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

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Soliz J, Thomsen JJ, Soulage C, Lundby C, Gassmann M. Sex-dependent regulation of hypoxic ventilation in mice and humans is mediated by erythropoietin. Am J Physiol Regul Integr Comp Physiol 296: R1837–R1846, 2009. First published March 25, 2009; doi:10.1152/ajpregu.90967.2008.—Acclimatization to hypoxic exposure relies on an elevated ventilation and erythropoietic activity. We recently proposed that erythropoietin (Epo) links both responses: apart from red blood cell production, cerebral and plasma Epo interact with the central and peripheral respiratory centers. Knowing that women cope better than men with reduced oxygen supply (as observed at high altitude), we analyzed the hypoxic ventilatory response in Epo-overexpressing transgenic male and female mice with high Epo levels in brain and plasma (Tg6) or in wild-type animals injected with recombinant human Epo (rhEpo). Exposure to moderate and severe hypoxia as well as to hyperoxia and injection of domperidone, a potent peripheral ventilatory stimulant, revealed that the presence of transgenic or rhEpo extensively increased the hypoxic ventilatory response in female mice compared with their corresponding male siblings. Alterations of catecholamines in the brain stem’s respiratory centers were also sex dependent. In a proof-of-concept study, human volunteers were intravenously injected with 5,000 units rhEpo and subsequently exposed to 10% oxygen. Compared with men, the hypoxic ventilatory response was significantly increased in women. We conclude that Epo exerts a sex-dependent impact on hypoxic ventilation improving the response in female mice and in women that most probably involves sexual hormones. Our data provides an explanation as to why women are less susceptible to hypoxia-associated syndromes than men.

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, neuronal 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 erythrocytosis). 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.
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. fR 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 hypoxic Dejours test (13) was performed in anesthetized 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 (\(\dot{V}O_2\), ml·min\(^{-1}\)·100 g\(^{-1}\); atmospheric temperature and pressure in dry air) and CO₂ production (\(\dot{V}CO_2\), 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.
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{C}O_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 (P\dot{C}O_2), oxygen tensions (P\dot{O}_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.

**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}, fR, and V_T were evaluated in female mice kept at basal resting conditions (Fig. 1A–C). Compared with WT, Tg6 females showed increased basal V\dot{E} due to elevated V_T rather than fR. 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 fR 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{C}O_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.
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 \( V_{\text{E}} \) and HVR of WT and transgenic females with males. Comparison of basal ventilation between female and male WT revealed higher \( V_{\text{E}} \) 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 \( f_R \) (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 \( V_{\text{E}} \), 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 \( f_R \) (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 \( f_R \) 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 \( f_R \) 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 \( f_R \) 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 \( D_2 \)-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 \( f_R \) (Fig. 4, B and C). On the other hand, domperidone application altered neither \( f_R \) nor \( V_T \) in Tg6 females (Fig. 4, E and F). Six differences were observed, however, when comparing the ventilatory response to hypoxia between domperidone-injected males and females. Basal \( V_{\text{E}} \) 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. The results do not show changes in basal ventilation during normoxia; however, an extensive increase in HVR was observed only in female but not in male mice (Fig. 6, A and D). The female HVR elevation was due to significant augmentation of both \( f_R \) and \( V_T \) (Fig. 6, E and F). On the other hand, male mice injected with Epo showed an alteration of the ventilatory pattern only at 6% \( O_2 \) (increased \( f_R \), but decreased \( V_T \)) (Fig. 6, B and D). These data suggest that in females the carotid body’s response to hypoxia is governed in part by an interaction between Epo and sexual hormones.

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...
catecholaminergic cell groups in WT and transgenic female animals. Our results show that catecholaminergic metabolism is altered in transgenic females. Compared with WT, Tg6 animals showed higher TH activity in A6 and A5 (Fig. 7, A and B) but lower NE content in A2C2 and A1C1 (Fig. 7, C and D). Because catecholamines in brain play a pivotal role in the modulation of HVR (25, 57, 58), these data suggest that cerebral Epo alters the catecholaminergic metabolism in the transgenic brain stem and thus, central catecholamines may also participate in the observed sex-dependent ventilatory changes.

Women, but not men, enhance their HVR upon injection of rhEpo. To determine whether our results obtained from animal studies reflect the situation in humans, we performed a small proof-of-concept study in young men and women for biometric data (see MATERIAL AND METHODS). Evaluation of the HVR occurred in volunteers exposed to 10% O2 for 15 min upon intravenous injection of rhEpo. We observed that increased plasma Epo concentration altered HVR in all tested subjects. However, while HVR was decreased in men due to a significant decrease of fR rather than VT (Fig. 8, A–C), HVR was augmented in women due to a significant increase of VT rather than fR (Fig. 8, G–I). The observed differences in ventilatory parameters between males and females were not due to changes in metabolism (Fig. 8, D–F, J–L) nor other blood parameters, including pH, PaCO2, PaO2, SaO2, K+, glucose, and hematocrit (Table 1). Because we do not expect significant amounts of Epo to cross the blood-brain barrier during the short period of time between the Epo injection and the hypoxic exposure, our results suggest that elevated Epo plasma levels influence the peripheral chemosensitivity not only in mice (as shown above) but also in humans.

**DISCUSSION**

This is the first demonstration that the “blood hormone” Epo modulates hypoxic ventilation in a sex-dependent manner. 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 V̇E by augmenting fR rather than VT. 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 V̇E of chemodenervated animals (55). In analogy, when basal V̇E and HVR were evaluated in male Tg6 (showing increased level of cerebral and plasma Epo), the V̇E response remained similar to that in corresponding WT animals, while the V̇E pattern was dramatically altered: fR was widely increased and VT 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 altered. Moreover, HVR was dramatically augmented.
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). This protocol was repeated using the same volunteers, with subjects receiving acute injection of 5,000 units of rhEpo prior to being acutely exposed to 10% O₂.

Fig. 8. Ventilatory and metabolic parameters evaluated in male and female human volunteers upon intravenous injection of Epo. Data recording was performed under normoxic conditions for 5 min. Oxygen concentration was then further reduced to 10% over the next 2 min, and recordings were performed for 15 min. 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% O₂. Subsequently pH, oxygen tension (PO₂), carbon dioxide (PCO₂), oxygen saturation (SO₂), 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.

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

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<td>rhEpo</td>
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<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% O₂. Subsequently pH, oxygen tension (PO₂), carbon dioxide (PCO₂), oxygen saturation (SO₂), 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. Respiratory center (4–6, 36) as well as on peripheral chemoreceptors (23, 29, 36, 63). How Epo and female sex hormones interact in neural tissue is under current investigation.

Several studies in humans revealed that female hormones are involved with numerous hypoxia-associated sicknesses and syndromes and show that women have a better capacity to adapt to hypoxia (20, 37, 66). As such, chronic mountain sickness, mainly characterized by excessive erythropoiesis and hypoventilation in highlanders, predominantly occurs in men and in postmenopausal women (28, 37). Likewise, data on other high-altitude breathing syndromes and illnesses, such as acute mountain sickness, high-altitude pulmonary edema, and high-altitude cerebral edema, also suggest a protective role for female sexual hormones (2). As expected, our proof-of-concept study in human subjects showed that HVR is also significantly 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.
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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 ovariectomizing female mice and reconstituting the hormonal loss. In view of clinical trials in which high-dose rhEpo is administered 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.

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