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Research Report

Growth restriction and the cerebral metabolic response to acute hypoxia of chick embryos in-ovo: A proton magnetic resonance spectroscopy study

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Abstract

Introduction: Perinatal brain injury is more common in growth-restricted (GR) than normally grown (NG) fetuses. This study addresses the hypothesis that chronic oxygen and substrate deprivation during pregnancy will engender an abnormal fetal cerebral metabolic response to acute hypoxia.
Method: Cerebral metabolite resonance amplitudes relative to that of creatine were measured by proton magnetic resonance spectroscopy in chick embryos on day 19 of incubation. Measurements were obtained before, during and after acute hypoxia (8% ambient oxygen concentration for 44 min) in NG and GR embryos (10% albumen extracted day 0 and 14% oxygen exposure from day 10 of incubation).
Results: In both NG and GR embryos, the cerebral lactate/creatine increased during acute hypoxia and slowly recovered after restoration of normoxia. However, the mean (±SD) increase in lactate/creatine was significantly less in GR compared to NG embryos (0.51 ± 0.36 vs. 0.94 ± 0.36; $P = 0.02$, t test). Alanine increased during acute hypoxia in NG but not GR embryos. Mean β-hydroxybutyrate was increased only in GR embryos (0.63 ± 0.01 vs. 0.22 ± 0.01; $P < 0.001$, ANOVA).
Conclusions: Acute hypoxia increases cerebral lactate and alanine in NG chick embryos; these increases are reduced by pre-exposure to substrate deprivation and chronic hypoxia.

Theme: Development and regeneration
Topic: Nutritional and pre-natal factors

Keywords: Growth; Acute hypoxia; Cerebral metabolism; Chick embryo; Lactate; Proton spectroscopy

1. Introduction

Fetal growth restriction (GR) is a common complication affecting up to 10% of pregnancies and is an important antenatal risk factor for neonatal encephalopathy [3] and cerebral palsy [5,55]. There is also an association between intrauterine growth restriction (IUGR) and white matter injury in very premature babies [37] and intrauterine or early neonatal death at later gestations [20].

A possible link between fetal substrate deprivation and abnormal neurological outcome lies in the effect of haemodynamic and metabolic perturbations on cerebral development. Fetal rat studies have demonstrated associations between GR and systemic metabolic perturbations such as hypoglycaemia, acidosis, hypercapnia and hypoxia [25,39,40]. Cordocentesis studies in humans have shown that small-for-gestational-age (SGA) fetuses are also relatively hypercapnic, hypoxic, hyperlacticaemic, acidotic and hypoglycaemic compared to appropriate for gestational age fetuses [17,38,48]. However, cerebral metabolism appears to remain surprisingly well preserved; growth-restricted rat

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fetuses have normal brain glucose and glycogen concentrations despite systemic hypoglycaemia [9,54], whilst SGA infants had similar phosphocreatine (PCr), adenosine triphosphate (ATP) and intracellular pH, measured using non-invasive phosphorus (^{31}P) magnetic resonance spectroscopy (MRS) to normal infants [2]. Circulatory adaptation in favour of essential organs like the brain, heart and adrenal glands in the presence of GR [52,16] acts to maintain cerebral oxygen delivery, and aerobic metabolism is further protected by increased oxygen extraction [19,44]. Further adaptive mechanisms include the ability of the fetal brain to use alternative energy substrates [30,19] and to reduce metabolic demand to balance oxygen requirement with delivery.

An additional way by which growth restriction could damage the developing brain is by impairing the fetal response to an acute hypoxic episode, as might be encountered by the growth-restricted human fetus during labour. In a previous study, we described how cerebral lactate and alanine, measured by proton (^{1}H) MRS in normally grown (NG) chick embryos in-ovo, studied on day 19 of incubation (normal incubation is 21 days), showed a significant rise during acute moderate hypoxia [41].

The chick embryo in-ovo was chosen because: 1) its cardiac output redistribution and heart rate responses to acute hypoxia are similar to those in the late gestation mammalian fetus [36,46,53]; 2) asymmetrical GR with maintenance of cerebral growth, similar to that in growth-restricted human fetuses, can be induced by reducing egg albumen at the start of incubation in a hypoxic atmosphere [35]; 3) placental absence removes maternal confounding factors, and fetal response can be studied in isolation; 4) the hypoxic insult can be accurately regulated by alteration of the ambient oxygen concentration; and 5) oxygen and carbon dioxide partial pressures in mammalian fetus and chick embryo are comparable [29]. However, compared with the mammalian fetus, myelination in the chick embryo starts earlier (on day 12, total incubation period 21 days) and progresses more rapidly.

The hypothesis addressed in the present study is that chronic reduced delivery of oxygen and substrate impairs the fetal response to subsequent acute hypoxic episodes with the potential consequences of neuronal and glial cell death and abnormal neurological development.

2. Methods and materials

2.1. Experimental preparation

Fertile White Leghorn hens' eggs were supplied from a large commercial flock (Henry Stewart and Co. Ltd., Louth, England) and stored at 10–12 °C for up to 7 days, when incubation was commenced (Polyhatch incubator, Brinsea Ltd, Sandford, N. Somerset, England). All eggs were weighed immediately prior to incubation.

Nineteen NG chick embryos were incubated at 38 °C in air (60% humidity) for 19 days. In 17 other embryos, GR was induced by removing 10% of egg albumen on day 0 (calculated from Eq. (1)) and chronic hypoxia during the latter period of incubation. This protocol was selected on the basis of previous studies in which the combination of albumen deprivation and hypoxia was associated with more severe overall growth restriction (but with maintenance of brain growth) than observed with either insult alone [35]. Typically, growth restriction was apparent by 15 days of incubation. In order to remove the albumen, the shell surface was cleaned with 100% ethanol, and albumen was withdrawn through a sterile 19G needle inserted horizontally and perpendicular to the surface through the pointed end of the eggshell. The hole in the shell was then sealed with cyanoacrylate glue (RS Components) and incubation commenced. On day 10 of incubation, eggs in the GR group were transferred to an incubator with an ambient oxygen concentration of 14% (chronic hypoxia) produced by a regulated air/nitrogen mixture flowing at 3 l/min sampled continuously by an oxygen analyser (Servomex 570A, Sussex, UK).

$$\text{Egg albumen volume} = (\text{Weight (g)} \times 0.7\ (\text{ml/g})) - 8.6\ \text{ml}. \quad (1)$$

Eq. (1): derived from [18].

On day 19, all embryos were anaesthetised with 5 mg of ketamine applied topically to the chorioallantoic membrane via a hole drilled into the air cell (at the blunt end of the egg). Ketamine was chosen because, compared with many anaesthetics, it has less effect on cerebral metabolic rate [41]. The egg was put in a sealed 150 ml plastic bag in which the oxygen concentration was accurately manipulated with a variable air/nitrogen mixture flowing at 3 l/min. The egg was kept warm by a neonatal blood pressure cuff, perfused with water at 50 °C and placed over the upper surface of the egg. Internal egg temperature was monitored using an optothermometer (Luxtron Corporation, USA) placed within the air cell.

Four groups of chick embryos were studied: NG subjected to acute hypoxia (n = 11); NG that remained in normoxia (n = 8); GR subjected to acute hypoxia (n = 9); and GR that remained in normoxia (n = 8).

2.2. ^{1}H MRS

In order to locate the chick embryo brain, the egg was centred in an inductively coupled (balanced–matched) elliptical surface coil (major axis 7 cm, minor axis 5.5 cm) made from a single loop of 6 mm copper tube. This was then positioned at the isocentre of a 7 T Biospec imaging spectrometer (Bruker Medizintechnik GmBH, Karlsruhe, Germany; 20 cm clear bore; ^{1}H frequency