Comparative Biochemistry and Physiology, Part A 203 (2017) 49–58

Contents lists available at ScienceDirect

Comparative Biochemistry and Physiology, Part A

journal homepage: www.elsevier.com/locate/cbpa

The contribution of heart rate to the oxygen consumption of the chicken embryo during cold- or hypoxia-hypometabolism

Satoko Tomita Ide, Ryoji Ide, Jacopo P. Mortola *

Dept. of Physiology, McGill Univ., 3655 Promenade Sir William Osler, room 1121, Montreal, Quebec H3G 1Y6, Canada

ARTICLE INFO

Article history:
Received 24 May 2016
Received in revised form 18 July 2016
Accepted 25 August 2016
Available online 30 August 2016

Keywords:
Cardiovascular control
Development
Hypometabolism
Hypoxia
Oxygen consumption
Prenatal

ABSTRACT

In embryos, cooling and hypoxia cause a decrease in oxygen consumption ($\dot{V}_{O_2}$); we asked what was the relative contribution of heart rate (HR) and of the 'not-HR' factor (the product of stroke volume and arterial-venous O_2 difference) to the drop in $\dot{V}_{O_2}$. Data of HR (with subcutaneous electrodes) and $\dot{V}_{O_2}$ (by an open-flow methodology) were collected simultaneously on chicken embryos close to end-incubation. Over the last four days of incubation (E16–E20) differences in HR contributed about 30% of the differences in resting $\dot{V}_{O_2}$ among embryos. At E20, progressive cooling from 38 to 8 °C decreased $\dot{V}_{O_2}$ entirely because of the decrease in HR, with minimal compensation of the 'not-HR' component. The same pattern during cooling occurred in younger embryos (age E16), in E20 embryos simultaneously exposed to hypoxia (15% O_2) and in E20 normoxic embryos which were incubated in hypoxia (15% O_2). Differently, in E20 embryos in normothermia, progressive hypoxia (15%, 10% or 5% O_2) lowered $\dot{V}_{O_2}$ largely because of the reduction in the 'not-HR' component. We conclude that at end incubation during hypometabolism the changes in HR contribute very differently to the decrease in $\dot{V}_{O_2}$, from about the totality of it during cold to only about 10–20% during hypoxia, depending on its severity. It follows that during cold-hypometabolism, but not during hypoxic hypometabolism, the changes in HR are a good index of the changes in $\dot{V}_{O_2}$. The close relationship between $\dot{V}_{O_2}$ and HR during cold-hypometabolism may permit estimates of the changes in $\dot{V}_{O_2}$ from the changes in HR in infants undergoing therapeutic hypothermia.

1. Introduction

With the sole exception of the first days of incubation, when oxygen diffusion fulfills the embryo's metabolic requirements (Cirotto and Arangi, 1989; Burggren et al., 2000; Mortola et al., 2010), the oxygen consumption ($\dot{V}_{O_2}$) of an embryo depends almost entirely on cardiovascular convection. Therefore, $\dot{V}_{O_2}$ is expressed by the Fick equation

$$\dot{V}_{O_2} = CO \cdot O_2(a\text{-}v) = (HR \cdot SV) \cdot O_2(a\text{-}v) \quad (1)$$

where CO is cardiac output (the product of heart rate HR and stroke volume SV) and O_2(a-v) stands for the arterial-venous difference in oxygen content. The question we are addressing in this study is to what extent changes in embryo's HR accommodate the changes in $\dot{V}_{O_2}$ that occur during the last days of incubation and those introduced by variations in temperature or oxygenation. The strength of the $\dot{V}_{O_2}$-HR relationship during prenatal development cannot be examined easily in fetal mammals, because of the technical difficulties in the chronic instrumentation of the mammalian fetus and because maternal, uterine and placental responses complicate the interpretation of the results. The chicken embryo is an experimental model that permits recordings and interventions during 'in egg' development and bypasses the interpretative issues of the mammalian preparations.

Differently from most of incubation when $\dot{V}_{O_2}$ drops linearly with ambient temperature (Ta), close to hatching a drop in Ta of a few degrees may not decrease $\dot{V}_{O_2}$ immediately, because of the onset of thermogenic mechanisms (Black and Burggren, 2004; Mortola and Labbè, 2005). Like $\dot{V}_{O_2}$, also HR during the last days of incubation is less sensitive to mild hypothermia (Tazawa and Nakagawa, 1985); hence, the possibility has been raised that the developmental changes in the $\dot{V}_{O_2}$ response to cold may reflect the fact that HR remained more stable against changes in Ta (Nechaeva, 2011). In such a case, one would predict a close relationship between the changes in HR and those of $\dot{V}_{O_2}$ not only in young embryos but also in embryos close to

* Corresponding author.
E-mail address: jacopo.mortola@mcgill.ca (J.P. Mortola).

http://dx.doi.org/10.1016/j.cbpa.2016.08.026
1095-6433/

hatching. However, to the best of our knowledge no study has measured these two variables (HR and $\dot{V}_{O_2}$) simultaneously during cooling in avian embryos; hence, no definite conclusions can be drawn regarding the strength of the $\dot{V}_{O_2}$-HR relationship.

With respect to the effect of acute hypoxia, the general tenet based on a large number of observations (reviewed in Mortola, 2009 and in Nechaeva, 2011) is that hypoxia lowers both HR and $\dot{V}_{O_2}$. A departure from this general pattern occurs at end-incubation (E20), when HR does not necessarily drop with hypoxia of brief duration (15% or 10% O_2 for a few min) (Crossley and Altimiras, 2000; Crossley et al., 2003), while $\dot{V}_{O_2}$ drops immediately (Mortola et al., 2012). It should follow, therefore, that at this age not the fall in HR but that of SV, O_2(a-v) or the fall of both is responsible for the drop in $\dot{V}_{O_2}$. Hence, differently from cold, in the case of hypoxia one could predict that changes in HR are poorly related to those of $\dot{V}_{O_2}$.

This study quantifies the $\dot{V}_{O_2}$-HR relationship in chicken embryos close to hatching under the experimental conditions of cooling and hypoxia, singly or combined, to evaluate the role of HR versus *not-HR* variables to the changes of $\dot{V}_{O_2}$. The term 'not-HR' lumps together the product SV · O_2(a-v), which simplifies Eq. (1) into

$$\dot{V}_{O_2}(\mu l/\min) = HR(\text{beats}/\min) \cdot \textit{not-HR}(\mu l/\text{beat}) \quad (2)$$

Measurements of the $\dot{V}_{O_2}$-HR relationship during cooling have been extended to a group of younger embryos (E16) and to embryos incubated in hypoxia, for different reasons. Although close to hatching (E20) some thermogenic response begins to be recognisable, even if very modest, in younger embryos it is completely absent. Hence, measurements at these two ages permit to consider the possibility that the $\dot{V}_{O_2}$-HR response during cooling may differ with the onset of the thermogenic capacity. Prenatal chronic hypoxia is of interest because it depresses cardiac function in humans and animals (Ostádalová et al., 1995; Hassan et al., 2013), including the chicken embryo (Jonker et al., 2015).

2. Methods

Experiments were performed on chicken (*Gallus gallus*) eggs of the White Leghorn variety purchased from a local supplier. Fresh egg weight was noted and incubation started at midday (embryonic day zero, E0). The incubator (Hova-Bator, Savannah, GA) was 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. The core of the experiments consisted in simultaneous measurements of heart rate (HR) and oxygen consumption ($\dot{V}_{O_2}$) in embryos during the last days of incubation, at various temperatures and levels of oxygenation.

2.1. Egg preparation

On the day of the experiment the air cell was identified by trans-illumination and its contour marked. A window (~1 cm^2) was opened through the eggshell at the blunted end of the egg over the air cell. First, the area was covered by adhesive tape, so that the drilling through the shell and the cutting of three of the four sides of the window would not produce shell fragments. Then, the shell window was folded back on its fourth side, giving access to the air cell. The inner membrane was pulled up and severed with forceps to expose the embryo, which at end incubation presents head and left wing just under the air cell. Hook wired electrodes were used to monitor the embryo's electrocardiogram (ECG). A thin wire was threaded through the cannula of a hypodermic needle and its 1–2 mm end, cleared from insulation, was folded back on the tip of the needle to form a hook. The needle-wire combination was gently inserted subcutaneously at the level of the embryo's upper back and left shoulder, if necessary with the help of a lighted magnifying lens. As the needle was slowly withdrawn, the hook end of the wire kept the electrode anchored to the tissue. The same procedure was for the remaining two electrodes, one of which acted as ground. If any significant bleeding occurred, the embryo was discarded. Finally, the portion of the eggshell lifted was returned to its original position to close the window, and carefully sealed by adhesive tape. The egg was positioned in a respirometer, which consisted in a 280-ml plastic container submerged under water and maintained at the desired temperature (37.5 °C) by a circulating water bath (Fig. 1).

2.2. Measurement of $\dot{V}_{O_2}$

The open flow methodology, adapted to the chicken embryo (Mortola and Labbè, 2005; Szdzuy et al., 2008), was chosen for the measurements of $\dot{V}_{O_2}$ and carbon dioxide production. Two tungsten-constantan thermocouples were in the respirometer for continuous monitoring of ambient temperature (Ta). A steady flow of 100 ml/min, under the control of a mass flow meter, passed through two openings in the lid of the respirometer (Fig. 1). 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 was dried through a column of anhydrous calcium sulphate (Drierite®). The inflow gas concentrations were monitored intermittently by momentarily bypassing the respirometer pathway. The inflow lid of the respirometer was connected to an impermeable 15 l bag filled with the gas mixture of interest. The time of first detection of a gas perturbation in the respirometer was measured by suddenly connecting the inflow line to CO_2; it averaged 40 s, while the time constant (63% of the full response) was 165 s. These response times were adequate for the purpose of our measurements, which occurred at step intervals of 15–20 min (Section 2.4.). The gas flow, O_2 and CO_2 concentrations were continuously displayed on a computer monitor. After mathematical correction of the gas fractional concentrations for the error introduced by a respiratory exchange ratio different from unity (Depocas and Hart, 1957; Mortola and Besterman, 2007), $\dot{V}_{O_2}$ (μl/min) corresponded to the product of flow rate and inflow-outflow O_2 concentration difference at standard temperature, pressure and dry conditions.

2.3. Measurement of HR

The wire electrodes were threaded through the lid of the respirometer and connected to an amplifier and filtering system. The ECG acquisition had hardware notch filter at 60 Hz; additional low- and high-pass filters and signal amplifications were chosen ad hoc to produce the best signal-to-noise ratio. The ECG was continuously displayed on an oscilloscope, in parallel with a loudspeaker. The time to sweep across the oscilloscope screen was 20 s, which corresponded to a speed of 6.5 mm/s, adequate to discriminate the beats even at the highest HR (Fig. 1). For each condition, at least three sweeps were recorded; from their average HR was calculated in beats/min.

2.4. Experimental protocols

Two experiments were performed to evaluate the strength of the $\dot{V}_{O_2}$-HR relationship during cooling and hypoxia.

Experiment 1 (cooling) aimed to establish the $\dot{V}_{O_2}$-HR relationship during cooling in normoxia (group A), in normoxia before the onset of thermogenesis (group B), in association with hypoxia (group C) and in normoxia on embryos that were exposed to hypoxia for a long portion of incubation (group D). Group A consisted of embryos at age E19–E20 (hereafter nominally "E20", N = 15), out of 20.5 days of total incubation duration. The embryos were at the internal pipping phase of the hatching process, when the thermogenic response to cold begins to be detectable (Szdzuy et al., 2008); none of them had initiated external pipping. By