

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

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**Mulkey, Daniel K., Richard A. Henderson III, James E. Olson, Robert W. Putnam, and Jay B. Dean.** 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  $\leq 10$  min of hyperbaric oxygen (HBO<sub>2</sub>;  $\leq 2,468$  Torr) stimulates solitary complex neurons. To better define the hyperoxic stimulus, we measured PO<sub>2</sub> in the solitary complex of 300- $\mu$ m-thick rat medullary slices, using polarographic carbon fiber microelectrodes, during perfusion with media having PO<sub>2</sub> values ranging from 156 to 2,468 Torr. Under control conditions, slices equilibrated with 95% O<sub>2</sub> at barometric pressure of 1 atmospheres absolute had minimum PO<sub>2</sub> values at their centers ( $291 \pm 20$  Torr) that were  $\sim 10$ -fold greater than PO<sub>2</sub> values measured in the intact central nervous system (10–34 Torr). During HBO<sub>2</sub>, PO<sub>2</sub> increased at the center of the slice from  $616 \pm 16$  to  $1,517 \pm 15$  Torr. Tissue oxygen consumption tended to decrease at medium PO<sub>2</sub>  $\geq 1,675$  Torr to levels not different from values measured at PO<sub>2</sub> found in all media in metabolically poisoned slices (2-deoxy-D-glucose and antimycin A). We conclude that control medium used in most brain slice studies is hyperoxic at normobaric pressure. During HBO<sub>2</sub>, slice PO<sub>2</sub> increases to levels that appear to reduce metabolism.

solitary complex; polarographic oxygen measurements; metabolism; reactive oxygen species; central nervous system oxygen toxicity

THE IN VITRO BRAIN SLICE PREPARATION has been used for more than 40 years to study neuronal excitability because it allows considerable control of the neuronal environment while retaining local neuronal circuitry (42). Brain slices are removed from blood supply and receive oxygen solely by diffusion from the nutrient medium that bathes the tissue. To ensure adequate oxygenation of cells lying deep to the slice surface, most brain slice studies use 95% O<sub>2</sub> to set the PO<sub>2</sub> in the control medium. At a barometric pressure (P<sub>B</sub>) of 1 atmosphere absolute (ATA),<sup>1</sup> this produces a PO<sub>2</sub> in the

nutrient medium of  $\sim 722$  Torr. Under this condition, brain slices remain viable for up to 8 h, based on electrophysiological criteria (12). Consequently, it has generally been assumed that PO<sub>2</sub> in the core of a submerged slice is adequate (40).

Several studies have reported tissue PO<sub>2</sub> values in brain slices measured with polarographic microelectrodes (3–5, 19–21, 28, 50, 56). These experiments were done to determine the slice thickness that optimized slice viability, as measured by extracellularly recorded field potentials, while ensuring that an anoxic tissue core was avoided (19), or, alternatively, to identify the level of oxygenation in the slice so that electrophysiological data could be correlated with tissue PO<sub>2</sub> during hypoxia (28, 50, 56) and hyperoxia (3–5, 56). Because PO<sub>2</sub> at any depth in a slice is determined by PO<sub>2</sub> of the perfusate, oxygen diffusion distance into the slice, and oxygen consumption ( $\dot{V}_{O_2}$ ), tissue PO<sub>2</sub> measurements were also used to determine  $\dot{V}_{O_2}$  (20, 21). Results from these studies varied, however, because of differences in slice thickness, central nervous system (CNS) regions, animal age, and orientation of slice surfaces relative to the supporting structure (nylon mesh vs. solid Plexiglas support) and the fluid-gas interface (interface slice vs. submerged slice) (4, 19, 28). Nevertheless, under control conditions, 300- to 450- $\mu$ m-thick brain slices had minimum PO<sub>2</sub> values that were consistently higher (19, 20, 28, 50) than those measured in the intact CNS (9, 23, 24, 27, 51, 61).

We studied the effects of hyperoxia, reactive oxygen species (ROS), and antioxidants on the electrophysiology of neurons in the solitary complex (12, 44–46), an important cardiorespiratory control center in the caudodorsal medulla oblongata (14, 18). The challenge of studying hyperoxia in rat brain slices, however, is that the standard control PO<sub>2</sub> of the medium used in this preparation is already hyperoxic at normobaric pressure (P<sub>B</sub> of  $\sim 1$  ATA). Increasing tissue PO<sub>2</sub> further requires increasing the P<sub>B</sub> of the slice and nutrient media together with a gas mixture containing a high fractional concentration of O<sub>2</sub> (FO<sub>2</sub>). Our initial find-

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<sup>1</sup>At sea level, P<sub>B</sub> is 1 ATA or 760 Torr. Other commonly used pressure equivalents include 0.099 MPa (SI unit), 14.7 pounds per inch<sup>2</sup>, and 1.01 bar.

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ings, under conditions of  $\leq 10$  min of hyperbaric oxygen ( $\text{HBO}_2$ ; i.e.,  $\text{F}_{\text{O}_2} = 95\text{--}98\%$   $\text{O}_2$  at  $\text{P}_\text{B}$  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  $\text{HBO}_2$  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  $\text{P}_{\text{O}_2}$  (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%  $\text{O}_2$  is different from neuronal activity recorded in medium equilibrated with 95%  $\text{O}_2$  (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%  $\text{O}_2$  (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  $\text{P}_{\text{O}_2}$  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  $\text{HBO}_2$  with known changes in tissue  $\text{P}_{\text{O}_2}$ . Moreover, we wanted to determine the degree of hyperoxia within our brain slice model under control conditions at normobaric pressure ( $\text{P}_\text{B}$  of  $\sim 1$  ATA). We hypothesize that tissue  $\text{P}_{\text{O}_2}$  will decrease in the solitary complex under control conditions (95%  $\text{O}_2$  at  $\text{P}_\text{B}$  of  $\sim 1$  ATA) with increasing tissue depth; however, tissue  $\text{P}_{\text{O}_2}$  at the center of the slice will remain hyperoxic compared with tissue  $\text{P}_{\text{O}_2}$  in the intact CNS. We also hypothesize that slice  $\text{P}_{\text{O}_2}$  will increase significantly during  $\text{HBO}_2$ . Finally, we hypothesize that tissue  $\dot{V}_{\text{O}_2}$  will decrease during  $\text{HBO}_2$  because it has previously been shown that increasing  $\text{P}_{\text{O}_2}$  of the perfusate from 150 to 600 Torr at normobaric pressure increased  $\dot{V}_{\text{O}_2}$  (4), whereas  $\text{HBO}_2$  reduced cellular  $\dot{V}_{\text{O}_2}$  (1, 11). A preliminary report of these data was previously published (47).

## METHODS

### *Pressure Terminology*

The partial pressure of oxygen is the product of  $\text{P}_\text{B}$  and  $\text{F}_{\text{O}_2}$ . When varying  $\text{P}_\text{B}$ , it is important to define the  $\text{P}_{\text{O}_2}$  of the perfusate and tissue slice relative to  $\text{P}_\text{B}$ , especially when  $\text{P}_\text{B}$  and  $\text{P}_{\text{O}_2}$  are independently manipulated (12). “Normobaric pressure” refers to ambient pressure measured in our laboratory with a mercury barometer; this was slightly less than normal  $\text{P}_\text{B}$  at sea level ( $\sim 1$  ATA or  $\sim 760$  Torr), typically ranging from 738 to 752 Torr.<sup>2</sup> “Hyperbaric pressure” refers to ambient pressure inside the hyperbaric chamber that is

greater than 1 ATA. “Normoxia” refers to slice  $\text{P}_{\text{O}_2}$  values that approximate values measured in vivo from rats that breathed air (20–21%  $\text{O}_2$ ) at normobaric pressure, i.e., CNS tissue  $\text{P}_{\text{O}_2}$  of  $\sim 10\text{--}34$  Torr (Table 1). “Normobaric hyperoxia” refers to slice  $\text{P}_{\text{O}_2}$  values greater than those measured in vivo from rats breathing air at  $\text{P}_\text{B} \sim 1$  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%  $\text{O}_2\text{--}5\%$   $\text{CO}_2$  at normobaric pressure; thus, under control conditions, the slice was exposed to hyperoxic medium; in this study, control medium  $\text{P}_{\text{O}_2}$  values were  $\sim 708$  Torr. “ $\text{HBO}_2$ ” in this report describes any perfusate with  $\text{P}_{\text{O}_2}$  of  $>760$  Torr or 1 ATA. In the present study, slices were exposed to three different  $\text{HBO}_2$  values depending on  $\text{P}_\text{B}$ , designated here in ATA after the dash (e.g.,  $\text{HBO}_2\text{--}2$  signifies hyperoxic medium at a  $\text{P}_\text{B}$  of 2 ATA). The  $\text{HBO}_2$   $\text{P}_{\text{O}_2}$  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  $\text{P}_\text{B}$  (31, 53, 59). After decapitation, the brain stem was isolated and submerged in ice-cold ( $4\text{--}6^\circ\text{C}$ ) aCSF of the following composition (in mM): 125 NaCl, 5 KCl, 1.3  $\text{MgSO}_4$ , 26  $\text{NaHCO}_3$ , 1.24  $\text{KH}_2\text{PO}_4$ , 2.4  $\text{CaCl}_2$ , 10 glucose at 300 mosM, pH of  $\sim 7.45$  and  $\text{P}_{\text{O}_2}$  of  $\sim 708$  Torr after equilibration with a 95%  $\text{O}_2\text{--}5\%$   $\text{CO}_2$  gas mixture at  $\text{P}_\text{B}$  of  $\sim 1$  ATA. Hyperoxia (22) and  $\text{HBO}_2$  (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  $\mu\text{m}$  starting from the obex and proceeding rostrally through the medulla oblongata. Slices were incubated in control medium at  $\sim 25^\circ\text{C}$  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  $37 \pm 0.3^\circ\text{C}$  by a servo-controlled two-channel temperature controller. The tissue chamber and electronic microdrive, which was used to maneuver the  $\text{P}_{\text{O}_2}$  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 stereoscope 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

<sup>2</sup>In Dayton, OH, the  $\text{P}_\text{B}$  averaged  $745 \pm 2$  (SE) Torr. When determining medium  $\text{P}_{\text{O}_2}$ , the vapor pressure of water, which is  $\sim 48$  Torr at  $37 \pm 0.3^\circ\text{C}$  (60), was not subtracted from  $\text{P}_\text{B}$ .

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

Study	In Vivo Model	CNS Region	P <sub>B</sub> , ATA	Inspired Gas, %O <sub>2</sub> , %CO <sub>2</sub>	P <sub>tO<sub>2</sub></sub> , Torr	P <sub>CSF<sub>O<sub>2</sub></sub></sub> , Torr	P <sub>aO<sub>2</sub></sub> , Torr
27	Adult rat, urethane	Cortex	1.0	Air	34 ± 4	33 ± 5	
			1.0	100, 0	90 ± 13	83 ± 23	
				95, 5	72 ± 12	120 ± 19	
			2.0	100, 0	244 ± 39	277 ± 50	
				95, 5	360 ± 51	402 ± 54	
			3.0	100, 0	452 ± 68	480 ± 80	
				95, 5	791 ± 51	718 ± 64	
			4.0	100, 0	643 ± 89	699 ± 106	
				95, 5	1,189 ± 195	1,053 ± 150	
				100, 0	917 ± 123	1,044 ± 131	
24	Adult rat, methoxyflurane	Globus pallidus	1.0	Air	11 ± 3.2		
			5.0	100, 0	620–1,250		
		Neostriatum	1.0	Air	19 ± 9		
			5.0	100, 0	490–1,010		
61	Adult rat, pentobarbital sodium	Parietal cortex	1.0	Air	12 ± 5		85 ± 4
			3.0	7, 0			150 ± 10
			3.0	100, 0			1,530 ± 24
9	Adult rat, urethane	Hypothalamus	1.0	Air	13.0 ± 3.3		
			1.0	100, 0	40.5 ± 5.9		
23	Human, anesthetized		1.0	Air		41 ± 3.4	92.7 ± 2

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. The range of CNS tissue  $P_{O_2}$  ( $P_{tO_2}$ ) during hyperbaric  $O_2$  ( $HBO_2$ ) is reported in some cases because of the transient responses observed. Typically,  $P_{tO_2}$  reached a maximum value at some point during  $HBO_2$  and then decreased slowly. This is most likely due to regional changes in cerebral blood flow (1). Adult rat hypothalamus values are based on average calculated for 19 different nuclei in and surrounding the hypothalamus; see Table 1 in Cater et al. (9).  $P_{CSF_{O_2}}$ , CSF  $P_{O_2}$ ;  $P_{aO_2}$ , arterial blood  $P_{O_2}$ .

effect [e.g., at  $P_B \geq 3$  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 oxy-

genated 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  $\mu m$ ), previously described by Jiang et al. (28). Electrodes were constructed by sealing an 8- $\mu m$ -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 M $\Omega$ ) 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

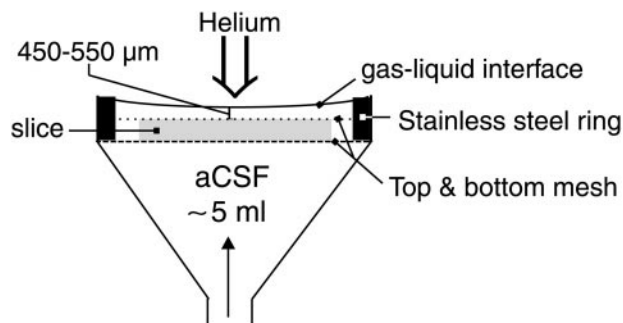


Fig. 1. Illustration of the brain slice orientation in relation to the flow of fresh oxygenated artificial cerebral spinal fluid (aCSF; not drawn to scale). Fresh aCSF enters the tissue chamber from the bottom and flows around and over the slice while a filter paper wick (not shown) draws it away from the surface.

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- $\mu\text{m}$  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  $\text{PO}_2$  was minimum (4, 19).

Absolute  $\text{PO}_2$  values presented here were obtained directly from continual  $\text{PO}_2$  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  $\sim 0.5$  h in the slice, probably as the result of tissue debris on the tip (10), and resulted in an offset of  $91 \pm 56$  Torr. If an offset developed in the measured  $\text{PO}_2$ , the presented values were the sum of both the measured  $\text{PO}_2$  plus any offset.

#### Metabolic Block Media

To minimize tissue  $\dot{V}\text{O}_2$ , 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 *c*<sub>1</sub> (29), was added to the 2DG aCSF to block metabolism of substrates other than glucose (e.g., lactate).

#### Data Collection and Analysis

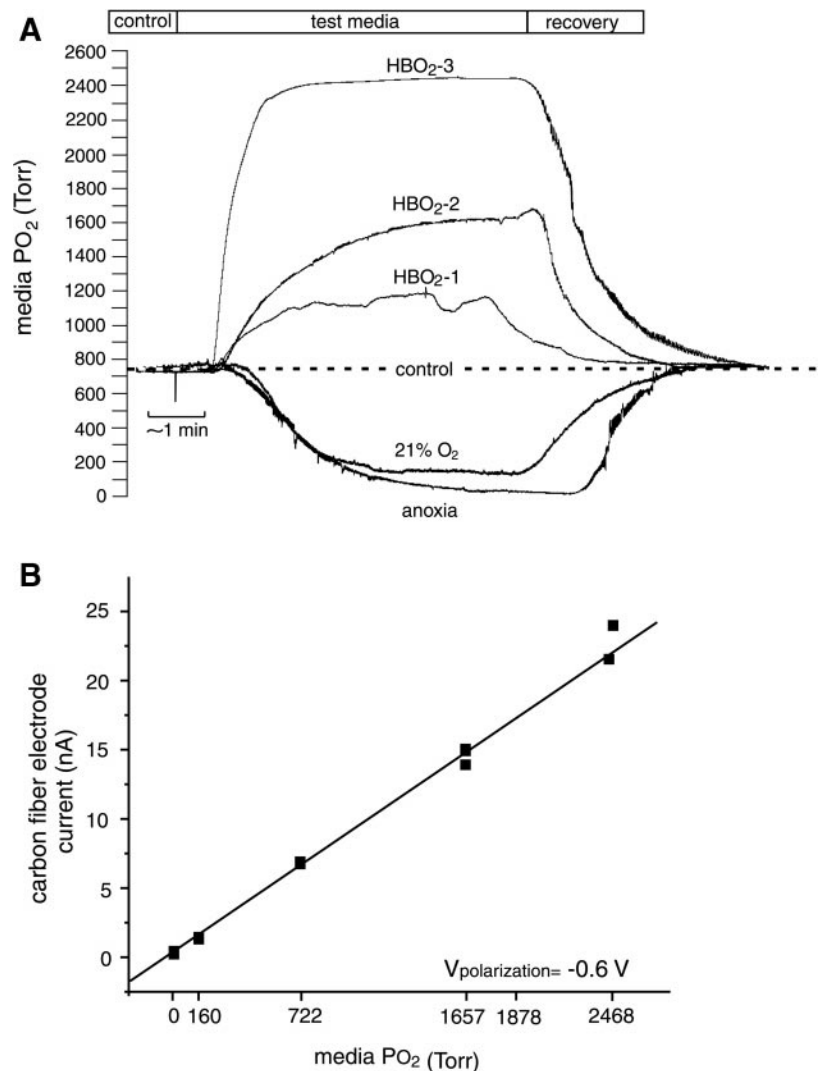
Data were collected and analyzed using a 486 PC and the AxoScope 7.0, Origin 5.0, and Mathematica software packages. Statistical significance was determined at  $P < 0.05$  by one-way ANOVA and multiple comparison tests (Tukey's or Newman-Keuls) or Student's *t*-test. Linear regressions were also compared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

## RESULTS

### $\text{PO}_2$ Measurements in the Tissue Bath

**$\text{PO}_2$  electrode calibrations.** Figure 2A shows five superimposed  $\text{PO}_2$  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  $\text{PO}_2$  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 ( $\text{PO}_2 \sim 710$  Torr) to aCSF with  $\text{PO}_2$  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  $\text{HBO}_2$ -1, where  $\text{HBO}_2$  signifies hyperbaric  $\text{O}_2$ ) were recorded at 1 atmospheres absolute (ATA), whereas the upper two traces ( $\text{HBO}_2$ -2 and  $\text{HBO}_2$ -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  $\text{PO}_2$ , excluding  $\text{HBO}_2$ -1. Carbon fiber electrode produced a current that was linearly proportional to  $\text{PO}_2$  from 0 to 2,468 Torr (9.6 pA/Torr,  $r^2 = 0.97$ ,  $n = 15$ ).



then switched to aCSF with  $P_{O_2}$  values of 0, 156, 1,200, 1,657, and 2,468 Torr to produce normobaric anoxia, 21% oxygen, HBO<sub>2</sub>-1 ( $P_B$  of 1 ATA), HBO<sub>2</sub>-2 ( $P_B$  of 2 ATA), and HBO<sub>2</sub>-3 ( $P_B$  of 3 ATA), respectively. During HBO<sub>2</sub>-1,  $P_{O_2}$  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  $P_{O_2}$  at both normobaric and hyperbaric pressure over a range of  $P_{O_2}$  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,  $P_{O_2}$  was measured as a function of aCSF depth into the tissue bath to determine the extent to which medium  $P_{O_2}$  dropped as a result of diffusion into the chamber atmosphere. The  $P_{O_2}$  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  $P_{O_2}$  values of ~708, 1,657, or 2,468 Torr. The electrode was then moved through the aCSF in 50- $\mu$ m steps until the recorded  $P_{O_2}$  reached a stable plateau. These measurements show that bath  $P_{O_2}$  in-

creased at depths into medium between 0 and ~450  $\mu$ m, 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  $P_{O_2}$  at 450  $\mu$ m, at each medium  $P_{O_2}$ , was similar. For instance, the steady-state  $P_{O_2}$  at depths of 100, 250, or 450  $\mu$ m, were ~20, 50, or 100%, respectively, of the maximum  $P_{O_2}$ . The independence of the relative oxygen diffusion gradient from medium  $P_{O_2}$  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  $P_{O_2}$  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  $P_{O_2}$  buildup.

#### *P<sub>O<sub>2</sub></sub> Measurements in the Slice*

**Regular aCSF.** A total of 38  $P_{O_2}$  profiles were made in 300- $\mu$ m-thick brain slices perfused with aCSF having  $P_{O_2}$  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  $\geq 500$   $\mu$ m from the gas-liquid interface. The recordings began while the electrode was positioned 200  $\mu$ m 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  $\mu$ m, based on the gas-liquid oxygen diffusion gradient (Fig. 3), assuming that the slice does not

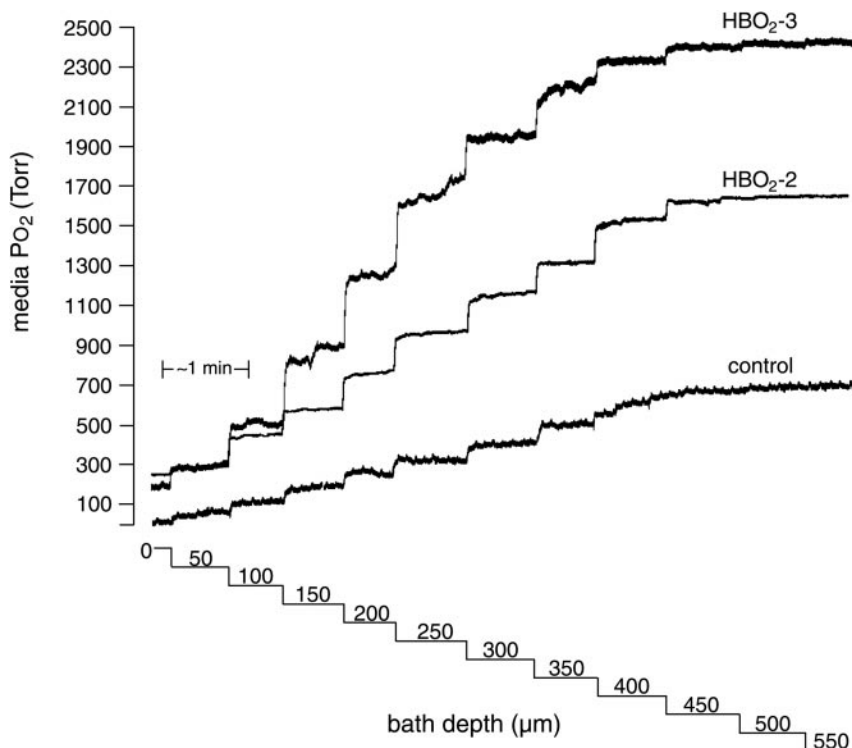


Fig. 3. An oxygen diffusion gradient was measured under control and hyperoxic conditions in the absence of a brain slice. Oxygen measurements were initiated while the oxygen electrode was positioned at the surface of the aCSF. Then, moving in 50- $\mu$ m increments, the electrode was driven into the aCSF, which flowed into the ~5-ml chamber at a rate of 2 ml/min. Medium  $P_{O_2}$  was lowest at the interface where  $O_2$  diffused into the gas phase of the overlying atmosphere. Medium  $P_{O_2}$  increased with depth reaching a maximum value at a depth of ~450  $\mu$ m for each medium. The results were similar for 2 additional trials using HBO<sub>2</sub>.

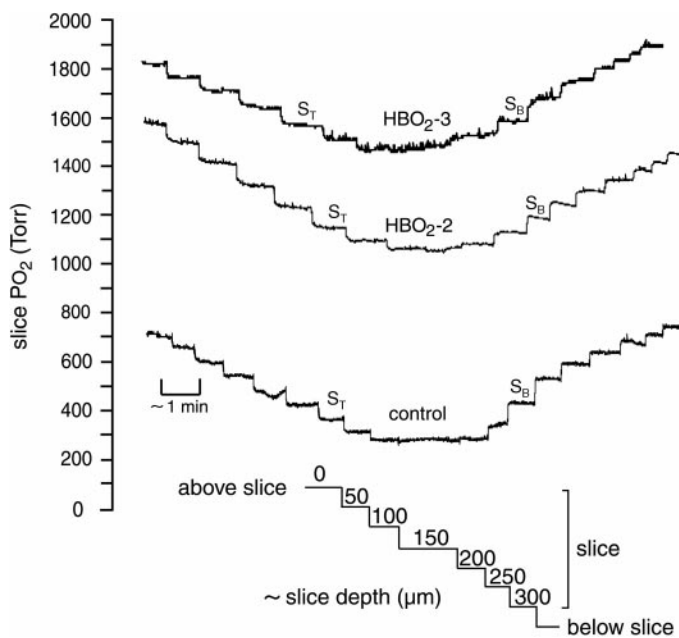


Fig. 4. Continual  $\text{PO}_2$  measurements were made through 300- $\mu\text{m}$ -thick brain slices equilibrated with control ( $\text{PO}_2 \sim 708$  Torr) or hyperoxic ( $\text{HBO}_2$ -2 and  $\text{HBO}_2$ -3) aCSF. For the top 2 traces, the perfusate was switched to hyperoxic medium and allowed to establish a new stable  $\text{PO}_2$  before the electrode was moved toward the tissue in 50- $\mu\text{m}$  steps. Profiles were initiated 200  $\mu\text{m}$  above the surface of slice. The designation “ $S_T$ ” signifies when the electrode was closest to the top surface of the slice; “ $S_B$ ” signifies when the electrode was closest to the slice bottom. With each step, the corresponding change in  $\text{PO}_2$  was allowed  $\sim 1$  min to stabilize before the next step. The depth scale under the  $\text{PO}_2$  traces corresponds to the control  $\text{PO}_2$  profile. Notice that  $\text{PO}_2$  declined above the slice and increased below the slice, thus indicating the presence of a diffusion layer at each surface of the slice.

influence oxygen diffusion into the chamber atmosphere. In contrast to Fig. 3,  $\text{PO}_2$  decreased in the aCSF as the oxygen electrode moved deeper into the bath toward the surface of the submerged tissue slice in Fig. 4, undoubtedly due to  $\dot{V}\text{O}_2$  by the slice. Once the oxygen electrode was in the slice,  $\text{PO}_2$  decreased further to a minimum at the slice core ( $\sim 150$   $\mu\text{m}$ ), after which tissue  $\text{PO}_2$  increased as the oxygen electrode approached the lower surface of the slice. Likewise,  $\text{PO}_2$  in the bath increased as the electrode moved away from the lower surface of the slice. This indicated that an oxygen diffusion layer in the aCSF ( $\sim 200$   $\mu\text{m}$  thick) was present around each surface of the tissue, with a magnitude roughly equivalent to the oxygen diffusion gradient in the first 100  $\mu\text{m}$  of tissue. Figure 5 summarizes the dynamics of oxygen diffusion in our submerged slice preparation and further illustrates the significant effect that these oxygen diffusion gradients have on setting  $\text{PO}_2$  in the aCSF and slice.

Tissue  $\text{PO}_2$  measured at the surface (0  $\mu\text{m}$ ) of the slice and at a maximum depth of 150  $\mu\text{m}$  were averaged to estimate mean tissue  $\text{PO}_2$  and plotted against medium  $\text{PO}_2$  in Fig. 6. Table 2 summarizes mean tissue  $\text{PO}_2$  plotted in Fig. 6 as well as values measured at a depth of 50 and 100  $\mu\text{m}$  into tissue. Increasing  $\text{PO}_2$  of the medium from 156 to 2,468 Torr increased tissue

$\text{PO}_2$  measured at 0 and 150  $\mu\text{m}$  depths proportionally; slopes of regression lines were  $0.65 \pm 0.02$  ( $r^2 = 0.998$ ,  $n = 38$ ) and  $0.66 \pm 0.04$  ( $r^2 = 0.997$ ,  $n = 38$ ) at the surface and core, respectively. However, mean tissue  $\text{PO}_2$  measured at 0 and 150  $\mu\text{m}$  were significantly different at medium  $\text{PO}_2$  values of 156, 708, and 1,200 Torr. As medium  $\text{PO}_2$  increased beyond 1,200 Torr, there was no statistical difference in mean  $\text{PO}_2$  measured at the tissue surface and tissue core. These results suggest slice  $\dot{V}\text{O}_2$  may have decreased during exposure to the higher levels of  $\text{HBO}_2$  (see *Metabolically Poisoned Tissue* below).

In our electrophysiological studies of how  $\text{HBO}_2$  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  $\text{PO}_2$ ; thus tissue  $\text{PO}_2$  measurements were made in tissue equilibrated with a constant control level of  $\text{PO}_2$  ( $\sim 708$  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  $\text{PO}_2$  was measured at a depth of 150  $\mu\text{m}$ . Results from these measurements are plotted in Fig. 6. Tissue  $\text{PO}_2$  at 2 and 3 ATA were not significantly different from  $\text{PO}_2$  measured at 1 ATA, and the slope of the regression line,  $0.04 \pm 0.04$  ( $r^2 = 0.772$ ,  $n = 3$ ), 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  $\text{PO}_2$  profiles were made in slices incubated at medium  $\text{PO}_2$  values ranging from 156 to 2,468 Torr in 2DG aCSF or 2DG plus antimycin A. This was done to determine how  $\dot{V}\text{O}_2$  affects slice  $\text{PO}_2$  (i.e., the magnitude of  $\text{PO}_2$  profiles between the slice surface and the core of the slice). The difference in  $\text{PO}_2$  measured at the slice surface (0  $\mu\text{m}$ ) to its core (150  $\mu\text{m}$ ) was defined as delta  $\text{PO}_2$  ( $\Delta\text{PO}_2$ ). By comparing the  $\Delta\text{PO}_2$  measured in metabolically active slices at different medium  $\text{PO}_2$  values with the same measurements made in metabolically poisoned slices, the  $\text{PO}_2$  dependence of  $\dot{V}\text{O}_2$  could be determined. Mean slice  $\text{PO}_2$  measured at 150  $\mu\text{m}$  in slices perfused with 2DG and 2DG plus antimycin A medium was linearly related to medium  $\text{PO}_2$ . 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  $\text{PO}_2$  was  $0.74 \pm 0.03$  ( $r^2 = 0.997$ ,  $n = 33$ ).

Figure 7A shows superimposed  $\text{PO}_2$  profiles, measured at 708 Torr, in regular and a metabolically poisoned brain slice. For comparison, mean  $\text{PO}_2$  values measured at depths of 0, 50, 100, and 150  $\mu\text{m}$  in metabolically poisoned slices are given in Table 2. In this example,  $\Delta\text{PO}_2$  was smaller in metabolically poisoned tissue ( $\Delta\text{PO}_2 = 66$  Torr) compared with metabolically active tissue ( $\Delta\text{PO}_2 = 116$  Torr). We considered the difference in  $\Delta\text{PO}_2$  between metabolically active tissue and 2DG tissue to be proportional to  $\dot{V}\text{O}_2$ . Consequently,  $\Delta\text{PO}_2$  in nonpoisoned tissue was used as an

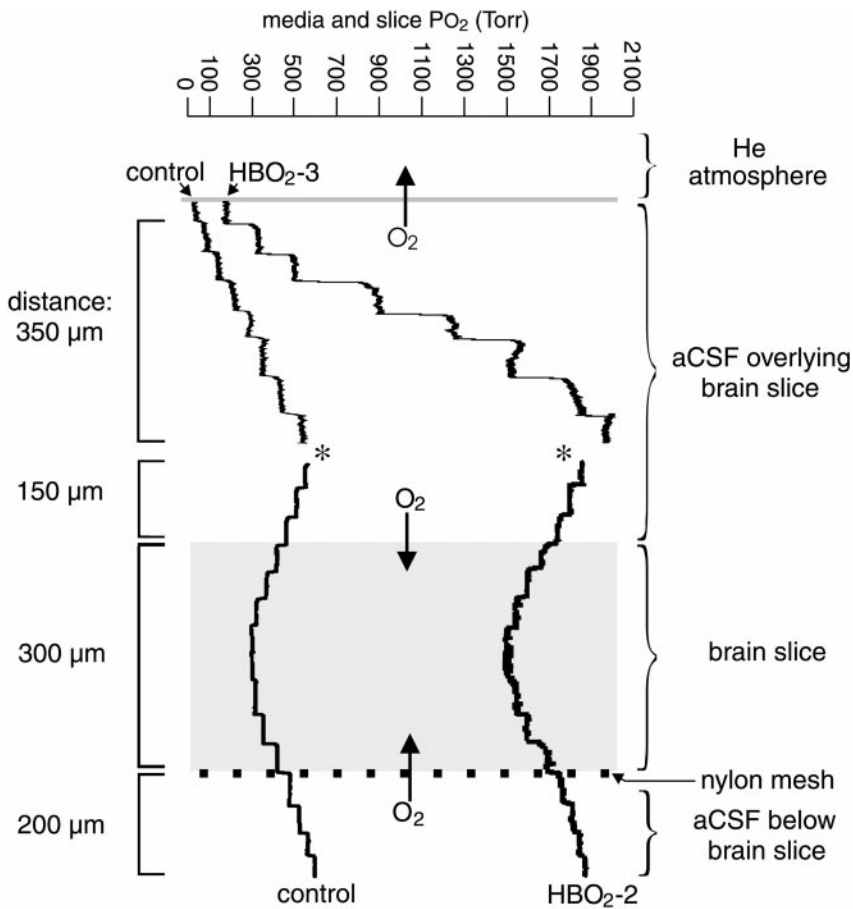


Fig. 5. PO<sub>2</sub> traces were plotted as a function of aCSF and slice depth. Traces of PO<sub>2</sub> made during control (708 Torr) and HBO<sub>2</sub>-3 (2,468 Torr) conditions show both PO<sub>2</sub> increasing with distance (50-μm steps) from the aCSF meniscus. As the electrode approached the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

indirect measure of  $\dot{V}O_2$  to gain insight as to how HBO<sub>2</sub> affects  $\dot{V}O_2$ . Mean  $\Delta PO_2$  values were measured in metabolically active and inactive tissue and plotted against medium PO<sub>2</sub> in Fig. 7B. Mean  $\Delta PO_2$  values measured in

metabolically poisoned slices did not vary significantly over the entire range of medium PO<sub>2</sub> values studied. This indicated that the effects of PO<sub>2</sub> on nonmetabolic forms of oxygen utilization (e.g., formation of ROS) were negli-

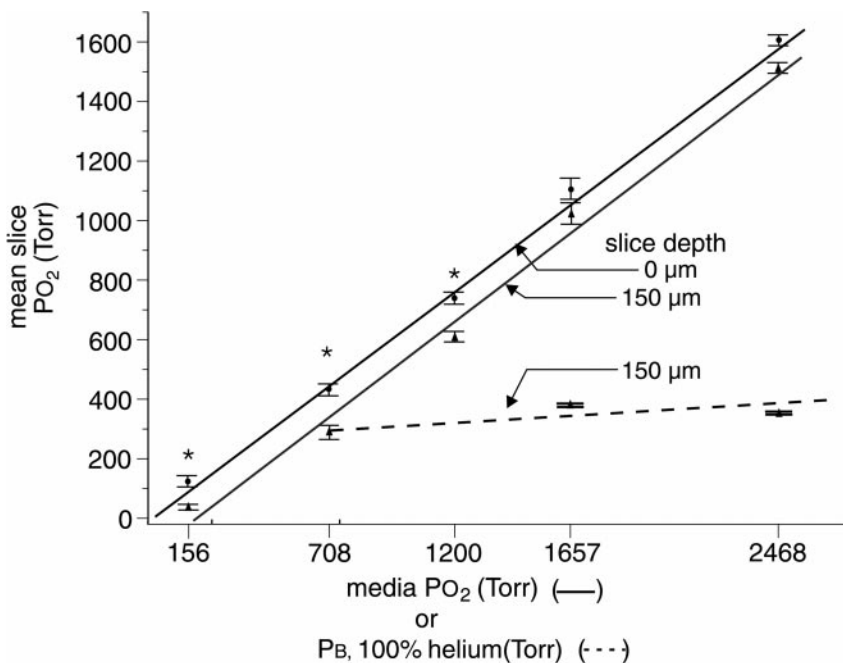


Fig. 6. Mean PO<sub>2</sub> ( $n = 3-13$ , see Table 1) measured in regular aCSF at the slice surface (0 μm) and at the center of the slice (150 μm) were plotted against media PO<sub>2</sub>. Oxygen tension measured at 0 μm differed significantly ( $*P < 0.05$ ) from values measured at 150 μm at PO<sub>2</sub> ≤ 1,200 Torr. At a constant PO<sub>2</sub> of ~708 Torr, slice PO<sub>2</sub> at 150 μm was measured in slices compressed with helium to PB of 1, 2, and 3 ATA, and these mean PO<sub>2</sub> values ( $n = 3$ ) were plotted as the dashed line. At ~708 Torr, PO<sub>2</sub> measured at 2 and 3 ATA were not significantly different from PO<sub>2</sub> measured at 1 ATA.

Table 2. Mean tissue  $P_{O_2}$  in regular aCSF and in aCSF with 2DG and antimycin A

Tissue Depth	Po <sub>2</sub> of Medium, Torr				
	156	708	1,200	1,657	2,468
<i>Regular aCSF</i>					
Surface	136 ± 17(3)	436 ± 21(13)	742 ± 21(3)	1,105 ± 40(8)	1,613 ± 16(6)
50 μm	79 ± 9(6)	368 ± 22(13)	700 ± 10(5)	1,058 ± 39(8)	1,570 ± 11(6)
100 μm	52 ± 9(4)	319 ± 24(13)	665 ± 18(5)	1,036 ± 41(8)	1,537 ± 13(6)
150 μm	40 ± 7*(6)	291 ± 20*(13)	616 ± 16*(5)	1,021 ± 40(8)	1,517 ± 15(6)
<i>2DG + antimycin A aCSF</i>					
Surface	117 ± 5(3)	607 ± 14(8)	955 ± 17(3)	1,207 ± 53(9)	1,828 ± 106(7)
50 μm	77 ± 12(6)	573 ± 16(8)	924 ± 22(3)	1,177 ± 53(9)	1,808 ± 107(7)
100 μm	49 ± 9(6)	550 ± 21(8)	893 ± 16(3)	1,158 ± 54(9)	1,783 ± 109(7)
150 μm	25 ± 9(6)	532 ± 23†(8)	876 ± 20†(3)	1,115 ± 47(11)	1,773 ± 107(7)

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

ble in this comparison. However,  $\Delta P_{O_2}$  measured in metabolically active slices at 708 Torr was significantly greater than  $\Delta P_{O_2}$  measured in metabolically poisoned slices; however, as medium  $P_{O_2}$  increased from 708 to 2,468 Torr,  $\Delta P_{O_2}$  values measured in metabolically active slices got smaller and more closely resembled profiles in metabolically poisoned slices, thus suggesting  $\dot{V}_{O_2}$  was reduced during exposure to HBO<sub>2</sub>.

The oxygen diffusion coefficient (*D*) and  $\dot{V}_{O_2}$  were calculated to better describe the dynamics of oxygen in our preparation over a broad range of  $P_{O_2}$  values as well as to quantify, if only by approximation, the relationship between  $\dot{V}_{O_2}$  and tissue  $P_{O_2}$  (see APPENDIX for details regarding the calculations of *D* and  $\dot{V}_{O_2}$ ). 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  $P_{O_2}$  over time when switching between two media with different non-zero  $P_{O_2}$  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  $1.3 \times 10^{-6}$  cm<sup>2</sup>/s. Our estimated *D* was smaller by about one-tenth than the *D* calculated from  $P_{O_2}$  measurements in 1,000-μm-thick slices of cat cortex equilibrated with a similar initial  $P_{O_2}$ ,  $1.54 \times 10^{-5}$  cm<sup>2</sup>/s (21).

$\dot{V}_{O_2}$  rate can be calculated from tissue oxygen profiles using Fick's second law of diffusion (20). With the use of boundary conditions of  $P_{O_2}$  at the surface of the slice (defined as  $P_0$ ) and at the bottom of the slice [defined as  $P_L$ ; thickness of the slice (*L*) = 300 μm], our slice  $P_{O_2}$  profiles at each medium  $P_{O_2}$  were fitted to the parabolic equation (20)

$$P_{O_2} = aX^2 - \left( aL + \frac{P_0 - P_L}{L} \right) x + P_0$$

where  $P_{O_2}$  is oxygen tension measured at depth *X* in a brain slice,  $a = \dot{V}_{O_2}/2DS$ , and *S* is the estimated solubility coefficient of oxygen in cat brain ( $1.89 \times 10^{-5}$  ml O<sub>2</sub>·cm<sup>-3</sup>·Torr<sup>-1</sup>) (21). Using our estimated *D* ( $1.3 \times 10^{-6}$  cm<sup>2</sup>/s), we calculated the  $\dot{V}_{O_2}$  (mean ± SE ml O<sub>2</sub>·cm<sup>-3</sup>·min<sup>-1</sup>) at each medium  $P_{O_2}$  to be  $1.4 \pm 0.17 \times 10^{-3}$  at 156 Torr,  $1.8 \pm 0.2 \times 10^{-3}$  at 708 Torr,

$1.9 \pm 0.32 \times 10^{-3}$  at 1,200 Torr,  $1.7 \pm 0.75 \times 10^{-3}$  at 1,657 Torr, and  $9.3 \pm 1.3 \times 10^{-4}$  at 2,468 Torr. Although  $\dot{V}_{O_2}$  at 1,200 and 1,657 Torr were not less than  $\dot{V}_{O_2}$  at 708 Torr (i.e.,  $\dot{V}_{O_2}$  and  $\Delta P_{O_2}$  were not well matched), a trend of decreasing  $\dot{V}_{O_2}$  was evident at 2,468 Torr. These results suggest  $\dot{V}_{O_2}$  was dependent on  $P_{O_2}$  during hyperoxia; therefore, we incorporated this assumption into our calculation of  $\dot{V}_{O_2}$  (see APPENDIX) and estimated  $\dot{V}_{O_2}$  at a constant depth by measuring the rate of change in  $P_{O_2}$  measured in a metabolically active slice exposed to different aCSF  $P_{O_2}$  values (8). Oxygen measurements were made at a constant depth of 150 μm in a brain slice while media  $P_{O_2}$  changed from ~708 to ~156 Torr or from ~708 to ~2,468 Torr. From these measurements, we estimated  $\dot{V}_{O_2}$  to be  $7.9 \times 10^{-5}$  and  $7.3 \times 10^{-6}$  ml O<sub>2</sub>·cm<sup>-3</sup>·min<sup>-1</sup>, respectively. Although the absolute values varied, both methods of determining  $\dot{V}_{O_2}$  showed that  $\dot{V}_{O_2}$  was consistently reduced under the more extreme hyperoxic conditions compared with control  $P_{O_2}$  values.

## DISCUSSION

Neuronal tissue  $P_{O_2}$  has been measured in the intact CNS during HBO<sub>2</sub> (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  $P_{O_2}$  gradients in brain slices during HBO<sub>2</sub>. We found that, under conventional brain slice control conditions (95% O<sub>2</sub>),  $P_{O_2}$  measured in the solitary complex decreased with increasing recording depth to a minimum  $P_{O_2}$  value at the core of the slice that was still ~10-fold higher than normal cerebral  $P_{O_2}$  in vivo, which has been reported to range from 10 to 34 Torr (Table 1). In fact, at an aCSF  $P_{O_2}$  of 708 Torr,  $P_{O_2}$  at the core of the slice approximated  $P_{O_2}$  measured in the CNS of rats breathing 100% oxygen at  $P_B$  of >2 ATA (27). Furthermore, tissue  $P_{O_2}$  increased linearly with aCSF  $P_{O_2}$  from 156 to 2,468 Torr, to levels that are known to result in CNS O<sub>2</sub> toxicity in whole animals (1, 25, 58). This range of HBO<sub>2</sub> has been reported to depolarize solitary complex neurons in brain stem slices after ≤10 min of exposure (12, 45, 46). At  $P_{O_2}$  values >1,200 Torr, the



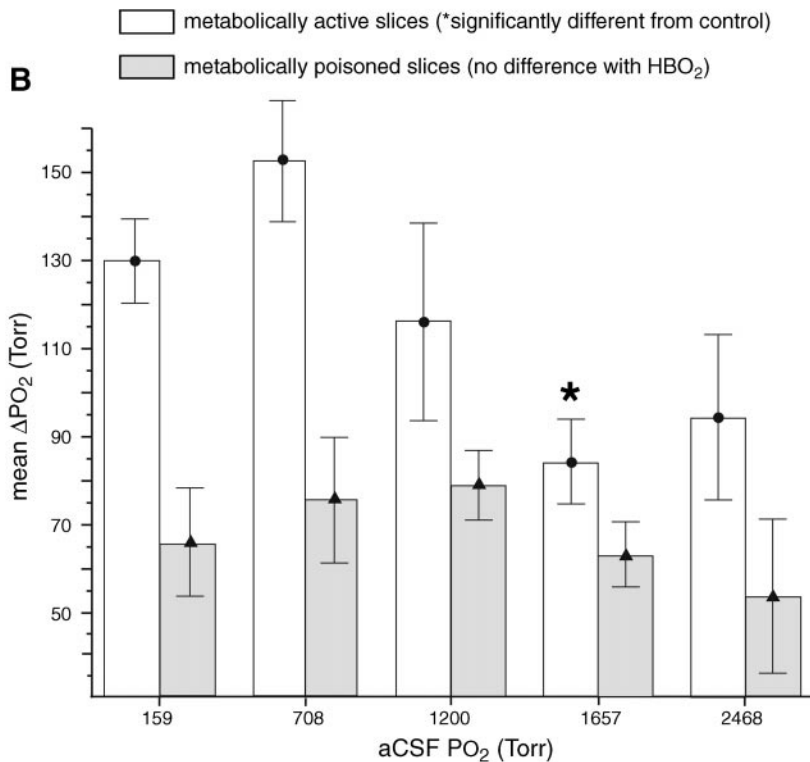
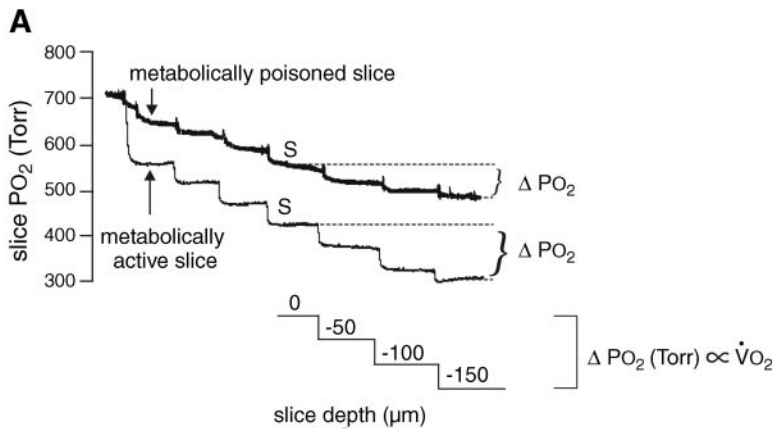


Fig. 7. A: 2 superimposed PO<sub>2</sub> profiles measured at ~708 Torr in a metabolically active and metabolically inactive slice. The difference in PO<sub>2</sub> from the slice surface (0 μm) to its core (150 μm) was defined as delta (Δ) PO<sub>2</sub>. B: ΔPO<sub>2</sub> measured in metabolically active tissue at a PO<sub>2</sub> of ~708 Torr was significantly greater ( $P < 0.05$ ) than ΔPO<sub>2</sub> in metabolically poisoned tissue at all PO<sub>2</sub> tested. During HBO<sub>2</sub>, the ΔPO<sub>2</sub> values measured in metabolically active brain slices were reduced to values that more closely resembled ΔPO<sub>2</sub> in metabolically poisoned slices. \*PO<sub>2</sub> measured in metabolically active slices at which the ΔPO<sub>2</sub> was significantly ( $P < 0.05$ ) smaller than ΔPO<sub>2</sub> measured at ~708 Torr. These results suggest that oxygen consumption is reduced by HBO<sub>2</sub>.

difference in PO<sub>2</sub> from the surface to tissue core (i.e., ΔPO<sub>2</sub>) decreased to the extent that ΔPO<sub>2</sub> measured in metabolically active tissue exposed to hyperoxic medium no longer differed from ΔPO<sub>2</sub> made in metabolically poisoned tissue. This difference was attributed to a reduction in  $\dot{V}O_2$ , suggesting that the higher levels of HBO<sub>2</sub> 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  $\dot{V}O_2$  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 PO<sub>2</sub> 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.

As was the case in previous studies (4, 19, 21), there was considerable variability in our  $P_{O_2}$  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  $P_{O_2}$  measurements,<sup>3</sup> 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  $P_{O_2}$ , 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  $P_B > 1$  ATA.

#### *P<sub>O<sub>2</sub></sub> Profiles in aCSF*

At the gas-liquid interface, oxygenated aCSF was in contact with an oxygen-free helium atmosphere. Oxygen, according to Le Châtelier's principle, will diffuse from the aCSF down its chemical gradient into the overlying chamber atmosphere, leaving behind a graded layer of  $P_{O_2}$  in the aCSF. As expected,  $P_{O_2}$  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  $\sim 450$   $\mu\text{m}$  regardless of the media  $P_{O_2}$ . In addition, our results indicate that submerged brain slices are oxygenated by the diffusion of oxygen from aCSF of approximately  $\pm 200$   $\mu\text{m}$  to the tissue surface. We observed that, at medium  $P_{O_2}$  values  $\geq 708$  Torr, oxygen diffusion into the brain slice resulted in a 35–40% drop in  $P_{O_2}$  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  $< 450$   $\mu\text{m}$  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  $> 450$   $\mu\text{m}$  deep to the bath surface.

#### *P<sub>O<sub>2</sub></sub> Profiles in Brain Slices*

Measurements of  $P_{O_2}$  through 300- $\mu\text{m}$ -thick brain slices exposed to medium  $P_{O_2}$  ranging from 156 to 2,468 Torr showed that, although oxygen tension decreased with increasing recording depth in tissue,  $P_{O_2}$  measured at the tissue surface (0  $\mu\text{m}$ ) and tissue core (150  $\mu\text{m}$ ) increased linearly as medium  $P_{O_2}$  increased. As expected, these results indicate that the oxygen diffusion coefficient in tissue did not change with medium  $P_{O_2}$  or diffusion distance. Furthermore, the magnitude of the oxygen gradient in aCSF from  $\leq 200$   $\mu\text{m}$  above or below the slice was roughly equivalent to the oxygen gradient in the outer 100- $\mu\text{m}$  layers of tissue. A similar observation was previously reported in the neonatal rat brain stem spinal cord preparation (52).

The majority of our tissue  $P_{O_2}$  profiles were symmetrical, with the minimum  $P_{O_2}$  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%  $O_2$ , reported that diffusion from the upper surface dominated and resulted in a minimum  $P_{O_2}$  near the bottom of the slice (28).

#### *Control P<sub>O<sub>2</sub></sub> at Normobaric Pressure*

When slices were submerged in aCSF equilibrated with 95%  $O_2$ , we measured a minimum  $P_{O_2}$  of  $291 \pm 83$  Torr at the center of the slice. A similar minimum  $P_{O_2}$  value of  $187.2 \pm 11$  Torr ( $n = 2$ ) was measured at a depth of 150  $\mu\text{m}$  in 320- $\mu\text{m}$ -thick guinea pig cortical slices submerged in aCSF equilibrated with 95%  $O_2$  at 1 ATA (19). Likewise, although variability between brain slice preparations and experimental parameters makes direct comparison of absolute slice  $P_{O_2}$  difficult, minimum control  $P_{O_2}$  values reported here were similar to values measured in 400- to 450- $\mu\text{m}$  thick brain slices positioned in the interface preparation; these values ranged from  $\sim 150$  to 280 Torr (28, 50).

Minimum  $P_{O_2}$  values measured in our slice preparation and in others (19, 28, 50) when incubated with conventional control solution (95%  $O_2$ ,  $P_B$  of 1 ATA) were  $\sim 10$ -fold greater than  $P_{O_2}$  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%  $O_2$  compared with 21%  $O_2$  at normobaric pressure (33, 54). Bingmann and colleagues (3, 5) found neurons in hippocampal slices incubated in  $\sim 21\%$   $O_2$  depolarized and increased their firing rate when exposed to 100%  $O_2$ , indicating that the high  $P_{O_2}$

<sup>3</sup>Lehmenkuhler et al. (37) reported that, when making  $P_{O_2}$  measurements in excitable tissue, slow tissue potential changes or direct current shifts resulting from oxygen-induced excitation may mimic changes in  $P_{O_2}$  by interfering with the polarizing voltage at the oxygen electrode.

of brain slice control medium (i.e., normobaric hyperoxia) can, in fact, alter cellular activity. The activity of hippocampal neurons in 21% O<sub>2</sub> was not considered a response to hypoxia (in the brain slice preparation, hypoxia is typically mimicked by F<sub>O<sub>2</sub></sub> values of 10–15% O<sub>2</sub> at P<sub>B</sub> of 1 ATA), however, because the activity of hippocampal neurons exposed to hypoxia was quite different (15, 36). Normobaric hyperoxia 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 P<sub>O<sub>2</sub></sub> has proven to be important for thin tissue preparations like neuronal cell cultures. For example, in cultures of neocortical and hippocampal neurons, the optimal medium P<sub>O<sub>2</sub></sub>, based on cell growth and viability, was determined to be 9% O<sub>2</sub> at P<sub>B</sub> 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 incubation medium (7). These results suggest that the optimal medium P<sub>O<sub>2</sub></sub> for an in vitro tissue preparation must balance tissue oxygen requirements with the otherwise toxic oxidative effects of excess oxygen.

Clearly, medium P<sub>O<sub>2</sub></sub> affects both neuronal viability and excitability. For this reason, it is important that in vitro experimental conditions match, as closely as possible, in vivo conditions (i.e., optimum F<sub>O<sub>2</sub></sub> of the perfusion medium should produce a P<sub>O<sub>2</sub></sub> at the core of the brain slice that ranges between 10 and 34 Torr). In our study, when medium P<sub>O<sub>2</sub></sub> was reduced from control to 21% O<sub>2</sub>, although slice surface P<sub>O<sub>2</sub></sub> was still hyperoxic, the minimum P<sub>O<sub>2</sub></sub> values, which averaged  $40 \pm 17$  Torr, more closely resembled P<sub>O<sub>2</sub></sub> values measured in the CNS and CSF of whole animals (Table 1). Alternatively, antioxidants can be added to the medium to provide protection from ROS when using 95% O<sub>2</sub> (6, 7, 34). Subsequent electrophysiological studies of solitary complex neurons in 300- $\mu$ m-thick brain slices are required to confirm, however, that cells remain viable and healthy in this preparation at this lower level of control P<sub>O<sub>2</sub></sub>.

### *Metabolically Poisoned Tissue*

$\dot{V}_{O_2}$  is another important factor that must be considered when determining the optimum brain slice control P<sub>O<sub>2</sub></sub>. Bingmann et al. (4) reported that cellular  $\dot{V}_{O_2}$  increased when the incubation medium P<sub>O<sub>2</sub></sub> of 300- $\mu$ m-thick hippocampal slices increased from 150 to 600 Torr. The authors suggested that, at normobaric pressure and a medium P<sub>O<sub>2</sub></sub> of 150 Torr, cell respiration was limited by oxygen availability such that an increase in medium P<sub>O<sub>2</sub></sub> resulted in an increase in  $\dot{V}_{O_2}$ . However, the extent to which brain slice  $\dot{V}_{O_2}$  is directly dependent on medium P<sub>O<sub>2</sub></sub> is not known under conditions of HBO<sub>2</sub>. For these reasons, we measured P<sub>O<sub>2</sub></sub> in brain slices equilibrated with aCSF having P<sub>O<sub>2</sub></sub> values that ranged from 156 to 2,468 Torr; then, for compar-

ison, P<sub>O<sub>2</sub></sub> measurements were repeated in metabolically poisoned tissue equilibrated with the same range of P<sub>O<sub>2</sub></sub> values. Oxygen profiles in metabolically active slices showed that, as P<sub>O<sub>2</sub></sub> increased from 708 to 1,657 Torr, the  $\Delta$ P<sub>O<sub>2</sub></sub> approached a minimum, at 1,657 Torr, that was significantly different from the  $\Delta$ P<sub>O<sub>2</sub></sub> at 708 Torr and not different from  $\Delta$ P<sub>O<sub>2</sub></sub> values in metabolically poisoned tissue. At P<sub>O<sub>2</sub></sub> greater than 1,657 Torr,  $\Delta$ P<sub>O<sub>2</sub></sub> values of oxygen profiles remained similar in magnitude to  $\Delta$ P<sub>O<sub>2</sub></sub> values made in metabolically poisoned tissue. In addition, calculated  $\dot{V}_{O_2}$  under control conditions (95% O<sub>2</sub>) was  $1.8 \pm 0.2 \times 10^{-3}$  ml O<sub>2</sub>·cm<sup>-3</sup>·min<sup>-1</sup>. A comparable  $\dot{V}_{O_2}$  of  $3.38 \pm 0.31 \times 10^{-2}$  ml O<sub>2</sub>·cm<sup>-3</sup>·min<sup>-1</sup> was measured in 500- $\mu$ m-thick slices of guinea pig olfactory cortex equilibrated with 95% O<sub>2</sub> (20). During HBO<sub>2</sub>-3,  $\dot{V}_{O_2}$  was reduced to  $9.3 \pm 1.3 \times 10^{-4}$  ml O<sub>2</sub>·cm<sup>-3</sup>·min<sup>-1</sup>. Furthermore, although absolute  $\dot{V}_{O_2}$  values varied, this trend was maintained when  $\dot{V}_{O_2}$  was assumed to be dependent on medium P<sub>O<sub>2</sub></sub> (see APPENDIX). Together, these results suggest that the higher levels of HBO<sub>2</sub> reduced metabolism in 300- $\mu$ m-thick slices.

The mechanism by which HBO<sub>2</sub> may reduce brain slice metabolism is not clear but likely involves the increased production of ROS and the oxidation of mitochondrial enzymes and/or cofactors, including  $\alpha$ -lipoic acid, cytochrome *c*, flavin nucleotides, and ubiquinone (1, 11). Furthermore, neuronal responses to HBO<sub>2</sub> depend on the duration of the HBO<sub>2</sub> exposure. Previous electrophysiological recordings show that short ( $\leq 10$  min) bouts of HBO<sub>2</sub> increase neuronal activity (12, 45, 46); it is well known that  $\dot{V}_{O_2}$  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 HBO<sub>2</sub> actually reduced  $\dot{V}_{O_2}$ . Future studies focusing on the details regarding the dose dependence of HBO<sub>2</sub> sensitivity may be necessary.

In conclusion, oxygen tension in the submerged brain slice during normobaric hyperoxia and HBO<sub>2</sub> was a complex function that was dependent on several experimental conditions, including ambient P<sub>O<sub>2</sub></sub>, depth of slice in the tissue bath, and  $\dot{V}_{O_2}$ . Our findings show that P<sub>O<sub>2</sub></sub> in the solitary complex of the 300- $\mu$ m-thick brain slice submerged in control medium (95% O<sub>2</sub> at P<sub>B</sub> of ~1 ATA) was hyperoxic compared with the in vivo CNS. When exposed to HBO<sub>2</sub>, tissue P<sub>O<sub>2</sub></sub> increased to oxygen tensions that corresponded with cerebral P<sub>O<sub>2</sub></sub> values measured in vivo under conditions that result in symptoms of CNS O<sub>2</sub> toxicity (1, 25, 58). Our results also suggest that metabolism decreased during high levels of HBO<sub>2</sub>, which was consistent with previous observations (1, 11) and suggests that there may be a metabolic component to the mechanism of HBO<sub>2</sub>-induced 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)

$$PO_2 \approx P_1 + \frac{4(P_0 - P_1)}{\pi} \sin\left(\frac{\pi X}{L}\right) \exp\left[-D\left(\frac{\pi}{L}\right)^2 t\right]$$

where  $P_0$  is the measured  $PO_2$  at a depth of 150  $\mu\text{m}$  in tissue equilibrated with a medium  $PO_2$  of 708 Torr,  $P_1$  is the measured  $PO_2$  at a depth of 150  $\mu\text{m}$  in tissue equilibrated with a medium  $PO_2$  of 156 Torr,  $PO_2$  is calculated to be the mean of  $P_0$  and  $P_1$ ,  $X$  is the recording depth (150  $\mu\text{m}$ ),  $L$  is tissue thickness (300  $\mu\text{m}$ ), and  $t$  is time, in seconds, to reach  $PO_2$ . We calculated  $D$  at a  $PO_2$  halfway between two steady-state conditions because, presumably,  $O_2$  flux would be maximum. We estimated  $D$  to be  $1.3 \times 10^{-6} \text{ cm}^2/\text{s}$ . Our estimated  $D$  was smaller than the  $D$  calculated from  $PO_2$  measurements in 1,000- $\mu\text{m}$ -thick slices of cat cortex equilibrated with a similar initial  $PO_2$ ,  $1.54 \times 10^{-5} \text{ cm}^2/\text{s}$  (21).

Our results suggest that slice  $\dot{V}O_2$  was dependent on  $PO_2$  of the bathing medium. By assuming that  $\dot{V}O_2$  was dependent on  $PO_2$ , we approximated  $\dot{V}O_2$  from  $PO_2$  measurements made at a depth of 150  $\mu\text{m}$  in a brain slice equilibrated with regular aCSF while switching medium  $PO_2$  from 708 to 156 or 2,468 Torr. With the same boundary conditions as before,  $\dot{V}O_2$  was approximated graphically from the equation (8)

$$\phi(\beta) \approx -PO_2 + \left(\frac{2P_1}{\sinh(\beta L)}\right) \sinh\left(\frac{\beta L}{2}\right) + \left[\frac{4P_0}{\pi} - \frac{4\pi P_1}{\pi^2 + (\beta L)^2} \cosh\left(\frac{\beta L}{2}\right)\right] \exp\left[-D\left(\frac{\pi}{L}\right)^2 t\right]$$

where  $\phi(\beta) = \beta = \sqrt{\dot{V}O_2/D}$ ,  $P_0$  is the measured  $PO_2$  at a depth of 150  $\mu\text{m}$  into a slice equilibrated with a medium  $PO_2$  of 708 Torr,  $P_1$  is the measured  $PO_2$  at a depth of 150  $\mu\text{m}$  into a slice equilibrated with either 156 or 2,468 Torr,  $PO_2$  is equal to the mean of  $P_0$  and  $P_1$ ,  $t$  is time, in seconds, to reach  $PO_2$ , and  $D$  is  $1.3 \times 10^{-6} \text{ cm}^2/\text{s}$ . To convert  $\dot{V}O_2$  to units of milliliters  $O_2$  per cubic centimeters per minute, the approximated  $\dot{V}O_2$  was multiplied by the oxygen solubility coefficient of cat brain,  $0.0144 \text{ ml } O_2 \cdot \text{cm}^3 \text{ tissue}^{-1} \cdot \text{atm}^{-1}$  (21). Switching medium  $PO_2$  from 708 to 156 or from 708 to 2,468 Torr resulted in estimated  $\dot{V}O_2$  of  $7.9 \times 10^{-5}$  or  $7.3 \times 10^{-6} \text{ ml } O_2 \cdot \text{cm}^3 \text{ tissue}^{-1} \cdot \text{min}^{-1}$ , respectively.

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## REFERENCES

1. **Balentine JD.** *Pathology of Oxygen Toxicity*. New York: Academic, 1982.
2. **Bennett P, Papahadjopoulos D, and Bangham A.** The effect of raised pressure of inert gases on phospholipid membranes. *Life Sci* 6: 2527–2533, 1967.
3. **Bingmann D and Kolde G.** Burst activity elicited in hippocampal slices by a rise of  $PO_2$ . *Pflügers Arch* 382: R38, 1979.
4. **Bingmann D and Kolde G.**  $PO_2$ -profiles in hippocampal slices of the guinea pig. *Exp Brain Res* 48: 89–96, 1982.
5. **Bingmann D, Kolde G, and Speckmann EJ.** Effects of elevated  $PO_2$  values in the superfusate on the neural activity in hippocampal slices. In: *Physiology and Pharmacology of Epilep-*
6. **Brahma B, Forman RE, Stewart EE, Nicholson C, and Rice ME.** Ascorbate inhibits edema in brain slices. *J Neurochem* 74: 1263–1270, 2000.
7. **Brewer GJ and Cotman CW.** Survival and growth of hippocampal neurons in defined medium at low density: advantages of a sandwich culture technique or low oxygen. *Brain Res* 494: 65–74, 1989.
8. **Carslaw HS and Jaeger JC.** *Conduction of Heat in Solids* (2nd ed.). London: Oxford University Press, 1959, p. 99–100.
9. **Cater D, Garattini S, Marina F, and Silver I.** Changes of oxygen tension in brain and somatic tissues induced by vasodilator and vasoconstrictor drugs. *Proc R Soc B* 155: 136–157, 1961.
10. **Cater D, Silver I, and Wilson G.** Apparatus and technique for the quantitative measurement of oxygen tension in living tissues. *Proc R Soc B* 151: 256–276, 1959.
11. **Chance B and Boveris A.** Hyperoxia and hydroperoxide metabolism. In: *Extrapulmonary Manifestations of Respiratory Disease. Lung Biology in Health and Disease*, edited by Robin E. New York: Marcel Dekker, 1978, vol. 8, p. 185–238.
12. **Dean JB and Mulkey DK.** Continuous intracellular recording from mammalian neurons exposed to hyperbaric helium, oxygen, or air. *J Appl Physiol* 89: 807–822, 2000.
13. **Dean JB, Mulkey DK, and Arehart JT.** Details on building a hyperbaric chamber for intracellular recording in brain tissue slices. Supplemental material from *J Appl Physiol*. [online] <http://jap.physiology.org/cgi/content/full/89/2/807/DC1>.
14. **Dekin MS, Getting PA, and Johnson SMB.** In vitro characterization of neurons in the ventral part of the nucleus tractus solitarius. I. Identification of neuronal types and repetitive firing properties. *J Neurophysiol* 58: 195–214, 1987.
15. **Donnelly D, Jiang C, and Haddad G.** Comparative responses of brain stem and hippocampal neurons to  $O_2$  deprivation: in vitro intracellular studies. *Am J Physiol Lung Cell Mol Physiol* 262: L549–L554, 1992.
16. **Etzion Y and Grossman Y.** Spontaneous  $Na^+$  and  $Ca^{2+}$  spike firing of cerebellar Purkinje neurons at high pressure. *Pflügers Arch* 437: 2276–2284, 1999.
17. **Fatt I.** *Polarographic Oxygen Sensors*. Boca Raton, FL: CRC, 1976, p. 63–96, 121–134.
18. **Feldman JL and Ellenberger HH.** Central coordination of respiratory and cardiovascular control in mammals. *Annu Rev Physiol* 50: 593–606, 1988.
19. **Fujii T, Baumgartl H, and Lubbers D.** Limiting section thickness of guinea pig olfactory cortical slices studied from tissue  $PO_2$  values and electrical activities. *Pflügers Arch* 393: 83–87, 1982.
20. **Fujii T, Buerk D, and Whalen W.** Activation energy in the mammalian brain slice as determined by oxygen micro-electrode measurements. *Jpn J Physiol* 31: 279–283, 1981.
21. **Ganfield R, Nair P, and Whalen W.** Mass transfer, storage, and utilization of  $O_2$  in cat cerebral cortex. *Am J Physiol* 219: 814–821, 1970.
22. **Gozal D.** Potentiation of hypoxic ventilatory response to hyperoxia in the conscious rat: putative role of nitric oxide. *J Appl Physiol* 85: 129–132, 1998.
23. **Gulati SC, Sood SC, Baili IM, and Kak VK.** Cerebral metabolism following brain injury I Acid-base and  $PO_2$  changes. *Acta Neurochir (Wien)* 53: 239–246, 1980.
24. **Hunt R, Blackburn J, Ogilvie R, and Balentine D.** Oxygen tension in the globus pallidus and neostriatum of unanesthetized rats during exposure to hyperbaric oxygen. *Exp Neurol* 62: 698–707, 1978.
25. **Jain KK.** *Textbook of Hyperbaric Medicine*. New York: Kirkland: Hogrefe and Huber, 1996, p. 63–78.
26. **Jamieson D.** Oxygen toxicity and reactive oxygen metabolites in mammals. *Free Radic Biol Med* 7: 87–108, 1989.
27. **Jamieson D and Van den Brenk HAS.** Measurement of oxygen tensions in cerebral tissues of rats exposed to high pressures of oxygen. *J Appl Physiol* 18: 869–876, 1962.

28. **Jiang C, Agulian S, and Haddad G.** O<sub>2</sub> tension in adult and neonatal brain slices under several experimental conditions. *Brain Res* 568: 159–164, 1991.
29. **Kaniuga Z, Bryla J, and Slater E.** Inhibitors around the antimycin-sensitive site in the respiratory chain. In: *Inhibitors, Tools in Cell Research*, edited by Bucher T and Sies H. New York: Springer-Verlag, 1969, p. 282–300.
30. **Kaplan R, Brighton C, Boytim M, Selzer M, Lee V, Spindler K, Silberberg D, and Black J.** Enhanced survival of rat neonatal cerebral cortical neurons at subatmospheric oxygen tensions in vitro. *Brain Res* 384: 199–203, 1986.
31. **Kendig JJ.** Nitrogen narcosis and pressure reversal of anesthetic effects in node of Ranvier. *Am J Physiol Cell Physiol* 246: C91–C95, 1984.
32. **Kohling R, Greiner C, Wolfer J, Wassmann H, and Speckmann E.** Optical monitoring of PO<sub>2</sub> changes and simultaneous recording of bioelectric activity in human and animal brain slices. *J Neurosci Methods* 85: 181–186, 1998.
33. **Kovachich G and Mishra O.** Lipid peroxidation in rat brain cortical slices as measured by the thiobarbituric acid test. *J Neurochem* 35: 1449–1452, 1980.
34. **Kovachich G and Mishra O.** The effect of ascorbic acid on malonaldehyde formation, K<sup>+</sup>, Na<sup>+</sup> and water content of brain slices. *Exp Brain Res* 50: 62–68, 1983.
35. **Kramer J, Nolan P, and Waldrop T.** In vitro responses of neurons in the periaqueductal gray to hypoxia and hypercapnia. *Brain Res* 835: 2197–203, 1999.
36. **Krnjevic K.** Early effect of hypoxia on brain cell function. *Croatian Med J* 40: 375–380, 1999.
37. **Lehmenkuhler A, Caspers H, and Speckmann EJ.** A method for simultaneous measurements of bioelectric activity and local tissue PO<sub>2</sub> in the CNS. In: *O<sub>2</sub> Transport to Tissue II*, edited by Grote RD, Reneau D, and Theus G. New York: Plenum, 1976, p. 3–7.
38. **Liberzon I, Arieli R, and Kerem D.** Attenuation of hypoxic ventilation by hyperbaric O<sub>2</sub>: effects of pressure and exposure time. *J Appl Physiol* 66: 851–856, 1989.
39. **Lipinski H and Bingmann D.** Diffusion in slice preparations bathed in unstirred solutions. *Brain Res* 473: 26–34, 1987.
40. **Lipton P and Whittingham T.** Energy metabolism and brain slice function. In: *Brain Slices*, edited by R. Dingledine. New York: Plenum, 1984, p. 113–153.
41. **Marsden CA, Joseph MH, Kruk ZL, Maidment NT, O'Neill RD, Schenk JO, and Stamford JA.** In vivo voltametry—present electrodes and methods. *Neuroscience* 25: 389–400, 1988.
42. **Mcilwain H.** Introduction: cerebral subsystems as biological entities. In: *Brain Slices*, edited by R. Dingledine. New York: Plenum, 1983, p. 1–5.
43. **Mokashi A and Lahiri S.** Aortic and carotid body chemoreception in prolonged hyperoxia in the cat. *Respir Physiol* 86: 233–243, 1991.
44. **Mulkey DK and Dean JB.** Effects of hyperbaric pressure on membrane potential of solitary complex neurons in rat brainstem slices (Abstract). *FASEB J* 13: A825, 1999.
45. **Mulkey DK and Dean JB.** Hyperbaric pressure and hyperbaric oxygen independently affect membrane potential of solitary complex neurons in rat brainstem slices (Abstract). *FASEB J* 14: A713, 2000.
46. **Mulkey DK, Henderson RA III, and Dean JB.** Hyperbaric oxygen depolarizes solitary complex neurons in tissue slices of rat medulla oblongata. In: *Oxygen Sensing: From Molecule to Man*, edited by Lahiri S, Prabhakar NR, and Forster II RE. New York: Kluwer Academic/Plenum, 2000, chapt. 46, p. 465–476.
47. **Mulkey DK, Henderson RA III, and Dean JB.** Oxygen tension profiles in nucleus tractus solitarius (NTS) in brain slices exposed to normobaric and hyperbaric oxygen (Abstract). *Soc Neurosci Abstr* 26: 425, 2000.
48. **Mulkey DK, Reich JL, and Dean JB.** Comparison of neuronal barosensitivity in the solitary complex to 1 to 8 atmospheres of helium or air (Abstract). *FASEB J* 13: A825, 1999.
49. **Nicoll RA and Madison DV.** General anesthetics hyperpolarize neurons in the vertebrate central nervous system. *Science* 217: 1055–1057, 1982.
50. **Nolan PC and Waldrop TG.** Ventrolateral medullary neurons show age-dependent depolarizations to hypoxia in vitro. *Dev Brain Res* 91: 111–120, 1996.
51. **Oglivie RW and Balentine DJ.** Oxygen tension in spinal cord gray matter during exposure to hyperbaric oxygen. *J Neurosurg* 43: 156–161, 1975.
52. **Okada Y, Mchenhoff K, Holtermann G, Acker H, and Scheid P.** Depth profiles of pH and PO<sub>2</sub> in the isolated brain stem-spinal cord of the neonatal rat. *Respir Physiol* 93: 315–326, 1993.
53. **Parmentier JL, Shrivastav BB, Bennett PB, and Wilson KM.** Effect of interaction of volatile anesthetics and high hydrostatic pressure on central neurons. *Undersea Biomed Res* 6: 75–91, 1979.
54. **Pellmar T.** Electrophysiological correlates of peroxide damage in guinea pig hippocampus in vitro. *Brain Res* 364: 377–381, 1986.
55. **Shibata M and Blatteis CM.** High perfusate PO<sub>2</sub> impairs thermosensitivity of hypothalamic thermosensitive neurons in slice preparations. *Brain Res Bull* 26: 467–471, 1991.
56. **Speckmann EJ, Bingmann D, Lehmenkuhler A, and Lipinski HG.** Changes of the bioelectrical activity and extracellular micromilieu in the central nervous system during variations of local oxygen pressure. In: *Oxygen Sensing in Tissue*, edited by Acker H. New York: Springer-Verlag, 1988, p. 179–191.
57. **Torbati D, Mokashi A, and Lahiri S.** Effects of acute hyperbaric oxygenation on respiratory control in cats. *J Appl Physiol* 67: 2351–2356, 1989.
58. **Van den Brenk HAS and Jamieson D.** Brain damage and paralysis in animals exposed to high pressure oxygen-pharmacological and biochemical observations. *Biochem Pharmacol* 13: 165–182, 1964.
59. **Wann KT and Southan AP.** The action of anesthetics and high pressure on neuronal discharge patterns. *Gen Pharmacol* 23: 993–1004, 1992.
60. **Weast R and Astle MJ (Eds.).** Pressure of aqueous vapor. In: *Handbook of Chemistry and Physics*. Boca Raton, FL: CRC, 1978, vol. 59, p. D232.
61. **Zhang J, Sam A, Klitzman B, and Piantadosi C.** Inhibition of nitric oxide synthase on brain oxygenation in anesthetized rats exposed to hyperbaric oxygen. *Undersea Hyperb Med* 22: 377–382, 1995.