

# Purine and Pyrimidine Metabolism and Electrocortical Brain Activity during Hypoxemia in Near-Term Lambs

SANDRA VAN OS, RONNEY DE ABREU, JEROEN HOPMAN, KARINA WETHLY, DJIEN LIEM, AND MARGOT VAN DE BOR

*Division of Neonatology [S.V.O., D.L., M.V.D.B.], Laboratory of Pediatrics and Neurology [R.D.A., K.W.], and Clinical Physics [J.H.], Department of Pediatrics, University Medical Center Nijmegen, 6500 HB Nijmegen, The Netherlands*

## ABSTRACT

Insufficient cerebral O<sub>2</sub> supply leads to brain cell damage and loss of brain cell function. The relationship between the severity of hypoxemic brain cell damage and the loss of electrocortical brain activity (ECBA), as measure of brain cell function, is not yet fully elucidated in near-term newborns. We hypothesized that there is a strong relationship between cerebral purine and pyrimidine metabolism, as measures of brain cell damage, and brain cell function during hypoxemia. Nine near-term lambs (term, 147 d) were delivered at 131 (range, 120-141) d of gestation. After a stabilization period, prolonged hypoxemia (fraction of inspired oxygen, 0.10; duration, 2.5 h) was induced. Mean values of carotid artery blood flow, as a measure of cerebral blood flow, and ECBA were calculated over the last 3 min of hypoxemia. At the end of the hypoxemic period, cerebral arterial and venous blood gases were determined and CSF was obtained. CSF from 11 normoxemic siblings was used for baseline values. HPLC was used to determine purine and pyrimidine metabolites in CSF, as measures of brain cell damage. Concentrations of purine and pyrimidine metabolites were significantly higher in hypoxemic

lambs than in their siblings, whereas ECBA was lower in hypoxemic lambs. Significant negative linear relationships were found between purine and pyrimidine metabolite concentrations and, respectively, cerebral O<sub>2</sub> supply, cerebral O<sub>2</sub> consumption, and ECBA. We conclude that brain cell function is related to concentrations of purine and pyrimidine metabolites in the CSF. Reduction of ECBA indeed reflects the measure of brain damage due to hypoxemia in near-term newborn lambs. (*Pediatr Res* 55: 1018-1025, 2004)

### Abbreviations

**CaO<sub>2</sub>**, content of arterial oxygen  
**CBF**, cerebral blood flow  
**CFM**, cerebral function monitor  
**CSF**, cerebrospinal fluid  
**Fio<sub>2</sub>**, fraction of inspired oxygen  
**ECBA**, electrocortical brain activity  
**MABP**, mean arterial blood pressure  
**Qcar**, flow in left carotid artery

Despite the increase in survival of preterm infants, long-term morbidity has not changed (1). Hypoxia-ischemia-related brain damage is an important contributor to perinatal mortality and long-term morbidity in survivors (2).

The immature brain is very vulnerable to disturbances in cerebral oxygenation and hemodynamics. Cerebral O<sub>2</sub> supply depends on both CaO<sub>2</sub> and CBF. Cerebral hypoxia is defined as an insufficient O<sub>2</sub> supply to the brain, resulting from either hypoxemia (decreased CaO<sub>2</sub>) or hypoperfusion (decreased CBF). During hypoxemia, the brain is considered to be protected adequately from injury by an increase in CBF to preserve cerebral O<sub>2</sub> supply and to stabilize brain metabolism,

unless cerebral ischemia occurs from supervening systemic hypotension. With the neuronal oxygen and glucose debts arising from severe hypoxemia, oxidative metabolism shifts to anaerobic glycolysis, with its inefficient generation of high-energy phosphate reserves, necessary to maintain cellular ionic gradients and other metabolic processes. However, when hypoxemia progresses, cellular energy failure ultimately occurs, which, if not promptly reversed, results in decreased neuronal viability and death of the cell (3).

During insufficient cerebral O<sub>2</sub> supply, an accumulation of purine metabolites, which are the degradation products from high-energy phosphate compounds (ATP, ADP, and AMP), will occur (4-6). Because the synthesis of uridine triphosphate (UTP) and cytidine triphosphate (CTP) also depends on the ATP level, it is therefore to be expected that the decline in ATP content during hypoxemia is also followed by an accumulation of pyrimidine catabolites, at the expense of UTP and CTP contents (7).

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Correspondence: Margot van de Bor, M.D., Ph.D., Department of Pediatrics 435, University Medical Center Nijmegen, P.O. Box 9101, 6500 HB Nijmegen, The Netherlands; e-mail: m.vandebor@cukz.umcn.nl

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It is known that measurements of purine and pyrimidine catabolites in the CSF can be used as an indicator for cerebral energy failure, or even as an early marker for brain cell damage (8). An early marker for brain cell damage due to hypoxemia might facilitate recognition of the neonate at risk for cerebral injury (9). However, purine and pyrimidine metabolites originating from damaged brain cells have to be determined in CSF, which has to be obtained invasively. In contrast, ECBA can be measured noninvasively and continuously in a clinical setting.

Energy failure in the brain, due to hypoxemia, leads to a blockade of neuronal synaptic function and reduced electrical firing of neurons. This is reflected in recordings of ECBA, which can reveal a general picture of the functional state of the brain, monitored by CFM (10). It has been reported that CFM correlates well with conventional multichannel EEG evaluation of cortical neuronal activity of neonates, except for the recognition of very short seizure activity patterns (11–16). Integrated EEG signals have been found to correlate with the number of firing neurons (17). A disadvantage of EEG is that it requires the presence of an expert interpreting the large data volumes. In an effort to solve this problem, various methods of compressing the EEG signal have been developed, the CFM being one of them. Noninvasive recording of electrocortical brain cell activity by means of EEG and CFM-like signals (e.g. ECBA) in the newborn period can be used as a measure for brain cell function (10). Moreover, abnormal tracings are related to neonatal death and in the survivors to neurodevelopmental outcome (16, 18–21). Although relationships between EEG and intracerebral hemorrhages and number of damaged brain structures are reported (18, 22–24), ECBA does not provide a measure of energy failure leading to brain cell damage. One of the major problems in the care for high-risk neonates is determining whether a baby's brain has suffered severe, irreversible damage after hypoxemia (25). The extent of the damage is important with respect to the reversibility of the compromised brain function. The relationship between hypoxic brain cell damage due to energy shortage, as measured by purine and pyrimidine metabolism, and brain cell function, as measured by ECBA, after near-term birth is, however, not yet fully elucidated.

The near-term born lamb was used to investigate whether ECBA can provide an adequate measure for brain cell damage due to hypoxemia. We hypothesized that there is a relationship between disturbed brain cell function due to hypoxic cell damage and the release of purine and pyrimidine metabolites in the CSF.

## METHODS

**Animal preparation and instrumentation.** Pregnant ewes of Dutch Texel breed were operated at 131 (mean; range, 120–141) d of gestation (term, 147 d) under general anesthesia with 3% isoflurane. After a polyvinyl catheter was inserted into the ewe's jugular vein, isoflurane anesthesia was replaced with infusion of 600 mg/h ketamine hydrochloride and 15 mg/h midazolam. A pregnant horn of the uterus was exposed through a midline incision in the ewe's abdomen, and a uterine incision was made over the fetal head of one lamb only. Siblings were

kept *in utero* and were not instrumented. They were used to determine baseline values of purine and pyrimidine concentrations in the CSF. The ewe was monitored throughout the experiment and was kept in an optimal ventilatory ( $P_{aO_2}$ , 10–15 kPa;  $P_{aCO_2}$ , 4–5 kPa; pH, 7.3–7.4) and circulatory condition (MABP, 100–120 mm Hg).

The fetus' head and right forelimb were delivered and an occluder was placed around the umbilical cord, but was not clamped yet. A polyvinyl catheter [outer diameter (OD) 2.1 mm] was placed in the right brachial vein for administration of ketamine hydrochloride (10 mg/kg/hr), glucose 5% (2 mL/kg/hr) and antibiotics (amoxicillin and gentamicin). Furthermore, the right brachial artery (polyvinyl catheter, OD: 2.1 mm, with its catheter tip in the arch of the aortae) and right jugular vein (polyurethane catheter, OD: 0.9 mm) were cannulated for measurement of the arterial blood pressure and arterial and venous blood gas sampling. The venous catheter was inserted in the cranial direction of the right jugular vein to obtain information on the venous cerebral compartment. Arterial and venous blood gases were analyzed with a blood gas analyzer (ABL 510, Radiometer Medical A/S, Copenhagen, Denmark). Oxygen saturation values were corrected for interspecies differences according to Nijland *et al.* (26). Arterial and venous blood pressures were measured with disposable transducers (Edwards Life Sciences BV, Los Angeles, CA, U.S.A.).

After exposing the left carotid artery, we applied an appropriately sized perivascular ultrasonic blood flow transducer (2SL, S or B, Transonic Systems Inc., Ithaca, NY, U.S.A.) to fit around the vessel to assess changes in  $Q_{car}$ . Changes in  $Q_{car}$  were used to assess changes in CBF, inasmuch as a close linear relationship between  $Q_{car}$  and CBF (determined with radioactive microspheres) was reported by Van Bel *et al.* (27).

Cerebral  $O_2$  supply and  $O_2$  consumption respectively were calculated as follows:

$$O_2\text{-supply (mL } O_2/\text{min)} = Q_{car} \times CaO_2,$$

$$O_2\text{-consumption (mL } O_2/\text{min)} = Q_{car} \times (CaO_2 - CvO_2),$$

with arterial (venous) oxygen content:

$$Ca(v)O_2 (\text{mL } O_2/\text{mL}) =$$

$$Sa(v)O_2 \times Hb (\text{g/dL}) \times 1.36 (\text{mL } O_2/\text{gHb}/100).$$

Two disposable subdermal needle electrodes for EEG recordings (Oxford Instruments BV, Gorinchem, The Netherlands) were positioned on the parietal regions of the skull, and one electrode on the occipital region as a reference. Thus, a CFM-recording was registered. Conventional CFM provides a semi-logarithmic amplitude distribution plot of a single-channel EEG through amplification, bandpass filtering (2–16 Hz), compression, rectification and smoothing. The signal is then recorded at slow speed and is very well suited for visual evaluation. Although many authors have tried to express electrical activity derived from the CFM-signal on a numerical scale, calculating means from a semi-logarithmic scale is not a logical step in signal analysis. Therefore, from a signal analytical point of view, we have used a slightly different

approach. We also used a 2–16 Hz band-filtered one-channel EEG. But, we calculated the (3 min) mean of the squared signal (mean power). The result is presented as the root from this signal. ECBA is thus calculated as the root mean square (RMS) value of a band-filtered (2–16 Hz) one-channel EEG (28) and is comparable with a voltage scale. Seizures were determined by visual inspection of the CFM recordings (Professor L.S. De Vries). The width of the band indicates the spontaneous moment-to-moment variations in voltage from minimum to maximum of cerebral electrical activity. The bandwidth was calculated during 3 min at the end of the baseline period by subtracting the minimum amplitude from the maximum amplitude. Minimum and maximum amplitudes were calculated as described by Viniker *et al.* (29).

**Experimental procedure.** After the preparation, the instrumented lamb was intubated. Ventilation was started using a continuous flow pressure controlled ventilator (Babylog 1 HF, Dräger, Lübeck, Germany). Ventilator settings were adjusted to obtain optimal blood gases.

When the instrumented lamb was in an optimal ventilatory ( $P_{aO_2}$ , 10–14 kPa;  $P_{aCO_2}$ , 4.5–6.0 kPa; pH, 7.3–7.4) and circulatory (MABP, 60–65 mm Hg) condition, the umbilical cord was clamped to mimic an extrauterine condition. Surfactant (Survanta, Ross Laboratories, Columbus, OH, U.S.A.) was administered if necessary to achieve optimal ventilation and oxygenation with a  $F_{iO_2}$  of 0.30. A stabilization period of 3 h was applied. Qcar and ECBA were recorded with a computer system and stored for further analysis (MIDAC, Biomedical Engineering Department, University Medical Center Nijmegen, Nijmegen, The Netherlands). At the end of the stabilization period, mean baseline values of Qcar and ECBA were calculated over 3 min in the instrumented lamb and cerebral arterial blood gases were obtained for calculation of baseline cerebral  $O_2$  supply and  $O_2$  consumption.

Thereafter, prolonged hypoxemia [ $F_{iO_2}$ , 0.10; duration, 2.5 h] was induced in the nine instrumented lambs by gradual reduction of the inspired oxygen concentration by mixing air with increasing amounts of nitrogen. At the end of the hypoxemic period, mean values of Qcar and ECBA were calculated again over 3 min and cerebral arterial blood gases were obtained for calculation of cerebral  $O_2$  supply and  $O_2$  consumption.

**Purine and pyrimidine metabolism.** Measurements of Qcar and ECBA could only be performed in the instrumented lambs. Instrumentation of the siblings was not feasible. In the instrumented lambs, CSF was obtained at the end of the hypoxemic period. Baseline CSF samples were obtained from the siblings. Because the ewes were kept in an optimal ventilatory and circulatory condition until the end of the experiment, we assumed that the siblings were in a normoxemic (fetal) situation at the time of CSF sampling. The purine and pyrimidine metabolite concentrations in the CSF of the siblings were considered to be similar to the baseline CSF purine and pyrimidine metabolite concentrations in the instrumented lambs, if baseline CSF could have been obtained in these lambs. Therefore we linked the CSF purine and pyrimidine metabolite concentrations of the siblings to the physiologic baseline values of the instrumented lambs.

CSF was obtained by puncture of the cisterna magna in the nine instrumented hypoxemic lambs and in the 11 (normoxemic) siblings. After anterior flexion of the neck, the needle was introduced through the foramen magnum at a point just below the nuchal ridge until the appearance of CSF. It was not always feasible to obtain CSF from both the instrumented hypoxemic lamb as from its sib.

Samples were immediately centrifuged (3000 rpm, 10 min) to eliminate any red blood cell contamination, fixed with 8 M perchloric acid, and homogenized gently. After centrifugation, the supernatant was neutralized with 4 M  $K_2HPO_4$ . The samples were frozen at  $-80^\circ C$  until analysis. A HPLC procedure with an Alltima C18 reversed-phase column, pore size 5  $\mu m$ , column size 250  $\times$  4.6 mm, (Alltech Associates, Deerfield, IL, U.S.A.) was used to determine purine metabolites (guanosine, inosine, hypoxanthine, xanthine) and pyrimidine metabolites (uridine, uracil, pseudo uridine) in the CSF as a measure of brain cell damage. Pseudo uridine is the most commonly modified nucleotide and is solely found in the RNA (30).

**Statistical analysis.** In the instrumented lambs, mean values were calculated for cerebral  $O_2$  supply, cerebral  $O_2$  consumption, and ECBA during the last 3 min of the baseline period and during the last 3 min of the hypoxemic period. The mean of left and right hemispheric ECBA was used for further analysis.

The data were expressed as median (range). Mann-Whitney *U* tests were used to compare baseline and hypoxemic blood gas values, physiologic variables, and metabolite concentrations.

Linear regression analysis shows to what extent the variability in the dependent variable can be attributed to different values of the independent variable (represented by  $R^2$ ) (31). Univariate linear regression analyses were used to test the relationships between metabolite concentrations and cerebral  $O_2$  supply, cerebral  $O_2$  consumption, and ECBA. Linear regression was forced through median baseline values.

Statistical analysis was performed with the SPSS statistical package (SPSS Inc., Chicago, IL, U.S.A.).

The study was approved by the Institutional Animal Care and Use Committee of the University of Nijmegen before implementation

## RESULTS

There were no statistically significant differences in birth weight and gender between the instrumented lambs and the siblings.

A total of nine hypoxemic CSF samples from the instrumented lambs and 11 baseline CSF samples from their siblings were analyzed for purine and pyrimidine metabolism. Guanosine was not detectable in three baseline and in three hypoxemic CSF samples. Inosine and uracil were not detectable in four baseline and two hypoxemic CSF samples. Xanthine was not detectable in one, pseudo uridine was not detectable in two, and uridine was not detectable in three hypoxemic CSF samples. Purine and pyrimidine metabolite concentrations were significantly higher at the end of prolonged hypoxemia (Table 1). Especially the concentrations of inosine, hypoxanthine, and uracil showed dramatic increases at the end of prolonged hypoxemia.

**Table 1.** Median (range) CSF purine and pyrimidine metabolite concentrations at baseline and at the end of prolonged hypoxemia

	Baseline values (n = 11)	Hypoxemia (n = 9)
Guanosine ( $\mu\text{M}$ )	0.52 (0.24–2.48)	5.60 (3.30–6.20) $\P$
Inosine ( $\mu\text{M}$ )	2.36 (1.08–11.68)	48.70 (24.10–63.80) $\P$
Hypoxanthine ( $\mu\text{M}$ )	13.92 (6.41–44.40)	120.50 (57.90–205.40) $\P$
Xanthine ( $\mu\text{M}$ )	3.86 (1.31–11.75)	24.00 (7.80–44.60) $\P$
Uridine ( $\mu\text{M}$ )	3.50 (0.50–7.55)	8.60 (6.70–29.50) $\P$
Pseudo uridine ( $\mu\text{M}$ )	5.60 (3.34–7.40)	7.00 (6.30–9.30) $\dagger$
Uracil ( $\mu\text{M}$ )	2.22 (0.25–35.00)	239.7 (134.60–479.60) $\P$

Mann-Whitney *U* test compared with baseline.

$\dagger p < 0.01$ ;  $\P p < 0.001$ .

Arterial oxygen saturation ( $\text{SaO}_2$ ),  $\text{Po}_2$  ( $\text{PaO}_2$ ), pH, MABP,  $\text{CaO}_2$ , cerebral  $\text{O}_2$  supply, cerebral  $\text{O}_2$  consumption, and ECBA were significantly lower at the end of prolonged hypoxemia than at baseline (Table 2).

Spearman correlation showed no significant relationship between Qcar and ECBA ( $r = 0.434$ ).

The relationships between cerebral  $\text{O}_2$  supply and cerebral  $\text{O}_2$  consumption and the purine and pyrimidine metabolite concentrations were statistically significant for all metabolites (Table 3).

A typical example of a CFM recording in baseline and hypoxemic conditions is provided in Figure 1. Median (range) CFM bandwidth was 17.0 (12.5–29.0)  $\mu\text{V}$  in baseline conditions. After prolonged hypoxemia, the CFM bandwidth in the interburst or interseizure intervals was not calculated, inasmuch as the tracings were nearly flat ( $< 5 \mu\text{V}$ ). After prolonged hypoxemia, a flat tracing with or without a few bursts was observed in four lambs, a burst suppression pattern was observed in two lambs, and a depressed background pattern with seizure activity was observed in three lambs.

ECBA was significantly negatively related to all purine and pyrimidine metabolite concentrations. The relationships between ECBA and the purine and pyrimidine metabolite concentrations are presented in Figure 2.

## DISCUSSION

We used the near-term newborn lamb to study the effects of hypoxemia on purine and pyrimidine concentrations in the CSF, as a measure of structural brain cell damage, and ECBA,

as a measure of functional brain cell damage, after near-term birth. To test the reversibility of structural and functional brain cell damage after hypoxemia was beyond the scope of this study.

Even today there is no consensus as to which animal model best describes human perinatal hypoxic-ischemic encephalopathy. Although the fetal sheep, the newborn lamb, and the piglet are comparable in size to human newborn infants, it has not been well established whether their brains can be compared with human newborn brains (32). However, this model was chosen because, in the fetal lamb brain, development in the last trimester of pregnancy is rather similar to that in the human fetus (33). Furthermore, the lamb model is applicable in fetal as well as neonatal studies and there is extensive experience with cerebral hemodynamic studies in this animal model (32, 33). This model is suitable for acute and subacute studies and the size of a near-term lamb is adequate to test and monitor multiple organ systems (32).

Because sampling of CSF in the instrumented lambs during both normoxemia and hypoxemia was not feasible, we used CSF of the siblings for baseline values. The ewes were monitored continuously and were in an optimal ventilatory and circulatory condition and the umbilical cords of the siblings were not obstructed during the experiments. Therefore, we assumed an optimal cerebral oxygenation and circulation of the siblings. We did not expect elevated concentrations of purine and pyrimidine metabolites due to brain cell damage under these physiologic fetal conditions in the siblings. This was confirmed by the low concentrations of purine and pyrimidine metabolites in the siblings. These concentrations were comparable to the baseline CSF purine concentrations in fetal lambs as observed by De Haan *et al.* (34). The umbilical cords of the instrumented lambs were clamped to mimic delivery. To our knowledge, the effect of delivery on the purine and pyrimidine metabolite concentrations is not known. However, we assume it to be negligible after a cesarean section, in particular after a stabilization period of 3 h.

Because we determined baseline values of purine and pyrimidine metabolite concentrations and baseline physiologic values in different pools of lambs (respectively in siblings and instrumented lambs), these baseline purine and pyrimidine metabolites and physiologic values could not be presented as

**Table 2.** Median (range) blood gas values and physiological variables in nine instrumented lambs during baseline and hypoxemic conditions

	Baseline values	Hypoxemia
$\text{SaO}_2$ (%)	100.00 (68.70–100.00)	10.47 (3.49–46.61) $\P$
$\text{PaO}_2$ (kPa)	15.68 (11.53–16.85)	1.57 (0.11–3.08) $\ddagger$
$\text{Paco}_2$ (kPa)	5.05 (3.18–7.96)	5.64 (3.32–11.01)
Ph	7.38 (7.23–7.46)	6.97 (6.68–7.41) $\dagger$
MABP (mm Hg)	61.45 (55.26–84.26)	24.83 (15.17–64.02) $\P$
$\text{CaO}_2$ (mL/dL)	12.38 (7.17–18.50)	1.35 (0.45–3.23) $\P$
Qcar (mL/min)	34.13 (22.07–55.84)	12.25 (0.00–95.09)
Cerebral $\text{O}_2$ supply (mL/min)	3.60 (2.98–7.79)	0.23 (0.00–1.30) $\P$
Cerebral $\text{O}_2$ consumption (mL/min)	0.84 (0.50–2.44)	0.18 (0.00–0.62) $\ddagger$
ECBA ( $\mu\text{V}$ )	10.28 (10.01–12.55)	3.63 (0.93–8.97) $\P$

Mann-Whitney *U* test compared with baseline.

$\dagger p < 0.01$ ;  $\ddagger p < 0.005$ ;  $\P p < 0.001$ .

**Table 3.** Square root ( $R^2$ ) values from univariate linear regression analyses with CSF purine and pyrimidine metabolite concentrations as the dependent and cerebral  $O_2$  supply and cerebral  $O_2$  consumption as the independent variables

	Cerebral $O_2$ supply (mL/min)	Cerebral $O_2$ consumption (mL/min)
Guanosine ( $\mu$ M)	0.90¶	0.87†
Inosine ( $\mu$ M)	0.90¶	0.85¶
Hypoxanthine ( $\mu$ M)	0.92¶	0.91¶
Xanthine ( $\mu$ M)	0.86¶	0.86¶
Uridine ( $\mu$ M)	0.96¶	0.93¶
Pseudo uridine ( $\mu$ M)	0.66*	0.52
Uracil ( $\mu$ M)	0.83‡	0.86‡

Analyses include all purine and pyrimidine concentrations and values of cerebral  $O_2$  supply and cerebral  $O_2$  consumption at the end of prolonged hypoxemia in the instrumented lambs. For calculation of  $R^2$ , linear regression lines were forced through fixed median baseline values. Significance of  $R^2$ : \*  $p < 0.05$ ; †  $p < 0.01$ ; ‡  $p < 0.005$ ; ¶  $p < 0.001$ .

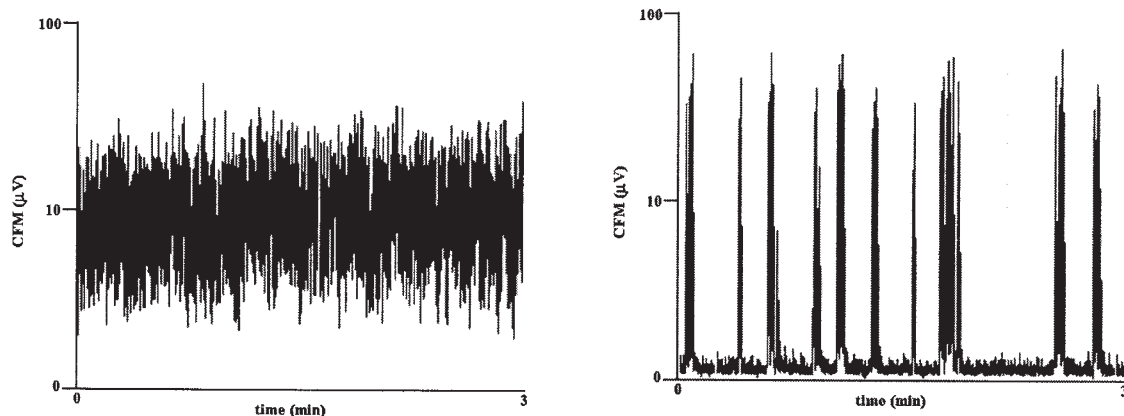
independent data points belonging to individual lambs. The baseline values of the purine and pyrimidine metabolite concentrations and of the physiologic variables are obtained during normoxemic conditions and can therefore be considered as given baseline data for this group of animals.

A standardized insult ( $F_{iO_2}$ , 0.10 for 2.5 h) resulted in a range in arterial oxygen concentrations. Alveolar hypoxemia is a very frequently used procedure in physiology to challenge control systems, receptors, and even cellular behavior. This procedure is based on the decrease in oxygen tension in the alveoli and arterial blood, and the decrease in Hb saturation, all leading to a decrease in  $Ca_{O_2}$  (35). Based upon this reasoning it could be proposed that the  $O_2$  supply to all body tissues would decrease equally and in direct proportion to the decrease in  $Ca_{O_2}$ . However, this is not the case inasmuch as there are compensatory mechanisms that maintain and redistribute the blood flow to vital organs, like heart and brain. In summary, a decrease in  $F_{iO_2}$  and  $Ca_{O_2}$  does not necessarily produce changes at neuronal level. Biologic variation between animals resulted in a range of  $Ca_{O_2}$  and cerebral  $O_2$  supply values; we used these values as the independent variables. This allowed us to compare cerebral electrical activity with oxygenation status.

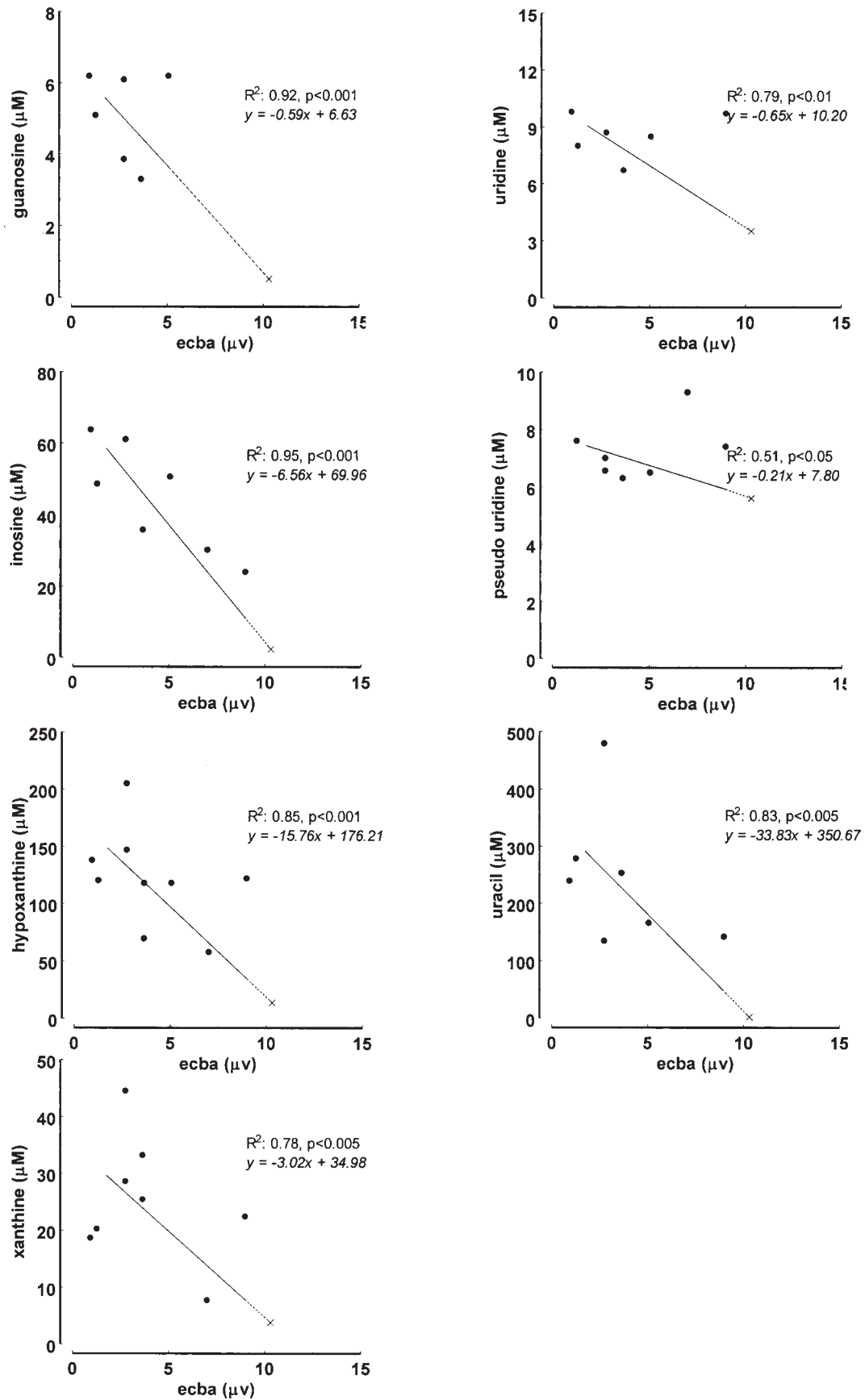
Hypoxia increases CBF adequately to maintain brain metabolism stable until cerebral ischemia supervenes owing to car-

diac depression and systemic hypotension (3). During hypoxemia, blood pressure will remain relatively stable as long as the myocardium is able to sustain cardiac output (36). However, if the myocardium fails, blood pressure will decrease below its baseline level, which we observed in our study at the end of prolonged hypoxemia. Inadequate CBF for maintenance of cerebral  $O_2$  supply shifts oxygen metabolism to anaerobic glycolysis and sets in motion a cascade of metabolic processes, leading to perinatal hypoxic-ischemic cerebral injury (37). The compensatory ability during prolonged hypoxemia to preserve cerebral  $O_2$  supply to meet the cellular needs for adequate brain cell function was not sufficient, as we also observed in a previous study (28). The lack of reactivity of the cerebral arterial vascular system to hypoxemia, and therefore the lack of sufficient compensatory hemodynamic properties, was reflected by the decrease in CBF during the hypoxemic period, although there was considerable interindividual variability. Szymonowicz *et al.* (38) observed that local cerebral perfusion in near term fetal lambs is not primarily determined by  $Ca_{O_2}$ . They found that regional CBF sensitivity to oxygenation increases with increasing maturity, except in the white matter, where regional CBF in the near-term fetus, is blood pressure dependent. Gunn *et al.* (24) showed in fetal sheep that neuronal damage due to asphyxia was strongly associated with the percentage of decrease in blood pressure during the insult but not with the degree of hypoxia.

Normal cerebral function is intimately related to adequate  $O_2$  supply and metabolism. During insufficient  $O_2$  supply, ATP is catabolized to AMP, IMP, adenosine, hypoxanthine, and xanthine and is ultimately excreted as uric acid. The formation of xanthine and uric acid by xanthine oxidase is a source of free radical formation, which is important for the development of ischemic and postischemic damage (39, 40). Elevated levels of hypoxanthine and xanthine in the brain are related to irreversible brain cell damage due to free radical formation after hypoxia (41). In addition, the increase of hypoxanthine and xanthine after asphyxia seems to be related to the loss of sensory evoked potentials, which indicates poorer cerebral function (6). During the breakdown of ATP, not only concentrations of hypoxanthine and xanthine in the CSF will increase, but also those of other purine metabolites, such as guanosine



**Figure 1.** A typical example of a CFM recording in baseline (*left*) and prolonged hypoxemic (*right*) conditions. Note that the recording speed is considerably higher than in the standard clinical CFM recordings (44).



**Figure 2.** Relationships between CSF purine and pyrimidine metabolite concentrations and ECBA. In each figure, purine and pyrimidine metabolite concentrations and ECBA values of the hypoxic lambs are presented as independent points. Median baseline values are presented as fixed data points (x) in the figures. Regression lines were forced through these fixed median baseline values.

and inosine, and pyrimidine metabolites, such as uridine and uracil, increase. We have shown that after prolonged hypoxemia, the CSF concentrations of purine and pyrimidine metabolites were significantly higher compared with the baseline values that were obtained in the control lambs. Furthermore, ECBA was significantly lower after prolonged hypoxemia compared with the values obtained at baseline conditions in the instrumented lambs. Combining these facts, we suggest that there exists a relationship between the increase in the concentrations of these metabolites and the decrease in brain cell function. This was supported by the linear regression analysis that showed that a large part of the variability of the CSF concentrations of the purine and pyrimidine metabolites could be attributed to the variability of ECBA. In adult animals exposed to hypoxia, the EEG indicates disturbed function before the breakdown of ATP (42) and a threshold-type relationship was suggested between electrophysiological function as measured by evoked potential and cortical blood flow (43). Thiringer *et al.* (6) found a close relationship between a gradual increase in fetal plasma concentrations of hypoxanthine and the deterioration of fetal evoked potentials. However, hypoxanthine was measured in plasma and might therefore have originated from other fetal organs.

After prolonged hypoxemia, the bandwidth of the CFM trace was significantly reduced when compared with baseline conditions because moment-to-moment variations in voltage of cerebral activity were decreased enormously. Epileptic seizures are relatively common in ill and distressed newborn infants. The seizures are often reactive and caused by transient disturbances in cerebral oxygenation, metabolism, and blood flow. Burst suppression patterns and electrocerebral inactivity, or extremely low-voltage patterns, are predictive of poor outcome (death or severe handicap) (44). The seizures and bursts did not have a large influence on the calculated mean ECBA values and differences in mean ECBA values between baseline and hypoxemic conditions were obvious, despite the seizures.

Much controversy still exists regarding the anesthetic effect of ketamine on CBF, metabolic rate of oxygen, and ECBA. However, the usage of an anesthetic agent is a prerequisite from an ethical point of view and, moreover, it prevents stress-induced activation of the brain (45). Burrows *et al.* (46) demonstrated that, in the presence of adequate ventilation, ketamine produces no significant cardiovascular effects in pre-term lambs. In general, anesthetic agents produce an alteration in the EEG and evoked responses consistent with their clinical effect on the CNS. Therefore, ketamine treatment may have influenced the recorded ECBA. However, other anesthetics may even have had a more profound effect on ECBA (47). An overall lack of depressant effect on the EEG has made ketamine a desirable agent for monitoring responses that are usually difficult to record under anesthesia (*e.g.* dermatomal evoked responses and transcranial motor evoked potential). Furthermore, if ketamine had caused an activation of cerebral function, this activation should have been paralleled by increases in CBF, according to Kochs *et al.* (48). Inasmuch as our baseline CBF values were not higher than the reported reference CBF values for near-term lambs (27, 49) and no significant relationship between Qcar and ECBA was ob-

served, we assume that ketamine has not influenced ECBA to a large extent. In the present study, recordings of ECBA (both baseline as at the end of prolonged hypoxemia) were made during the same depth of anesthesia. Therefore, changes between baseline and hypoxemic values cannot be attributed to an anesthetic effect, but to changes in oxygenation.

Purine and pyrimidine metabolite concentrations are known to increase after a severe reduction in cerebral O<sub>2</sub> supply in the fetus (6, 34, 50–53), newborn (7, 54), and adult (5, 41, 53, 55). However, it appears that there is a difference between the immature and the mature brain in the relationship between the breakdown of ATP and cerebral function during hypoxemia. In the fetal brain, impairment of cerebral function coincided with a gradual breakdown of ATP, whereas in the adult brain cerebral function is already observed before breakdown of ATP. Thiringer *et al.* (6) suggested that this difference corresponds to different rates of reduction in cerebral O<sub>2</sub> consumption during hypoxia in fetal and adult animals. In the immature animal, O<sub>2</sub> consumption rapidly decreases with falling arterial oxygenation, especially in combination with acidemia (56), whereas in adult animals (42) and humans (57) no reduction in cerebral O<sub>2</sub> consumption takes place until very low oxygenation values are reached. Therefore, the concentrations of purine and pyrimidine metabolites will increase more rapidly in the immature than in the mature animals.

We have demonstrated that brain cell damage, as measured by purine and pyrimidine metabolite concentrations in CSF, increases and brain cell function, as measured by ECBA, decreases after prolonged hypoxemia in near-term lambs and that variations in these variables are related. Combining these findings, we conclude that decreases in brain cell function are related to increases in concentrations of purine and pyrimidine metabolites in CSF and that ECBA therefore most likely reflects the measure of brain cell damage due to prolonged hypoxemia in the near-term neonate.

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