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The hypometabolic response to repeated or prolonged hypoxic episodes in the chicken embryo

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ABSTRACT

Hypoxia (hx) in embryos causes a drop in oxygen consumption ($\dot{V}_{O_2}$) that rapidly recovers upon return to normoxia. We asked whether or not this pattern varies with the embryo's hypoxic history. The $\dot{V}_{O_2}$ of chicken embryos in the middle (E12) or at end-incubation (E19) was measured by an open-flow methodology during 15-min epochs of moderate (15% O_2) or severe hx (10% O_2). Each hx-epoch was repeated or alternated with air by various modalities (air-hx-air-hx-air-hx-air, air-2·hx-air-2·hx-air, air-5·hx-air), in randomized sequences. The hx drop in $\dot{V}_{O_2}$ was larger with severe than with moderate hx; however, in either case, its magnitude was essentially independent of the preceding hx history. E19 embryos had hx drops in $\dot{V}_{O_2}$ of the same magnitude whether their incubation was in air or in moderate hx from E4 to E19. A different protocol (air-12·hx-air) gave variable results; with moderate hx, the $\dot{V}_{O_2}$ response was similar to that of the other hx regimes. Differently, with severe hx most embryos progressively decreased $\dot{V}_{O_2}$ and eventually died. We interpret these data on the basis of what is known on the 'compensatory partitioning' between costs of growth and maintenance. With moderate hx presumably each episode caused an energy shortfall absorbed entirely by the blunted growth. Hypoxic events of this type, therefore, should have no long-term functional effects other than those related to the small birth weight. Differently, the aerobic energy shortfall with severe hypoxia probably impinged on some maintenance functions and became incompatible with survival.

1. Introduction

Since the early observations in human infants (Cross et al., 1958) the drop in oxygen consumption ($\dot{V}_{O_2}$) has been recognized to be part of the response to hypoxia of many mammalian species (Hill, 1959; Taylor, 1960; Mortola et al., 1989; Frappell et al., 1992). In newborns, hypoxic hypometabolism can be particularly pronounced and has been extensively studied for its implications on ventilatory and thermal control, the newborn's survival and therapeutic interventions (reviewed in Mortola, 2004).

Like postnatally, hypoxia lowers $\dot{V}_{O_2}$ also prenatally. In humans as in other mammalian species the occurrence of hypometabolism is inferred indirectly from the fact that maternal hypoxia during gestation blunts the growth of the fetus (Mortola et al., 2000). In avian eggs, the embryo's $\dot{V}_{O_2}$ can be measured directly more easily than in the mammalian fetus and is not confounded by maternal, placental and uterine responses. In chicken embryos acute (15–30 min) epochs of moderate (15% O_2) or severe hypoxia (10% O_2) decreased $\dot{V}_{O_2}$ (Bjønnes et al., 1987; Ar et al., 1991; Tazawa et al., 1992). Then, upon return to normoxia, $\dot{V}_{O_2}$ did not overshoot significantly above the pre-hypoxic level (Mortola and Besterman, 2007; Mortola et al., 2012), meaning that anaerobic energy sources did not compensate the shortfall in aerobic energy production and that the hypoxic decrease in $\dot{V}_{O_2}$ reflected the decrease in metabolic rate ('hypoxic hypometabolism').

Whether or not the magnitudes of the hypometabolic response and of the post-hypoxic recovery persist unaltered with repeated episodes of hypoxia has received only superficial attention, although the question has mechanistic and clinical interest. In humans, short episodes of fetal hypoxia of maternal origin can be a recurring phenomenon, like during labor or in cases of pre-eclampsia, maternal infection or cigarette smoking (Hutter et al., 2010), and intermittent hypoxia can produce damage more severe than sustained hypoxia (Tan et al., 1999). The only information we have on the metabolic response to repeated hypoxic episodes originates from postnatal observations. In 1-3-day old and 2-week old rabbits, $\dot{V}_{O_2}$ dropped by a similar magnitude for each of five successive 10-min exposures to 10% O_2 (Trippenbach, 1994). In a previous experiment on neonatal rabbits, the drop in $\dot{V}_{O_2}$ remained

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stable for up to 4 h of hypoxia (Blatteis, 1964). Similar observations prenatally have not been done and their outcome may depend on which embryonic functions are curtailed during the hypoxic drop in $\dot{V}_{O_2}$. During embryonic development the major energetic cost is for body growth and maintenance (Mortola and Cooney, 2008; Rombough, 2011; Mortola et al., 2013). If the reduction in growth was the only function curtailed by hypoxia, it is conceivable that the hypometabolic response could repeat itself similarly for each recurring episode until, eventually, the uneven development of the various organs may compromise survival (Sant'Anna and Mortola, 2003; Azzam and Mortola, 2007). Indeed, chicken embryos can sustain moderate hypoxia (15% O_2) for a large fraction of incubation and hatch successfully, albeit with small body weight during fetal growth and a delayed development of some regulatory processes (Azzam et al., 2007; Szdzuy and Mortola, 2007; Mortola, 2009). Differently, if the energy shortfall tapped into the maintenance component of the energy budget, the magnitude of the hypoxic drop in $\dot{V}_{O_2}$ and of its post-hypoxic recovery may depend on which organ functions have been compromised; this, in turn, could influence the metabolic response to the following hypoxic episodes. As embryonic development progresses the maintenance component becomes a larger fraction of resting $\dot{V}_{O_2}$, because the cost of maintenance increases with body size (Mortola and Cooney, 2008; Rombough, 2011). Therefore, whether or not the hypoxic hypometabolic response varies with the number of hypoxic episodes should depend on at least two variables, the age of the embryo (which influences the proportion of the energy budget partitioned between growth and maintenance) and the degree of hypoxia (which influences the magnitude of the energy shortfall).

The current study on chicken embryos investigates to what extent the hypoxic drop in $\dot{V}_{O_2}$ and its post-hypoxic recovery vary depending on the hypoxic history, that is, the succession and duration of the preceding hypoxic episodes. We have considered two periods of incubation. The end of incubation is of special interest because the embryo often is O_2-limited, owing to the fact that its rising metabolic requirements are not met by corresponding increases in O_2 availability (Høiby et al., 1983; Tazawa et al., 1992; Mortola, 2009); hence, the hypometabolic effects of hypoxia are expected to be larger than earlier in incubation. We have repeated the same observations at mid incubation, when the chorioallantoic membrane is complete but the embryo is only 20% of its final weight (Mortola and Al Awam, 2010); at this time, therefore, the embryo's O_2 needs are small by comparison to its O_2 availability.

2. Methods

Experiments were conducted on chicken (*Gallus gallus*) eggs of the White Leghorn variety purchased from a local supplier. After noting the fresh weight, the eggs were placed in incubators (Hova-Bator, Savannah, GA) set at the temperature of 37.5 °C and 60% relative humidity, both monitored by a data logger (Hobo®, Onset Computer Corp., Bourne, MA), with a 90° egg rotation eight times a day. Incubation started at midday (embryonic day E0).

2.1. Measurements of $\dot{V}_{O_2}$

Oxygen consumption ($\dot{V}_{O_2}$) and carbon dioxide production were measured by an open flow methodology adapted to the chicken embryo (Mortola and Labbè, 2005; Szdzuy et al., 2008). The egg was placed inside a respirometer, which consisted in a 120-ml plastic container almost entirely submerged into water and maintained at the desired temperature (37.5 °C) by a circulating water bath. The temperature (Ta) of the respirometer was collected by telemetry via a transmitter powered by an energizer-receiver unit (4000E, Minimitter, Sunriver, OR). A flow of 100 ml/min, under the control of a mass flow meter (Sable Systems International Fox, Henderson, NV), continuously passed through two openings in the lid of the respirometer and was maintained by a negative pressure pump located downstream the circuitry. The outflow O_2 and CO_2 concentrations were recorded continuously by calibrated gas analyzers (Sable Systems International Fox, Henderson, NV) arranged in series, after the gas had passed through a drying column (anhydrous calcium sulfate, Drierite®, Hammond, Xenia, OH); the inflow concentrations were monitored intermittently. The inflow lid of the respirometer was connected to an impermeable 15 l or 30 l bag filled with the gas mixture of interest. The wash-out time of the respirometer (with the egg inside) and connecting tubings was measured as the time needed to detect a rapidly injected bolus of CO_2; it was ~20 s. The gas flow, O_2 and CO_2 concentrations and Ta were continuously displayed on a computer monitor. The gas fractional concentrations were mathematically corrected for the error introduced by a respiratory exchange ratio different from unity (Depocas and Hart, 1957; Mortola and Besterman, 2007); then, $\dot{V}_{O_2}$ corresponded to the product of flow rate and inflow-outflow O_2 concentration difference. The $\dot{V}_{O_2}$ values were calculated at standard temperature, pressure and dry conditions in μl/min.

2.2. Protocols

A first set of experiments (protocol 1) was performed on 24 embryos divided into four groups according to age and level of hypoxia (hx). They were studied at embryonic day E12 (N = 12) or at E19-E20 (N = 12), out of 20.5 days of incubation length; none of the embryos of the older group had entered the internal pipping phase at the beginning of the measurements. Embryos were subjected to either moderate hx (nominal 15% O_2; N = 6 per age group) or severe hx (nominal 10% O_2; N = 6 per age group); hence, no embryo was studied at more than one age and one hx level. The egg was left in the respirometer at least one hour to reach temperature equilibration (~37.5 °C) and gas exchange stability. One protocol consisted of three runs of seven 15-min epochs each. The sequence of the epochs was air-hx-air-hx-air-hx-air (run 1), air-hx-hx-air-hx-hx-air (run 2), air-hx-hx-hx-hx-hx-air (run 3). The order of the three runs alternated systematically among embryos to eliminate the possibility of carry-over effects. Because each run lasted 15·7 = 105 min, the whole recording period was 5 h and 15 min long. During this time, the $\dot{V}_{O_2}$ of embryos close to term remains approximately constant (Mortola and Al Awam, 2010), likely because of the limitation in O_2 availability. Differently, at E12 the embryo is in a phase of rapid growth, and over the period of one run (105 min) its $\dot{V}_{O_2}$ is expected to increase by 3.5% (Mortola and Cooney, 2008; Mortola and Al Awam, 2010). This estimate was confirmed in a separate group of E12 embryos (N = 6) exposed to air for the duration of one full run (105 min); their $\dot{V}_{O_2}$ increased by 3.3% (±0.9). This increase has implications on the interpretation of some results (see Section 3.1).

A different protocol (protocol 2) was conducted on a new set of E12 (N = 15) and E19 embryos (N = 12). In this case hx (either ~15% O_2 or ~10% O_2) was maintained for twelve 15-min epochs (3 h), preceded and followed by an epoch in air.

Lastly (protocol 3), measurements of the $\dot{V}_{O_2}$ response to an acute episode of hx (10% O_2 for 75 min, preceded and followed by 15 min in air, like run 3 of protocol 1) were conducted on E19 embryos (N = 6) maintained in 15% O_2 for almost the whole incubation (E4-E19). The chronic hx of this set of eggs was obtained by bleeding the incubator with a stream of warm and humidified N_2, under the control of a precision flow-meter, to maintain the O_2 concentration at 15%, continuously checked by an O_2 analyzer and displayed on a computer monitor (Ferner and Mortola, 2009).