Sex-dependent regulation of hypoxic ventilation in mice and humans is mediated by erythropoietin

Jorge Soliz,1 Jonas Juhl Thomsen,2 Christophe Soulage,3 Carsten Lundby,2,4 and Max Gassmann1

1Institute of Veterinary Physiology, Vetsuisse Faculty, and Zurich Center for Integrative Human Physiology, University of Zurich, Zurich, Switzerland; 2Copenhagen Muscle Research Centre, Copenhagen, Denmark; 3Laboratoire de Physiologie Intégrative Cellulaire et Moléculaire, Villeurbanne Cedex, France; and 4Department of Sport Science, University of Århus, Denmark

Submitted 28 November 2008; accepted in final form 20 March 2009

Erythropoietin (Epo) is an oxygen-dependent cytokine first described as a blood hormone able to increase the number of red blood cells. During the last years, however, it became obvious that Epo is a pluripotent cytokine synthesized in several tissues including the mouse and human brain (14, 42). Subsequent investigation demonstrated that under pathological (mostly ischemic) conditions, Epo has a potent neuroprotective role (9, 19, 21, 22), safeguarding the affected cerebral tissue via mechanisms involving antiapoptotic (61), anticytotoxic (44), and antioxidative (33) pathways. As these stress situations (apoptosis, cytotoxicity, and oxidative stress) also occur during physiological hypoxia (e.g., at high altitude), we hypothesized that apart from its pathological implications, neural Epo also exerts a physiological function by controlling hypoxic ventilation. Indeed, we provided data showing that Epo participates in the regulation of acute and chronic ventilatory responses to hypoxia (55). In mouse brain, we provided convincing evidence that Epo stimulates ventilation by binding of the Epo receptor (EpoR), the latter being located in the brain stem respiratory areas (55) and that soluble EpoR, a negative regulator of Epo’s binding to its EpoR, abolishes the ventilatory acclimatization to hypoxia (54). In parallel, coherent with its embryonic neural origin, we described that EpoR is expressed in carotid body glomus cells, and intravenous injection of recombinant human Epo (rhEpo) alters the ventilatory pattern of wild-type (WT) male mice exposed to hypoxia (55). Taken together, these recent results showed for the first time that neuronal respiratory and erythropoietic systems are tightly connected, obviously playing complementary roles in improving tissue oxygenation during hypoxia.

On the other hand, several studies proposed that the neural respiratory network is one of the major sites for sex-dependent regulation of ventilatory control upon exposure to hypoxia. Women and female animals cope better than men and male animals when exposed to decreased oxygen partial pressure at high altitude (28, 29, 34, 50). This observation is of particular importance because women are known to be less susceptible to a number of hypoxia-associated syndromes at sea level (emphysema, chronic bronchitis, cystic fibrosis, neonatal asphyxia, infant respiratory distress syndrome, and others) as well as at altitude (chronic mountain sickness, sudden infant death syndrome, excessive erythropoiesis). Because the mechanism underlying these processes remains poorly understood, we aimed to define the impact of central and peripheral Epo on basal minute ventilation (Ve) and on the hypoxic ventilatory response (HVR) of female mice. To this end, we used transgenic mice (Tg6) that show constitutive overexpression of human Epo in brain and lung, the later leading to increased plasma Epo levels (23). In addition, because carotid bodies are known to respond in a sex-dependent manner (29, 63), WT female mice were intravenously injected with rhEpo to investigate Epo’s impact, specifically on these peripherally located chemosensitive cells. Finally, to test whether Epo’s effect on the HVR occurs in humans as well, rhEpo was also intravenously applied to human volunteers of both sexes before inhaling 10% O2. Our results show that the impact of Epo on ventilation is a sex-dependent process, with females having a higher sensitivity to Epo. Thus, these results suggest that Epo is a critical factor contributing to the greater ability of females to cope with hypoxic environments and have lower susceptibility to hypoxia-associated sickness and syndromes.

Address for reprint requests and other correspondence: M. Gassmann, Institute of Veterinary Physiology, Vetsuisse Faculty, Univ. of Zurich, and Zurich Center for Integrative Human Physiology (ZIHP), Winterthurerstrasse 260, CH-8057 Zurich, Switzerland (e-mail: maxg@access.uzh.ch).

http://www.ajpregu.org 0363-6119/09 $8.00 Copyright © 2009 the American Physiological Society R1837
MATERIAL AND METHODS

Transgenic animals. Epo overexpressing transgenic mouse lines were generated by microinjection of human Epo cDNA driven by the human platelet-derived growth factor (PDGF) B-chain promoter into the pronuclei of fertilized oocytes derived from B6C3 hybrid mice (51). One resulting transgenic mouse line TgN(PDGFBEPO)321ZbZ (Tg6) showed increased Epo levels in plasma (12-fold compared with WT) and brain (26-fold compared with WT), accompanied by a doubled hematocrit value and 25.3 ± 0.7 (g/dl) hemoglobin concentration (24, 64). This transgenic mouse line was backcrossed to C57Bl/6 mice for more than 12 generations by mating heterozygous Tg6 males to WT C57Bl/6 female mice. Half of the offspring was heterozygous for the transgene, while the other half was WT and thus was used as control (8). Knowing that aged Tg6 mice show organ degeneration processes at later stages of life (24), we used exclusively transgenic and WT mice at 3–4 months of age. Animal experimentation was performed in accordance with the Swiss animal protection laws and institutional guidelines. Permission was given by the corresponding authorities (Veterinäramt des Kantons Zürich).

Ventilatory measurements in mice. Respiration was monitored by whole body flow-through plethysmography as previously described (55, 56). Briefly, mice were placed in a 600-ml chamber continuously supplied with airflow at 0.7–0.8 l/min using flow restrictors. VE was calculated as the product of tidal volume (VT) and respiratory frequency (fR) and normalized to 100 g body wt (i.e., ml·min⁻¹·100 g⁻¹). As soon as the animal was familiarized with the plethysmographic chamber (about 1 h), measurements of baseline ventilation (normoxia, 21% O₂) and hypoxic ventilation were performed. Acute hypoxia was achieved by flushing air balanced in N₂ using a gas-mixing pump (Digamix, model M302 a-F; H Wösthoff, Bochum, Germany). The fraction of inspired O₂ (FiO₂) in the chamber was gradually decreased from 21% to 10% O₂ over 15 min. Respiratory recordings at 10% O₂ were performed for 20 min. The oxygen concentration in the chamber was then further reduced to 6% over the next 15 min, and recordings were performed for 20 min in the severely hypoxic environment. At the end of each experiment, body weight was routinely measured to express VT in milliliters per 100 grams in body temperature and pressure, saturated (conversion factor 1.091 for 22°C) conditions. IR was defined as number of respirations per minute. Hemoglobin was quantified using standard methodology and body temperature in normoxia, and hypoxia was measured using a rectal thermocouple (Fluke). Note that the measurements of the ventilatory response to hypoxia in male and female mice were performed side by side, thus allowing us to compare the male’s ventilatory response (56) with the female’s as shown in Fig. 2.

To avoid any movement of the animals during the brief exposure to 100% O₂, the hyperoxic Dejours test (13) was performed in anaesthetized mice. Two minutes after injecting urethane solution (1.2 g/kg body wt ip), mice showed regular ventilation and normal fR. Baseline respiration was recorded while animals breathed 21% O₂ for 20 s. The plethysmographic chamber was then quickly saturated with 100% O₂, and the decline of ventilation was recorded over 20 s. Respiratory variables were analyzed, and the magnitude of the transient ventilatory decline was calculated as the difference between baseline and hyperoxic respiration parameters. Note that due to its minimal effect on fR and cardiac dynamics, urethane is commonly used in experiments evaluating the respiratory response (7, 27, 30). In addition, the use of urethane does not alter the acid-base status in experimental animals (11, 31).

Fig. 1. Determination of basal ventilation, hypoxic ventilatory response (HVR), body temperature, and metabolism in wild-type (WT) and transgenic (Tg6) female mice. A–C: normoxic basal ventilation. D–F: hypoxia was achieved in 2 steps of 15 min-gradual reduction of FiO₂ (represented by ▲); first step from 21% to 10% O₂ and second step from 10% to 6% O₂. Minute ventilation (VE), respiratory frequency (fR), and tidal volume (VT) were evaluated in WT control and in Tg6 mice over 20 min at 10% and at 6% O₂. Determination of body temperature (G), oxygen consumption (VO₂) (H) and carbon dioxide production (VCO₂) (I) in WT and Tg6 mice. *P < 0.05 Tg6 vs. WT, at same fraction of inspired O₂. Animals per group: n = 9–12.
Baseline ventilation in normoxia and ventilatory response to hypoxia (10% and 6% O2) were also evaluated 1–2 h after injection of domperidone (1 mg/kg ip, kindly provided by Janssen-Cilag; dissolved in 0.9% saline solution with 1 equivalent of tartaric acid). Note that according to the provider, domperidone is a highly specific peripheral D2-dopaminergic receptor antagonist that does not cross the blood-brain barrier. Control animals were injected with similar volumes of 0.9% NaCl.

rhEpo (2,000 U/kg body wt; Cilag, Switzerland) was intravenously injected into WT mice via the tail vein after its heat dilation using an infrared lamp (100W light; during 2 min). Subsequently, ventilatory response was evaluated at normoxia and 10% and 6% O2 as described above. Control WT animals received an injection of saline.

An open-circuit system allowed measurement of O2 consumption (\(V\dot{O}_2\), ml·min\(^{-1}\)·100 g\(^{-1}\)) and CO2 production (\(V\dot{CO}_2\), ml·min\(^{-1}\)·100 g\(^{-1}\)) during normoxia and hypoxia (10% and 6% O2). Female mice were placed in a chamber where a steady 0.2 l/min flow of air was maintained. The fractions of O2 and CO2 at the inflow and the outflow of the chamber were measured by O2 and CO2 analyzers (Qubit Systems, Kingston, Ontario).

Quantification of catecholamines in brain stem. Catecholamines were evaluated in successive transverse brain stem sections as previously described (55, 56). In brief, the catecholaminergic cell groups A6 and A5 (in pons) and A1C1 and A2C1 (in medulla) were dissected from the brain stem. A different group of animals were used to determine norepinephrine (NE) content or tyrosine hydroxylase (TH) activity, the latter analysis requiring a previous injection of 3-hydroxybenzylhydrazine dihydrochloride (NSD 1015; 75 mg/kg body wt ip in saline solution; Sigma, St. Louis, MO). Twenty minutes after injection, animals were decapitated, and the enzymatic activity of TH was indirectly evaluated by measuring the accumulation of L-dihydroxyphenylalanine (l-DOPA) during 20 min, following the blockade of DOPA decarboxylase with NSD 1015. Both NE and l-DOPA were quantified by HPLC coupled with electrochemical detection as described earlier (28). The mobile phase consisted of 0.1 M potassium phosphate buffer pH 3.0 containing 0.15 mM disodic EDTA at a flow rate of 0.8 ml/min. DOPA was measured at +0.65 V. The detection limit, calculated by doubling the noise ratios and expressed in picomoles of injected amounts, was < 0.03 pmol and the intra-assay coefficient was 0.2%.

Ventilatory measurements in humans. Thirteen healthy men (age 27 ± 4 yr, height 186 ± 4 cm, weight 82 ± 6 kg) and seven women (age 24 ± 3 yr, height 168 ± 5 cm, weight 61 ± 7 kg; no hormonal contraceptives used) volunteers participated in the study, which was approved by the local Danish ethical committee of the communities of Denmark.

![Fig. 2. Comparison of basal ventilation and HVR between WT and Tg6 female and male mice. Ventilation at normoxic (A–C), moderate hypoxic at 10% O2 (D–F), and severe hypoxic at 6% O2 (G–I) exposure are compared between WT and Tg6 female and male mice. Bars represent the average of the corresponding parameters during 20-min exposure time. Raw data of male mice were obtained from earlier experiments (55, 56). *P < 0.05 females vs. males, same mouse line. Animals per group: n = 10.](http://ajpregu.physiology.org/DownloadedFrom/)
Copenhagen and Frederiksberg and conformed to the Declaration of Helsinki. After receiving information, subjects gave their written consent to participate. On the experimental day, subjects reported to the laboratory at 0800. Throughout the experiment, subjects were in the supine position. Two hours after catheterization (vein flow in antecubital vein), ventilatory ($V\dot{E}$, $V_T$, and $f_R$) and metabolic ($V\dot{O}_2$ and $V\dot{CO}_2$) parameters were measured continuously (Quark b2; Cosmed, Rome, Italy). Before each test, ambient conditions were measured, and the gas analyzer and the flowmeter were then calibrated with high precision gases and a 3-liter calibration syringe, respectively. After 15 min of familiarization, measurements were completed under normoxic conditions for 5 min. The oxygen concentration was then reduced to 10% over the next 2 min, and recordings were performed for 15 min. Arterialized capillary blood samples were taken from a preheated earlobe during normoxia and after 15 min of exposure to hypoxia. During normoxia and hypoxia, blood was sampled anaerobically in heparinized capillary tubes and immediately analyzed for oxygen saturation ($SO_2$) (model OSM3 hemoxymeter; Radiometer, Copenhagen Denmark), and blood pH, carbon dioxide ($PCO_2$), oxygen tensions ($PO_2$), potassium ($K^+$), and glucose (ABL5; Radiometer). Hematocrit was determined by centrifuging designated capillary tubes. This protocol was repeated in the same subjects a second time after they received an acute injection of 5,000 units rhEpo prior to being acutely exposed to 10% $O_2$ for 15 min.

Statistical analysis. Analysis was performed using the StatView software (Abacus Concepts, Berkeley, CA). The reported values are means ± SD. For simple measurements, data were analyzed by one-way ANOVA followed by a post hoc protected least significant difference Fisher test. For hypoxic ventilation responses, data were analyzed by two-way ANOVA for repeated measurements. Differences were considered significant at $P < 0.05$.

RESULTS

Transgenic female mice show enhanced HVR. Ventilation of Tg6 female mice that showed a 26-fold constitutive increase in Epo concentration in the brain was compared with WT control siblings. In a first step, $V\dot{E}$, $f_R$, and $V_T$ were evaluated in female mice kept at basal resting conditions (Fig. 1 A–C). Compared with WT, Tg6 females showed increased basal $V\dot{E}$ due to elevated $V_T$ rather than $f_R$. Determination of the HVR was performed under either moderate (10% $O_2$) or severe (6% $O_2$) conditions of normobaric hypoxia. Similar to the observation in normoxia (21% $O_2$), the HVR of Tg6 females was higher than the one of WT at both, 10% and 6% $O_2$ (Fig. 1D). In contrast to the data observed in normoxia, this increase was due to significant elevation in $f_R$ rather than in $V_T$ (Fig. 1, E and F). Changes in ventilation were not due to differences in body temperature (Fig. 1G). No differences were noticed in oxygen consumption ($V\dot{O}_2$) and carbon dioxide production ($V\dot{CO}_2$) between WT and Tg6 animals. Taken together, these data provide evidence that elevated central and/or circulating Epo concentrations influence normoxic and hypoxic ventilation in female mice.

![Fig. 3. Peripheral chemosensitivity of males vs. females to $O_2$. Decline of $V\dot{E}$, $V_T$, and $f_R$ upon transition from 21% to 100% $O_2$ (Dejours test). The experiment was performed in urethane-anaesthetized mice. Tg6 mice were more sensitive to alteration of $O_2$ concentration in arterial blood than WT mice. Data are means ± SD for $n = 7–9$ animals per group. *$P < 0.001$. Tg6 vs. WT, same sex; $\Phi$ $P < 0.001$ females vs. males, same mouse line.](http://ajpregu.physiology.org/)

![Fig. 4. Ventilatory response of female mice in normoxia and acute hypoxia upon intraperitoneal injection of domperidone (NaCl, ●, domperidone, ○). Basal ventilation was evaluated 1–2 h after injection of domperidone. Hypoxia was achieved with a gradual reduction of $FIO_2$ (●); from 21% to 10% $O_2$ (over 15 min) and from 10% to 6% $O_2$ (over 15 min). HVR was evaluated during 20 min at 10% and at 6% $O_2$. Control animals were injected with an equal volume of 0.9% NaCl. *$P < 0.001$. Animals per group: $n = 6–8$.](http://ajpregu.physiology.org/)

Fig. 3. Peripheral chemosensitivity of males vs. females to $O_2$. Decline of $V\dot{E}$, $V_T$, and $f_R$ upon transition from 21% to 100% $O_2$ (Dejours test). The experiment was performed in urethane-anaesthetized mice. Tg6 mice were more sensitive to alteration of $O_2$ concentration in arterial blood than WT mice. Data are means ± SD for $n = 7–9$ animals per group. *$P < 0.001$. Tg6 vs. WT, same sex; $\Phi$ $P < 0.001$ females vs. males, same mouse line.

Fig. 4. Ventilatory response of female mice in normoxia and acute hypoxia upon intraperitoneal injection of domperidone (NaCl, ●, domperidone, ○). Basal ventilation was evaluated 1–2 h after injection of domperidone. Hypoxia was achieved with a gradual reduction of $FIO_2$ (●); from 21% to 10% $O_2$ (over 15 min) and from 10% to 6% $O_2$ (over 15 min). HVR was evaluated during 20 min at 10% and at 6% $O_2$. Control animals were injected with an equal volume of 0.9% NaCl. *$P < 0.001$. Animals per group: $n = 6–8$. 
Sexual dimorphism in the HVR. Knowing that the ventilatory response is sex-dependent with female mice having better capacity to adapt to hypoxia (28, 29, 34, 50), we compared \( V_E \) and HVR of WT and transgenic females with males. Comparison of basal ventilation between male and female WT revealed higher \( V_E \) in females (Fig. 2A, white bars). Interestingly, when exposed to 10% and 6% of hypoxia, the HVR did not differ between the sexes in WT animals (Fig. 2, D and G, white bars), despite the \( fR \) (but not the \( V_T \)) being increased in females (Fig. 2, B, E, and H, white bars). In contrast, elevated Epo levels in brain and plasma led to dramatic changes in the HVR of female Tg6 mice. In addition to basal \( V_E \), the ventilatory response to severe hypoxia was also increased in Tg6 females compared with Tg6 males (Fig. 2, A and G, black bars). This elevation was a result of increased \( V_T \) (Fig. 2, C, F, and I, black bars) rather than \( fR \) (Fig. 2, B, E, and H, black bars). In summary, these data show an Epo-mediated sex-dependent regulation of ventilation, thus suggesting an interaction between Epo and sexual hormones in the control of both normoxic and hypoxic ventilation.

Dejours test indicates higher sensitivity to hyperoxia in female WT and Tg6 mice. The carotid body has been proposed as one of the major sites for sex-dependent control of ventilation under acute and chronic hypoxic stimulation (28, 29, 63). To evaluate the carotid body’s sensitivity to \( O_2 \) changes in arterial blood, the transient ventilatory decline in response to a brief period of hyperoxia (Dejours test) was measured. The hyperoxia-induced ventilatory decline was higher in WT females compared with WT males (Fig. 3A, white bars). This difference observed in WT animals was due to \( fR \) decline rather than changes in \( V_T \) (Fig. 3, B and C). Even more pronounced, Epo-overexpressing Tg6 mice showed higher ventilatory decline in females compared with males (Fig. 3A, black bars), that was due to higher decline in both \( fR \) and \( V_T \) (Fig. 3, B and C, black bars). Different responses to hyperoxia were also evident when comparing the Dejours test results within females only: the hyperoxia-induced ventilatory decline was significantly higher in transgenic female mice compared with WT females (Fig. 3A, compare females only). These changes were due to altered \( fR \) and \( V_T \) in Tg6 animals (Fig. 3, B and C, compare females only).

Domperidone does not increase ventilatory response in Tg6 females. Domperidone is a highly specific peripheral D2-dopaminergic receptor-antagonist that induces carotid sinus nerve discharge, thereby increasing ventilation (26, 29, 56). While intraperitoneal injection of domperidone increased female WT basal ventilation and HVR (Fig. 4A), Tg6 female ventilation was not altered at all, during normoxia or hypoxia after domperidone injection (Fig. 4D). The observed increase of ventilation in normoxic and hypoxic WT females was due to the stimulation of \( V_T \) rather than \( fR \) (Fig. 4, B and C). On the other hand, domperidone application altered neither \( fR \) nor \( V_T \) in Tg6 females (Fig. 4, E and F). Sex differences were observed, however, when comparing the ventilatory response to hypoxia between domperidone-injected males and females. Basal \( V_E \) and HVR (at 10% and 6% \( O_2 \)) of WT and transgenic females were always higher than ventilation in the corresponding males (Fig. 5, A–C).

Plasma Epo stimulates HVR. In view of the fact that both the Dejours test and the injection of domperidone suggested a sex-dependent interaction of Epo with the carotid body, we evaluated the HVR in WT mice after an acute intravenous injection of rhEpo (2,000 U/kg). Control animals were injected with an equal volume of 0.9% NaCl. Bars at 10% and 6% \( O_2 \) represent the average of corresponding parameters during 20-min exposure time. *\( P < 0.05 \) females vs. males.

Transgenic females show altered catecholaminergic content and activity in brain stem cells. In a last set of experiments using mice, we evaluated the TH activity and the NE content in pons (A6 and A5) and medullary (A2C2 and A1C1) brain stem
corticosterone concentrations were increased in female mice (55) but not in male mice (55, 56). Consequently, the increase in the V工具E response was more clearly observed in female Tg6 (showing increased level of Epo) and female Tg6 siblings than male Tg6 (Table 1). Taken together, these observations strongly support the notion that Epo influences HVR, at least in mice.

As mentioned above, all experiments were performed in male animals. However, because HVR is known to be sex dependent (28, 29) we performed similar experiments using female mice, either WT (upon injection with Epo) or Tg6 animals. Indeed, compared with WT control females or male Tg6 siblings, female Tg6 mice overexpressing transgenic Epo and those administrated exogenous Epo showed extensive changes in ventilatory responses to hypoxia. Overall, both basal ventilation and the ventilatory response to moderate and severe hypoxia were dramatically augmented.

In addition, our results suggest that the observed impact of Epo on the neuronal control of female ventilation appears to act at both the central respiratory centers in the brain stem, as well as with the carotid bodies. We observed that when exposed to acute and severe hypoxia (6% O2), cerebral Epo increased V工具E by augmenting fR rather than V工具T. Moreover, transection of carotid sinus nerves (chemodenervation) led to life-threatening apneas and gasping patterns in WT males exposed to 6% O2, while the presence of cerebral Epo resulted in sustained V工具E of chemodenervated animals (55). In analogy, when basal V工具E and HVR were evaluated in male Tg6 (showing increased level of cerebral and plasma Epo), the V工具E response remained similar to that in corresponding WT animals, while the V工具T pattern was dramatically altered: fR was widely increased and V工具T extensively decreased (56). Taken together, these observations strongly support the notion that Epo influences HVR, at least in mice.

Moreover, Epo’s effect on ventilation in hypoxic humans is shown for the first time.

Recently, we reported that HVR is modulated by exogenous Epo in WT mice and confirmed this observation using two transgenic mouse lines that constitutively overexpress Epo in a hypoxia-independent manner (54–56). Of note, all these data were collected in male WT and transgenic mice. Briefly, we provided convincing evidence that Epo modulates HVR by interacting with the respiratory centers in the brain stem as well as with the carotid bodies. We observed that when exposed to acute and severe hypoxia (6% O2), cerebral Epo increased V工具E by augmenting fR rather than V工具T. Moreover, transection of carotid sinus nerves (chemodenervation) led to life-threatening apneas and gasping patterns in WT males exposed to 6% O2, while the presence of cerebral Epo resulted in sustained V工具E of chemodenervated animals (55). In analogy, when basal V工具E and HVR were evaluated in male Tg6 (showing increased level of cerebral and plasma Epo), the V工具E response remained similar to that in corresponding WT animals, while the V工具T pattern was dramatically altered: fR was widely increased and V工具T extensively decreased (56). Taken together, these observations strongly support the notion that Epo influences HVR, at least in mice.

As mentioned above, all experiments were performed in male animals. However, because HVR is known to be sex dependent (28, 29) we performed similar experiments using female mice, either WT (upon injection with Epo) or Tg6 animals. Indeed, compared with WT control females or male Tg6 siblings, female Tg6 mice overexpressing transgenic Epo and those administrated exogenous Epo showed extensive changes in ventilatory responses to hypoxia. Overall, both basal ventilation and the ventilatory response to moderate and severe hypoxia were dramatically augmented.

In addition, our results suggest that the observed impact of Epo on the neuronal control of female ventilation appears to act at both the central respiratory centers in the brain stem, as well as with the carotid body cells. Within the central
respiratory centers we observed that, compared with WT females, Tg6 females showed higher TH activity in A6 and A5 pons cell groups, while NE stores were less in medullar A2C2 and A1C1 cells. This expression pattern is different than the one reported in Tg6 males where the catecholaminergic content was only increased in A5 cells (56). Because catecholaminergic cells are potent modulators of ventilation in hypoxic conditions (25, 57, 58) and Epo modulates the release of catecholamines in cells with neuronal characteristics (33, 43, 61, 65), these results suggest that Epo in brain participates in the sex-dependent regulation of the HVR.

In a next step, we focused on the peripheral chemoreceptors that are represented by the carotid bodies. Carotid body glomus cells are specialized in translating the stimulus of lowered blood oxygen tension into corrective cardiorespiratory and autonomic reflexes (35). As carotid bodies originate from the embryonic ectodermal layer (32), it was not surprising to find expression of EpoR in these chemosensitive cells (55). In support of this finding, it was demonstrated that a reliable model of carotid body cells, the PC12 cells, express EpoR (1, 33) and, moreover, release dopamine under Epo stimulation (33, 43, 61, 65). Accordingly, we observed here that, compared with corresponding WT animals, the peripheral chemosensitivity to oxygen (Dejours test) was significantly higher in Tg6 mice. This suggests that plasma Epo also modulates ventilation through the peripheral respiratory center. Additionally, our results show the hyperoxia-induced ventilatory decline in Tg6 female animals was extensively larger compared with their Tg6 male siblings. Therefore, these data indicate that plasma Epo’s impact on the carotid body is sex dependent. Furthermore, the inhibition of domperidone-induced hyperventilation in Tg6 animals demonstrates that the elevated plasma Epo level depleted the carotid body stores of catecholamines, as domperidone is a highly selective peripheral D2-dopaminergic antagonist (29).

Based on the notion that an intact blood-brain barrier prevents larger glycosylated molecules, such as Epo, to enter the brain (10, 40, 41), we measured HVR (10% and 6% O2) in female and male WT mice following intravenous injection of 2,000 U/kg of rhEPO. This experiment allowed us to study the sex-dependent impact of Epo on carotid bodies omitting the influence of cerebrally produced Epo. Interestingly, while no differences were observed in male mice during hypoxia (10% or 6%), to our surprise, Epo-injected female WT animals experienced a remarkable increase of hypoxic ventilation during both moderate (10% O2) and severe (6% O2) hypoxia. These results support the hypothesis that peripheral stimulation of hypoxic ventilation by plasma Epo is much stronger in females than in males. In summary, our results provide strong evidence that circulating Epo affects carotid body cells in a sex-specific manner.

It is tempting to speculate that female sex hormones are involved in the sex-dependent control of ventilation by Epo. Note that ovarian steroids can influence the expression of oxygen-dependent genes such as renal Epo, vascular endothelial growth factor, endothelin-1, nitric oxide synthases, and hypoxia-inducible factor-1 (3, 15, 46 – 48, 52, 60). Additionally, estradiol and progesterone exert neuroprotection by interacting with molecules such as IL-1 and ERK, similar to what was proposed for Epo (12, 59, 67). Moreover, steroid sex hormones also modulate the expression of catecholamines in several tissues including brain (45, 53). Concerning ventilation, progesterone and estradiol are potent ventilatory stimulants acting at the central
Measurements were performed in the same volunteers with or without receiving acute injection of 5,000 units of recombinant human erythropoietin (rhEpo).

Table 1. Blood parameters evaluated in male and female human volunteers upon acute intravenous injection of rhEpo

<table>
<thead>
<tr>
<th></th>
<th>pH</th>
<th>Hct, %</th>
<th>( \text{P}_{\text{CO}_2} ) mmHg</th>
<th>( \text{P}_{\text{O}_2} ) mmHg</th>
<th>( \text{S}_{\text{O}_2} ) %</th>
<th>( K^+ ), mmol</th>
<th>Glu, mmol</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Men</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nx</td>
<td>7.403±0.012</td>
<td>47.0±2.9</td>
<td>41.6±2.6</td>
<td>72.5±4.3</td>
<td>96.8±0.6</td>
<td>3.9±0.1</td>
<td>5.1±0.3</td>
</tr>
<tr>
<td>Hx</td>
<td>7.440±0.019</td>
<td>46.8±3.9</td>
<td>35.4±1.9*</td>
<td>34.2±2.6*</td>
<td>76.2±4.5*</td>
<td>3.8±0.1</td>
<td>5.1±0.2</td>
</tr>
<tr>
<td>rhEpo</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nx</td>
<td>7.399±0.076</td>
<td>46.5±2.9</td>
<td>39.4±4.7</td>
<td>75.6±10.4</td>
<td>97.2±1.2</td>
<td>3.9±0.1</td>
<td>5.1±0.2</td>
</tr>
<tr>
<td>Hx</td>
<td>7.415±0.070</td>
<td>45.9±2.3</td>
<td>36.6±3.1</td>
<td>35.4±2.8*</td>
<td>78.2±3.7*</td>
<td>3.8±0.1</td>
<td>5.1±0.1</td>
</tr>
<tr>
<td><strong>Women</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nx</td>
<td>7.402±0.076</td>
<td>43.0±2.7</td>
<td>38.7±5.1</td>
<td>77.2±10.2</td>
<td>97.1±1.3</td>
<td>3.9±0.1</td>
<td>5.1±0.2</td>
</tr>
<tr>
<td>Hx</td>
<td>7.407±0.073</td>
<td>42.8±2.4</td>
<td>37.4±2.4</td>
<td>35.4±3.0*</td>
<td>77.3±2.8*</td>
<td>3.8±0.1</td>
<td>5.1±0.1</td>
</tr>
<tr>
<td>rhEpo</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nx</td>
<td>7.418±0.015</td>
<td>43.0±1.7</td>
<td>39.8±1.7</td>
<td>74.9±3.6</td>
<td>96.7±0.2</td>
<td>3.8±0.05</td>
<td>5.1±0.1</td>
</tr>
<tr>
<td>Hx</td>
<td>7.386±0.038</td>
<td>43.4±0.9</td>
<td>38.2±1.1</td>
<td>34.9±0.9*</td>
<td>76.9±2.5*</td>
<td>3.8±0.05</td>
<td>5.1±0.1</td>
</tr>
</tbody>
</table>

Values are means ± SD; n = 13 men and 7 women. Blood was sampled under normoxia (Nx) and after 15 min of hypoxia (Hx) at 10% \( \text{O}_2 \). Subsequently pH, oxygen tension (\( \text{P}_{\text{O}_2} \)), carbon dioxide (\( \text{P}_{\text{CO}_2} \)), oxygen saturation (\( \text{S}_{\text{O}_2} \)), potassium (\( K^+ \)), glucose (Glu), and hematocrit (Hct) were immediately analyzed. Measurements were performed in the same volunteers with or without receiving acute injection of 5,000 units of recombinant human erythropoietin (rhEpo).

* \( P < 0.05 \) Hx vs. Nx from same parameter.

Perspectives and Significance

Our results indicate that Epo exerts a sex-dependent impact on the ventilatory response to acute hypoxia in both mice and humans. Increased in women after acute intravenous administration of rhEpo. We found, however, that similar rhEpo intravenous injection blunted the HVR in men. Interestingly, Epo is also endogenously synthesized in testicular cells (38), and testosterone inhibits the hypercapnic ventilatory response in primates (17), indirectly modulates breathing in male cats, and modulates the carotid sinus nerve response to hypoxia (62). Accordingly, the blunted HVR observed in men following Epo injection implies that Epo may have a specific impact in the basal level of testosterone and/or in cells expressing testosterone receptors.
humans. Our data suggest that the Epo-dependent ventilatory sex dimorphism occurs both at the central (brain stem respiratory centers) as well as at the peripheral (carotid bodies) level and that catecholamines in brain stem and glomus cells are implicated in this process. Moreover, we provide convincing evidence that female sex hormones are involved in the mechanism(s) controlling the ventilatory response of females exposed to hypoxic conditions. At present, we are conducting follow-up studies by ovarectomizing female mice and reconstituting the hormonal loss. In view of clinical trials in which high-dose rhEpo is administrated to adults (16, 39, 49) and preterm infants (18) are becoming frequent, we strongly suggest consideration of the sexual dimorphisms described here when analyzing data obtained in those patients.

ACKNOWLEDGMENTS

The authors thank Stephan Keller for technical help, as well as Vincent Joseph for discussion.

GRANTS

The present study was supported by grants from the Roche Foundation for Anemia Research (RoFAR), Forschungskredit der Universität Zürich, and the Swiss National Science Foundation.

REFERENCES

35. Leon-Velarde F, Ramos MA, Hernandez JA, De Idaquez D, Munoz LS, Gaffo A, Cordova S, Durand D, Monge C. The role of menopause...
R1846

Epo MEDIATES SEX-DEPENDENT VENTILATION


