Available online at www.sciencedirect.com
ScienceDirect
Comparative Biochemistry and Physiology, Part A 148 (2007) 761 – 768
CBP
www.elsevier.com/locate/cbpa

Respiratory and cardiovascular responses to acute hypoxia and hyperoxia in internally pipped chicken embryos ☆

Stephanie Sbong, Edward M. Dzialowski *

Department of Biological Sciences, University of North Texas, P.O. Box 305220, Denton, TX 76203, USA

Received 31 January 2007; received in revised form 9 March 2007; accepted 10 March 2007
Available online 16 March 2007

Abstract

During the first day of hatching, the developing chicken embryo internally pips the air cell and relies on both the lungs and chorioallantoic membrane (CAM) for gas exchange. Our objective in this study was to examine respiratory and cardiovascular responses to acute changes in oxygen at the air cell or the rest of the egg during internal pipping. We measured lung ($\dot{V}_{O_2lung}$) and CAM ($\dot{V}_{O_2CAM}$) oxygen consumption independently before and after 60 min exposure to combinations of hypoxia, hyperoxia, and normoxia to the air cell and the remaining egg. Significant changes in $\dot{V}_{O_2total}$ were only observed with combined egg and air cell hypoxia (decreased $\dot{V}_{O_2total}$) or egg hyperoxia and air cell hypoxia (increased $\dot{V}_{O_2total}$). In response to the different O_2 treatments, a change in $\dot{V}_{O_2lung}$ was compensated by an inverse change in $\dot{V}_{O_2CAM}$ of similar magnitude. To test for the underlying mechanism, we focused on ventilation and cardiovascular responses during hypoxic and hyperoxic air cell exposure. Ventilation frequency and minute ventilation (V_E) were unaffected by changes in air cell O_2, but tidal volume (V_T) increased during hypoxia. Both V_T and V_E decreased significantly in response to decreased P_{CO_2}. The right-to-left shunt of blood away from the lungs increased significantly during hypoxic air cell exposure and decreased significantly during hyperoxic exposure. These results demonstrate the internally pipped embryo's ability to control the site of gas exchange by means of altering blood flow between the lungs and CAM.

Keywords: Chicken; Egg; Oxygen consumption; Hypoxia; Hyperoxia; Chorioallantoic membrane; Lungs; Ductus arteriosus

1. Introduction

The chicken embryo utilizes two modes of gas exchange prior to hatching. During the prenatal period, the transfer of oxygen and carbon dioxide occurs at the chorioallantoic membrane (CAM). On day 19 of incubation, the embryo internally pips by piercing the air cell inner membrane with its beak and begins lung ventilation on air cell gas. This air cell gas is both hypoxic and hypercapnic (Visschedijk, 1968b; Wangensteen and Rahn, 1970/71). During internal pipping, oxygen uptake at the lungs ($\dot{V}_{O_2lung}$) accounts for between 27 and 39% of the total oxygen uptake of the egg ($\dot{V}_{O_2total}$; Visschedijk, 1968a; Menna and Mortola, 2002). Up to 12 hours after internal pipping, the embryo breaks the eggshell with its beak during external pipping and the contribution of the lungs to $\dot{V}_{O_2total}$ increases to 77% (Menna and Mortola, 2002). Upon hatching, all oxygen uptake occurs by lung ventilation (Rahn et al., 1985; Visschedijk, 1968a).

Associated with the switch in the site of gas exchange during hatching is a shift in the pattern of blood flow. Prior to internal pipping, the embryo has interatrial foramina and two patent ductus arteriosi that allow for a right-to-left shunt of blood flow away from the non-ventilating lungs and towards the CAM and systemic tissues (White, 1974; Tazawa and Takenaka 1985; Rahn et al., 1985). With the initiation of internal pipping, right atrial and ventricle output flowing to the lungs increases and output flowing to the systemic tissues and CAM decreases. At this point, approximately 40% of the right atrial outflow passes through the pulmonary arteries to the lungs while 60% bypasses the lungs through the ductus arteriosi and interatrial foramina (Rahn et al., 1985). As hatching progresses, blood flow through the ductus arteriosi decreases such that during external pipping

☆ First presented in the symposium on Developmental Transitions in Respiratory Physiology at the first International Congress of Respiratory Biology in Bad Honnef, Germany, August 2006.

* Corresponding author. Tel.: +1 940 565 3631; fax: +1 940 565 3821.
E-mail address: edzial@unt.edu (E.M. Dzialowski).

1095-6433/$ - see front matter © 2007 Elsevier Inc. All rights reserved.
doi:10.1016/j.cbpa.2007.03.013

75% of the blood leaving the right atria flows through the pulmonary arteries to the lungs (Rahn et al., 1985). Thus, throughout incubation, internal pipping, and to a lesser extent external pipping, the embryo has a functioning right-to-left shunt of blood. This right-to-left shunt may provide the internally pipped embryo with the ability to alter blood flow between the lungs and CAM under conditions such as hypoxia or hyperoxia.

It is well known that hypoxia and hyperoxia can have profound effects on the physiology and morphology of the development of the avian embryo *in ovo* (Stock et al., 1983; Stock and Metcalfe, 1987; Dzialowski et al., 2002; Chan and Burggren, 2005). However, very little is known about the physiological response of the chicken embryo when exposed to acute or chronic changes in oxygen availability during the transition from CAM to lung respiration during hatching. The studies to date have focused on the externally pipped stage of this paranatal period. In response to 15% oxygen, $\dot{V}_{O_2total}$ of externally pipped embryos decreased by 10% in one study (Tazawa et al., 1992) and increased by 10% in another (Menna and Mortola, 2003). Much larger and consistent decreases in $\dot{V}_{O_2total}$ occurred in response to 10% and 11% oxygen (Tazawa et al., 1992; Menna and Mortola, 2003; Mortola and Labbè, 2005). In response to hyperoxia, the $\dot{V}_{O_2total}$ of externally pipped embryos either increased (Tazawa et al., 1992) or did not change (Mortola, 2004). Thus, during the externally pipped stage mild hypoxia or hyperoxia has little influence on $\dot{V}_{O_2total}$. With a functional right-to-left shunt, the contribution of the lungs and CAM to $\dot{V}_{O_2total}$ in the internally pipped embryo could vary in response to changes in air cell gas content.

An additional mechanism available to internally and externally pipped embryos to influence $\dot{V}_{O_2lung}$ is the alteration of lung ventilation patterns. Externally pipped embryos increased tidal volume (V_T) with a relatively constant ventilation frequency (f) in response to ventilating their lungs with hypoxic and hypercapnic gas (Menna and Mortola, 2003). It is possible that the internally pipped embryo may have the ability to alter $\dot{V}_{O_2lung}$ by changing ventilation patterns much like the externally pipped embryo to help compensate for changes in air cell O_2 content.

Here we examined the contribution of the lungs and CAM to $\dot{V}_{O_2total}$ in the internally pipped chicken embryo when exposed to hypoxia or hyperoxia at the air cell and/or the rest of the egg. Additionally, we examined potential mechanisms by which internally pipped chicken embryos may control the site of gas exchange when exposed to air cell hypoxia and hyperoxia. To do this, we measured ventilation and blood flow patterns in internally pipped embryos exposed to hypoxia or hyperoxia at the air cell. We found that the internally pipped embryo altered the contribution of the lungs and CAM to $\dot{V}_{O_2total}$ by altering the right-to-left shunt of blood rather than by changing ventilation patterns.

2. Materials and methods

2.1. Incubation

White leghorn (*Gallus gallus*) eggs were obtained from Texas A&M University and used within one week of arrival. Eggs were incubated in a circulated air incubator (GQF Model 1502) with a relative humidity of 70% at 37.5 °C. The eggs were automatically turned every 4 h.

2.2. Oxygen consumption

Oxygen consumption was measured independently at the air cell ($\dot{V}_{O_2lung}$) and the rest of the egg ($\dot{V}_{O_2CAM}$) in internally pipped chicken embryos. Eggs were considered internally pipped if we were able to see the embryo's beak in the air cell when candling the egg. To isolate the air cell, we candled the egg and outlined the air cell with a sharpie marker. Two small holes were made in the shell above the air cell using an 18 gauge needle and short pieces of PE190 tubing were inserted into the holes. The entire shell above the air cell was then painted with Kerr Dental Wax, sealing the PE190 tubing into place. This allowed us to use the air cell as a mini respirometer and regulate the O_2 and CO_2 content flowing through the air cell. The egg was sealed in a respirometer jar (236 mL) with ports for gas flow into and out of the chamber. The PE190 tubing coming from the air cell was attached to 18 gauge needles that were glued into the top of the jar allowing for flow of the mixed gas through the air cell, independent of the rest of the egg. The gas content of the air flowing into the air cell and the egg chamber were controlled with two mass flow control systems (Porter Mass Flow and MFC-2, Sable Systems). The initial gas flowing though the air cell was composed of 12% O_2, 5% CO_2, and 83% N_2 and was considered to be normoxic for air cell gas (Wangensteen and Rahn, 1970/71). The flow rate through the egg respirometer and the air cell was set to approximately 50 mL min^{-1} and was measured with a Flowbar-1 Mass Flow Meter (Sable Systems).

Internally pipped eggs were allowed to equilibrate for 1 h at the normoxic conditions, followed by a measurement of inflow and outflow oxygen content using a Sable Systems Field Oxygen Analysis System. Prior to measurement with the O_2 analyzer, the gas was passed through columns of drierite, ascarite, and drierite. The output from the O_2 analyzer was recorded with an ADInstruments Powerlab 8SP and Chart 5 for later analysis. Following the initial measurements of O_2 at the air cell and the rest of the egg, the gas content was altered. Eight combinations of hypoxia and hyperoxia to the air cell and/or egg were tested on eight sets of eggs and are listed in Table 1. Carbon dioxide to the air cell was maintained at 5% during all treatments. After changing the O_2 content of the gas, the eggs were allowed to equilibrate for 1 h at the new O_2 levels and the inflow and outflow O_2 was recorded again. Oxygen consumption at the air cell and the rest of the eggs were calculated using the equation of Hill (1972) and expressed as mL O_2 min^{-1}. Total oxygen consumption of the egg ($\dot{V}_{O_2total}$) was calculated as $\dot{V}_{O_2lung}$ plus $\dot{V}_{O_2CAM}$. All oxygen consumption values were corrected to standard temperature, pressure and dry (STPD).

2.3. Ventilation

Ventilation frequency (f) and tidal volume (V_T) were measured with a pneumotachograph connected to a Validyne pressure transducer using methods adapted from Glass et al. (1978).