Dopaminergic metabolism in carotid bodies and high-altitude acclimatization in female rats

VINCENT JOSEPH,[1] JORGE SOLIZ,[2] RUDDY SORIA,[3] JACQUELINE PEQUIGNOT,[4]
ROLAND FAVIER,[4] HILDE SPIELVOGEL,[3] AND JEAN MARC PEQUIGNOT[4]
[1]*Centre d'Étude des Rythmes Biologiques, Université Libre de Bruxelles-Hôpital Erasme,*
B-1070 Brussels, Belgium; [2]*Physiologisches Institut, Universität Zürich-Irchel,*
CH-8057 Zurich, Switzerland; [3]*Instituto Boliviano de Biología de Altura, Embajada de Francia,*
La Paz, Bolivia; and [4]*Laboratoire de Physiologie des Régulations Energétiques, Cellulaires*
et Moléculaires, Centre National de la Recherche Scientifique et Faculté de Médecine,
Université Claude Bernard, Unité Mixte de Recherche 5123, F-69373 Lyon, France

Joseph, Vincent, Jorge Soliz, Ruddy Soria, Jacqueline Pequignot, Roland Favier, Hilde Spielvogel, and Jean Marc Pequignot. Dopaminergic metabolism in carotid bodies and high-altitude acclimatization in female rats. *Am J Physiol Regulatory Integrative Comp Physiol* 282: R765–R773, 2002; 10.1152/ajpregu.00398.2001.—We tested the hypothesis that ovarian steroids stimulate breathing through a dopaminergic mechanism in the carotid bodies. In ovariectomized female rats raised at sea level, domperidone, a peripheral D_2-receptor antagonist, increased ventilation in normoxia (minute ventilation = +55%) and acute hypoxia (+32%). This effect disappeared after 10 daily injections of ovarian steroids (progesterone + estradiol). At high altitude (3,600 m, Bolivian Institute for High-Altitude Biology-IBBA, La Paz, Bolivia), neutered females had higher carotid body tyrosine hydroxylase activity (the rate-limiting enzyme for catecholamine synthesis: +129%) and dopamine utilization (+150%), lower minute ventilation (−30%) and hypoxic ventilatory response (−57%), and higher hematocrit (+18%) and Hb concentration (+21%) than intact female rats. Consistent signs of arterial pulmonary hypertension (right ventricular hypertrophy) also appeared in ovariectomized females. None of these parameters was affected by gonadectomy in males. Our results show that ovarian steroids stimulate breathing by lowering a peripheral dopaminergic inhibitory drive. This process may partially explain the deacclimatization of postmenopausal women at high altitude.

hypoxia; ovarian steroids; chronic mountain sickness

MONGE'S DISEASE, or chronic mountain sickness (CMS), has been recognized for many years as a major risk for high-altitude natives. Its major features are elevated hematocrit (Hct) and hemoglobin (Hb) concentration, chronic alveolar hypoventilation associated with low arterial O_2 saturation, and low hypoxic ventilatory responsiveness (15, 22). Pulmonary arterial hypertension and right heart hypertrophy are also classically associated with CMS and may ultimately lead to right heart failure. Most of the patients are young to old adult men who have been living for years above 3,000 m of altitude, whereas women are usually protected until they reach menopause. After menopause, women living at high altitude are submitted to a deacclimatization process that includes an increase of blood Hb concentration and Hct and a decrease of arterial O_2 saturation (16, 17).

Most of the human and animal studies carried out to better understand the pathophysiological factors of CMS have focused on the implication of gonadal steroids (17, 20): progesterone associated with estrogens has been found to be effective in the treatment of CMS by stimulating minute ventilation (14). More recently, some studies carried out on cats or rats pointed out the carotid body to be one of the major sites for gender differentiation of ventilatory control under acute or chronic hypoxic stimulation (13, 24), but the underlying mechanisms of this hormonal stimulation remain poorly understood.

Among the various neuromodulators synthesized by glomic cells in the carotid bodies and released under hypoxemic challenge, dopamine (DA) is found at high concentration and has been recognized as a potent inhibitory neuromodulator of carotid body chemotransduction (7, 8, 10, 29).

Our hypothesis is that ovarian steroids can modulate breathing by reducing a dopaminergic drive in the carotid bodies. To test this hypothesis, we performed two complementary studies. First we studied, at sea level, the effect of domperidone in ovariectomized female rats before and after repeated hormonal injections (progesterone + estradiol). Because domperidone is a highly specific peripheral D_2-dopaminergic receptor antagonist and potent ventilatory stimulant drug acting directly on carotid sinus nerve discharge (10, 25,

---

28, 29), we hypothesized that before hormonal treatment domperidone should stimulate resting minute ventilation and/or hypoxic ventilatory response (HVR) and that this influence should be reduced after repeated hormonal injections.

The second part of this study was performed in rats permanently living at high altitude (Bolivian Institute for High-Altitude Biology-IBBA, La Paz, Bolivia; 3,600 m). Under such conditions, there is a marked hyperventilation and stimulation of the catecholaminergic activity in the carotid bodies with a notable gender dimorphism for both variables (13, 24). We hypothesized that endogenous ovarian steroids specifically modulate the dopaminergic metabolism in carotid bodies. To test this hypothesis, we measured the activity of tyrosine hydroxylase (TH), as well as the utilization rate (% of the content used per hour) of norepinephrine (NE) and DA, in the carotid body of intact or neutered female rats. Resting minute ventilation, HVR, Hct, Hb concentration, and right ventricular (RV) hypertrophy, a direct reflection of pulmonary arterial hypertension (21), which are all relevant outcomes for health at high altitude, were also determined in these animals. Male rats were also added in this study to test the gender specificity of the response to gonadectomy.

Our results are consistent with the hypothesis that ovarian steroids stimulate breathing, at least partially, by lowering a peripheral dopaminergic inhibitory drive that is likely to originate from the carotid bodies. The implication of this finding for high-altitude dwellers is discussed.

MATERIALS AND METHODS

Sea-Level Studies

General experimental design. Ovariectomized Sprague-Dawley female rats were purchased from IFFA-CREDO (l'Arbresle, France) and installed under standardized conditions in an animal room for 1 mo (Lyon, France, altitude 150 m). The animal room was climatized at $24 \pm 1°C$ with a 12:12-h light-dark cycle, and animals had free access to standard chow and water. Basal ventilatory measurements (see below) and HVR (10% O_2; 20 min) measurements were made in ovariectomized females after intraperitoneal saline injection. The same animals were used 48 h later to assess the effect of domperidone on resting minute ventilation and HVR (1 mg/kg ip provided by Janssen-Cilag; $n = 9$; domperidone was dissolved in saline solution with 1 equivalent of tartaric acid). Every measurement was taken between 1 and 2 h after the corresponding injection. The females were then treated for 10 consecutive days with progesterone (1 mg/day; Sigma) and 17β-estradiol (0.05 mg/day; Sigma; subcutaneous injection of hormones in sesame oil), and the same procedure was repeated to measure HVR after saline and domperidone injections ($n = 8$). 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.

Basal ventilation and HVR. Ventilation was measured in awake unrestrained rats using a barometric plethysmograph chamber. 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, CA). The pressure signal was calibrated by 10 consecutive injections of an adequate volume of air (1 ml) into the animal box and by recording the related changes in pressure. Tidal volume (V_t; ml), respiratory frequency (F_r; breaths/min), minute ventilation corrected for body weight ($\dot{V}_{E100}$; $ml \cdot min^{-1} \cdot 100 g^{-1}$), and V_t-to-body weight (BW) ratio (V_t/BW; ml/100 g) were calculated from breath-by-breath computer analysis of the spirogram over 30–50 consecutive breaths using standard methods (1). The temperature inside the chamber was set around 24–25°C and permanently checked on a digital thermometer ($\pm0.1°C$) during measurements. Atmospheric pressure for correction of the plethysmographic pressure signal was read at the beginning of each measurement. On the basis of the previous demonstration that changes in body temperature related to gender or hormonal status do not affect HVR or minute ventilation in rats (19), body temperature was considered to be 37°C (1) for all groups. Time periods for collecting the ventilatory data lasted for 30 s to 1 min, and during this time the temperature and CO_2 level within the box remained unaltered. Measurements were performed at least in triplicate, and no significant differences were found between the first and the last measurement. The mean of these values was defined as the basal level of ventilation.

After resting minute ventilation measurement, the inlet tube of the plethysmograph was derived from the hypoxic gas mixture. The O_2 percentage within the animal chamber was rapidly decreased to the desired level (10% O_2 in N_2). Ventilatory measurements were done 20 min after the onset of the hypoxic exposure.

High-Altitude Studies

Animals: general housing conditions and operative procedures. Sprague-Dawley rats were reared in La Paz, Bolivia, at the altitude of 3,600 m [mean barometric pressure (P_b) = 495–500 mmHg]. The high-altitude 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). The animal room was climatized at $24 \pm 1°C$ with a 12:12-h light-dark cycle, and animals had free access to standard chow and water.

Female rats were mated with adult males, left undisturbed for 2 wk, and then checked daily for pregnancy and separated in individual cages a few days before delivery. Gonadectomy was performed under cold anesthesia (induced by total immersion in ice during 4–5 min, one of the best choices for anesthesia of rat pups; Ref. 5a) in 1-wk-old rats. In females, ovaries were removed by a bilateral dorsal incision, and in males testes were removed from the abdominal cavity by a slight incision at the bottom of the abdomen. Postoperative care included a progressive return to ambient temperature that allowed complete recovery within 10–15 min after surgery. Rat pups were left with their mothers until weaning (on postnatal *day 21*), when they were separated according to their status and gender (4–6 animals/cage). Control animals were undisturbed until weaning. All measurements were carried out during the 12th wk of life. Different groups of animals, born to different mothers, were used for noninvasive (ventilatory measurements) and invasive (hematological status, cardiac weight, and neurochemical measurements) studies to avoid possible interactions between the hypoxic challenge done to measure HVR and catecholaminergic metabolism in the carotid bodies. All measures including control and gonadectomized animals were carried out within a period of 2 mo.

Basal ventilation and HVR. Resting minute ventilation was measured as described above. After basal ventilation measurement, the inlet tube of the plethysmograph was derived from the hypoxic gas mixture. The O_2 percentage within the animal chamber was gradually decreased and set at the desired level (12% O_2 in N_2). Ventilatory measurements were taken 10 min after the onset of hypoxic exposure.

Hematological status and cardiac weight. Rats were anesthetized by an intraperitoneal injection of pentobarbital sodium (60 mg/kg body wt; Sanofi Santé Animale). Blood samples were drawn by cardiac puncture into a heparinized tube, Hct (%) was measured by a microtechnique method, and the Hb concentration was determined by using the Hemocue (AB Ångelholm, Sweden) field spectrophotometer. After blood sampling, the chest was opened and the heart was dissected out. The whole heart was rapidly washed in a saline solution and weighed. The atria were separated from the ventricles, the RV was cut off from the left ventricle (LV), and the septum was left as a part of the LV. The RV and the LV + septum were immediately weighed.

Estimation of in vivo catecholaminergic activity in the carotid bodies. The rate of catecholamine biosynthesis was assessed from in vivo TH activity, estimated by measuring L-DOPA accumulation after the inhibition of L-amino acid decarboxylase by 3-hydroxybenzylhydrazine dihydrochloride (NSD-1015; Sigma; Ref. 5). NSD-1015 was injected intraperitoneally (75 mg/kg in saline solution) 20 min before the animal was killed as already described (12, 13).

After cardiac puncture for hematological determinations and cardiac dissection, the carotid bodies were rapidly removed in a solution of perchloric acid (0.1 M) and disodium EDTA (1 mg/ml), frozen in liquid nitrogen, and stored at −80°C. Further analyses were done in France (Lyon). Samples from Bolivia were shipped in dry ice and arrived in the French laboratory (and stored at −80°C) in 24 h. L-DOPA, DA, and NE were assayed by high-performance liquid chromatography coupled with electrochemical detection. The mobile phase consisted of 0.1 M potassium phosphate buffer, pH 3.0, containing 0.15 mM disodium EDTA. The flow rate was 0.8 ml/min. L-DOPA was measured at +0.65 V. The detection limit, calculated by doubling the noise ratio and expressed in terms of picomoles of injected amounts, was <0.03 pmol, and the intra-assay coefficient was 0.2%.

The TH activity was expressed as picomoles of L-DOPA formed per 20 min per carotid body. This assay has been recognized as a valuable tool to assess catecholaminergic activity in the carotid body because the minute size of this organ does not allow one to measure total protein content. An index of total catecholamine synthesis to total, or specific, catecholamine content of the structure was calculated by expressing catecholamine synthesis per hour (TH activity/20 min × 3) and by dividing it by the total amount of catecholamine present in the structure (DA + NE) or by the amount of the specific catecholamine (DA or NE). Results are expressed as the percentage of the amine content renewed per hour. In a steady state of catecholamine utilization rate, this corresponds to the percentage of total content of amine used per hour in the structure.

Statistical Analyses

All analyses were done with Statview software (Abacus Concepts, Berkeley, CA). For simple measurements, data were analyzed by one-way ANOVA followed by a post hoc Fisher's protected least significant difference (PLSD) test. For HVR, data were analyzed by two-way ANOVA for repeated measures. If a global effect or a significant interaction appeared between groups on the general ANOVA, data were analyzed for factorial measures by a PLSD post-ANOVA test. The results of the PLSD are indicated by symbols placed on the corresponding point in Figs. 1–6 ($P < 0.05$). All data are presented as means ± SE. The level of significance for all analyses was set at 0.05.

RESULTS

Sea-Level Studies

Effects of domperidone on minute ventilation and HVR in ovariectomized females. In this study we used ovariectomized females as controls before hormonal treatment. In ovariectomized females, domperidone injection resulted in an increase of resting $\dot{V}_{E100}$ (+39%; 59.4 ± 2.6 ml·min^{-1}·100 g^{-1} after domperidone injection vs. 42.6 ± 5.1 ml·min^{-1}·100 g^{-1} after saline injection; $P = 0.01$) and V_t/BW (+46%; 0.70 ± 0.03 vs. 0.48 ± 0.07 ml/100 g, $P < 0.05$). During hypoxic exposure, $\dot{V}_{E100}$, and V_t/BW were also elevated after domperidone injection compared with saline injection (+32 and +39%, respectively; Fig. 1).

A negative correlation ($r^2 = 0.80$; $P = 0.001$) was found between the resting minute ventilation measured after saline injection and the effect of domperi-
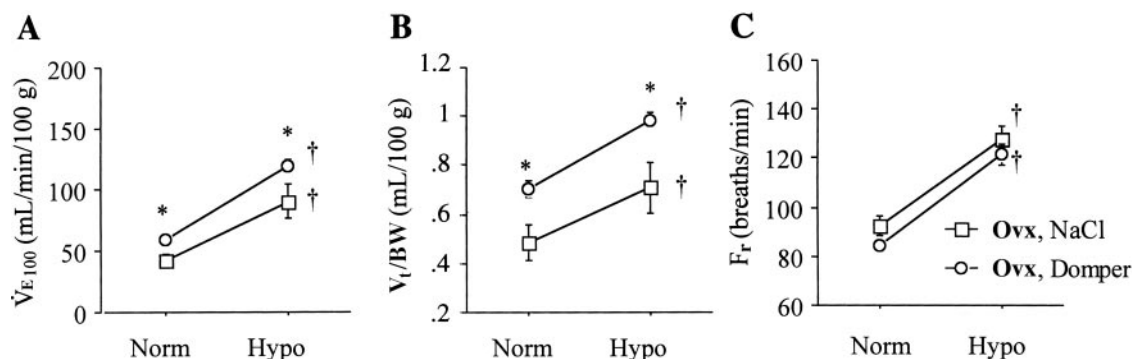

Fig. 1. Basal ventilatory parameters and hypoxic ventilatory response (HVR) in ovariectomized (Ovx) females at sea level after NaCl or domperidone (Domper) injections. Minute ventilation corrected for body weight ($\dot{V}_{E100}$; *A*), tidal volume (V_t)-to-body weight (BW) ratio (V_t/BW; *B*), and respiratory frequency (F_r; *C*) in normoxia (Norm) and after 20 min of hypoxic exposure (10% O_2; Hypo) are shown. Values are expressed as means ± SE. *$P < 0.05$, domperidone vs. saline. †$P < 0.05$, hypoxia vs. normoxia.
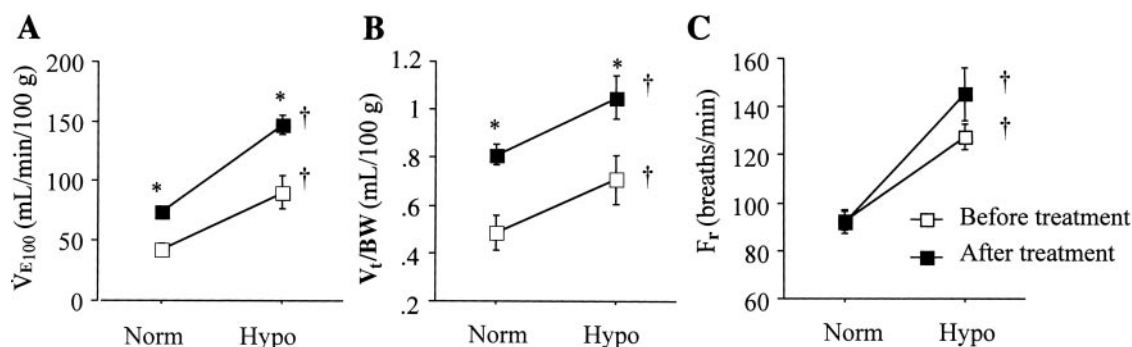
Fig. 2. Effects of 10 days of hormonal treatment in Ovx females on basal ventilatory parameters and HVR. $\dot{V}_{E100}$ (*A*), V_t/BW (*B*), and F_r (*C*) in normoxia and after 20 min of hypoxic exposure (10% O_2) are shown. Values are expressed as means $\pm$ SE. *$P < 0.05$, after vs. before treatment. †$P < 0.05$, hypoxia vs. normoxia.

done (assessed as the difference between $\dot{V}_{E100}$ after domperidone injection and $\dot{V}_{E100}$ after saline injection) for each animal (see Fig. 4*A*). Similar correlations were found for V_t/BW ($r^2 = 0.85$; $P < 0.001$) and F_r ($r^2 = 0.95$; $P < 0.0001$) under normoxia (see Fig. 4*A*).

Effects of hormonal treatment on minute ventilation and HVR. After 10 days of daily hormonal injection, females had a lower BW (-9.5%; 297 ± 6 vs. 328 ± 6 g, $P < 0.001$), higher resting $\dot{V}_{E100}$ ($+74\%$; 74.2 ± 4.0 vs. 42.6 ± 5.1 ml·min^{-1}·100 g^{-1}; $P < 0.001$), and higher V_t ($+55\%$; 2.42 ± 0.15 vs. 1.56 ± 0.25 ml, $P = 0.01$) and V_t/BW ($+68\%$; 0.81 ± 0.04 vs. 0.48 ± 0.07 ml/100 g, $P < 0.01$; Fig. 2). F_r was unaffected by repeated hormonal injections [92.1 ± 5.0 vs. 92.3 ± 4.0 breaths/min; not significant (NS)]. When exposed to hypoxia, the gain of the ventilatory response, defined as the difference between the values of respiratory parameters after 20 min of hypoxia and the normoxic values, was more elevated in treated females, for $\dot{V}_{E100}$ ($+51\%$; 72.3 ± 5.9 vs. 47.8 ± 11.4 ml·min^{-1}·100 g^{-1}, $P < 0.05$) but not for F_r (52.3 ± 7.9 vs. 35.1 ± 6.3 breaths/min, $P = 0.07$), V_t (0.73 ± 0.23 vs. 0.72 ± 0.21 ml; NS), or V_t/BW (0.24 ± 0.08 vs. 0.22 ± 0.07 ml/100 g; NS).

Effects of domperidone on minute ventilation and HVR after hormonal treatment. After domperidone injection in treated females, there was a slight decrease of $\dot{V}_{E100}$ (-17%; 61.7 ± 3.3 vs. 74.2 ± 4.2 ml·min^{-1}·100 g^{-1}, $P < 0.05$) and F_r (-13%; 80.1 ± 1.3 vs. 92.1 ± 5.0 breaths/min, $P < 0.05$). V_t and V_t/BW were not affected

by domperidone [2.33 ± 0.14 vs. 2.42 ± 0.15 ml (NS) and 0.77 ± 0.05 vs. 0.81 ± 0.04 ml/100 g (NS)], and domperidone had no effect on the HVR after hormonal treatment (Fig. 3). A negative correlation between the values in normoxia after saline injection and the effect of domperidone was also apparent after hormonal treatment for $\dot{V}_{E100}$ ($r^2 = 0.62$, $P < 0.05$), V_t/BW ($r^2 = 0.72$; $P < 0.01$), and F_r ($r^2 = 0.99$, $P < 0.0001$; Fig. 4*B*).

High-Altitude Studies

Catecholaminergic activity in the carotid bodies. In intact animals, all indexes of catecholaminergic activity (TH activity, amine content, and utilization rate index) were lower (between 30 and 60%) in females than in males (Table 1). Ovariectomized females had higher TH activity ($+129\%$, $P < 0.01$) and NE content ($+55\%$, $P < 0.01$) than intact ones, whereas DA content was unaffected by ovariectomy (-14%, NS, Table 1). The calculated index of total catecholamine utilization was higher ($+88\%$, $P < 0.01$) in ovariectomized females than in intact ones. The index of individual catecholamine utilization revealed that DA utilization was enhanced ($+150\%$, $P < 0.001$), while NE utilization was left unchanged ($+20\%$, NS, Table 1). In males, the only effect observed after orchidectomy was a lower DA content (-31%, $P < 0.0001$) vs. intact males. As a result of this differential effect of gonadectomy in males and females, no difference in the metabolism of
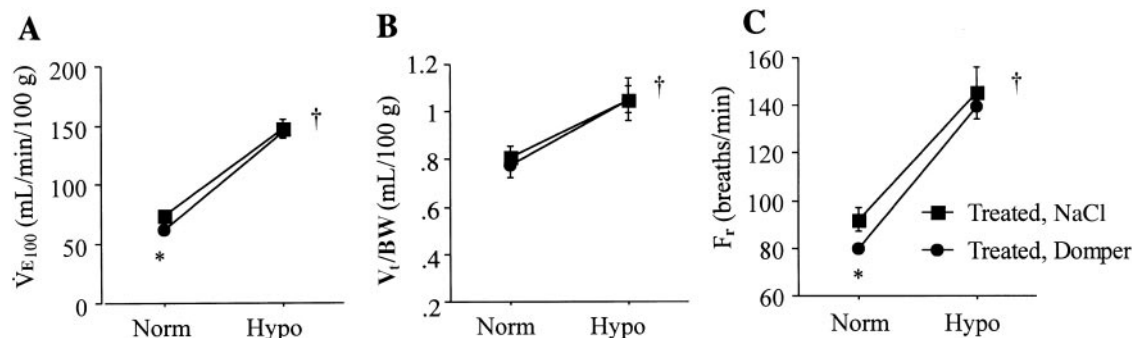

Fig. 3. Basal ventilatory parameters and HVR in Ovx females after 10 days of hormonal injections, following NaCl or domperidone. $\dot{V}_{E100}$ (*A*), V_t/BW (*B*), and F_r (*C*) in normoxia and after 20 min of hypoxic exposure are shown. Values are expressed as means $\pm$ SE. *$P < 0.05$, domperidone vs. saline. †$P < 0.05$, hypoxia vs. normoxia.
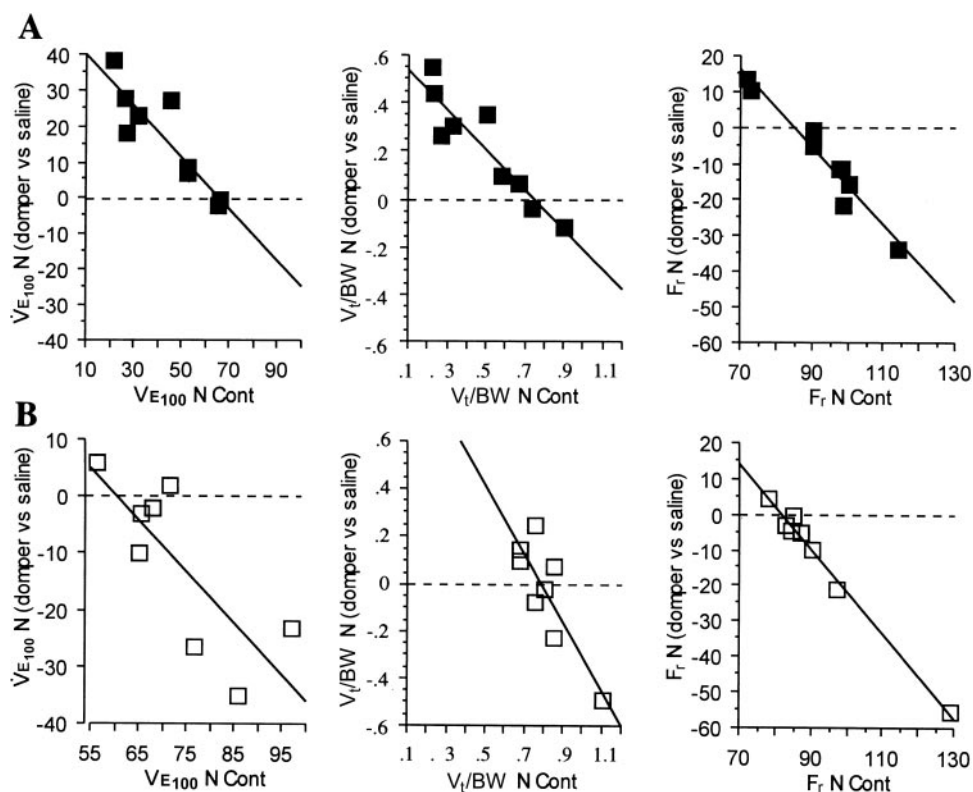
Fig. 4. Correlation between the basal level of ventilatory parameters [normoxic value (N) after saline injection; x-axis] and the effects of domperidone injection (value after domperidone injection − value after saline injection; y-axis) before (filled boxes; *A*) and after (open boxes; *B*) hormonal treatment. For each graph, horizontal dotted line represents no effect of domperidone; points above this line indicate stimulation, and points below the line indicate inhibition of the corresponding parameter. Cont, control.

catecholamine was observed in the carotid body between neutered males and females (Table 1).

Resting minute ventilation. Ovariectomized females weighed more (+19%) than intact females (209 ± 6 vs. 176 ± 6 g, $P < 0.001$) but had a lower (−16%) V_t (1.46 ± 0.08 ml) than control females (1.72 ± 0.10 ml, $P < 0.05$), which resulted in a reduction (−28%) of V_t/BW (0.70 ± 0.04 vs. 0.98 ± 0.10 ml/100 g, $P < 0.001$; Fig. 5) and $\dot{V}_{E100}$ (−29%; 101 ± 10 vs. 143 ± 11 ml·min^{-1}·100 g^{-1}, $P < 0.01$; Fig. 5) compared with intact females. F_r was not different between intact

Table 1. *In vivo TH activity and NE and DA content in the CBs of control or neutered males and females living at high altitude*

CBs	Control		Neutered	
	M (12)	F (12)	M (15)	F (11)
TH activity	3.87 ± 0.5	1.40 ± 0.1*	3.30 ± 0.3	3.20 ± 0.5†
NE content	155 ± 13	97 ± 10*	124 ± 11	150 ± 16†
DA content	312 ± 16	212 ± 12*	214 ± 14†	183 ± 20
(NE + DA) utilization	2.50 ± 0.3	1.49 ± 0.2*	2.97 ± 0.3	2.80 ± 0.3†
NE utilization	7.53 ± 0.8	5.10 ± 1.0*	8.20 ± 0.7	6.13 ± 0.5
DA utilization	3.95 ± 0.7	2.15 ± 0.4*	4.80 ± 0.5	5.37 ± 0.8†

Values are means ± SE; nos. in parentheses are no. of animals/group. Tyrosine hydroxylase (TH) activity is expressed as pmol of L-DOPA formed per 20 min per carotid body (CB) after intraperitoneal injection of NSD-1015. Norepinephrine (NE) and dopamine (DA) contents are expressed as pmol/CB, and utilization indexes are expressed as percentage of content synthesized per hour. *$P < 0.05$: gender difference observed in the same experimental group; †$P < 0.05$: effect of castration for males (M) or females (F).
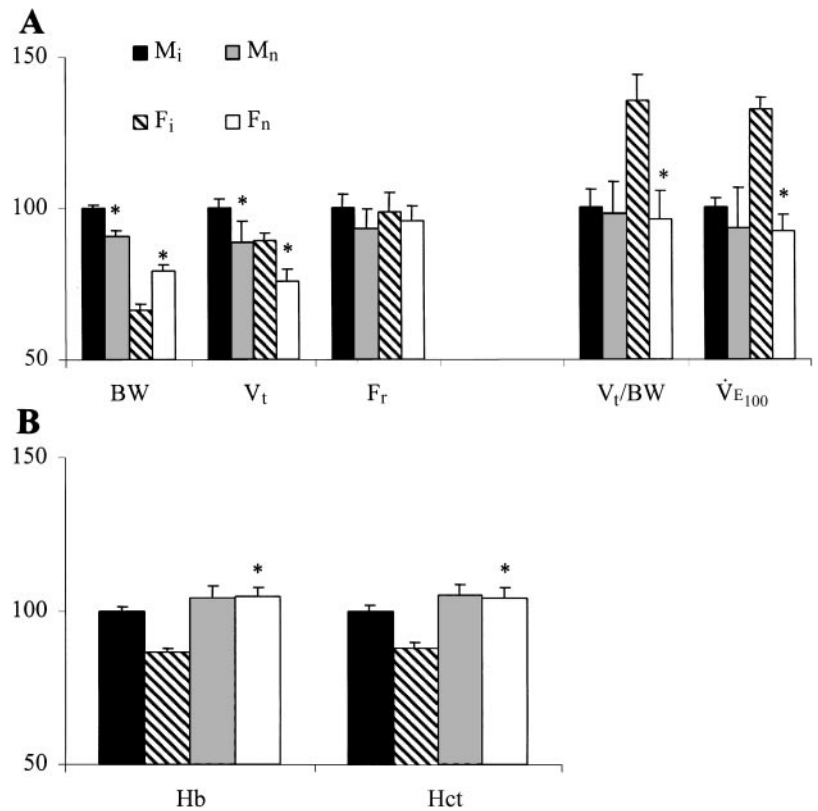
(148 ± 9 breaths/min) and ovariectomized females (142 ± 8 breaths/min). In males, the effect of gonadectomy was limited to V_t, castrated males having lower (−11%) V_t than intact ones (1.71 ± 0.04 vs. 1.93 ± 0.06 ml, $P < 0.05$), but the relation of V_t to BW was maintained (0.71 ± 0.03 vs. 0.73 ± 0.02 ml/100 g, respectively; NS; Fig. 5). Therefore, the changes in V_t seem to be directly related to changes in BW in neutered males compared with control (−10%; 240 ± 5 vs. 264 ± 8 g, $P = 0.001$; Fig. 5).

HVR. Ovariectomized females had a lower ability to increase the absolute value of $\dot{V}_{E100}$ under acute hypoxic exposure (150 ± 22 ml·min^{-1}·100 g^{-1}) compared with all other groups (intact females, 259 ± 15; intact males, 234 ± 20; neutered males, 221 ± 20 ml·min^{-1}·100 g^{-1}, $P = 0.01$; Fig. 6). The gain of the response (difference between normoxia and hypoxia) in ovariectomized females (50 ± 16 ml·min^{-1}·100 g^{-1}) was also decreased compared with other groups (intact females, 116 ± 16; intact males, 125 ± 14; neutered males, 121 ± 13 ml·min^{-1}·100 g^{-1}, $P < 0.01$). The decrease of HVR was mediated by a reduced ability to increase V_t under acute hypoxic exposure in ovariectomized females (Fig. 6).

Hematological parameters. Hct and Hb concentration were not different between control (51.2 ± 0.9% and 18.1 ± 0.2 g/dl) and neutered males (53.5 ± 1.8% and 18.8 ± 0.7 g/dl; NS for both values). However, ovariectomized females had higher Hct (53.1 ± 1.9%) and Hb concentration (18.9 ± 0.6 g/dl) than control females (44.8 ± 1.0% and 15.6 ± 0.3 g/dl, respectively; $P < 0.001$; Fig. 5).

Fig. 5. Ventilatory (*A*) and hematological parameters (*B*) of intact (M_i, F_i) or neutered males and females (M_n, F_n) living at high altitude. *A*: BW, V_t, F_r, V_t/BW, and resting $\dot{V}E_{100}$ are shown. Values are expressed as percentage of value for M_i. Number of animals for each group: M_i, $n = 9$; M_n, $n = 8$; F_i, $n = 5$; F_n, $n = 7$. *B*: Hb concentration (g/dl) and hematocrit (Hct; %). Values are expressed as percentage of value for M_i (means ± SE). Number of animals for each group: M_i, $n = 12$; M_n, $n = 11$; F_i, $n = 12$; F_n, $n = 11$. *$P < 0.05$ vs. intact counterpart.

Cardiac hypertrophy. No difference in cardiac weight was observed between control males and females (Table 2). In males gonadectomy resulted in an increased heart weight-to-BW ratio (+27%; $P < 0.05$) and (LV + S)-to-BW ratio (+23%; $P < 0.05$, Table 2), but no RV hypertrophy appeared in neutered males compared with control (Table 2). In females ovariectomy resulted in a specific hypertrophy of the RV: RV-to-(LV + S) ratio was 40% higher in ovariectomized than in control females ($P = 0.0001$, Table 2).

DISCUSSION

The main finding of this study is that in ovariectomized females raised at sea level, a peripheral dopaminergic drive inhibits ventilation under normoxia or
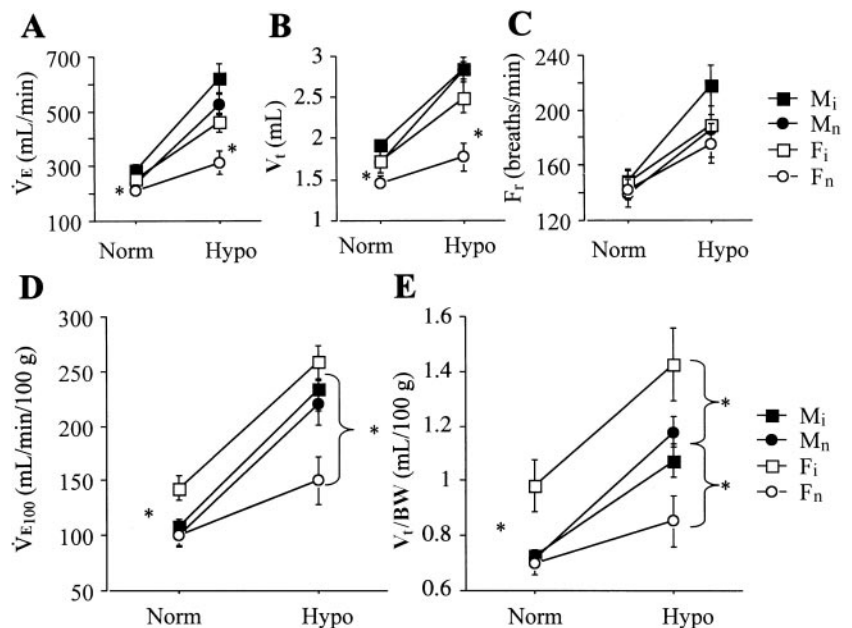

Fig. 6. HVR of M_i ($n = 9$), M_n ($n = 8$), F_i ($n = 5$), and F_n ($n = 7$) living at high altitude. $\dot{V}E$ (*A*), V_t (*B*), F_r (*C*), $\dot{V}E_{100}$ (*D*), and V_t/BW (*E*) in normoxia and after 10 min of hypoxia are shown. *$P < 0.05$, F_n vs. F_i or indicated groups.

Table 2. *Cardiac parameters of control or neutered males and females living at high altitude*

	Control		Neutered	
	M (9)	F (7)	M (8)	F (7)
BW, g	262 ± 4	$172 \pm 3*$	$206 \pm 10†$	$204 \pm 4†$
Heart wt, g	1.37 ± 0.08	$0.89 \pm 0.03*$	1.33 ± 0.06	$1.68 \pm 0.10*†$
Heart wt/BW, %	0.52 ± 0.03	0.52 ± 0.02	$0.66 \pm 0.05†$	$0.82 \pm 0.05*†$
RV/(LV + S), %	0.37 ± 0.01	0.35 ± 0.01	0.43 ± 0.03	$0.49 \pm 0.03†$
(LV + S)/BW, %	0.31 ± 0.02	0.34 ± 0.01	$0.38 \pm 0.02†$	$0.47 \pm 0.03*†$

Values are means $\pm$ SE; nos. in parentheses are no. of animals/group. RV and LV, right and left ventricle, respectively; S, septum; BW, body weight. $*P < 0.05$: gender difference observed in the same experimental group; $†P < 0.05$: effect of castration for males or females.

acute hypoxia and that this inhibitory drive disappears after repeated injections of ovarian steroids for 10 days. This mechanism appears to be physiologically relevant for female rats at high altitude: DA utilization index in carotid bodies was 2.5 times higher in ovariectomized females compared with control. This was associated with a deacclimatization process in ovariectomized females living at high altitude, i.e., lower minute ventilation, higher Hct, higher Hb concentration, and right heart hypertrophy. This finding is strikingly similar to the clinical observations associated with menopause in high-altitude women (16, 17).

Methodological Considerations

At sea level, we used ovariectomized females as control animals to study the effects of hormonal injections on peripheral dopaminergic influence on breathing and HVR. The dose of domperidone that we used (1 mg/kg) has already been shown to be effective (9) and corresponded to the recommendations made by the provider of the drug. The doses for hormonal treatment were also chosen with regard to previous works and recommendations. The corresponding effects on BW, resting minute ventilation, and HVR were as expected.

The high-altitude study was carried out in a strain of rats that have been bred at high altitude for at least 15–20 generations (Bolivian Institute for High-Altitude Biology, La Paz, 3,600 m, mean $P_b = 495–500$ mmHg). A complete study concerning these animals and some physiological and neurochemical changes that characterized them has been previously published (13). In the present study, we used a chronic model of hormonal deficiency; orchidectomy and ovariectomy were performed in 1-wk-old rats, and the effect of the surgery was assessed in 3-mo-old animals. No hormonal titration was done after death; however, various points indicated that the surgery resulted in a chronic hormonal deficiency in those animals. Males were checked during growth to verify the absence of external genital organs, and females were checked during dissection to verify the absence of the internal genital tract. Furthermore, lower BW in neutered males vs. controls and higher BW of ovariectomized females vs. controls underlined chronic hormonal deficiency. A confounding point of the design of this study is that control females were not checked for period of the estrous cycle; therefore, the exact hormonal status of these animals cannot be identified. Nevertheless, the estrous cycle in rats is of short duration (4 days) compared with the typical time course of the hormonal ventilatory effects, which is usually around 7–10 days (23). According to this discrepancy, the control females may be considered as being in a state of chronic hormonal stimulation, and possible short-term effects are likely to be minor compared with the long-term hormonal effects.

Ovarian Steroids Modulate an Inhibitory Dopaminergic Tone in the Carotid Bodies to Stimulate Breathing

DA, which is synthesized and stored in carotid body type I cells, is recognized as the most abundant and potent neuromodulator influencing global nerve ending response under hypoxemic challenge. When released from carotid body type I cells under hypoxemic stimulation, DA acts through low-affinity excitatory postsynaptic and high-affinity inhibitory presynaptic D_2 receptors (7). Despite this discrepancy, in physiological conditions DA is recognized as a potent inhibitor of resting ventilation and HVR, as increase of catecholaminergic activity in carotid body type I cells has been related to an inhibition of HVR, both under acute and chronic hypoxic exposure (7, 12, 27).

In the present study, we used TH activity as a direct reflection of catecholamine biosynthesis rate, as has been previously done (12, 13). Nevertheless, the carotid body is also a noradrenergic structure, and NE released by glomic cells and by sympathetic nerve endings under hypoxemic stimulation is active on hypoxic chemosensitivity (6, 7, 18). Therefore, a complete understanding of the catecholaminergic modifications after gonadectomy needed a better discrimination between dopaminergic and noradrenergic metabolisms. For this reason, we calculated distinct indexes of utilization for DA and NE in the carotid body. They are expressed as percentage of specific catecholamine synthesized per hour. If we assume that we are in steady conditions, this specific index of synthesis is similar to the utilization of the amine in the structure, i.e., the turnover rate.

Domperidone, a highly selective peripheral D_2-dopaminergic antagonist, has been reported as a tool to assess the dopaminergic tone on carotid sinus nerve activity under normoxia or hypoxia (10, 28, 29) and has also been used to study in vivo the peripheral dopaminergic tone on the control of breathing (9, 25, 26). In the present study, the inhibitory effect of repeated ovarian steroid injections in ovariectomized females on domperidone-induced hyperventilation demonstrates that ovarian steroids are able to abolish the dopaminergic inhibitory influence on breathing. At high altitude, the neurochemical results are consistent with a

specific control of dopaminergic metabolism in the carotid bodies by ovarian steroids.

Evidence for central mechanisms in the control of breathing after chronic hormonal treatment has also been reported (2–4), and some of our results after the hormonal treatment at sea level may also be explained by a central effect and/or by changes affecting other neurochemical processes in the carotid bodies (for example, the enhanced hypoxic response of $\dot{V}_{E100}$ after hormonal treatment was not affected by domperidone). Nevertheless, the elimination of the peripheral dopaminergic inhibitory drive on breathing after hormonal treatment clearly shows that ovarian steroids are able to act on peripheral dopaminergic metabolism to stimulate breathing.

Perspectives

In the present study, the hematological and ventilatory data obtained in neutered females are strikingly similar to the findings of F. León-Velarde and coworkers (16, 17) in high-altitude postmenopausal women (increased Hct, impaired ventilation, and lower O_2 arterial saturation). According to these results, it may be hypothesized that the sequence leading to CMS in postmenopausal women at high altitude includes a gradual increase in carotid body TH activity and DA utilization after menopause. This increased dopaminergic activity in the carotid bodies limits the efficiency of the chemosensitive hypoxic stimulation on ventilation. As a result, a more severe hypoxemia appears, the Hct rises, and pulmonary hypertension and right heart hypertrophy are enhanced. The conditions for development of CMS in susceptible women are no longer masked by the protective effects of ovarian steroids, and the occurrence of the disease increases in high-altitude postmenopausal women (16, 17).

This mechanism may also explain the alveolar hypoventilation (15) and the low hypoxic peripheral chemosensitivity (22) associated with CMS in men, and it can be hypothesized that these ventilatory disturbances are linked to an elevated dopaminergic metabolism in the carotid body. Accordingly, a possible strategy for therapy of CMS in high-altitude residents may aim at limiting the dopaminergic neuromodulation of carotid body chemosensitivity as has already been done in animals (9, 25) and men (11) with domperidone.

REFERENCES

1. **Bartlett D and Tenney SM.** Control of breathing in experimental anemia. *Respir Physiol* 10: 384–395, 1970.
2. **Bayliss DA, Cidlowski JA, and Millhorn DE.** The stimulation of respiration by progesterone in ovariectomized cat is mediated by an estrogen-dependent hypothalamic mechanism requiring gene expression. *Endocrinology* 126: 519–527, 1990.
3. **Bayliss DA and Millhorn DE.** Central neural mechanisms of progesterone actions: application to the respiratory system. *J Appl Physiol* 73: 393–404, 1992.
4. **Bayliss DA, Millhorn DE, Gallman EA, and Cidlowski JA.** Progesterone stimulates ventilation through a central nervous system steroid receptor-mediated mechanism in the cat. *Proc Natl Acad Sci USA* 84: 7788–7792, 1987.
5. **Carlsson A, Davis JN, Kehr W, Lindqvist M, and Atack CV.** Simultaneous measurement of tyrosine and tryptophan hydroxylase activities in brain in vivo using an inhibitor of the aromatic amino acid decarboxylase. *Naunyn Schmiedebergs Arch Pharmacol* 275: 153–158, 1972.
5a. **Danneman PS and Mandrell TD.** Evaluation of five agents/methods for anesthesia of neonatal rats. *Lab Anim Sci* 47: 386–395, 1997.
6. **Folgering HJ, Ponte J, and Sadig T.** Adrenergic mechanisms and chemoreception in the carotid body of the cat and rabbit. *J Physiol (Lond)* 325: 1–21, 1982.
7. **Gonzalez C, Almaraz L, Obeso A, and Rigual R.** Carotid body chemoreceptors: from natural stimuli to sensory discharges. *Physiol Rev* 74: 829–898, 1994.
8. **Gonzalez C, Lopez-Lopez JR, Obeso A, Perez-Garcia MT, and Rocher A.** Cellular mechanisms of chemoreception in the carotid body. *Respir Physiol* 102: 137–147, 1995.
9. **Huey KA, Brown IP, Jordan MC, and Powell FL.** Changes in dopamine D_2-receptor modulation of the hypoxic ventilatory response with chronic hypoxia. *Respir Physiol* 123: 177–187, 2000.
10. **Iturriaga R, Larrain C, and Zapata P.** Effects of dopaminergic blockade upon carotid chemosensory activity and its hypoxia-induced excitation. *Brain Res* 663: 145–154, 1994.
11. **Javaheri S and Guerra LF.** Effects of domperidone and medroxyprogesterone acetate on ventilation in man. *Respir Physiol* 81: 359–370, 1990.
12. **Joseph V, Dalmaz Y, Cottet-Emard JM, and Pequignot JM.** Dexamethasone's influence on tyrosine hydroxylase activity in the chemoreflex pathway and on the hypoxic ventilatory response. *Pflügers Arch* 435: 834–839, 1998.
13. **Joseph V, Soliz J, Pequignot J, Sempore B, Cottet-Emard JM, Dalmaz Y, Favier R, Spielvogel H, and Pequignot JM.** 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.
14. **Kryger M, McCullough RE, Collins DD, Scoggin CH, Weil JV, and Grover RF.** Treatment of excessive polycythemia of high altitude with respiratory stimulant drugs. *Am Rev Respir Dis* 117: 455–464, 1978.
15. **Kryger M, McCullough RE, Doekel R, Collins DD, Weil JV, and Grover RF.** Excessive polycythemia of high altitude: role of ventilatory drive and lung disease. *Am Rev Respir Dis* 118: 659–666, 1978.
16. **León-Velarde F, Ramos MA, Hernández JA, De Idiáquez D, Muñoz LS, Gaffo A, Córdova S, Durand D, and Monge CC.** The role of menopause in the development of chronic mountain sickness. *Am J Physiol Regulatory Integrative Comp Physiol* 272: R90–R94, 1997.
17. **León-Velarde F, Rivera-Chira M, Tapia R, Huicho L, and Monge-CC.** Relationship of ovarian hormones to hypoxemia in women residents of 4,300 m. *Am J Physiol Regulatory Integrative Comp Physiol* 280: R488–R493, 2001.
18. **Milsom WK and Sadig T.** Interaction between norepinephrine and hypoxia on carotid body chemoreception in rabbits. *J Appl Physiol* 55: 1893–1898, 1983.
19. **Mortola J and Saiki C.** Ventilatory response to hypoxia in rats: gender differences. *Respir Physiol* 106: 21–34, 1996.
20. **Ou LC, Sardella GL, Leiter JC, Brinck-Johnsen, and Smith RP.** Role of sex hormones in the development of chronic mountain sickness in rats. *J Appl Physiol* 77: 427–433, 1994.
21. **Rabinovitch M, Gamble WJ, Miettinen OL, and Reid L.** Age and sex influence on pulmonary hypertension of chronic hypoxia and on recovery. *Am J Physiol Heart Circ Physiol* 240: H62–H72, 1981.

22. **Severinghaus JW, Bainton GR, and Carcelen A.** Respiratory insensitivity to hypoxia in chronically hypoxic man. *Respir Physiol* 1: 308–334, 1966.

23. **Skatrud JB, Dempsey JA, and Kaiser DG.** Ventilatory response to medroxyprogesterone acetate in normal subject: time course and mechanism. *J Appl Physiol* 44: 939–944, 1978.

24. **Tatsumi K, Pickett CK, Jacoby CR, Weil JV, and Moore LG.** Role of endogenous female hormones in hypoxic chemosensitivity. *J Appl Physiol* 83: 1706–1710, 1997.

25. **Tatsumi K, Pickett CK, and Weil JV.** Decreased carotid body hypoxic sensitivity in chronic hypoxia: role of dopamine. *Respir Physiol* 101: 47–57, 1995.

26. **Tatsumi K, Pickett CK, and Weil JV.** Possible role of dopamine in ventilatory acclimatization to high altitude. *Respir Physiol* 99: 63–73, 1995.

27. **Wach RA, Bee D, and Barer GR.** Dopamine and ventilatory effects of hypoxia and almitrine in chronically hypoxic rats. *J Appl Physiol* 67: 186–192, 1989.

28. **Zapata P, Iturriaga R, and Larrain C.** Domperidone as a tool to assess the role of dopamine within carotid body chemoreception. *Adv Exp Med Biol* 410: 291–297, 1996.

29. **Zapata P and Torrealba F.** Blockade of dopamine-induced chemosensory inhibition by domperidone. *Neurosci Lett* 51: 359–364, 1984.