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Progesterone increases hypoxic ventilatory response and reduces apneas in newborn rats

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Abstract

We hypothesized that progesterone may enhance the hypoxic ventilatory response and reduce the occurrence of apneas in newborn male rats. We studied 10-day-old rats chronically exposed to progesterone (Prog) or vehicle through the milk of lactating mothers. Respiratory and metabolic recordings were performed using whole body plethysmography under normoxia and during hypoxic exposure (10% O_2—30 min). While progesterone did not alter baseline breathing and metabolic rate, it increased hypoxic ventilatory response particularly by limiting the magnitude of the ventilatory roll-off during the second phase of the hypoxic ventilatory response (i.e. following 5 min of exposure). In parallel, progesterone lowered the number of spontaneous apneas and drastically reduced the occurrence of post-sigh apneas during hypoxic exposure by limiting the time of the post-sigh expiratory pause. Following domperidone injection (used to block peripheral D2 dopamine receptor), minute ventilation increased in Veh pups and the number of spontaneous apneas decreased. These responses were not observed in Prog pups, suggesting that progesterone reduces peripheral dopaminergic inhibition on breathing. We conclude that progesterone is a potent stimulant of hypoxic ventilatory response in newborn rats and effectively reduces the occurrence of apneas.

Keywords: Control of breathing; Newborn; Progesterone; Dopamine; Domperidone

1. Introduction

Progesterone has long been shown to exert a critical role on respiratory control in adults, acting both in the central nervous system (Bayliss et al., 1987, 1990) and on the peripheral chemoreceptors (Hannhart et al., 1989, 1990; Tatsumi et al., 1997). Combined with estradiol, progesterone reduces dopamine synthesis in peripheral chemoreceptors and abolishes dopaminergic inhibition on breathing mediated by D2 dopamine receptors (Joseph et al., 2002). Recently, progesterone has been shown to reduce the occurrence of apneas in adult rats (Yamazaki et al., 2005), while epidemiological studies have demonstrated the protective role of ovarian steroids against sleep-apneas in adult humans (Shahar et al., 2003; Young et al., 2003). Throughout gestation, the placenta synthesizes important amounts of progesterone resulting in elevated plasma levels both in human (Trotter and Pohlandt, 2000) and rat foetuses (Ward and Weisz, 1984), but the potential implications of this hormonal exposure on respiratory control development are unknown. These aspects may have some important implications since preterm delivery is commonly associated with threatening respiratory instabilities (i.e. apneas, Razi et al., 2002) or sudden infant death syndrome (Sorenson and Becker, 2003), which are both linked to impaired functions of respiratory control network, including peripheral chemoreceptors (Holgert et al., 1995; Al-Matary et al., 2004; Gauda et al., 2004). On the other hand, preterm birth also produces a chronic deprivation from placental steroids exposure, mainly progesterone and estradiol (Trotter et al., 1999, 2001), which may be a factor contributing to the development of respiratory instabilities in neonates.

Accordingly, we tested the hypothesis that chronic exposure to progesterone during postnatal days 1–10 in rats (a period that summarizes late foetal development of hypoxic ventilatory response in humans, Bissonnette, 2000) may: (i) improve hypoxic ventilatory response, (ii) reduce apneic events and (iii) reduce dopaminergic inhibition on breathing mediated by D2 dopamine receptors. Our results are consistent with these hypothesis, thus pointing-out progesterone as an important actor on respiratory control in neonates.

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2. Methods

2.1. Animals

All procedures have been approved by the local committee of animal care and use of Laval University and were in accordance with the guidelines of the Canadian Council on Animal Care.

Twenty-four to 36 h following birth, lactating mothers were slightly anesthetized under halothane and implanted (s.c.) with a subcutaneous osmotic pump (Alzet osmotic pumps, Cupertino, CA, USA) filled with 2 ml propylene glycol containing vehicle alone ($n = 7$, propylene glycol), or progesterone ($n = 7$, Prog—1 mg). Each pump delivered a regular flow of 140 μl/day during 14 days (i.e. 0,07 mg progesterone/day). All rats have been studied at postnatal day 10 and only males were used to avoid the potential confounding influence of gender on hypoxic ventilatory response in prepubertal rats (Mortola and Saiki, 1996). Pups from one litter for each group were sacrificed with an anesthetic overdose (ketamine/xylasine) and a sample of blood was drawn by cardiac puncture for ELISA assays of total plasma progesterone (performed accordingly to the manufacturer instructions—ALPCO Diagnostics, Salem, NH, USA).

2.2. Respiratory recordings

All respiratory recordings were performed in freely behaving, unrestrained rat pups on the 10th postnatal day by whole body flow-through plethysmography (Emka technologies, Paris, France) using a method derived from the pressure plethysmograph described by Bartlett and Tenney (Bartlett and Tenney, 1970). The recording chamber included a built-in reference chamber and was permanently flushed with fresh air at a constant flow rate. The temperature inside the chamber was fixed at 32.0 °C by a temperature control loop (Physitemp, Clifton, New Jersey, USA). Following adequate calibration of the chamber, temperature and relative humidity were constantly monitored during recordings and barometric pressure was read on a barometer for correction of tidal volume using the standard equation after integration of the flow signal by the Emka software (Bartlett and Tenney, 1970; Doan et al., 2004). During recordings, sub-samplings of inlet and outlet gas were dried and directed towards a dual channel oxygen analyzer (S-3A/II AEI technologies) and a carbon dioxide analyzer (CD3A, AEI technologies), the flow through the chamber was continuously recorded by a mass flowmeter (TSI series 4140 mass flowmeter—TSI, Shoreview, MN, USA). All signals (from the plethysmograph, gas analyzers and flowmeter) were directed towards a computer for storage and online calculation of respiratory parameters, CO_2 production ($\%CO_{2_{out}} \times$ flow) and O_2 consumption ($[O_{2_{in}} - O_{2_{out}}] \times$ flow) using the IOX software (Emka Technologies).

2.3. Hypoxic ventilatory response

Pups from three Veh and four Prog dams were used in this study. Following a period of habituation of 10–15 min, the chamber was opened to measure rectal temperature (mice thermocouple rectal probe—Physitemp) and the animal was replaced in the chamber for an additional 10 min before the onset of recordings. When the animal laid quiet in the chamber, normoxic recordings were performed for 15–20 min, then inflowing air was switched to the hypoxic gas mixture and maintained at 10% O_2 for 30 min. At the end of the recordings, the chamber was opened and rectal temperature immediately measured. Hypoxic ventilatory response was either assessed as the relative changes of respiratory variables between normoxia and the mean of the last 5 min of exposure, or as minute-by-minute changes of respiratory variables during the first 10 min of exposure.

2.4. Respiratory response to peripheral D2 dopamine receptors antagonist

Pups from three Veh and three Prog dams (that were not used for the previous study) were used in this study. On the day of recordings, rat pups received a saline vehicle injection (i.p., 50 μl) before entering the recording chamber. When the animal laid quiet in the chamber, respiratory recordings were performed for 15–20 min, rectal temperature was measured and the animal received an i.p. injection of domperidone (10 mg/kg in 50 μl saline – Sigma–Aldrich – highly specific and strictly peripheral D2 dopamine receptor antagonist), before being returned to his nursing mother. Two hours following injection (plasmatic domperidone half-life being around 7–9 h), the pup was replaced in the recording chamber, allowed 10–15 min of habituation and respiratory recordings were performed under similar conditions. At the end of the experiments, all pups were sacrificed with an anesthetic overdose (ketamine/xylasine).

2.5. Apnea index

All respiratory recordings were analyzed on a computer display to report the apneic index as the number of apneas per hour. We used standard criteria (Mendelson et al., 1988) to identify apneas as an absence of flow for at least two normal respiratory cycles, corresponding to a period of 700 ms in normoxia and 500 ms in hypoxia. As classically reported, two types of apneas were distinguished, spontaneous apneas and post-sighs apneas preceded by a breath with amplitude at least twice the resting tidal volume. Since some sighs did not resulted in standard apneas as defined above, we also report the total number of sighs and the proportion of sighs leading to apneas under normoxia and hypoxia. Fig. 1 shows typical recordings of spontaneous apnea in normoxia, a sigh followed by a significant apnea (in hypoxia), and a sigh followed by a respiratory pause that did not exceeded 500 ms (also in hypoxia).

2.6. Statistical analysis

All responses (to hypoxia or domperidone injection) were tested using a two-way ANOVA analysis for repeated measures, followed by one-way factorial analyses to test group effects. All values are mean ± S.E.M., and the level of significance was set at 0.05. All analyses have been done with the Statview 5.0 or JMP 5.1.2 softwares.