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Gender differentiation of the chemoreflex during growth at high altitude: functional and neurochemical studies

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Joseph, V., J. Soliz, J. Pequignot, B. Semporé, J. M. Cottet-Emard, Y. Dalmaz, R. Favier, H. Spielvogel, and J. M. Pequignot. Gender differentiation of the chemoreflex during growth at high altitude: functional and neurochemical studies. *Am J Physiol Regulatory Integrative Comp Physiol* 278: R806–R816, 2000.—The effect of chronic hypoxia on gender differences in physiology and neurochemistry of chemosensory pathways was studied in prepubertal and adult rats living at sea level (SL; Lyon, France) or at high altitude (HA; La Paz, Bolivia, 3,600 m). HA adult rats had higher hematocrit (Ht%), Hb concentration, resting ventilatory rate (Ve_{100}), and higher tyrosine hydroxylase (TH) activity in carotid bodies (CB) than SL animals. At HA and SL, adult females had lower Ht% (46.0 ± 0.8 vs. 50.4 ± 0.6% at HA, $P < 0.05$ and 43.8 ± 0.9 vs. 47.1 ± 0.8% at SL, $P < 0.05$) and Hb (16.1 ± 0.3 vs. 17.7 ± 0.2 g/dl at HA, $P < 0.05$ and 14.5 ± 0.3 vs. 15.6 ± 0.1 g/dl at SL, $P < 0.05$) than males. Females had higher Ve_{100} [170 ± 19 vs. 109 ± 7 $ml \cdot min^{-1} \cdot 100\ g^{-1}$ at HA, $P < 0.05$ and 50 ± 3 vs. 40 ± 2 $ml \cdot min^{-1} \cdot 100\ g^{-1}$ at SL, not significant (NS)] and lower CB-TH activity (1.40 ± 0.2 vs. 3.87 ± 0.6 pmol/20 min at HA, $P < 0.05$ and 0.52 ± 0.1 vs. 0.68 ± 0.1 pmol/20 min at SL; NS) than males at HA only. The onset of hypoxic ventilatory response during development was delayed at HA. Prepubertal HA females had higher Ve_{100} than males (2 wk old, +47%) and higher CB-TH activity (3 wk old, +51%). Medullary noradrenergic groups were sex dimorphic during development at SL. Rats raised at HA had a drop of TH activity between the second and the third postnatal week in all medullary groups. In conclusion, our data support the hypothesis that the CB is the major site for sexual differentiation of the ventilatory control. Ventilatory differences appeared before puberty, and the animals bred at HA had profound alterations in the developmental process of the chemoreflex and its neural pathways. Some of these alterations are under dependence of the sex of the animal, and there is an important interaction between gender and the hypoxic environmental condition during the developmental period.

development; carotid bodies; brain stem noradrenergic neurons

GENDER-RELATED DIFFERENCES in the physiological responses to hypoxia have been reported by several studies that demonstrated a better capacity of women and female rats to adapt to hypoxia (23, 30). This observation is of particular importance in pathophysiology because women are less susceptible to a number of hypoxia-associated syndromes both in their infancy and at adulthood. For example, sudden infant death syndrome is more frequent in boys than in girls (25) and at adulthood, sleep apneas are generally restricted to men (8).

Classic symptoms of chronic mountain sickness include a life-threatening elevation of hematocrit (Ht%), associated with hypoventilation and low arterial oxygen saturation, and occur almost exclusively in adult male subjects living at high altitude and in postmenopausal women (24). Because progesterone and estrogen are potent ventilatory stimulants and have been successfully used to reduce excessive erythrocytosis induced by hypoxia both in humans (24) and rats (13), it has been suggested that these hormones improve the oxygen supply to the erythropoietin-synthesizing renal cells and thus reduce the hypoxic hematopoiesis. Supporting this proposal is the evidence showing that exogenous administrated ovarian hormones enhance phrenic nerve discharge and the hypoxic ventilatory responsiveness (HVR) through an action on central site located in the hypothalamus (3), whereas endogenous hormones stimulate the chemoreflex drive through a direct action on peripheral arterial chemoreceptors (33). Recent findings from our laboratory provided further evidence supporting these views by showing that both gender and sex steroids may control the catecholamine activity in carotid bodies (CB) and discrete brain stem noradrenergic areas involved in the chemoafferent pathway and cardiorespiratory control, thus providing a neurochemical basis for sex-related differences in acclimatization to hypoxia (13, 30). Catecholaminergic brain stem areas implicated in ventilatory control and cardiorespiratory responses to hypoxia accumulate tyrosine hydroxylase (TH), the rate-limiting enzyme in catecholamine biosynthesis, mRNA, and protein in response to long-term hypoxia (11). Hypoxia also stimulates dopamine production and enhances TH mRNA expression in the CB type I cells, which

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monitor the reduced O_2 tension and elicit the associated cardiorespiratory responses (10).

In a recent study, Mortola and Saiki (26) showed that prepubertal females raised at sea level (SL) had a higher hypoxic ventilatory response than males. Sustained neonatal hypoxia is known to alter the maturation of chemoreceptor pathway (12) and to produce long-term effects on respiratory functions (29). Accordingly, our hypothesis was that the sexual dimorphism of physiological responses to hypoxia may appear well before puberty and can be influenced by chronic hypoxic exposure during the maturational phase of the chemoreflex pathway. To test this hypothesis, the time course of developmental changes in chemoreflex activity was studied from 1 wk of age to adulthood in male and female rats that were exposed either to normoxia or to hypoxia throughout their lives. The chemoreflex activity was assessed by measuring the HVR and the in vivo TH activity in the CB and noradrenergic brain stem cell groups involved in the cardiorespiratory responses to hypoxia.

MATERIALS AND METHODS

Animals

Sprague-Dawley rats were reared in La Paz, Bolivia, at the altitude of 3,600 m (mean Pb = 495–500 mmHg). The high-altitude (HA) native rats are descendants of a lineage implanted in the Bolivian Institute for High Altitude Biology (IBBA) since 1992 (originally purchased from Iffa-Credo, L'Arbresle, France). Sea level (SL) controls were Sprague-Dawley rats reared in Lyon, France (160 m; mean Pb = 750 mmHg; rats purchased from Iffa-Credo). All animals were housed and bred in similar conditions in France and Bolivia. The animal room was climatized at 24 ± 1°C with a 12:12-h light-dark cycle and allowed free access to standard chow and water. All experiments were carried out according to the ethical principles laid down by the French (Ministry of Agriculture) and European Union Council Directives for care of laboratory animals.

General Experimental Design

To work in similar conditions between the two laboratories, all physiological measurements were carried out in France and Bolivia using similar instruments, all ventilatory measurements were done by the same observer. Neurochemical analyses for CBs and brain stem cell groups (punching procedure and HPLC analysis) were done in France. Samples from Bolivia were shipped in dry ice and arrived in the French laboratory (and stored at −80°C) in <24 h.

Developmental Studies

Mated female rats were checked daily for pregnancy, then separated in individual cages a few days before delivery. Randomized groups of rats from different litters (8–12 pups/litter) were assigned for ventilatory or hematological studies (7–12 rats in each group; see Figs. 1–6 for further details). Rat pups were separated from their mother after postnatal *day 21*. Measurements were done at 1, 2, 3, 4, 5, and 12 wk of age. The same groups of animals were followed at various ages for developmental ventilatory studies, whereas different groups of each age were used for hematological and neurochemical determinations. For adult animals, different groups of rats were used for ventilation and hematological/neurochemical studies.

Hematological Status

Rats were anesthetized by an intraperitoneal injection of pentobarbital sodium [0.6 mg/kg body wt (BW); Sanofi Santé Animale]. Blood samples were drawn by cardiac puncture in anesthetized rats into a heparinized tube. Ht% was measured by a microtechnique method. The Hb concentration was determined by using the Hemocue (Hemocue, AB Ängelholm, Sweden) field spectrophotometer.

Basal Ventilation

Ventilation was measured in awake unrestrained rats using a set of barometric plethysmograph chambers. The chamber volume varied from 0.2 to 5.4 liters according to the age-related BW. The temperature inside the chamber was set around 24°C for adult rats and 28–30°C for rat pups. Once the animal was quiet, the inlet and outlet tubes of the animal chamber were closed and pressure fluctuations related to breathing were recorded with a differential pressure transducer (Celesco, California). The pressure signal was calibrated by injecting an adequate volume of air (from 50 μl to 1 ml according to the chamber volume) into the animal box and by recording the related changes in pressure. Tidal volume (Vt), respiratory frequency (F_r), minute ventilation ($\dot{V}_E$) corrected for BW and Vt-to-BW ratio were calculated from breath-by-breath computer analysis of the spirogram over 30–50 consecutive breaths using standard methods (2). Time periods for collecting the ventilatory data lasted from 30 s to 1 min. During this time, the temperature and CO_2 level within the box remained unaltered. Measurements were performed in triplicate, and no significant differences were found between the first and the last measurement. The mean of these three values was considered the basal level of ventilation.

HVR

After basal ventilation measurement, the inlet tube of the plethysmograph was derived on gas mixtures allowing both hyperoxic or hypoxic flushing of the cage. Equivalent levels of partial pressure of inspired O_2 ($P_{I_{O_2}}$) were used at high and low altitudes. The hyperoxic test was followed by different levels of hypoxic stimulation ($P_{I_{O_2}}$ from 250 to 43 mmHg). During each test, the O_2 percentage within the animal chamber was continuously monitored and set at the desired level. The box was flushed with the test gas for 5 min before measurement of ventilation. According to the mathematical model of HVR, the slope parameter A was calculated by plotting $\dot{V}_E$ vs. $1/P_{I_{O_2}}$. The following equation was obtained: $\dot{V}_E(ml \cdot min^{-1} \cdot 100\ g^{-1}) = V_o + A/P_{I_{O_2}}$; where V_o is the asymptote for ventilation and A determines the slope of the curve. In this model, a strong HVR is related to a high A coefficient.

Estimation of in Vivo TH Activity

The rate of catecholamine biosynthesis was estimated from in vivo TH activity, estimated by measuring L-dihydroxyphenylalanine (DOPA) accumulation after the inhibition of L-amino acid decarboxylase by 3-hydroxybenzylhydrazine dihydrochloride (NSD) 1015 (Sigma Chemical, St. Louis, MO) (20). NSD 1015 was injected intraperitoneally (75 mg/kg in saline solution) 20 min before death. TH activity was expressed as picomoles DOPA formed per 20 min per pair of central structures or per CB.

Tissue Dissection and Catecholamine Assay

After cardiac puncture for hematological determination, rats were killed by cervical dislocation. The CBs and the brain

were rapidly removed, frozen in liquid nitrogen, and stored at −80°C. CBs were stored in a solution of perchloric acid (0.1 M) and disodic EDTA (1 mg/ml). The brain stem was cut into serial frontal slices 320 μm in thickness for young rats (1–4 wk of age) and 480 μm for adult rats. The noradrenergic cell groups A2, A5, and A6 (locus ceruleus) were punched out according to the dissection procedure described by Soulier et al. (32). To separate the area receiving chemosensory inputs from the area to which barosensory fibers project (17), the A2 cell group was divided into two portions, respectively, caudal ($A2_C$) and rostral ($A2_R$) to the calamus scriptorius.

DOPA was assayed by HPLC coupled with electrochemical detection. The mobile phase consisted in 0.1 M potassium phosphate buffer pH 3.0 containing 0.15 mM disodic EDTA. The flow rate was 0.8 ml/min. DOPA was measured at +0.65 V. The detection limit, calculated by doubling the noise ratios and expressed in term of picomoles of injected amounts, was <0.03 pmol, and the intraassay coefficient was 0.2%.

Statistical Analysis

All the values reported are means ± SE. For statistical comparisons of group means of adult animals, a two-way ANOVA [grouping factors were sex and location (HA or SL)] was used followed by a post hoc test (protected least significant difference of Fisher). For statistical comparisons of group means during growth, a three-way ANOVA [grouping factors were age, sex, and location (HA or SL)] was used followed by a post hoc test (protected least significant difference of Fisher). The level of significance was set at 5%.

RESULTS

Influence of Gender and HA in Adult Rats

The gender-related differences in hematology, ventilation, and catecholamine activity were investigated in 12-wk-old rats reared at low and high altitudes. All physiological parameters studied are shown in Table 1.

Animal Weight

BWs of males and females at HA was lower than SL control animals (Table 1). BWs of SL males was 1.6 times greater than SL females; this difference was not altered in rats raised at HA (Table 1).

Hematological Parameters

Rats raised at HA exhibit higher Ht% and Hb concentration than SL rats (Table 1). In HA males, Ht% and Hb were, respectively, 7% and 13.5% higher than in SL males. In HA females, the increases in Ht% and Hb were, respectively, 5% and 11%. Ht% and Hb concentration were higher in males than in females both in animals living at SL and HA (Table 1). The mean relative differences of Ht% and Hb between males and females were slightly enhanced at HA (9.6% vs. 7.5% and 9.4% vs. 7.6%, respectively).

Resting Basal Ventilation

Rats raised at HA had a higher ventilatory rate than SL rats (+172% in males; +240% in females; Table 1). The basal ventilation rate in HA animals was enhanced by an increase of F_r (+75% in males; +70% in females; Table 1) and Vt/BW ratio (+66% in males; +84% in females; Table 1). A notable increase of Vt was observed in female rats at HA compared with SL rats (+34%) but not in males (Table 1).

Whereas only minor gender-related differences were found in ventilatory rate of SL rats, a strong sexual dimorphism existed at HA (Table 1). Ventilatory rate of SL males and females was not different. SL females had lower Vt values than males (−20%) but higher Vt/BW ratio (+ 32%). HA females had a resting basal ventilation 56% higher than their male counterparts and higher Vt/BW ratio (+47%), whereas Vt was not different between males and females at HA. No differences in F_r values were found between males and females at SL or HA.

Hypoxic Ventilatory Response

The shape parameter *A* was higher in rats raised at HA. SL females had higher HVR than their male counterparts (+39%), whereas no gender-related differences of HVR were found at HA (Table 1).

Catecholaminergic Activity

All the results for neurochemical studies are shown in Tables 2 (CBs) and 3 (brain stem noradrenergic cell groups).

CBs

In rats raised at HA, the stimulating effect of HA on the in vivo TH activity in CBs was sex dependent: the

Table 1. *Physiological parameters of male and female high altitude and sea level adult rats*

	SL		HA	
	M	F	M	F
Weight, g	403 ± 3 (8)	251 ± 4* (6)	265 ± 3† (7)	172 ± 5*† (9)
Hb, g/dl	15.6 ± 0.1 (12)	14.5 ± 0.3* (12)	17.7 ± 0.2† (12)	16.1 ± 0.3*† (11)
Ht%	47.1 ± 0.8 (12)	43.8 ± 0.9* (12)	50.4 ± 0.6† (12)	46.0 ± 0.8*† (11)
Ve_{100}, ml/min/100 g	40 ± 2 (8)	50 ± 3 (6)	109 ± 7† (7)	170 ± 19*† (9)
*F*r, min^{-1}	91.3 ± 6 (8)	88.6 ± 3.4 (6)	160 ± 7† (7)	151 ± 12† (9)
Vt, ml	1.79 ± 0.1 (8)	1.44 ± 0.1* (6)	1.83 ± 0.1 (7)	1.93 ± 0.1† (9)
Vt/BW, ml/kg	4.4 ± 0.3 (8)	5.8 ± 0.5* (6)	7.3 ± 0.2† (7)	10.7 ± 0.9*† (9)
A of HVR	3.1 ± 0.3 (8)	4.3 ± 0.4* (6)	8.9 ± 0.6† (7)	10.2 ± 0.6† (9)

Data are expressed as means ± SE for rats 12 wk of age. M, males; F, females; HA, high altitude; SL, sea level; BW, body weight; Ht%, hematocrit; Ve_{100}, ventilatory rate; *F*r, ventilatory frequency; Vt, tidal volume; Vt/BW, tidal volume to body weight ratio; *A*, shape parameter of hypoxic ventilatory response (HVR). The number of animals used is indicated for each group (*n*). *$P < 0.05$ M vs. F at the same altitude; †$P <$ 0.05 HA vs. SL rats of the same gender.

Table 2. *In vivo TH activity, NE and DA content in the CBs of M and F HA and SL rats*

	TH Activity	NE	DA
SL			
M (12)	0.68 ± 0.1	6.33 ± 0.5	7.24 ± 0.8
F (12)	0.52 ± 0.1	5.50 ± 0.5	3.86 ± 0.4*
HA			
M (12)	3.87 ± 0.6†	155 ± 13†	312 ± 16†
F (11)	1.40 ± 0.2*†	97 ± 10*†	212 ± 12*†

Data are expressed as means ± SE. Tyrosine hydroxylase (TH) activity is expressed as picomoles of L-dihydroxyphenylalanine (DOPA) formed per 20 min per carotid body (CB) after intraperitoneal injection of 3-hydroxybenzylhydrazine dihydrochloride 1015. The number of animals used is indicated for each group (*n*). *$P < 0.05$ M vs. F at the same altitude; †$P < 0.05$ HA vs. SL rats of the same gender.

TH activity of HA females was 2.5 times higher than in SL controls, whereas this factor was equal to 5.6 in males (Table 2). As the TH activity in CBs of SL rats showed no gender-related differences, the sex-dependent effect in HA rats resulted in a higher TH activity in male than in female rats (Table 2).

SL females had a lower CB content of DOPA than SL males; the norepinephrine content and TH activity showed no gender-related differences in SL animals (Table 2).

Noradrenergic Brain Stem Cell Groups

In vivo TH activity showed no hypoxic-dependent variations in the noradrenergic cell groups punched out from the brain stem of adult rats, except for the A6 cell group, which showed a higher TH activity in HA males compared with SL male rats. No gender-related differences appeared at SL. At HA, only the A6 cell group showed a marked gender-related difference of TH activity, males having higher TH activity than females (+38%).

Influence of Gender and HA in Developing Rats

The gender-related differences in hematology, ventilation, and TH activity during development were investigated in rats of various ages (from 1 to 5 wk reared at low and high altitudes).

Table 3. *In vivo TH activity in brain stem catecholaminergic cell groups*

	$A2_c$	$A2_r$	A5	A6
SL				
M (12)	4.23 ± 0.4	4.36 ± 0.6	1.78 ± 0.2	12.0 ± 1.2
F (12)	3.74 ± 0.4	4.33 ± 0.5	1.47 ± 0.2	12.1 ± 1.5
HA				
M (12)	3.84 ± 0.5	3.64 ± 0.4	1.43 ± 0.1	15.2 ± 1.1†
F (11)	3.16 ± 0.4	4.27 ± 0.5	1.38 ± 0.2	11.0 ± 1.3*

Data are expressed as means ± SE. Caudal and rostral subsets of A2 ($A2_c$, $A2_r$), A5, and A6 (locus ceruleus) of M and F HA and SL rats. TH activity is expressed as picomoles of DOPA formed per 20 min per pair of cell groups. The number of animals used is indicated for each group (*n*). *$P < 0.05$ males vs. females at the same altitude; †$P < 0.05$ HA vs. SL rats of the same gender.

BW During Growth

The BW of HA rats was lower than their SL counterparts throughout life. Gender-related differences appeared only in rats after puberty both at SL or HA (Fig. 1). The growth rate was slightly altered in HA rats between the second and the third postnatal week as shown by the semilogarithmic plotting of BW vs. age (Fig. 1).

Hematological Parameters During Growth

The Ht% in SL rats remained low until 3 wk of age then increased between 3 and 4 wk (Fig. 2*A*). In rats living at HA, the elevation of Ht% occurred after the second postnatal week, then Ht% increased up to the fourth postnatal week, being higher than in SL controls from 3 wk to adulthood. Around puberty (5-wk-old animals), Ht% was higher in HA males than in females.

The evolution of Hb concentration was similar in HA and SL rats. It remained low until 3 wk then increased abruptly between the third and the fifth postnatal

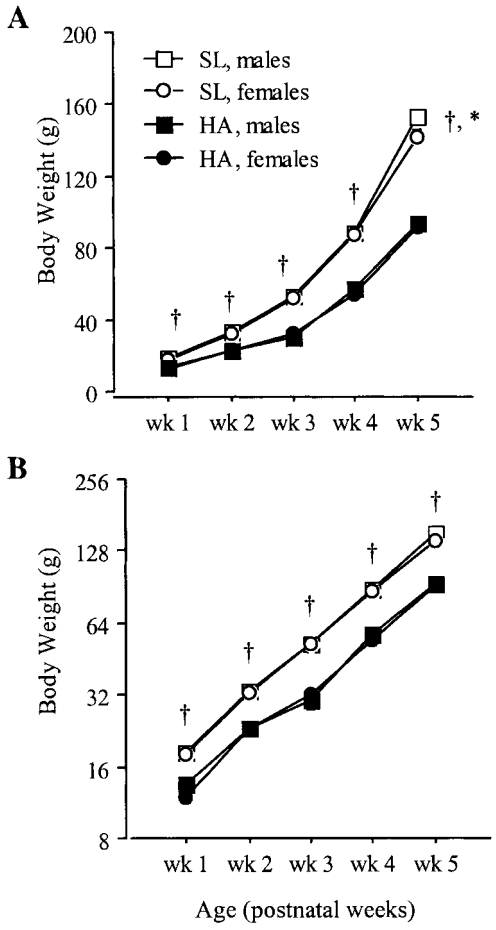

Fig. 1. Evolution of body weight in male and female high altitude (HA) and sea level (SL) rats during growth. Body weights (g) are expressed as means ± SE as a function of age (wk). *A*: linear plot. *B*: semilogarithmic plot. *$P < 0.05$ males vs. females at same altitude. †$P < 0.05$ HA vs. SL rats for both male and female rats. Number of animals used (males to females) at SL for each age (*weeks 1–5*), respectively, is 7:7, 9:7, 8:8, 6:6, and 8:8 and 7:7, 7:7, 7:8, 7:7, and 10:8, respectively, at HA.

week. After puberty (5 wk), Hb was higher in HA males than in females.

Resting Basal Ventilation During Growth

The ventilatory rate of 1-wk-old rats was similar in all groups. Then a striking elevation of ventilation appeared and reached a peak in 2-wk-old HA rats and decreased rapidly between the second and fourth postnatal weeks (Fig. 3). In SL control rats, ventilation rate remained constant between 1 and 3 wk of age, then slightly decreased from 3 wk to adulthood. A strong sexual dimorphism appeared in HA rats both before and after puberty: ventilation rate of 2-wk-old females was 47% higher than in the male counterparts; later (at 3, 4, and 5 wk of age), this difference was abolished, then reappeared in adult rats (Fig. 3). No gender-related differences appeared in SL controls. The gender-related ventilatory differences observed in prepubertal HA rats was due to a differential control of Vt and ventilatory frequency, Vt/BW ratio was higher in females than males at 2 wk of age (17.1 ± 0.4 vs. 13.7 ± 0.9 ml/kg; $P < 0.05$). F_r was also higher in females than males (218 ± 7 vs. 187 ± 13 breaths/min; $P < 0.05$).

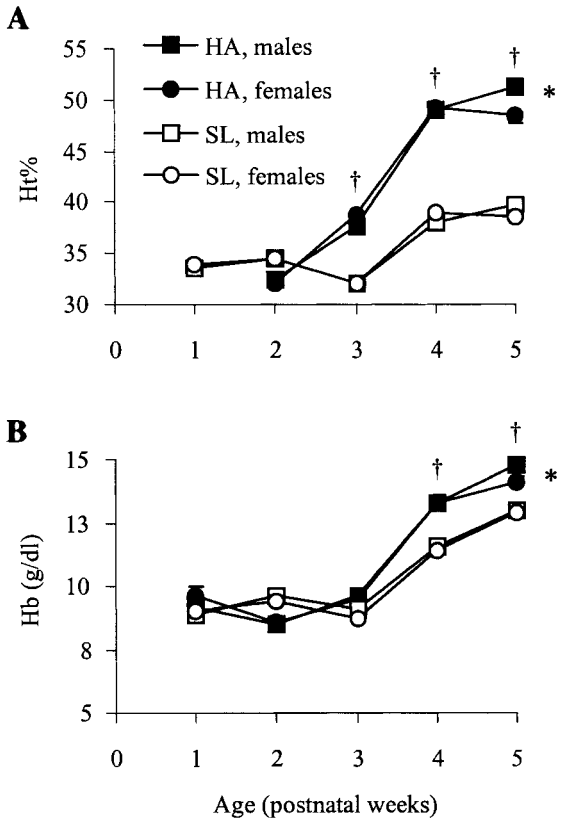

Fig. 2. Evolution of hematological factors in male and female HA and SL rats during growth. Hb concentration (g/dl) and hematocrit (Ht%) as a function of age (wk). Results are expressed as means ± SE. *$P <$ 0.05 males vs. females at same altitude. †$P < 0.05$ HA vs. SL rats of same gender. Number of animals used (males to females) at SL for each age (*weeks 1–5*), respectively, is 12:12, 12:12, 12:12, 12:12, and 12:12 and 12:12, 10:10, 12:12, 12:12, and 12:12, respectively, at HA.

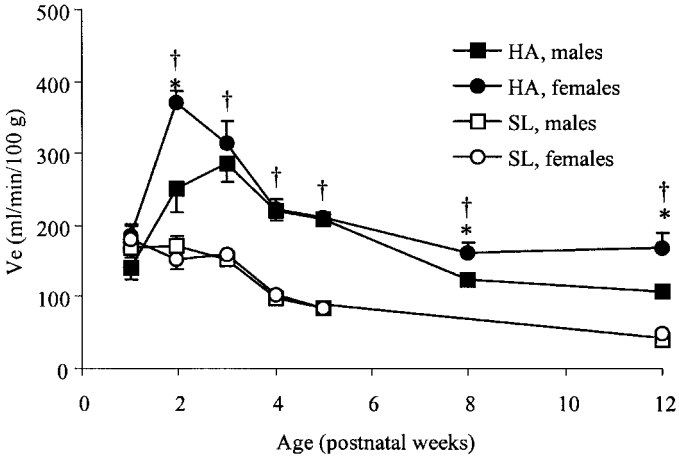

Fig. 3. Evolution of resting ventilatory rate of male and female HA and SL rats during growth. Minute ventilation ($\dot{V}_E$; $ml \cdot min^{-1} \cdot 100\ g^{-1}$) as a function of age (wk). Results are expressed as means ± SE. *$P < 0.05$ males vs. females at same altitude. †$P < 0.05$ HA vs. SL rats of same gender. Number of animals used (males to females) at SL for each age (*weeks 1–5*), respectively, is 7:7, 9:7, 8:8, 6:6, and 8:8 and 7:7, 7:7, 7:8, 7:7, and 10:8, respectively, at HA.

Hypoxic Ventilatory Response During Growth

In SL and HA 1-wk-old rats, the pattern of ventilatory response to hypoxia was characterized by a progressive decline of ventilatory rate after the decline of $P_{I_{O_2}}$ in the animal chamber (Fig. 4*A*); in these conditions, the shape parameter *A* of HVR was not calculated. The classical hyperbolic HVR curve appeared at 2 wk of age in SL, but not in HA rats. At this time, HA rats still showed as a general pattern of HVR, a strong inhibition of Ve after the decrease of $P_{I_{O_2}}$ (Fig. 4*B*). The classical hyperbolic HVR curve appeared at 3 wk of age in HA rats (Fig. 4*C*).

HVR was age dependent: the maximal response was observed between 3 and 4 wk of age and thereafter declined until adulthood (Table 4). Despite a marked tendency of SL females to display a higher HVR since 3 wk of age, this difference reached significant levels only in 5-wk-old SL rats (Table 4).

Catecholamine Metabolism During Growth

CBs. Contrary to what we observed for ventilation and hematological status, the effect of the hypoxic environment on catecholamine metabolism in the CBs appeared in the 1-wk-old rats: TH activity and dopamine content were higher in 1 wk-old HA than SL rats regardless of the gender (Fig. 5).

The TH activity in the CBs showed a gender dependence that can be divided in two distinct phases (Fig. 5): in prepubertal HA rats, an increase in TH activity was observed between the second and the third postnatal weeks, no gender-related differences being observed in 1- and 2-wk-old HA rats. At 3 wk of age, females had a higher TH activity than males. From 3 to 5 wk, the TH activity in the CBs of males increased, whereas it

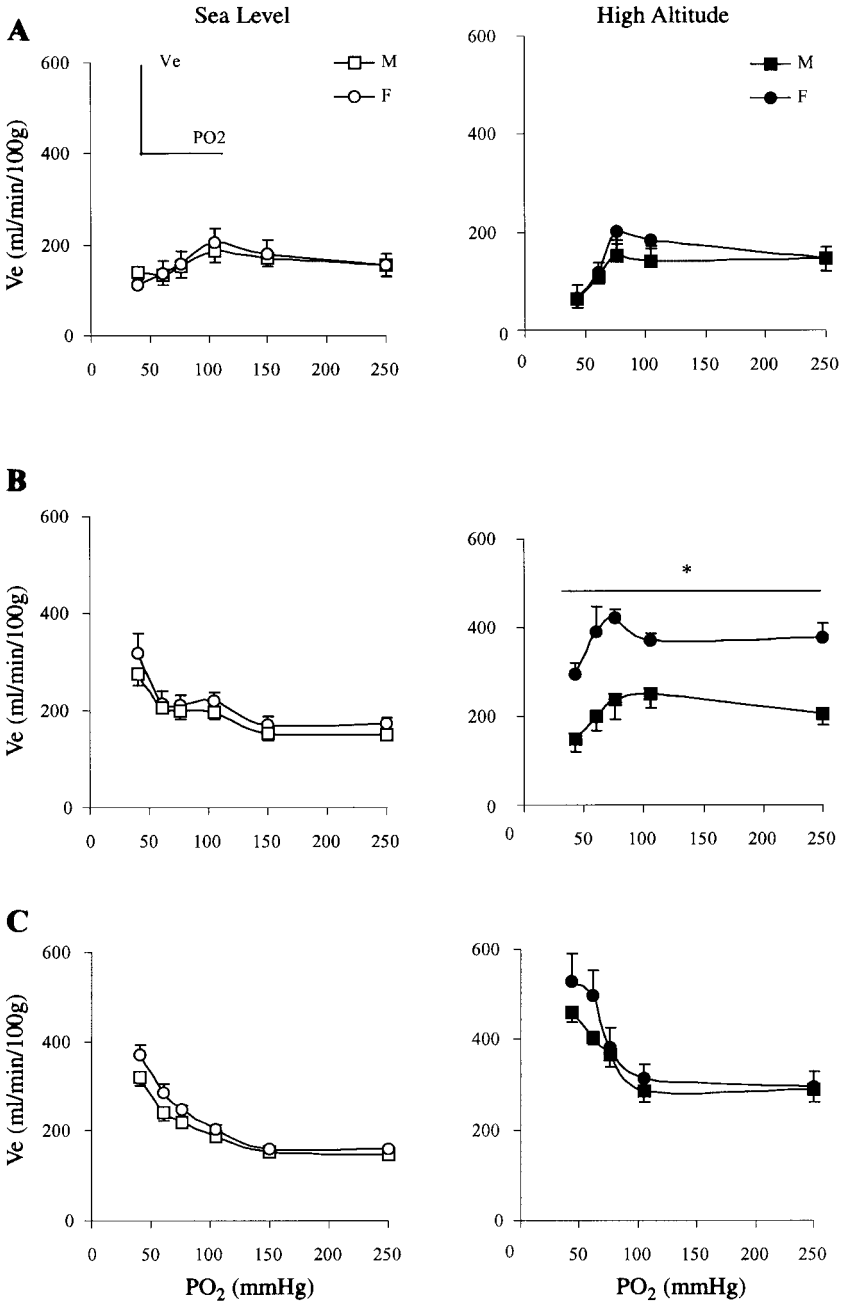

Fig. 4. Hypoxic ventilatory response of male and female, HA and SL rats between first and third postnatal week (*A*: *week 1*; *B*: *week 2*; *C*: *week 3*). $\dot{V}_E$ ($ml \cdot min^{-1} \cdot 100\ g^{-1}$) vs. partial pressure of inspired O_2 ($P_{I_{O_2}}$; mmHg). Results are expressed as means ± SE. In all groups, global effect of $P_{I_{O_2}}$ was significant ($P < 0.05$). *Significant overall effect of gender (males vs. females at every P_{O_2}). Number of animals used (males to females) at SL for each age (*weeks 1–5*), respectively, is 7:7, 9:7, 8:8, 6:6, and 8:8 and 7:7, 7:7, 7:8, 7:7, and 10:8, respectively, at HA.

remained stable in females. As a result of these differences in the maturation process in HA rats, adult males had a higher TH activity in the CBs than their female counterparts (Fig. 5).

TH activity in CBs of SL rats increased during the first 5 postnatal wk and showed no gender-related differences during growth (Fig. 5). There was a rapid increase of catecholamine content in the CB at HA during the first postnatal weeks, 3-wk-old females having higher norepinephrine and dopamine content than males (Fig. 5).

Noradrenergic brain stem cell groups. TH activity was measured in discrete brain stem areas from 2 to 5 wk of age in male and female HA and SL rats. Brain stem cell groups from 1-wk-old rats were not punched out because of the lack of precision and reliability due to the size of the brain.

At HA, all brain stem cell groups had a similar pattern of maturation that included a drop of TH activity between the second and the third postnatal weeks. After this period, a continuous rise of TH activity appeared. In SL rats, a decrease of TH activity between the second and the third postnatal weeks in brain stem noradrenergic neurons was present only in female rats in A5 and A6 cell groups (Fig. 6).

Some gender related differences appeared in $A2_C$, A5, and A6 cell groups during growth, but not in $A2_R$. SL females had lower TH activity than SL males at 3 and 4

Table 4. *Evolution of shape parameter A of HVR ($ml \cdot min^{-1} \cdot 100\ g^{-1} \cdot mmHg \times 10^{-3}$) response in M and F HA and SL rats during growth*

Age, postnatal weeks	SL		HA	
	M	F	M	F
2	6.1 ± 1.6 (9)	5.4 ± 1.2 (7)	—	—
3	8.1 ± 0.7 (8)	9.3 ± 1.0 (8)	9.9 ± 1.3 (7)	11.7 ± 2.2 (8)
4	5.4 ± 1.2 (8)	7.1 ± 0.7 (8)	12.6 ± 1.8† (7)	12.2 ± 2.1† (7)
5	4.0 ± 0.6 (8)	5.3 ± 0.5* (8)	12.2 ± 1.1† (10)	10.2 ± 0.7† (8)

Results are expressed as means ± SE. The number of animals used is indicated for each group (*n*). *$P < 0.05$ males vs. females at the same altitude; †$P < 0.05$ HA vs. SL rats of the same gender.

wk of age and lower TH activity in $A2_C$ than males at 4 wk of age (Fig. 6). At HA, 2-wk-old females had higher TH activity in $A2_C$ than males (Fig. 6).

DISCUSSION

Studies in HA rats were conducted in descendants of a lineage of Sprague-Dawley rats reared in La Paz, Bolivia, at the altitude of 3,600 m (mean Pb = 495–500 mmHg) since 1992. We estimate that during this period, 15–20 generations of rats were bred in La Paz. It may be hypothesized that a natural selection has occurred. As far as we are aware, such breeding conditions of laboratory animals are unique in the field of HA physiology, and the rats that we studied should be considered as a unique altitude population. This idea is supported by the hematological data of our rats: the Ht% from rats that recently arrived in the HA laboratory is generally higher (around 60% 10 wk after arrival) than the Ht% reported in the descendants of the rats that arrived in La Paz in 1992 (present data: Ht% around 50% for adult males at HA).

Physiological acclimatization to HA was evidenced by a strong stimulation of resting ventilatory rate and HVR as well as hematological adjustment. Some of these variables showed a strong sexual dimorphism in prepubertal and/or adult rats at HA. The maturation pattern of catecholaminergic activity in the structures of the chemoreflex pathway, i.e., CB and brain stem cell groups, were strongly modified at HA, and some of these modifications were gender dependent.

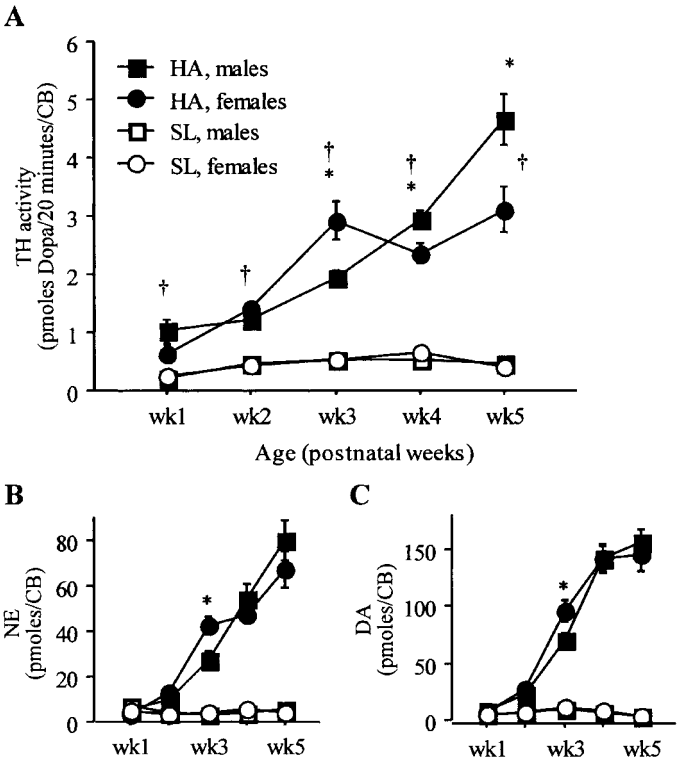

Fig. 5. Evolution of in vivo tyrosine hydroxylase (TH) activity (*A*), norepinephrine (NE; *B*) and dopamine (DA; *C*) in carotid bodies (CB) of male and female HA and SL rats during growth. TH activity is expressed as picomoles of DOPA formed per 20 min per CB. NE and DA content are expressed as picomoles per CB as a function of age (wk). Results are expressed as means ± SE. *$P < 0.05$ males vs. females at same altitude. †$P < 0.05$ HA vs. SL rats for both genders. Number of animals used (males to females) at SL for each age (*weeks 1–5*), respectively, is 12:12, 12:12, 12:12, 12:12, and 12:12 and 12:12, 10:10, 12:12, 12:12, and 12:12, respectively, at HA.

Influence of Gender and HA in Adult Rats

Physiological studies. Acclimatization to HA was different in male and female rats. Some of the sex-related differences observed were shared both by HA and SL animals, whereas others appeared only in rats raised at HA. Females showed reduced Ht% and Hb values compared with males both in the HA and SL groups. In HA females, the lower hematological status was associated with a higher ventilation rate than in males. The higher ventilatory rate of HA females was related to a greater HVR of SL females.

Gender-related differences concerning the hematological status and ventilation in HA populations or animals chronically exposed to hypoxia have already been noted (23, 30). In HA populations, men and postmenopausal women (24) may be subject to chronic mountain sickness. It has been proposed, and it is widely assumed, that progesterone acts as a ventilatory stimulant to protect women from chronic mountain sickness (23, 24) and HA rats from excessive polycythemia (13, 30). This proposition is consistent with the fact that chronic mountain sickness in men or postmenopausal women is generally associated with low arterial oxygen saturation and hypoventilation syndrome (24). In the present work, the hematological differences between males and females found in HA and SL rats were associated with a gender-related difference of resting ventilation at HA, but not at SL. Progesterone acts both on peripheral chemoreceptors (33) and on central site (3) to improve alveolar ventilation and HVR. More recently, it was suggested that in HA rats, ovarian steroids may modulate the catecholamine utilization rate in CBs and discrete brain stem areas implicated in cardiorespiratory control under hypoxia (13, 30). On the other hand, almitrine, another ventilatory stimulant, increases ventilatory rate in HA rats without any effects on hematological status (13). Progesterone is also a repressor of expression of the erythropoietin (Epo) gene (18); thus the mechanism by which progesterone is able to protect females from excessive polycythemia at HA is likely to

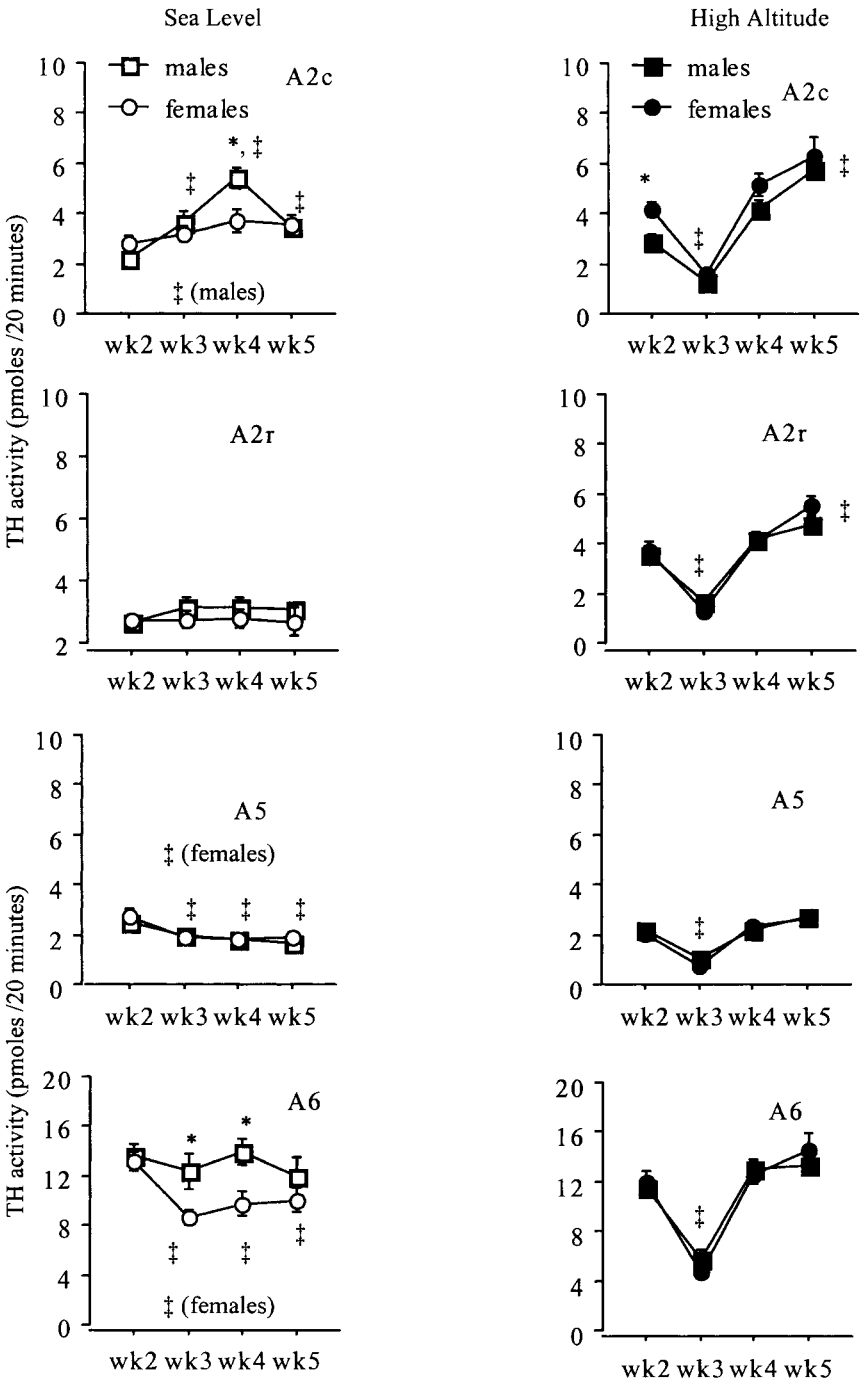

Fig. 6. Evolution of in vivo TH activity (TH activity) in noradrenergic brain stem nucleus A2 (caudal and rostral part), A5 and A6, of male and female SL (*left*) and HA (*right*) rats. TH activity is expressed as picomoles of DOPA formed per 20 min per pair of structure. $*P < 0.05$ males vs. females at same altitude. $‡P < 0.05$ vs. 2-wk-old rats, significant effect for males or females only if indicated. Number of animals used (males to females) at SL for each age (*weeks 1–5*), respectively, is 12:12, 12:12, 12:12, 12:12, and 12:12 and 12:12, 10:10, 12:12, 12:12, and 12:12, respectively, at HA.

implicate a complex interaction between ventilation and control of Epo metabolism.

Neurochemical Studies

General considerations. Chemosensitive type I cells in the CBs play a major role in ventilatory adjustment to hypoxia. The afferents from the CB project to the caudal part of the nucleus tractus solitarius, which is reciprocally connected with pontine areas involved in cardiorespiratory regulations. Each of these brain stem regions contains major clusters of noradrenergic neurons (the A2 cell group in the caudal nucleus tractus solitarius, A5 in the ventrolateral pons, and A6 in the locus ceruleus). The A2 cell group is involved in the control of respiration through its connections with the adjacent dorsal respiratory group. Respiratory neurons of the dorsal respiratory group possess adrenergic receptors, and iontopheric applications of epinephrine, norepinephrine, isoproterenol, or clonidine depress their discharge (6). Furthermore the A2 cell group provides one of the main catecholaminergic innervations of the hypothalamic paraventricular and supraoptic nuclei involved in neuroendocrine regulations (9). A5 is involved in cardiorespiratory and autonomic regulation,

whereas the A6 cell group is the main source of innervation of the cerebral cortex and is involved in general attention, arousal, and learning capacities.

In the CB, dopamine is recognized as the most abundant and potent neuromodulator influencing chemosensitivity. At this level, dopamine released from CB Type I cells under hypoxemic stimulation acts through low-affinity excitatory postsynaptic and high-affinity inhibitor presynaptic D2 receptors (15). Although dopamine is considered as one of the most putative excitatory neurotransmitters in the CB, physiological increase of catecholaminergic activity in CB type I cells has been related to an inhibition of hypoxic ventilatory response, both in acute and chronic hypoxic exposure (15, 20, 34). In the present study, we used TH activity as a direct reflection of catecholamine biosynthesis rate. TH is the rate-limiting enzyme of catecholamine biosynthesis and TH activity is commonly used as an index of neuronal activity. The reliability of this neurochemical technique and of the punching dissection method has been discussed in a previous article (20).

At HA, the CB is hypertrophied and the number of fibroblasts is augmented as is the capillary density. A specific effect of chronic hypoxic exposure has also been described on CB type I cells (4, 10, 15). Thus the enhanced catecholamine content at HA is a direct reflection of the regulation that occurs on glomic cells rather than on the other cellular population (see also Refs. 13, 16, 20, 30).

TH activity and catecholamine content of the CB were higher in HA than in SL rats. The increased catecholamine metabolism in CBs may be partially related to the hyperplasia of type I cells during long-term exposure to hypoxia (4) and, for another part, to the stimulating effect of oxygen deprivation on TH mRNA synthesis and stability (10). Furthermore, a marked sexual dimorphism in HA rats appeared in TH activity and catecholamine content of CBs and was also found to a lesser extent in SL rats. HA female adult rats had a reduced TH activity in CBs compared with males. At SL, the only difference concerned the dopamine content in CBs, which was lower in females than in males.

According to the modulating effects of dopamine in the CBs, we propose that the gender-related differences in basal ventilation rate (HA animals only), Vt/BW ratio (HA and SL animals), and HVR (SL animals only) may have a common neurochemical basis implying gender-related differences in the control of catecholamine synthesis in the CBs. A recent study by Tatsumi et al. (33) showed that the principal action site of endogenous progesterone on HVR was restricted to the CBs. Our data reinforce this finding, showing that the major gender-related differences of catecholamine metabolism in HA and, to a lesser extent, in SL rats, concerned the CBs and not the central noradrenergic areas implicated in the chemoreflex pathways.

The only gender-related differences at sea level or HA in the noradrenergic brain stem cell groups were found in the A6 group of HA rats, males having higher TH activity than females. In a previous article, we reported that gender-related differences of norepinephrine utilization rate appeared in the brain stem cell groups of HA rats (30). In brain stem noradrenergic cell groups, >90% of total TH protein are found in neuronal cell bodies rather than in synaptic nerve endings from neurons that project to the studied group (14), whereas norepinephrine release within each cell group reflects the synaptic activity of axons from neurons located in different regions of the brain stem. Thus the two biochemical markers (TH activity and norepinephrine utilization rate) reflect different levels of control of the catecholaminergic metabolism within a defined area. A gender-related difference of catecholamine metabolism in the brain stem cell groups cannot be excluded, even if we were unable to show it with the DOPA accumulation method, and might participate in the gender-related ventilatory differences. Finally, some mechanical respiratory factors were recently shown to be different between male and female rats at SL (26) and may take part in the gender differences we observed in HVR at SL.

Developmental studies. No gender-related differences appeared in the hematological status in prepubertal rats. This observation strongly suggests that a fully functional secretion state of gonadal hormones is necessary to set up the differences observed at adulthood.

On the other hand, an important sexual dimorphism appeared before puberty both in ventilatory control and catecholamine metabolism in the chemoreflex pathway. HA females showed higher ventilation rate, Vt, and TH activity in $A2_C$ than males at 2 wk of age and higher TH activity in CBs at 3 wk of age. These differences were triggered by HA exposure and were undetectable in SL rats. Recently, Mortola and Saiki (26) reported that prepubertal female rats had higher HVR (corrected for O_2 consumption) than their male counterparts. In our study, SL prepubertal rats showed no gender-related differences of HVR. The reasons for this discrepancy may lay in the differences between our respective protocols [single stimulation at 10% O_2 for 10–15 min and correction of $\dot{V}_E$ by $\dot{V}_{O_2}$ (26); gradual stimulation at various $F_{I_{O_2}}$ without correction for $\dot{V}_{O_2}$ (present work)]. Nevertheless, the fact that in our study, the 2-wk-old HA females had greater resting ventilatory rate than males may be due to a higher hypoxic sensitivity of prepubertal females that we were unable to detect with our protocol.

At birth, there is a sudden rise in arterial oxygen pressure, the chemoreceptors become silent, and the chemoreflex, which is active in fetal lambs (7), is weak or absent at birth in lambs and rats (7, 16). Then, there is a gradual resetting of the CB chemosensitivity to the postnatal blood gas status, the adultlike response to hypoxia being observed after the second postnatal week in rats (12, 21), lambs, cats, and dogs (see Ref. 21). The arterial O_2 pressure is a key element in the sequence leading to the resetting of the CB as chronic hypoxia from birth delays the postnatal resetting of the chemoreflex (12). Our results are consistent with this finding, showing that ventilatory rate of the 1-wk-old HA rats did not differ from SL animals and that the onset of HVR was delayed in HA rats. Furthermore, in this

study, some gender-related differences in the biochemical and functional activity of the chemoreflex pathway appeared before puberty in HA rats.

The maturation pattern of TH activity in brain stem noradrenergic neurons was profoundly altered in rats raised at HA. The main alteration was a marked reduction of TH activity in young rats between the second and the third postnatal week, followed by a rise of TH activity that reached a higher level in HA rats than in their SL counterparts (4 and 5 wk of age). At SL, there was also a drop of TH activity between the second and the third postnatal weeks in the A5 and A6 cell groups of SL females only. Such an alteration in TH expression during growth, in terms of TH protein amount and total number of TH expressing cell bodies in the locus ceruleus (A6 cell group) of young male rats raised at SL, has been reported to occur around the third postnatal week (5). A decrease of TH activity in hypothalamic neurons was reported to occur between the second and the third postnatal weeks in SL females but not in males (1). Such events may be related to programmed cell death that occurs naturally during postnatal development in the central nervous system and/or to the existence of a population of quiescent cells that would exhibit a TH phenotype only in a determined period during development (5). The present results show that an early gender influence appeared at the biochemical level in the locus ceruleus and other brain stem cell groups and may be associated with changes in the steroid-sensitive cell number during development (31).

At HA, the drop in TH activity at the third postnatal week was generalized, suggesting that chronic hypoxia during development induces profound neuronal alterations in noradrenergic brain stem cell groups regardless of the gender. At birth and up to the second postnatal week, the rat central nervous system relies essentially on anaerobic metabolism. Within this period, anaerobic glycolysis plays a prominent role in neural metabolic reactions to hypoxia and neurons have higher resistance to hypoxic/ischemic injury (28). Thereafter, there is a metabolic shift toward the adult aerobic pattern. In response to acute hypoxia during this critical period, there is a severe decrease of brain ATP, which is more pronounced than in the preceding or following postnatal periods (19). Thus we hypothesize that in chronic hypoxia, there is a sudden neural metabolic challenge that is likely to interfere with the synthesis and activity of proteins in the developing neurons. During the same postnatal period, we observed a dramatic increase in ventilatory rate and slower growth rate of the HA rats. Even if we cannot assess that this hyperpnea is associated with a higher metabolic demand, we may hypothesize that there is an overall metabolic effect of chronic hypoxia during this sensitive developmental period.

In conclusion, it appears that chronic hypoxic exposure induces profound alterations in the developmental process of the ventilatory chemoreflex and of the chemosensitive neural pathways. Some of these alterations are dependent on the sex of the animal, and there is an important interaction between gender and the hypoxic environmental condition during the developmental period.

Perspectives

Living at HA (>2,500 m) concerns 140,000,000 people around the world (35). The effects of chronic hypoxia in newborns are likely to differ from what is observed in adults as they may influence the differentiation and growing processes of early life.

Recent studies indicate that human babies at HA are submitted to an important drop of arterial oxygen saturation after birth; subacute infantile mountain sickness may occur in such conditions [pulmonary hypertension and right-heart failure (27)]. Sudden Infant Death Syndrome, an unexplained cardiorespiratory arrest of the newborn, is more frequent in boys than girls (25) and has been found to be enhanced at HA (22). Thus our results are likely to be of importance and may highlight the fact that gender differences in physiological responses to HA appear not only in adult mammals but also in prepubertal animals. A broad range of HA-linked pathological disorders may be related to such early differentiation.

From a fundamental point of view, our results may confer a neurochemical basis to the statement made by Nyakas that "in the developing neurons, the energy for structural differentiation processes, such as axon and dendrite elongation or synapse formation, is probably higher compared with what is necessary for synaptic signal transmission" and that "hypoxia, especially of a chronic type, might interfere with the augmented synthesis of structural proteins and other macromolecules" (28). The drop of TH activity in the brain stem that appeared in this sensible period may be a reflection of such alterations. If future studies are able to confirm this hypothesis, our knowledge of physiological reactions to hypoxia of the newborn should be greatly enhanced.

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