Effects of hypoxia and hypocapnia on brain redox balance in ducks

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BICKLER, PHILIP E., SHIN OK KOH, AND JOHN W. SEVERINGHAUS. *Effects of hypoxia and hypocapnia on brain redox balance in ducks.* Am. J. Physiol. 257 (Regulatory Integrative Comp. Physiol. 26): R132–R135, 1989.—Low arterial CO_2 tension (Pa_{CO_2}) experienced by birds during high-altitude flight may result in cerebral vasoconstriction with reduced cerebral O_2 delivery. To test this, brain redox balance and blood volume were studied during severe hypocapnia (Pa_{CO_2} 11–20 mmHg) in ducks. Cerebrocortical redox balance, measured as relative [NADH], and blood volume were measured simultaneously with a fiber-optic fluorometer-reflectometer. Cerebrocortical blood volume (an index of blood flow) fell nearly linearly with Pa_{CO_2} during severe hypocapnia, even during severe hypoxemia. Cerebrocortical redox balance was shifted toward reduction of NADH ([NADH] increased) by both hypoxemia and hypocapnia. If hypocapnia causes similar changes in brain blood flow during high-altitude flight, tissue hypoxia will be exacerbated. Tolerance of brain tissue hypoxia during flight may be an important adaptation in high-flying birds.

NADH; avian brain; fiber-optic fluorometry; cerebral blood flow

EXTREME HIGH-ALTITUDE FLIGHT in birds must be accompanied by hypoxia and hypocapnia. If the cerebral vasculature of mammals and birds react similarly to CO_2, severe hypocapnia will result in reduced cerebral blood flow and O_2 delivery. Neural function in birds during hypocapnic hypoxia may be preserved by mechanisms acting to preserve cerebral oxygen delivery (e.g., blood flow, Bohr effects) or by the hypoxia tolerance of brain tissue. Grubb et al. (4, 5) suggested that oxygen delivery to the avian brain was maintained during hypocapnia by the following mechanisms: *1*) a relatively independent relationship between arterial partial pressure of CO_2 (Pa_{CO_2}) and cerebral blood flow (CBF) during hypoxia, and 2) a Bohr effect that results in relatively unchanged tissue O_2 delivery during hypocapnic hypoxia. Clues to the mechanisms of hypoxia tolerance in birds were discovered by Bryan and Jones (2), who found that brain redox balance and electroencephalogram (EEG) during anoxia were maintained several times longer in ducks than chickens but that both species were equally dependent on tissue Po_2 to maintain the redox state. Maintenance of CBF and O_2 delivery may thus be the crucial element in maintaining brain function. It is not known how the potential vasoconstriction of severe hypocapnia in combination with severe hypoxia, as may accompany high-altitude flight, affects the delivery of O_2 to the avian brain. These data are crucial in interpreting the significance of changes in CBF and the Bohr effect in determining brain tissue oxygenation. We approached this problem by simultaneously measuring brain intracellular redox balance and brain tissue blood flow to better understand the relationship of tissue oxygenation, hypoxia tolerance, and CBF in birds.

METHODS

Cerebrocortical NADH and blood volume were measured by the technique of in vivo fiber-optic fluorometry-reflectometry. In this technique, developed by Chance et al. (3) and Mayevsky (9), mitochondrial NADH redox balance is detected as an indirect measure of intracellular oxygenation. When illuminated at 366 nm, the reduced nucleotide NADH fluoresces at a wavelength of 450 nm, whereas the oxidized NAD^+ does not (3). Because hemoglobin in the tissue region illuminated by the fiber-optic probe absorbs some of the incident light, the amount of light reflected back into the fiber-optic light guide decreases when there is a larger volume of blood in the region 0–3 mm beyond the probe. Changes in the intensity of the reflected light can be used to determine changes in cerebrocortical blood volume (6), which are proportional to changes in cerebrocortical blood flow (11).

Animal preparation. Approval for these experiments was obtained from the University of California, San Francisco, Committee on Animal Research. Anesthesia was induced in six adult Muscovy ducks (*Carina moschata*), weighing 2.0–3.0 kg, by in intravenous injection of 25 mg/kg pentobarbital sodium. Arterial and venous catheters were placed in wing vessels so that blood samples could be obtained, blood pressure could be monitored continuously, and drugs could be administered. The trachea was intubated, and tidal ventilation was begun with 100% O_2. Pancuronium ($1\ mg \cdot kg^{-1} \cdot h^{-1}$) was infused for muscle paralysis. Anesthesia for the surgery and measurements was maintained thereafter by additional 10–50 mg iv doses of pentobarbital every 30–50 min. Inspired and end-tidal gas samples from a catheter in the endotracheal tube were analyzed on-line with a Perkin-Elmer 1100 mass spectrometer that has a built-in peak detector. Current values of inspired and end-tidal Po_2 and Pco_2 were monitored with the aid of a PDP 11/44 computer. A thermistor cloacal temperature probe was connected

to a servo-control unit with a heating pad to maintain core temperature at 39°C.

After endotracheal intubation, the posterior thoracic and anterior abdominal air sacs were cannulated bilaterally to allow unidirectional ventilation of the lungs. The surgical approach is described by Grubb et al. (5). Care was taken to create a surgical anastomosis between the abdominal and posterior thoracic air sacs to allow for low-resistance flow through the lungs. Incurrent gas flow was ~800–1,500 ml/min and was humidified and metered through separate rotameter flowmeters for each lung. The fractional composition of O_2, CO_2, and N_2 in the incurrent gas mixture was controlled by a gas-mixing manifold. Expired gas was vented through the interclavicular air sac. On initiation of unidirectional gas flow, the endotracheal tube was plugged.

The head of the bird was then clamped in a stable position. The cranium was exposed in the midline from the rostral margin of the orbits to the nuchal line. The scalp was retracted and a burr drill was used to make a 0.6-cm craniotomy. The dura was incised and retracted. Cotton pledgets soaked in mock cerebrospinal fluid (CSF) solution were then applied to the brain cortex to protect it until the fiber-optic fluorescence probe was in place.

In vivo fiber-optic fluorometry. Cerebrocortical NADH fluorescence and ultraviolet reflectance were measured with a CF-1 fiber-optic flurometer/reflectometer (Johnson Research Foundation, University of Pennsylvania) originally designed and described by Chance et al. (3) and Jobsis et al. (7). A 100-W air-cooled Hg vapor arc lamp served as a light source. Excitation light at 366 ± 1 nm was obtained with a Corning 5874 filter. The light then traversed the central fibers of a fiber-optic cable whose proximal end was trifurcated and whose distal tip (0.5 cm) was in direct contact with the cerebrocortical surface. Circumferential fibers in the cable returned light to two filtered photomultiplier tubes (366 and 450 nm).

Artifactual changes in NADH fluorescence that arose from changes in incident light intensity and from blood in the detection region were handled by the compensation method of Jobsis et al. (7) and Harbig et al. (6). The correction is based on the fact that artifactual contributions are proportional to the magnitude of the reflected light. The proportionality constant for that relationship is determined as follows. A transient dilution of cerebrocortical blood is caused by the rapid injection of 0.3–0.07 ml of normal saline into the ipsilateral carotid artery. The hemodilution transiently increases the light intensity in both the fluorescence and reflectance channels. Compensated fluorescence (CF) is defined by assuming that the true cerebrocortical [NADH] did not change. The correction factor k is the ratio of the hemodilution-induced fluorescence and reflectance changes, and the true change in [NADH] is then calculated as $\Delta CF = \Delta F - k(\Delta R)$, where ΔF and ΔR are the changes in the signals measured at 450 and 366 nm, respectively.

Output voltages of the two photomultiplier tubes were calibrated by equating the reflectance and fluorescence output signals during 100% O_2 breathing at a Pa_{CO_2} of 35–40 mmHg ($NADH/NAD^+$ redox couple 100% oxidized). The percentage of the NADH pool in the oxidized state was estimated by expressing the signal relative to the signal when the pool is fully reduced, i.e., after 3–4 min of anoxia. Output signals were computer digitized and stored for later analysis.

Effects of CO_2 and O_2 on blood volume and NADH. The birds were ventilated with 100% O_2 during the surgical preparation. Relative cerebrocortical blood volume and [NADH] were then studied at different steady-state levels of Pa_{CO_2} and arterial O_2 partial pressure (Pa_{O_2}). Blood gas changes were made by changing the composition of the gas perfusing the lungs. Measurements of blood volume and [NADH] were made during steady-state periods (usually ~10 min after changing gas composition). The experimental protocol involved measurements of cerebrocortical blood volume (CBV) and [NADH] at different levels of Pa_{CO_2} for perfusion gas O_2 fractions of 1.0, 0.5, 0.21, 0.14, 0.10, 0.07, and 0.05.

RESULTS

Measurements of cerebrocortical [NADH] and blood volume were obtained in six ducks. A total of 113 different steady-state periods were studied.

Table 1 summarizes blood gas and pH values obtained for different perfusion gas compositions. Changes in perfusion gas produced rapid [half-time ($t_{1/2}$) ~1 min] changes in Pa_{CO_2} and Pa_{O_2}. Pa_{CO_2} was controlled from 11–130 mmHg and P_{O_2} from 14 to 300 mmHg.

Relative CBV increased with Pa_{CO_2} during hypoxia, normoxia, and hyperoxia (Fig. 1). CBV at fractional concentration of O_2 in inspired gas (FI_{O_2}) 0.5 was nearly identical to that at a FI_{O_2} of 0.21. The increase in CBV with falling O_2 tension was greater with more severe hypoxia. In each of the six birds studied, the slope of CBV/Pa_{CO_2} relationship generally decreased slightly above a Pa_{CO_2} of ~40 mmHg. However, this decrease in slope was statistically significant only for FI_{O_2} values of 0.50 and 0.21 ($P > 0.05$, t test for difference between slopes of least-squares linear regression equations for portions of curves above and below 40 mmHg). No plateau level was found even at Pa_{CO_2} levels between 100 and 130 mmHg. Even under conditions of extreme hypoxia and hypocapnia (Pa_{O_2} 10–25 mmHg, Pa_{CO_2} 10–20 mmHg) further reduction in Pa_{CO_2} still produced substantial reductions in CBV.

Increased intracellular oxygenation, as measured by

TABLE 1. *Relationship of O_2 fraction of lung perfusion gas and CO_2 fraction of mixed expired gas to arterial pH, P_{CO_2}, and P_{O_2} in unidirectionally ventilated ducks*

FI_{O_2}	Pa_{O_2}	$F\bar{E}_{CO_2}$	Pa_{CO_2}	pH_a
0.5	227±42 (24)	0–0.02	16.2±5.9 (27)	7.66±0.12 (27)
0.21	103±12 (32)	0.021–0.05	31.2±4.5 (17)	7.45±0.06 (17)
0.14	69±9 (9)	0.051–0.10	48.0±8.1 (21)	7.32±0.06 (21)
0.10	59±5 (12)	0.101–0.15	77.4±11.8 (17)	7.15±0.08 (17)
0.05	29±1 (6)	0.15	116.6±14.5 (16)	7.01±0.07 (16)
0.025	14±2 (4)			

Values are means ± SD. No. of determinations given in parentheses. Values are corrected to 37°C. FI_{O_2} and $F\bar{E}_{CO_2}$, fractional concentrations of inspired and expired gases, respectively; Pa_{O_2} and Pa_{CO_2}, arterial O_2 and CO_2 partial pressures, respectively; pH_a, arterial pH.

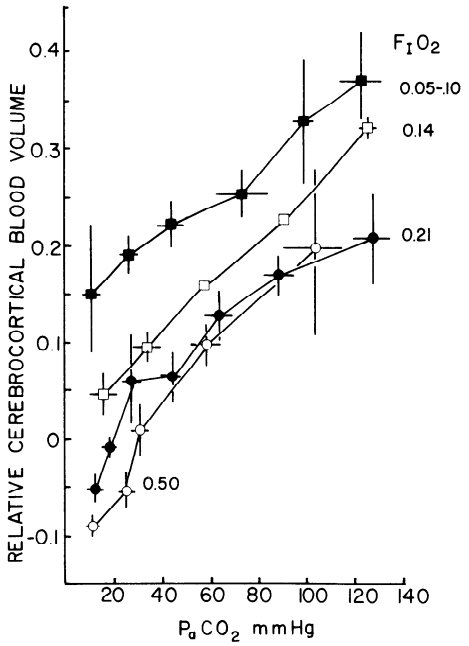

FIG. 1. Relationship of relative cerebrocortical blood volume to arterial PCO_2 (Pa_{CO_2}) at different levels of oxygenation in six ducks. Data represent means ± SD for both Pa_{CO_2} and cerebrocortical blood volume. Numbers adjacent to curves indicate fractional concentration of O_2 in inspired gas (FI_{O_2}). See Table 1 for grouped Pa_{O_2}, Pa_{CO_2}, and pH values.

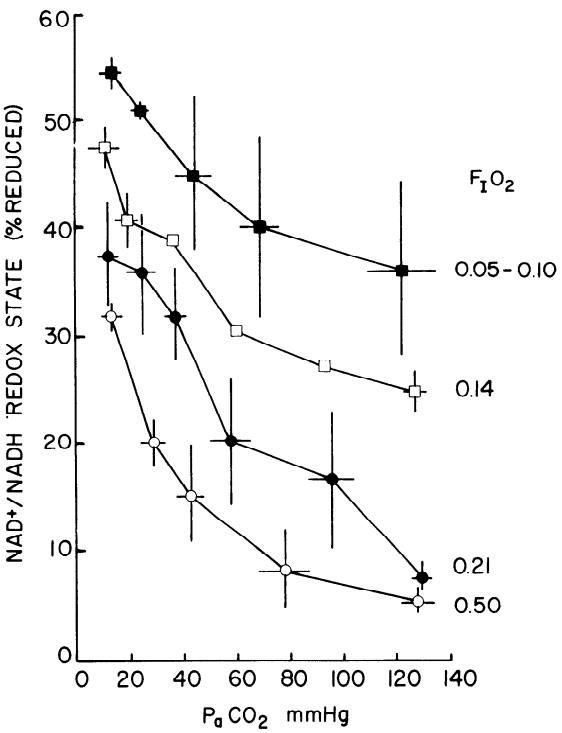

FIG. 2. Relationship of redox state of cerebrocortical NADH to Pa_{CO_2} at different inspired O_2 levels. Data represent means ± SD for 6 ducks. Points without SD bars are single data points. See Table 1 for grouped values of blood gases and pH for different ventilation gas mixtures and Fig. 1 for abbreviations.

falling levels of reduced nicotinamide adenine dinucleotide were seen with rising Pa_{CO_2} (Fig. 2). Higher NADH levels were seen with the perfusion of progressively more hypoxic gas mixtures. The relationship of Pa_{CO_2} to NADH was similar during hypoxia, normoxia, and hyperoxia.

The redox state of cerebrocortical NADH was estimated by expressing [NADH] relative to levels found during 85% O_2-15% CO_2 ventilation (assumed 100% oxidized) and during anoxia (assumed 100% reduced). The NAD^+/NADH redox pair is ~65% oxidized at a Pa_{CO_2} of 35 mmHg and a Pa_{O_2} of 100 mmHg.

DISCUSSION

Our data are derived from events in the superficial gray matter of cortex. We have assumed that cerebral blood volume is proportional to flow. As shown previously in mammals, flow correlates well with volume (11) and, furthermore, reflectometrically measured volume correlates well with CBF measured by a variety of techniques (9).

The in vivo fluorometric technique provides information on relative CBV and [NADH]. Absolute values for blood volume and [NADH] cannot be obtained from this technique. However, a good correlation between fluorometrically measured changes in NADH and chemically measured NADH has been demonstrated in neural tissue (6). In our laboratory, good correlation was found between fluorometrically determined NADH and perturbations induced by changes in blood pressure, hemorrhage, arterial oxygenation and CO_2 levels, and administration of acetazolamide (increased CBF) or cyanide (1). Raw reflectance and fluorescence signals from the duck cortex were very similar to those shown in Bickler et al. (1).

The relationship of cerebrocortical blood volume to Pa_{CO_2} found in our ducks is similar to the relationship found previously by our group in rabbits (1) and to the relationship of CBF to Pa_{CO_2} seen in mammals as measured by the xenon clearance technique (9). Our findings are significantly different from those found in previous studies in ducks (4, 5). We observed no plateau of cerebral blood volume at low Pa_{CO_2} in any of the six birds studied. A further difference between our findings and that of Grubb et al. (4, 5) is that the slope of our cerebral blood volume-Pa_{CO_2} relationship during normoxia or hyperoxia tended to be less steep above 40 mmHg.

The reasons for differences between our findings and those of Grubb et al. (5, 6) may be several. First, the xenon washout technique used by Grubb et al. (5, 6) inevitably combines some flow from both white and gray brain matter despite isolation of washout kinetics into fast and slow components. Another recently described error in the xenon washout technique is the so-called "falling flow phenomenon" (8) that underestimates CBF if data collection is extended beyond 1 min. This error is larger at low flow rates, so the shape of the Pa_{CO_2}/CBF relationship can be altered. As shown in Fig. 1 of Grubb et al. (4), most of the data are found well beyond the 1-min mark.

Our data show that the brain of ducks becomes more hypoxic with extreme hypocapnia. A causal relationship probably exists between that observation and the simultaneously measured fall in CBV. Our results are in clear contradiction to the proposition that the hypocapnic

brain is better supplied with O_2 than is the normocapnic brain (5). The Bohr effect is apparently insufficient to promote maintenance of intracellular O_2 tension-redox balance when blood flow falls with extreme hypocapnia.

Extrapolating these data to conditions probably occurring during high-altitude flight suggests that the avian brain becomes more hypoxemic as arterial CO_2 tension falls with the hyperventilation of hypoxia and exercise. The extremes of blood CO_2 and O_2 tension occurring during flight are not known, but based on data from exercising humans on the summit of Mount Everest (12), arterial P_{CO_2} is certainly <15 mmHg, and P_{O_2} is probably ~30 mmHg. Extrapolation of our data to flight conditions is of course hazardous, in particular because of the large differences in cardiac output between conditions of anesthesia and maximal exercise. It is tempting to speculate that the avian brain is hypoxia tolerant and may rely in part on anaerobic metabolism or hypometabolism during extreme hypoxia and hypocapnia. However, Bryan and Jones (2) found that EEG activity (presumably reflecting total brain ATP production, whether anerobic or aerobic) had a similar relationship to tissue redox balance in hypoxia-resistant (ducks) and hypoxia-sensitive birds (chickens). Measurements of cardiac output and O_2 delivery to the brain during simulated flight conditions may be necessary to clarify these relationships. We are led to doubt, however, that special avian adaptations of CBF regulation and O_2 delivery during severe hypocapnic hypoxia contribute to the adaptations for high-altitude flight in birds.

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