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Author: Hampson NB

Imprint:

Neil Hampson (neil.hampson@vmmc.org)
42306 N Caledonia Way
Anthem, AZ

Phone: **206-817-9945**

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Near-infrared optical responses in feline brain and skeletal muscle tissues during respiratory acid–base imbalance

Neil B. Hampson and Claude A. Piantadosi

Department of Medicine, Virginia Mason Medical Center, Seattle, WA 98111 (U.S.A.) and Department of Medicine, Duke University Medical Center, Durham, NC 27710 (U.S.A.)

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The effects of hyper- and hypocapnia on oxidative metabolism were evaluated by near-infrared (NIR) multiwavelength spectroscopy in intact brain and skeletal muscle tissues of the anesthetized cat. A 3-wavelength NIR algorithm was used to monitor cytochrome a,a_3 oxidation state, regional blood volume, and tissue oxyhemoglobin and O_2 stores simultaneously in brain and muscle in ventilated animals. Incremental hypercapnia was produced in 10 cats by raising arterial pCO_2 from 27.0 ± 1.3 to 95.1 ± 1.9 mmHg with inspired CO_2. Hypercapnia produced progressive increases in cerebral HbO_2, blood volume, and cytochrome a,a_3 oxidation state ($P < 0.01$). In contrast, CO_2 simultaneously decreased all 3 NIR parameters in intact hindlimb muscles ($P < 0.01$). Blood volume changes during hypercapnia correlated with changes in blood flow measured qualitatively by intravascular injections of indocyanine green dye. Hypocapnia produced by hyperventilation in 8 cats lowered p_aCO_2 from 28.5 ± 0.4 to 13.5 ± 0.5 mmHg. Hypocapnia decreased cerebral HbO_2, blood volume, and cytochrome a,a_3 redox level ($P < 0.05$), but NIR changes were not seen in skeletal muscle. These experiments demonstrate preferential distribution of oxygen to brain during hypercapnia and the ability of NIR spectroscopy to assess regional oxygenation in multiple tissues non-invasively.

INTRODUCTION

Near infrared (NIR) multiwavelength spectroscopy is a non-invasive technique developed for in vivo assessment of tissue oxygen sufficiency[6]. It has been used to continuously monitor regional blood volume changes, heme saturation, and the reduction–oxidation (redox) state of cytochrome a,a_3 in intact tissues[4,6,7,12]. Cytochrome a,a_3 (cytochrome *c* oxidase), the terminal member of the mitochondrial respiratory chain, contains two copper atoms; one copper varies its optical density in the NIR region as a function of the redox state of the enzyme complex. Cytochrome a,a_3 redox state changes in response to the availability of oxygen, substrate, and ADP. In brain, cytochrome a,a_3 redox state correlates with local tissue oxygen tension in vivo, and the redox level of the enzyme in skeletal muscle has been shown to vary with hindlimb blood flow and oxygen consumption[2]. The present study was designed to test the sensitivity of NIR signals in detecting differences in oxygen availability in the two tissues during respiratory acid–base imbalance. Utilizing the powerful effects of carbon dioxide on cerebral vasculature[10] and our recent findings that CO_2 can decrease the oxygenation of resting skeletal muscle[3], we have measured changes in oxygen availability by NIR simultaneously in intact brain and hindlimb skeletal muscle during graded hypercapnia and hypocapnia in the anesthetized cat.

MATERIALS AND METHODS

Adult cats of both sexes were anesthetized with intraperitoneal injections of sodium pentobarbital (38 mg/kg). A tracheal tube was inserted and polyethylene cannulae were placed in the left brachial artery and the right femoral artery and vein. The brachial catheter was used for arterial blood sampling for glucose determinations (Chemstrip bG, Biodynamics) and p_aO_2, p_aCO_2, and pH measurements (Instrumentation Laboratory, model 513 pH/blood gas analyzer). Femoral artery blood pressure was monitored continuously with a pressure transducer (Statham Model P23 DC). Animals were paralyzed with gallamine triethiodide (5 mg/kg i.v.) and mechanically ventilated using a positive pressure respirator at a frequency of 20 breaths/min. Tidal volume was adjusted during initial ventilation with room air to provide a p_aCO_2 near 28 mm Hg[15]. Anesthesia and muscular paralysis were maintained by constant intravenous infusion of sodium pentobarbital (5 mg·kg^{-1}·h^{-1}) and gallamine triethiodide (3.75 mg·kg^{-1}·h^{-1}) in $D_{10}W$ (total infused volume 1 cl/h). Rectal temperature was maintained near 37 °C with external heating. The hindlimb was immobilized and the hair removed for NIR monitoring of the intact skeletal muscles as previously described[3].

The head was secured in a stereotaxic device and the scalp reflected to expose both parietal bones. Two platinum needle electrodes (Grass Instruments) were inserted into the fascia for continuous recording of a bipolar EEG.

Spectrophotometric measurements were obtained from brain and muscle using a 3-wavelength differential spectrophotometer designed to monitor two tissues simultaneously. Near-infrared light of 3 wavelengths (775, 815 and 904 nm) was produced with grating

Correspondence: N.B. Hampson, Virginia Mason Clinic, 1100 Ninth Avenue, Seattle, WA 98111, U.S.A.

monochromators (Instruments S.A.), pulsed with a slotted chopping wheel, and conducted to the tissues by means of two separate fiberoptic bundles. In the case of skeletal muscle, the hindlimb muscles (primarily biceps plus gracilis) were transilluminated transcutaneously by applying the fiberoptic bundle gently to the skin and conducting transmitted light from the opposite side of the muscles to a photomultiplier tube (Hammamatsu R936). For brain monitoring, the fiberoptic bundle was coupled directly to the intact skull with silicon gel (Math Associates). Reflected light was collected from the brain by a second fiberoptic bundle placed against the skull at approximately 60° from the first and conducted to a second photomultiplier tube. The photocurrents generated were integrated in sequence for each wavelength and stored for signal processing. Derivation of hemoglobin (Hb, HbO_2), myoglobin (Mb, MbO_2) (muscle only), and cytochrome a,a_3 copper signals was performed using algorithms generated during development of the method[6]. These algorithms allow independent monitoring of these signals, with little interference of one chromophore by the other chromophores being monitored[13]. Blood volume changes in the muscle were assessed by assuming that the concentration of Mb + MbO_2 was constant during the time course of the experiment. Thus, addition of the two optical signals provides a measurement of relative tissue blood hemoglobin volume (tBV). Optical data are reported as variations in density (v.d.) instead of true optical density changes because the algorithms used weighting coefficients which have slightly different scattering contributions at each of the 3 measuring wavelengths. Variations in density and absolute concentration cannot be related at present because of unquantified variables such as optical path length. Relative changes in concentration caused by experimental manipulations can be compared between animals with the understanding that the biophysical variables are affected by minor differences in the geometric arrangement of the optical fibers and will therefore contribute to the statistical variability of the data. Additional details of the NIR method have been published previously[3,4,6,12].

Baseline physiological parameters were measured while the animals were ventilated with room air. After establishing stable optical baselines, 10 animals were exposed to progressive hypercapnia at CO_2 concentrations of 3%, 5%, 10% and 15% with constant inspired oxygen concentration (20% O_2, balance N_2). Each CO_2 exposure of 15 min in duration was sufficient for stabilization of the optical signals at new steady states. In 8 separate experiments, hypocapnia was produced by increasing ventilatory frequency to a rate sufficient to lower arterial pCO_2 to a level less than half of control. In some of the hypercapnia experiments, signal interpretation was verified qualitatively by serial injections of indocyanine green (Cardio-Green, Becton-Dickinson), a non-diffusible dye that absorbs near-infrared light at 805 nm. Boluses of 0.4 ml of dye (0.5 mg/ml in saline) were rapidly injected into the inferior vena cava and clearance curves in brain and muscle recorded. Statistical analysis of the data was performed using one-way ANOVA and paired *t*-testing.

RESULTS

Ventilation with carbon dioxide at increasing concentrations resulted in progressive hypercapnia (Table I). A mean arterial pCO_2 of 95.1 ± 1.9 mmHg (mean ± S.E.) was reached with 15% inspired CO_2 in the 10 cats. This was associated with a progressive fall in arterial pH from 7.38 ± 0.01 to 6.96 ± 0.01 and a rise in mean arterial blood pressure from 100 ± 9 to 150 ± 13 mmHg. Core temperature, blood glucose and arterial pO_2 remained stable.

Hypercapnia was associated with progressive changes in all NIR signals derived from both brain and muscle tissue. Fig. 1 shows NIR responses recorded from the tissues of a typical animal. Hypercapnia caused an increase in the relative amount of muscle deoxyhemoglobin plus deoxymyoglobin (tHb + Mb), a simultaneous decrease in oxyhemoglobin plus oxymyoglobin ($tHbO_2$ + MbO_2), and a decrease in the oxidation level of cytochrome a,a_3 in the intact muscle tissue. These responses were in contrast to the effects of hypercapnia on brain cortex. Increasing arterial pCO_2 increased the relative amounts of oxyhemoglobin ($tHbO_2$) and oxidized cytochrome a,a_3 in the brain. Cerebral deoxyhemoglobin (tHb) had a biphasic response, characterized by an early decrease followed by an increase in tHb.

Summarized NIR optical data from 10 hypercapnia experiments are presented in Figs. 2–4. In intact muscle (Fig. 2), significant increases in tHb + Mb and decreases in $tHbO_2$ + MbO_2 occurred at each level of hypercapnia tested ($P < 0.02$). Tissue blood volume [(tHb + Mb) + ($tHbO_2$ + MbO_2)] in the muscle fell with each level of hypercapnia, and the decrease was greatest during ventilation with 15% CO_2 ($P < 0.001$). The contrasting responses of brain hemoglobin to hypercapnia are displayed in Fig. 3. Hypercapnia increased the relative amount of cerebral $tHbO_2$ ($P < 0.001$) until p_aCO_2 values were approximately 45 mmHg, after which there

TABLE I

Arterial blood gas tensions, pH, and mean arterial blood pressure in respiratory acid-base disturbances in cats

Values are mean ± S.E.M. for each group of animals. Values noted by * are significantly different from preceding baseline value.

Experimental group	*pHa*	*p_aCO_2 (mmHg)*	*p_aO_2 (mmHg)*	*MABP (mmHg)*
Hypercapnic acidosis (n = 10)				
Baseline	7.38 ± 0.01	27.0 ± 1.3	102.1 ± 4.0	100 ± 9
3% CO_2 15 min	7.27 ± 0.02*	35.5 ± 1.4*	105.6 ± 3.8	110 ± 11*
5% CO_2 15 min	7.20 ± 0.01*	44.7 ± 1.4*	105.7 ± 2.7	117 ± 11*
10% CO_2 15 min	7.07 ± 0.01*	64.7 ± 2.6*	106.4 ± 2.2	127 ± 11*
15% CO_2 15 min	6.96 ± 0.01*	95.1 ± 1.9*	104.2 ± 2.3	150 ± 13*
Hypocapnic alkalosis (n = 8)				
Baseline	7.39 ± 0.01	28.5 ± 0.4	102.3 ± 1.8	115 ± 11
Hyperventilation	7.60 ± 0.02*	13.5 ± 0.5*	124.2 ± 2.7*	91 ± 10*

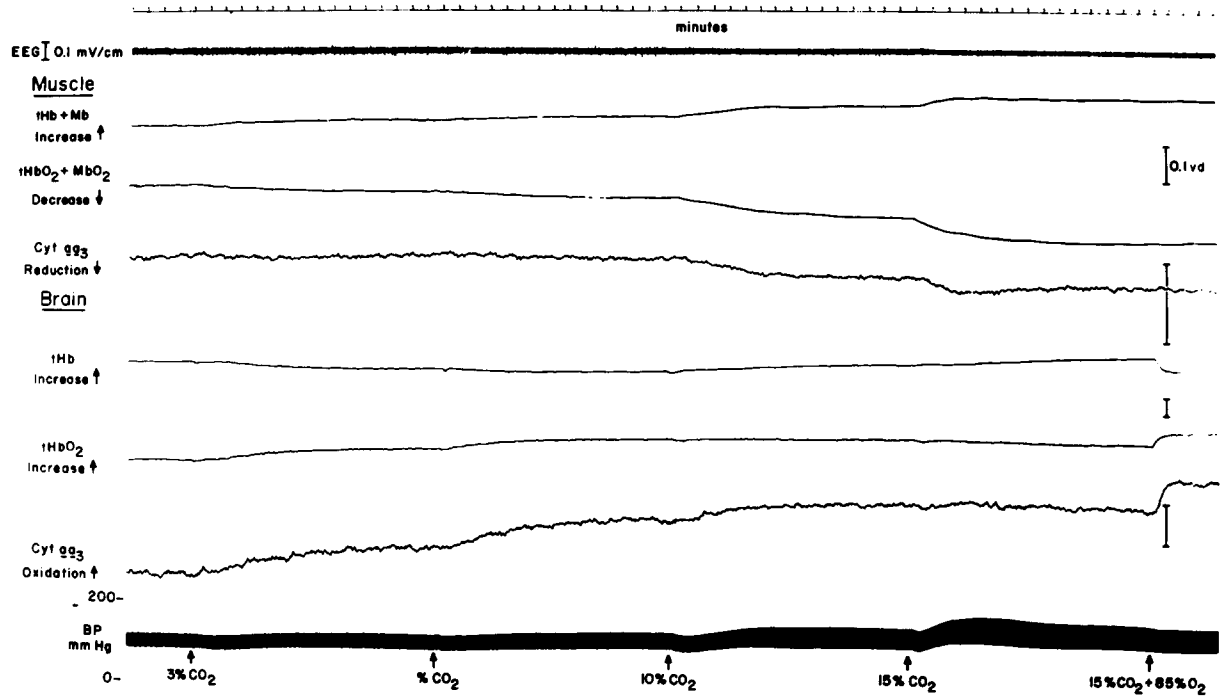

Fig. 1. Near-infrared optical responses to progressive hypercapnia in the brain and intact hindlimb musculature of the anesthetized cat. In the muscle, hypercapnia increased the reduction level of cytochrome a,a_3 (Cyt a,a_3), increased the amount of deoxyhemoglobin plus deoxymyoglobin (tHb + Mb), and decreased the amount of oxyhemoglobin plus oxymyoglobin ($tHbO_2 + MbO_2$). In simultaneously monitored brain tissue, the relative amount of oxyhemoglobin ($tHbO_2$) increased to a greater degree than deoxyhemoglobin (tHb) and this was accompanied by an increase in the oxidation level of cerebral cytochrome a,a_3. Other abbreviations: v.d. = variation in density; BP = arterial blood pressure.

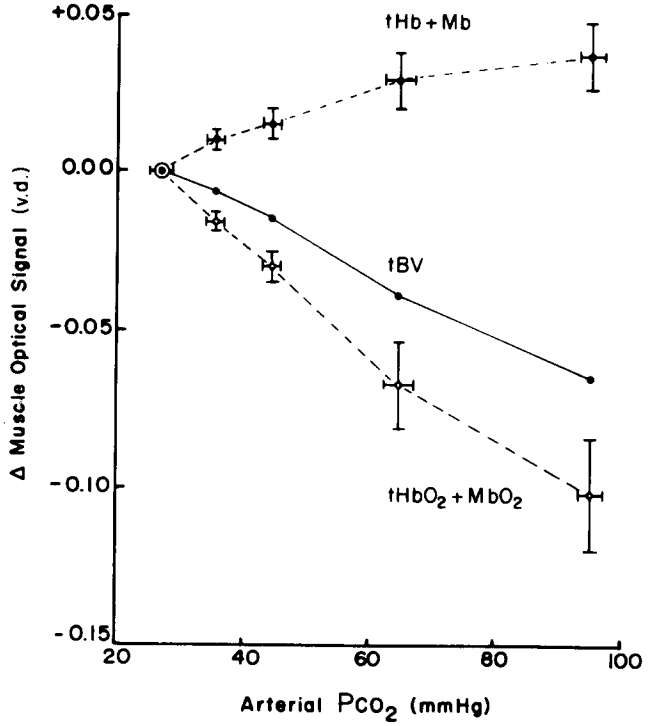

Fig. 2. Change in tissue deoxyhemoglobin plus deoxymyoglobin, oxyhemoglobin plus oxymyoglobin, and blood volume (tBV) in intact muscle as a function of hypercapnia. Data are mean ± S.E. for 10 cats. All values are significantly different ($P < 0.05$) from control. For definition of abbreviations, see Fig. 1.

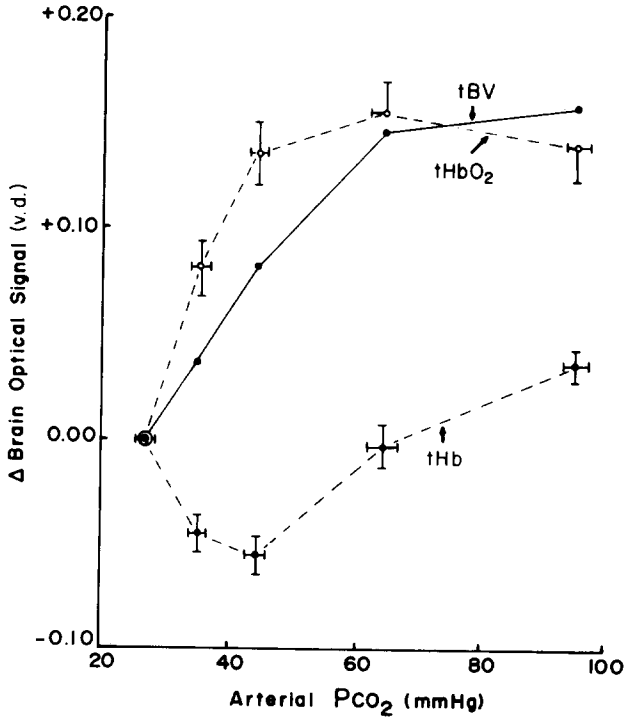

Fig. 3. Change in tissue oxyhemoglobin, deoxyhemoglobin, and blood volume in intact brain as a function of hypercapnia. Data are mean ± S.E. for 10 cats. All values are significantly different ($P < 0.05$) from control except tHb at $p_aCO_2 = 65$ mmHg. For definition of abbreviations, see Fig. 1.

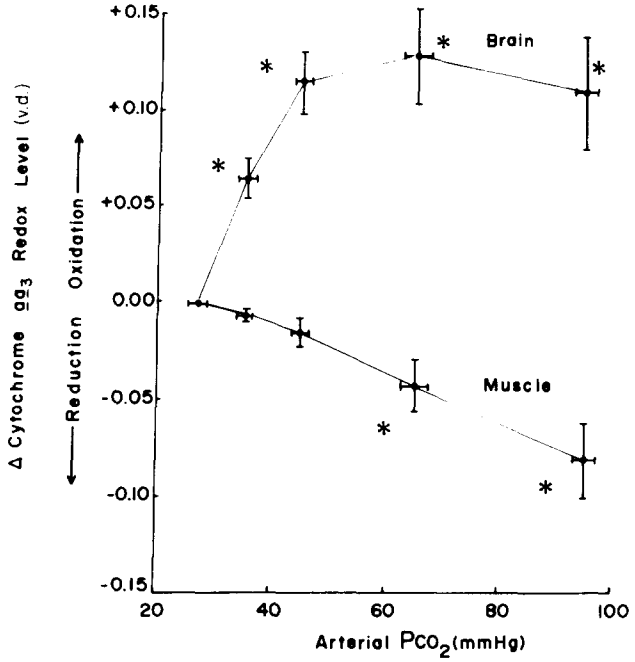

Fig. 4. Change in cytochrome a,a_3 redox level in simultaneously monitored brain and muscle tissues as a function of increasing arterial pCO_2. Data are mean ± S.E. for 10 cats. Hypercapnia resulted in an increase in the oxidation level of cerebral cytochrome a,a_3 and an opposite effect in muscle. Changes were significantly different ($P < 0.05$) from baseline at the points indicated by *.

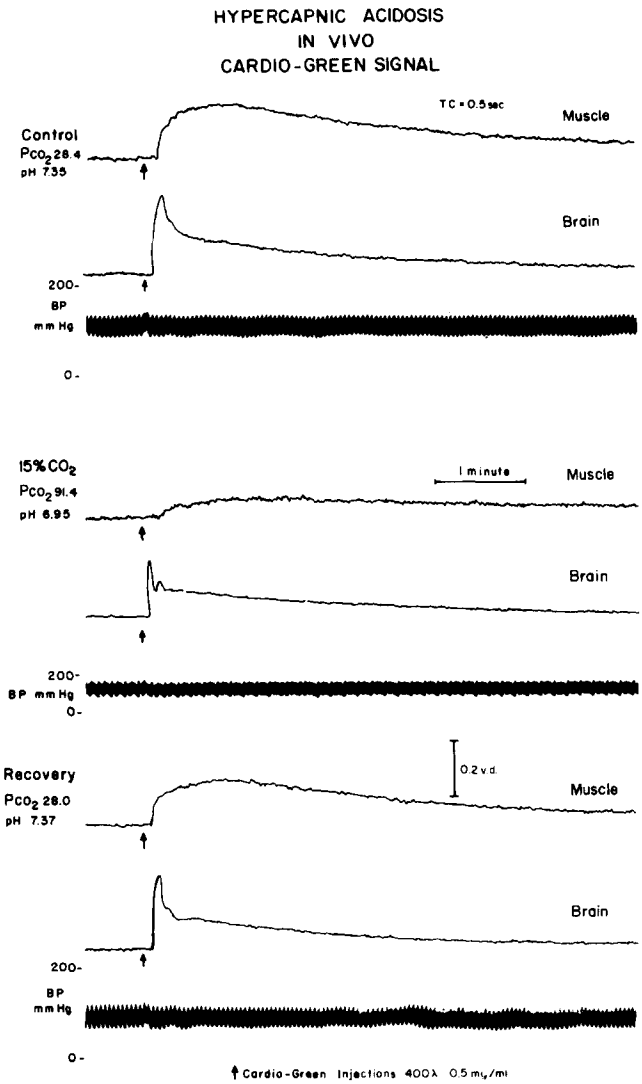

Fig. 5. Indocyanine green dye clearance curves in the intact brain and hindlimb muscles of the anesthetized cat during normocapnia, hypercapnia, and recovery. Note that hypercapnia was associated with diminished dye circulation through the muscle and augmented dye flow through brain. Abbreviations: TC = time constant; v.d. = variation in density.

was no further response. The tHb, however, decreased initially, followed by an increase above baseline level during extreme hypercapnia ($P < 0.001$). The sum of the two hemoglobin signals (tBV) demonstrated a progressive increase in cerebral blood volume with graded hypercapnia. The divergent changes in oxygen delivery to intact muscle and brain during hypercapnia were accompanied by compatible changes in cytochrome a,a_3 redox level in the two tissues (Fig. 4). The decrease in the cytochrome a,a_3 redox level in muscle caused by CO_2 was accompanied closely by a relative decrease in blood volume while brain cytochrome a,a_3 redox level increased to a more oxidized state during hypercapnia ($P < 0.001$). The cytochrome response followed the increase in cerebral $tHbO_2$ most closely.

The tissue blood signals during hypercapnia were validated qualitatively by recording indocyanine green dye clearance curves in muscle and brain. Fig. 5 shows dye transit and clearance from the two tissues during normocapnia, hypercapnia and recovery. Dye transit time in muscle increases (blood flow decreases) with hypercapnia, while transit time in brain decreases (blood flow increases), in agreement with the changes in blood volume previously described.

The NIR responses to hypercapnia were compared to responses during hypocapnia by hyperventilating 8 animals acutely to reduce arterial pCO_2 to 12–15 mmHg. Hyperventilation resulted in a small drop in mean arterial blood pressure and an increase in pHa from 7.39 ± 0.01 to 7.60 ± 0.02 (see Table I). No significant changes were seen in any optical parameter in the muscle of 8 animals as p_aCO_2 was lowered from a mean of 28.5 ± 0.4 to 13.5 ± 0.5 mmHg. In the brain, however, hypocapnia was associated with a decrease in the relative amount of $tHbO_2$ and a smaller increase in tHb, resulting in a net decrease in tissue blood volume. These changes were accompanied by a decrease in the redox level of cerebral cytochrome a,a_3 ($P < 0.05$). NIR signals from a typical hypocapnia experiment are shown in Fig. 6.

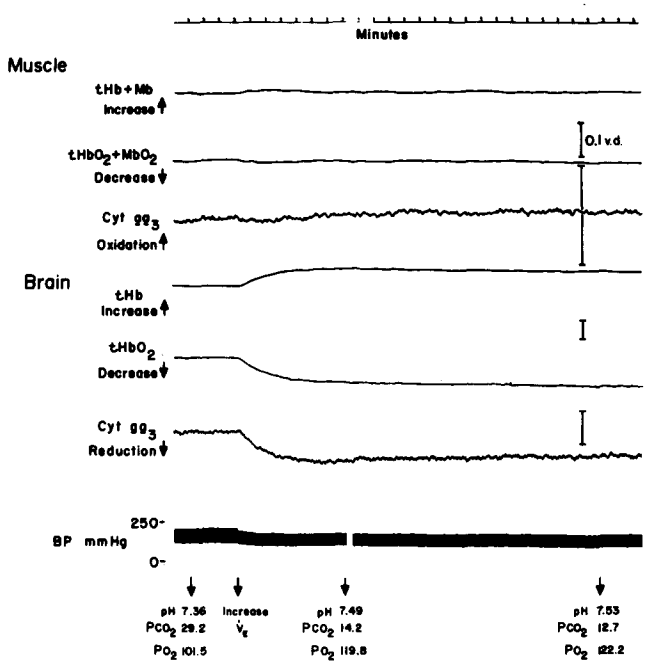

Fig. 6. Near-infrared optical responses to hypocapnia in the intact brain and hindlimb musculature of the anesthetized cat. Hypocapnia caused no appreciable change in muscle deoxyhemoglobin plus deoxymyoglobin, oxyhemoglobin plus oxymyoglobin, or cytochrome a,a_3 redox level. In the brain, tissue deoxyhemoglobin increased while oxyhemoglobin and the redox level of cytochrome a,a_3 both decreased. For definition of abbreviations, see Fig. 1.

DISCUSSION

These experiments demonstrate not only changes in oxygen distribution to intact muscle and brain tissues accompanying changes in arterial pCO_2, but also the utility of NIR spectroscopy to monitor these responses simultaneously in multiple tissues in a non-invasive fashion. Hypercapnia is a potent stimulus for cerebral vasodilation, while hypocapnia causes vasoconstriction. These perturbations result in opposite effects on cerebral blood flow and oxygen delivery[10]. Conventional methods for monitoring such changes in tissue oxygen delivery are typically invasive or relatively slow compared to NIR spectroscopy. The optical technique is a rapid, sensitive method for continuously monitoring changes in tissue blood volume, oxygenation, and flow. Additionally, it provides information about mitochondrial oxygen availability in the monitored tissue by assessing changes in the oxidation state of the optically active copper moiety of cytochrome a,a_3.

Hypercapnia in these experiments was associated with a definite increase in brain cytochrome a,a_3 oxidation level, demonstrating increased oxygen availability to cerebrocortical mitochondria in situ. The current results indicate that the NIR absorption band of cerebrocortical cytochrome a,a_3 is approximately 50% reduced at rest, a value in close agreement with previous work using visible wavelengths to monitor brain cytochrome a,a_3 in rats[16]. Earlier studies using differential spectrophotometry in the visible wavelength range to monitor absorbance changes of the cytochrome a heme in cats during hypercapnia also reported oxidation of cerebral cytochrome a,a_3 (refs. 5, 14). Because light in the visible region of the spectrum penetrates tissue poorly, exposure of the cerebral cortex was necessary in those experiments, either by acute craniotomy or by implantation of a cranial window. The NIR technique measures changes in brain cytochrome a,a_3 redox level directly through intact skull of larger animals during hypercapnia, thus avoiding brain edema and cerebrovascular responses that may be associated with exposure of the cortex.

Hypercapnia produces effects in intact skeletal muscle opposite to those observed in brain. This finding indicates regional redistribution of oxygen delivery away from intact muscle and to brain during acute CO_2 inhalation. The mechanisms for this response are probably related to (1) direct stimulation of carotid chemoreceptors by CO_2 resulting in sympathetic vasoconstriction in skeletal muscle[11] and (2) a direct effect of CO_2 on cerebrovascular smooth muscle mediated by changes in extracellular fluid pH[10]. Previous investigations, also in anesthetized cats, have demonstrated that changes in cerebral vessels induced by alterations in p_aCO_2 can be explained by the effects of CO_2 locally, and that remote mechanisms of vasoactivity of CO_2 are not necessary[8].

The NIR cytochrome responses from muscle during CO_2 exposure are consistent with tissue pO_2 measurements of cat muscle by microelectrode which showed significant decreases in tissue pO_2 during hypercapnia[17]. We have previously found that the reduction in blood flow and oxygen delivery to skeletal muscle during hypercapnia is mediated directly by CO_2 and not by decreased pH, as metabolic acidosis of a similar degree does not induce these muscle responses. The CO_2 effect in muscle also can be blocked by pretreatment with the sympatholytic agent, bretylium tosylate, lending support to the chemosympathetic mechanism. These experiments have been previously reported with a review of acid–base effects on skeletal muscle oxygenation[3].

The responses to hypocapnia reported here also corroborate previous findings that the effect of CO_2 on cerebral blood flow is a continuum in either direction from normocapnia, mediated presumably by its effect on cerebrospinal fluid pH[10]. Decreases in arterial pCO_2, however, had no substantial effect on muscle oxygen delivery. Apparently, only upward excursions in p_aCO_2 significantly change muscle oxygenation in the anesthetized cat. The reasons for the latter finding are not entirely clear, although activation of vasodilator fibers in

muscle by pulmonary inflation during hypocapnia may be important in this species as it is in others[1].

The biphasic response of cerebral deoxyhemoglobin during progressive hypercapnia was somewhat unexpected. The physiologic implications are not entirely clear, but the increase in cerebral tHb seen during the more extreme levels of hypercapnia may be contributed to by at least two mechanisms. First, hypercapnia of this degree may be altering tissue oxygen utilization in addition to increasing cerebral blood flow. If cerebral oxygen consumption increased, more deoxyhemoglobin molecules would be seen at the postcapillary level at any cerebral blood flow. Secondly, the reciprocal changes in the $tHbO_2$ and tHb measured during the change from 10% to 15% inspired CO_2 may be partially explained by a rightward shift in the oxygen dissociation curve of hemoglobin induced by progressive hypercapnia and acidosis.

In summary, NIR spectroscopy is capable of rapid non-invasive assessment of changes in oxygen sufficiency in multiple tissues continuously. Further experimental application of the technology in animal and human models of health and disease will be necessary to determine its longterm capability and limitations, and hopefully, improve our understanding of normal physiologic mechanisms regulating regional oxygen transport and utilization. Long-range extension of the technique to clinical medicine can be envisioned in two major settings: (1) disease processes where systemic oxygen delivery is limited and the regional effects of therapy must be assessed, e.g. inotropic therapy for cardiac failure, and (2) whenever early detection of regional hypoxia or ischemia would allow intervention to prevent injury to hypoxia-sensitive tissue such as the brain, e.g. during general anesthesia or surgery.

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