Cerebral oxygen availability by NIR spectroscopy during transient hypoxia in humans

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HAMPSON, N. B., E. M. CAMPORESI, B. W. STOLP, R. E. MOON, J. E. SHOOK, J. A. GRIEBEL, AND C. A. PIANTADOSI. *Cerebral oxygen availability by NIR spectroscopy during transient hypoxia in humans.* J. Appl. Physiol. 69(3): 907–913, 1990.—The effects of mild hypoxia on brain oxyhemoglobin, cytochrome a,a_3 redox status, and cerebral blood volume were studied using near-infrared spectroscopy in eight healthy volunteers. Incremental hypoxia reaching 70% arterial O_2 saturation was produced in normocapnia [end-tidal P_{CO_2} ($P_{ET_{CO_2}}$) 36.9 ± 2.6 to 34.9 ± 3.4 Torr] or hypocapnia ($P_{ET_{CO_2}}$ 32.8 ± 0.6 to 23.7 ± 0.6 Torr) by an 8-min rebreathing technique and regulation of inspired CO_2. Normocapnic hypoxia was characterized by progressive reductions in arterial P_{O_2} (Pa_{O_2}, 89.1 ± 3.5 to 34.1 ± 0.1 Torr) with stable $P_{ET_{CO_2}}$, arterial P_{CO_2} (Pa_{CO_2}), and arterial pH and resulted in increases in heart rate (35%) systolic blood pressure (14%), and minute ventilation (5-fold). Hypocapnic hypoxia resulted in progressively decreasing Pa_{O_2} (100.2 ± 3.6 to 28.9 ± 0.1 Torr), with progressive reduction in Pa_{CO_2} (39.0 ± 1.6 to 27.3 ± 1.9 Torr), and an increase in arterial pH (7.41 ± 0.02 to 7.53 ± 0.03), heart rate (61%), and ventilation (3-fold). In the brain, hypoxia resulted in a steady decline of cerebral oxyhemoglobin content and a decrease in oxidized cytochrome a,a_3. Significantly greater loss of oxidized cytochrome a,a_3 occurred for a given decrease in oxyhemoglobin during hypocapnic hypoxia relative to normocapnic hypoxia. Total blood volume response during hypoxia also was significantly attenuated by hypocapnia, because the increase in volume was only half that of normocapnic subjects. We conclude that cytochrome a,a_3 oxidation level in vivo decreases at mild levels of hypoxia. Pa_{CO_2} is an important determinant of brain oxygenation, because it modulates ventilatory, cardiovascular, and cerebral O_2 delivery responses to hypoxia.

brain; cytochrome a,a_3; oxyhemoglobin; deoxyhemoglobin; blood volume; normocapnia; hypocapnia

CEREBRAL METABOLIC INTEGRITY is dependent precariously on arterial O_2 delivery for production of ATP by mitochondrial respiration; even brief disruptions in O_2 supply can result in metabolic failure and cell death. Ventilatory events that threaten brain oxygenation are generally accommodated by changes in cerebral vascular resistance and consequential alterations in cerebral blood flow in an attempt to restore O_2 delivery. Cerebral hemodynamics, however, may not accurately predict impairment of cerebral metabolism (4, 17). A better indicator of cerebral O_2 sufficiency may be the reduction-oxidation (redox) state of cytochrome a,a_3, the concentration of which directly reflects the availability and utilization of O_2 at intraneuronal sites of oxidative phosphorylation in the mitochondrial electron transport system. Cytochrome a,a_3, the terminal enzyme in the electron transport chain located in the inner mitochondrial membrane, catalyzes the transfer of electrons from ferricytochrome c to molecular oxygen, resulting in formation of water and generation of the high-energy product ATP (26). Because of the tight coupling between neuronal activity and oxidative metabolism, cytochrome oxidase is generally accepted as an endogenous marker of neuronal activity (27–29).

Noninvasive measurement of cytochrome a,a_3 redox levels in brain tissue and oxy/deoxyhemoglobin in small cerebral blood vessels and capillaries has become feasible over the past few years by use of near-infrared (NIR) wavelengths of light (12). NIR spectroscopy noninvasively detects relative changes in the concentrations of oxyhemoglobin, deoxyhemoglobin, and oxidized cytochrome a,a_3 by measuring changes in absorption at specific wavelengths of NIR light. The porphyrin moieties of oxyhemoglobin and deoxyhemoglobin and the oxidized copper moiety of cytochrome a,a_3 have different absorption spectra in the NIR region (700–1,000 nm), allowing independent measurement of each molecular species. The degree of oxygenation of hemoglobin reflects the O_2 concentration in the circulatory system, whereas oxidation of cytochrome a,a_3 reflects that of the intramitochondrial space (24). We have previously shown that NIR spectroscopy can detect changes in cerebral oxygenation in animals including cats (14, 19) and rats (14) and in isolated mitochondrial preparations in vitro (14). NIR spectroscopy also has been used successfully to monitor skeletal muscle oxygenation in animals (8) and adult humans (9) and cerebral oxygenation in small premature infants (1, 30). NIR spectroscopy has been utilized in preliminary studies to assess cerebral oxygenation in humans (3, 7), and it has promise as an early warning system for the prevention of cerebral anoxia and subsequent neurological damage (21).

Our goals in this study were *1*) to investigate the sensitivity of NIR spectroscopy as an indicator of cerebral O_2 deprivation with an experimental model of hypoxia in normal volunteers, *2*) to characterize the effects of hypocapnia of hypoxia-induced changes in brain oxygenation and metabolism, and *3*) to identify other physiological alterations that may correlate with or predict

brain O_2 or CO_2 deficits. We induced hypoxia with or without hypocapnia by rebreathing techniques and manipulation of inspired P_{CO_2}. During each challenge to cerebral oxygenation, we measured a range of pertinent cardiovascular and ventilatory variables in addition to the NIR optical parameters.

METHODS

Subjects. This study was approved by the Duke University Institutional Review Board. Eight normal healthy adult male volunteers (28–40 yr old) gave written informed consent to participate in all studies. All subjects were nonsmokers. Each subject was familiarized with the experimental environment and procedures before testing.

Experimental protocol. Cerebral oxygenation plus cardiovascular and ventilatory function were monitored simultaneously during progressive hypoxia in the presence of either graded hypocapnia or normocapnia. Subjects were seated comfortably, and intra-arterial catheters were inserted into the radial artery under local anesthesia (1% lidocaine) for blood sampling. Subjects were fitted with standard electrocardiogram (ECG) leads and a finger oximeter. Two optical fiber bundles (transmitting and receiving, ~4 cm apart) were placed firmly against the scalp over one of the frontal bones for NIR spectroscopy. During testing, the subjects wore a nose clip and breathed through a mouthpiece connected to a 13-liter bag-in-the-box closed rebreathing circuit. Rebreathing of expired air resulted in linear reductions of inspired O_2 and consequent reductions in arterial O_2 saturation (Sa_{O_2}) as measured by finger oximetry. Testing was terminated at an Sa_{O_2} of 70% measured by the finger oximeter (~6–9 min). Hypoxic challenges were accompanied by either normocapnia or progressive hypocapnia, while a CO_2 scrubber allowed for precise control of the CO_2 level within the rebreathing system. Graded hypocapnia developed during rebreathing if CO_2 was continuously cleared by the CO_2 scrubber. Alternatively, normocapnia was maintained during rebreathing if the CO_2 scrubber was bypassed almost completely. Each subject received both ventilatory challenges in fixed order (hypocapnic hypoxia then normocapnic hypoxia) 30 min apart.

The following variables were recorded continuously on a Gould (model 2800S) chart recorder during each trial: end-tidal CO_2 ($P_{ET_{CO_2}}$; Datex capnograph, model 223), Sa_{O_2} (Nellcor pulse oximeter, model N-100), tidal volume (V_T; Validyne no. 3 pneumotach with pressure transducer, model MP45-7), and respiratory rate. Minute ventilation ($\dot{V}_E$) was calculated as $\dot{V}_E = (V_T \times \text{respiratory rate})$ and corrected for BTPS. We also recorded heart rate and systemic pressures from the ECG and arterial pressure trace. Arterial blood samples were collected anaerobically and analyzed for arterial P_{O_2} (Pa_{O_2}), arterial P_{CO_2} (Pa_{CO_2}), and pH with an Instrumentation Laboratory pH-blood gas analyzer (model 813) calibrated before each experiment. Hemoglobin O_2 saturation and carboxyhemoglobin levels in arterial blood were measured by CO oximetry (Instrumentation Laboratory model 482).

Cerebral oxygenation measurements. Relative changes in concentrations of oxidized cytochrome a,a_3 in brain tissue (t) and deoxyhemoglobin (tHb) and oxyhemoglobin ($tHbO_2$) in small cerebral blood vessels and capillaries were detected by NIR spectroscopy; brain tissue relative blood (hemoglobin) volume was calculated as $tBV = [tHb] + [tHbO_2]$. The laser-based spectroscope constructed in our laboratory has been described previously (9). Briefly, NIR light (700–900 nm) transmitted through an optical fiber bundle penetrated the underlying tissues including skin and bone. In the tissue, NIR light is absorbed by the oxidized copper atoms of cytochrome a,a_3 and by the iron-porphyrin complexes of tHb and $tHbO_2$. Changes in the concentration of either of these molecules can be detected from changes in the amount of reflected light. For each measurement, the transmitting fiber bundle pulsed four consecutive wavelengths of NIR light (775, 810, 870, and 904 nm), and reflected light was collected in the receiving bundle and transmitted to a photomultiplier tube (Hamamatsu model R936). Concentrations of tHb, $tHbO_2$, and cytochrome a,a_3 were derived from algorithms of the transmittance-reflection relationships at these wavelengths as previously described (9). The time constant for the spectrophotometer was 5 s for all studies. The NIR data are reported only as variations in density (vd) because absolute concentrations cannot be ascertained reliably by this method. The vd are proportional to changes in concentration, and 1 vd unit is defined as a 10-fold change in a signal computed through its algorithm.

Data analysis. Data presented herein represent the pooled values (means ± SE) from all subjects ($n = 8$) at progressive degrees of rebreathing-induced hypoxia. Baseline (control) O_2 saturation was 97%, and hypoxic data are presented at consecutive 3% steps in Sa_{O_2} reduction (i.e., 94, 91, . . .70%). Significant differences were identified by analysis of variance and paired t tests.

RESULTS

The rebreathing maneuvers resulted in progressive hypoxia, producing reductions in finger O_2 saturation. At the onset of rebreathing, finger blood was ~97% saturated, and unless otherwise specified, 97% Sa_{O_2} was the control for statistical comparison with decreasing levels of hemoglobin saturation. All subjects attained Sa_{O_2} of 70% (the maximal test limit) within 9 min. The mean time to 70% Sa_{O_2} was 6.1 min for hypocapnic hypoxia and 6.8 min for normocapnic hypoxia. We measured O_2 tensions and hemoglobin saturation in arterial blood (Pa_{O_2}) at 97, 85, and 70% Sa_{O_2} to validate the pulse oximetry readings as reliable indicators of hypoxemia. Reductions in arterial O_2 content paralleled the drop in Sa_{O_2}, although the hypocapnic protocol produced a significantly greater decrease in Pa_{O_2} than the normocapnic protocol (Table 1). In the normocapnic-hypoxic protocol, subjects rebreathed their exhaled CO_2, which allowed them to maintain constant levels of CO_2 in the expirate ($P_{ET_{CO_2}}$, Fig. 1) and in arterial blood (Table 1). In the hypocapnic-hypoxic protocol, subjects rebreathed expired gas that had been shunted through a CO_2 scrubber, resulting in progressive declines in $P_{ET_{CO_2}}$ (Fig. 1) and Pa_{CO_2} (Table 1). Arterial pH reflected these changes in Pa_{CO_2} as the hypocapnic group developed respiratory alkalosis, whereas the normocapnic group maintained

TABLE 1. *Changes in arterial gases and pH during rebreathing under normocapnic and hypocapnic conditions*

Sa_{O_2},* %	Normocapnic Hypoxia				Hypocapnic Hypoxia			
	Pa_{O_2}, Torr	Pa_{CO_2}, Torr	pH	Sa_{O_2},† %	Pa_{O_2}, Torr	Pa_{CO_2}, Torr	pH	Sa_{O_2},† %
97	89.1±3.5	39.2±1.0	7.40±0.01	97.0±0.2	100.2±3.6‡	39.0±1.6	7.41±0.02	97.3±0.2
86	44.2±1.0§	38.9±1.0	7.41±0.01	83.7±1.2	38.5±1.0‡§	29.4±2.1‡§	7.50±0.03‡§	82.7±1.2
70	34.1±0.07§	38.5±1.3	7.41±0.02	70.2±1.4	28.9±0.06‡§	27.3±1.9‡§	7.53±0.03‡§	69.2±1.6

Values are means ± SE of 8 subjects. * Pulse oximeter. † Co-oximeter. ‡ Significant difference from corresponding normocapnic hypoxia, $P < 0.05$. § Significant difference from control (3% Δsaturation), $P < 0.05$.

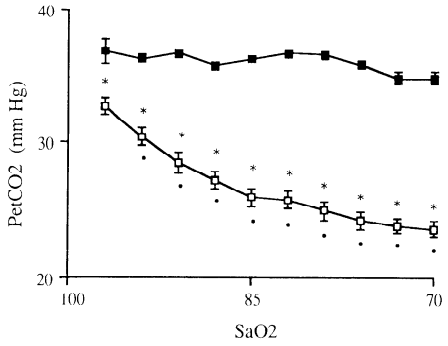

FIG. 1. Comparison of PET_{CO_2} in normocapnic-hypoxic (■) and hypocapnic-hypoxic (□) subjects during progressive hypoxia. Each point represents mean ± SE of 8 subjects. • Significantly different from control (3% Δsaturation), $P < 0.01$. * Significant difference between normocapnic- and hypocapnic-hypoxic subjects $P < 0.05$.

normal pH (Table 1). After each ventilatory challenge, the volunteers were asked to describe their experiences during rebreathing. The most common symptoms were light-headedness, a "dimming of lights," nausea, and restlessness. Normocapnic subjects also reported more intense dyspnea.

The effects of normocapnia and hypocapnia on the ventilatory response to hypoxia are presented in Fig. 2. Respiratory rate increased with each drop in Sa_{O_2} in both trials (Fig. 2*A*). Normocapnic subjects showed increasing VT with decreasing Sa_{O_2}, although VT of hypocapnic subjects did not increase above control levels (Fig. 2*B*). At 70% Sa_{O_2}, $\dot{V}E$ increased fivefold in normocapnic-hypoxic subjects and nearly threefold in hypocapnic-hypoxic subjects relative to control (Fig. 2*C*).

The effects of normocapnia and hypocapnia on the cardiovascular response to hypoxia are compared in Fig. 3. Although heart rate increased in both trials during progressive hypoxia, hypocarbic subjects manifested greater increases relative to normocarbic subjects (Fig. 3*A*). Peak heart rates, which occurred at 70% Sa_{O_2}, were increased by 35% in normocapnic subjects and by 61% in hypocapnic subjects. Also normocapnic subjects had an increase in systolic blood pressure of 14% during hypoxia, whereas systolic pressure in hypocapnic subjects remained unchanged (Fig. 3*B*). Diastolic pressures were also unchanged in both protocols (Fig. 3*C*). Blood pressure variables are presented in Fig. 3 as percentage of control values because of large intersubject variability in absolute blood pressure responses, although the resting pressures of all subjects were normal.

Parameters of cerebral oxygenation measured by NIR spectroscopy are expressed as vd of brain $tHbO_2$, tHb, tissue blood volume, and oxidized cytochrome a,a_3 relative to control because the method does not estimate absolute concentrations. Therefore, measurements at 97% Sa_{O_2} were set at zero change, and relative increases or decreases in concentration were calculated as positive or negative vd, respectively. Because the 97% Sa_{O_2} values were arbitrarily set at 0 and were used as the standard for estimating change at lower levels of saturation, we did not use 97% as the control for statistical comparisons. Instead, we used the vd measured between 97 and 94% Sa_{O_2} as the control value for statistical comparisons at

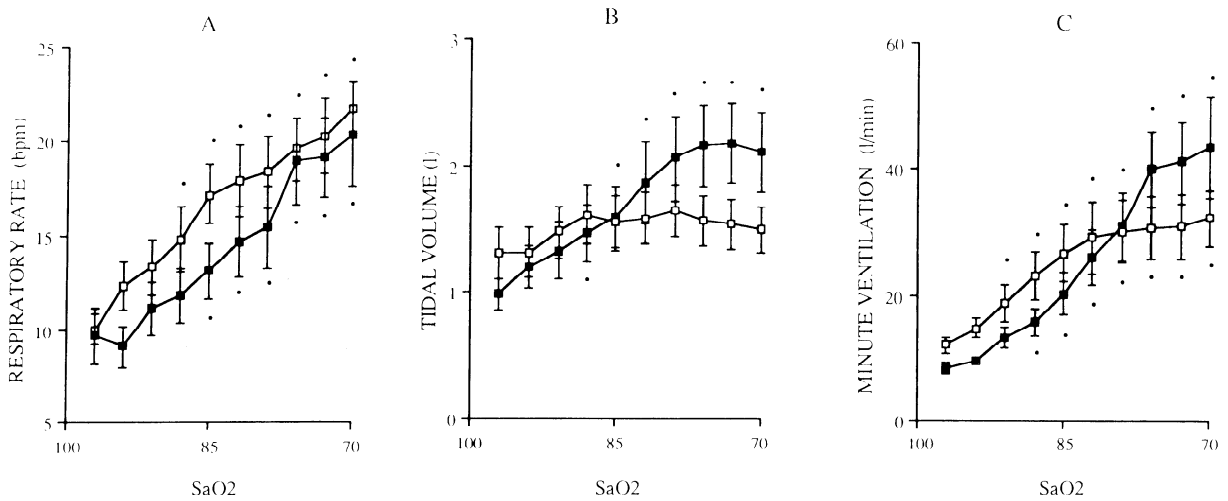

FIG. 2. Comparison of normocapnic-hypoxic (■) and hypocapnic-hypoxic (□) subjects in terms of respiratory rate (*A*), VT (*B*), and $\dot{V}E$ (*C*) during progressive hypoxia. Each point represents mean ± SE of 8 subjects. • Significantly different from control (3% Δsaturation), $P < 0.05$.

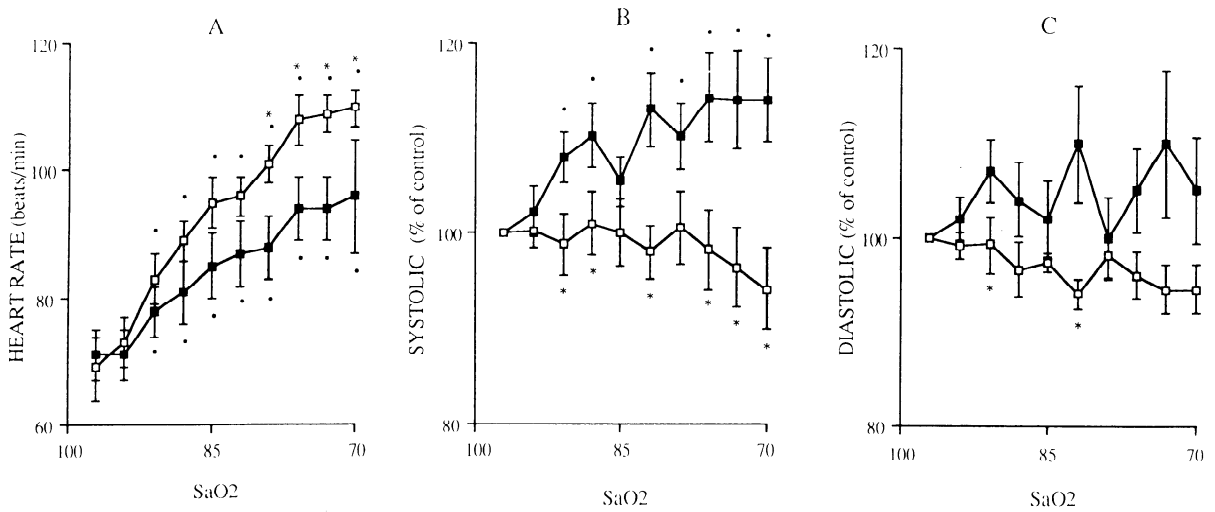

FIG. 3. Comparison of normocapnic-hypoxic (■) and hypocapnic-hypoxic (□) subjects in terms of heart rate (*A*), systolic pressure (*B*), and diastolic pressure (*C*) during progressive hypoxia. Each point represents the mean ± SE of 8 subjects. • Significantly different from control (3% Δsaturation), $P < 0.05$. * Significant difference between normocapnic- and hypocapnic-hypoxic subjects, $P < 0.05$.

lower levels of oxyhemoglobin saturation. Although this method precludes detection of possible significant changes from control at the initial 3% drop in Sa_{O_2}, it provides a more rigorous test of significant differences for the lower values of saturation. Relative changes in brain $tHbO_2$ and tHb are compared in Fig. 4. In both protocols, hypoxia resulted in progressive increases in tHb (Fig. 4*A*) and decreases in $tHbO_2$ (Fig. 4*B*). The hypocapnic hypoxia trial showed greater decreases in $tHbO_2$ at 94–82% Sa_{O_2} relative to the normocapnic hypoxia trial. At 79–70% Sa_{O_2}, both protocols produced similar decrements in $tHbO_2$. Brain tissue blood volume increased steadily during hypoxia in both trials, but the increase in normocapnic-hypoxic subjects exceeded that of the hypocapnic subjects, especially at the more severe degrees of desaturation (i.e., 79–70% Sa_{O_2}) (Fig. 4*C*). At 70% Sa_{O_2}, the brain tissue blood volume increase in normocapnic hypoxia trials averaged twice that of hypocapnic hypoxia trials.

As $tHbO_2$ declined, so did the amount of oxidized cytochrome a,a_3 (Fig. 5). In both protocols, the initial significant decrease from control values (94% Sa_{O_2}) in cytochrome a,a_3 occurred at 88% Sa_{O_2}, whereas the significant reduction in oxyhemoglobin initially occurred at 91% Sa_{O_2}. Even though using 94% Sa_{O_2} as the statistical control decreases the probability of detecting the actual percentage of saturation at which significant changes first occurred, it appears reasonable to assume that significant reductions in brain $tHbO_2$ preceded significant reductions in oxidized cytochrome a,a_3. At 70% Sa_{O_2}, the oxidized cytochrome a,a_3 signal of hypocapnic-hypoxic subjects was lower than that of normocapnic-hypoxic subjects.

DISCUSSION

In our subjects, hypoxia stimulated cardiovascular and ventilatory function, but despite these responses, brain oxygenation and cytochrome a,a_3 redox levels declined rapidly whether hypoxia was induced in the presence of normocapnia or hypocapnia. Rebreathing under hypocapnic conditions caused the subjects to incur greater reductions in Pa_{O_2} relative to rebreathing under normocapnic conditions for equivalent reductions in arterial hemoglobin saturation. This was probably a consequence of the Bohr effect, whereby decreasing CO_2 and increasing pH increases the affinity of hemoglobin for O_2 (2). Thus the normocapnic hypoxia challenge was characterized by progressive reductions in Pa_{O_2} with stable PET_{CO_2}, Pa_{CO_2}, and arterial pH, whereas the hypocapnic hypoxia challenge was characterized by simultaneous progressive reductions in Pa_{O_2}, PET_{CO_2}, and Pa_{O_2} and increasing arterial pH. The maximal reduction in Sa_{O_2} produced Pa_{O_2} <40 Torr, which may be considered mild hypoxia (21). This mild hypoxia, however, was physiologically significant as exemplified by the perceptions of the subjects.

Cardiovascular responses to hypoxia were altered differently by hypocapnia and normocapnia. Hypoxia stimulated heart rate in both groups, but hypocapnic subjects developed more tachycardia than the normocapnic-hypoxic subjects. In normocarbic hypoxia systolic blood pressure increased but remained stable under hypocapnic conditions. This difference probably represents differences in peripheral vascular resistance modulated by CO_2. Both hypoxic challenges stimulated ventilation, and the quantity of inspired CO_2 was only a minor determinant of the ventilatory response. In summary, normocapnic hypoxia increased heart rate, systolic blood pressure, and $\dot{V}E$. In comparison, hypocapnic hypoxia produced greater accelerations in heart rate, with lesser effect on ventilatory volumes and blood pressure.

Reductions in brain oxygenation became apparent at early stages in the development of hypoxia and worsened rapidly. Cerebral oxyhemoglobin was especially sensitive to hypoxia, because decreases in brain oxyhemoglobin were apparent after even a 3% drop in Sa_{O_2}. Hypocapnic-hypoxic subjects manifested significantly greater reductions in brain oxyhemoglobin than normocapnic subjects at milder levels of hypoxia, i.e., 94–82% Sa_{O_2}, although

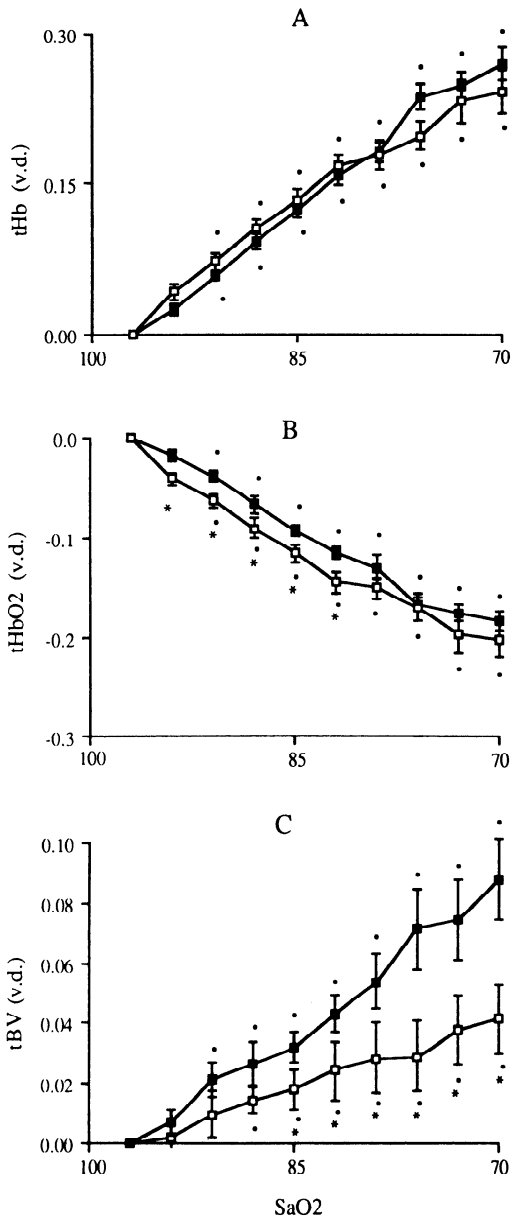

FIG. 4. Variations in density (vd) in brain deoxyhemoglobin (tHb, *A*), oxyhemoglobin ($tHbO_2$, *B*), and brain tissue blood volume (tBV, *C*) in normocapnic-hypoxic (■) and hypocapnic-hypoxic (□) subjects during progressive hypoxia. Each point represents mean ± SE of 8 subjects. Negative values represent relative decreases in quantity of $tHbO_2$. • Significantly different from control (6% Δsaturation), $P < 0.05$. * Significant difference between normocapnic- and hypocapnic-hypoxic subjects, $P < 0.05$.

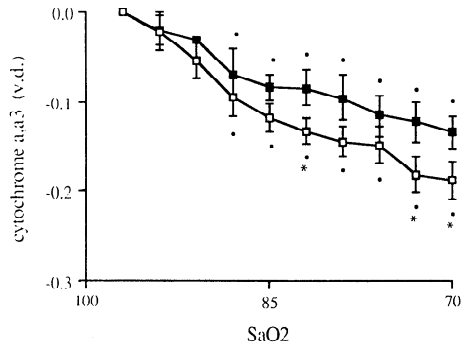

FIG. 5. Variations in density (vd) in oxidized cytochrome a,a_3 in brains of normocapnic-hypoxic (■) and hypocapnic-hypoxic (□) subjects during progressive hypoxia. Each point represents mean ± SE of 8 subjects. Negative values represent relative decreases in quantity of oxidized cytochrome a,a_3. • Significantly different from control, $P < 0.05$. * Significant difference between normocapnic- and hypocapnic-hypoxic subjects, $P < 0.05$.

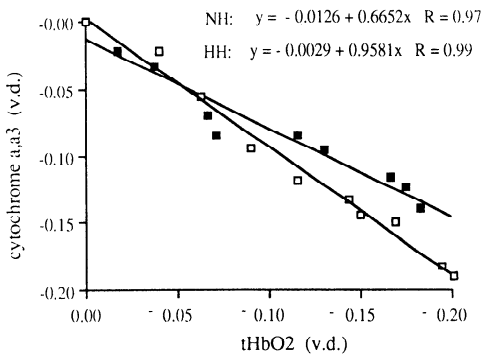

FIG. 6. Relationship between $tHbO_2$ and oxidized cytochrome a,a_3 in normocapnic-hypoxic (NH, ■) and hypocapnic-hypoxic (HH, □) subjects. Negative values represent relative decreases in quantity of $tHbO_2$ or oxidized cytochrome a,a_3. Each point represents mean ± SE of 8 subjects.

equal degrees of hemoglobin deoxygenation occurred at more severe levels of hypoxia, i.e., 79–70% Sa_{O_2}.

Decreases in cerebral oxyhemoglobin led to progressive decreases in O_2 availability to brain mitochondria as shown by progressive conversion of the oxidized copper of cytochrome a,a_3 to its reduced state. The oxidation state of the enzyme was less sensitive to hypoxia than hemoglobin, because significant change from control did not occur until Sa_{O_2} reached ~88%. Hypocapnic-hypoxic subjects developed significantly greater decreases in oxidized cytochrome a,a_3 relative to normocapnic subjects, which became apparent at 85% Sa_{O_2}. The relationships between $tHbO_2$ and cytochrome a,a_3 in normocapnia and hypocapnia are compared in Fig. 6. The difference in slopes determined by linear regression analysis indicates that cerebral cytochrome a,a_3 was more reduced with equivalent decreases in $tHbO_2$ during hypocapnia relative to normocapnic conditions. The changes in oxidized cytochrome a,a_3 during the two rebreathing protocols also closely paralleled the difference in the brain blood volume responses. Although cerebral blood volume signal increased in both groups during hypoxia, normocapnic subjects had a significantly greater increase in tissue blood volume than hypocapnic subjects. This divergence in brain tissue blood volume grew in magnitude with reductions in Sa_{O_2} below 85%.

The effects of hypocapnia on brain oxidation during hypoxia indicate that hypoxia plus hypocapnia incurs greater deficits in brain oxygenation than hypoxia alone. CO_2 therefore seems to be a key factor in determining the degree of cerebral oxidation under O_2-deprived conditions. In addition to differences in brain hemoglobin volume, this effect may be related in part to the alkaline Bohr effect, where the loss of CO_2 leads to a decline in carbonic acid and hence an increase in pH (2). The increase in pH increases the affinity of hemoglobin for O_2 and therefore impairs the release of O_2 to the tissues. Because the internal milieu of the mitochondria already has a higher pH than the surrounding cytoplasm, the

loss of hydrogen ions also may have significant effect there. CO_2 has previously been reported to strongly influence the oxidation state of the brain, because hypercapnia promotes oxygenation of cerebral hemoglobin and oxidation of cytochrome a,a_3 in cats (10, 13) and rats (23). The pro-oxygenation effect of high CO_2 also may be related to its enhancement of cerebral vasodilation and increased blood flow (12). Our findings that low CO_2 worsens brain oxygenation, together with previous findings that high CO_2 promotes cerebral oxygenation, confirm that CO_2 levels influence brain oxidation state.

Hypoxia was a potent stimulus to cerebral blood volume as measured by NIR spectroscopy. By inference, blood flow increased in both protocols in this study, in agreement with previous experimental measurements of cerebral blood flow (16). The lesser increase in brain blood volume seen in hypocapnia compared with normocapnia also was not surprising, because low Pa_{CO_2} increases cerebral vascular resistance, which in turn decreases blood flow (20, 25). The smaller increase in brain tissue blood volume of hypocapnic-hypoxic subjects was probably the result of interplay between the opposing forces of hypocapnia and hypoxia. In addition, Grubb et al. (6) showed that increasing arterial blood pressure increased the CO_2 reactivity of cerebral vasculature, resulting in relatively greater increases in brain blood volume in monkeys. This effect may also contribute to the greater increase in brain tissue blood volume noted in our normocapnic subjects, as systolic pressure increased during hypoxia, whereas it did not change in hypocapnic subjects. These findings support the hypothesis that hypoxia plus hypocapnia incurs greater brain O_2 deficits than hypoxia alone.

In this study, decreases in brain oxyhemoglobin was a sensitive indicator of imminent alterations in brain oxidative metabolism during the progression of hypoxia. The change in brain oxyhemoglobin preceded changes in heart rate, blood pressure, or ventilation and presumably was responsible for stimulating these functions. Changes in Pa_{O_2}, Sa_{O_2}, and brain oxyhemoglobin were correlated and decreases in cytochrome a,a_3 oxidation levels were detected after oxyhemoglobin had changed. Brazy and colleagues (1) also observed that changes in cytochrome a,a_3 reduction began to occur faster after repeated hypoxic episodes; cytochrome a,a_3 reduction then occurred simultaneously with loss of oxyhemoglobin.

The relationship between O_2 availability and the metabolic rate for O_2 has provoked physiologists for decades. Early studies indicated that a critical Pa_{O_2} existed in the brain where O_2 deprivation first depressed mitochondrial electron transport (11). Later, more sophisticated studies determined that the concept of a critical Po_2 did not apply to the cerebral cortex (13). Changes in cytochrome a,a_3 oxidation detected after deoxygenation of hemoglobin in this study also suggest that there is a "cushion" between depletion of available O_2 and impairment of brain aerobic metabolism.

In summary, we have shown that NIR spectroscopy is a capable technique for monitoring cerebral O_2 availability in adults. NIR spectroscopy was a more sensitive indicator of cerebral oxidative impairment than any of the other physiological parameters measured, and the technique demonstrated that hypoxia results in rapid decreases in brain oxygenation and mitochondrial oxidation-reduction state. Although the NIR technique does not detect discretely localized oxidative changes in the brain, as can be done with invasive techniques (5, 28), the ability to measure rapid changes in oxidative function of populations of neurons noninvasively is advantageous. High localized detection methods may miss important trends, as cerebral perfusion is discretely regulated through local changes in microvasculature (22). In addition, the distribution of cytochrome a,a_3 within single neurons is not homogenous but rather is preferentially located in dendrites (15), the site of greatest metabolic activity within the neuron (18). Because NIR spectroscopy can rapidly detect minor changes in cerebral oxygenation, it should prove useful in early detection of cerebral hypoxia.

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Received 12 May 1989; accepted in final form 4 May 1990.

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