Ventilatory responses to acute and chronic hypoxia are altered in female but not male Paskin-deficient mice

Jorge Soliz,1 Christophe Soulage,2 Emanuela Borter,3 Martha Tissot van Patot,4 and Max Gassmann1

1Institute of Veterinary Physiology, Vetsuisse Faculty, and Zurich Center for Integrative Human Physiology (ZIHP), University of Zurich, Zurich, Switzerland; 2Laboratoire de Physiologie Intégrative Cellulaire et Moléculaire, UMR Centre National de la Recherche Scientifique 5123 Villeurbanne, France; 3Institute of Physiology and ZIHP, University of Zürich, Zürich, Switzerland; and 4Department of Anesthesiology, University of Colorado Denver Health Sciences Center, Denver, Colorado

Submitted 9 December 2007; accepted in final form 21 May 2008

THE PER-ARNT-SIM (PAS) DOMAIN regulates the function of many intracellular signaling cascades in response to both extrinsic and intrinsic stimuli, including hypoxia (40). In prokaryotes, such as archaea, bacteria, and some plant species, the PAS domain senses a wide range of primary physiological parameters, including light intensity, oxygen partial pressure, and redox state (53). The sensing potential of the PAS protein becomes functional when the PAS domain is associated with a protein kinase, thereby forming a PAS kinase termed PASKIN. This molecular arrangement links sensing processes with signaling cascades (17). Apart from lower forms of life, PASKIN is also ubiquitously expressed in mammalian cells (17, 40) and is especially highly expressed in tubuli seminiferi (22). PASKIN is encoded by an evolutionarily conserved single-copy gene that was the first PAS protein containing a kinase in mammals (17). The human and mouse PASKIN genes show a conserved intron-exon structure and share their promoter regions with another ubiquitously expressed gene that encodes a regulator of protein phosphatase-1 (17, 22). The mammalian PASKIN contains two PAS domains (PAS A and PAS B), as well as a serine/threonine kinase domain, which is related to AMP-activated protein kinase (6). This architecture closely resembles the PAS domain formed in the oxygen sensor termed FixL of nitrogen-fixing Rhizobium species. FixL contains a heme group within its PAS domain that upon oxygen binding inhibits the histidine kinase domain. In contrast, under low oxygen conditions, kinase activity is derepressed and activates FixJ, the master transcriptional inducer of genes involved in nitrogen fixation (6). These data point toward a possible implication of mammalian PASKIN in oxygen-dependent processes.

We have studied central and peripheral ventilatory responses to acute and chronic hypoxia in male and female mice, with particular regard to oxygen-sensing mechanisms (46, 47). Increasing ventilation is the first response to acute reduction of environmental oxygen (hypoxic ventilatory response, HVR) (25), and acclimatization to chronic hypoxia (occurring within several hours to months) results in a progressive, time-dependent increase in baseline ventilation, a process termed ventilatory acclimatization to hypoxia (VAH) (37). While peripheral chemotransmission (39) modulated by central activity (15, 48, 49) is primarily implicated in the stimulation of ventilation during acute hypoxia, increasing evidence suggests that transcriptional regulation of genes is a pivotal mechanism underlying long-term adaptations to chronic hypoxia (8, 30, 43). Because PASKIN might be involved in oxygen sensing and presumably in transcriptional regulation (6, 22), we hypothesize that PASKIN plays a role in the neural control of hypoxic ventilation. We studied hypoxic ventilation upon acute and chronic hypoxic exposure of adult PASKIN homozygous deficient mice (termed Paskin−/−). We evaluated ventilation in both male and female animals, because several reports showed that women and female rats have a better capacity to adapt to hypoxia (20, 21, 26, 36). Women have shown to have less susceptibility to a number of hypoxia-associated syndromes in
infancy and adulthood, such us sudden infant death syndrome (3, 20, 27), sleep apneas, and chronic mountain sickness (27, 34). Accordingly, we found that HVR and VAH are largely altered in female, but not male mice.

Because catecholamines play an important role in the modulation of hypoxic ventilation (15, 48, 49), we also evaluated tyrosine hydroxylase (TH) activity in central catecholaminergic regions. Similarly, intravenous injection of domperidone allowed the determination of the dopaminergic status in carotid glomus cells. Finally, the Dejours test (10) was used to measure the carotid body sensitivity to oxygen changes in arterial blood. These studies revealed that changes at central, but not peripheral, respiratory network sites are responsible for the alterations observed in gender-dependent hypoxic ventilation. In summary, our results imply that PASKIN is involved in the gender-dependent regulation of oxygen homeostasis in mammals.

MATERIAL AND METHODS

Animals. Experiments were performed on wild-type (WT) and Paskin−/− male and female mice that were a gift from Dr. Roland H. Wenger (Institute of Physiology, University of Zurich, Switzerland). Note that Paskin−/− mice do not display an obvious phenotype and have normal fertility and life span (22). Mice were maintained in pathogen-free barrier facility under 12:12-h light-dark cycle and were fed ad libitum. All experiments were performed in 3- to 4-mo-old males and females, which were backcrossed to C57BL/6 for 5–10 generations (mean body weight is shown in Table 1). All animal experiments conformed to the Guide for Care and Use of laboratory animals published by the U.S. National Institutes of Health (NIH publication No. 85-23, revised 1996) and institutional guidelines were approved by the Kantonalen Veterinärmst Zurich.

Ventilatory measurements by plethysmography. Respiration was monitored by the whole body flow-through plethysmography technique, as previously described (46, 47). Briefly, mice were placed in a 600-ml chamber continuously supplied with airflow at 0.7–0.8 l/min using flow restrictors. Ventilation (VE) was calculated as the product of tidal volume (VT) and respiratory frequency (fR) and normalized to a 600-ml chamber continuously supplied with airflow at 0.7–0.8 l/min.

Table 1. Basal ventilatory parameters during normoxia and during normoxia following chronic hypoxia (3 days at 10% O2)

<table>
<thead>
<tr>
<th></th>
<th>Normoxia</th>
<th>Normoxia After Chronic Hypoxia</th>
<th>Normoxia</th>
<th>Normoxia After Chronic Hypoxia</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>WT</td>
<td>Paskin−/−</td>
<td>WT</td>
<td>Paskin−/−</td>
</tr>
<tr>
<td>Body weight, g</td>
<td>32.7±2.06</td>
<td>32.7±2.06</td>
<td>32.7±2.06</td>
<td>32.7±2.06</td>
</tr>
<tr>
<td>Hemoglobin, g/dl</td>
<td>33.5±3.86</td>
<td>33.5±3.86</td>
<td>33.5±3.86</td>
<td>33.5±3.86</td>
</tr>
<tr>
<td>VT, ml·min⁻¹·100 g⁻¹</td>
<td>28.4±2.08</td>
<td>28.4±2.08</td>
<td>28.4±2.08</td>
<td>28.4±2.08</td>
</tr>
<tr>
<td>fR, responses/min</td>
<td>28.2±2.39</td>
<td>28.2±2.39</td>
<td>28.2±2.39</td>
<td>28.2±2.39</td>
</tr>
<tr>
<td>VT, ml/100 g</td>
<td>13.0±0.9</td>
<td>13.0±0.9</td>
<td>13.0±0.9</td>
<td>13.0±0.9</td>
</tr>
</tbody>
</table>

WT, wild type; fR, respiratory frequency; VT, tidal volume. *P < 0.05 Paskin−/− vs. WT at normoxia or at normoxia following chronic hypoxia. †P < 0.05 female vs. male at given ventilatory parameter.
Quantitation of TH activity in brain stem catecholaminergic cell groups. Catecholaminergic cell groups were obtained from successive coronal brain stem sections (60 μm thick) according to a mouse brain atlas (35), as described previously (52). In brief, A6 and A5 (in pons) and A1C1 and A2C1 (in medulla oblongata) were dissected from the brain stem. TH activity required a previous injection of 3-hydroxybenzylhydrazine dihydrochloride (NSD 1015; 75 mg/kg ip in saline solution; Sigma Chemical, 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 DOPA decarboxylase with NSD 1015. L-DOPA was quantified by HPLC coupled with electrochemical detection, as described earlier (20, 46). 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%.

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 Fisher’s protected least significant difference test. For hypoxic ventilation responses, data were analyzed by two-way ANOVA for repeated measurements. Differences were considered significant at $P < 0.05$.

RESULTS

Ventilatory responses to normoxia and acute hypoxia. Minute ventilation (Ve), respiratory frequency (fR), and tidal volume (VT) were evaluated in male and female WT and Paskin−/− mice. Basal resting conditions (normoxia, 21% O2) were not different between Paskin−/− and WT mice within the same gender. However, basal ventilation of WT and Paskin−/− females was significantly higher compared with corresponding males (Table 1). After basal measurements, mice were acutely and sequentially exposed to two levels of normobaric hypoxia (10% O2 and 8% O2) for 20 min each (Fig. 1, A–C and 1, G–I, respectively). Compared with corresponding WT controls, male Paskin−/− mice showed an increased HVR only during the first minute of exposure to 8% O2 (Fig. 1, A–C). In contrast, female Paskin−/− mice showed a significant increase of HVR both throughout 10% and at 8% O2 (Fig. 1G), compared with WT females. This increase was due to augmentation of both fR (Fig. 1H) and VT (Fig. 1I). Normalizing the HVR to baseline measurements and expressing HVR as a percent change from baseline clearly demonstrates HVR differences between male and female WT and Paskin−/− mice. As shown in Fig. 2A, the percent rise of HVR in Paskin−/− males is higher than the corresponding WT male control during the first 10 min of exposure.

Fig. 1. Baseline ventilation and hypoxic ventilatory response under acute and after chronic hypoxia in WT and Paskin−/− male (A–F) and female (G–L) mice. Ventilation (Ve), respiratory frequency (fR), and tidal volume (VT) were measured at normoxia (21% O2) and hypoxia (10% and 8% O2), during acute exposure (male A, B, C; female G, H, I) and after 3 days of acclimatization to chronic hypoxia (10% O2 in a hypoxic chamber; male D, E, F; female J, K, L). Following normoxic baseline ventilation (21% O2), hypoxia was achieved in two sequential steps of 15-min gradual reduction of FIO2 (represented by §); first step from 21% to 10%, and second step from 10% to 8% O2. Ventilatory responses to hypoxia were evaluated during 20 min at 10% and 6% O2. *$P < 0.05$. Data are presented as means (SD) for n = 16–18 per group.
Fig. 2. Acute and chronic hypoxic ventilatory response normalized to baseline measures in wild-type (WT) and Paskin⁻/⁻ mice. Basal ventilation for all of the groups is represented as a dotted line at 100%. Hypoxic ventilatory responses (HVR) were then normalized to baseline. The black triangles (△) represent the 15-min gradual reduction of FIO₂ achieved in two sequential steps from 21% to 10%, and from 10% to 8% O₂. *P < 0.05. Data are presented as means (SD) for n = 16–18 per group.

acute exposure to 8% O₂. The greatest response to 10% and 8% of acute hypoxia in male and female mice is reached during the first minutes of hypoxia. Over the following 10 min, the classic decline in ventilation referred to as the hypoxic ventilatory decline (HVD) or “roll off” is observed (37). The HVD in WT and Paskin⁻/⁻ male mice is similar at 10% O₂, but it is slower in Paskin⁻/⁻ at 8% O₂ (Fig. 2A). In contrast, female Paskin⁻/⁻ animals showed slower HVR than corresponding WT control animals, both at 10% and 8% O₂ (Fig. 2C).

Effect of ventilatory acclimatization to chronic hypoxia. Chronic hypoxia induces ventilatory acclimatization (VAH) that is manifested by a large increase in normoxic ventilation (38), as well as by an augmented response to “subsequent” acute hypoxia (HVR) (23). After 3 days of exposure to normobaric hypoxia (FiO₂ 10%), the hemoglobin concentration increased in WT and Paskin⁻/⁻ mice to similar levels in male and female mice (Table 1). In addition, a significant increase of basal ventilation (measured at 21% O₂) was observed in WT and Paskin⁻/⁻ mice of both genders, thus showing that chronic hypoxia induced, as expected, ventilatory acclimatization (Table 1). However, despite this increase in ventilation, two important differences are observed. Female normoxic ventilation after acclimatization in WT and Paskin⁻/⁻ mice was higher than corresponding male mice (Table 1). Second, Paskin⁻/⁻ female normoxic ventilation after acclimatization was significantly lower than the corresponding control WT females. These observations are indicative of an impaired ability to acclimate in Paskin⁻/⁻ females (Table 1).

HVR evaluated after ventilatory acclimatization showed similar values between WT and Paskin⁻/⁻ males, except for a significant increase of V˙E and fR in the first minute of exposure to 8% O₂ (Fig. 1, D and E). Remarkably, however, compared with the controls, Paskin⁻/⁻ females showed decreased HVR (absolute values), thus confirming that Paskin⁻/⁻ females have impaired capability to reach ventilatory acclimatization (Fig. 1J). The observed alterations were due to significantly decreased VT rather than fR (Fig. 1, K and L). When normalizing the HVR to baseline measurements, the net increase in ventilation is similar in WT and Paskin⁻/⁻ female mice (Fig. 2, B and D).

Finally, HVD after acclimatization to hypoxia was similar between WT and Paskin⁻/⁻ males or females (Fig. 2, B and D). Taken together, these data suggest that PASKIN is implicated in the neural control of hypoxic ventilation in a gender-dependent manner.

Metabolism measurements upon acute and chronic hypoxia. The effects of body temperature on the mechanical properties of the respiratory system are crucial for interpretation of the ventilatory responses (33). We evaluated the body temperature of WT and Paskin⁻/⁻ mice, upon acute and chronic hypoxia (Fig. 3, A, D, G, J). The results showed no differences of body temperature during normoxic or hypoxic conditions between WT and Paskin⁻/⁻ male and female mice. However, exposure to hypoxia resulted in a quick decrease of body temperature in WT and Paskin⁻/⁻ mice (hypometabolism) (42). Next, to evaluate as to whether changes observed in ventilation were related to metabolic alterations, we measured the oxygen consumption (V˙O₂) and carbon dioxide production (V˙CO₂) upon acute and chronic exposure to hypoxia. As expected, V˙O₂ and V˙CO₂ significantly decreased when FiO₂ was reduced from...
21% to 10% and from 10% to 8% O₂, yet no differences were found in \( V\dot{O}_2 \) and \( V\dot{CO}_2 \) between WT and Paskin\(^{−/−}\) male (Fig. 3, B, C, E, F) or female mice (Fig. 3, H, I, K, L), both during acute or after chronic hypoxia.

**Evaluation of TH activity in brain stem cell groups.** Catecholaminergic cell groups in the brain stem respiratory centers play an important role in the modulation of the HVR (15, 48, 49). Similarly, changes in neuronal catecholaminergic metabolism might fine tune the increasing ventilatory output in long-term hypoxia (48). Here, we evaluated the TH activity in pontine (A6 and A5) and medullary (A2C2 and A1C1) brain stem catecholaminergic cell groups in WT and Paskin\(^{−/−}\) male and female animals. Our results show that catecholaminergic metabolism is altered in mice lacking PASKIN. Compared with WT, Paskin\(^{−/−}\) male mice had lower TH activity in A6, A5, and A2C2, but not in A1C1 (Fig. 4A). In contrast, compared with WT controls, higher TH activity was found in all of the catecholaminergic cell groups of Paskin\(^{−/−}\) female mice (Fig. 4B).

**Impact of domperidone injection in WT and Paskin\(^{−/−}\) animals.** Domperidone is a highly specific peripheral \(D_2\)-dopaminergic receptor-antagonist that induces carotid sinus nerve discharge, thereby increasing ventilation (18, 21, 47). Our results revealed that domperidone injection increased HVR in WT and Paskin\(^{−/−}\) mice similarly (Fig. 5), thus implying that there are no differences in the catecholaminergic machinery between the WT and Paskin\(^{−/−}\) carotid body cells.

**Evaluation of carotid body sensitivity to arterial oxygen (Dejours test).** The magnitude of the transient ventilatory decline in response to a brief period of hyperoxia (Dejours test) is used as an index of carotid body sensitivity to oxygen changes in arterial blood (23). The Dejours test was applied to WT and Paskin\(^{−/−}\) animals. While our results indicated a tendency toward lower ventilatory decline in Paskin\(^{−/−}\) carotid bodies, these differences were not significant, in male or female mice (Fig. 6).

**DISCUSSION**

The major findings of the present study are 1) HVR upon acute and after chronic hypoxia showed only minor differences between Paskin\(^{−/−}\) and WT male mice. 2) Female Paskin\(^{−/−}\) animals however, compared with corresponding female control WT, showed significant higher HVR to acute hypoxia, but attenuated HVR after chronic hypoxia. 3) These ventilatory differences in male and female Paskin\(^{−/−}\) mice appear to be due to changes at central rather than at peripheral respiratory centers.

**Methodological considerations.** The normoxic and hypoxic ventilatory measurements were conducted in a plethysmographic device that allows the animals to be unrestrained and unanesthetized. The advantage of this system is that it alleviates the effects that anesthesia may have on breathing and allows monitoring of body metabolism (O₂ consumption and CO₂ production) during normoxia and hypoxia (23, 30, 46, 47).
However, the disadvantage of this procedure is that it does not permit tracking of blood pressure or arterial gases. To overcome this limitation, \( \dot{V}O_2 \) and \( \dot{V}CO_2 \) were recorded and data were combined with the metabolic values, thus providing an indirect measure of arterial gas status. Considering, for example, that arterial CO2 pressure is proportional to the metabolic rate and inversely proportional to ventilation (32), our data suggest that normoxic and hypoxic arterial blood gases were similar between corresponding male and female mice groups. The hyperoxic test, according to Dejours (10), was performed in urethane (1.2 g/kg) anesthetized mice. As such, the movement of the animals was avoided when they were rapidly

Fig. 4. In vivo tyrosine hydroxylase (TH) activity in catecholaminergic groups in the brain stem (A1C1, A2C2 in the medulla oblongata; A5 and A6 in the pons) determined by HPLC in WT and Paskin\(^{-/-}\) mice. TH activity is expressed as picomoles of DOPA formed in 20 min following blockage of DOPA decarboxylase. *\( P < 0.05 \). \( n = 9–11 \) per group.

Fig. 5. Ventilatory responses in normoxia and acute hypoxia upon intraperitoneal injection of domperidone in WT and Paskin\(^{-/-}\) mice. Baseline ventilation was evaluated after 1–2 h after intraperitoneal 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 8% \( O_2 \) (over 15 min). HVR was evaluated during 20 min at 10% and at 8% \( O_2 \). Control animals were injected with an equal volume of 0.9% NaCl. *\( P < 0.05 \). \( n = 8–9 \) per group.
exposed to 100% O2. As urethane is a toxic compound (31), a different set of animals (n = 8–9 per group) was used for performing this experiment. Note that, because of its minimal effect on respiratory frequency and cardiac dynamics, urethane is commonly used in experiments concerning the respiratory response (4, 19, 23). In addition, the use of urethane does not alter the acid-base status in experimental animals (7, 24).

The role of PASKIN in male mice. We observed that baseline ventilation, ventilatory response to acute hypoxia (HVR), and acclimatization to chronic hypoxia (VAH) were only slightly different between WT and Paskin−/− male mice. Compared with WT mice, Paskin−/− males had greater ventilation in the first minute following 8% hypoxic response, both upon acute hypoxia (Fig. 1A) and after chronic hypoxia (Fig. 1D). Because this difference was a consequence of increased fR and VT under acute hypoxia (Fig. 1, B and C), only fR was increased after chronic hypoxia (Fig. 1, E and F). Although changes in body temperature and variations on VO2 and VCO2 can account for the alteration of hypoxic ventilation (33), we found that metabolic variables do not contribute to the observed alterations in the ventilatory drive.

In addition to the cells controlling respiratory rhythmogenesis in the brain stem, it is known that the neighboring catecholaminergic groups (ponsine and medullary) are able to adjust the stimulatory input to the brain stem (5). In keeping with this, it was found that catecholaminergic cells are potent modulators of ventilation in hypoxia (15, 48, 49). Lower catecholamine content in A6 and A5 decreases fR (12, 15), and lower catecholamine content in A2C2 is associated with augmentation of fR (9). In the current study, Paskin−/− males had significantly decreased TH activity in A6, A5, and A2C2 compared with WT control mice (fig. 4A), but no corresponding changes in ventilation. Together, these results suggest that catecholaminergic metabolism in Paskin−/− mice is decreased in specific central respiratory areas but may be simultaneously compensated in other brain stem areas. Currently, we are not able to discriminate which specific areas are affected by PASKIN protein.

In parallel to central regulation, carotid bodies (the main organs sensing arterial decrease of oxygen pressure and mediating the integrated cardiorespiratory responses to hypoxemia) can also alter the ventilatory responses to hypoxia. However, our studies of the dopaminergic activity (by injection of domperidone) and chemosensitivity (Dejours test) in glomus cells showed no differences between male WT and Paskin−/− carotid bodies. In accordance with these findings, it has been reported that PASKIN mRNA levels were detected in several tissues, including brain (6, 16), but undetectable in carotid bodies (51).

Note that hypoxic ventilation has a biphasic response. The hypoxic ventilation response is classically shaped by an initial ventilatory peak followed by a ventilatory decline, which is termed hypoxic ventilatory decline (HVD) (13). The normalization of the HVR data to baseline measurements (Fig. 2A) showed that at 8% of acute hypoxia, Paskin−/− HVD was slower than the corresponding WT male controls. HVD, however, was similar in both groups after chronic hypoxia (Fig. 2B). The HVD component of hypoxic ventilation is not fully understood, however, it is postulated to represent a peripheral chemoreceptor desensitization and/or the expression/repression of other centrally mediated mechanisms (2, 50). Interestingly, it was shown previously that the kinetics of the HVD can be centrally altered by the inhibition of γ-glutamyl transpeptidase, which is a critical precursor of S-nitrosoglutathione in brain stem (28). As we did not observe any differences between male WT and Paskin−/− carotid bodies, our results suggest that following a short period of sustained acute hypoxia, HVD is altered centrally, and that PASKIN is involved in the modulation of HVD.

On the other hand, it is relevant to mention that despite the fact that PASKIN is ubiquitously expressed, it is markedly upregulated in postmeiotic germ cells during spermatogenesis. Because critical testosterone secretion in male rodents occurs during the period restricted to the late fetal and early postnatal life, the early testosterone surge may be altered in Paskin−/− mice. Perinatal testosterone secretion acts as a masculinizing factor for male sexual behavior, thus influencing the development of related brain and spinal nuclei (29, 44). Moreover, the alteration of perinatal testosterone secretion inhibits the hypercapnic ventilatory response in infant primates (14). Furthermore, the neonatal testosterone surge is involved in the gender differentiation of resting minute ventilation in prepubertal rats under chronic hypoxic exposure (20, 21). However, previous studies reported no differences of Paskin−/− mice regarding fertility, sperm production, reproduction, and development (6, 22, 51). These observations suggest that the perinatal secretion of testosterone in Paskin−/− mice is similar to WT controls, and in consequence, the HVR should not have been altered by this hormone. Indeed, our results show that in male mice, PASKIN induces only small differences in ventilatory re-
responses to hypoxia, implying that PASKIN is not critical in the regulation of the male ventilatory response to hypoxia.

The role of PASKIN in female mice. Pathophysiological response to hypoxia is far more prevalent in males than females, as evidenced by sudden infant death syndrome, sleep apneas, and acute and chronic altitude illnesses. As such, knowing that the hypoxic ventilatory responses have been reported to be gender dependent (20, 21, 36), we also evaluated the minute ventilation and hypoxic ventilatory responses in WT and Paskin−/− female mice. In contrast to males, female animals showed large ventilatory differences in response to acute and chronic hypoxia. When hypoxia was applied acutely, Paskin−/− female mice significantly increased HVR compared with WT controls, via elevated fR and VT (Fig. 1, G–I). Similar to the observation in males, body temperature, VO₂, and VCO₂ were comparable between WT and Paskin−/− female mice. Considering that VO₂ and VCO₂ reflect the levels of arterial blood gases [e.g., the arterial gas pressure is proportional to the metabolic rate, and inversely proportional to minute ventilation, as described by the equation of alveolar gas (32)], we assume that arterial gases were similar between both mouse groups. Taken together, these results suggest that PASKIN regulates ventilation by a direct interaction with centers located in the neural respiratory network. In line with this hypothesis, we found that Paskin−/− females showed significant alteration in the central regulation of catecholaminergic metabolism in brain stem (Fig. 3B). Moreover, as peripheral regulation via carotid bodies was similar in both WT and Paskin−/− lines, it is tempting to suggest that PASKIN protein in female mice modulates, apart from central catecholaminergic groups, other respiratory cells in the rostroventrolateral medulla. Since PASKIN mRNA levels were detected in brain (6, 17), a detailed investigation of PASKIN expression in brain stem respiratory centers is planned.

When acute HVR was normalized to baseline measurements, considerable alterations were observed in the kinetics of HVD. The Paskin−/− HVD was significantly slower than that in corresponding WT mice. HVR had returned to baseline in WT mice within 20 min of hypoxic exposure, yet HVR had only declined by ∼50% in Paskin−/− females (Fig. 2C), similar to male Paskin−/− mice. There were no physiological differences between WT and Paskin−/− carotid body responses, suggesting that PASKIN is involved in the modulation of HVR and that HVD-PASKIN modulation occurs at the central rather than the peripheral level.

Although Paskin−/− female animals had higher acute HVR (Fig. 1, G–I), they were unable to attain ventilatory acclimatization to 3-day chronic hypoxia (VAH; Fig. 1, J–L), WT, but not Paskin−/−, mice showed a large increase in normoxic ventilation after chronic exposure to hypoxia, which is a hallmark of VAH (38), due to increased VT rather than fR. Similar ventilatory patterns were reported in the hypoxic acclimatization of humans (11) and rats (1). Furthermore, HVR (absolute value) subsequent to chronic hypoxia was markedly augmented in WT but not in Paskin−/− female mice, a finding that is also consistent with earlier reports on mice (23, 45) and other experimental animals (38). Likewise, note that when the HVR values were normalized to baseline measurements (Fig. 2D), the percent change in HVR was similar between WT and Paskin−/− female mice. Thus, despite attenuated VAH during chronic hypoxia in Paskin−/− female mice, the magnitude of HVR response was similar in Paskin−/− and WT mice. Because the initial increase of hypoxic ventilation depends on the activity of the peripheral chemoreceptors (23), these data suggest that carotid body response to chronic hypoxia in Paskin−/− animals was not different from WT animals. However, in contrast to our observations during acute hypoxia, after chronic hypoxia, HVD was similar between WT and Paskin−/− mice.

In addition, because the metabolic parameters did not differ after chronic hypoxia (Fig. 2, J–L), metabolically mediated alterations in these variables do not account for the lack of ventilatory acclimatization in mutant mice. Also, because carotid body chemosensitivity was similar between WT and Paskin−/− lines, it is unlikely that the peripheral chemosensors account for the blunted ventilatory acclimatization. Alternatively, impaired processing of central regulation may also depress the respiratory motor output during the acclimatization. In this sense, the alteration in brain stem catecholaminergic metabolism mentioned above suggests that the attenuated VAH results from central rather than peripheral respiratory centers.

PASKIN and sexual dimorphism. The primary function of the respiratory system is to regulate lung ventilation and ensure adequate blood gas homeostasis. Accordingly, numerous factors are able to modulate the respiratory motor output and adapt its activity to the different states and functions of the organism. Hormonal factors, classically involved in endocrine regulation, also participate in the modulation of the neural respiratory control, either indirectly (i.e., changes in metabolic rate) or through a direct action on one or various cellular components of the respiratory controller network. As such, sex hormones strongly participate in setting up the gender-specific differences, which are especially evident in hypoxic respiratory physiology (20). As mentioned above, PASKIN is ubiquitously expressed at rather low levels (17), however, surprisingly, very high levels of PASKIN expression are detected in mouse testis during spermatogenesis (22). With no evidence of higher testosterone concentrations in these animals, PASKIN expression and function are more likely directly linked to the gender-related physiology, via interaction with sex-dependent gene expression and/or gonadal hormones. This interaction may explain, at least in part, the gender differences that we observed in the present study. In addition, it was previously reported that PASKIN in yeast controls and connects the balance of fuel consumption/storage to protein synthesis (41). The acute hypoxic response also includes a quick displacement of fuel for promoting a sudden increase in ventilation, which may be part of the explanation for the higher HVR observed in female Paskin−/− mice. Moreover, the studies of PASKIN in yeast evidenced that under stress conditions (nutrient restriction combined with temperature), PASKIN kinase activity results in downregulation of protein synthesis and carbohydrate storage (41). As such, it is tempting to speculate that under hypoxic stress, the absence of PASKIN in Paskin−/− mice helps, at least the female gender, to have an elevated response to acute hypoxia. However, the cost of the energetic imbalance probably attenuated the capacity of Paskin−/− females to fully achieve the ventilatory acclimatization to hypoxia. In summary, it is clear that more experimentation is needed in female
Paskin−/− gender, to better understand the relationship of PASKIN with sex hormones and its impact on gender-dependent physiological processes such as ventilation.

Central and peripheral oxygen sensors control ventilation in a gender-dependent manner. Acute and chronic hypoxia produce elevations in ventilation that are variably due to elevations of tidal volume (VT) and frequency of respiration (fR). Our data indicate that in female mice, PASKIN centrally mediates increased ventilation in response to acute hypoxia via VT and fR increased basal ventilation and via VT in response to chronic hypoxia. Some of PASKIN’s centrally mediated effects are associated with impaired catecholaminergic signaling in pontine and medullary respiratory centers. However, PASKIN has little effect on hypoxic ventilatory responses in male mice. Thus, PASKIN centrally mediates hypoxic ventilatory responses in a gender-dependent manner.

**Perspectives and Significance**

A gender-specific component is recognized as an important part of the etiology of hypoxia-mediated ventilatory diseases. Such conditions include sudden infant death syndrome, acute and chronic mountain sickness, and high-altitude pulmonary edema and sleep apneas, which occur predominantly in men and in postmenopausal women (3, 20, 27). The etiology of these diseases is not known, and it would be of great value to define the pathways involved in the gender-dependent ventilatory processes. PASKIN is the mammalian homolog of FixL, the oxygen sensor molecule of *Rizobium* species. In the present work, we show for the first time that PASKIN is implicated in the neural regulation of the hypoxic ventilatory response. Using WT and Paskin−/− mice, we have determined that PASKIN plays a greater role in female rather than male ventilation, primarily via modulating central control of breathing.

**ACKNOWLEDGMENTS**

The authors thank Stephan Keller for technical help, as well as R. H. Wenger for discussion and proofreading the manuscript.

**GRANTS**

The present study was supported by grants from the Forschungskredit der Universität Zürich (to J. Soliz) as well as by the Swiss National Foundation and the European Union projects, Pulmotension and EUROXY (to M. Gassmann).

**REFERENCES**


