Cerebral Energy Metabolism and Oxygen State during Hypoxia in Neonate and Adult Dogs

S. NIOKA, B. CHANCE, D. S. SMITH, A. MAYEVSKY, M. P. REILLY,
C. ALTER, AND T. ASAKURA

*Department of Biochemistry & Biophysics [S.N., B.C., A.M., C.A.], Department of Anesthesiology [D.S.S.],
University of Pennsylvania, School of Medicine, and Division of Hematology, The Children's Hospital of
Philadelphia [M.P.R., T.A.], Philadelphia, Pennsylvania 19104*

ABSTRACT. The relationship between a noninvasive determination of relative oxygen saturation of Hb circulating in brain tissue (StO_2) and energy metabolism was investigated with respect to age [dogs in three age groups (0 to 6-d-old, 7- to 21-d-old, and adults)] and to severity of brain hypoxia using double beam spectroscopy of Hb deoxygenation and nuclear magnetic resonance spectroscopy of energy metabolism. The *in vivo* oxy-Hb dissociation was determined from the relationship between StO_2 curve in the adult dog brain and sagittal sinus oxygen partial pressure during graded hypoxemia and found to be sigmoidal with an oxygen dissociation constant of 26.6 mm Hg. This agreed with an *in vitro* determination for oxygen dissociation constant of 28.2 mm Hg in adult dog red cells. The arterial oxygen pressure at which brain StO_2 was reduced by 50% was shifted toward the right with increasing age (22.2, 33.8, and 40.8 mm Hg, respectively). This correlated with an *in vitro* oxygen dissociation constant of red cell Hb of 17.0, 22.3, and 28.2 mm Hg in the three age groups, respectively. The phosphocreatine-inorganic phosphate ratio (PCr/ P_i) was used to relate changes in cellular energy metabolism during hypoxia with changes in StO_2 . There was no change in PCr/ P_i when StO_2 had decreased to 50% of the control value. However, when the brain StO_2 had decreased to between 7 and 15%, a reduction of PCr/ P_i to 50% of the normoxic value occurred. The StO_2 at which PCr/ P_i falls should be considered a critical level of hypoxemia inasmuch as it represents a threshold of energy failure. There was no significant age dependence in the relationship between StO_2 and PCr/ P_i . Neonates had much smaller intravascular oxygen gradients than those in adults at a critical level of hypoxemia. However, compared with adults, the 1- to 3-wk-old age group requires a similar O_2 availability and extraction for brain metabolic survival. (*Pediatr Res* 28: 54-62, 1990)

Abbreviations

StO_2 , relative oxygen saturation of Hb circulating in tissue
 SAO_2 , arterial oxygen saturation of Hb
 SVO_2 , venous oxygen saturation of Hb
 PaO_2 , arterial oxygen pressure
 Pa_{50} , PaO_2 at which StO_2 is 50%
 P_{50} , oxygen dissociation constant
PCr, phosphocreatine
 P_i , inorganic phosphate
PCr/ P_i , phosphocreatine-inorganic phosphate ratio

pHi, intracellular pH

PvO_2 , sagittal sinus oxygen partial pressure

$PtvO_2$, oxygen pressure in brain vasculature

^{31}P NMR, phosphorus nuclear magnetic resonance

FiO_2 , fraction of inspired oxygen

Adult brain tissue appears to be considerably more sensitive to hypoxia or ischemia (1, 2) than neonatal brain tissue, which appears to tolerate relatively long periods of oxygen deprivation (3-6). For example, the duration of tolerance to anoxia in neonatal dogs is 10 times longer than in adult dogs (4). This age-dependent tolerance to anoxia may be related to various factors including the oxygen affinities of fetal and adult Hb, oxygen availability, oxygen consumption, oxygen gradients, and mitochondrial capacity. However, because of difficulties in measuring oxygen partial pressure in the tissue vasculature, no prior work has been done to elucidate the relationship between the oxygen state of the vasculature and the high neonatal tolerance to hypoxia. In studying cellular oxygen states, two primary considerations, oxygen delivery and oxidative phosphorylation, are key elements to be monitored. To determine oxygen delivery, we have studied the StO_2 in the adult and neonate using double beam spectroscopy (7-9), inasmuch as this gives a global evaluation of oxygen delivery in relation to tissue oxygen demand. To measure the oxidative phosphorylation, we used ^{31}P NMR to determine PCr/ P_i , which is proportional to phosphorylation potential (10) and is also a monitor of oxygen deprivation (1, 11). The primary objective of our study was to investigate the relationship between the deoxygenation of high affinity fetal Hb and the diminution of PCr and phosphorylation potential that occurs during systemic hypoxemia.

The fundamental equation for maintenance of life as defined by Bernard (12) and Cannon (13) is the steady state, which can be expressed as the equality of energy conservation between generation of cell energy and its use for cell function. The greater the surfeit of supply compared with demand, the smaller the risk of a hypoxic catastrophe. Some features of neonatal metabolism resist hypoxia. Neonates exhibit a high mitochondrial cytochrome aa_3 turnover for ATP generation, particularly at low oxygen tensions (14) and low ATP consumption (15). A critical factor that may enable the neonate brain to resist hypoxia is its ability to function at a low phosphorylation potential (PCr/ P_i = 1) (11). The concentration of mitochondrial enzymes for ATP generation increases with age (16) and, as a consequence, there is increased mitochondrial electron transport, ATP production, and capacity for oxygen consumption (15, 17, 18). Thus maturation leads to an increase in phosphorylation potential, and the electrical activity increases as neuronal activity is needed. Oxygen gradients may also increase and this change may be a key in the differential sensitivity to neurologic damage between neonates and adults.

Received December 11, 1989; accepted March 7, 1990.

Correspondence: Shoko Nioka, Department of Biochemistry & Biophysics, University of Pennsylvania, School of Medicine, Philadelphia, PA 19104.

Supported in part by NIH grants HR 34004, NS 23859, and NS 22881. D.S. Smith was supported by HL 07286.

MATERIALS AND METHODS

Animal Preparation and Experimental Protocol. The studies were approved by the institutional Animal Care and Use Committee. Eight adult dogs were anesthetized with 2% isoflurane (1% during the experiment), and 16 puppies (0- to 6- and 7- to 21-d-old) were anesthetized with fentanyl, 50 µg/kg, droperidol, 2.5 mg/kg (both given intraperitoneally), and N₂O. Though different anesthetics were used in adults and puppies, both agents were used in relatively low doses. The animals were intubated or tracheotomized and mechanically ventilated. Body temperature was maintained with heating blankets and monitored using a nasopharyngeal probe. Mean arterial blood pressure was measured through a catheter in the femoral artery. A slow infusion of 5% dextrose in half normal saline was maintained during the experiment. In all subjects, arterial blood was obtained to measure PaO₂ and pH at each level of hypoxemia. In adult dogs, sagittal sinus blood samples were obtained to measure PvO₂ and SvO₂. For these measurements, an 18- to 20-gauge cannula was inserted into the anterior sagittal sinus through a burr hole in front of the occipital process. To minimize extracranial blood contamination of the samples, blood flow from the posterior part of the sagittal sinus was blocked by a small piece of surgical placed just posterior to the catheter (19). The blood from the anterior sagittal sinus flowed into a calibrated container and was returned to the animal with a pump. The ³¹P NMR findings suggested that there was no interference with brain oxygenation due to this procedure.

Graded hypoxemia was induced from an FiO₂ of 0.21 by decrements of 0.02. PCr/P_i was determined at 4-min intervals. When PCr/P_i began to decrease, the FiO₂ was decremented more slowly to avoid an abrupt further decrease of PCr/P_i. If PCr/P_i started to decrease abruptly, or mean arterial blood pressure declined to lower than 55 and 30 mm Hg in adults and puppies, respectively, epinephrine was infused at a rate of 3 to 10 µg/kg/min to maintain a steady state of energy metabolism. Sodium bicarbonate (1 M) was administered to keep blood base deficit less than 5 mEq/L.

Measurement Techniques. Double beam spectroscopy. A Johnson Foundation dual wavelength air turbine spectrometer (U. of Penn., Philadelphia, PA) using a Hamamatsu R928 photomultiplier (Hamamatsu, Japan) and a JY-type curved holographic grating monochromator at 850 and 782 nm (resolution 0.5 nm) was used to determine oxy- and deoxy-Hb changes for studies *in* and *in vitro*. The light source was a 50 W halogen lamp. For studies *in vivo*, the light source and photomultiplier were coupled to the brain by 3-mm diameter glass fiber bundles that were attached to the bare skull 1 cm apart at a 35–45° angle with respect to each other. Preliminary studies demonstrated that this distance between the two light guides is necessary to provide increased light through the brain surface vasculature, which is more abundant in this area than inside the brain. The skull thickness in the adult was thinned to 2 mm to produce a skull-to-brain distance similar to that of neonates. When the light guide was separated by 5 cm, as in previous studies, a contribution by cytochrome oxidase to the signal was observed (20). By these operations, the incident light intensity distribution was focused on the vasculature in all age groups and the contribution of cytochrome oxidase signal at 850 nm was minimized (Nioka S, unpublished observations).

The absorbance was calculated using the control (normoxia) light intensity as a reference. Because 782 and 850 nm are isobestic points, Hb oxygen saturation can be calculated by the following equation with the assumption that the path lengths of light at 850 and 782 nm are equal (21).

$$OD_{850-782} = L (\Delta\epsilon) [(HbO_2) - (dHb)]$$

where OD₈₅₀₋₇₈₂ is the difference between OD at 850 nm and that at 782 nm; L is the light path length; Δε is the difference of extinction coefficients between oxy- and deoxy-Hb at 850 nm

(Δε is also equal to the difference of the extinction coefficients of deoxy- and oxy-Hb at 782 nm); HbO₂ is oxy-Hb; and dHb is deoxy-Hb. This equation shows that OD₈₅₀₋₇₈₂ measures [HbO₂] [which is the term for oxygen saturation of Hb (StO₂) if total Hb concentration [THb] is constant].

The procedure for determining StO₂ is as follows: At an FiO₂ of 0.33, the light intensities at 850 and 782 nm were equalized with a balancing circuit. Therefore, this condition was used to normalize the reference light intensity. The highest OD₈₅₀₋₇₈₂ was obtained during the recovery period after hypoxia when FiO₂ was 1.0 and was defined as 100% StO₂ (this value was considerably higher than that obtained with FiO₂ of 1.0 without prior hypoxia). Previous experiments in this laboratory indicated that at this time period a very small deoxy-Hb peak was detectable at 760 nm. All data points were normalized to the 100% StO₂ and the lowest StO₂ at FiO₂ = 0 (obtained at the end of the study). These two points of calibration produce a relative value for oxygen saturation of Hb (values of StO₂) in the brain. This value of StO₂ was correlated with SvO₂ and SaO₂, and thus shown to be closely related to oxygen saturation in the brain. The OD of the sum of the absorbance at 850 and 782 nm is a function of total Hb concentration and can be used to estimate changes in total Hb concentration.

We performed studies to measure Hb oxygen saturation *in vitro* using the same dual wavelength system to estimate the error of our *in vivo* StO₂ measurements. For these studies, the light guides were attached to a container (2.5 cm diameter by 3 cm depth), the inside of which was blackened. A 5% yeast suspension was used as a scattering media to model the scattering of brain tissue. This particular concentration of yeast had been previously determined to have light-scattering properties similar to the brain (22). Fully oxygenated dog red blood cells were added to the yeast solution and the changes in light intensity at 782 to 850 nm were measured as oxygen saturation decreased from 100 to 0%. Control data from blood-free yeast solutions showed that there was no absorbance change as oxygen concentration was decreased, demonstrating that the optical changes were only related to changes in Hb saturation. Our experiments showed that OD₈₅₀₋₇₈₂ increased by 11 and 37% when the hematocrit of red cell suspension was increased from 5 to 10 and 20%, respectively. Assuming that the mean red cell volume in the brain tissue is 5% (23, 24) and the maximum increase in blood volume is 2-fold, the maximal error for the calculation of StO₂ would be 11%.

NMR spectroscopy. A 2.1-Tesla NMR spectroscopy system (Otsuka-Electronics, Havertown, PA) was used to measure PCr and P_i. A two-turn copper surface coil (2.5 cm) was placed on the top of the skull. Shimming established a mean value of 0.2 ppm for the half-height line width in the water peak. A near 90° flip angle pulse was optimized between 40 and 60 µs for every dog. The radio frequency pulse at 35.8 MHz was transmitted and received with the same coil and the pulse interval was 4 s. After Fourier transformation, the spectra were phased and curve fitted to determine the areas of PCr and P_i (Fig. 1). PCr/P_i was calculated from the ratio of the area of the PCr and P_i peaks and the ratio of the saturation factors of PCr and P_i. The saturation factors for 4-s acquisition intervals were obtained from the spectra using 20-s intervals, which provided unsaturated spectra. The PCr/P_i data was normalized to the ratio of PCr/P_i obtained during the control period. pH_i was calculated from the chemical shift of P_i referred to PCr (25). The ADP was calculated from creatine kinase equilibrium (26).

Measurement of blood gases and calculations of oxygen saturation of Hb *in vitro*. Blood gas tension and pH were measured with a Radiometer ABL2 (Copenhagen, Denmark). The blood Hb oxygen saturation curve was obtained with a Hemox Analyzer (TCS Medical Products Co., Huntington Valley, PA) (27) using blood taken from the femoral vein in all age groups. The oxygen saturation of Hb in the arterial and venous blood was