Acute and chronic exposure to hypoxia alters ventilatory pattern but not minute ventilation of mice overexpressing erythropoietin

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Soliz J, Soulage C, Hermann DM, Gassmann M. Acute and chronic exposure to hypoxia alters ventilatory pattern but not minute ventilation of mice overexpressing erythropoietin. Am J Physiol Regul Integr Comp Physiol 293: R1702–R1710, 2007. First published July 25, 2007; doi:10.1152/ajpregu.00350.2007.—Apart from enhancing red blood cell production, erythropoietin (Epo) has been shown to modulate the ventilatory response to reduced oxygen supply. Both functions are crucial for the organism to cope with increased oxygen demand. In the present work, we analyzed the impact of Epo and the resulting excessive erythropoiesis in the neural control of normoxic and hypoxic ventilation. To this end, we used our transgenic mouse line (Tg6) that shows high levels of human Epo in brain and plasma, the latter leading to a hematocrit of ~80%. Interestingly, while normoxic and hypoxic ventilation in Tg6 mice was similar to WT mice, Tg6 mice showed an increased respiratory frequency but a decreased tidal volume. Knowing that Epo modulates catecholaminergic activity, the altered catecholaminergic metabolism measured in brain stem suggested that the increased respiratory frequency in Tg6 mice was related to the overexpression of Epo in brain. In the periphery, higher response to hyperoxia (Dejours test), as well as reduced tyrosine hydroxylase activity in carotid bodies, revealed a higher chemosensitivity to oxygen in transgenic mice. Moreover, in line with the decreased activity of the rate-limiting enzyme for dopamine synthesis, the intraperitoneal injection of a highly specific dopamine receptor antagonist, domperidone, did not stimulate hypoxic ventilatory response in Tg6 mice. These results suggest that high Epo plasma levels modulate the carotid body’s chemotransduction. All together, these findings are relevant for understanding the cross-talk between the ventilatory and erythropoietic systems exposed to hypoxia.

carotid body; brain stem; ventilatory control

UPON HYPOXIC EXPOSURE, ERYTHROPOIETIN (Epo) synthesis in kidney is accelerated, resulting in augmented Epo plasma levels and leading to enhanced globin synthesis and increased hematocrit levels (10, 40). Epo also has several nonerythropoietic functions (12). As such, Epo was recently identified as a key factor controlling the hypoxic ventilatory response (HVR) (39). We recently reported that Epo receptor (EpoR) is present in peripheral chemoreceptors and central respiratory areas controlling ventilation (Ve), including glomus cells in carotid bodies (the primary sensory organ that monitors arterial oxygen), NK-1R substance preceptor immunopositive neurons in the pre-Bötzing complex (proposed as the generator of respiratory rhythm), and the nucleus tractus solitarii in dorsal brain stem (relays input from peripheral chemoreceptors to the central respiratory areas) (39). Furthermore, it was demonstrated that cerebrally produced Epo per se influences the central respiratory activity, thereby increasing the respiratory frequency (fR) in response to hypoxia. Recently, we demonstrated that intracerebroventricular infusion of soluble EpoR, a negative regulator of Epo binding to the EpoR, abolishes the ventilatory acclimatization to chronic hypoxia (38).

Apart from enhancing Ve, erythrocytosis is also necessary to increase oxygen-carrying capacity during exposure to chronic hypoxia. As such, physiological erythrocytosis develops in individuals living at a high altitude. Enhanced erythrocytosis is also found in sports, the most prominent case being that of an outstanding Olympic endurance athlete (19) harboring an autosomal dominant erythrocytosis (33) that resulted in hematocrit levels up to 68%. Despite these beneficial effects, erythrocytosis may have a pathological impact. This occurs in lowlanders as a consequence of numerous respiratory disorders and in some highlanders when high altitude hypoxia pathologically stimulates dramatic increases in hemoglobin resulting in chronic mountain sickness (35). It is believed that CMS is the consequence of a ventilatory deacclimatization to hypoxia, leading to central hypoventilation, severe hypoxemia, increased erythropoiesis, and excessive erythrocytosis (27, 35). In turn, excessive erythrocytosis leads to life-threatening cyanosis, hyperemia, increased viscosity, thrombosis, and pulmonary hypertension in humans and mouse (13, 27, 35, 43).

Because Epo and the adaptation to erythrocytosis are of general interest in sport medicine and are significant factors concerning life at high altitudes, we studied the normoxic and HVR in a transgenic mice (Tg6) that due to constitutive overexpression of human Epo in brain and lung (13) develop chronic erythrocytosis (36, 44). Despite Tg6 mice showing hematocrit values up to 80–90%, we found no differences in minute Ve between Tg6 and control [wild-type (WT)] mice during acute and chronic hypoxia. Nevertheless, Tg6 mice showed dramatic changes in the ventilatory pattern with enlarged fR but decreased tidal volume (VT), both under acute and chronic hypoxic conditions. These findings suggest that elevated Epo levels in brain and blood are important factors regulating oxygen homeostasis in conditions of restricted oxygen supply.

MATERIAL AND METHODS

Transgenic animals. The Epo-overexpressing transgenic mouse line was generated by microinjection of human Epo cDNA driven by the human platelet-derived growth factor (PDGF) B-chain

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promoter into the pronuclei of fertilized oocytes derived from B6C3 hybrid mice (36). The 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 (48). This erythropoietic line was backcrossed to C57BL/6 mice for more than 12 generations by mating hemizygous males to WT C57BL/6 females. Half of the offspring were hemizygous for the transgene, while the other half were WT and thus were used as control animals. All experiments were performed in 3- to 4-mo-old male mice. Animal experimental protocols were approved by the Veterinäranstalt des KF Zürich.

Ventilatory measurements by plethysmography. Respiration was monitored by the whole body flow through plethysmography technique as previously described (39). Briefly, mice were placed in a 600-ml chamber continuously supplied with airflow at 0.7–0.8 l/min using flow restrictors. \( V_E \) was calculated as the product of VT and 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 (1–1 h), measurements of baseline \( V_E \) (normoxia, 21% \( O_2 \)) and hypoxic \( V_E \) were performed. Acute hypoxia was achieved by flushing air balanced in \( N_2 \) using a gas-mixing pump (Digamix type M302a-F; H. Wösthoff). The fraction of inspired \( O_2 \) (\( F_{I O_2} \)) in the chamber was gradually decreased from 21% to 10% \( O_2 \) over 15 min. Respiratory recordings at 10% \( O_2 \) 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 more hypoxic environment. At the end of each experiment, body weight was routinely measured to express \( V_T \) in milliliters per 100 g in body temperature and pressure (saturated) conditions. fR was defined as respirations per minute. Hemoglobin was quantified using standard methodology, and body temperature in normoxia and hypoxia was measured using a rectal thermocouple (Fluke). The same measurements of \( V_E \) and temperature were performed in animals that previously were exposed to chronic hypoxia (see below).

Chronic exposure to hypoxia was performed as described by Prabhakar and colleagues (20, 24). Briefly, mice were housed in a homemade hypoxic chamber connected to a gas-mixing pump (Digamix type M302 a-F; H. Wösthoff). The chamber oxygen concentration was gradually reduced from room air to 10% over 30 min and maintained at 10% for 3 days. During this period, mice were allowed free access to food and water. One hour after returning the mice to room air, baseline \( V_E \) and HVR at 10% and 6% \( O_2 \) were recorded by plethysmography as described above.

To avoid any movement of the animals during the brief exposure to 100% \( O_2 \), the hypoxic test [Dejours test (7)] was performed in anesthetized mice. Two minutes after injecting urethane solution (1.2 g/kg) mice showed regular \( V_E \) and normal fR. Baseline respiration was recorded while animals breathed 21% \( O_2 \) for 20 s. The plethysmographic chamber was then quickly saturated with 100% \( O_2 \), and the decline of \( V_E \) was recorded over 20 s. Respiratory variables were analyzed, and the magnitude of the transient \( V_E \) decline was calculated as the difference between baseline and hypoxic respiration parameters.

Baseline \( V_E \) in normoxia and \( V_E \) response to hypoxia (10% and 6% \( O_2 \)) were 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 agonist that does not cross the blood-brain barrier. Control animals were injected with similar volumes of 0.9% NaCl.

An open-circuit system allowed measurement of \( O_2 \) consumption (\( \dot{V}O_2 \), ml·min\(^{-1}·100\) g\(^{-1}\); atmospheric temperature and pressure in dry air) and \( CO_2 \) production (\( \dot{V}CO_2 \), ml·min\(^{-1}·100\) g\(^{-1}\)) in normoxia and hypoxia (10% and 6% \( O_2 \)). Mice were placed in a chamber where a steady 0.2 l/min flow of air was maintained. The fractions of \( O_2 \) and \( CO_2 \) at the inflow and the outflow of the chamber were measured by \( O_2 \) and \( CO_2 \) analyzers (Quibit Systems, Kingston, Ontario).

Quantitation of catecholamines in brain stem and carotid bodies. Catecholaminergic cell groups were obtained from successive transverse brain stem sections (60-μm thick), according to a mouse brain atlas (30) as described previously (47). In brief, A6 and A5 (in pons) and A1C1 and A2C1 (in caudal region) were dissected from the brain stem. Different animals were used to determine norepinephrine (NE) content (in brain stem slices) or tyrosine hydroxylase (TH) activity (in brain stem slices and in carotid bodies), the latter analysis requiring a previous injection of 3-hydroxybenzylhydrazine dihydrochloride (NSD 1015; 75 mg/kg 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) over 20 min, following the blockade of L-DOPA decarboxylase with NSD 1015. Both NE and L-DOPA were quantified by HPLC coupled with electrochemical detection as described earlier (17). 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. L-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%.

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 \( V_E \) responses, data were analyzed by two-way ANOVA for repeated measurements. Differences were considered significant at \( P < 0.05 \).

RESULTS

Basal \( V_E \) and metabolic parameters showed no differences in transgenic and WT mice. Minute \( V_E \), fR, and VT, as well as rectal temperature and metabolic variables, were evaluated in transgenic Tg6 and WT control mice under basal resting conditions (normoxia, 21% \( O_2 \); Table 1). Neither the minute \( V_E \) nor the ventilatory pattern, i.e., VT and fR were found to be different between the two strains in a normoxic environment. Additionally, no differences were noticed in metabolic parameters, such as body temperature, oxygen consumption, or carbon dioxide production (Table 1 and Fig. 1, F–H, 21% \( O_2 \)).

Tg6 mice show higher fR but lower VT upon exposure to acute hypoxia. The first attempt to regulate oxygen homeostasis during hypoxia is hyperventilation (31). We measured the \( V_E \) response in unanesthetized WT and transgenic mice to two levels of normobaric hypoxia (10% \( O_2 \) and 6% \( O_2 \)). HVR was similar between WT and transgenic male mice (Fig. 1A). However, the \( V_E \) pattern of Tg6 mice was substantially affected; while fR of moderate (10% \( O_2 \) and severe hypoxia (6% \( O_2 \) were significantly augmented (Fig. 1B; \( P < 0.0001 \)). VT was extensively decreased (Fig. 1C; \( P < 0.001 \)). The comparison of the relative proportions of fR and VT in the HVR clearly shows that VT is the main contributor in the hypoxic response of WT mice during hypoxia (Fig. 1D). On the contrary, the fR is the major provider for the HVR in transgenic animals (Fig. 1E). Under our experimental conditions, body temperature, oxygen consumption, or carbon dioxide production (Fig. 1, F–H) were similar, thus the change in the metabolic drive cannot account for the observed changes in breathing pattern.

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WT mice exhibited similar minute V\(_{\text{E}}\) (Fig. 2), Tg6 mice showed higher fR (Fig. 2) during acute hypoxic conditions, ventilatory acclimatization to hypoxia occurred (Table 1 and Figs. 2 and 3). Knowing that increased catecholamine levels in brain stem (39) suggest that the basic neurochemistry of the peripheral chemoreceptor is altered in Tg6 animals and that high Epo plasma levels are implicated in this chemosensory rearrangement.

Transgenic mice show altered breathing patterns after acclimatization to hypoxia. Long-term hypoxic exposure leads to a progressive increase in minute V\(_{\text{E}}\) that is termed ventilatory acclimatization to hypoxia (32). In mice this process is completed after 3 days (24, 29). Accordingly, transgenic and WT control mice were exposed to 10% O\(_2\) for 3 days, and then returned to normoxia to evaluate V\(_{\text{E}}\) during normoxia and acute hypoxia. Both transgenic and WT mice showed similar increases in the level of V\(_{\text{E}}\) during normoxia, indicating that ventilatory acclimatization to hypoxia occurred (Table 1 and Fig. 2, F–H, 21% O\(_2\)). During the hypoxic challenges, Tg6 and WT mice exhibited similar minute V\(_{\text{E}}\) (Fig. 2A), but show differences in terms of hypoxic V\(_{\text{E}}\) pattern. In agreement with the measurements performed under acute hypoxic conditions, Tg6 mice showed higher fR (Fig. 2B) and lower VT (Fig. 2C) responses to hypoxia compared with WT control animals. The comparison of the relative proportions of fR and VT in the HVR show that after chronic hypoxia in both WT and Tg6 mice, HVR is mainly supported by VT rather than fR; however, the contribution of VT to WT ventilatory response is higher in WT compared with transgenic mice (Fig. 2, D and E). As observed previously, alteration in body temperature or metabolic rate (Fig. 2, F–H) did not change and thus cannot account for the observed alterations in the V\(_{\text{E}}\) pattern.

Transgenic animals had altered catecholaminergic brain stem levels. We recently reported that the overexpression of Epo in neuronal cells alter catecholamine activity in respiratory-related brain stem areas (39). In that work, we also showed the presence of EpoRs on brain stem catecholamine neurons in WT mice. Because the Tg6 mouse line overexpresses 26-fold more Epo in brain compared with WT, we hypothesized that these animals would exhibit an altered level of catecholamines in the brain stem respiratory areas, which could contribute to the observed changes in V\(_{\text{E}}\). Our previous work strongly suggested that Epo stimulation of fR during hypoxia is, at least in part, mediated by an effect on catecholaminergic synthesis in the brain stem (39). Thus, we evaluated TH activity and the NE content in pontial A6 and A5 and medullar A1C1 and A2C2 brain stem catecholaminergic groups. Compared with WT mice, we found that transgenic Tg6 animals showed higher TH activity and NE content in A5 cell group only (Fig. 3B). In contrast, the catecholamine synthesis in A6, A1C1, and A2C2 cell groups showed no differences in either mouse lines (Fig. 3, A, C, and D).

Table 1. Metabolic parameters

<table>
<thead>
<tr>
<th>Type of mice</th>
<th>Normoxia</th>
<th>Normoxia After Chronic Hypoxia</th>
</tr>
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<tbody>
<tr>
<td>Number of mice used</td>
<td>10</td>
<td>9</td>
</tr>
<tr>
<td>Body weight, g</td>
<td>27.9±1.7</td>
<td>26.3±2.3</td>
</tr>
<tr>
<td>Body temperature, °C</td>
<td>37.7±0.67</td>
<td>36.7±0.23</td>
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<tr>
<td>Hemoglobin, g/dl</td>
<td>13.8±0.80</td>
<td>16.7±0.80</td>
</tr>
<tr>
<td>V(_{\text{E}}), ml·min(^{-1})·100 g(^{-1})</td>
<td>86.5±4.69</td>
<td>93.5±7.09</td>
</tr>
<tr>
<td>fR, resp/min</td>
<td>143.1±8.60</td>
<td>157.7±4.80</td>
</tr>
<tr>
<td>VT, ml/100 g</td>
<td>0.61±0.02</td>
<td>0.59±0.4</td>
</tr>
<tr>
<td>VO(_2), ml·min(^{-1})·100 g(^{-1})</td>
<td>10.2±0.79</td>
<td>9.5±0.61</td>
</tr>
<tr>
<td>VCO(_2), ml·min(^{-1})·100 g(^{-1})</td>
<td>9.3±0.58</td>
<td>8.7±0.78</td>
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WT, wild-type mice; Tg6, transgenic mouse line TgN(PDGFBEPO)321IzhZ; V\(_{\text{E}}\), ventilation; VT, tidal volume; fR, respiratory frequency; VO\(_2\), O\(_2\) consumption; VCO\(_2\), CO\(_2\) production.
In the present study, the impact of high Epo levels and excessive erythrocytosis on respiratory responses and ventilatory acclimatization to hypoxia were examined. We used a transgenic mouse line showing constitutive high levels of human Epo in brain (26-fold/WT) and plasma (12-fold/WT). The hematocrit values of these mice was 80–90% (36, 43, 44). Despite these remarkable characteristics, our results showed that normoxic and hypoxic V̇E in transgenic mice were similar to WT control. Tg6 mice however, showed marked alteration in the ventilatory pattern upon hypoxic exposure. fR in Tg6 mice was significantly higher, while VT decreased. In line with earlier work, our results suggest that the elevated cerebral Epo levels enhance fR in hypoxia and that Epo plasma levels participate in the modulation of the carotid body chemotransduction.

Following our previously published methodology (39), our experimental protocol included ventilatory measurements during normoxia, moderate hypoxia (10% O₂), and severe hypoxia (6% O₂). These experiments were conducted in unanesthetized and unrestrained mice. The advantage of this protocol is that it alleviated the effects that anesthesia may have had on breathing and allowed us to monitor changes in body metabolism that are known to occur during hypoxia (21, 24, 38). Changes in blood
pressure and arterial gases could not be monitored in unanesthetized mice; nevertheless VO₂ and VCO₂ were evaluated. VO₂ and VCO₂ values, together with the VE data, gave a reliable indication of potential differences between the tested groups. Considering, for example, that arterial CO₂ pressure is proportional to the metabolic rate and inversely proportional to VE, our results imply that arterial blood gases in normoxia and hypoxia were similar between both mouse groups.

Compared with WT mice, minute VE under normoxia, as well as the HVR, during acute and after chronic hypoxia was not altered in Tg6 mice. What triggers the neural control of VE in Tg6 mice—high Epo levels in brain and circulation or the elevated hemoglobin concentration? A direct impact of hematocrit on the neural control of VE has not been reported so far. As blood viscosity rose exponentially with the augmentation of hematocrit (6), we expected an exponential increase of pulmonary vascular resistance (28). Interestingly, however, despite hematocrit values of 80%, blood pressure, heart rate, and cardiac output in Tg6 mice were not abnormal (36). As shown earlier, the adaptive mechanisms to excessive erythrocytosis in Tg6 mice involve increased activity of endothelial nitric oxide (NO) synthase (eNOS). Thus, despite a concomitant increase of the potent vasoconstrictor endothelin-1 (34), the eNOS-mediated enhanced NO synthesis in Tg6 mice results in gen-

Fig. 2. Basal VE and VE response to hypoxia measured in Wt and Tg6 mice acclimatized to chronic hypoxia (3 days at 10% O₂). After acclimatization to hypoxia, animals were returned to room air and basal VE was evaluated. Hypoxia was achieved with a gradual reduction of FIO₂ (black triangle); from 21% to 10% O₂ (over 15 min) and from 10% to 6% O₂ (over 15 min). Hypoxic ventilatory response (HVR) was evaluated during 20 min at 10% and at 6% O₂ (A–C). The relative mean proportions of fR and VT were calculated and represented in bars (D and E). After chronic hypoxic VT is the major component of HVR both in Wt and Tg6 mice; however, VT contribution is higher in Wt compared with transgenics. Metabolic parameters; body temperature (F), VO₂ (G), and VCO₂ (H) were also determined in Wt and Tg6 mice. *P < 0.001. Data are means ± SD for n = 10 animals per group.
eralized peripheral vasodilatation (36). In parallel, one should keep in mind that elevation of the NO metabolism in the lung was also found in Tibetans and Bolivian Aymara as adaptive mechanism to hypoxia (2). Another adaptive mechanism to excessive erythrocytosis that most probably contributes to normal $V\dot{E}$ in Tg6 mice is the subtle increase of blood viscosity that is due to an increased flexibility of the transgenic erythrocytes (5, 43). We also speculate that increased flexibility of the red blood cells in Tg6 animals contributes to normal oxygen uptake and delivery. As such, we observed no differences in $V_{O2}$ and $V_{CO2}$ between Tg6 and control WT mice. Taken together, these observations do not support the notion that elevated hematocrit level influences the neuronal regulation of $V_{E}$.

On the other hand, despite no differences in minute $V_{E}$, the ventilatory pattern was remarkably altered. While not significant, somewhat higher $fR$ and lower VT are already observed under normoxic conditions, and hypoxia potentiated these differences. During hypoxic $V_{E}$ fR increased significantly, while VT decreased. We reported earlier that cerebral Epo enhanced fR under hypoxic conditions (39). We also showed recently that bilateral transsection of carotid sinus nerve (chemodenervation) in WT mice leads to dramatic respiratory depression during hypoxia, but high levels of brain Epo main-

Fig. 3. In vivo tyrosine hydroxylase (TH) activity and norepinephrine (NE) levels in brain stem catecholaminergic cell groups (A1C1 and A2C2 in the medulla oblongata; A5 and A6 in the pons) were determined by HPLC. TH activity is expressed as picomoles of $L$-dihydroxyphenylalanine ($L$-DOPA) formed in 20 min following blockade of $L$-DOPA decarboxylase. Significant differences were found in the A5 cell group. *$P < 0.05$; $n = 10–12$ animals per group.

Fig. 4. Peripheral chemosensitivity to oxygen evaluated by a hyperoxic test and TH activity in carotid bodies. Ventilation ($V_{E}$), VT, and fR declined (transition from 21% to 100% $O_2$) in response to hyperoxic testing (Dejours test). The experiment was performed in urethane-anesthetized Wt and Tg6 mice. Tg6 mice were more sensitive to hyperoxia. Data are means ± SD for $n = 8$ animals per group. *$P < 0.001$. TH activity is expressed as picomoles of $L$-DOPA accumulated in 20 min following blockade of $L$-DOPA decarboxylase using 3-hydroxybenzylhydrazine dihydrochloride (NSD 1015). Noradrenaline levels were determined using HPLC coupled to electrochemical detection. *$P < 0.05$. Data are means ± SD for $n = 10–12$ animals per group.
tained fR despite the absence of signal information coming from the peripheral chemoreceptors (39). Because Tg6 mice also overexpress Epo in brain (26-fold/WT), it is tempting to attribute the higher hypoxic fR to local presence of Epo in brain. In addition, our previous results strongly suggested that Epo increased fR by altering the activity in brain stem catecholaminergic centers. High content of NE in A5 group cells increases the hypoxic fR (8, 14). In line with these observations, we found that Tg6 mice showed significant increases of TH activity and NE content in A5 cells compared with WT control mice (Fig. 3B). In conclusion, the present results, together with previous data (39), support the hypothesis that increased cerebral Epo level modulates the catecholamine synthesis in brain stem. Once exposed to hypoxia, this alteration affects the ventilatory response via increasing the fR. Despite the tight correlation between HVR and catecholaminergic metabolism in brain stem, we do not discard other possible mechanisms by which cerebral Epo modulates the hypoxic Ve.

Apart from stimulating erythropoiesis (10, 40), plasma Epo affects hypoxic Ve most probably by interacting with carotid body glomus cells. Carotid bodies are the main peripheral sensors responding to decreased oxygen content and mediate the integrated cardiorespiratory responses to hypoxemia. We demonstrated earlier that intravenous injection of recombinant human Epo in WT mice induced a significant alteration in breathing pattern in response to severe hypoxia (39). An increased fR and a profoundly decreased VT, compared with vehicle-injected mice, suggested that plasma Epo levels participated in the breathing control. The present results using Tg6 mice support this hypothesis. Moreover, reduced activity of TH (the rate-limiting enzyme for dopamine synthesis in carotid bodies) in transgenic carotid bodies, suggest that the inhibitory function of dopamine is reduced in transgenic animals. In line with these results, we found that Tg6 mice exhibited a higher peripheral chemosensitivity as indicated by the response to the hyperoxic challenge (Dejours test). The greater ventilatory decline observed during hyperoxia implies that transgenic carotid bodies exert a stronger tonicity, and this effect is most probably due to direct stimulation by plasma Epo.

Despite good correlations between elevated central and plasma Epo levels and acute and chronic hypoxic breathing patterns in Tg6 mice, we do not discard other factors that may contribute to this process. Indeed, as mentioned earlier we showed that as a consequence of excessive erythrocytosis, Tg6 animals elevate expression of eNOS by threefold (36). The
generalized vasodilation produced by eNOS activity prevents Tg6 animals from cardiovascular dysfunction, hypertension, and thromboembolic complication (36). However, the eNOS-mediated production of NO also impacts on the peripheral respiratory centers in hypoxia (42, 45, 46). As such, our data do not allow discrimination between the effect produced by NO and plasma Epo. However, as Epo is a potent factor leading to the release of catecholamines in PC12 cells, a reliable model of peripheral chemosensitive cells (22, 26, 41, 49), it is reasonable to suggest that Epo and not NO is the responsible factor of the catecholamine alteration observed in carotid bodies. The combined impact of Epo and NO in the peripheral respiratory centers is a matter of current investigation.

Very recently, the specificity of the commercially available anti-EpoR-antibodies has been challenged (11). As regarding neuronal cells, however, we and others have described the presence of EpoR mRNA in the brain of several mammals, including humans, by RT-PCR (3, 4, 9, 23, 25, 37); 2) the presence of specific Epo binding sites in the brain by using radiolabeled Epo (9); 3) the impact of Epo on PC12 cells (a reliable cell model of peripheral chemosensitive cells) (1, 26, 41, 49); and 4) the inhibition of the ventilatory acclimatization to chronic hypoxia in WT mice by intracerebroventricular infusion of soluble EpoR (38). Thus, there is convincing evidence that EpoR is expressed in neural cells.

In conclusion, the ventilatory response to acute and chronic hypoxia remains similar between Tg6 and WT animals. In contrast, the ventilatory pattern was significantly altered—fR was widely increased and VT was extensively decreased in Tg6 compared with WT mice. In line with previous findings, our results imply that enhanced cerebral Epo levels stimulate fR in response to hypoxia. On the other hand, the modulation of the hypoxic ventilatory pattern in Tg6 carotid bodies might be a consequence of several factors, among them the high Epo plasma levels being a major player. These results support the hypothesis that enhanced levels of cerebral and plasma Epo are important factors in the regulation of oxygen homeostasis and, moreover, might have decisive implications in the ventilatory acclimatization and deacclimatization process to high altitude hypoxia.

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HYPOXIC VENTILATION IN MICE OVEREXpressING ERYTHROPOIETIN