Monitoring of the Intracranial Hemodynamics and Oxygenation during and after Hyperventilation in Newborn Rabbits with Near-Infrared Spectroscopy

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ABSTRACT. Oxyhemoglobin, deoxyhemoglobin, total hemoglobin, and oxidized cytochrome *aa*₃ were monitored, blood pressure and heart rate were continuously recorded, and cerebral hemodynamic changes were analyzed during and after hyperventilation (arterial CO₂ tension < 2.67 kPa) and/or hyperoxemia (100% O₂ inhalation) in 11- to 12-d-old rabbits. Oxyhemoglobin, deoxyhemoglobin, total hemoglobin, and oxidized cytochrome *aa*₃ were monitored by means of near-infrared spectroscopy. Near-infrared spectroscopy ideally demonstrated decreases in oxyhemoglobin and total hemoglobin and an increase in deoxyhemoglobin during hyperventilation and a return to the previous values after hyperventilation. Cytochrome *aa*₃ decreased during hyperventilation. On hyperventilation with 100% O₂ inhalation, however, cytochrome *aa*₃ was not reduced, although the changes in oxyhemoglobin and total hemoglobin were more significant. This reduction of cerebral oxygenation during hyperventilation without the administration of oxygen may induce hypoxic-ischemic brain damage. Noninvasive monitoring of cerebral oxygenation and hemodynamics, for which near-infrared spectroscopy is useful, is a requisite for the prevention of brain injury caused by severe hypocarbia in hyperventilation therapy, (*Pediatr Res* 35: 334–338, 1994)

Abbreviations

HbO₂, cerebral oxyhemoglobin concentration
HbR, cerebral deoxyhemoglobin concentration
HbT, total cerebral hemoglobin concentration
Cyt *aa*₃, cerebral oxidized cytochrome *aa*₃ concentration
NIRS, near-infrared spectroscopy
PaCO₂, arterial CO₂ tension
PaO₂, arterial O₂ tension
CBV, total cerebral blood volume
CMRO₂, cerebral metabolic rate of oxygen
CBF, cerebral blood flow

hypertension and asphyxia in many patients in neonatal intensive care units, concern has been raised as to how much decreased CBF during hyperventilation could cause cerebral hypoxia. Previous studies have demonstrated significant decreases in cerebral tissue PO₂ during hyperventilation (6–8). However, inadvertent hypocarbia may occur when premature infants have mechanical ventilation for transport by ambulance from the place of birth to the neonatal intensive care unit because infants do not usually have any form of carbon dioxide monitoring during transport. In neonatal intensive care units, assisted ventilation is occasionally required for patients with relatively mild pulmonary damage (e.g. central apnea, mild respiratory distress after surfactant replacement therapy). Moreover, previous clinical and neuropathologic studies have suggested that hypocarbia is one of the important factors in the pathogenesis of leukomalacia (9), multicystic encephalomalacia (10), and pontosubicular necrosis (11) in neonates. In addition, there have been several reports that neurodevelopmental deficits may be caused by severe hypocarbia in preterm infants (12). Therefore, neonatologists must pay close attention to hypocarbia in such newborn infants. Continuous recording of intracranial oxygenation and hemodynamics during hyperventilation is important.

Noninvasive monitoring with NIRS provides continuous information on the intracranial hemodynamics, tissue oxygenation, and cell metabolism through measurement of HbO₂, HbR, HbT, and Cyt *aa*₃, which is the terminal step in mitochondrial respiration (13, 14). This method was only recently applied in human neonatal care units (15, 16).

Rabbit brain, the rabbit being a rather immature species at birth, is a suitable neonatal model for measuring CBF (17). Histologically, the brains of 11- to 12-d-old newborn rabbits have a thin subependymal germinal layer and sparse myelination in the white matter of the cerebrum, and physiologically, immature electrical activity remains until 10 to 15 d after birth (18). Therefore, we regard these rabbits as being developmentally similar in maturity to human neonates.

The aim of this study is to clarify the dynamic changes in the cerebral circulation and oxygenation during hyperventilation in newborn rabbits with NIRS.

MATERIALS AND METHODS

Twenty-two newborn rabbits (11 to 12 d old), weighing 100–180 g, were anesthetized through inhalation of diethyl ether during all operative procedures. Tracheostomy and intubation were performed, and each rabbit underwent ventilation (Harvard Rodentventilator Model 683, Harvard Apparatus Co., Natick, MA). Pancronium bromide (0.1–0.2 mg/kg) was injected intraperitoneally to obtain paralysis. NIRS (NIR-1000, Hamamatsu

Hypocarbia is a known depressant of CBF (1–5). Because hyperventilation is a common practice for persistent pulmonary

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Photonics KK, Hamamatsu Corp., Middlesex, NJ) monitoring was started at least 30 min after a steady heart rate and steady arterial blood pressure had been attained. Initially, the animals underwent ventilation at a rate of approximately 30 cycles/min. The arterial blood pressure and heart rate were continuously monitored through the femoral artery (San-ei Amplifier Case 7903 and Gould H.B. disposable pressure monitoring kit, Gould, Santa Clara, CA). Microsamples (200 μ L) of arterial blood were taken to measure the blood gas and hemoglobin concentrations (Ciba-Corning 288, Ciba-Geigy, Summit, NJ).

The concentration changes of HbO₂, HbR, HbT, and Cyt aa₃ were continuously measured in 22 rabbits using NIRS, which involves near-infrared light at six wavelengths (780, 808, 830, 847, 867, and 911 nm). A flexible fiberoptic bundle conveyed the near-infrared light from laser diodes to the head. The end of the fiber bundle (the optode) was applied to the scalp. The interoptode distance was 2.5 cm, which was fixed in all experiments. The room lights were turned off, and monitoring was performed in a dark room. In this study, changes in HbO₂, HbR, HbT, and Cyt aa₃ were expressed as relative variation because we could not determine the optical path length.

The rabbits were divided into four groups: group HV, HO, HH, and C. In group HV, after the first blood sampling for blood gas analysis and hemoglobin concentration measurement and after baseline values determination in a normoxic and normocarbic steady state for at least 30 min, hyperventilation was started at a rate of approximately 80 cycles/min with a fixed tidal volume to produce hypocarbia of below 2.67 kPa PaCO₂. The second blood sampling was performed in a normoxic and hypocarbic steady state for at least 30 min, and then the hyperventilation was stopped. Thereafter, the third measurement was performed in a normoxic and normocarbic steady state for at least 30 min. In group HO, hyperoxia was induced by 100% O₂ inhalation, and the second measurement was performed in a hyperoxic and normocarbic steady state for at least 30 min. In group HH with hyperventilation with hyperoxia, the second measurement was performed in a hyperoxic and hypocarbic steady state for at least 30 min. The first and third measurements were performed in the same state as in group HV. Group C served as normoxic and normocarbic controls to assess the stability of this animal preparation. They were treated in a similar manner as the experimental groups but had no loading.

The results are presented as mean values $\pm$ SD. Statistical analysis of the mean blood pressure, hemoglobin concentration, and arterial blood gas data was performed by means of *t* test. The Mann-Whitney U test was also used in statistical analysis of the NIRS data.

RESULTS

Table 1 shows an outline of the data for the heart rate, mean arterial pressure, blood Hb, and blood gas analysis before, during, and after the experimental loading in each group. There were significantly higher pH and lower PaCO₂ values during hyperventilation in groups HV (pH 7.711 ± 0.059 , PaCO₂ 1.76 ± 0.29 kPa) and HH (pH 7.750 ± 0.024 , PaCO₂ 1.69 ± 0.27 kPa), and higher PaO₂ values during 100% O₂ inhalation in groups HO (PaO₂ 38.19 ± 7.20 kPa) and HH (PaO₂ 33.65 ± 8.67 kPa) than in the control group. The mean arterial blood pressure did not show a significant difference at any time between any of the experimental and control groups, although it was lower during than before hyperventilation in group HV. Blood gas analysis also did not reveal significant difference at any time after loading between the experimental and control groups.

Figure 1 presents typical NIRS data for each experiment. An obvious difference exists between hyperventilation (A), and hyperventilation with 100% O₂ inhalation (C) in that there is no reduction during hyperventilation with 100% O₂ inhalation, although the reduced state of cytochrome oxidase is observed during hyperventilation.

Figure 2 shows changes in the concentrations of HbO₂, HbR, HbT, and Cyt aa₃. In group HV, HbO₂ decreased with the passage of time during hyperventilation, and the reduction persisted after loading. HbR increased with time during hyperventilation and gradually recovered to within normal range after hyperventilation. HbT showed a pattern similar to HbO₂. Cyt aa₃ also decreased during hyperventilation but after that gradually increased to within normal range. In group HO, HbO₂ slightly increased during 100% O₂ inhalation, but the increase was not statistically significant. HbR markedly decreased during loading and after that rapidly increased to the preloading level. HbT showed a tendency to decrease, but Cyt aa₃ did not show any changes during or after 100% O₂ loading. In group HH, HbO₂ and HbT decreased during loading and then increased. The reduction of HbT was more significant in group HH. However, HbR was slightly increased during loading and continued to increase after the loading. The oxygenation of Cyt aa₃ remained in the control range.

DISCUSSION

This study demonstrated that passive hyperventilation causes gradual reductions of HbO₂, HbT, and Cyt aa₃ and an increase in HbR, although systemic oxygenation remains within normal range. However, hyperventilation with 100% O₂ inhalation does not cause any reduction of Cyt aa₃ despite marked reduction of HbO₂ and HbT. The 100% O₂ inhalation causes decreases in HbR and HbT and a slight increase in HbO₂ but no change in Cyt aa₃. Because HbT reflects CBV (16), hypocarbia and hyperoxemia cause the reduction of CBV.

Although hypocarbia and hyperoxemia cause a decrease in CBF, the CBF response varies depending on the levels of hypocarbia, systemic oxygenation, and loss of cerebral pressure-flow autoregulation in brain damage, and the maturity of the animals. The decrease in CBF during passive hyperventilation was 36% in human adults (PaCO₂ 5.73 to 3.07 kPa) (4), 40% in the cerebrum of newborn piglets (PaCO₂ range of <2.0 kPa) (3), and 51% (PaCO₂ 5.33 to 3.33 kPa) and 69% (PaCO₂ 5.33 to 2.0 kPa) in the cerebral gray matter of a newborn dog (2). On the other hand, the changes in CBF with 100% O₂ inhalation are in general agreement with the study by Kennedy *et al.* (19), which involved the ¹⁴C-antipyrine autoradiographic method in newborn dogs. They showed that 100% O₂ lowered CBF by 25% in 2-d-old beagle puppies, but at 3 wk of age the effect was much less (13%). A decrease of 15% in CBF has also been reported in human adults and preterm infants (20, 21).

The relationship between the decrease in CBF and cerebral biochemical metabolism during hyperventilation is complex. Severe hypocarbia has been found to increase the lactate concentration in cerebral tissue and cerebrospinal fluid (6, 7, 22). It is uncertain whether this increase is due to mild tissue hypoxia or alkalosis-induced activation of the phosphofructokinase pathway, which is unrelated to hypoxia (23). Controversy exists as to whether the production of lactate is a result of anaerobic glycolysis. Reuter and Disney (2) observed that severe hypocarbia in newborn dogs causes a 15% decrease in CMRO₂. In another study, newborn piglets exhibited a decrease in CMRO₂ after 30 min of hyperventilation (24). This evidence suggests that the decrease in CMRO₂ during respiratory alkalosis might be caused by a decrease in cerebral oxygen demand and an increase in anaerobic glycolysis against cerebral hypoxia. However, studies on both adult animals and human beings suggest that CMRO₂ is maintained at a constant level during severe hypocarbia (4, 5). Therefore, neonatal cerebral metabolism in hypocarbia may differ from that in adults. In addition, several biochemical and ³¹-phosphorus magnetic resonance spectroscopic (22, 25) studies did not demonstrate any change in the ATP or phosphocreatine level during hyperventilation in adults. However, in newborn piglets, profound tissue hypoxia with membrane insufficiency

Table 1. Changes in heart rate, mean arterial blood pressure, Hb concentration, and arterial blood gas analysis before, during, and after hyperventilation, hyperoxemia, and hyperventilation with hyperoxemia, with NIRS and in controls*

	n	HR (bpm)	MAP (mm Hg)	Hb (g/L)	pH	Paco ₂ (kPa)	Pao ₂ (kPa)	HCO ₃ (mmol/dL)
Before								
HV-nir	7	363.4 ± 19.2	55.4 ± 6.4	118 ± 15	7.425 ± 0.037	4.85 ± 0.51	17.00 ± 4.45	23.8 ± 1.3
HO-nir	5	369.0 ± 18.2	46.6 ± 8.0	125 ± 23	7.490 ± 0.050	4.37 ± 0.72	16.19 ± 4.20	24.7 ± 2.9
HH-nir	5	347.4 ± 30.5	49.8 ± 6.6	126 ± 15	7.460 ± 0.031	4.45 ± 0.55	18.49 ± 3.15	23.6 ± 1.7
C	5	345.4 ± 37.2	51.0 ± 5.7	104 ± 22	7.457 ± 0.036	4.33 ± 0.64	19.48 ± 2.12	22.9 ± 2.7
During								
HV-nir	7	346.3 ± 21.3	45.1 ± 6.6†	111 ± 14	7.711 ± 0.059†‡	1.76 ± 0.29†‡	20.90 ± 1.00	16.5 ± 1.3†‡
HO-nir	5	370.0 ± 25.4	46.0 ± 8.2	124 ± 24	7.510 ± 0.058	4.17 ± 0.63	38.19 ± 7.20†‡	24.8 ± 2.2
HH-nir	5	337.4 ± 38.0	41.6 ± 7.3	126 ± 10	7.750 ± 0.024†‡	1.69 ± 0.27†‡	33.65 ± 8.67†‡	17.6 ± 2.4†‡
C	5	353.8 ± 32.1	47.2 ± 6.8	100 ± 29	7.502 ± 0.046	4.07 ± 0.56	19.03 ± 2.93	23.9 ± 3.1
After								
HV-nir	7	356.6 ± 24.7	51.3 ± 6.8	107 ± 13†	7.428 ± 0.030	4.36 ± 0.41†	13.61 ± 3.59	21.5 ± 0.9†
HO-nir	5	358.0 ± 19.2	45.2 ± 6.6	115 ± 22	7.467 ± 0.046	4.47 ± 0.85	13.76 ± 4.00	24.0 ± 2.2
HH-nir	5	335.2 ± 27.2	46.0 ± 6.5	119 ± 12	7.479 ± 0.044	3.93 ± 0.45	16.33 ± 3.41	21.9 ± 2.1
C	5	351.2 ± 38.3	48.0 ± 10.6	98 ± 22	7.503 ± 0.054	4.08 ± 0.44	17.33 ± 3.20	24.0 ± 2.9

* Values are means ± SD. HR, heart rate; MAP, mean arterial blood pressure; HCO₃, bicarbonate ion; HV-nir, hyperventilation; HO-nir, hyperoxemia; HH-nir, hyperventilation with hyperoxemia; C, controls.

† Significantly lower than the preceding value; $p < 0.01$, paired t test.

‡ Significantly lower than the control value; $p < 0.01$, unpaired t test.

Fig. 1. Typical NIRS data for each experiment. A, hyperventilation; B, 100% O₂ inhalation; and C, hyperventilation with 100% O₂ inhalation. O, HbO₂; □, HbR; ■, HbT; ●, Cyt aa₃.

and decreased high-energy compounds was observed during severe hypocarbia (Paco₂ = 1.07–1.47 kPa) (26).

The present study seems to establish directly that hypocarbia causes cerebral tissue mitochondrial hypoxia (anaerobic metabolism ensuing) in newborn rabbits. Kogure *et al.* (27) also reported that the increased NADH/NAD⁺ ratio in severe hypocarbia suggested stagnation of electrical flow if the metabolic impairment is in the mitochondrial system. Also, hyperventilation causes slowing of waves in an EEG, which resembles the alterations seen during hypoxic hypoxia. Also, hyperventilation during the inhalation of hyperbaric 100% O₂ eliminates this EEG slowing (28). Moreover, the cerebral surface Po₂ drops during hyperventilation but not during hyperventilation with 100% O₂ inhalation (6). In our study, Cyt aa₃ never decreased during hyperventilation with 100% O₂ inhalation, although it decreased during hyperventilation with air. Thus, metabolic abnormalities, including both brain tissue and cerebrospinal fluid lactate increases with hyperventilation with air, may be mitigated by hyperventilation during hyperbaric, hyperoxic ventilation (29, 30). Considering the decreased CMRO₂, reduced oxygen de-

mand, and increased anaerobic glycolysis during hyperventilation, the decrease in Cyt aa₃ may be related to an increase in aerobic glycolysis. Therefore, further studies are required to define the significance of NIRS parameters, including the neuropathologic correlation with hypocarbia (*i.e.* leukomalacia and/or pontosubicular necrosis).

In conclusion, NIRS monitoring in hyperventilation provides important information on the cerebral oxygenation, blood volume, and cell metabolism in neonates. During hyperventilation with room air, NIRS demonstrated decreases in HbO₂, HbT, and Cyt aa₃ and an increase in HbR. On hyperventilation with 100% O₂ inhalation, HbO₂ and HbT tended to decrease more markedly but oxygenation of Cyt aa₃ remained within normal range. The reduction in cerebral oxygenation may induce ischemic brain damage. Our data demonstrated that hypocarbia results in cerebral mitochondrial hypoxia consistent with its known electroencephalographic and metabolic effects. NIRS can demonstrate the changes in the total cerebral arterial and venous blood volume, cerebral venous retention, and cerebral tissue oxygenation. In addition, anaerobic and aerobic metabolism may be

Fig. 2. Comparison of each parameter of NIRS between hyperventilation (HV, ●), 100% O₂ inhalation (HO, ■), and hyperventilation with 100% O₂ inhalation (HH, ○). Arrows labeled A and B indicate starting and stopping of loading. A, Changes in the concentration of HbO₂; B, changes in the concentration of HbR; C, changes in the concentration of HbT; and D, changes in the concentration of Cyt aa₃. The slanted lines in each panel indicate the variation of the control (mean ± SD), and bars indicate SD. ★. Significantly lower than the control value, $p < 0.05$; Mann-Whitney U test.

reflected by HbO₂ and Cyt aa₃ on NIRS. NIRS is available for the noninvasive monitoring of cerebral oxygenation, which may be important during hyperventilation therapy.

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