Developmental Changes in Newborn Lamb Brain Mitochondrial Activity and Postasphyxial Lipid Peroxidation (43892)

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Abstract. **Previously, our laboratory has demonstrated inhibition of mitochondrial state 3 (ADP-dependent) respiration 5 min after resuscitation from an asphyxial insult in lambs less than 3 days of age. Older lambs were resistant to this transient mitochondrial dysfunction. This study was designed to examine if age-related differences in baseline state 3 mitochondrial respiration, electron transport chain activity, or susceptibility to oxygen free radical-mediated lipid peroxidation were related to the previously observed differences in postasphyxial mitochondrial respiration. Mitochondrial respiration was measured in 24 nonasphyxiated control lambs aged 1–10 days using four different substrates. Electron transport chain activity was assessed in 15 of these lambs, and lipid peroxidation measured as conjugated diene production was measured in 11 of these lambs. These lambs were all ventilated to maintain normal blood gases for a time period equal to the length of the hypoxic insult in asphyxiated lambs (see below), after which samples of brain were removed for isolation of mitochondria. A second group of 11 lambs (seven ≤3 days of age and four >3 days of age) were asphyxiated. The insult was a 75-to-90-min episode of hypoxia and hypercarbia that resulted in bradycardia and systemic hypotension over the final 15 min of the insult. At the end of asphyxia, the lambs were resuscitated and returned to control ventilator settings. Samples of brain were removed 5 min after resuscitation. Postasphyxia electron transport chain activity and lipid peroxidation were measured. All measurements described above were done in both nonsynaptic (primarily glial in origin) and synaptic mitochondria. State 3 mitochondrial respiration varied significantly with age, decreasing by an average of 41.2% ± 11.1% (mean ± SEM) from Day 2 to Day 5–6 and then increasing back to levels similar to Day 2 by Day 8–10 in nonsynaptic mitochondria. State 3 respiration in synaptic mitochondria decreased 60.6% ± 5.2% from Day 2 to Day 5–6 before returning to levels similar to Day 2 by Day 8–10. Resting (nonADP-dependent) state 4 respiration demonstrated similar developmental patterns. Electron transport chain activities did not vary with age in the nonasphyxiated control animals. In addition, an asphyxial insult did not diminish electron transport chain activities in either lambs ≤3 days old or those >3 days of age. In the ≤3-day-old animals, lipid peroxidation (measured as conjugated diene production) was 0.755 ± 0.19 (Abs 233/mg lipid; mean ± SEM) in controls and 1.31 ± 0.28 postasphyxia ($P < 0.03$) in nonsynaptic mitochondria. Synaptic mitochondria conjugated diene productions were 0.561 ± 0.05 and 1.369 ± 0.27 ($P < 0.004$), respectively. No differences in conjugated diene production between control and postasphyxia lambs were present in animals >3 days old. These data are consistent with the conclusion that lambs ≤3 days old were more susceptible to oxygen free radical-mediated lipid peroxidation than those >3 days of age.** [P.S.E.B.M. 1995, Vol 209]

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There are many possible mechanisms of postasphyxia cellular dysfunction. One of these mechanisms is energy failure due to mitochondria dysfunction. In a variety of adult animal models, mitochondrial oxidative function has been shown to be extremely sensitive to hypoxic/ischemic insults (1–5). Restoration of function during recirculation has been dependent primarily on the duration and type of ischemia (complete versus incomplete) (1, 2, 4, 5). Previously, our laboratory has demonstrated inhibition of mitochondrial state 3 respiration 5 min after resuscitation from an asphyxial insult in lambs less than 3 days of age (6). By 2 hr after the event, mitochondrial respiration had returned to baseline indicating only transient mitochondrial dysfunction. Of interest is that lambs greater than 3 days of age appeared to demonstrate resistance to even transient mitochondrial dysfunction. This resistance of mitochondrial respiration is in sharp contrast to the concept of greater resistance to the effects of asphyxia in more immature members of a species (7–10).

One possible explanation for the lack of inhibition of mitochondrial respiration seen in the >3-day-old animals is the lower baseline level of respiration compared with that demonstrated in younger lambs (6). Mela and colleagues have reported similar data in both brain and heart mitochondria. Newborns had higher state 3 respiratory rates while at the same time the levels of a number of measured cytochromes were lower than in adult animals (11, 12). It is possible that a lower baseline respiration rate with higher cytochrome levels may render mitochondria intrinsically more resistant to asphyxia than with higher rates of respiration and lower cytochrome levels. Another possibility to consider is that the younger lamb may be more susceptible to oxygen free radical damage (perhaps to the membrane bound electron transport chain). *In vitro* studies have demonstrated inhibition of respiratory activity similar to that reported following transient ischemia in mitochondria exposed to oxygen radicals (13). Furthermore, *in vitro,* an increase in free radicals leading to membrane lipid peroxidation has been shown to effect electron transport chain enzymes (in particular complex 1), which could lower state 3 respiration (14).

This investigation was designed to address possible mechanisms of transient mitochondrial dysfunction observed in newborn lambs less than 3 days of age. The first hypothesis examined was that developmental differences in either electron transport chain activity or baseline levels of mitochondrial respiration are related to the transient inhibition of state 3 mitochondrial respiration observed in lambs <3 days of age. The second hypothesis examined was that greater susceptibility to oxygen free radical mediated lipid peroxidation of mitochondrial membranes in younger lambs contributes to transient inhibition of state 3 mitochondrial respiration.

Materials and Methods

Thirty-five newborn lambs 1–10 days old were operated upon under intravenous Ketamine anesthesia (100 mg as an initial dose followed by 20 mg every 15 min). Polyvinyl chloride catheters (0.034 in i.d. × 0.054 in o.d.; Martech Medical Products, Lansdale, PA) were placed into the abdominal aorta via each femoral artery and into the inferior vena cava via a femoral vein. A 1-cm diameter burr hole in the skull down to the dura mater was drilled over the parietal region to facilitate brain removal at the study's conclusion. The burr hole was filled with bone wax, and the skin was closed with a running 3-O suture. The lambs were allowed 24 hr to recover prior to study. During this time, animals were standing and nursing normally with no evidence of pain or distress.

On the day of study, the lambs were anesthetized with fentanyl (20 μg/kg followed by 10 μg/kg/hr infusion), paralyzed with 0.1 mg/kg pancuronium and ventilated with an infant ventilator (Bird Co., Palm Springs, CA) with a gas mixture providing a $Pa0_2$ of 80–120 mm Hg and a $PaCO_2$ of 35–40 mm Hg. Adequacy of analgesia during the study was documented with continuous monitoring of heart rate and blood pressure.

Twenty four of the lambs were used as nonasphyxiated (control) lambs, divided by age into six groups: 2, 3, 4, 5–6, 7, 8–10 days, respectively. These control lambs were maintained at normal blood gas levels for 4 hr. $Pa0_2$, $PaCO_2$, pH, and arterial oxygen content ($Ca0_2$) were measured every 30 min over the 4-hr period; heart rate and blood pressure were recorded. All physiologic parameters remained stable over the 4-hr study period. After the last blood sample, an additional 20 μg/kg dose of fentanyl was administered and a 5-to-7-g brain sample was removed from the parietal cortex for isolation of mitochondria. The brain sample contained predominantly gray matter, but no effort was made to remove all white matter tissue. Lambs were then sacrificed immediately with Pentobarbital Sodium Euthanasia Injection C-II (Anthony Products Co., Aracadia, CA).

Another group of 11 lambs (seven that were 24–48 hr of age and four that were 4–7 days of age) were anesthetized, intubated, and ventilated as described above prior to undergoing an asphyxial insult (15). $Pa0_2$ was lowered to 15–22 mm Hg, $Ca0_2$ to 1.5–2.5 vol%, and $PaCO_2$ was increased to 60–70 mm Hg by decreasing the ventilator rate to 6–10 breaths/min and adjusting the gas mixture to 10% 0_2 and 90% N_2. The total duration of the insult was 75–90 min. Over the final 10–15 min, the lambs became bradycardic (heart rate <100 bpm) and hypotensive (mean arterial blood

pressure 20–35 mm Hg). The episode of asphyxia was terminated before cardiac arrest, when it was still possible to resuscitate the lambs without the use of cardiotonic drugs. The lambs were then returned to baseline ventilator settings and $Fi0_2$. Brain material was removed as above for isolation of mitochondria 5 min after the end of the asphyxial episode. Total study duration for this group of 11 animals was the same as for the control lambs.

Blood samples for $Pa0_2$, $PaCO_2$, pH and $Ca0_2$ were withdrawn anaerobically into heparinized Natalson glass pipettes from the abdominal aorta catheter. $P0_2$, PCO_2, and pH were measured at 39.5°C using the Radiometer ABL 330 blood gas analyzer (Radiometer, Copenhagen, Denmark). Blood hemoglobin concentration and oxyhemoglobin saturation were measured colorimetrically in duplicate by a hemoximeter (Radiometer), and $Ca0_2$ was calculated as the product of hemoglobin concentration and oxyhemoglobin saturation. Blood pressure (referenced to the right atrium) and heart rate were continuously monitored in the abdominal aorta (Gould Instruments, Oxford, CA).

Brain nonsynaptic mitochondria (primarily glial in origin) (16) and synaptic mitochondria were isolated by a modification of the method of Lai and Clark (17). Brain tissue for mitochondria isolation was homogenized on ice immediately in a small volume of buffer composed of 0.32 *M* sucrose, 10 m*M* Tris base, and 2 m*M* EDTA. After an initial separation, centrifuging (Sorval, Newtown, CT) twice at 1200*g* (saving the supernatants) and centrifuging the combined supernatants once at 14,460*g*, the pellet was resuspended in buffer to a volume of 5 ml. This material was layered over a step gradient of 7 ml 7.5% Ficoll solution and 7 ml 10% Ficoll solution. Ultracentrifugation (Beckman Instruments, Inc., Fullerton, CA) was then performed for 37 min at 100,000*g*. Myelin was at the first interface, synaptic mitochondria were at the second interface, and nonsynaptic mitochondrial were in the pellet. The mitochondrial fractions were then purified and resuspended in assay buffer containing 225 m*M* mannitol, 75 m*M* sucrose, 10 m*M* Tris-HCl, 20 m*M* KCl, and 5 m*M* potassium phosphate at pH 7.4. Concentration of mitochondrial protein in the final suspension was determined by the method of Lowry *et al.* (18).

Brain polarographic assays with a Clark electrode (Yellow Springs Instrument Co., Yellow Springs, OH) were performed at 25°C as previously described (19–21) in the nonasphyxiated group of lambs. Substrates assayed were glutamate, α-ketoglutarate, pyruvate/malate, and succinate; all at 5 m*M* concentration. The amount of ADP added to the assay chamber was determined by ultraviolet absorbance. Absorbance was read at 259 nm against a buffer reference using a molar extinction coefficient for ADP of $15.4 \times 10^3/M/cm$.

For assay of mitochondrial electron transport chain complexes, freshly isolated mitochondria were frozen at −70°C in 0.22 *M* sucrose, 0.07 *M* mannitol, 0.03 *M* EDTA, 0.005 *M* MOPS (pH 7.4) with 50% glycerol as a cryoprotective agent. Complex I (NADH-ubiquinol oxidoreductase) was assayed spectrophotometrically in sonicated mitochondria as the rotenone sensitive (2 μ*M*) oxidation of NADH in the presence of 2 m*M* NaN_3 using the ubiquinol analog, Q1 (Eisai, Tokyo), as the electron acceptor (22). Complexes II and III were assayed as succinate:cytochrome c oxidoreductase activity (23). Complex IV activity was assayed spectrophotometrically as cyanide sensitive cytochrome c oxidase activity in cholate solubilized mitochondria (22, 24).

Mitochondrial lipid peroxidation was assessed by measuring lipid-conjugated dienes, as previously described (25). Lipids were extracted with chloroform/methanol (2:1) dried under a stream of oxygen free nitrogen, redissolved in 1.5 ml of cyclohexone, and analyzed for absorbance between 220 and 275 nm. Aliquots (250 μl) from each sample were analyzed for total lipid content (26). Absorbance measurements were normalized to a denominator of 1 mg lipid/ml cyclohexane. Lipid-conjugated dienes were expressed as the absorbance at 233 nm/mg lipid.

Results of polarographic assays were calculated according to the method of Estabrook (27). State 4 respiratory rate (Fig. 1) was the consumption of 0_2 by mitochondria in the presence of substrate. State 3 respiratory rate (Fig. 1) was the consumption of 0_2 by mitochondria in the presence of substrate after the addition of ADP. The respiratory control ratio (RCR) was the ratio of state 3 to state 4 respiration. The ADP:0 was calculated as the ratio of the amount of ADP added to the reaction chamber and the total 0_2 consumed. State 3 and state 4 respiratory rates, RCR, ADP:0 and electron transport chain protein activities

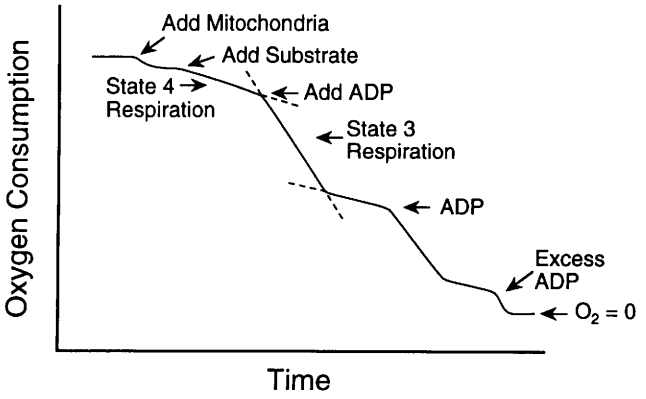

Figure 1. Mitochondrial respiration. State 4 respiratory rate is the consumption of oxygen by mitochondria (nano-atoms O_2/mg mitochondrial protein/min) in the presence of substrate. State 3 respiration is the consumption of oxygen in the presence of substrate after the addition of ADP. When all ADP is converted to ATP, respiration returns to state 4 respiratory rate oxygen consumption.

were compared among the different age groups by one-way analysis of variance and t tests using Bonferroni's correction for multiple comparisons (28). Conjugated diene production was compared between asphyxia and nonasphyxia groups using an unpaired t test.

Results

Tables I through IV present state 3 and state 4 mitochondrial respiration in the different age groups for nonsynaptic and synaptic mitochondria. A consistent pattern is demonstrated with state 3 respiratory rates declining to a low point on Day 5–6 with an increase back to levels similar to the first 4 days of life on Day 8–10. For most substrates tested, state 3 respiration was significantly decreased on Day 5–6 compared with Day 2–4; (40%–54% for nonsynaptic and 40%–62% for synaptic). No significant differences were noted over time for state 4 respiratory rates. However, a trend demonstrating a similar pattern as seen with state 3 respiration was seen with resting respiration lowest on Day 5–6. The RCR's in nonsynaptic mitochondria did not differ over the age range studied for all four substrates (Table V). In synaptic mitochondria, no significant differences were seen with glutamate or α-ketoglutarate. RCR for pyruvate was significantly higher on Day 3 than other time points. ADP:0 ratio did not vary with any substrate in either nonsynaptic or synaptic mitochondria (Table VI).

Electron transport protein complex data are presented in Table VII). No significant differences were noted among control animals over the first 10 days of life. In addition, activity was not affected by a prior asphyxial insult in lambs less than 3 days of age.

Figure 2 presents conjugated diene production in control and asphyxiated animals, broken down by age. In asphyxiated animals ≤3 days of age, conjugated diene production was increased compared with controls in both nonsynaptic and synaptic mitochondria while no differences were present in animals greater than 3 days of age.

Discussion

The newborn lamb postasphyxia model has been utilized to describe physiologic and biochemical abnormalities after a standardized asphyxial insult (6, 15). The biochemical abnormality described was a transient inhibition of mitochondrial state 3 (ADP dependent) respiratory activity (6). This finding was age dependent in that state 3 respiration was inhibited immediately postasphyxia in animals who were less than 3 days of age and not in those greater than 3 days of age. These findings were not felt to be indicative of uncoupling of mitochondrial respiration in that the anticipated increase in state 4 respiratory rate was not seen. In addition, substrates entering the electron transport chain through both complex I (NAD-linked) and complex II (FAD-linked) were effected to the same extent. The latter finding was felt to be consistent with either a defect further down the electron transport chain or with a depression of the various dehydrogenases involved in substrate oxygenation. The data in the present study demonstrated that electron transport chain complex activities did not vary with age (over the first 10 days of life) and were uneffected by asphyxia. This finding is consistent with the hypothesis that postasphyxial mitochondrial dysfunction is due to diminshed dehydrogenase activity. Data from adult rats supports this notion in that reductions of pyruvate-supported respiration is related specifically to selective reductions in the activity of the pyruvate dehydrogenase complex activity rather than reductions of electron transport chain activity (29–31). Of note, no significant change was seen in the activity α-ketoglutarate dehydrogenase or succinate supported respiration (29–31). However, against the hypothesis of diminished dehydrogenase activity, in particular if the mechanism is related to oxidative damage, is that the dehydrogenases are not uniformly membrane bound. An alternative explanation for diminished post asphyxia state 3 respiration is an effect on the ATP synthetase or the ADP/ATP translocase, either directly or due to a decrease in matrix adenine nucleotide content (32, 33).

Developmental changes over the first 10 days of life in baseline mitochondrial respiratory activity also do not explain the previously observed differences in sensitivity to asphyxia in lambs less than versus greater than 3 days of age. State 3 respiratory rates do vary considerably over the first 10 days of life. The present data are consistent with previous observations

Table I. Nonsynaptic Mitochondria State 3 Respiration at Different Ages

	2 Days	3 Days	4 Days	5–6 Days	7 Days	8–10 Days
Glutamate	214.8 ± 24.2	238.6 ± 27.2	205.9 ± 23.8	121.7 ± 8.4[a,b,c]	144.8 ± 19.8[d]	243.8 ± 37.1
Pyruvate	238.3 ± 22.2	190.0 ± 22.8	213.9 ± 23.7	125.9 ± 8.2[a,b,c]	115.8 ± 14.2[a,c,d,e]	176.2 ± 19.9[f]
α KG	116.3 ± 12.2	161.7 ± 26.1	163.3 ± 13.4	98.2 ± 19.7	102.1 ± 24.8	114.3 ± 18.7
Succinate	224.3 ± 24.3	201.2 ± 32.3	227.9 ± 9.2	105.2 ± 12.3[a,b]	121.1 ± 15.5[c,d]	167.2 ± 24.7

Note. Values are mean ± SEM nanoatoms O_2/mg mitochondrial protein/min; $n = 4$ in each age group. α KG = α-ketoglutarate. For glutamate: [a]$P < 0.02$ vs 2 days, [b]$P < 0.005$ vs 3 days, 8–10 days, [c]$P < 0.03$ vs 4 days, [d]$P < 0.02$ vs 3 days, 8–10 days. For pyruvate: [a]$P < 0.001$ vs 2 days, [b]$P < 0.05$ vs 3 days, [c]$P < 0.005$ vs 4 days, [d]$P < 0.02$ vs 3 days, [e]$P < 0.05$ vs 8–10 days, [f]$P < 0.05$ vs 2 days. For succinate: [a]$P < 0.001$ vs 2 days, 4 days, [b]$P < 0.005$ vs 3 days, [c]$P < 0.005$ vs 2 days, 4 days, [d]$P < 0.02$ vs 3 days.

Table II. Nonsynaptic Mitochondria State 4 Respiration at Different Ages

	2 Days	3 Days	4 Days	5–6 Days	7 Days	8–10 Days
Glutamate	38.5 ± 3.7	39.1 ± 8.3	28.7 ± 7.8	25.4 ± 3.0	24.4 ± 6.6	50.6 ± 13.4
Pyruvate	64.6 ± 9.7	34.7 ± 6.8	38.2 ± 8.4	32.0 ± 13.5	26.4 ± 5.4	45.8 ± 7.7
α KG	29.3 ± 5.6	32.9 ± 4.0	28.8 ± 5.8	20.7 ± 2.5	24.1 ± 2.3	33.7 ± 5.7
Succinate	85.2 ± 12.0	89.7 ± 12.0	80.5 ± 11.9	56.8 ± 12.0	56.0 ± 21.2	96.3 ± 23.2

Note. Values are mean ± SEM nanoatoms O_2/mg mitochondrial protein/min; $n = 4$ in each age group. α KG = α-ketoglutarate.

Table III. Synaptic Mitochondria State 3 Respiration at Different Ages

	2 Days	3 Days	4 Days	5–6 Days	7 Days	8–10 Days
Glutamate	155.3 ± 13.7	165.4 ± 20.3	170.5 ± 26.7	99.9 ± 16.2	102.0 ± 20.1	155.8 ± 17.9
Pyruvate	144.1 ± 21.0	174.7 ± 25.8	148.4 ± 20.0	72.6 ± 7.0[a,b]	88.4 ± 15.9[c,d]	126.8 ± 21.5
α KG	86.1 ± 15.0[a]	122.0 ± 2.2	151.4 ± 18.6	57.9 ± 8.5[b,c]	80.8 ± 21.0[d]	84.3 ± 14.7[e]

Note. Values are mean ± SEM nanoatoms O_2/mg mitochondrial protein/min; $n = 4$ in each age group. α KG = α-ketoglutarate. For pyruvate; [a]$P < 0.02$ vs 2 days, 4 days, [b]$P < 0.002$ vs 3 days, [c]$P < 0.006$ vs 3 days, [d]$P < 0.05$ vs 4 days. For α KG; [a]$P < 0.01$ vs 4 days, [b]$P < 0.007$ vs 3 days, [c]$P < 0.001$ vs 4 days, [d]$P < 0.006$ vs 4 days, [e]$P < 0.01$ vs 4 days.

Table IV. Synaptic Mitochondria State 4 Respiration at Different Ages

	2 Days	3 Days	4 Days	5–6 Days	7 Days	8–10 Days
Glutamate	38.1 ± 2.6	26.9 ± 2.4	22.9 ± 7.3	31.7 ± 6.1	25.4 ± 3.8	30.1 ± 3.9
Pyruvate	44.0 ± 5.3	28.2 ± 5.5	25.5 ± 6.4	21.7 ± 4.6	28.2 ± 1.7	33.6 ± 5.3
α KG	32.1 ± 4.7	27.2 ± 1.9	29.1 ± 5.4	23.1 ± 5.4	24.3 ± 2.2	22.1 ± 2.8

Note. Values are mean ± SEM nanoatoms O_2/mg mitochondrial protein/min; $n = 4$ in each age group. α KG = α-ketoglutarate.

Table V. Respiratory Control Ratio (RCR) at Different Ages

	2 Days	3 Days	4 Days	5–6 Days	7 Days	8–10 Days
Nonsynaptic						
Glutamate	4.2 ± 1.4	6.4 ± 0.6	7.6 ± 0.5	5.7 ± 1.0	5.7 ± 0.5	4.7 ± 0.5
Pyruvate	3.3 ± 0.3	5.0 ± 0.5	6.5 ± 0.9	5.0 ± 1.4	4.1 ± 0.5	3.8 ± 0.4
α KG	3.6 ± 0.2	5.5 ± 0.6	5.6 ± 0.5	6.3 ± 1.6	4.8 ± 0.7	3.2 ± 0.4
Succinate	2.1 ± 0.2	2.2 ± 0.4	3.0 ± 0.3	2.0 ± 0.3	2.3 ± 0.2	2.2 ± 0.3
Synaptic						
Glutamate	4.2 ± 0.6	6.0 ± 1.3	6.2 ± 1.0	4.3 ± 1.0	3.9 ± 0.3	4.9 ± 0.8
Pyruvate	3.3 ± 0.3	7.0 ± 0.2[a]	4.5 ± 0.9	2.9 ± 0.4	3.2 ± 0.6	3.7 ± 0.7
α KG	3.2 ± 0.6	4.2 ± 0.6	5.3 ± 0.5	3.3 ± 0.7	3.3 ± 0.7	3.9 ± 0.7

Note. Values are mean ± SEM; $n = 4$ in each age group. α KG = α-ketoglutarate.
[a] $P < 0.008$ vs 2 days, $P < 0.02$ vs 4 days, $P < 0.006$ vs 5–6 days, $P < 0.006$ vs 7 days, $P < 0.002$ vs 8–10 days.

of Mela *et al.* in newborn puppies in which state 3 respiratory activities of isolated heart mitochondria decreased progressively over the first 4 days of life with a nadir on Day 4 before increasing again over Day 5–10 of life (12). This pattern of respiratory activity in isolated lamb brain mitochondria over the first 10 days of life cannot explain the resistance of older animals' mitochondria to asphyxia. The changes described represent a biologic continuum with no break point at 3 days of age. Baseline state 3 respiration is lower at 5–7 days of age, but is similar at 8–10 days of age to respiratory rates in animals less than 3 days of age.

The major difference demonstrated in this study between young and older animals was the degree of postasphyxia mitochondrial lipid peroxidation present in animals ≤3 days of age. Lipid peroxidation was significantly increased compared with controls in young, but not older, animals. Previously, we have demonstrated the role of oxygen free radicals in post asphyxia cerebral hypoperfusion in the newborn lamb (34). In addition, oxygen free radicals have been shown in an *in vitro* model to inhibit mitochondrial respiration after ischemia in adult rats (13). The results of this particular investigation are consistent with our results in that the oxygen free radical-mediated inhibition of state 3 respiration did not appear related to inhibition in the cytochrome region of the respiratory chain. The data in the present study do not provide an explanation for the decreased resistance of the young animals to oxygen free radical-mediated lipid peroxidation nor the site of injury. It is possible that the mechanism of injury is due to decreased levels of ox-

Table VI. ADP:O Ratio at Different Ages

	2 Days	3 Days	4 Days	5–6 Days	7 Days	8–10 Days
Nonsynaptic						
Glutamate	2.9 ± 0.2	2.7 ± 0.2	2.9 ± 0.2	2.6 ± 0.4	3.2 ± 0.1	2.9 ± 0.2
Pyruvate	3.0 ± 0.2	2.6 ± 0.3	3.1 ± 0.3	3.0 ± 0.4	3.4 ± 0.2	2.9 ± 0.4
α KG	3.3 ± 0.3	2.6 ± 0.2	3.2 ± 0.3	2.8 ± 0.5	3.3 ± 0.1	3.0 ± 0.4
Succinate	2.2 ± 0.2	2.3 ± 0.4	2.8 ± 0.2	2.2 ± 0.3	2.4 ± 0.2	2.1 ± 0.1
Synaptic						
Glutamate	2.6 ± 0.1	2.6 ± 0.2	2.7 ± 0.2	2.8 ± 0.3	3.0 ± 0.3	3.0 ± 0.3
Pyruvate	2.8 ± 0.2	2.7 ± 0.2	3.1 ± 0.3	3.1 ± 0.7	3.4 ± 0.1	3.3 ± 0.3
α KG	3.3 ± 0.3	2.7 ± 0.1	3.3 ± 0.4	2.9 ± 0.5	3.5 ± 0.2	3.6 ± 0.4

Note. Values are mean ± SEM μmoles ADP added/nanoatoms of O_2 consumed; $n = 4$ in each age group. α KG = α-ketoglutarate.

Table VII. Electron Transport Chain Activity at Different Ages and with Asphyxia

	2–4 Days	5–7 Days	8–10 Days	Asphyxia
Complex I NSM[a]	0.347 ± 0.08	0.285 ± 0.08	0.406 ± 0.06	0.469 ± 0.09
Complex II & III NSM[a]	0.776 ± 0.09	0.524 ± 0.10	0.765 ± 0.10	0.963 ± 0.12
Complex IV NSM[b]	0.630 ± 0.15	0.435 ± 0.07	0.632 ± 0.08	0.808 ± 0.14
Complex I SM[a]	0.533 ± 0.05	0.556 ± 0.07	0.446 ± 0.07	0.565 ± 0.04
Complex II & III SM[a]	0.788 ± 0.07	0.800 ± 0.17	0.939 ± 0.12	0.854 ± 0.10
Complex IV SM[b]	0.727 ± 0.09	0.493 ± 0.15	0.766 ± 0.10	0.696 ± 0.09

Note. NSM = nonsynaptic mitochondria; SM = synaptic mitochondria. $n = 5$ in each of the nonasphyxia groups, and $n = 7$ in the asphyxia group (all ≤3 days of age).
[a] Values are mean ± SEM micromoles/mg mitochondrial protein/min.
[b] Values are mean ± SEM first order rate constant/mg mitochondrial protein/min.

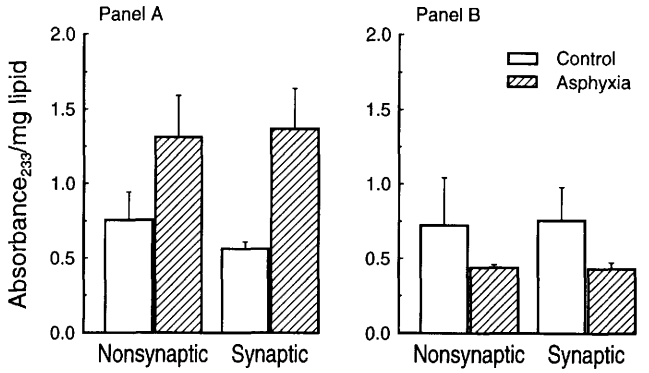

Figure 2. Lamb brain mitochondrial conjugated dienes (all values mean ± SEM). (A) Lambs fewer than 3 days old ($n = 7$ in each group) with conjugated diene production increased after asphyxia in nonsynaptic ($P < 0.03$ compared with control) and synaptic ($P < 0.004$) mitochondria. (B) Lambs more than 3 days old ($n = 4$ in each group). No significant differences in conjugated diene production were noted in nonsynaptic or synaptic mitochondria.

ygen free radical scavenging enzymes in the younger lambs. This hypothesis is supported by data demonstrating developmental changes in superoxide dismutase and catalase in the lung (35) and by changes with maturity of mitochondrial superoxide dismutase in heart, kidney, and liver (36).

Alternatively, our data could also be explained by the ability of the younger animals to transiently decrease state 3 respiration as a protective mechanism. This could occur by a temporary inhibition of ATP synthetase or by a transient loss of adenine nucleotides. This could potentially confer protection against hypoxia consistent with earlier reports (7–10).

The present investigation does not address the source of oxygen free radical production. However, recent data using rat liver mitochondria subjected to *in vivo* ischemia reperfusion demonstrate that the mitochondria themselves are the likely source of increased production of free radical species (38). Ordinarily, mitochondria are well protected against endogenously generated superoxide anion and hydrogen peroxide since these are continuously formed by the electron transport chain. During ischemia, the lack of oxgyen to act as an electron acceptor and an inhibition of electron transfer leads to a high reduction of the components of the respiratory chain. With reoxygenation, a burst of superoxide anion occurs owing to increased auto-oxidation of the major intramitochondrial sources of superoxide anion.

The data about the possible role of oxygen free radicals in the genesis of transient mitochondrial respiratory inhibition considered in conjunction with the documented role of oxygen free radicals in postasphyxia hypoperfusion in the lamb (34) provide evidence that ischemia reperfusion injury may contribute to postasphyxial brain injury in neonates. If oxygen free radical-mediated injury is confirmed as an important mechanism in postasphyxial injury in neonates, therapeutic approaches utilizing scavenging enzymes or the cyclooxygenase inhibitor indomethacin may hold promise (34, 37).

In summary, we have presented data on developmental changes over the first 10 days of life in mitochondrial state 3 respiratory activity in nonsynaptic and synaptic mitochondria in the newborn lamb. Neither these developmental changes in state 3 respiration nor electron transport chain activity provide an explanation for differences in response to asphyxia of mitochondria in lambs less than and greater than 3 days of age. Mitochondria of older lambs do appear more resistant to oxygen free radical-mediated lipid peroxidation than mitochondria in younger lambs. However, the current study is only descriptive in design, so the relationship between lipid peroxidation and inhibition of state 3 respiration in young lambs can only be considered an association. Furthermore, the question of why more conjugated dienes are formed in the younger lambs was not specifically addressed, and it is only speculation that this finding is related to diminished activity of oxygen free of radical scavenging enzymes.

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