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Acute regulation of hematocrit and acid–base balance in chicken embryos in response to severe intrinsic hypercapnic hypoxia

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ABSTRACT

The regulation of blood acid–base balance and hematology in day 15 chicken embryos in response to partial water submersion (with egg's air cell in air) and complete submersion producing severe intrinsic hypercapnic hypoxia and recovery in air was studied. The acid–base disturbance during submersion was characterized by initial rapid respiratory changes and then superseded by metabolic processes, resulting in a large progressive hysteresis. Throughout submersion and recovery, blood lactate concentration changed swiftly along with the changes in bicarbonate concentration ($[HCO_3^-]$), indicating that anaerobic glycolysis determined overall acid–base disturbances. Both partial and complete submersion produced large, rapid increases in hematocrit through proportional increases in mean corpuscular volume and red blood cell concentration. Death ensued once the internal pool of O_2 was exhausted and/or the acid–base disturbance became too severe for survival (i.e., $[HCO_3^-]_a < \sim 10\ mmol\ L^{-1}$). However, embryos recovered from acid–base and hematological disturbances within 120 min recovery in air after short bouts of complete (20 min) or partial (60 min) submersion, suggesting that shorter severe intrinsic hypercapnic hypoxia does not compromise viability of embryos.

1. Introduction

In chicken embryos both pure hypoxia and hypercapnic hypoxia induce acid–base disruption and change hematocrit (Hct) and red blood cell concentration ([RBC]) (e.g., Ackerman, 1970; Tazawa et al., 1971, 1981, 2012; Tazawa, 1982; Baumann et al., 1983; Nakazawa and Tazawa, 1988; Dzialowski et al., 2002; Andrewartha et al., 2011; Burggren et al., 2012; Mueller et al., 2013). Hct increases in response to decreasing O_2 concentration ($[O_2]$). Whether or not CO_2 was present, day 15 (d15) embryos exposed to moderate (e.g., 15% O_2) extrinsic hypoxia for 2–6 h increased Hct due to an increase in red blood cell volume (mean corpuscular volume, MCV) only. However, by 24 h Hct was elevated due to an increase in both MCV and [RBC] (Burggren et al., 2012; Mueller et al., 2013). Exposure to more severe extrinsic hypoxia (10% O_2) or hypercapnic hypoxia (5% CO_2, 10% O_2) increased Hct though MCV alone, with [RBC] remaining unchanged in d15 embryos (Tazawa et al., 2012). Creating moderate intrinsic hypoxia by covering partially the eggshell over the air cell for 24 h increased Hct through increasing MCV in d16 embryos. However, the increase in Hct in d19 embryos was due to an increase in both MCV and [RBC] (Tazawa et al., 1988).

In embryos exposed to these altered gaseous environments, acid–base balance is influenced by both O_2 and CO_2 in a dose- (concentration-) dependent fashion. Increased lactate (La^-) production accompanied by uncompensated metabolic acidosis resulted from extrinsic severe hypoxia (10% O_2), whereas severely hypercapnic hypoxic embryos additionally underwent an uncompensated respiratory acidosis (Tazawa et al., 2012). Time plays an important role in the progression of these acid–base disturbances. For example, d15 embryos experienced respiratory acidosis after 2 h of moderate hypercapnic hypoxia (5% CO_2, 15% O_2) exposure. By 6 h the acidosis was partially compensated by metabolic alkalosis. However, at 24 h the partial metabolic compensation had discontinued and $[La^-]$ decreased (Burggren et al., 2012; Mueller et al., 2013). Similarly, respiratory acidosis resulted in d16 embryos 10 min after intrinsic hypoxia had been induced by covering ~¼ of their eggshells with a gas impermeable material (Tazawa, 1981). By 1–6 h the respiratory acidosis was partially compensated by a metabolic alkalosis that continued for more than 24 h, in contrast to embryos exposed to extrinsic hypercapnic hypoxia (Burggren et al., 2012; Mueller et al., 2013).

Collectively, these experiments show that the different blood acid–base and hematological responses observed depend upon the severity and length of the hypercapnic hypoxia and whether it is produced intrinsically or extrinsically. The physiological effects of reducing gas exchange across the entire eggshell have not yet been examined and there are no data on embryos facing prolonged, severe intrinsic hypercapnic hypoxia. Water submersion

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greatly reduces gas exchange across the eggshell as a result of much lower O_2 capacitance and lower gas diffusion coefficient of water compared with air. This creates a severe, intrinsically hypoxic and hypercapnic environment for the embryo, with the CO_2 accumulation inside the egg disrupting acid–base balance and blood hematology. Prolonged submersion of course results in death of the embryo, but during short-term submersion, it is likely that the air cell prolongs survival by enabling a limited amount of gas exchange with the air above the water line.

Although the eggs of domestic chickens are unlikely to be submersed, many avian embryos are potentially vulnerable to brief or long-term flooding due to being incubated in nests on the ground, along the shore-line or floating in water bodies, (e.g., Rounds et al., 2004; Wilson and Peach, 2006; Sotherland et al., 1984; Shriver et al., 2007). Further, water submersion provides a simple, effective method for disrupting gas exchange through the eggshell pores and producing an intrinsically hypoxic, hypercapnic environment. Thus, this study aims to characterize the physiological effects of severe intrinsic hypercapnic hypoxia in chicken embryos produced by water submersion, which is a potential stressor for many avian embryos. Specifically, we used partial or complete water submersion to characterize the dose-dependent nature and dynamics of partially or fully blocking gas exchange on the survival length and progression of the acid–base and blood hematology disturbance. We hypothesize that blood hematology and acid–base disturbances in embryos experiencing severe intrinsic hypercapnic hypoxia produced by water submersion, and the mechanisms used to partially mitigate these disturbances, will differ from the physiological disturbances resulting from exposure to severe extrinsic hypercapnic hypoxia produced by modification of the surrounding gaseous environment. We further hypothesize that partial submersion will extend survival time (cf. complete submersion) by partially mitigating these disturbances.

2. Materials and methods

2.1. Egg incubation

Eggs of the White Leghorn (mainly Lohmann) strain of chickens (*Gallus gallus*) were obtained weekly from Texas A&M University (College Station, Texas, USA). Eggs were weighed (±0.01 g) and then incubated at a temperature of 37.5 ± 0.1 °C and relative humidity of ~55% in a forced draught incubator (model 1502, G.Q.F. Manuf. Co., GA, USA). The eggs were placed vertically on an automatic turning tray rotating the eggs every 3 h. On d14 of incubation, the eggs were candled to locate the allantoic vein and a site over the vein was marked on the eggshell. The eggs were moved into a desk-top incubator (Hova-Bator 1590, G.Q.F Manuf.) at 37.5 °C on the following day.

2.2. Protocols

The effects of intrinsic hypercapnic hypoxia on acid–base and hematology regulation were investigated by blocking gas exchange across the eggshell via submerging eggs in water or by wrapping eggs in Parafilm® (Laboratory film M, American National Can). Embryos were subjected to either a short submersion (or wrapping) and recovery protocol that allowed 100% embryonic survival or a survival submersion protocol to determine how long embryos could tolerate intrinsic hypercapnic hypoxia. Both protocols were conducted at 37.5 °C to facilitate comparisons with previously published work with extrinsic hypoxia with or without CO_2 (e.g., Burggren et al., 2012; Tazawa et al., 2012; Mueller et al., 2013).

2.2.1. Parafilm® wrapping

Eggs were wrapped in a sheet of Parafilm® and returned to the desk-top incubator at 37.5 °C (except eggs sampled at time 0). Blood was collected from embryos prior (control), immediately after (time 0: sampled within 2 min), and 10, 30 and 60 min after Parafilm® wrapping (Table 1). Blood samples were then analyzed for blood gas variables (Pco_2, pH, $[HCO_3^-]$), Hct and osmolality (Osm, mmol kg^{-1}) (see Section 2.3). Embryos in the recovery protocol were wrapped in Parafilm® for 60 min and the same variables were then measured during recovery (eggs unwrapped) at time 0, 10, 30, 60 and 120 min (Table 1).

Blood gas variables, Hct and Osm were additionally measured from embryos in the survival protocol which were sampled every 10 min during 60–140 min Parafilm®-wrapping (Table 1).

2.2.2. Partial water submersion (air cell at the water's surface)

Eggs placed into the water bath (Model 3545, Lab-Line Instrument, USA) at 37.5 °C naturally floated with the air cell up and part of the eggshell protruding into the air above the water bath. At the time of blood collection, the egg was removed from the water and, while still moist, the entire egg minus the area underlying the air cell was immediately wrapped with Parafilm® to maintain the internal environmental conditions during the brief (<2 min) blood sampling process. During the short submersion protocol the following variables were measured: blood gas variables (Pco_2, pH, $[HCO_3^-]$), hematological respiratory variables with mean corpuscular indices (Hct, [RBC]), hemoglobin concentration ([Hb], g%), MCV, mean corpuscular hemoglobin (MCH, pg) and mean corpuscular hemoglobin concentration ([MCHb], g%), Osm and lactate concentration ($[La^-]$, mmol L^{-1}). All of these variables were measured prior to (control) and 10, 20, 30 and 60 min following partial water submersion (Table 1). Embryos in the recovery protocol were partially submersed for 60 min, placed back in air in the desk-top incubator and the same variables were then measured during recovery in air at 0, 10, 30, 60 and 120 min (Table 1).

Blood gas variables, hematological respiratory variables, Osm and $[La^-]$ were additionally measured from embryos in the survival protocol which were sampled every 10 min during 60–140 min partial submersion (Table 1).

2.2.3. Complete water submersion

Eggs were completely submersed to block gas exchange across the entire eggshell. A plastic sheet and weights were used to hold the eggs completely underwater. The partial submersion protocol (Section 2.2.2) was then followed, but the entire surface of the egg (including the air cell) was wrapped with Parafilm® immediately after removal from the water bath, to maintain experimental conditions during blood sampling.

During the short submersion protocol the blood gas variables, hematological respiratory variables, Osm and $[La^-]$ were measured prior to (control) and after 10, 20 and 30 min complete water submersion (Table 1). Embryos in the recovery protocol were completely submersed for 20 min, placed back in air in the desk-top incubator and the same variables were then measured during recovery at 0, 10, 30, 60 and 120 min (Table 1).

Blood gas variables, hematological respiratory variables, Osm and $[La^-]$ were additionally measured from embryos in the survival protocol which were sampled every 10 min during 20–70 min complete submersion (Table 1) based on preliminary embryonic survival data.

2.3. Blood collection and analysis

Blood was collected following previously published procedures (Burggren et al., 2012; Tazawa et al., 2012). Briefly, a 6–8 mm diameter region of the eggshell was removed and an underlying allantoic