Oxygen measurements in brain stem slices exposed to normobaric hyperoxia and hyperbaric oxygen

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Oxygen measurements in brain stem slices exposed to normobaric hyperoxia and hyperbaric oxygen. J Appl Physiol 90: 1887–1899, 2001.—We previously reported (J Appl Physiol 89: 807–822, 2000) that ∼10 min of hyperbaric oxygen (HBO2; ≤2,468 Torr) stimulates solitary complex neurons. To better define the hyperoxic stimulus, we measured PO2 in the solitary complex of 300-μm-thick rat medullary slices, using polarographic carbon fiber microelectrodes, during perfusion with media having PO2 values ranging from 156 to 2,468 Torr. Under control conditions, slices equilibrated with 95% O2 at barometric pressure of 1 atmosphere absolute (ATA), this produces a PO2 in the standard control PO2 of the medium used in this preparation is already hyperoxic at normobaric pressure. During HBO2, slice PO2 increases to levels that appear to reduce metabolism.

Solitary complex; polarographic oxygen measurements; metabolism; reactive oxygen species; central nervous system oxygen toxicity.
ings, under conditions of ≤10 min of hyperbaric oxygen (HBO2; i.e., Po2 = 95–98% O2 at Ps of 2.4–3.3 ATA), indicate a subpopulation of neurons in the solitary complex that are depolarized, exhibit increased firing rate, and, typically, have decreased membrane conductance (12, 45–46). However, these neuronal responses to HBO2 may be blunted because slices recorded under control conditions are already hyperoxic. Moreover, we are concerned that under control conditions neuronal activity is altered by the high Po2 (3, 5, 56) and increased exposure to ROS (26, 33, 54, 55). Previous electrophysiological studies in brain slices found that neuronal activity recorded in medium equilibrated with 21% O2 is different from neuronal activity recorded in medium equilibrated with 95% O2 (3, 5, 56). Investigators have proposed that these differences in excitability seen with 21% oxygen are not due to hypoxia, which is typically studied using 10–15% O2 (35), but rather to normobaric hyperoxia with 95% oxygen (3, 5, 56), possibly by an increased production of ROS (26).

The goal of the present study was to measure Po2 in both perfusion media and the solitary complex in slices prepared from weaned and adult rats under the same experimental conditions used in our electrophysiology studies. In doing so, we will be able to correlate changes in neuronal excitability recorded during HBO2 with known changes in tissue Po2. Moreover, we wanted to determine the degree of hyperoxia within our brain slice model under control conditions at normobaric pressure (Ps of ~1 ATA). We hypothesize that tissue Po2 will decrease in the solitary complex under control conditions (95% O2 at Ps of ~1 ATA) with increasing tissue depth; however, tissue Po2 at the center of the slice will remain hyperoxic compared with tissue Po2 in the intact CNS. We also hypothesize that slice Po2 will increase significantly during HBO2. Finally, we hypothesize that tissue VO2 will decrease during HBO2 because it has previously been shown that increasing Po2 of the perfusate from 150 to 600 Torr at normobaric pressure increased VO2 (4), whereas HBO2 reduced cellular VO2 (1, 11). A preliminary report of these data was previously published (47).

METHODS

Pressure Terminology

The partial pressure of oxygen is the product of Ps and Po2. When varying Ps, it is important to define the Po2 of the perfusate and tissue slice relative to Ps, especially when Ps and Po2 are independently manipulated (12). Normobaric pressure refers to ambient pressure measured in our laboratory with a mercury barometer; this was slightly less than normal Ps at sea level (~1 ATA or ~760 Torr), typically ranging from 739 to 752 Torr.2 Hyperbaric pressure refers to ambient pressure inside the hyperbaric chamber that is greater than 1 ATA. "Normoxia" refers to slice Po2 values that approximate values measured in vivo from rats that breathed air (20–21% O2) at normobaric pressure, i.e., CNS tissue Po2 of ~10–34 Torr (Table 1). "Normobaric hyperoxia" refers to slice Po2 values greater than those measured in vivo from rats breathing air at Ps ~1 ATA, i.e., >34 Torr (Table 1). Conventional brain slice control medium, including the artificial cerebral spinal fluid (aCSF) used in this study, was equilibrated with 95% O2-5% CO2 at normobaric pressure; thus, under control conditions, the slice was exposed to hyperoxic medium; in this study, control medium Po2 values were ~708 Torr. HBO2 in this report describes any perfusate with Po2 of >760 Torr or 1 ATA. In the present study, slices were exposed to three different HBO2 values depending on Ps, designated here in ATA after the dash (e.g., HBO2 signifies hyperoxic medium at a Ps of 2 ATA). The HBO2 Po2 values used were 1,200, 1,675, and 2,468 Torr. In this way, tissue can be exposed to hyperoxia at both normobaric pressure and hyperbaric pressure.

Brain Slices and Control Media

Slices were prepared from weaned and adult Sprague-Dawley rats as previously described (12). Anesthesia was not used because of the depressant actions these agents have on neurons (49) and their reported antagonistic interactions with elevated Ps (31, 53, 59). After decapitation, the brain stem was isolated and submerged in ice-cold (4–6°C) aCSF of the following composition (in mM): 125 NaCl, 5 KCl, 1.3 MgSO4, 26 NaHCO3, 1.24 KH2PO4, 2.4 CaCl2, 10 glucose at 300 mosM, pH of ~7.45 and Po2 of ~708 Torr after equilibration with a 95% O2-5% CO2 gas mixture at Ps of ~1 ATA. Hyperoxia (22) and HBO2 (38, 57) both affect central respiratory control; therefore, we chose to study the effects of oxygen on a part of the brain involved in respiratory control, namely, the solitary complex. Transverse slices were cut at 300 μm starting from the oxb and proceeding rostrally through the medulla oblongata. Slices were incubated in control medium at ~25°C for at least 1 h before one was selected and transferred to a tissue chamber inside the hyperbaric chamber (12). Brain slices typically remained viable for electrophysiological studies under these conditions for up to 8 h (12).

Hyperbaric Chamber

A detailed description of the hyperbaric chamber, sample cylinders, and tissue chamber are given elsewhere (13). Briefly, the hyperbaric chamber has a maximum working pressure of 65 ATA. Within the hyperbaric chamber, tissue was submerged in aCSF that was delivered at a rate of 2 ml/min using one of two high-pressure liquid chromatography (HPLC) pumps. The brain slice rested on a fine-mesh nylon grid and was stabilized by placing a large-mesh nylon grid over the top surface (Fig. 1). Temperature of the tissue bath and air above the preparation was regulated at 37 ± 0.3°C by a servo-controlled two-channel temperature controller. The tissue chamber and electronic microdrive, which was used to maneuver the Po2 electrode by remote control, and various other equipment items were positioned on a retractable sled for easy access when the hyperbaric chamber was opened. Once the equipment sled was pushed in and the chamber door was sealed, the tissue slice and oxygen electrode were visualized using an externally mounted stereo scope positioned over a window in the top of the chamber. As in previous studies (12, 16, 44–48, 59), pure helium was used to hydrostatically compress the tissue bath and, hence, the brain slice. Helium is inert and of low solubility in aqueous and lipid media (2), thus helium has no partial pressure.

2 In Dayton, OH, the Ps averaged 745 ± 2 (SE) Torr. When determining medium Po2, the vapor pressure of water, which is ~48 Torr at 37°C (60), was not subtracted from Ps.
Table 1. Oxygen electrode measurements of $P_{O_2}$ in CNS tissue, cerebrospinal fluid, and arterial blood in anesthetized rats and humans breathing air, $O_2$, and $CO_2$ gas mixtures at normobaric pressure and hyperbaric pressure

<table>
<thead>
<tr>
<th>Study</th>
<th>In Vivo Model</th>
<th>CNS Region</th>
<th>$P_B$, ATA</th>
<th>Inspired Gas, %$O_2$, %$CO_2$</th>
<th>$P_{O_2}$, Torr</th>
<th>$P_{CSF_{O_2}}$, Torr</th>
<th>$P_{A_{O_2}}$, Torr</th>
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<tr>
<td>27</td>
<td>Adult rat, urethane</td>
<td>Cortex</td>
<td>1.0</td>
<td>Air</td>
<td>$34 \pm 4$</td>
<td>$33 \pm 5$</td>
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<tr>
<td></td>
<td></td>
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<td>1.0</td>
<td>100, 0</td>
<td>$30 \pm 13$</td>
<td>$83 \pm 23$</td>
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<td></td>
<td></td>
<td></td>
<td>1.0</td>
<td>95, 5</td>
<td>$72 \pm 12$</td>
<td>$120 \pm 19$</td>
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</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>2.0</td>
<td>100, 0</td>
<td>$244 \pm 39$</td>
<td>$277 \pm 50$</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>3.0</td>
<td>100, 5</td>
<td>$360 \pm 51$</td>
<td>$402 \pm 54$</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>3.0</td>
<td>100, 0</td>
<td>$452 \pm 68$</td>
<td>$480 \pm 80$</td>
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<td></td>
<td></td>
<td></td>
<td>4.0</td>
<td>100, 5</td>
<td>$791 \pm 51$</td>
<td>$718 \pm 64$</td>
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<td>5.0</td>
<td>100, 0</td>
<td>$917 \pm 123$</td>
<td>$1,044 \pm 131$</td>
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<tr>
<td>24</td>
<td>Adult rat, methoxyflurane</td>
<td>Globus pallidus</td>
<td>1.0</td>
<td>Air</td>
<td>$11 \pm 3.2$</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>5.0</td>
<td>100, 0</td>
<td>$620–1,250$</td>
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<tr>
<td>61</td>
<td>Adult rat, pentobarbital</td>
<td>Parietal cortex</td>
<td>1.0</td>
<td>Air</td>
<td>$12 \pm 5$</td>
<td>$85 \pm 4$</td>
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<tr>
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<td></td>
<td></td>
<td>3.0</td>
<td>7, 0</td>
<td>$150 \pm 10$</td>
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<td></td>
<td></td>
<td>3.0</td>
<td>100, 0</td>
<td>$1,530 \pm 24$</td>
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<td></td>
</tr>
<tr>
<td>9</td>
<td>Adult rat, urethane</td>
<td>Hypothalamus</td>
<td>1.0</td>
<td>Air</td>
<td>$13.0 \pm 3.3$</td>
<td></td>
<td></td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>1.0</td>
<td>100, 0</td>
<td>$40.5 \pm 5.9$</td>
<td></td>
<td></td>
</tr>
<tr>
<td>23</td>
<td>Human, anesthetized</td>
<td></td>
<td>1.0</td>
<td>Air</td>
<td>$41 \pm 3.4$</td>
<td>$92.7 \pm 2$</td>
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</table>

Values for various $P_{O_2}$ are reported as means ± SE (either from the references given or calculated by the present authors from the data tabulated in the original article). Table 1 includes only studies that reported absolute values of $P_{O_2}$ for central nervous system (CNS) tissue or cerebral spinal fluid (CSF). $P_B$, barometric pressure or ambient pressure inside room or hyperbaric chamber. One atmosphere absolute (1 ATA) is equivalent to 760 Torr (sea level). Most studies that do not use a hyperbaric chamber do not report $P_B$ under normobaric conditions. In constructing this table, however, $P_B = 1$ ATA was assumed by the authors. Air refers to breathing 20–21% $O_2$ and balance $N_2$ gas mixture.

Effect [e.g., at $P_B = 3$ ATA, nitrogen can act as an anesthetic (31)] over the range of ambient pressures used in our study. Before compression, room air was purged from the chamber atmosphere and replaced with 100% helium; the chamber was then compressed at a rate of 2 atm/min.

Test Conditions

Equilibrating aCSF at $P_B$ of ~1 ATA and 37°C with 95% $N_2$ or 95% air (balance $CO_2$) resulted in media with $P_{O_2}$ values of ~0 and ~156 Torr, respectively. Hyperbaric oxygenated medium (i.e., medium that has been supersaturated with oxygen) was made by equilibrating aCSF with 98.3% $O_2$ at 1.6, 2.2, or 3.3 ATA in separate high-pressure sample cylinders (1-liter volume) to produce corresponding medium $P_{O_2}$ values of ~1,200, 1,657, and 2,468 Torr. No attempt was made to keep $P_{CO_2}$ constant at $P_{O_2}$ of 1,200 and 1,657 Torr; however, it is possible to do so by reducing the fractional concentration of $CO_2$ with increased $P_B$ (12). A pressure differential of 0.13–0.6 ATA was used to deliver hyperoxic aCSF to the tissue chamber. A high-pressure solenoid valve (General Valve, Fairfield, NJ) was used to rapidly select between control and hyperoxic medium such that perfusate flow rate was not significantly disrupted.

Oxygen Measurements

Oxygen was measured using a carbon fiber needle electrode (tip outer diameter of ~10 μm), previously described by Jiang et al. (28). Electrodes were constructed by sealing an 8-μm-diameter carbon fiber (Alfa Aesar, Ward Hill, MA) at the tip of a glass pipette (MTW150–6, World Precision Instruments, Sarasota, FL) using Duco cement. The other end of the fiber was attached to a copper wire using graphite conductive adhesive (Alpha Aesar) and connected to the input of a polarographic oxygen amplifier (A-M Systems, model 1900). A ~0.6-V potential was imposed between the oxygen electrode and a low-resistance (~1.0 MΩ) Ag/AgCl reference that was in contact with the tissue bath via a potassium gluconate agar bridge (12). Oxygen electrodes were typically calibrated before and after each profile in aCSF equilibrated
with 21 and 95% oxygen; only electrodes that showed a 3.5- to 4.5-fold current difference between media were used (typical slope varied between 5–10 pA/Torr).

Oxygen profiles were made by lowering the oxygen electrode in 50-μm steps perpendicular to the tissue surface. Recording depth in tissue was approximated two ways: 1) surface depth was determined by moving the electrode down in small steps and then moving it laterally until the tip of the electrode touched the tissue, as seen by a bowing of the electrode shank during lateral movement; and 2) core tissue depth was identified as the depth at which PO2 was minimum (4, 19).

Absolute PO2 values presented here were obtained directly from continual PO2 recordings stored as AxoScope records (Axon Instruments, Foster City, CA) and/or on magnetic tape (Vetter PCM recorder model 400, Rebersburg, PA). Approximately one-half of the electrodes used developed drift after more than ~0.5 h in the slice, probably as the result of tissue debris on the tip (10), and resulted in an offset of 91 ± 56 Torr. If an offset developed in the measured PO2, the presented values were the sum of both the measured PO2 plus any offset.

Metabolic Block Media

To minimize tissue VO2, all metabolizable glucose in the aCSF was replaced with 1 mM 2-deoxy-D-glucose (2DG; Sigma Chemical, St. Louis, MO). Oxygen measurements also were made in 2DG medium supplemented with 9.1 nM antimycin A (Sigma Chemical). Antimycin A, an antibiotic that blocks electron flow from cytochrome b to c1 (29), was added to the 2DG aCSF to block metabolism of substrates other than glucose (e.g., lactate).

Data Collection and Analysis

Data were collected and analyzed using a 486 PC and the AxoScope 7.0, Origin 5.0, and Mathematica software packages. Statistical significance was determined at P < 0.05 by one-way ANOVA and multiple comparison tests (Tukey’s or Newman-Keuls) or Student’s t-test. Linear regressions were also compared using analysis of covariance. Data are presented as means ± SE.

RESULTS

PO2 Measurements in the Tissue Bath

PO2 electrode calibrations. Figure 2A shows five superimposed PO2 traces made with the oxygen electrode submerged deep into the tissue bath in the absence of a brain slice. The recordings were initiated in control aCSF with a PO2 of 708 Torr. The perfusate source was

Fig. 2. A: continuous traces of oxygen partial pressure measured deep in the tissue bath while switching aCSF from control (PO2 ~710 Torr) to aCSF with PO2 values of 0, 156, 1,200, 1,657, and 2,468 Torr. All recordings were made at 37°C and at constant barometric pressure (Pb). Bottom 3 traces (anoxia, 21% oxygen, and HBO2-1, where HBO2 signifies hyperbaric O2) were recorded at 1 atmospheres absolute (ATA), whereas the upper two traces (HBO2-2 and HBO2-3) were recorded while the chamber pressure was held at 2 and 3 ATA, respectively. B: triplicate individual current values measured during the plateau phase of each medium PO2, excluding HBO2-1. Carbon fiber electrode produced a current that was linearly proportional to PO2 from 0 to 2,468 Torr (9.6 pA/Torr, r² = 0.97, n = 15).
then switched to aCSF with PO₂ values of 0, 156, 1,200, 1,657, and 2,468 Torr to produce normobaric anoxia, 21% oxygen, HBO₂-1 (PB of 1 ATA), HBO₂-2 (PB of 2 ATA), and HBO₂-3 (PB of 3 ATA), respectively. During HBO₂-1, PO₂ recordings were less stable because, as the pressure differential between the medium and tissue chamber approached 2:1, small oxygen bubbles would form in the aCSF inflow line and tissue bath, which disrupted the perfusate flow rate and aCSF meniscus in the tissue chamber. When the control medium was switched to one of the test media, a short delay in the electrode response was observed due to the dead space between the medium reservoirs and tissue chamber. Figure 2B shows that the polarographic electrode current measured at the plateau phase of each curve in Fig. 2A was linearly proportional to medium PO₂ at both normobaric and hyperbaric pressure over a range of PO₂ values from 0 to 2,468 Torr.

Gas-liquid oxygen diffusion gradient. At an interface between gas and liquid media with dissimilar oxygen tensions, oxygen will diffuse down its chemical gradient. In our submerged slice preparation, oxygenated aCSF was in contact with an anoxic gaseous atmosphere (100% helium). Consequently, PO₂ was measured as a function of aCSF depth into the tissue bath to determine the extent to which medium PO₂ dropped as a result of diffusion into the chamber atmosphere. The PO₂ measurements shown in Fig. 3 were made in the tissue bath without a brain slice present. The recordings were initiated at the tissue bath surface in aCSF, with PO₂ values of ~708, 1,657, or 2,468 Torr. The electrode was then moved through the aCSF in 50-μm steps until the recorded PO₂ reached a stable plateau. These measurements show that bath PO₂ increased at depths into medium between 0 and ~450 μm, thus signifying the presence of an oxygen diffusion layer that was probably due to a loss of oxygen from the aCSF to the chamber atmosphere. The relative oxygen gradient, expressed as a percentage of the maximum PO₂ at 450 μm, at each medium PO₂, was similar. For instance, the steady-state PO₂ at depths of 100, 250, or 450 μm, were ~20, 50, or 100%, respectively, of the maximum PO₂. The independence of the relative oxygen diffusion gradient from medium PO₂ may result from the configuration of our perfusion system. Fresh oxygenated aCSF is delivered to the tissue chamber from the bottom where it flows up toward the surface. We assumed that PO₂ of the chamber atmosphere remained negligible since the hyperbaric chamber volume was considerably larger (72 liters) than the tissue chamber volume (~5 ml) and the frequent flushing of the hyperbaric chamber atmosphere with fresh helium gas further minimized any PO₂ buildup.

**PO₂ Measurements in the Slice**

Regular aCSF. A total of 38 PO₂ profiles were made in 300-μm-thick brain slices perfused with aCSF having PO₂ values that ranged from 156 to 2,468 Torr. Examples of individual profiles made at 708, 1,657, and 2,468 Torr are shown in Fig. 4. The brain slice was positioned ~500 μm from the gas-liquid interface. The recordings began while the electrode was positioned 200 μm above the tissue surface. Although the distance of the initial recording position from the surface of the bath was not measured directly, it was estimated to be 250–350 μm, based on the gas-liquid oxygen diffusion gradient (Fig. 3), assuming that the slice does not...
PO$_2$ measured at 0 and 150 $\mu$m depths proportionally; slopes of regression lines were 0.65 ± 0.02 ($r^2 = 0.998$, $n = 38$) and 0.66 ± 0.04 ($r^2 = 0.997$, $n = 38$) at the surface and core, respectively. However, mean tissue PO$_2$ measured at 0 and 150 $\mu$m were significantly different at medium PO$_2$ values of 156, 708, and 1,200 Torr. As medium PO$_2$ increased beyond 1,200 Torr, there was no statistical difference in mean PO$_2$ measured at the tissue surface and tissue core. These results suggest slice VO$_2$ may have decreased during exposure to the higher levels of HBO$_2$ (see Metabolically Poisoned Tissue below).

In our electrophysiological studies of how HBO$_2$ affects neuronal excitability (12, 45, 46), it was important to differentiate the effects of pressure per se (i.e., hyperbaric helium) from those of high PO$_2$; thus tissue PO$_2$ measurements were made in tissue equilibrated with a constant control level of PO$_2$ (~708 Torr) at P$_B$ of 1, 2, and 3 ATA. The hyperbaric chamber was compressed with 100% helium and allowed 2–5 min for equilibration before tissue PO$_2$ was measured at a depth of 150 $\mu$m. Results from these measurements are plotted in Fig. 6. Tissue PO$_2$ at 2 and 3 ATA were not significantly different from PO$_2$ measured at 1 ATA, and the slope of the regression line, 0.04 ± 0.04 ($r^2 = 0.772$, $n = 3$), was not significantly different from zero. These results indicate that the effect of pressure per se can be differentiated from increased oxygen tension. It also indicates that 2 and 3 ATA of pressure do not affect oxygen diffusion or utilization (12, 47).

Metabolically poisoned tissue. A series of PO$_2$ profiles were made in slices incubated at medium PO$_2$ values ranging from 156 to 2,468 Torr in 2DG aCSF or 2DG plus antimycin A. This was done to determine how VO$_2$ affects slice PO$_2$ (i.e., the magnitude of PO$_2$ profiles between the slice surface and the core of the slice). The difference in PO$_2$ measured at the slice surface (0 $\mu$m) to its core (150 $\mu$m) was defined as delta PO$_2$ (ΔPO$_2$). By comparing the ΔPO$_2$ measured in metabolically active slices at different medium PO$_2$ values with the same measurements made in metabolically poisoned slices, the PO$_2$ dependence of VO$_2$ could be determined. Mean slice PO$_2$ measured at 150 $\mu$m in slices perfused with 2DG and 2DG plus antimycin A medium was linearly related to medium PO$_2$. No significant difference existed between the slopes of regression lines for each data set; therefore, the 2DG data and antimycin A supplemented 2DG data were pooled. The slope of pooled data vs. medium PO$_2$ was 0.74 ± 0.03 ($r^2 = 0.997$, $n = 33$).

Figure 7A shows superimposed PO$_2$ profiles, measured at 708 Torr, in regular and a metabolically poisoned brain slice. For comparison, mean PO$_2$ values measured at depths of 0, 50, 100, and 150 $\mu$m in metabolically poisoned slices are given in Table 2. In this example, ΔPO$_2$ was smaller in metabolically poisoned tissue (ΔPO$_2$ = 66 Torr) compared with metabolically active tissue (ΔPO$_2$ = 116 Torr). We considered the difference in ΔPO$_2$ between metabolically active tissue and 2DG tissue to be proportional to VO$_2$. Consequently, ΔPO$_2$ in nonpoisoned tissue was used as an
indirect measure of $\dot{V}O_2$ to gain insight as to how HBO$_2$ affects $\dot{V}O_2$. Mean $\Delta P_{O2}$ values were measured in metabolically active and inactive tissue and plotted against medium $P_{O2}$ in Fig. 7B. Mean $\Delta P_{O2}$ values measured in metabolically poisoned slices did not vary significantly over the entire range of medium $P_{O2}$ values studied. This indicated that the effects of $P_{O2}$ on nonmetabolic forms of oxygen utilization (e.g., formation of ROS) were negligi-

Fig. 5. $P_{O2}$ traces were plotted as a function of aCSF and slice depth. Traces of $P_{O2}$ made during control (708 Torr) and HBO$_2$-3 (2,468 Torr) conditions show bath $P_{O2}$ increasing with distance (50-$\mu$m steps) from the aCSF meniscus. As the electrode approached the surface of the slice, $P_{O2}$ began decreasing. *Not a continuous recording. Arrows at the He gas-aCSF and slice-aCSF interfaces indicate the direction of oxygen diffusion. Measured $P_{O2}$ reached a relative minimum at the slice core, after which $P_{O2}$ increased as the electrode passed through and away from the bottom of the brain slice. Typically, $P_{O2}$ recordings were terminated ~200 $\mu$m past the bottom of the slice.

Fig. 6. Mean $P_{O2}$ ($n = 3–13$, see Table 1) measured in regular aCSF at the slice surface (0 $\mu$m) and at the center of the slice (150 $\mu$m) were plotted against media $P_{O2}$. Oxygen tension measured at 0 $\mu$m differed significantly ($^{*} P < 0.05$) from values measured at 150 $\mu$m at $P_{O2} \leq 1,200$ Torr. At a constant $P_{O2}$ of ~708 Torr, slice $P_{O2}$ at 150 $\mu$m was measured in slices compressed with helium to $P_s$ of 1, 2, and 3 ATA, and these mean $P_{O2}$ values ($n = 3$) were plotted as the dashed line. At ~708 Torr, $P_{O2}$ measured at 2 and 3 ATA were not significantly different from $P_{O2}$ measured at 1 ATA.
Table 2. Mean tissue Po2 in regular aCSF and in aCSF with 2DG and antimycin A

<table>
<thead>
<tr>
<th>Tissue Depth</th>
<th>Po2 of Medium, Torr</th>
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<tbody>
<tr>
<td></td>
<td>156</td>
</tr>
<tr>
<td>Surface</td>
<td>136 ± 17(3)</td>
</tr>
<tr>
<td>50 μm</td>
<td>79 ± 9(6)</td>
</tr>
<tr>
<td>100 μm</td>
<td>52 ± 9(4)</td>
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<tr>
<td>150 μm</td>
<td>40 ± 7(6)</td>
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</table>

<table>
<thead>
<tr>
<th></th>
<th>2DG + antimycin A aCSF</th>
</tr>
</thead>
<tbody>
<tr>
<td>Surface</td>
<td>117 ± 5(3)</td>
</tr>
<tr>
<td>50 μm</td>
<td>77 ± 12(6)</td>
</tr>
<tr>
<td>100 μm</td>
<td>49 ± 9(6)</td>
</tr>
<tr>
<td>150 μm</td>
<td>25 ± 9(6)</td>
</tr>
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</table>

Values are means ± SE; n (in parentheses) = no. of slices. 2DG, 2-deoxy-D-glucose. *P < 0.05, surface PtO2 at 0 μm is greater than core PtO2 at 150 μm; †P < 0.05; core PtO2 at 150 μm is greater in 2DG-antimycin A aCSF than core PtO2 at 150 μm in regular aCSF.

The oxygen diffusion coefficient (D) and VO2 were calculated to better describe the dynamics of oxygen in our preparation over a broad range of PO2 values as well as to quantify, if only by approximation, the relationship between VO2 and tissue PO2 (see APPENDIX for details regarding the calculations of D and VO2). During measurements of oxygen at a constant depth of 150 μm in a metabolically poisoned brain slice, D was determined by measuring the change in PO2 over time when switching between two media with different non-zero PO2 values (8, 17), in this case 708 and 156 Torr. With the assumption that all forms of oxygen utilization were constant, D was estimated to be 1.3 × 10^-6 cm²/s. Our estimated D was smaller by about one-tenth than the D calculated from PO2 measurements in 1,000-μm-thick slices of cat cortex equilibrated with a similar initial PO2, 1.54 × 10^-5 cm²/s (21).

VO2 rate can be calculated from tissue oxygen profiles using Fick’s second law of diffusion (20). With the use of boundary conditions of PO2 at the surface of the slice (defined as P0) and at the bottom of the slice [defined as PL; thickness of the slice (L) = 300 μm], our slice PO2 profiles at each medium PO2 were fitted to the parabolic equation (20)

\[ PO2 = aX^2 - \left( aL + \frac{P_0 - P_L}{L} \right) x + P_0 \]

where PO2 is oxygen tension measured at depth X in a brain slice, a = VO2/2DS, and S is the estimated solubility coefficient of oxygen in cat brain (1.89 × 10^-5 ml O2·cm⁻³·Torr⁻¹) (21). Using our estimated D (1.3 × 10^-6 cm²/s), we calculated the VO2 (mean ± SE ml O2·cm⁻³·min⁻¹) at each medium PO2 to be 1.4 ± 0.17 × 10^-3 at 156 Torr, 1.8 ± 0.2 × 10^-3 at 708 Torr, 1.9 ± 0.32 × 10^-3 at 1,200 Torr, 1.7 ± 0.75 × 10^-3 at 1,657 Torr, and 9.3 ± 1.3 × 10^-4 at 2,468 Torr. Although VO2 at 1,200 and 1,657 Torr were not less than VO2 at 708 Torr (i.e., VO2 and aPO2 were not well matched), a trend of decreasing VO2 was evident at 2,468 Torr. These results suggest VO2 was dependent on PO2 during hyperoxia; therefore, we incorporated this assumption into our calculation of VO2 (see APPENDIX) and estimated VO2 at a constant depth by measuring the rate of change in PO2 measured in a metabolically active slice exposed to different aCSF PO2 values (8). Oxygen measurements were made at a constant depth of 150 μm in a brain slice while media PO2 changed from ~708 to ~156 Torr or from ~708 to ~2,468 Torr. From these measurements, we estimated VO2 to be 7.9 × 10^-5 and 7.3 × 10^-6 ml O2·cm⁻³·min⁻¹, respectively. Although the absolute values varied, both methods of determining VO2 showed that VO2 was consistently reduced under the more extreme hypoxic conditions compared with control PO2 values.

**DISCUSSION**

Neuronal tissue PO2 has been measured in the intact CNS during HBO2 (24, 27, 51, 61), and in brain slices at normobaric pressure (3–5, 19–21, 28, 50, 56); however, this is the first study to systematically study PO2 gradients in brain slices during HBO2. We found that, under conventional brain slice control conditions (95% O2), PO2 measured in the solitary complex decreased with increasing recording depth to a minimum PO2 value at the core of the slice that was still ~10-fold higher than normal cerebral PO2 in vivo, which has been reported to range from 10 to 34 Torr (Table 1). In fact, at an aCSF PO2 of 708 Torr, PO2 at the core of the slice approximated PO2 measured in the CNS of rats breathing 100% oxygen at Ps of >2 ATA (27). Furthermore, tissue PO2 increased linearly with aCSF PO2 from 156 to 2,468 Torr, to levels that are known to result in CNS O2 toxicity in whole animals (1, 25, 58). This range of HBO2 has been reported to depolarize solitary complex neurons in brain stem slices after ~10 min of exposure (12, 45, 46). At PO2 values >1,200 Torr, the
difference in $P_O_2$ from the surface to tissue core (i.e., $\Delta P_O_2$) decreased to the extent that $\Delta P_O_2$ measured in metabolically active tissue exposed to hyperoxic medium no longer differed from $\Delta P_O_2$ made in metabolically poisoned tissue. This difference was attributed to a reduction in $V_\dot{O}_2$, suggesting that the higher levels of HBO$_2$ may decrease cellular respiration in brain slices, as previously reported (1, 11).

**Critique of Methods**

Initially, we measured oxygen with platinum needle electrodes (12); however, these electrodes showed poor resolution between 50-µm steps in tissue (not shown). This resolution problem likely resulted from the high rate of $V_\dot{O}_2$ by the electrode, as signified by the large current generated per Torr oxygen (7.87 nA/Torr), which then depleted oxygen from the area around the electrode tip, thus blunting the $P_O_2$ difference per 50 µm. Therefore, we switched to carbon fiber electrodes of the type typically used for the voltametric detection of neurotransmitters and metabolites around synapses (41). Polarographic electrodes can measure oxygen at a polarization potential of $-0.6$ V with minimal interference from oxidizable substances (e.g., neurotransmitters are typically oxidized at potentials of 0.2–0.8 V), and, because these electrodes are small and of high resistance, they are ideal for measuring oxygen in brain slices (28). Only carbon fiber electrodes were used in this study; as illustrated in Fig. 2B, these electrodes produced a current that was linearly proportional to oxygen concentration at both normobaric and hyperbaric pressure.

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**Fig. 7.** A: 2 superimposed $P_O_2$ profiles measured at $-708$ Torr in a metabolically active and metabolically inactive slice. The difference in $P_O_2$ from the slice surface (0 µm) to its core (150 µm) was defined as delta ($\Delta$) $P_O_2$. B: $\Delta P_O_2$ measured in metabolically active tissue at a $P_O_2$ of $-708$ Torr was significantly greater ($P < 0.05$) than $\Delta P_O_2$ in metabolically poisoned tissue at all $P_O_2$ tested. During HBO$_2$, the $\Delta P_O_2$ values measured in metabolically active brain slices were reduced to values that more closely resembled $\Delta P_O_2$ in metabolically poisoned slices. $P_O_2$ measured in metabolically active slices at which the $\Delta P_O_2$ was significantly ($P < 0.05$) smaller than $\Delta P_O_2$ measured at $-708$ Torr. These results suggest that oxygen consumption is reduced by HBO$_2$.
As was the case in previous studies (4, 19, 21), there was considerable variability in our Po2 measurements. This variability likely resulted from error in the estimated tissue depth due to tissue dimpling as the electrode penetrated the slice, uneven brain slice thickness, or tissue debris on the electrode tip that reduced the tip surface available for the reduction of oxygen (10). Likewise, tissue debris on the electrode tip likely accounted for offsets that occurred in about one-half of the electrodes used. Slow tissue potential changes or direct current shifts, which can influence Po2 measurements,3 were presumed to be small in the solitary complex during exposure to the same hyperoxic conditions, compared with the −0.6-V polarizing potential (12, 45, 46). Furthermore, it has also been shown that, when using a low-resistance remote reference, the effects of any slow tissue potential changes on the polarizing voltage were negligible (37). More stable methods of measuring Po2, such as with a Clark-style oxygen electrode (17) or the optical phosphorescence method (32), were not used because of the constraints of doing such measurements inside a hyperbaric chamber at Ps >1 ATA.

**Po2 Profiles in aCSF**

At the gas-liquid interface, oxygenated aCSF was in contact with an oxygen-free helium atmosphere. Oxygen, according to Le Chatelier’s principle, will diffuse from the aCSF down its chemical gradient into the overlying chamber atmosphere, leaving behind a graded layer of Po2 in the aCSF. As expected, Po2 was minimum at the aCSF surface and increased with increasing depth of aCSF. However, the depth at which a measurable diffusional loss of oxygen to the chamber atmosphere no longer occurred was consistently ~450 μm regardless of the media Po2. In addition, our results indicate that submerged brain slices are oxygenated by the diffusion of oxygen from aCSF of approximately ±200 μm to the tissue surface. We observed that, at medium Po2 values ≥708 Torr, oxygen diffusion into the brain slice resulted in a 35–40% drop in Po2 from bulk aCSF to slice surface. Previous studies have noted similar diffusion layers, sometimes referred to as unstirred layers in brain slices (4, 19, 21, 39) and in the brain stem spinal cord preparation (52).

If we assume that the oxygen diffusion gradient in the bath is identical with or without the tissue slice present, then these results suggest that oxygenation of the top surface of the slice is limited by the following two factors: 1) the diffusion of oxygen into the helium atmosphere and 2) the depth of the aCSF overlying the slice. Thus maintaining <450 μm of perfusate over the slice could potentially limit oxygenation at the upper surface of the slice. In our preparation, however, the brain slice was always positioned >450 μm deep to the bath surface.

**Po2 Profiles in Brain Slices**

Measurements of Po2 through 300-μm-thick brain slices exposed to medium Po2 ranging from 156 to 2,468 Torr showed that, although oxygen tension decreased with increasing recording depth in tissue, Po2 measured at the tissue surface (0 μm) and tissue core (150 μm) increased linearly as medium Po2 increased. As expected, these results indicate that the oxygen diffusion coefficient in tissue did not change with medium Po2 or diffusion distance. Furthermore, the magnitude of the oxygen gradient in aCSF from ~200 μm above or below the slice was roughly equivalent to the oxygen gradient in the outer 100-μm layers of tissue. A similar observation was previously reported in the neonatal rat brain stem spinal cord preparation (52).

The majority of our tissue Po2 profiles were symmetrical, with the minimum Po2 value measured approximately at the center of the slice. Some studies conducted at normobaric pressure came to similar conclusions (19–21). In contrast, investigators who used the interface slice preparation, with an overlying atmosphere of 95% O2, reported that diffusion from the upper surface dominated and resulted in a minimum Po2 near the bottom of the slice (28).

**Control Po2 at Normobaric Pressure**

When slices were submerged in aCSF equilibrated with 95% O2, we measured a minimum Po2 of 291 ± 83 Torr at the center of the slice. A similar minimum Po2 value of 187.2 ± 11 Torr (n = 2) was measured at a depth of 150 μm in 320-μm-thick guinea pig cortical slices submerged in aCSF equilibrated with 95% O2 at 1 ATA (19). Likewise, although variability between brain slice preparations and experimental parameters makes direct comparison of absolute slice Po2 difficult, minimum control Po2 values reported here were similar to values measured in 400- to 450-μm-thick brain slices positioned in the interface preparation; these values ranged from ~150 to 280 Torr (28, 50).

Minimum Po2 values measured in our slice preparation and in others (19, 28, 50) when incubated with conventional control solution (95% O2, Ps of 1 ATA) were ~10-fold greater than Po2 values measured in vivo (9, 23, 24, 27, 51, 61). This indicates that most brain slice studies are performed under hyperoxic conditions at normobaric pressure, thus raising the concern that neuronal activity may be affected by an increased production of ROS. It has been shown that the degree of tissue damage resulting from lipid peroxidation was significantly increased in brain slices incubated in 95% O2 compared with 21% O2 at normobaric pressure (33, 54). Bingmann and colleagues (3, 5) found neurons in hippocampal slices incubated in ~21% O2 depolarized and increased their firing rate when exposed to 100% O2, indicating that the high Po2

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3 Lehmenkuhler et al. (37) reported that, when making Po2 measurements in excitable tissue, slow tissue potential changes or direct current shifts resulting from oxygen-induced excitation may mimic changes in Po2 by interfering with the polarizing voltage at the oxygen electrode.
of brain slice control medium (i.e., normobaric hyper-
oxia) can, in fact, alter cellular activity. The activity of
hippocampal neurons in 21% O₂ was not considered a
response to hypoxia (in the brain slice preparation,
hypoxia is typically mimicked by Fₒ₂ values of 10–15%
O₂ at Ps of 1 ATA), however, because the activity of
hippocampal neurons exposed to hypoxia was quite
different (15, 36). Normobaric hypoxia has also been
shown to alter neuronal function in hypothalamic
slices (55) and in the carotid body (43), and these
responses were attributed to increased ROS.

Characterization of an optimal medium Pₒ₂ has
proven to be important for thin tissue preparations like
neuronal cell cultures. For example, in cultures of
neocortical and hippocampal neurons, the optimal me-
dium Pₒ₂, based on cell growth and viability, was
determined to be 9% O₂ at Ps of 1 ATA (~68 Torr) (7,
30). Neuritogenesis of cultured hippocampal neurons
was also improved by the addition of antioxidants
(vitamin E, vitamin A, and linolenate) to the incuba-
tion medium (7). These results suggest that the opti-
mal medium Pₒ₂ for an in vitro tissue preparation
must balance tissue oxygen requirements with the
otherwise toxic oxidative effects of excess oxygen.

Clearly, medium Pₒ₂ affects both neuronal viability
and excitability. For this reason, it is important that in
vitro experimental conditions match, as closely as pos-
sible, in vivo conditions (i.e., optimum Fₒ₂ of the per-
fusion medium should produce a Pₒ₂ at the core of the
brain slice that ranges between 10 and 34 Torr). In our
study, when medium Pₒ₂ was reduced from control to
21% O₂, although slice surface Pₒ₂ was still hyperoxic,
the minimum Pₒ₂ values, which averaged 40 ± 17
Torr, more closely resembled Pₒ₂ values measured in
the CNS and CSF of whole animals (Table 1). Alterna-
tively, antioxidants can be added to the medium to
provide protection from ROS when using 95% O₂ (6, 7,
34). Subsequent electrophysiological studies of solitary
complex neurons in 300-µm-thick brain slices are re-
quired to confirm, however, that cells remain viable
and healthy in this preparation at this lower level of
control Pₒ₂.

**Metabolically Poisoned Tissue**

Vₒ₂ is another important factor that must be consid-
ered when determining the optimum brain slice control
Pₒ₂. Bingmann et al. (4) reported that cellular Vₒ₂
increased when the incubation medium Pₒ₂ of 300-µm-
 thick hippocampal slices increased from 150 to 600
Torr. The authors suggested that, at normobaric oxy-
 gen pressure and a medium Pₒ₂ of 150 Torr, cell respiration
was limited by oxygen availability such that an in-
crease in medium Pₒ₂ resulted in an increase in Vₒ₂.

However, the extent to which brain slice Vₒ₂ is directly
dependent on medium Pₒ₂ is not known under condi-
tions of HBO₂. For these reasons, we measured Pₒ₂ in
brain slices equilibrated with aCSF having Pₒ₂ values
that ranged from 156 to 2,468 Torr; then, for compar-
ison, Vₒ₂ measurements were repeated in metabolically
poisoned tissue equilibrated with the same range of
Pₒ₂ values. Oxygen profiles in metabolically active
slices showed that, as Pₒ₂ increased from 708 to 1,657
Torr, the ΔPₒ₂ approached a minimum, at 1,657 Torr,
that was significantly different from the ΔPₒ₂ at 708
Torr and not different from ΔPₒ₂ values in metabolically
poisoned tissue. At Pₒ₂ greater than 1,657 Torr,
ΔPₒ₂ values of oxygen profiles remained similar in
magnitude to ΔPₒ₂ values made in metabolically poi-
soned tissue. In addition, calculated Vₒ₂ under control
conditions (95% O₂) was 1.8 ± 0.2 × 10⁻³ ml
O₂·cm⁻³·min⁻¹. A comparable Vₒ₂ of 3.38 ± 0.31 × 10⁻²
ml O₂·cm⁻³·min⁻¹ was measured in 500-µm-
 thick slices of guinea pig olfactory cortex equilibrated
with 95% O₂ (20). During HBO₂-3, Vₒ₂ was reduced to
9.3 ± 1.3 × 10⁻⁴ ml O₂·cm⁻³·min⁻¹. Furthermore,
although absolute Vₒ₂ values varied, this trend was
maintained when Vₒ₂ was assumed to be dependent on
medium Pₒ₂ (see **Appendix**). Together, these results
suggest that the higher levels of HBO₂ reduced metab-
olism in 300-µm-thick slices.

The mechanism by which HBO₂ may reduce brain
slice metabolism is not clear but likely involves the
increased production of ROS and the oxidation of mit-
ochondrial enzymes and/or cofactors, including a-
li-poic acid, cytochrome c, flavin nucleotides, and ubiqui-
none (1, 11). Furthermore, neuronal responses to
HBO₂ depend on the duration of the HBO₂ exposure.
Previous electrophysiological recordings show that
short (~10 min) bouts of HBO₂ increase neuronal ac-
tivity (12, 45, 46); it is well known that Vₒ₂ increases
in conjunction with neuronal activity (42); however, in
this study, we presented evidence suggesting that 30
min or more of exposure to the same HBO₂ actually
reduced Vₒ₂. Future studies focusing on the details
regarding the dose dependence of HBO₂ sensitivity
may be necessary.

In conclusion, oxygen tension in the submerged
brain slice during normobaric hyperoxia and HBO₂
was a complex function that was dependent on several
experimental conditions, including ambient Pₒ₂, depth
of slice in the tissue bath, and Vₒ₂. Our findings show
that Pₒ₂ in the solitary complex of the 300-µm-
 thick brain slice submerged in control medium (95% O₂ at Ps
of ~1 ATA) was hyperoxic compared with the in vivo
CNS. When exposed to HBO₂, tissue Pₒ₂ increased to
oxygen tensions that corresponded with cerebral Pₒ₂
values measured in vivo under conditions that result in
symptoms of CNS O₂ toxicity (1, 25, 58). Our results
also suggest that metabolism decreased during high
levels of HBO₂, which was consistent with previous
observations (1, 11) and suggests that there may be a
metabolic component to the mechanism of HBO₂-in-
duced neuronal sensitivity.

**Appendix**

By assuming one-dimensional diffusion and uniform
boundary conditions across the surface of the slice, D can be
approximated by the solution to Fick’s second law of diffusion (8)

$$P_{O_2} = P_1 + \frac{4(P_0 - P_1)}{\pi} \sin \left( \frac{\pi X}{L} \right) \exp \left[ -D \frac{\pi^2}{L^2} t \right]$$

where $P_0$ is the measured $P_{O_2}$ at a depth of 150 $\mu$m in tissue equilibrated with a medium $P_{O_2}$ of 708 Torr, $P_1$ is the measured $P_{O_2}$ at a depth of 150 $\mu$m in tissue equilibrated with a medium $P_{O_2}$ of 156 Torr, $P_{O_2}$ is calculated to be the mean of $P_0$ and $P_1$, $X$ is the recording depth (150 $\mu$m), $L$ is tissue thickness (300 $\mu$m), and $t$ is time, in seconds, to reach $P_{O_2}$. We calculated $D$ at a $P_{O_2}$ halfway between two steady-state conditions because, presumably, $O_2$ flux would be maximum. We estimated $D$ to be $1.3 \times 10^{-6}$ cm$^2$/s. Our estimated $D$ was smaller than the $D$ calculated from $P_{O_2}$ measurements in 1,000-$\mu$m-thick slices of cat cortex equilibrated with a similar initial $P_{O_2}$, 1.54 $\times 10^{-5}$ cm$^2$/s (21).

Our results suggest that slice $V_{O_2}$ was dependent on $P_{O_2}$ of the bathing medium. By assuming that $V_{O_2}$ was dependent on $P_{O_2}$, we approximated $V_{O_2}$ from $P_{O_2}$ measurements made at a depth of 150 $\mu$m in a brain slice equilibrated with regular aCSF while switching medium $P_{O_2}$ from 708 to 156 or 2,468 Torr. With the same boundary conditions as before, $V_{O_2}$ was approximated graphically from the equation (8)

$$\phi(\beta) = -P_{O_2} + \left( \frac{2P_1}{\sinh(\beta L)} \right) \sinh \left( \frac{\beta L}{2} \right) + \frac{4P_0}{\pi} - \frac{4P_1}{\pi} \cosh \left( \frac{\beta L}{2} \right) \exp \left[ -D \frac{\pi^2}{L^2} t \right]$$

where $\phi(\beta) = \beta = \sqrt{4D_t}$. $P_0$ is the measured $P_{O_2}$ at a depth of 150 $\mu$m into a slice equilibrated with a medium $P_{O_2}$ of 708 Torr, $P_1$ is the measured $P_{O_2}$ at a depth of 150 $\mu$m into a slice equilibrated with either 156 or 2,468 Torr, $P_{O_2}$ is equal to the mean of $P_0$ and $P_1$, $t$ is time, in seconds, to reach $P_{O_2}$, and $D$ is $1.3 \times 10^{-6}$ cm$^2$/s. To convert $V_{O_2}$ to units of milliliters $O_2$ per cubic centimeters per minute, the approximated $V_{O_2}$ was multiplied by the oxygen solubility coefficient of cat brain, 0.0144 ml $O_2$-cm$^{-3}$ tissue$^{-1}$-atm$^{-1}$ (21). Switching medium $P_{O_2}$ from 708 to 156 or from 708 to 2,468 Torr resulted in estimated $V_{O_2}$ of $7.9 \times 10^{-5}$ or $7.3 \times 10^{-6}$ ml $O_2$-cm$^{-3}$ tissue$^{-1}$-min$^{-1}$, respectively.

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