Critical oxygen tension in rat brain: a combined $^{31}$P-NMR and EPR oximetry study

ELLIS L. ROLETT, ALI AZZAWI, KE JIAN LIU, MARTIN N. YONGBI, HAROLD M. SWARTZ, AND JEFF F. DUNN

Departments of 1Medicine and 2Radiology, Nuclear Magnetic Resonance and Electron Paramagnetic Resonance Research Centers, Dartmouth-Hitchcock Medical Center, Hanover, New Hampshire 03755

Received 1 April 1999; accepted in final form 21 January 2000

The brain is a highly aerobic organ, requiring sufficient flux of oxygen for mitochondria to accept electrons generated during the production of ATP. In a resting human, the brain receives ~12% of the cardiac output and accounts for almost one-fifth of the total body metabolism, although it constitutes only 2% of total body weight (47). Although oxygen deprivation may be the cause of brain cell death during either hypoxia or ischemia, it is still unclear what constitutes a dangerously low oxygen level within the substance of the brain itself. Early energy failure is signaled by a shift to anaerobic glycolysis with a buildup of NADH and lactate (9). Severe hypoxia is known to cause intracellular acidosis and reduced tissue levels of phosphocreatine (PCr) and ATP (4, 33).

The present study was conducted to test the hypothesis that there is a critical cortical oxygen tension (P_{O_{2,ct}}) below which homeostasis for cellular energetics begins to fail. We employed parallel studies of brain oxygenation and energetics in anesthetized rats to characterize metabolic changes during hypoxic hypoxia in vivo. Tissue oxygen tension (P_{O_{2,t}}) was measured by electron paramagnetic resonance (EPR) oximetry (26), and energetics were measured using $^{31}$P-nuclear magnetic resonance ($^{31}$P-NMR) spectroscopy (2). The results of these experiments support the concept that oxygen concentration is normally above the apparent Michaelis constant (K_m) for mitochondria and that oxygen becomes a regulatory substrate for oxidative metabolism only when P_{O_{2,t}} is reduced to a critical level.

MATERIALS AND METHODS

Animal preparation. Male albino CD rats (Charles River Laboratories, Wilmington, MA), fed ad libitum and averaging 360 g in weight, were anesthetized with a single intramuscular injection of ketamine (8–10 mg/100 g) and xylazine (1.1–1.4 mg/100 g), which produced surgical anesthesia throughout the measurements. Animals were intubated with PE-240 tubing by tracheal cutdown and connected to a small animal ventilator (model SAR-830/P, CWE, Ardmore, PA) via Tygon tubing (5-mm bore diameter, 4.5-m length). The long tubing permitted controlled ventilation inside the NMR magnet. Ventilatory parameters were 120–140 cc · min$^{-1}$ · 100 g$^{-1}$ at a rate of 100 breaths/min and inspiratory time of 300 ms, resulting in a peak net inspiratory pressure of 10 ± 0.5 mmHg. A data acquisition system (model MP100WS, Biopac Systems, Goleta, CA) was employed to monitor airway pressure, electrocardiogram, temperature, and blood pressure during experiments. Core temperature, monitored by rectal thermostor, was maintained at a constant level between 37.5 and 38°C with a circulating water system.

Rats were ventilated successively at inspired oxygen fractions (F_{IO_{2}}) of 30, 15, and 10%. The desired F_{IO_{2}} was achieved by mixing different proportions of 100% oxygen, room air, and 100% nitrogen with a clinical anesthesia system (Ohmeda Unitrol, Madison, WI) and was continuously monitored with a calibrated oxygen analyzer (Datex Capnomac II, Helsinki, Finland). Levels of hypoxia below an F_{IO_{2}} of 10% could not routinely be achieved because of excessive hypotension.

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Address for reprint requests and other correspondence: E. Rolett, Dartmouth Medical School, Hinman Box 7500, Hanover, NH 03755

(E-mail: ellis.rolett@dartmouth.edu).

http://www.ajpregu.org 0363-6119/00 $5.00 Copyright © 2000 the American Physiological Society
levels by reducing FIO2 from 30 to 15% and then to 10%. steadystate hypoxia was induced for 30 min at each of two the data points for each FIO2 level. FIO2 was dropped to the
returned to 30%, with acquisition of additional spectra.

Arterial blood gases. In one group of animals, femoral arterial cannulation was performed for the measurement of arterial blood gases and intra-arterial blood pressure. Arterial pH and blood gas levels were measured in duplicate at each FIO2 with the use of a pH/blood gas analyzer (model 238, Ciba-Corning, Medfield, MA).

31P-NMR spectroscopy. Studies were performed in a second group with the use of a 7-T 12-cm horizontal bore system (SMIS, Surrey, UK) with a two-turn, 11 × 15-mm radiofrequency (RF) surface coil positioned over the dorsum of the skull. The signal from a surface coil is localized to a volume approximately defined by the coil radius (2). Taking into account the surface coil geometry, positioning of the coil, and skull thickness, it is estimated that the volume of interest in the present study extended to a depth of 3.5 mm in the brain. Consequently, the NMR signal should have emanated predominantly from cortical tissue (34) in proportion to the populations of neurons and glial cells. The metabolites measured by 31P-NMR spectroscopy are intracellular (2, 4). Spectra were collected with 10-kHz sweep width, 60 averages, repetition time 4 s, and 90° adiabatic RF pulse (for increased RF homogeneity). Animals were supported in the prone position in a Plexiglas holder sized to the magnet bore. Nonmagnetic electrodes (Vermont Medical, Bellows Falls, VT) taped to the foot pads and connected to shielded cables were used to monitor the electrocardiogram. After measurements at an FIO2 of 10%, in three animals in this group the FIO2 was

Sequential 60-s scans were obtained throughout the hypoxia protocol described above, and adjacent pairs of scans were averaged for each data point. The final steady-state values at each FIO2 level were used for data analysis. After the dead-space time in the gas delivery system was subtracted, the halftime of the change in PtO2 to the new steady state was obtained by fitting the data with a semilogarithmic function with the use of Cricket Graph Software. At the end of the hypoxia sequence in four animals in this set, the FIO2 was returned to 30%, and additional spectra were acquired.

The electrocardiogram, heart rate, blood pressure (tail-cuff method, Kent Scientific, Litchfield, CT), and temperature were monitored during the EPR experiments. The tail-cuff
blood pressure method was previously validated against direct arterial blood pressure measurements in our laboratory. Blood pressure measurements in our laboratory.

**Histological examination of LiPc crystal implants.** Brains from three animals (two at 3 days after implant and one at 8 days after implant) were fixed by perfusion with 3.7% formalin in phosphate buffer before removal for subsequent histological study of the LiPc crystal implant site. Serial 10-μm-thick sections containing LiPc crystals were stained with hematoxylin and eosin and examined under light microscopy (Fig. 2).

**Data analysis.** With the exception of mean arterial blood pressure (MABP) and heart rate, data obtained from each of the three animal groups were analyzed separately. At each FIO2 level, MABP measurements by the tail-cuff and direct arterial methods were not statistically different and were combined for analysis. Similarly, there was no statistical difference among heart rate values in the three animal groups when compared at the same FIO2 level, and consequently these data were pooled for further analysis. Because scaling of 31P-NMR spectra varied among studies, it was not possible to average absolute measurements of the phosphate resonances from different animals. For that reason, 31P-NMR resonances were normalized (FIO2 30% = 1.0) to compare spectral peaks in the three FIO2 groups. All data were tested for differences among groups using one-way ANOVA for repeated measures (SPSS Software). If the ANOVA demonstrated a significant effect at the P = 0.05 level, then pairwise comparisons between FIO2 levels were performed using the Scheffé’s test. All data are expressed as means ± SE.

**RESULTS**

Heart rate, blood pressure, and blood gas responses to decreasing FIO2. Reductions in FIO2 from 30 to 15% and 10% were characterized by arterial hypoxemia and no significant change in arterial pH (Table 1). Arterial blood oxygen tension (PaO2) decreased proportionately with FIO2. The regression equation for this relationship is: PaO2 = 5.38 × FIO2 - 17.84, where PaO2 is in millimeters mercury and FIO2 is in percent (r = 0.995, P = 0.0001). Mechanical ventilation completely suppressed spontaneous respirations during hypoxia. Heart rate and electrocardiogram were unchanged during hypoxia, whereas MABP was significantly reduced at an FIO2 of 10% (Table 1). The absence of a heart rate change during hypoxia may be attributed to blunting of the arousal reflex by ketamine (43) and controlled ventilation (13).

**EPR oximetry results.** PtO2 values during controlled ventilation at each level of FIO2 are listed in Table 1. Baseline PtO2 values in individual animals bore no relationship to the age of the crystal implant. With each downward adjustment in FIO2 in the ventilated animals, PtO2 plateaued at a new level within 8–10 min (Fig. 3A). The decline in PtO2 followed first-order kinetics. The halftimes for the changes from FIO2 30 to 15% and from FIO2 15 to 10% were 4.7 ± 0.4 and 4.8 ± 0.3 min, respectively. The relationship between PtO2 and FIO2 can be fit with the linear regression equation PtO2 = 0.42 × FIO2 + 2.59, where PtO2 is in millimeters mercury and FIO2 is in percent (r = 1.000, P = 0.0001). The relationship between PtO2 and PaO2 is shown in Fig. 3B and is well approximated by a polynomial function.

**31P-NMR results.** The cortical metabolic responses to hypoxia are shown in Table 2 and Fig. 4. There was no change in phosphate metabolites or pH between an FIO2 of 30 and 15%. With a further decrease in FIO2 to 10%, significant changes in pH, P, 2NTP, and PCr were observed. Mean pH was 7.13 ± 0.01 at both FIO2 30% and FIO2 15% and was 6.95 ± 0.02 at FIO2 10%. The changes in pH, and the PCr/Pi ratio are plotted against PtO2 in Fig. 4.

The mobile phosphate fraction (Table 2) and phosphomonoester (PME) fraction (not shown) were not affected by hypoxia. The observation that the sum of the peaks of PME, Pi, PCr, and NTP remained relatively constant at the three FIO2 levels suggests a con-

---

**Fig. 2.** Photomicrograph of an 8-day-old LiPc implant stained with hematoxylin and eosin. A: low-power view of crystal and surrounding cerebral cortex; 100-μm scale bar. The crystal was partially shelled out during cutting of the section. B: higher-power view of same section as A showing crystal and immediately adjacent tissue; 50-μm scale bar. Numerous capillaries and several venules are visible. Normal appearing neuronal and glial cells are present together with occasional lymphocytes and macrophages containing crystal fragments. The crystal is surrounded by a thin fibrous capsule.
Table 1. Physiological variables at three FIO2 levels

<table>
<thead>
<tr>
<th>FIO2</th>
<th>n</th>
<th>30%</th>
<th>15%</th>
<th>10%</th>
</tr>
</thead>
<tbody>
<tr>
<td>HR (beats/min)</td>
<td>20</td>
<td>284 ± 7</td>
<td>269 ± 8</td>
<td>271 ± 7</td>
</tr>
<tr>
<td>MABP (mmHg)</td>
<td>12</td>
<td>90.0 ± 4.7</td>
<td>77.4 ± 4.6</td>
<td>59.6 ± 6.0</td>
</tr>
<tr>
<td>PaO2 (mmHg)</td>
<td>5</td>
<td>145.1 ± 11.7</td>
<td>56.5 ± 4.4</td>
<td>40.7 ± 2.3</td>
</tr>
<tr>
<td>P aCO2 (mmHg)</td>
<td>5</td>
<td>45.2 ± 10.2</td>
<td>41.5 ± 7.4</td>
<td>33.6 ± 4.4</td>
</tr>
<tr>
<td>pH</td>
<td>5</td>
<td>7.36 ± 0.04</td>
<td>7.30 ± 0.04</td>
<td>7.30 ± 0.02</td>
</tr>
<tr>
<td>P tO2 (mmHg)</td>
<td>7</td>
<td>15.1 ± 1.8</td>
<td>8.8 ± 0.4</td>
<td>6.8 ± 0.3</td>
</tr>
</tbody>
</table>

Values are means ± SE. HR, heart rate; MABP, mean arterial blood pressure; PaO2 and P aCO2, arterial blood partial O2 pressure and partial CO2 pressure; pH, arterial blood pH; PtO2, tissue PO2. PtO2 was obtained by electron paramagnetic resonance oximetry. *FIO2 10% value differs significantly from 15% and 30% values, P < 0.001. †FIO2 10% and 15% values differ significantly from the 30% value, P < 0.01. §FIO2 10% and 15% values differ significantly from the 30% value, P < 0.01. \‡FIO2 10% and 15% values differ significantly from the 30% value, P < 0.01.

Table 2. Brain biochemical measurements at three FIO2 levels

<table>
<thead>
<tr>
<th>FIO2</th>
<th>n</th>
<th>30%</th>
<th>15%</th>
<th>10%</th>
</tr>
</thead>
<tbody>
<tr>
<td>ΣNTP fraction</td>
<td>8</td>
<td>0.95 ± 0.02</td>
<td>0.92 ± 0.03</td>
<td></td>
</tr>
<tr>
<td>PCr fraction</td>
<td>8</td>
<td>0.98 ± 0.01</td>
<td>0.92 ± 0.02</td>
<td></td>
</tr>
<tr>
<td>Pi fraction</td>
<td>8</td>
<td>1.01 ± 0.03</td>
<td>1.36 ± 0.11</td>
<td></td>
</tr>
<tr>
<td>Mobile PO4 fraction</td>
<td>8</td>
<td>0.97 ± 0.01</td>
<td>0.98 ± 0.02</td>
<td></td>
</tr>
<tr>
<td>pH</td>
<td>8</td>
<td>7.13 ± 0.01</td>
<td>7.13 ± 0.01</td>
<td>6.95 ± 0.02</td>
</tr>
</tbody>
</table>

Values are means ± SE. ΣNTP, nucleotide triphosphate pool; PCr, phosphocreatine; pH, intracellular pH. ΣNTP, P cr, Pi, and pH were obtained by magnetic resonance spectroscopy. Mobile PO 4 is the sum of P, P cr, NTP, and phosphomonooester resonances. ΣNTP, P cr, P i, and mobile PO 4 are reported as fractions of the FIO2 30% value. *FIO2 10% value differs significantly from 30% value, P < 0.05. †FIO2 10% value differs significantly from 15% and 30% values, P < 0.05. \‡FIO2 10% value differs significantly from 15% and 30% values, P < 0.01. §FIO2 10% value differs significantly from 15% and 30% values, P < 0.01.

Recovery to FIO2 30% from hypoxia. After a 30-min exposure to an FIO2 of 15% followed by another 30-min exposure to an FIO2 of 10%, four animals in the EPR oximetry group and three animals in the 31P-NMR group were returned to an FIO2 of 30% for 10 min. PtO2, pH, Pi, and P cr showed a prompt and complete recovery to the prehypoxia levels. The prehypoxia and post-hypoxia values, respectively, for PtO2 were 15.0 ± 2.5 and 15.4 ± 2.4 mmHg and for pH were 7.14 ± 0.01 and 7.16 ± 0.02.

Histological results. None of the slices demonstrated edema and focal hemorrhage in the region of LiPc crystals was uncommon (Fig. 2A). In no case did hemorrhage encompass crystals. There were intact capillaries, neurons, and glia cells adjacent to the crystals in all sections where crystals were identified (Fig. 2B).

DISCUSSION

The relationship between PtO2 and intracellular energetics is complex and difficult to determine but has significant implications for our understanding of metabolic regulation in the brain. Of particular interest is whether there is a “critical PO2” at which sudden metabolic shifts occur or whether the changes in metabolism during progressive hypoxia are proportional to changes in PtO2. This study was designed to address this question and to determine the PO2 crit in the brain under one set of specific physiological conditions.

Measurement of cortical PtO2 by EPR oximetry. The novel feature of the present study was the measure-
ment of cortical PtO$_2$ for comparison with pH$_i$, P$_i$, and high energy phosphates during graded hypoxia in the intact rat. The technique of EPR oximetry was chosen because repeated measurements of PtO$_2$ may be performed noninvasively after the initial trauma of crystal implantation. Values obtained by EPR oximetry are from sites that are deeper in the cortex than those sampled by surface fluorescence and more definable than those obtained with the use of near-infrared spectroscopy (NIRS). Because LiPc crystals, which were used as the EPR material, are deposited interstitially, the EPR method measures interstitial rather than intracellular Po$_2$. LiPc crystals have a rapid response time, stable calibration, and high sensitivity at low values of PtO$_2$ and are not affected by changes in pH or redox conditions (27). Histological evidence from the present study and previously published from this laboratory (20) demonstrated intact cortical tissue in direct contact with the LiPc crystals (Fig. 2). Both the histological results and the rapid change to a new steady-state Pt$_o$ level with each alteration in F$_O_2$ (Fig. 3A) argue against a significant disruption of the capillary supply or the presence of an important barrier to oxygen diffusion between the capillary bed and LiPc crystals.

Measurements of Pt$_O_2$ with the use of polarographic microelectrodes have demonstrated significant variations between regions of the brain and within the cortex itself (14, 25, 29). This spatial heterogeneity may reflect differences in metabolic rate, perfusion rate, capillary density, and proportions of glial cells and neurons (14). Moreover, within a specific volume of brain cortex, Pt$_O_2$ declines as a function of the radial distance from microvessels (46). This suggests that studies performed with small microelectrode tip diameters (e.g., 2–6 μm) will yield more variable Pt$_O_2$ values than studies done with larger tip diameters (e.g., 25–100 μm). In an earlier study from this laboratory, comparison of polarographic and EPR measurements of Pt$_O_2$ by positioning a Clark-type microelectrode (50-75-μm tip diameter) and LiPc crystals in close proximity to each other demonstrated remarkably close agreement in brain, skeletal muscle, and tumor tissue (18).

In the present study, NMR images demonstrated that LiPc crystals were distributed along a 1- to 2-mm needle track within the cortex (Fig. 1). This leads us to predict that the Pt$_O_2$ values presented in this study are closer to mean cortical interstitial Po$_2$ than are Po$_2$ values obtained by single microelectrodes.

**Definition of critical Po$_2$ in brain.** There is no universally agreed upon definition of Po$_2$crit. In our case, we have defined it as the Pt$_O_2$ that correlates with well-defined perturbations in the homeostasis of oxidative metabolism as measured by pH$_i$ and high-energy phosphate compounds. Declining pH$_i$ and PCr/P$_i$ ratio are used as indicators of increased lactic acid production and falling phosphorylation potential (1, 19, 33). The absolute value for Po$_2$crit may be tissue specific and depend on oxygen demand, oxygen supply, and aerobic capacity.

It should be pointed out that Po$_2$crit as defined in this paper does not necessarily cause brain cell damage or death. The metabolic changes of hypoxia were rapidly reversible with reoxygenation in a subset of the animals studied. This evidence for metabolic recovery suggests that the hypoxic stress did not irreversibly impair mitochondrial function. Moreover, the conservation of the mobile phosphate pool is evidence that the acute hypoxic stress imposed in this study did not compromise cellular integrity. In another study on unanesthetized neonatal rats, a 50% reduction in brain ATP content, produced by a combination of ischemia and prolonged hypoxia, was required before histological damage became apparent in half of the animals (48).

**Value for Po$_2$crit.** The observed changes in pH$_i$ and PCr/P$_i$ ratio at an F$_O_2$ of 10% in the present study are evidence for a Po$_2$crit between Pt$_O_2$ 6.8 and 8.8 mmHg in the rat cortex under the experimental conditions employed in this study. The drop in brain pH$_i$ can primarily be attributed to local cerebral metabolic acidosis in the absence of a significant change in arterial blood pH (Table 1). The sharp break point in the relationships between Pt$_O_2$, and pH$_i$, P$_i$ fraction, and PCr/P$_i$ ratio was most likely the result of perturbed oxidative metabolism, accelerated glycolysis, and excess lactate formation.

Although we observed a decrease in both pH$_i$ and PCr/P$_i$ at an F$_O_2$ of 10% and an average Pa$_O_2$ of 40.7 mmHg, studies from other laboratories in which graded hypoxia was employed indicate that these two metabolic indexes do not initially change at the same Pa$_O_2$ (4, 33, 44). In these earlier studies, a decline in pH$_i$ first became apparent at Pa$_O_2$ values ranging between 45 and 30 mmHg, depending on species and experimental conditions. Only with a further decrease below a Pa$_O_2$ of 25–30 mmHg and a pH$_i$ below 6.95 were there appreciable changes in brain concentrations of PCr and P$_i$. Studies of graded hypoxia in which tissue lactate was measured confirm that lactate rises before a change in PCr (4, 38). Thus it can be stated with reasonable certainty that metabolic acidosis is an earlier indicator of perturbed oxidative metabolism than are falling PCr and NTP levels.

Using sagittal sinus blood Po$_2$ as a surrogate for brain Pt$_O_2$ during acute hypoxia in isoflurane-N$_2$O anesthetized dogs, Nioka et al. (33) reported Po$_2$ values of 21 mmHg when significant reductions in PCr/P$_i$ were first observed and 15.4 mmHg at a point when average brain pH$_i$ had declined to 6.95. These Po$_2$ values are considerably greater than the Pt$_O_2$ value of 6.8 mmHg observed at a pH$_i$ of 6.95 in our study. This discrepancy may be due in part to differences in species, anesthesia, and experimental conditions. Other factors may also contribute to the discrepancy. Pt$_O_2$ reflects a complex relationship among arterial Po$_2$, local blood flow, capillary distribution, tissue permeability for oxygen, diffusion distances, and oxygen utilization rate. Sagittal sinus blood Po$_2$ would be expected to exceed Pt$_O_2$ if capillary blood Po$_2$ does not fully equilibrate with tissue Po$_2$. In mechanically ven-
Our data are consistent with those of other workers in demonstrating that tissue oxygen, by whatever method used for detection, can decrease substantially before significant changes occur in the energetic status of the brain. Biochemical measurements of PCr and lactate did not show a change in the brains of N2O-anesthetized rats until PaO2 fell below 40–45 mmHg (38). In studies in dogs, large reductions in cortical hemoglobin oxygen saturation and sagittal sinus blood PO2 were required before significant changes occurred in NTP, PCr, pH i, and PCr/Pi ratio as measured by 31P-NMR (32, 33). In those studies, the PCr/Pi ratio and CMRO2 fell only after arterial Po2 and sagittal sinus blood Po2 had dropped below 40 and 23 mmHg, respectively (33). These in vivo results are supported by in vitro observations. In cell suspensions, the rate of ATP synthesis remained constant over a wide range of oxygen concentrations (49). In both isolated mitochondria and renal tubule suspensions, the redox state of cytochromes was similarly unchanged until the oxygen supply was significantly limited (8, 50).

In summary, with the experimental protocol used in the present study, a PaO2crit was determined to be present between 6.8 and 8.8 mmHg. Moreover, it is postulated that pH i is an earlier indicator of PaO2crit than is PCr/Pi.

Metabolic regulation during acute hypoxia. A major question in metabolic regulation is whether oxygen concentration at the site of cytochrome-c oxidase is within the range that would allow oxygen to be a regulatory substrate. NIRS studies of cytochrome oxidation states are mixed on this subject. Earlier data showing that cytochrome oxidation varies continuously with changes in PaO2 (24) may be unreliable because of difficulties in differentiating cytochrome from hemoglobin spectra (12, 30). Other studies demonstrate that cytochrome oxidation state is relatively constant over a wide range of oxygen tensions, indicating that oxygen concentration is above the Km for oxygen at the cytochromes (12, 35, 42, 44).

We have documented that PtO2 can decline significantly before brain metabolism reacts with metabolic acidosis and a decline in high energy phosphate compounds. On the assumption that tissue oxygen solubility did not change, this observation suggests that oxygen concentration at the mitochondria normally is well above the saturation level of cytochrome-c oxidase. This statement is supported by the fact that the apparent Km of oxygen for oxidative phosphorylation in isolated mitochondria is very low, signifying that intracellular oxygen tension would have to be reduced markedly to be rate limiting for oxidative phosphorylation (40, 50).

The current study does not fix a value for PaO2crit. Gnaiger et al. (17) have summarized data that indicate that the extracellular value of PaO2 for half-maximum respiration in isolated mitochondria (P50) is five to ten times greater than mitochondrial P50. This difference depends on the oxygen diffusion gradient between the extracellular space and mitochondria. From this estimate, we calculate that the PaO2crit corresponding to a PtO2 of 6.8 mmHg in the present study would lie between 0.7 and 1.4 mmHg. This is within the range of some reports for the apparent Km for O2 in isolated mitochondria (50), although higher than others (40).

The findings of this study are consistent with the hypothesis that there is a PaO2crit above which oxidative phosphorylation is not sensitive to changes in PtO2. It is argued that oxygen is not a regulatory substrate for metabolism during normoxia because pH i and high-energy phosphates are insensitive to changes in PtO2 over a wide range of PaO2. Accumulated evidence suggests that compensatory increases in cerebral blood flow and oxygen extraction maintain oxygen flux over a relatively wide range of decreasing PtO2. Our data indicate that oxygen concentration is declining but remains above the saturation level for mitochondrial cytochrome-c oxidase. As hypoxia becomes more profound, we postulate that cellular oxygen concentration approaches the apparent Km for oxygen, and therefore oxygen becomes a regulatory substrate. This stage of hypoxia is characterized by an accelerated glycolytic rate and the onset of lactic acidosis. Even at this level of hypoxia, however, the metabolic effects of acute cerebral hypoxia are rapidly reversible with reoxygenation.

Potential limitations of the study. It is likely that PtO2 was affected by anesthesia and variations in cerebral blood flow as well as by changes in PtO2 in the present study. The net effect of the experimental conditions on cerebral blood flow in this study is unknown. The peak inspiratory pressure of 10 mmHg during controlled ventilation could have increased intrathoracic pressure sufficiently to impair venous return and thereby reduce cardiac output and cerebral blood flow. The ketamine-xylazine regimen was chosen because it produces stable anesthesia, maintains higher blood pressure than inhalation anesthetics, and does not abolish autoregulatory cerebrovascular dilation (15, 28). This anesthetic regimen, however, may have contributed importantly to the comparatively low baseline PtO2 observed in this study. Ketamine is known to stimulate cerebral metabolism while decreasing cerebral blood flow (3). In previous reports from this laboratory, cerebral PtO2 measured by EPR oximetry in spontaneously breathing rats was highest in the absence of anesthesia (34 mmHg), intermediary with isoflurane or halothane anesthesia (23–28 mmHg), and lowest with ketamine-xylazine anesthesia (16 mmHg), despite a considerably higher blood pressure with the last (26, 28). In another report from this laboratory, continuous monitoring by EPR oximetry demonstrated a decline in cerebral PtO2 from 29.8 in unanesthetized rats to 11.8 mmHg after induction with ketamine-xylazine (18).

During hypoxia, cerebral vasodilation and lowering of MABP in all likelihood would further influence cerebral blood flow. Hypoxia is known to increase cere-
bral blood flow as long as MABP is sufficiently high (4, 6, 10, 21). Cerebral blood flow has been shown to increase at PaO2 values below 40 mmHg despite declining MABP below 55 mmHg (4). Although PaO2 and MABP did not fall below 40 and 50 mmHg, respectively, in the present study, cerebrovascular autoregulation may have been insufficient to maintain cerebral blood flow at FiO2 of 10%. If this were the case, a declining cerebral blood flow may have further compromised oxygen delivery to the brain.

Because of the likelihood that the relationship between PaO2 and energetic status was influenced by the experimental conditions of the study, it should be stressed that the PaO2 - PtO2 relationship depicted in Fig. 3B probably would differ quantitatively if MABP had been supported pharmacologically or if another anesthetic agent had been employed. Nevertheless, these limitations do not detract from the fundamental observation that PtO2 can be lowered considerably before significant changes occur in PCr, NTP, or pHi.

In conclusion, we therefore propose that at least two phases of metabolic regulation exist during progressive hypoxia. In the first, or physiological response phase, we postulate that oxygen concentration at the cytochrome complex is far enough above the apparent KmA for oxygen (or Po2crit), despite a declining intracellular Po2, that cytochrome redox state and high-energy phosphate production are relatively unchanged. In this phase, compensatory increases in cerebral blood flow and oxygen extraction sustain oxygen delivery and mitochondrial respiration so that oxygen is not a regulatory substrate.

In the second phase, with a further decline in intracellular oxygen concentration, oxygen becomes a regulatory substrate. This phase is characterized by anaerobic lactic acid production, declining pHi, and rising Pi. Although the regulatory step for an increased glycolytic rate is unknown, the postulated stimulus is a drop in oxygen concentration to a level approximating the apparent KmA for oxygen for mitochondrial respiration. Below this level, the mitochondrial redox state is reduced. Po2crit defined in these terms should be associated with a reduction of cellular redox systems wherein the cytoplasmatic NADH/NAD+ ratio is increased (7), a larger fraction of cytochrome-c oxidase is reduced (16, 23, 37, 40), and allosteric modulation of cytochrome-c oxidase may occur (22).

Perspectives

There is a lack of consensus about whether oxidative metabolism is sensitive to tissue oxygenation under normal conditions. This study supports the concept that brain metabolism is insensitive to tissue oxygenation over a wide range of declining Po2, presumably because oxygen delivery is maintained through compensatory physiological mechanisms beginning with circulatory adjustments. We postulate, therefore, that respiratory enzymes are in a largely oxidized state over a wide range of Po2, and that cytochrome oxidase is insensitive to changes in oxygen until a narrow critical range of Po2 is reached. Our experimental results indicate that enzymes of the respiratory chain become reduced and brain energy homeostasis begins to fail when tissue Po2 declines to this critical range. This leads to the conclusion that there is a definable critical Po2 below which oxidative metabolism is perturbed. These observations have implications for studies of the regulation of oxidative metabolism, for studies of progressive hypoxia (e.g., high altitude physiology), and for the interpretation of tissue Po2 data in the clinical setting.

We gratefully acknowledge P. Jack Hoopes for performing the histological analysis.

This work was supported in part by The G. Harold and Leila Y. Mathers Charitable Foundation and by a National Institutes of Health Grant RO1-CANS-67431.

Present address of A. Azzawi: SMIS Limited, Alan Turing Road, Surrey Research Park, Guildford, Surrey GU2 5YP, UK.

Present address of M. N. Yongbi: LDRR-Clinical Center (Rm B1N256), NIH, Bethesda, MD 20892.

REFERENCES
