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Dynamics of metabolic compensation and hematological changes in chicken (*Gallus gallus*) embryos exposed to hypercapnia with varying oxygen

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ABSTRACT

In day 15 chicken embryos, we determined the time course responses of acid–base balance and hematological respiratory variables during 24 h exposure to 15, 20, 40 or 90% O_2, in the presence of 5% CO_2. Hypercapnic respiratory acidosis was initially (2 h) only slightly (~20%) compensated by metabolic alkalosis in normoxic/hyperoxic embryos. After 6 h, respiratory acidosis was partially (~40–50%) compensated not only in normoxic/hyperoxic embryos, but also in hypoxic embryos. However, partial metabolic compensation in 15% O_2 could not be preserved after 24 h. Preservation of metabolic compensation required oxygen concentration ($[O_2]$) above 20%, but the magnitude of partial metabolic compensation was unrelated to $[O_2]$. Hematocrit (Hct), together with mean corpuscular volume (MCV), markedly increased in hypercapnic hypoxia, and was maintained at 24 h due to a subsequent increase in red blood cell concentration ([RBC]). In contrast, Hct, together with MCV, decreased in hypercapnic normoxia/hyperoxia accompanied by a subsequent decrease in [RBC] at 24 h. Regulation of variables takes place similarly irrespective of environmental $[O_2]$ above 20%, matching acid–base regulation.

1. Introduction

Avian embryos developing within the eggshell are not able to employ convective ventilation, and the resultant prolonged period of diffusion-only gas exchange results in unique acid–base balance. For instance, exposure to a hyperoxic normocapnic (i.e., 40% O_2, 0% CO_2) environment for 24 h induces respiratory acidosis in late chicken embryos due to increased CO_2 production against the backdrop of a fixed eggshell gas conductance during development (Tazawa, 1986; Tazawa et al., 1992; Burggren et al., 2012).

In chicken eggs, natural variability of eggshell gas conductance is quite large (Tazawa et al., 1983; Visschedijk et al., 1985). Water vapor conductance of 395 eggs obtained from a commercial hatchery ranged from ~7 mg d^{-1} $Torr^{-1}$ to ~33 mg d^{-1} $Torr^{-1}$, resulting in wide variations of P_{CO_2} from 18 Torr to 54 Torr, in the perichorioallantoic air space on d16–d19 of incubation (Visschedijk et al., 1985). In d16 embryos, arterialized blood P_{CO_2} varies naturally from ~30 Torr to ~55 Torr in eggs selected for widely varying conductance (Tazawa et al., 1983). Accordingly, exposure to extrinsic CO_2 around 5–10% is not an unrealistic imposition on chicken embryos. Some bird species lay eggs in a burrow nest where developing embryos are exposed to a hypercanic (and hypoxic) environment and therefore avian embryos may potentially tolerate hypercapnia. In fact, 20 min exposure to 21% CO_2 with 21% O_2 or even with 10% O_2 is not fatal in d14 chicken embryos (Tazawa, 1981b). Day 15 embryos survive exposure to 10% CO_2, 20% O_2 for 24 h with acid–base balance returned to the control state after 2 h recovery in air (H. Tazawa, unpublished data). Likewise, d15 embryos survive 2 h exposure to 5% CO_2, 10% O_2 producing severe metabolic acidosis with $[HCO_3^-]$ of ~10 mmol L^{-1} (Tazawa et al., 2012). Therefore, avian embryos are a favorable model to study acid–base and hematological regulation in animals confronting extraordinary environmental respiratory gas challenges.

Respiratory acidosis of chicken embryos, induced by either CO_2 treatment or by decreasing eggshell gas conductance due to exposure of eggs to SF_6–O_2 gas mixture, is partially compensated by an increase in blood bicarbonate concentration ($[HCO_3^-]$) (Tazawa et al., 1981; Tazawa, 1982). Acid–base status for embryos exposed to 9% CO_2, or for embryos with the eggshell partially covered by gas-impermeable material, also shows metabolic compensation for respiratory acidosis, due to an increase in $[HCO_3^-]$ (Dawes and Simkiss, 1971; Tazawa et al., 1971b). When the eggshell is partially covered by impermeable material, blood hematocrit (Hct) (and calculated hemoglobin concentration ([Hb])) increases (Tazawa et al., 1971b, 1988; Nakazawa and Tazawa, 1988). Even in chicken eggs selected for widely varying eggshell gas conductance, partial compensation for blood pH occurs due to non-respiratory changes in $[HCO_3^-]$, because the variations of pH with P_{CO_2} are smaller than predicted for true plasma (Tazawa et al., 1983). Unique acid–base balance responses also occur in chicken embryos after 24 h exposure to hypercapnic hypoxia (5% CO_2, 15% O_2) and

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hypercapnic normoxia (5% CO_2, 20% O_2) (Burggren et al., 2012). In day 15 (d15) embryos, hypercapnic hypoxia caused uncompensated respiratory acidosis after 24 h exposure, while respiratory acidosis caused by hypercapnic normoxia was partially compensated by metabolic alkalosis after 24 h exposure. Because the CO_2 concentration ($[CO_2]$) was the same in both hypercapnic groups, O_2 concentration ($[O_2]$) appears to be a key factor in compensation of respiratory acidosis. In addition to acid–base responses determined after 24 h exposure (Burggren et al., 2012), respiratory disturbances of blood acid–base balance in chicken embryos begin soon after extrinsic gas alterations, with partial metabolic compensation reaching equilibrium within 3–6 h (Tazawa, 1981a, 1982, 1986; Tazawa et al., 1981). Such time-dependent responses of acid–base balance and associated hematological respiratory variables have not been previously investigated for chicken embryos exposed to hypercapnia with altered $[O_2]$.

It is apparent from the aforementioned emerging studies that isolated examination of acid–base balance, hematology or blood O_2 alone, rather than in concert, allows only fragmented understanding of their very complex interactions and dynamics during embryonic development. To address the roles of $[O_2]$, hematology and gas exchange perturbation in acid–base responses, this study examines the time course of acid–base balance changes in d15 embryos exposed to varying $[O_2]$ (i.e., 15, 20, 40 and 90%) in the presence of increased $[CO_2]$ (5%). In addition to the previous study showing acid–base responses and Hct regulation after 24 h exposure to moderate hypoxia (15% O_2, with or without 5% CO_2) (Burggren et al., 2012), we also investigated acid–base responses and Hct regulation in embryos exposed to severe hypoxia (10% O_2, with or without 5% CO_2) (Tazawa et al., 2012). While moderate hypoxia produced metabolic acidosis with a slight increase in lactate concentration ($[La^-]$), which accounted for ~1/6th of the decrease in $[HCO_3^-]$ (Burggren et al., 2012), metabolic acidosis produced by severe hypoxia (and hypercapnic hypoxia (5% CO_2, 10% O_2)) was accompanied by predominant increase in $[La^-]$ which matched the decrease in $[HCO_3^-]$ (Tazawa et al., 2012). Accordingly, a mechanism causing metabolic acidosis differs between moderate (15%) and severe (10%) hypoxia. While anaerobic glycolysis determines the metabolic alterations (i.e., $[HCO_3^-]$) and acid–base status during severe hypoxia, there is a possibility that $[HCO_3^-]$ may change to compensate for acidosis within 24 h (e.g., 3–6 h) of moderate hypoxia and hypercapnic hypoxia (5% CO_2, 15% O_2) as in embryos exposed to hypercapnic normoxia (4% CO_2, 21% O_2) (Tazawa, 1982) or to 20% O_2 balanced by SF_6 (Tazawa et al., 1981). That is, a mechanism responding to moderate hypoxia and hypercapnic hypoxia may be the same as to hypercapnic normoxia, although the magnitude of responses may depend on $[O_2]$. Thus, we predict that metabolic compensation will occur even in hypoxic (15% O_2) embryos during time-course of 24 h responses, and that compensation will increase in a dose-related fashion as $[O_2]$ rises to normoxia (20% O_2) and beyond to hyperoxia (40% and 90% O_2). Because Hb serves as a non-carbonate buffer in blood, with an increasing concentration during the last half of incubation (e.g., Tazawa et al., 2011), there may also be a possible contribution of [Hb] to the increase in non-respiratory $[HCO_3^-]$ in metabolic compensation for respiratory acidosis during 24 h responses. Consequently, we also undertook time course measurements of hematological respiratory variables, including Hct, [Hb], red blood cell concentration ([RBC]), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration ([MCHb]) and osmolality (Osm), in embryos challenged by extrinsic alterations of $[O_2]$ in the presence of 5% CO_2. By examining multiple hematological variables we may identify the mechanisms underlying acid–base compensation.

2. Materials and methods

2.1. Egg incubation and exposure to gas mixtures

Fertile eggs of the domestic chicken (*Gallus gallus*) were obtained from a hatchery at Texas A&M University (College Station, Texas, USA). Eggs were numbered, weighed (± 0.01 g) by an electronic balance upon arrival and set in an incubator within the week according to an experimental schedule. Temperature of the incubator (model 1502, G.Q.F. Manuf. Co., USA) was kept at 37.5 ± 0.1 °C with a relative humidity of approximately 55%. Eggs were turned automatically every 3 h until d14.

On d14 of incubation, eggs were candled to locate an allantoic vein and the eggshell over the vein was marked. The eggs were divided into "control" eggs and "gas–exposed" eggs, and moved to a second desk-top incubator (1588 Electr. Hova-Bator, G.Q.F. Manuf. Co., USA) warmed to 37.5 °C. Half of the incubator space was filled by a 3.78-L gas exposure bag to hold the gas exposed eggs as described previously (Burggren et al., 2012), while a cardboard egg stand held control eggs in the other half. The gas exposure bag was ventilated with one of four gas mixtures: 15, 20, 40 or 90% O_2, all with 5% CO_2 balanced by N_2. Gas mixtures were created with a Wösthoff gas mixing pump (oHG, Bochum, Germany) at a rate of 600 ml/min (Burggren et al., 2012). Gas exposed eggs were either placed in the bag at 12:00 on d14 of incubation (24 h exposure) or they were exposed to the gas mixtures for 2 h or 6 h on d15 (2 h or 6 h exposure). Accordingly, the experiments were carried out for four gas exposure groups; group 1 = hypercapnic hypoxia (5% CO_2, 15% O_2/80% N_2), group 2 = hypercapnic normoxia (5% CO_2, 20% O_2/75% N_2), group 3 = hypercapnic hyperoxia (5% CO_2, 40% O_2/55% N_2), and group 4 = hypercapnic hyperoxia (5% CO_2, 90% O_2/5% N_2). Each group included four sub-groups; control (0 h), 2 h, 6 h and 24 h exposure.

2.2. Blood collection and analysis

Approximately 0.4 mL of blood was collected from the allantoic vein of the control and gas exposed embryos on d15. Immediately after removal from the incubator, gas exposed eggs were wrapped loosely in aluminum foil to allow for a greater gas reservoir between the foil and the eggshell, preserving the blood gases during rapid blood collection (<2 min since removal from the incubator), as described by Burggren et al. (2012). In order to minimize a dead space between needle and syringe, we used a syringe on which a needle was directly fixed. Accordingly, collected blood had to be emptied once into a conic-ended plastic vial. To minimize contact of blood with air, a tip of the needle was placed at the bottom of the vial and the blood was gently transferred into the vial so that the blood would move gently from the bottom to the upper surface. For measurement, the blood was withdrawn from the bottom of the vial. Because the upper surface of blood contacted air, we made a preliminary measurement of blood gas variables to estimate the effect of air contact and assured that careful treatment of blood increased accuracy of the measurement and did not negate the measurement.

Because the blood collected from the allantoic vein was arterialized by chorioallantoic capillaries (Piiper et al., 1980), measured variables represent arterial values (annotated by subscript a) corresponding to adult pulmonary venous blood. Blood was immediately analyzed for pH_a, $[HCO_3^-]_a$ (mmol L^{-1}) and P_{CO_2} (mmHg) by a blood gas system (ABL5, Radiometer Medical A/S, Denmark). The relationship between pH_a and $[HCO_3^-]_a$ was depicted on a Davenport (pH_a–$[HCO_3^-]_a$) diagram, which was previously constructed by plotting P_{CO_2} isopleths calculated from the Henderson-Hasselbalch equation using a CO_2 solubility factor of 0.0308 mmol L^{-1} $mmHg^{-1}$ and a serum carbonic acid pK′ varying with pH (Severinghaus