

Prenatal blockade of estradiol synthesis impairs respiratory and metabolic responses to hypoxia in newborn and adult rats

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Doan, V. D., S. Gagnon, and V. Joseph. Prenatal blockade of estradiol synthesis impairs respiratory and metabolic responses to hypoxia in newborn and adult rats. *Am J Physiol Regul Integr Comp Physiol* 287: R612–R618, 2004. First published May 13, 2004; 10.1152/ajpregu.00627.2003.—We tested the hypothesis that estradiol modifies respiratory control in pregnant rats and participates in the development of respiratory chemoreflexes in fetuses. Pregnant rats ($n = 12$) received daily subcutaneous injections of vehicle (Veh, $n = 6$) or 4-androsten-4-ol-3,17-dione acetate (ATD; inhibitor of estradiol synthesis; $n = 6$; 5 mg/day in vehicle) from gestational day 16 (G16) to delivery. Baseline ventilation (whole body plethysmography) and metabolic rate [oxygen consumption ($\dot{V}O_2$)] were determined at G14 and G20, in pups [on postnatal day 3 (P3) and P20] and in adult rats (on P70) born to Veh- or ATD-treated mothers. Hypoxic chemoreflex was assessed in P3 rats by acute exposure to 60% O_2 and in P20 or P70 rats by moderate hypoxia (12% O_2 , 30 min). ATD treatment reduced circulating estradiol in pregnant dams at G20 without producing changes in the circulating level of estradiol precursors (testosterone and androstenedione). ATD-treated dams showed impaired respiratory adjustment to late gestation. Pups born to ATD mothers had higher resting $\dot{V}O_2$ (+23% at P3, +21% at P20), respiratory frequency (+15% at P3, +12% at P20), and minute ventilation (+11% at P3, +18% at P20) than pups from Veh mothers. Respiratory decrease during acute hyperoxic exposure at P3 was -9.7% in Veh ($P < 0.05$ vs. room air) and only -2.6% ($P =$ not significant) in ATD pups. In P20 ATD rats, hypoxic ventilatory response was attenuated compared with Veh. In P20 and P70 rats, the drop of $\dot{V}O_2$ in hypoxia (-31% in P70, $P < 0.0001$) was not observed in ATD rats. We conclude that estradiol secreted during late gestation is necessary for respiratory adjustment to pregnancy and is required for adequate development of respiratory and metabolic control in the offspring.

hypoxic ventilatory response; prenatal estradiol synthesis; aromatase inhibitor; development

OVARIAN STEROIDS, including progesterone and estradiol, have long been recognized as specific factors able to modulate normoxic breathing and hypoxic chemoreflexes by acting both on the central nervous system (4–6) and on the dopaminergic signaling pathway in the peripheral chemoreceptors (21, 22, 40). These animal models are clinically relevant because the combined action of estradiol and progesterone on respiratory control is thought to reduce the occurrence of respiratory disorders such as sleep-disordered breathing (10, 36, 38, 47) and high-altitude deacclimatization (22, 28) in women.

During pregnancy, the circulating levels of estradiol and progesterone in maternal circulation increase by ~ 100 -fold (33) as a result of the endocrine activity of the placenta (in

primate; Ref. 24) or ovaries (in rodents; Ref. 1), influencing both maternal and fetal physiology (16, 34). Additionally, estradiol synthesized directly in specific fetal brain neurons (i.e., dopaminergic nigrostriatal neurons) is a potent autocrine regulator of neural growth and survival, involving both classical (nuclear) and nonclassical (membrane bound) estradiol receptors (26).

Former studies emphasized the relationship between pregnancy-related hormonal secretion, increased minute ventilation ($\dot{V}E$), and enhanced respiratory chemoreflexes observed during late gestation in humans (33). However, the role of estradiol exposure on the respiratory control of the fetus and newborn remains unknown. This particular aspect may be of clinical importance as preterm and extreme preterm births are characterized both by respiratory disorders (19, 27) and chronic deficit of estradiol normally provided by the endocrine placenta (41–43). Thus we hypothesized that gestational blockade of estradiol synthesis in rats would impair maternal respiratory adjustment to pregnancy and potentially disrupt the adequate development of respiratory control in newborn rats. We used a highly specific pharmacological blocker of estradiol synthesis [4-androsten-4-ol-3,17 dione acetate (ATD), a potent inhibitor of *P*-450 aromatase enzyme, the key enzyme for estradiol synthesis] (37) in pregnant female rats to effectively reduce the circulating level of estradiol during the last days of gestations. Our results are consistent with a yet unrecognized role of endogenous estradiol for adequate development of ventilatory control in newborn rats.

MATERIALS AND METHODS

Animal treatment. All experiments have been approved by the local committee (Laval University) of animal care and use. On the day of estrus (determined on the basis of daily vaginal smears), female Sprague-Dawley rats ($n = 12$; purchased from Charles River Canada) were put overnight with mating males. Pregnancy was confirmed after observation of spermatozooids in the vaginal smear the next day [considered as day 0 of gestation; G0] and weight gain during the subsequent days. Pregnant female rats were housed separately and left undisturbed until G14, when they were weighed and baseline ventilation and oxygen consumption ($\dot{V}O_2$) were assessed (see below). Forty-eight hours later (G16), pregnant dams were divided into two groups receiving daily subcutaneous injections (500 μ l) of vehicle (Veh; $n = 6$, 10% ethanol in propylene glycol) or the inhibitor of estradiol synthesis, ATD ($n = 6$; 5 mg/day in vehicle, Sigma-Aldrich) until delivery. At G20, females were weighed, and baseline respiratory and metabolic measurements were repeated.

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Table 1. Circulating levels of estradiol, testosterone, and androstenedione assessed by gas chromatography at gestational day 20 in Veh- and ATD-treated mothers

| | Veh | ATD |
|------------------------|-------------|---------------------------|
| Estradiol, pg/ml | 28.1 ± 2.6 | Non detectable (<5 pg/ml) |
| Testosterone, ng/ml | 0.40 ± 0.08 | 0.34 ± 0.08 |
| Androstenedione, ng/ml | 0.40 ± 0.08 | 0.56 ± 0.06 |

Values are means ± SE. Since estradiol values in the 4-androsten-4-ol-3,17-dione acetate (ATD)-treated group were below the detection threshold (5 pg/ml), statistical analysis was not performed for estradiol. Veh, vehicle.

After respiratory recordings, females were briefly anesthetized under halothane, and a sample of blood (500 µl) was drawn from the jugular vein. To assess the efficiency of ATD treatment, hormonal assays were done by gas chromatography-mass spectrometry using negative chemical ionization to determine circulating levels of estradiol and its precursors (testosterone, androstenedione; Laboratory of Clinical Endocrinology, CHUL, University Laval, Quebec, Canada). Steroids were extracted from serum by liquid-liquid and solid-phase extraction, and derivatization reactions were performed to improve chromatographic and detection response as previously described (7).

At birth, male pups from all litters were kept for further analysis; females pups were used to normalize the size of each litter to 12 pups to avoid litter size effects on postnatal growth. All the animals (males and females) were weighed between 24 and 36 h after birth, and pups were raised with their natural mothers until weaning.

Respiratory recordings in pregnant dams and offspring. Respiratory recordings were performed by whole body plethysmography (Emka Technologies, Paris, France) using a method derived from the pressure plethysmograph described by Bartlett and Tenney (3), with chambers of different size for adults and pups. The recording chamber included a built-in reference chamber and was permanently flushed with fresh air (or the desired gas mixture; see below). The Emka chamber allowed measurements of flow related to respiratory gas exchanges in the chamber. After adequate calibration of the chamber, all measurements were corrected with the standard equation (3) to allow determination of tidal volume after integration of the flow signal by the Emka software. Temperature and relative humidity inside the chamber were constantly monitored during recordings, and barometric pressure was read from a barometer. For 3-day-old rats, body temperature was considered to be stable at 35°C with a mean ambient temperature of 30.3 ± 0.08°C inside the recording chamber.

The inlet air was circulated at a regular flow of 400 ml/min [postnatal day 3 (P3)], 600 ml/min (weaning), or 1,500 ml/min (adults) through a dedicated oxygen sensor (s103 O₂ analyzer, Qubit-systems, Kingston, ON, Canada) and a subsampling pump and drying column allowed the outlet flow to be monitored by a second oxygen sensor for determination of $\dot{V}O_2$ [(%O₂ in - %O₂ out) × flow]. The oxygen sensors were calibrated daily before each experiments. The outlet-subsampling pump was switched on only for metabolic measurements, which lasted 2–3 min and were initiated after 10–15 min of stable ventilatory recordings and at the end of the hypoxic exposure. For each animal, at the end of the hypoxic exposure, O₂% values were read once the animal had been removed from the chamber to ensure adequate baseline readings in hypoxia. All signals (ventilation and O₂%) were recorded and stored on a computer for later analysis.

Basal ventilation and respiratory responses to hyperoxia in newborn pups (Dejours test). Respiratory measurements were done in 3-day-old (P3) male pups born to Veh (n = 15)- or ATD-treated (n = 18) mothers. After a period of habituation in the recording chamber (15–30 min) and at least 10 min of stable baseline recordings, the inlet flow was derived from a hyperoxic gas mixture, which allowed a rapid exposure to 60% O₂ in the chamber. Respiratory changes were analyzed during the first 20 s of hyperoxic exposure. This procedure,

classically referred to as “Test de Dejours” (18, 23), is highly effective to address the sensitivity of peripheral chemoreceptors in vivo.

In weanling and adult rats, the same procedure for baseline recordings was repeated, and then the inlet flow was derived from a hypoxic gas mixture allowing rapid exposure to 12% O₂ for 30 min. The respiratory response to hypoxia was analyzed minute by minute during the first 10 min of exposure and as the average of the last 5 min of exposure. Rectal temperature was measured before the onset of normoxic recordings and at the end of the hypoxic exposure with a thermocouple thermometer. We used a total of 15 and 18 weanling rats from Veh- and ATD-treated dams, respectively, and 11 and 15 adults from Veh and ATD dams.

Statistical analysis. For simple measurements (1 factor), data were analyzed by one-way ANOVA. For longitudinal comparisons, data were analyzed by two-way ANOVA for repeated measures. If a global effect or significant interaction appeared between groups on the general ANOVA, data were analyzed for factorial measures by a post hoc Fisher’s protected least significant difference test. The results are indicated in the corresponding tables and by symbols in the figures. All values are means ± SE, and the level of significance was set at 0.05.

RESULTS

Blockade of estradiol synthesis impairs respiratory adaptation during late gestation in female rats. The ATD treatment reduced circulating estradiol measured at G20 in pregnant dams to undetectable levels (<5 pg/ml) without producing significant increases in the circulating level of the substrates of the P-450 aromatase, i.e., testosterone and androstenedione (see Table 1). Between G14 and G20, Veh- and ATD-treated dams had similar increase in body weight (from 363 ± 11 to 432 ± 14 g in Veh-treated and 377 ± 6 to 452 ± 9 g in ATD-treated dams), but the normal increase of tidal volume that takes place during late gestation in Veh-treated mothers (from 1.85 ± 0.09 to 2.35 ± 0.07 ml at G14 and G20, respectively) was blocked in ATD-treated dams (1.96 ± 0.10 ml at G14 and 1.91 ± 0.13 ml at G20, Fig. 1). Nonetheless, G20 ATD-treated dams maintained \dot{V}_E (37.8 ± 2.9 ml·min⁻¹·100 g⁻¹) at a level similar to Veh-treated mothers (41.0 ± 0.5 ml·min⁻¹·100 g⁻¹) by slightly increasing respiratory frequency (from 81.3 ± 2.5 at G14 to 90.1 ± 6.4 breaths/min at G20), while at the same time Veh-treated dams slightly decreased their respiratory frequency (from 82.7 ± 3.8 to 75.2 ± 1.6 breaths/min).

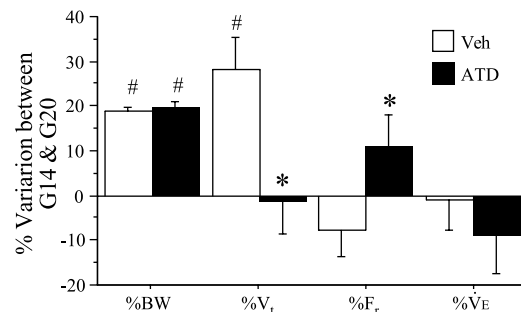


Fig. 1. Evolution of respiratory parameters between gestational days 14 and 20 in dams treated with vehicle (Veh) or 4-androsten-4-ol-3,17-dione acetate (ATD). BW, body weight; V_t, tidal volume; F_r, respiratory frequency; V_E, minute ventilation. Results are expressed as means ± SE of %changes between gestational day 14 (G14) and G20 calculated for each dam. # P < 0.05, G20 vs. G14; *P < 0.05, ATD vs. Veh.

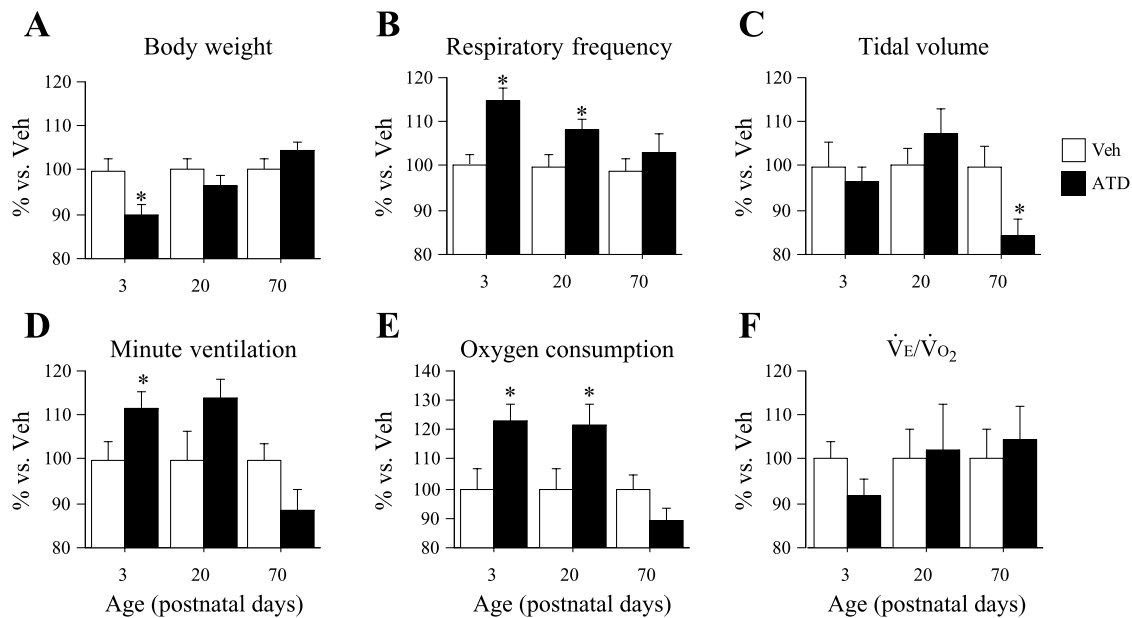


Fig. 2. Effects of prenatal ATD on baseline values for body weight (A), respiratory frequency (B), tidal volume (C), minute ventilation (D), oxygen consumption ($\dot{V}O_2$; E), and $\dot{V}_E/\dot{V}O_2$ ratio in 3-, 20-, or 70-day-old rats. For each age, values from rats born to Veh dams are considered 100% (mean \pm SE). * $P < 0.05$, ATD vs. Veh.

There was a similar decrease of $\dot{V}O_2$ between G14 and G20 in Veh (from 2.74 ± 0.16 to 2.14 ± 0.26 $\text{ml}\cdot\text{min}^{-1}\cdot 100$ g^{-1} , $P < 0.05$) and ATD (from 2.60 ± 0.25 to 2.00 ± 0.13 $\text{ml}\cdot\text{min}^{-1}\cdot 100$ g^{-1} , $P < 0.05$), leading to a similar increase of $\dot{V}_E/\dot{V}O_2$ between G14 and G20 for the two groups of dams (from 15.8 ± 1.3 to 20.1 ± 2.3 for Veh and from 15.5 ± 1.2 to 18.9 ± 0.9 for ATD dams). At birth, males and females born to ATD dams had reduced body weight (6.49 ± 0.17 vs. 6.96 ± 0.11 g for males, 6.29 ± 0.08 vs. 6.81 ± 0.11 g for females, $P < 0.0001$, ATD vs. Veh for both gender).

Gestational blockade of estradiol synthesis produces long-lasting alterations of respiratory and metabolic control in normoxic control conditions. Metabolic and respiratory results under normoxic conditions in rats born to ATD- and Veh-treated mothers are compiled in Fig. 2, A–F. Three-day-old male pups born to mothers treated with ATD had a lower body weight (8.53 ± 0.22 g; $n = 18$) compared with pups from Veh-treated dams (9.50 ± 0.23 g; $n = 15$; $P = 0.006$, Fig. 2A) that was no longer apparent in weanling (ATD 50.1 ± 1.4 g vs. Veh 52.2 ± 1.4 g) and adult rats (ATD 476 ± 10 g vs. Veh 468 ± 12 g; Fig. 2A). Compared with rats born to Veh-treated mothers, rats from ATD mothers presented higher respiratory frequency at 3 and 20 days of age (171 ± 5 vs. 149 ± 4 and 148 ± 3 vs. 132 ± 4 breaths/min, $P = 0.002$ for both ages, Fig. 2B), higher \dot{V}_E in 3-day-old rats (157 ± 6 vs. 141 ± 6 $\text{ml}\cdot\text{min}^{-1}\cdot 100$ g^{-1} , $P = 0.05$, Fig. 2D) and a tendency toward higher level in 20-day-old rats also (126 ± 4 vs. 100 ± 7 $\text{ml}\cdot\text{min}^{-1}\cdot 100$ g^{-1} , $P = 0.07$), and increased $\dot{V}O_2$ at 3 and 20 days of age (7.38 ± 0.35 vs. 6.00 ± 0.43 and 4.43 ± 0.24 vs. 3.65 ± 0.24 $\text{ml}\cdot\text{min}^{-1}\cdot 100$ g^{-1} ; $P = 0.02$ and 0.04 , respectively, Fig. 2E). The corresponding $\dot{V}_E/\dot{V}O_2$ was not altered at any age after ATD treatment (Fig. 2F). Adult rats born to ATD-treated dams had lower tidal volume under normoxia than rats from Veh-treated mothers (0.32 ± 0.01 vs. 0.39 ± 0.02 ml/100 g, $P = 0.008$, Fig. 2C), but they had similar \dot{V}_E

(35.1 ± 2.5 vs. 39.4 ± 1.1 $\text{ml}\cdot\text{min}^{-1}\cdot 100$ g^{-1} ; $P = 0.2$, Fig. 2D), $\dot{V}O_2$ (2.08 ± 0.10 vs. 2.33 ± 0.10 $\text{ml}\cdot\text{min}^{-1}\cdot 100$ g^{-1} ; $P = 0.1$, Fig. 2E), and $\dot{V}_E/\dot{V}O_2$ ratio (18.3 ± 1.6 vs. 17.7 ± 1.6 ; $P = 0.8$, Fig. 2F).

Gestational blockade of estradiol synthesis produces long-lasting alterations of hypoxic ventilatory and metabolic responses. When exposed to a brief hyperoxic period (Dejours test), 3-day-old pups born to Veh-treated mothers decreased \dot{V}_E (from 142 ± 6 to 127 ± 3 $\text{ml}\cdot\text{min}^{-1}\cdot 100$ g^{-1} , $P < 0.02$) and respiratory frequency (from 148 ± 4 to 117 ± 4 breaths/min, $P < 0.0001$), while ATD pups had a reduced response (\dot{V}_E from 159 ± 6 to 154 ± 6 $\text{ml}\cdot\text{min}^{-1}\cdot 100$ g^{-1} , $P =$ not significant; respiratory frequency from 172 ± 5 to 148 ± 4 , $P < 0.0001$) compared with Veh (see Fig. 3).

In weanling rats, the hypoxic ventilatory response was blunted by prenatal blockade of estradiol synthesis (Figs. 4 and 5); this pattern was not apparent in adults (Figs. 4 and 5). At the end of the hypoxic exposure (12% O_2 ; 30 min), weanling rats

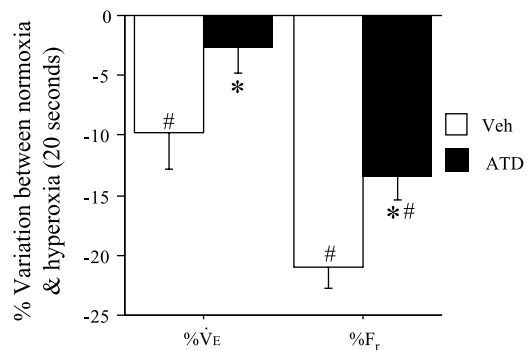


Fig. 3. Percent changes of respiratory variables (\dot{V}_E and F_T) during the first 20 s of hyperoxic exposure in newborn rats born to Veh- or ATD-treated mothers. # $P < 0.05$, hyperoxia vs. normoxia; * $P < 0.05$, ATD vs. Veh.

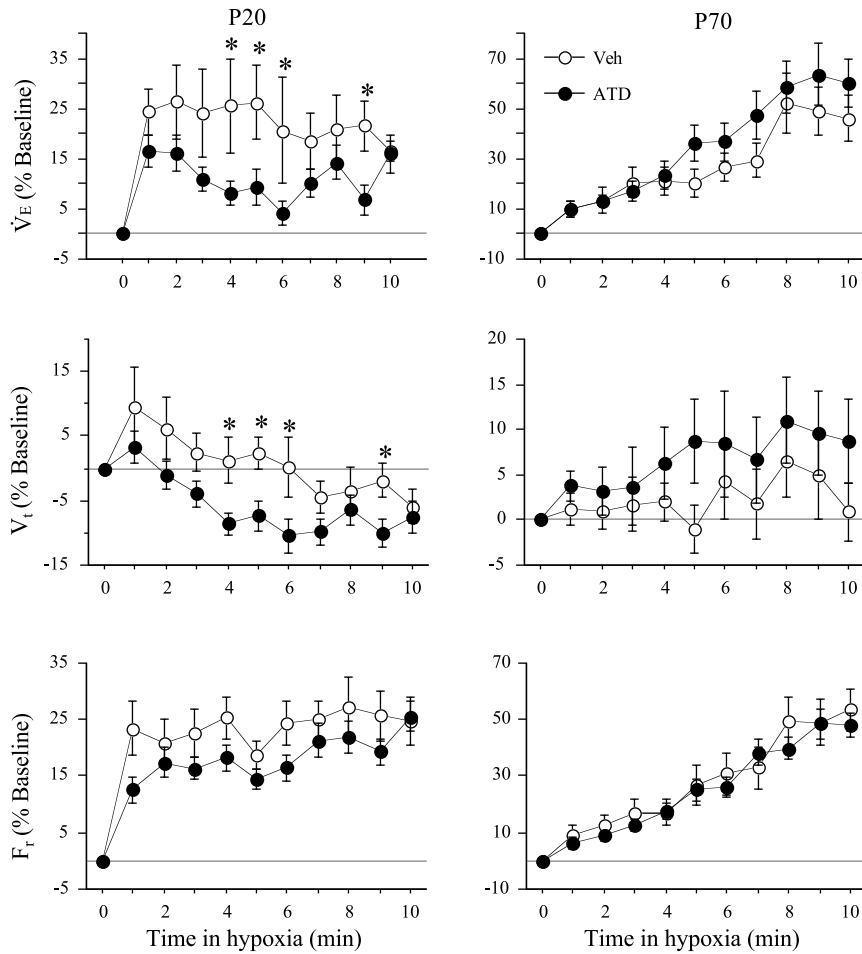


Fig. 4. Normalized minute-by-minute hypoxic ventilatory response in weanling [postnatal day 20 (P20), left] and adult rats (P70, right) born to Veh- or ATD-treated mothers during 10 min of hypoxic exposure (12% O₂). Ve (ml·min⁻¹·100 g⁻¹), Vi (ml/100 g), and Fr (breaths/min) are expressed as % of baseline values. All values are means ± SE. *P < 0.05, ATD vs. Veh.

born to Veh-treated mothers showed the classical decrease of $\dot{V}O_2$ (from 3.65 ± 0.24 ml·min⁻¹·100 g⁻¹ to 2.87 ± 0.33 , $P < 0.02$, Fig. 5), but this effect was not apparent in rats born to ATD dams (from 4.43 ± 0.24 to 4.04 ± 0.21 , $P = 0.14$); the resulting $\dot{V}E/\dot{V}O_2$ at the end of the hypoxic exposure was lower in pups from ATD-treated mothers vs. pups from Veh-treated mothers (35.7 ± 2.4 vs. 51.4 ± 6.1 , $P = 0.009$, Fig. 5). The same pattern was observed in adult rats for $\dot{V}O_2$ (decrease from 2.33 ± 0.10 to 1.65 ± 0.10 ml·min⁻¹·100 g⁻¹ in Veh rats, $P < 0.001$ and from 2.08 ± 0.10 to 1.94 ± 0.10 ml·min⁻¹·100 g⁻¹ for ATD rats; $P =$ not significant), and $\dot{V}E/\dot{V}O_2$ (increased from 16.8 ± 1.3 to 41.9 ± 3.3 in Veh and from 17.3 ± 1.1 to 30.6 ± 1.5 in ATD rats, $P < 0.002$ Veh vs. ATD in hypoxia). There was no difference in rectal temperature between ATD and Veh rats in normoxia at weaning ($36.9 \pm 0.1^\circ\text{C}$ in both

groups) or in adults ($38.1 \pm 0.1^\circ\text{C}$ in both groups), and the magnitude of rectal temperature drop after 30 min of hypoxic exposure was similar in ATD or Veh rats ($\sim 1^\circ\text{C}$).

DISCUSSION

The results of this study are consistent with the admitted hypothesis that the increase of circulating estradiol during late gestation participates in the respiratory adjustments observed during late pregnancy (13, 33) and demonstrate that fetal exposure to estradiol is required for normal development of respiratory and metabolic control. In newborn, weanling, and adult rats born to ATD-treated mothers, the alterations of respiratory and metabolic control included 1) increased resting $\dot{V}O_2$, 2) increased respiratory frequency and $\dot{V}E$, 3) decreased

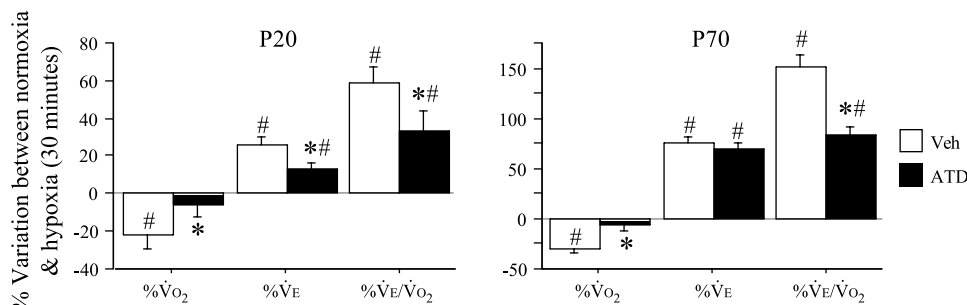


Fig. 5. Percent changes of metabolic and respiratory parameters between normoxia and hypoxia exposure (12% O₂; 30 min) in weanling (P20; left) and adult (P70; right) rats born to Veh- or ATD-treated dams. $\dot{V}O_2$ (ml·min⁻¹·100 g⁻¹), $\dot{V}E$ (ml·min⁻¹·100 g⁻¹), and $\dot{V}E/\dot{V}O_2$ ratio. # $P < 0.05$, hypoxia vs. normoxia; * $P < 0.05$, ATD vs. Veh.

sensitivity of the peripheral chemoreceptors (assessed by Dejours test in newborn rats), and 4) altered metabolic response to hypoxia in weanling and adult rats. These effects reflect the potential diversity of action of steroid hormones such as estradiol on fetal respiratory and metabolic control development.

Methodological considerations. We used a derivate (ATD) of androstenedione that has been specifically designed as a potent and selective inhibitor of *P*-450 aromatase enzyme (the key enzyme for estradiol synthesis) and that irreversibly binds to the active site of the enzyme as a competitor to endogenous substrate (37). The *P*-450 aromatase enzyme is a cytochrome protein converting testosterone to estradiol and androstenedione to estrone and is expressed at high level during late gestation in the placenta of primate (24), in the ovaries of rodent (1), and in some discrete brain areas of rat fetuses, where estradiol acts as a specific developmental factor (26, 44). ATD is highly effective in reducing the circulating levels of estradiol (12, 37) as well as immunostaining of brain aromatase (17) and aromatase mRNA concentration in hypothalamic preoptic area in quail (2). Because ATD binds irreversibly to the aromatase, recovery of complete estradiol synthesis is not completed until new aromatase protein is synthesized (37). In humans, after a single injection of an aromatase inhibitor showing similar properties (4-hydroxyandrostene-3,17-dione, 500 mg), the level of circulating estradiol is decreased by 50% for at least 7 consecutive days (12). Thus, if ATD crosses the placenta, it is highly probable that its effect will be maintained for several postnatal days in rat pups. Our own results show that in pregnant dams, ATD can be used to reduce the circulating levels of estradiol without producing an increase in the circulating levels of androstenedione and testosterone, the direct precursors for estradiol synthesis. On the other hand, this pharmacological model does not allow us to differentiate between the influences of maternal estradiol furnished to the fetus and in situ estradiol synthesis in fetal neurons (see below for further details). Nonetheless, this model is particularly interesting because it describes for the first time the role of endogenously secreted estradiol for the development of respiratory reflexes. Whether this is related to maternofetal estradiol transfer or depends on local (i.e., fetal) estradiol synthesis will require further experiments.

Effects of ATD in pregnant dams. Pregnant dams treated with ATD demonstrated consistent signs of impaired respiratory adjustments, including lower tidal volume compared with Veh-treated mothers, which is in good agreement with previous studies showing that ovarian steroids stimulate breathing almost exclusively by increasing tidal volume (22, 25, 35). This effect is mediated through central areas, including hypothalamic nuclei (6), and by a reduction of the dopaminergic inhibitory drive at the level of the peripheral chemoreceptors (22). The central facilitation of breathing by ovarian steroids requires the induction of progesterone receptors by estradiol in central areas (4–6). The present results clearly emphasize the critical importance of the stimulatory effects of estradiol for respiratory adjustments during late gestation in rats. Contrastingly, pups born to ATD-treated dams had specific alterations, thus revealing that estradiol synthesis is an important factor for adequate respiratory development.

Effects of ATD in pups. Three-day-old male pups born to treated mothers had increased metabolic rate and elevated

respiratory frequency and \dot{V}_E compared with pups from Veh-treated dams. The similar \dot{V}_E/\dot{V}_{O_2} ratio in treated pups compared with controls indicates that the direct drive of basal metabolic rate on breathing was not affected by prenatal hormonal blockade. Therefore, the increased \dot{V}_E likely results from the higher metabolic drive observed in treated pups. On the other hand, the decreased respiratory responses to acute hyperoxia (in newborn) and hypoxia (in weanling rats) in ATD pups strongly suggests that the responsiveness of the peripheral chemoreceptors to hypoxia has been altered. Nonetheless, and because a metabolic effect on respiratory integration cannot be excluded at that time, we will require more direct evidence to sustain (or not) this hypothesis. The metabolic and ventilatory alterations under normoxic or hypoxic conditions persisted until weaning (respiratory) and adulthood (metabolic), suggesting that these were not short-lasting effects of estradiol deficit, but rather long-term (or permanent) alterations of the respiratory and metabolic regulatory systems.

Developmental regulation of respiratory and metabolic control by estradiol. Direct fetal exposure to estradiol can occur either as the result of placental transfer from maternal circulation to the fetus or from local synthesis of estradiol in fetal brain. *P*-450 aromatase mRNA is expressed at high levels in the ovaries of pregnant female rats starting from G8 (1). In the fetal brain, immunohistochemical staining of *P*-450 aromatase protein appears at G13 in the hypothalamic preoptic area and is sustained at high level in hypothalamic nuclei from G16 to P7 in rats (44, 45). During this developmental window, testosterone (the endogenous precursor for estradiol synthesis by *P*-450 aromatase) can be provided to the fetus by the activity of testes in males (16) or as the result of local neurosteroid synthesis (48). Recent clues have accumulated showing that prenatal estradiol plays a crucial role in fetal development: estradiol receptors are expressed in numerous tissues in middle-gestation human fetuses (11), estradiol accelerates fetal lung maturation by stimulation of surfactant phospholipids synthesis (34), and, in fetal mice lacking the β -isoform of estrogen receptor, survival and migration of cortical neurons are both severely impaired during late gestation (46). Estradiol receptors (divided into nuclear and membrane bound) are located in a variety of neural populations, including hypothalamic and brain stem monoaminergic nuclei (see Refs. 8, 31, 32), which participate in ventilatory control under normoxic or hypoxic exposure (9, 14, 15, 20, 29) and may also contribute to the respiratory changes observed after prenatal blockade of estradiol synthesis.

Nonetheless, and because estradiol exerts a wide variety of action on different targets during development, it is difficult for the moment to propose a mechanistic framework to explain our results. The normal pattern of ventilation is generated in the pre-Bötzing complex of the lower brain stem (39) and is modulated by a variety of neural and visceral afferent messages emerging from central and peripheral chemoreceptors, pulmonary receptors (30), and suprapontic nuclei located for example within the hypothalamus (15). While the results of the acute hyperoxic exposure in newborn rats suggest an impairment of peripheral chemoreceptor activity in pups born to ATD-treated dams, we presently cannot further speculate on the origins of respiratory and metabolic changes observed in rats born to ATD-treated dams.

Our present findings may have valuable clinical implications. Recent studies emphasized the potential therapeutic benefits of replacement of circulating estradiol and progesterone at normal levels (such as observed in utero or immediately after delivery) in extreme preterm infants that are otherwise chronically deprived from the hormonal placental milieu (41–43). As discussed above, at that time our model cannot be used to discriminate between the maternofetal transfer and local fetal synthesis of estradiol on respiratory control system but describes for the first time the important developmental role of adequate prenatal estradiol synthesis for respiratory and metabolic control development.

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