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Hypercapnic thresholds for embryonic acid–base metabolic compensation and hematological regulation during CO_2 challenges in layer and broiler chicken strains

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ABSTRACT

Time specific acid–base metabolic compensation and responses of hematological respiratory variables were measured in day 15 layer (Hyline) and broiler (Cornish Rock) chicken embryos during acute hypercapnic challenges (3, 6, 10 and 20% CO_2). Control acid–base status and hematology differed between two strains. Broiler embryos were relatively respiratory acidotic and had higher hematocrit (Hct) and hemoglobin concentration. The partial metabolic compensation for respiratory acidosis produced by ≤10% CO_2 exposures occurred in proportion to CO_2 concentrations in both strains, but metabolic compensation for 20% CO_2 respiratory acidosis was depressed at 2, 6 and 24 h, particularly in broiler embryos. Exposure to ≤10% CO_2 induced the same hematological responses across CO_2 concentrations; i.e., Hct and mean corpuscular volume (MCV) increased while RBC concentration remained unchanged. In response to 20% CO_2 exposure, Hct and MCV increased dramatically in both stains. Consequently, altered acid–base and hematology responses to 20% CO_2 exposure compared to ≤10% CO_2 suggest that the hypercapnic threshold to compensation for acidosis and regulation of hematology is >10% CO_2.

1. Introduction

Gas exchange of an avian embryo developing inside its egg takes place by molecular gas diffusion through a porous eggshell between the environment and the blood in the chorioallantoic capillaries. Consequently, the diffusion of respiratory gases is governed by gas conductance of the eggshell. In chickens, the eggshell gas conductance is quite variable between eggs, causing large variations of P_{CO_2} in the air cell, and thus blood P_{CO_2} (Tazawa et al., 1983a; Visschedijk et al., 1985). In addition, some bird species lay eggs in burrow nests where embryos can experience a hypercapnic and hypoxic environment (White et al., 1978; Wickler and Marsh, 1981; Boggs et al., 1983). Therefore, in general, it is presumed that avian embryos should be able to demonstrate a high tolerance to hypercapnic environments. Indeed, exposures to 5–20% CO_2 has been used as an investigative tool in studies of reserve capacity of physiological functions of late chicken embryos (Dawes and Simkiss, 1971; Tazawa, 1981; Andrewartha et al., 2011; Burggren et al., 2012; Tazawa et al., 2012; Mueller et al., 2013, 2014b). Unlike respiratory acidosis experienced in pulmonary breathers, the respiratory acidosis that bird embryos experience in altered CO_2 environments cannot be compensated by convective ventilation. Instead, metabolic compensation due to an increase in bicarbonate concentration ($[HCO_3^-]$) alone functions efficiently for embryos if they are exposed to ≤10% CO_2. Embryos of Lohmann White Leghorn chicken (hereafter referred to as Lohmann), subjected to environmental hypercapnia, were capable of regulating respiratory acidosis with partial metabolic compensation during exposures to ≤10% CO_2 on days 14–15 (d14–15) of incubation (Mueller et al., 2014b). Partial metabolic compensation during 24 h exposure occurred in proportion to CO_2 concentration ($[CO_2]$), and thus percent compensation was the same irrespective of $[CO_2]$. Similarly, responses of hematological respiratory variables typically occurred independently of environmental $[CO_2]$ ≤10% (Mueller et al., 2014b). However, there were slight but significant differences in how red blood cell volume (mean corpuscular volume, MCV) and red blood cell concentration ([RBC]) contributed to the decrease in hematocrit (Hct) when embryos were exposed to 1–6% CO_2 compared to 10% CO_2. Likewise, the change in mean corpuscular hemoglobin concentration ([MCHb]) was different between 1–6% CO_2 and 10% CO_2 during early period after the onset of exposure. Hence, a major change in hematological regulation may occur as $[CO_2]$ climbs above 6% and beyond.

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Based on these previous findings, we hypothesized that in chicken embryos a threshold exists above 10% at which both metabolic compensation for hypercapnic respiratory acidosis and the associated response of hematological respiratory variables are modified. We examined acid–base and hematological responses to $[CO_2] > 10\%$ as well as $\leq 10\%$ in an attempt to define this hypercapnic threshold. Importantly, we have also examined whether such acid–base regulation and hematological changes in response to CO_2 differed between different chicken strains. Besides the selected traits of fast growth or high egg-laying capacity, other important variables including growth and development, body temperature, oxygen consumption, heart rate and cardiovascular regulation differ between chicken strains (Janke et al., 2004; Ohta et al., 2004; Chwalibog et al., 2007; Yoneta et al., 2007; Everaert et al., 2008; Druyan, 2010; Ho et al., 2011; Crossley and Altimiras, 2012). Such differences between strains may account for variations in results reported in different studies. For instance, the reported time of onset of cholinergic chronotropic control of HR in embryos differs between studies (Höchel et al., 1998; Crossley and Altimiras, 2000; Crossley et al., 2003b; Aubert et al., 2004; Chiba et al., 2004; Yoneta et al., 2006) and may be due to the different strains used in each study (Crossley et al., 2003a, 2003b). In light of these potential physiological differences, we examined the time-specific responses of acid–base balance and hematological respiratory variables at d15 of both Hyline White Leghorn layer and Cornish Rock broiler embryos, each exposed to altered $[CO_2]$ with 20% O_2.

2. Materials and methods

2.1. Egg incubation and exposure to hypercapnia

Eggs of the Hyline layer chicken (hereafter referred to as Hyline) were transported once a week from a hatchery at Texas A&M University (College Station, Texas, USA) to the laboratory (University of North Texas, Denton, Texas) in April, May and June. Cornish Rock broiler eggs (hereafter referred to as broiler) were obtained weekly from a local hatchery in June and July. The eggs were lightly washed in water with a sponge to remove extraneous materials that may hinder gas exchange through the eggshell. On the day of incubation, eggs were weighed to 0.01 g on an electronic balance, large (>70 g) and small (<45 g) eggs were excluded and 30 eggs were numbered and set at 12:00 in an incubator (model 1502, G.Q.F. Manuf. Co.). Temperature and relative humidity of the incubator were kept at 37.5 ± 0.1 °C and ~55%, respectively, and the eggs were turned automatically every 3 h.

On d13 of incubation, 30 eggs were candled to identify living embryos and an allantoic vein was marked for blood collection. On d14, viable eggs among 30 eggs were randomly divided into control eggs (not subjected CO_2 exposure) and CO_2-exposed eggs. All eggs were moved to a cardboard egg stand in a desk-top incubator (1588 Electr. Hova-Bator, G.Q.F. Manuf. Co.) maintained at 37.5 °C. A 3.78-L gas exposure bag that could accommodate up to 16 eggs was placed within the desktop incubator. The gas exposure bag was ventilated with gas mixtures provided by a Wösthoff gas mixing pump (oHG, Bochum, Germany), as described previously (Burggren et al., 2012; Tazawa et al., 2012). Eggs were exposed to either 3, 6, 10 or 20% CO_2 with 20% O_2, balanced by N_2. According to the experimental schedule, gas-exposed eggs were either placed in the bag at 12:00 on d14 for 24 h exposure, or were exposed to the gas mixture for 2 or 6 h in the bag on d15 (2 or 6 h exposure). To investigate recovery from hypercapnic exposures, eggs exposed to one of the above gas mixtures for 24 h on d15 were returned from the gas exposure bag to the cardboard egg stand in the incubator, allowing recovery in air for 2 or 6 h. The above procedures were repeated during a period of 2–3 months to obtain sufficient data on embryos exposed to the four CO_2 concentrations (3, 6, 10 and 20%) at six time sequences (control (0 h), 2, 6 and 24 h exposure and 2 and 6 h recovery).

A subset of 20 fresh Hyline and broiler eggs was weighed, the yolk removed and the yolk weighed to 0.01 g. A different subset of 20 fresh eggs was boiled after egg mass was determined and boiled yolk mass was measured to check that the determination of fresh yolk mass was accurate.

2.2. Blood collection and analysis

Blood could not be collected while the eggs were in the gas exposure bag, so we temporarily wrapped the egg in aluminum foil to preserve the blood gases immediately after removal from the exposure bag and during blood sampling which took less than 2 min (Burggren et al., 2012). Approximately 0.4 mL of allantoic vein blood was collected into a 1 mL heparinized plastic syringe. Blood was gently emptied into a 1.5 mL plastic vial and immediately analyzed for pH_a, $[HCO_3^-]_a$ (mmol L^{-1}) and P_{aCO_2} (mmHg) with a blood gas system (ABL5, Radiometer Medical A/S, Denmark). Because the blood collected from the allantoic vein was arterialized by passage through the chorioallantoic capillaries (Piiper et al., 1980; Tazawa, 1980), measured variables represent arterial values (given by subscript a) corresponding to adult pulmonary venous blood. The relationship between pH_a and $[HCO_3^-]_a$ was depicted on a Davenport (pH–$[HCO_3^-]$) diagram as reported previously (Burggren et al., 2012; Mueller et al., 2014b). A buffer line was drawn on the Davenport diagram to indicate the buffer value of −16 mmol L^{-1} pH^{-1} (Burggren et al., 2012).

The remaining blood was well stirred in the vial, and was then measured for [RBC] (10^6 cells μL^{-1}) and hemoglobin concentration ([Hb], g%) by a hematology analyzer (Coulter Analyzer A^c T, Beckman, USA), Hct (±0.1%) in duplication by a centrifuge (Readacrit Centrifuge, Becton Dickinson, USA) and osmolality (Osm, mmol kg^{-1}) by a vapor pressure osmometer (5520 Vapro, Wescor Inc., USA). Duplicate Hct measurements were averaged for each individual embryo. [RBC] was determined by a Coulter Analyzer, and values corrected based on standard [RBC] determinations using a hematometer (Tazawa et al., 2011). Mean corpuscular indices; MCV (μm^3), mean corpuscular hemoglobin (MCH, pg) and [MCHb] (g%) were calculated from Hct, [RBC] and [Hb]; i.e., MCV = 10·Hct/[RBC], MCH = 10·[Hb]/[RBC] and [MCHb] = 100·[Hb]/Hct. Lactate concentration ($[La^-]$, mmol L^{-1}) was determined by a Nova Lactate Plus Meter (Nova Biomedical, MA, USA).

After blood analyses, embryos were euthanized by cold exposure, removed from the shell, separated from the extra-embryonic membranes, blotted to remove excess fluid and weighed for body mass (BM) to 0.01 g on an electronic balance.

2.3. Statistical analysis

All data were tested for normality and equal variance and parametric ANOVA or ANOVA on ranks were used where appropriate. Differences in mean values of egg mass, embryo BM, Osm and acid–base and hematological variables between controls for the two strains were examined by un-paired Student's *t*-test. Differences in mean values of variables across gas exposure times and between different gas treatments were examined using a two-way ANOVA with all pairwise multiple comparisons by the Holm–Sidak test. Comparison of $[La^-]$ between different gas exposure times at each CO_2 was made by a one-way ANOVA with an un-paired Student's *t*-test used for comparison between Hyline and broiler at each exposure time. The significance level was $P < 0.05$. All data were presented as mean ± 1 S.E.M.