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Ventilatory chemosensitivity and thermogenesis of the chicken hatchling after embryonic hypercapnia

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ABSTRACT

Hypoxia during incubation results in hatchlings with a reduced thermogenic capacity and a blunted ventilatory ($\dot{V}_E$) chemosensitivity (Szdzuy, K., Mortola, J.P., 2007b. Ventilatory chemosensitivity of the 1-day-old chicken hatchling after embryonic hypoxia. Am. J. Physiol. (Regul. Integr. Comp. Physiol.) 293, R1640–R1649). We asked if similar effects occurred with embryonic hypercapnia, that is, with a non-hypoxic sustained stimulation of the chemoreceptors. White Leghorn chicken eggs were incubated at 38 °C either in air (controls, C) or in 4% CO_2 from embryonic day 5 (4% CO_2), hatching included. The 4% CO_2 embryos hatched about 12 h later than C, with similar body weight. On the day of hatching the thermogenic capacity, assessed from the changes in oxygen consumption ($\dot{V}_{O_2}$) during 1 h at 30 °C, increased from the early (about 3 h old) to the late hours (about 20 h old), and was similar between 4% CO_2 and C. Ventilatory chemosensitivity was evaluated from the changes in ($\dot{V}_E$) and in ventilatory equivalent ($\dot{V}_E/\dot{V}_{O_2}$) during acute hypoxia (15 and 10% O_2, 20 min each) or hypercapnia (2 and 4% CO_2, 20 min each). Both at the early and late hours ($\dot{V}_E$) chemosensitivity was lower in 4% CO_2 than in C. The $\dot{V}_E/\dot{V}_{O_2}$ responses of 4% CO_2 in hypoxia and hypercapnia averaged, respectively, about 45 and 60% of C. A separate set of eggs incubated in 2% CO_2 gave results qualitatively intermediate between C and 4% CO_2. We conclude that prenatal hypercapnia does not compromise the newborn's thermogenesis, but, like hypoxia, affects the development of respiratory control, resulting in a blunted chemosensitivity.

1. Introduction

Perinatal development in conditions of altered oxygen environments can cause long-lasting alterations of the ventilatory chemosensitivity (Carroll, 2003, for review; Bavis et al., 2006). Neonatal exposure to chronic hypoxia (Okubo and Mortola, 1988, 1990), intermittent hypoxia (Reeves et al., 2006) or hyperoxia (Ling et al., 1996, 1997a) causes a reduction in the hypoxic ventilatory response of rats that appears to be very long-lasting (Ling et al., 1998; Fuller et al., 2002). Also life-long at high altitude can be characterised by an attenuated hypoxic ventilatory response (Brutsaert, 2007) that may persist years after return to sea level (Lahiri et al., 1976; Moore, 2000). Also chicken hatchlings born after incubation in hypoxia (15% O_2) had a blunted ventilatory response to both hypoxia and hypercapnia (Szdzuy and Mortola, 2007b). In addition, they presented a decreased thermogenic capacity (Azzam et al., 2007), similarly to infants at high altitude (Frappell et al., 1998). This latter aspect is important because the hypometabolism obtained through the decreased thermogenesis is a primary mechanism to hyperventilate, that is, to increase the ventilation-metabolism ratio (Mortola and Gautier, 1995; Mortola, 1999).

Various mechanisms could be responsible for these effects on chemosensitivity following perinatal changes in oxygenation, and modifications of the chemoreceptor function are among the most probable candidates (Ling et al., 1997b; Carroll, 2003). Hence, one may expect that not only hypoxia, but also hypercapnia, that is, a non-hypoxic sustained stimulus of the chemoreceptors, may have some impact on the normal development of ventilatory chemosensitivity. Some studies have considered the effects of early exposure to hypercapnia on the development of the ventilatory response to hypercapnia, but the results are not uniform. Adult rats maintained in hypercapnia during the perinatal period presented only a minor, and short-lasting, attenuation of their hypercapnic ventilatory response (Rezzonico and Mortola, 1989; Bavis et al., 2006), or no effects at all (Birchard et al., 1984). By contrast, adult zebra finches and Japanese quails exposed to 2% CO_2 during the embryonic period had an attenuated ventilatory response to hypercapnia. However, this effect was present only in some groups of the study population and only for high level of hypercapnia (Williams and Kilgore, 1992; Bavis and Kilgore, 2001). No study has examined the

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effects of embryonic hypercapnia on the development of the ventilatory response to hypoxia. Accordingly, the purpose of the current study was to examine the effects of hypercapnia maintained during the embryonic period on the ventilatory responses to hypoxia and to hypercapnia of the newborn and the metabolic contribution to these responses. Thermogenesis (i.e., the metabolic response to an abrupt drop in ambient temperature) was also measured as an independent assessment of the development of metabolic control. The avian embryo was chosen as experimental model to circumvent some of the interpretative issues introduced by mammalian models, in which maternal and placental responses can by themselves have a major impact on fetal development (Fernandez-Cano, 1958; Moore et al., 1986).

2. Methods

Freshly laid fertilized eggs of White Leghorn chickens (*Gallus gallus*) were obtained from a local supplier. The eggs were weighed and placed in incubators (Hova-Bator, Savanah, GA, USA), starting around midday (embryonic day E0). The incubators maintained a steady temperature of 38 °C and 60% relative humidity, and provided a 45° egg rotation four times a day. At first, all eggs were incubated in normocapnic conditions. Then, at E5, they were separated into two groups. Some continued in normocapnia (0% CO_2), others were transferred into an incubator kept at 4% CO_2 (on average 3.53% ± 0.88 S.D., including the times of incubator openings for cleaning and eggs transfer). These two groups will be referred to as, respectively, controls, (C) and 4% CO_2-incubation (4% CO_2). The desired level of hypercapnia was obtained by leaking a small stream of warmed and humidified CO_2 into the incubator from a pressurised tank, under the control of a flowmeter. The CO_2 concentration within the incubator was continuously sampled and analyzed by a calibrated fuel cell gas analyzer (CA-1B CO_2 analyzer, Sable Systems Int., Las Vegas, NV) and displayed on a computer monitor. A data logger (HOBO, Onset Computer Corporation, Bourne, MA) inside the incubator monitored the incubation conditions, by collecting the temperature and humidity values every 10 min.

Experiments were conducted on the hatchlings on the day of hatching (H1), either within the first 8 h (<8 h, *Early*) or later during the day (12–24 h, *Late*). They consisted in the metabolic and ventilatory responses to hypercapnia and hypoxia. In separate groups of C and 4% CO_2 hatchlings thermogenesis was measured as an independent assessment of metabolic control in normoxic conditions.

Egg weights at the start of incubation and the body weight (W) of the hatchlings were measured on a digital scale; the time of hatching was also noted. Body temperature was measured by gently inserting a fine tungsten–constantan thermocouple connected to a digital thermometer (Omega 871A, Omega Engineering Inc., Stamford, CT) for about 15 mm into the cloaca. These temperature measurements were taken in air and at the end of the hypoxic or hypercapnic exposures, and before and after the thermogenesis test.

2.1. Gaseous metabolism

Metabolic rate was measured by indirect calorimetry (oxygen consumption $\dot{V}_{O_2}$ and carbon dioxide production, $\dot{V}_{CO_2}$), with an open-flow methodology (Frappell et al., 1992) adapted to the chicken embryo (Menna and Mortola, 2002, 2003). The hatchling was placed in a respirometer, which consisted of a 300-ml plastic container maintained at the desired temperature by a water bath. A steady gas flow of 150–200 ml/min was continuously delivered through the respirometer, under the control of a precision needle–valve flowmeter. After passing through a drying column, the inflowing and outflowing gases were monitored by calibrated gas analyzers (O_2 analyzer: Foxbox, Sable Systems Int., Las Vegas, NV, or OM-11, Beckman; CO_2 analyzer: CD-3A, AEI Technologies, Naperville, IL) arranged in series. Outflowing gases were monitored continuously, while the inflowing gases were sampled intermittently to test the stability of the analyzers. The output of the analyzers was displayed on a computer monitor during on-line acquisition. $\dot{V}_{O_2}$ and $\dot{V}_{CO_2}$ were computed from the flow rate and the inflow–outflow concentration difference. The values calculated at standard temperature, pressure, and dry conditions are presented in ml/min.

2.2. Thermogenic response to cold

After 30-min acclimatization to the respirometer, gaseous metabolism was measured continuously for additional 30 min at 39 °C, and for 1 h following an abrupt drop in water bath temperature to 30 °C. $\dot{V}_{O_2}$ and $\dot{V}_{CO_2}$ were computed as averages of 10 min time intervals. To analyze the thermogenic responses, first, for each animal, a plot of $\dot{V}_{O_2}$ versus time was constructed beginning from the first 30 min at 39 °C until the end of the 1-h exposure to 30 °C. The graph, plotted on fixed co-ordinates, was scanned and the areas under the warm and the cold parts of the curve were digitally computed (SigmaScan, Jandel Scientific). The area (ml) divided by the time duration (min) gave the average $\dot{V}_{O_2}$ (ml/min) at 39 and 30 °C; the ratio between the latter and the former represented the average magnitude of the thermogenic response to cold (Szdzuy et al., 2008).

2.3. Pulmonary ventilation

The breathing pattern was measured with the barometric technique originally proposed by Drorbaugh and Fenn (1955). It consists of recording the changes in chamber pressure generated during breathing, as the inspired air gains heat and humidity in its passage from the chamber to the animal's airways. In the case of embryos or hatchlings some modifications were needed to make sure that the animal was at its normal ambient temperature (about 37.5 °C) while the system retained a temperature gradient between chamber and animal sufficiently large to guarantee the accuracy of the signal (Mortola and Frappell, 1998). Full details of the methodology adapted to the hatchling, of its verification, and its application in combination with measurements of gaseous metabolism have been given elsewhere (Szdzuy and Mortola, 2007a). In essence, the animal chamber is separated into two sections, a smaller compartment acting as 'nest', of about 100 ml, where the hatchling was positioned, and a larger outer compartment of about 200 ml. The nest temperature, controlled by a water bath, was monitored by telemetry. Three polyethylene tubes passed through the lead of the container. Two of these lines were the inflow and outflow ports for continuous flushing with the desired gas mixture. The third line was for the recording of the pressure oscillations related to breathing, via a sensitive pressure transducer. The volume calibration K of the whole chamber was obtained by injecting a known volume with a micro-syringe (V_{cal}) and recording the corresponding change in pressure (P_{cal}); $K = V_{cal}/P_{cal}$. Hence, tidal volume (V_T) was calculated from K, temperature and water vapor pressure of the hatchling and the corresponding values of the chamber (Drorbaugh and Fenn, 1955; Szdzuy and Mortola, 2007a).

The inflow line to the chamber was connected to air or to a gas impermeable 10-l bag for the delivery of hypoxic (10 or 15% O_2) or hypercapnic (2 or 4% CO_2) mixtures. The outflow line was connected to a suction pump, which maintained a steady flow of 150 ml/min, arranged in series with O_2 and CO_2 gas analyzer. For the recording of the breathing pattern, the flow through the