Hemodynamic and metabolic responses to moderate asphyxia in brain and skeletal muscle of late-gestation fetal sheep

J. P. NEWMAN, J. P., D. M. PEEBLES, S. R. G. HARDING, R. SPRINGETT, and M. A. HANSON. Hemodynamic and metabolic responses to moderate asphyxia in brain and skeletal muscle of late-gestation fetal sheep. J. Appl. Physiol. 88: 82–90, 2000.—The purpose of this study was to investigate metabolic and hemodynamic responses in two fetal tissues, hindlimb muscle and brain, to an episode of acute moderate asphyxia. Near-infrared spectroscopy was used to measure changes in total hemoglobin concentration ([Hb]) and the redox state of cytochrome oxidase (COX) simultaneously in the brain and hindlimb of near-term unanesthetized fetal sheep in utero. Oxygen delivery (DO2) to, and consumption (VO2) by, each tissue was derived from the arteriovenous difference in oxygen content and blood flow, measured by implanted flow probes. One hour of moderate asphyxia (n = 11), caused by occlusion of the maternal common internal iliac artery, led to a significant fall in DO2 to both tissues and to a significant drop in VO2 by the head. This was associated with an initial fall in redox state COX in the leg but an increase in the brain. [Hb], and therefore blood volume, fell in the leg and increased in the brain. These data suggest the presence of a fetal metabolic response to hypoxia, which, in the brain, occurs rapidly and could be neuroprotective.

Fetal survival after perinatal hypoxia-ischemia depends in part on maintaining a balance between oxygen supply and demand. It is well established that at extreme levels of hypoxemia the fall in oxygen delivery (DO2) inhibits aerobic metabolism and eventually leads to a fall in the concentration of high-energy phosphates in all tissues. However, this represents the final stage. Before this, during more moderate reductions in DO2, there is evidence that the fetus is capable of mounting a complex response involving compensatory vascular and metabolic mechanisms (outlined below), which, at least in the short term, might preserve oxygenation and function in essential organs such as the heart and brain (1, 3, 23, 30).

The late-gestation fetal sheep mounts a rapid response to an episode of acute hypoxia with bradycardia and redistribution of combined ventricular output to the heart, brain, and adrenal glands at the expense of the fetal carcass (7, 12, 23, 43). The response is initiated by arterial chemoreflexes and then augmented by an increase in plasma catecholamines, ACTH, cortisol, and possibly by arginine vasopressin and angiotensin (9, 23, 31, 46). Therefore, a fall in arterial oxygen content further results in a response that reduces DO2 to organs such as the gut and carcass, while protecting the heart, adrenals, and brain.

The metabolic consequences of this vascular redistribution have not been described very extensively. Data from cultured fetal myocytes, in which oxygen consumption (VO2) falls linearly as PO2 is decreased, suggest a close relationship between DO2 and VO2 (4). This could reflect either a passive fall in metabolic rate as a result of decreased oxygen availability or an active mechanism whereby VO2 decreases as part of a metabolic response to hypoxia. The fact that VO2 falls over a substantially higher range of PO2 than that at which aerobic metabolism fails in the adult suggests the latter. Data from the newborn dog in vivo also show that the fall in VO2 during hypoxia is not due to a limitation of oxygen availability, but rather suggests metabolic regulation (45).

To investigate further the relationship between oxygen availability and tissue metabolism in both the fetal brain and muscle, we used near-infrared spectroscopy (NIRS). Advances in spectroscopic techniques using near-infrared light (660–1,000 nm) make it possible to obtain continuous, quantifiable measurements of changes in the concentrations of the oxygen-sensitive chromophores oxyhemoglobin ([HbO2]), deoxyhemoglobin ([Hb]), and the redox state of cytochrome oxidase (COX) from tissues such as the intact fetal brain or muscle (2, 19, 36, 42, 54). The redox state of COX, the terminal enzyme in the oxidative phosphorylation pathway, has been shown to be affected by changes in oxygen availability as well as the flow of reducing equivalents down the phosphorylation pathway and the rate of ATP turnover (14, 18). Hence, changes in the concentration of oxidized COX ([COX+]), measured by NIRS, will reflect changes in the function of the oxidative phosphorylation pathway, the main component of aerobic metabolism.

The purpose of this study was to investigate the hypothesis that a period of acute asphyxia, induced by partial occlusion of the maternal common internal iliac artery (CI1A), would lead to changes in cerebral hemodynamics and metabolism, as a result of which the redox state of COX would be maintained. Conversely, a
similar insult would cause reduction in COX in the hindlimb.

METHODS

Surgical Preparation

All work was conducted in accordance with the Animals (Scientific Procedures) Act (1986). Eleven ewes bearing singleton near-term fetuses (~123-days gestation) were anesthetized by intrajugular injection of 1.0 g of thiopentone sodium (Rhône Mérieux, Tallaght, Dublin, Ireland). Maintenance was achieved by using 2–3% halothane (Mallinckrodt Veterinary, Uxbridge, UK) administered by an Ohmeda closed-circuit anesthetic machine (Ohmeda, Englewood, NJ); anesthetic depth was determined by noting of corneal reflex, degree of pupillary constriction, and heart and respiratory rates. Additional monitoring was provided by use of an Ohmeda 525 RGM arterial saturation monitor. After preparation of the ewe, surgery was performed under aseptic conditions.

Surgical Procedure

Fitting the occluder. The maternal abdomen was opened by using a low, midline incision, and the maternal aorta was palpated at the level of the bifurcation of the CI1A and dissected free of connective tissue by blunt dissection. A tunnel was dissected behind the CI1A by using long instruments and the U bend of a custom-made mechanical screw occluder (donated by Dr. L. Bennet, Univ. of Auckland, NZ) inserted.

Fetal Surgery

The fetal hindlimbs were exposed through a uterine incision. Femoral artery and vein catheters were inserted and, on the contralateral side, a femoral artery flow probe (3R, Transonic Systems, Ithaca, NY) was placed. Infrared optodes were placed subcutaneously over the thigh muscle. The optodes were sutured into the muscle in a custom-made black rubber probe holder to prevent movement artifact. The skin incisions were closed, the hindlimbs were replaced in the uterus, and the uterine wall was closed in two layers.

The head and one upper limb were exteriorized in the same manner as for the hindlimbs. Catheters were placed in the carotid artery and jugular vein on one side such that their tips were close to the heart. On the contralateral side, a Transonic flow probe (as before) was placed around the carotid artery. Burr holes were made in the skull overlying the parasagittal cortex, and electrodes (Cooner Wire, Chatsworth, CA) were placed on the dura to allow monitoring of electrocortical activity. The burr holes were sealed by using rubber caps and cyanoacrylate. Infrared optodes were placed on the skull overlying the parasagittal cortex and held firmly in place by a custom-made black rubber holder that was sutured to the edges of the skin incision. The apposition of optodes to the scalp was improved further when the scalp incision was closed over the holder and optodes. A pair of electrodes was sewn onto the chest wall for monitoring of a fetal electrocardiogram. The fetus was replaced, and the uterus was closed as described above. The maternal abdomen was closed in two layers, and the ewe was allowed to recover after placement of a catheter in the maternal recurrent metatarsal vein for administration of antibiotics.

Antibiotic Regime

Before surgery, the ewe was given prophylactic streptomycin (1 g im). After surgery the antibiotic regime was benzyl penicillin (150 mg to the amniotic cavity, 300 mg iv to the ewe, and 150 mg to the fetus iv for 5 days) and gentamicin (40 mg to the amniotic cavity, 40 mg to the ewe iv for 2 days).

Experimental Protocol

Experiments were performed on either day 2 or 3 after surgery. Cardiovascular, blood-gas, and NIRS data were collected for 1 h before, during, and 1 h after occlusion of the maternal CI1A. Fetal asphyxia was induced by maximally tightening the screw of the mechanical occluder for 1 h.

Arterial, central venous, and amniotic pressure lines were connected to pressure transducers (SensoNor 840, SensoNor, Horten, Norway) and then into amplifiers (Digitimer, Welwyn Garden City, UK). Arterial and central venous pressures were recorded for amniotic pressure and monitored by use of MacLab software (AD Instruments). Other biophysical variables (fetal heart rate, carotid blood flow, and femoral blood flow) were monitored by use of MacLab software in a similar way and saved to optical disk for later analysis. The electrocorticogram waveform was monitored solely to confirm normal cerebral function by the presence of sleep cycling before the onset of hypoxia. Electrocorticogram data were not saved for later analysis.

Blood samples were collected for 3 h during the experimental period at the following times relative to occlusion: −55, −30, −5, +5, +30, +55, +75, +95, and +115 min. At each time point, 0.5-ml samples were collected from carotid artery, jugular vein, and femoral artery and vein. Samples were immediately tested for gases and electrolytes (YSI Electrolytes 14008–01 and Co-Oximeter 482, Instrumentation Laboratories) and for glucose and lactate (YSI 2300 STAT Plus), the values being corrected to the fetal temperature of 39.5°C.

DO2 values were calculated as the product of either carotid flow and carotid arterial oxygen content (CaO2), corrected to 39.5°C, or from femoral flow and femoral arterial oxygen content. V02 values were calculated in a similar manner by using, in addition, data for the appropriate venous oxygen content (CV02) and Fick’s law, i.e., VO2 = Q · (CaO2 − CV02), where Q is carotid or femoral blood flow (ml/min).

Collection of Near-Infrared Spectra

NIRS data were collected by using a near-infrared spectrometer purpose-built by The University College London Department of Medical Physics and Bioengineering (R. Springett). A filtered white-light source (Oriel Instruments, Stratford, CT) provided light in the near-infrared part of the spectrum, which was transmitted to the fetal head and leg via specially designed and made fiber-optic bundles (Schott). Unabsorbed light falling on the receiving optodes was transmitted to the spectrometer (Jobin Yvon SPex 270M, Groupe Instrumentation, Longjumeau, France) by another set of optical fibers, and an absorption spectrum was collected for the head and the leg. The near-infrared collection period was set to give an initial signal amplitude of ~100,000 photon counts. This represented a compromise, whereby sufficient signal was received to maximize signal-to-noise ratio while preventing saturation with light if tissue absorption fell during the experiment. The exposure time was therefore set at the beginning of each experiment and then held constant for that experiment. Exposure times ranged from 10 to 30 s in the 11 experiments performed in this study.
Table 1. Blood-gas data from 10 fetal sheep during 1 h of maternal common internal iliac artery occlusion

<table>
<thead>
<tr>
<th>Time</th>
<th>PO2, Torr</th>
<th>PCO2, Torr</th>
<th>pH</th>
<th>Hct, %</th>
<th>SaO2, %</th>
<th>Glucose, mmol/l</th>
<th>Lactate, mmol/l</th>
</tr>
</thead>
<tbody>
<tr>
<td>-55 min</td>
<td>23.10 ± 1.04</td>
<td>47.53 ± 1.03</td>
<td>7.33 ± 0.01</td>
<td>29.60 ± 1.19</td>
<td>72.02 ± 2.87</td>
<td>0.78 ± 0.08</td>
<td>0.78 ± 0.09</td>
</tr>
<tr>
<td>-5 min</td>
<td>22.20 ± 0.80</td>
<td>46.50 ± 0.98</td>
<td>7.33 ± 0.01</td>
<td>28.50 ± 1.67</td>
<td>68.55 ± 2.82</td>
<td>0.70 ± 0.11</td>
<td>0.83 ± 0.15</td>
</tr>
<tr>
<td>+5 min</td>
<td>12.60 ± 0.92</td>
<td>51.06 ± 5.62</td>
<td>7.24 ± 0.03</td>
<td>31.30 ± 1.13</td>
<td>33.72 ± 4.28</td>
<td>1.04 ± 0.13</td>
<td>1.56 ± 0.46</td>
</tr>
<tr>
<td>+55 min</td>
<td>14.30 ± 1.97</td>
<td>60.10 ± 3.79</td>
<td>7.06 ± 0.06</td>
<td>31.90 ± 1.11</td>
<td>28.68 ± 6.09</td>
<td>1.37 ± 0.21*</td>
<td>5.95 ± 0.91</td>
</tr>
<tr>
<td>+115 min</td>
<td>21.89 ± 1.40</td>
<td>50.46 ± 3.30</td>
<td>7.18 ± 0.05*</td>
<td>29.70 ± 1.57</td>
<td>66.16 ± 3.28</td>
<td>0.97 ± 0.06</td>
<td>5.57 ± 1.17*</td>
</tr>
</tbody>
</table>

Values are means ± SE. Hct, hematocrit; SaO2, arterial O2 saturation. Times shown are relative to onset of occlusion (time 0). Statistical analysis was by t-test compared with time = –5 min. *P < 0.05; †P < 0.02; ‡P = 0.002. §P < 0.0001.

NIRS Data Analysis

Spectra from head and leg were immediately saved to disk for later analysis. To obtain absolute changes in chromophore concentration, a difference spectrum was first generated from each raw absorption spectrum, as the arithmetic difference between each spectrum and the reference spectrum taken at the start of the experiment. Each difference spectrum was then fitted between 780 and 900 nm to previously determined individual chromophore absorption spectra by using a least squares multilinear regression algorithm (37, 53). Residual changes in optical density, not accounted for in the fitting process, were analyzed to look for large or systematic changes that might indicate the presence of another chromophore not included in the algorithm. Optical pathlength was obtained by using second-order differential analysis from the 840-nm water absorption feature (16).

Data for Δ[HbO2] and Δ[Hb] are absolute changes in concentration (in µmol/l) from a zero control set at the start of the experiment. However, because the amount of cytochrome oxidase present is fixed over the time scale of the experiment, changes in COX represent changes in the amount of the oxidized enzyme present relative to the start of the experiment and not to changes in enzyme levels. To get an impression of the magnitude of changes in COX observed relative to control values, COX was fully reduced just before culling in several experiments by infusion of sodium cyanide (NaCN; 10 mg·ml⁻¹·min⁻¹). A value for changes in total hemoglobin concentration (Δ[HbO2] + Δ[Hb]) can be generated from the sum of Δ[HbO2] and Δ[Hb] at any time point. T Hb is related to blood volume through the hematocrit.

Data Presentation and Statistical Methods

NIRS data were obtained simultaneously from the brain and hindlimb of 10 fetal sheep. In addition, there was one sheep for which the leg only was monitored. Thus for control and insult periods, n = 10 (head) and 11 (hindlimb). Two sheep failed to recover from the insult and died between reversal of the insult and the end of the experiment; thus in the recovery period, n = 8 (head) and 9 (leg). All data are expressed as means ± SE. Time points taken for analysis are, except where specifically noted, –55 and –5, then +5, +55, and +115 min relative to the start of occlusion. Student’s unpaired t-test was used to compare values at each time point relative to those at –55 min. Statistical significance is taken as P ≤ 0.05.

RESULTS

Blood Gases and Acid-Base Status

Changes in blood gases, pH, and glucose and lactate concentrations, on the basis of samples from the carotid artery, are shown in Table 1. The maternal CIIA occlusion resulted in a moderate asphyxial challenge with a minimum fetal pH of 7.06 ± 0.06, arterial PO2 (Pao2) of 12.60 ± 0.92 Torr, and a maximum arterial PCO2 (Paco2) of 60.10 ± 3.79 Torr.

Cardiovascular Responses

Fetal hemodynamic data are shown in Table 2. During asphyxia there was a transient bradycardia but no significant increase in either mean arterial pressure (MAP) or carotid blood flow. However, femoral flow fell significantly and remained low for the duration of the insult, recovering slowly after reversal of occlusion.

Table 2. Values for fetal heart rate, mean arterial pressure, carotid blood flow, and femoral artery blood flow during a 1-h occlusion of the maternal common internal iliac artery

<table>
<thead>
<tr>
<th>Time</th>
<th>HR, beats/min</th>
<th>MAP, mmHg</th>
<th>CBF, ml/min</th>
<th>FBF, ml/min</th>
</tr>
</thead>
<tbody>
<tr>
<td>-55 min</td>
<td>165 ± 7</td>
<td>42.3 ± 2.8</td>
<td>57.7 ± 7.9</td>
<td>27.5 ± 7.3</td>
</tr>
<tr>
<td>-5 min</td>
<td>177 ± 7</td>
<td>41.8 ± 3.1</td>
<td>58.9 ± 6.4</td>
<td>34.0 ± 10.5</td>
</tr>
<tr>
<td>+5 min</td>
<td>111 ± 7†</td>
<td>45.6 ± 2.6</td>
<td>52.8 ± 7.8</td>
<td>17.1 ± 9.2*</td>
</tr>
<tr>
<td>+55 min</td>
<td>160 ± 13</td>
<td>43.3 ± 3.6</td>
<td>57.9 ± 11.2</td>
<td>24.2 ± 11.3</td>
</tr>
<tr>
<td>+115 min</td>
<td>199 ± 7</td>
<td>46.4 ± 2.1</td>
<td>69.8 ± 8.8*</td>
<td>27.5 ± 4.7</td>
</tr>
</tbody>
</table>

Values are means ± SE at occlusion –55, –5, (control), +5, +55, and +115 min (i.e., 55 min after reversal of the occlusion). HR, heart rate; MAP, mean arterial pressure; CBF and FBF, carotid and femoral artery blood flow, respectively. *P < 0.05, †P < 0.001 (ANOVA; each variable vs. –5 min).
At 55 min after reversal of the insult, DO$_2$ values for both head and leg were not significantly different from control. The percent fall in DO$_2$ to the hindlimb was greater than to the brain at 15 min (85 vs. 61%, respectively).

D$_{[\text{HbO}_2]}$ and D$_{[\text{Hb}]}$ vs. control, measured by NIRS in brain and hindlimb, are shown in Fig. 2. Immediately after occlusion of the CIIA, [HbO$_2$] fell rapidly within the brain by 19.8 ± 2.5 µmol/l at +5 min (P < 0.001 vs. control), whereas there was a rapid reciprocal rise in [Hb] of 28.6 ± 3.7 µmol/l (P < 0.001). These findings indicate a fall in cerebral oxygen saturation, coincident with the fall in DO$_2$. With reversal of occlusion, [HbO$_2$] returned to control levels. Although [Hb] also fell toward control levels, it remained elevated at 55 min postreversal (P = 0.05).

In the hindlimb there was a similar rapid fall in [HbO$_2$] of 22.2 ± 2.7 µmol/l (P < 0.001) with occlusion, although this was not associated with an immediate increase in [Hb] as was observed in the brain (Fig. 3). Instead, [Hb] increased slowly to a maximum of 13.3 ± 4.2 µmol/l at +55 min (P < 0.05). The attenuated rise in [Hb] is related to the fall in blood volume seen in the hindlimb during the insult (see Fig. 3 below). After reversal of occlusion, both [HbO$_2$] and [Hb] slowly recovered toward, but did not reach, control by 55 min of recovery. Again, these findings indicate that hemoglobin oxygen saturation was lower than control values, even after a considerable period of recovery.

D$_{[\text{tHb}]}$

During the occlusion period the cerebral [tHb] increased to 23.16 ± 3.27 µmol/l at +55 min, reaching plateau levels after ~20 min (Fig. 2). Cerebral [tHb] returned to control by 55 min of recovery. In contrast,
[THb] fell initially in the hindlimb by 21.44 ± 2.80 µmol/l at +5 min, although it then started to return to control values, which were achieved by +55 min (Fig. 3). These findings are indicative of a large increase in cerebral blood volume during the insult and a fall in the blood volume within the hindlimb, coincident with the initial fall in femoral arterial blood flow (see Table 2 above).

Changes in oxidized [COX]

After the onset of occlusion, when DO₂ had fallen, cerebral COX became more oxidized, with a gradual increase in the oxidized [COX] to maximum values of 0.98 ± 0.19 µmol/l at +55 min (Fig. 2, P < 0.001). With the return to normoxia the levels fell and in fact were significantly lower than control at 55 min postreversal (P = 0.058). In contrast, occlusion was associated with a rapid reduction in COX in the hindlimb, with oxidized COX falling by 1.08 ± 0.27 µmol/l at +5 min (Figure 3). After this, COX slowly became oxidized but was still reduced, compared with control levels, after 55 min of recovery.

Optical Data

The raw data shown in Fig. 4 describe a typical response to infusion of 10 mg·ml⁻¹·min⁻¹ NaCN on cerebral [HbO₂], [Hb], and [COX]. NaCN causes a maximum fall of 0.96 µmol/l. This occurs despite Δ[HbO₂] and Δ[Hb]. No further reduction of COX was seen during terminal deoxygenation occurring at the end of the experiment.

To exclude the possibility of an artifact arising from the presence of a chromophore not accounted for, or of optical pathlength changes, further optical tests were performed on the data. Analysis of residuals left after fitting the data showed no significant or systematic change during hypoxia.

Control values in the brain were 4.5 ± 1.8 (sum of mean squares) vs. 6.4 ± 1.7 m OD during hypoxia (P = 0.48). Similar values were obtained in the leg: 6.9 ± 2.2 (control) vs. 7.9 ± 2.7 m OD (hypoxia) (P = 0.76). Similarly, optical pathlength at 840 nm did not change significantly in the brain during the insult, being 12.0 ± 2.8 (SD) cm before and 12.3 ± 3.8 cm during the period of hypoxia (P = 0.84). The optical pathlength in the hindlimb was 11.2 ± 1.9 cm before and 11.9 ± 2.0 cm during hypoxia (P = 0.53).

DISCUSSION

The mammalian fetus may be exposed to episodes of hypoxia both before and during labor (28, 42, 51). Some vertebrate species that are exposed to prolonged periods of reduced oxygen supply, such as the freshwater turtle and diving seal, have developed complex compensatory mechanisms to ensure intact brain survival (8, 22, 25, 26, 35). In such “hypoxia-tolerant” species the traditional view, that oxygen deprivation at a cellular level leads to energy depletion and that this deficit is taken up by the activation of ATP-generating anaerobic pathways, is an oversimplification. In fact, the twin strategies of reducing energy turnover and maximizing the efficiency of ATP production mean that supply and demand are balanced, and ATP levels stay stable (13). It is reasonable to expect that the late-gestation fetus might behave in a similar manner. The data presented
The muscle data do show that as femoral blood flow falls, so does blood volume, reflecting chemoreflex-mediated peripheral vasoconstriction. A different situation is observed in the brain, where cerebral blood volume increases rapidly, despite the fact that blood flow is unchanged. It is important to realize that NIRS measures Δ[tHb] occurring in all the vascular compartments, so that the increase in cerebral blood volume observed here could be primarily venous rather than arterial. Certainly, increased afterload as a result of peripheral vasoconstriction could cause increased atrial filling pressure, increased venous pressure, and venous congestion. Because the relationship between blood volume and [Hb] depends on the hematocrit, it would not be correct to deduce that an increase in cerebral blood volume occurred from the increase in [tHb] if there were a simultaneous increase in hematocrit. However, the small size of the observed change in hematocrit makes this explanation unlikely.

This study describes changes in the redox state of COX, measured by NIRS. COX is the terminal enzyme of the mitochondrial electron transport chain and is responsible for 90% of VO2. It contains two heme Fe and two Cu centers, the redox state of which reflect changes in 1) the supply of reducing equivalents to the respiratory chain, 2) the ATP concentration, and 3) the cellular oxygen concentration (14, 15, 48, 52). Of particular interest is the CuA center of the enzyme. This is the penultimate electron acceptor of the transport chain and is responsible for 90% of the near-infrared spectrum attributed to COX. The CuA center has a characteristic absorption peak in the near-infrared region that is sensitive to its redox state; the oxidized enzyme has a significantly greater absorbance at 830 nm than the reduced enzyme. It is thus possible to detect changes in the CuA redox state in vivo by NIRS. Although sensitive to all the factors listed above, the greatest changes are those accompanying severe decreases in DO2 to the mitochondria, when the CuA center becomes highly reduced.

After the onset of hypoxemia, there was a rapid and significant fall in DO2 to the hind leg. This was associated with a reduction of the CuA center with a mean fall in oxidized [COX] of 1.1 ± 0.3 μmol/l. Therefore, in hindlimb muscle the redox state of COX does appear to be sensitive to changes in DO2. However, these data also show a return of the oxidized [COX] toward control despite the persistent fall in DO2. This might reflect similar adaptive metabolic processes to those observed in the brain.

Despite a fall in DO2 to the brain during occlusion, there was a gradual increase in the redox state of COX, which returned to control values with normoxia. Although other authors using NIRS have reported small increases in the redox state of COX in the human brain in vivo, this has been interpreted as the consequence of an increase in DO2, not a decrease as reported here (21). There are little comparable fetal data. Using NIRS in the fetal sheep brain, Marks et al. (36) reported a decrease in the redox state of COX during bilateral occlusion of the carotid arteries. However, this was a more severe insult to that used in this study. Several studies in the neonatal piglet have also demonstrated increases in the redox state of COX like those seen here, during moderate, but not severe, hypoxemia (34, 50). More normally, studies in neonatal animals and humans show reductions in COX redox state during a fall in DO2. Such studies have demonstrated that this precedes changes in electrocortical activity (27), correlates closely with reductions in the concentration of high-energy phosphorus metabolites measured simultaneously with nuclear magnetic resonance spectroscopy (38, 50), and predicts neurological outcome in children after cardiac surgery (20, 40). Although these studies are not strictly comparable to ours, being neonatal rather than fetal and investigating the effects of anoxia
rather than hypoxia, they at least demonstrate that changes in the redox state of COX measured by NIRS correlate with other markers of cell metabolism. There are, however, few pointers as to the nature of the metabolic processes responsible for the redox changes reported here.

The control of respiration is complex and depends on a variety of factors, including the size of the system studied (i.e., cell, mitochondria, organ), conditions such as the rate of ATP use, and the particular organ studied (6). However, the idea that the rate of ATP use is linked to respiration and ATP synthesis by mass action in all tissues, as proposed by Chance and Williams (10) in 1955, still remains the basic mechanism of control and has a greater effect on respiratory function than, for instance, substrate availability. The hypothesis raised by our data would therefore be that, as a result of a moderate reduction in fetal cerebral DO2, cerebral metabolism decreases and ATP turnover is reduced. This could decrease oxidative phosphorylation in several ways: through an inhibitory effect of ATP on the TCA cycle and by causing a rise in NADH+, which, in turn, will inhibit the flow of reducing equivalents. If the rate of electron transfer down the mitochondrial electron transfer chain falls, the CuA moiety will become oxidized. In addition, nitric oxide, which is known to increase in the brain during hypoxia, has been shown to decrease VO2 by inhibition of complex I and IV of the oxidative phosphorylation pathway. This could have a similar effect on COX redox state (11).

It may be relevant that there is a rapid fourfold increase in plasma adenosine levels in the fetus during hypoxia and presumably a much greater increase in the brain. Adenosine, as well as causing cerebral vasodilation, is known to decrease cerebral VO2 by depressing excitatory neurotransmission and causing neuronal hyperpolarization (24, 32, 33, 41, 44, 47, 49). Further research is required, using adenosine agonists and antagonists acting at specific receptors, to determine the effect of adenosine on fetal cerebral metabolism and, in particular, the redox state of COX.

The interpretation of the optical signal attributed to COX is made more complex by the fact that [COX] is only 5% that of hemoglobin. This gives rise to the potential for “cross talk” between the two signals, especially when there are large changes in hemoglobin concentration, as reported here. To address such concerns, the following criteria, previously recommended for analyzing this signal (18), have been applied to our data. 1) The algorithm we used (37, 53) was designed for the adult human brain, but its components, such as the near-infrared spectrum of COX and wavelength dependence of optical pathlength, have subsequently been checked in the perfluorocarbon-perfused as well as blood-perfused neonatal piglet brain (14) and found to be identical. 2) Changes in the optical pathlength at 840 nm, determined by using the water peak method (16) and measured during hypoxia, were small and made little difference to calculated changes in oxidized COX when included. 3) Because the charge-coupled-device system used in these experiments is multiwave-length, in contrast to the 3- to 4-wavelength spectrometers commercially available, it is possible to achieve more accurate data fitting and thus better multi-component deconvolution of the individual hemoglobin and cytochrome signals. Proof of this is provided by analysis of the residuals, which shows that in our data systematic error is low. 4) A further condition is that the measured changes should be of a reasonable magnitude, i.e., not larger than the absolute [COX] in the tissue. Absolute quantification of the COX protein has not been performed in fetal sheep brain or muscle, but values of 5.5 and 3.2 μmol/l have been described in adult rat brain and the neonatal piglet, respectively (5, 17). It is likely that the values in the near-term fetal sheep may be slightly lower as the brain is less developed and has a lower metabolic rate. However, applying neonatal values would suggest changes in the oxidized [COX] of ~30% total concentration. 5) Finally, the relative sizes of the hemoglobin and cytochrome signals could give rise to cross talk, where the cytochrome signal is partially affected during large changes in hemoglobin concentration. It is therefore reassuring that during administration of NaCN, the COX redox state falls and remains reduced despite subsequent large changes in the concentrations of the other chromophores present (Fig. 4). These data suggest that the signal from COX is independent of those from HbO2, Hb, and THb.

In summary, these data show that, by using NIRS, it is possible to detect fundamentally different responses to 1 h of hypoxemia in fetal sheep brain and hindlimb muscle. They suggest that a coordinated pattern of response occurs in the brain that involves both vascular and metabolic components. Further studies are now necessary to determine the mechanisms involved and to see whether the response is neuroprotective.

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