR OFF) and upon return to room air (AIR ON) in conscious male and female C57BL6 mice. *Left-hand panels:* frequency of breathing (upper), tidal volume (middle) and ventilation (bottom). *Middle panels:* inspiratory Time (upper), expiratory time (middle), end inspiratory pause (bottom). *Right-hand panels:* tidal volume/inspiratory time (upper), peak inspiratory flow (middle), peak expiratory flow (bottom). The stippled horizontal line denotes the average resting values before exposure to hypoxia-hypercapnia. Data are presented as mean $\pm$ SEM. There were 14 mice in each group.

Fig. 2. Changes in ventilatory parameters during hypoxic challenge (10% O₂, 90% N₂ for 15 min) and following return to room-air (normoxia) in conscious male and female C57BL6 mice. *Left-hand panels:* frequency of breathing (upper), tidal volume (middle) and ventilation (bottom). *Right-hand panels:* inspiratory Time (upper), expiratory time (middle), and end inspiratory pause (bottom). The stippled horizontal lines denote the average resting values before exposure to hypoxia. Data are presented as mean $\pm$ SEM. There were 12 mice in each group.

Fig. 3. Changes in ventilatory parameters during hypoxic challenge (10% O₂, 90% N₂ for 15 min) and following return to room-air (normoxia) in conscious male and female C57BL6 mice. *Left-hand panels:* tidal volume/inspiratory time (upper), peak inspiratory flow (middle), peak expiratory flow (bottom). *Right-hand panels:* EF₅₀ (upper), relaxation time (middle), R_p (bottom). The stippled horizontal lines denote the average resting values before exposure to hypoxia. Data are presented as mean $\pm$ SEM. There were 12 mice in each group.

Table 1

Definition of ventilatory parameters

Parameter	Abbreviation, units	Definition
Frequency	fR, breaths/minute	Number of breaths per minute
Tidal Volume	V _T , mls	Volume of air per breath
Ventilation	V̇, mls/minute	Frequency × tidal volume
Inspiratory Time	TI, sec	Actual time of inspiration
Expiratory Time	TE, sec	Actual time of expiration
End Inspiratory Pause	EIP, msec	Pause between the end of inspiration and the start of expiration
Tidal Volume/Inspiratory Time	V _T /TI, mls/sec	Index of inspiratory drive *
Peak Inspiratory Flow	PIF, mls/sec	Peak air-flow velocity during inspiration
Peak Expiratory Flow	PEF, mls/sec	Peak air-flow velocity during expiration
Expiratory Flow ₅₀	EF ₅₀ , mls/sec	Air-flow when 50% of tidal volume is expired
Relaxation Time	TR, sec	Time for expiration to decrease to 36% maximum
R _{pef}	R _{pef}	Rate of achieving peak expiratory flow [R _{pef} = (time to PEF)/TE]

* See Moss et al., 2006.

Table 2

Body weights and ages of mice

Study	Gender	N	Weight, grams	Age, days
Hypoxia-Hypercapnia	Male	14	22.7 ± 0.2	82 ± 2
	Female	14	21.6 ± 0.2*	96 ± 2*
Hypoxia	Male	12	23.2 ± 0.6	84 ± 3
	Female	12	21.8 ± 0.6*	102 ± 2*

The data are presented as mean ± SEM. $P < 0.05$, females versus males.

Table 3

Resting ventilatory parameters in male and female mice subsequently exposed to the hypoxic-hypercapnic challenge

Parameter	Male	Female
Frequency, breaths/min	210 ± 5	201 ± 4
Tidal Volume, mls	0.27 ± 0.01	0.25 ± 0.01
Minute Volume, mls/min	58 ± 3	50 ± 3
Inspiratory Time, sec	0.10 ± 0.01	0.11 ± 0.01
Expiratory Time, sec	0.19 ± 0.01	0.21 ± 0.01
End Inspiratory Pause, msec	4.5 ± 0.2	4.4 ± 0.2
Tidal Volume/Inspiratory Time, mls/sec	3.0 ± 0.2	2.3 ± 0.1 *
Peak Inspiratory Flow, mls/sec	5.5 ± 0.4	4.1 ± 0.2 *
Peak Expiratory Flow, mls/sec	3.6 ± 0.3	2.9 ± 0.2 *

The data are presented as mean ± SEM.

* $P < 0.05$, age of females versus age of males.

* $P < 0.05$, female *versus* male.

Table 4

Cumulative responses upon exposure to a Hypoxic-Hypercapnic environment

Parameter	Arithmetic change		% change	
	Male	Female	Male	Female
Frequency, (breaths/min) $\times$ min	+1851 $\pm$ 99 *	+2016 $\pm$ 101 *	+60 $\pm$ 4 *	+67 $\pm$ 4 *
Tidal Volume, (mls) $\times$ min	+1.43 $\pm$ 0.21 *	+1.06 $\pm$ 0.11 *	+35 $\pm$ 5 *	+28 $\pm$ 3 *
Minute Volume, (mls/min) $\times$ min	+1053 $\pm$ 111 *	+906 $\pm$ 72 *	+123 $\pm$ 14 *	+119 $\pm$ 8 *
Inspiratory Time, (sec) $\times$ min	-0.28 $\pm$ 0.05 *	-0.41 $\pm$ 0.07 *	-17 $\pm$ 3 *	-23 $\pm$ 3 *
Expiratory Time, (sec) $\times$ min	-1.30 $\pm$ 0.12 *	-1.57 $\pm$ 0.13 *	-43 $\pm$ 2 *	-49 $\pm$ 2 *
End Inspiratory Pause, (msec) $\times$ min	+5.5 $\pm$ 1.0 *	+5.3 $\pm$ 0.8 *	+8.2 $\pm$ 1.5 *	+7.9 $\pm$ 1.2 *
Tidal Volume/Inspiratory Time, (mls/sec) $\times$ min	+28.3 $\pm$ 3.7 *	+25.5 $\pm$ 3.2 *	+70 $\pm$ 11 *	+79 $\pm$ 11 *
Peak Inspiratory Flow, (mls/sec) $\times$ min	+33.4 $\pm$ 6.6 *	+33.1 $\pm$ 4.7 *	+46 $\pm$ 9 *	+60 $\pm$ 11 *
Peak Expiratory Flow, (mls/sec) $\times$ min	+69.8 $\pm$ 6.4 *	+54.3 $\pm$ 5.5 *	+136 $\pm$ 14 *	+127 $\pm$ 12 *

The data are presented as mean $\pm$ SEM.

* $P < 0.05$, significant change from Pre. There were no-between-group differences in any parameter ($P < 0.05$, for all comparisons).

Baseline values and maximal responses in male and female C57Bl6 mice during hypoxia and subsequent return to normoxia

Table 5

Parameter	Pre		Hypoxia maximum		Normoxia maximum	
	Male	Female	Male	Female	Male	Female
Frequency (breaths/min)	222 ± 4	229 ± 3	306 ± 16 [†]	335 ± 10 [†]	358 ± 19 [†]	289 ± 22 ^{†,‡}
Tidal volume (TV, mls)	0.26 ± 0.02	0.23 ± 0.02	0.36 ± 0.02 [†]	0.29 ± 0.02 [†]	0.34 ± 0.03 [†]	0.24 ± 0.02 ^{†,‡}
Minute volume (mls/min)	58 ± 6	51 ± 6	112 ± 9 [†]	100 ± 7 [†]	129 ± 10 [†]	69 ± 7 ^{†,‡}
Inspiratory time (Ti, sec)	0.09 ± 0.006	0.10 ± 0.005	0.07 ± 0.004 [†]	0.07 ± 0.004 [†]	0.06 ± 0.01 [†]	0.07 ± 0.01 [†]
Expiratory time (sec)	0.19 ± 0.02	0.17 ± 0.02	0.14 ± 0.01 [†]	0.12 ± 0.01 [†]	0.14 ± 0.02 [†]	0.14 ± 0.01 [*]
End inspiratory pause (msec)	4.5 ± 0.2	4.8 ± 0.2	4.4 ± 0.4	4.5 ± 0.4	4.5 ± 0.06	4.5 ± 0.04
TV/Ti (mls/sec)	3.0 ± 0.2	2.3 ± 0.2 [*]	4.9 ± 0.5 [†]	4.1 ± 0.4 [†]	5.7 ± 0.5 [†]	3.3 ± 0.4 ^{†,‡}
Peak inspiratory flow (mls/sec)	4.8 ± 0.5	3.5 ± 0.4 [*]	7.9 ± 0.5 [†]	7.2 ± 0.6 [†]	9.9 ± 0.6 [†]	6.5 ± 0.7 ^{†,‡}
Peak expiratory flow (mls/sec)	3.7 ± 0.3	2.9 ± 0.2 [*]	6.1 ± 0.7 [†]	5.3 ± 0.3 [†]	7.6 ± 0.6 [†]	4.5 ± 0.4 ^{†,‡}
EF ₅₀ (mls/sec)	0.18 ± 0.02	0.15 ± 0.02	0.28 ± 0.04 [†]	0.27 ± 0.02 [†]	0.41 ± 0.05 [†]	0.22 ± 0.02 ^{†,‡}
Relaxation time (sec)	0.09 ± 0.01	0.07 ± 0.01	0.07 ± 0.01 [†]	0.08 ± 0.01 [†]	0.06 ± 0.01 [†]	0.08 ± 0.01 [†]
Rpef	0.12 ± 0.01	0.13 ± 0.01	0.20 ± 0.04 [†]	0.25 ± 0.04 [†]	0.16 ± 0.02	0.10 ± 0.01 ^{†,‡}

Data are shown as mean ± SEM. There were 12 mice in each group.

^{*} $P < 0.05$, female *versus* male pre hypoxia values.

[†] $P < 0.05$, significant change from Pre.

[‡] $P < 0.05$, female *versus* male post-hypoxia (normoxia) values.

Table 6
Cumulative responses of male and female C57Bl6 mice during exposure to hypoxia and subsequent normoxia

Parameter	Hypoxia		Normoxia	
	Male	Female	Male	Female
Frequency (breaths/min) $\times$ min	+562 $\pm$ 63 [*]	+469 $\pm$ 56 [*]	+1230 $\pm$ 278 [*]	+144 $\pm$ 23 ^{*,†}
Tidal volume (mls) $\times$ min	+0.88 $\pm$ 0.10 [*]	+0.19 $\pm$ 0.06 ^{*,†}	+0.28 $\pm$ 0.04 [*]	-0.10 $\pm$ 0.06 [†]
Minute volume (mls/min) $\times$ min	+382 $\pm$ 29 [*]	+180 $\pm$ 22 ^{*,†}	+521 $\pm$ 63 [*]	+37 $\pm$ 8 ^{*,†}
Inspiratory time (sec) $\times$ min	-0.04 $\pm$ 0.01 [*]	-0.08 $\pm$ 0.02 [*]	-0.18 $\pm$ 0.02 [*]	-0.13 $\pm$ 0.02 [*]
Expiratory time (sec) $\times$ min	-0.49 $\pm$ 0.07 [*]	-0.10 $\pm$ 0.04 [†]	-0.67 $\pm$ 0.12 [*]	+0.02 $\pm$ 0.13 [†]
End inspiratory pause (msec) $\times$ min	-0.23 $\pm$ 0.11	-2.67 $\pm$ 0.43 ^{*,†}	-0.38 $\pm$ 0.21	-0.52 $\pm$ 0.27
Tidal Volume/Inspiratory Time (mls/sec) $\times$ min	+10.1 $\pm$ 1.7 [*]	+4.4 $\pm$ 0.6 ^{*,†}	+13.3 $\pm$ 2.2 [*]	+1.8 $\pm$ 0.4 ^{*,†}
Peak inspiratory flow (mls/sec) $\times$ min	+22 $\pm$ 3 [*]	+21 $\pm$ 4 [*]	+34 $\pm$ 4 [*]	+14 $\pm$ 3 ^{*,†}
Peak Expiratory flow (mls/sec) $\times$ min	+24 $\pm$ 3 [*]	+18 $\pm$ 2 [*]	+23 $\pm$ 4 [*]	+8 $\pm$ 2 ^{*,†}
EF ₅₀ (mls/sec) $\times$ min	+0.58 $\pm$ 0.08 [*]	+0.54 $\pm$ 0.07 [*]	+1.16 $\pm$ 0.22 [*]	+0.23 $\pm$ 0.11 [†]
Relaxation time (sec) $\times$ min	-0.07 $\pm$ 0.01 [*]	+0.02 $\pm$ 0.02 [†]	-0.28 $\pm$ 0.06 [*]	-0.02 $\pm$ 0.04 [†]
R _{pef} $\times$ min	+0.25 $\pm$ 0.06 [*]	+0.42 $\pm$ 0.07 [*]	+0.32 $\pm$ 0.17	-0.04 $\pm$ 0.13

Data are shown as mean $\pm$ SEM. There were 12 mice in each group.

^{*} $P < 0.05$, significant cumulative response.

[†] $P < 0.05$, female *versus* male.

Table 7

Comparisons of baseline values and ventilatory responses in female (F) and male (M) mice

Parameter	Baseline	*Hypoxia	*Post-Hypoxia
Frequency	F = M	Increase: F = M	Increase: F < M
Tidal volume	F = M	Increase: F < M	Increase: F < M
Minute volume	F = M	Increase: F < M	Increase: F < M
Inspiratory time	F = M	Decrease: F = M	Decrease: F = M
Expiratory time	F = M	Decrease: F < M	Decrease: F < M
End inspiratory pause	F < M	Decrease: F < M	Decrease: F = M
Tidal volume/Inspiratory time	F < M	Increase: F < M	Increase: F < M
Peak inspiratory flow	F < M	Increase: F = M	Increase: F < M
Peak expiratory flow	F < M	Increase: F = M	Increase: F < M
EF ₅₀	F = M	Increase: F = M	Increase: F < M
Relaxation time	F = M	Decrease: F < M	Decrease: F < M
R _{pef}	F = M	Increase: F = M	No change: F = M

The pre and post-Hypoxia designations are based on cumulative responses (see Table 5).