Increased brain capillaries in chronic hypoxia


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Boero, Jaime A., Jonathan Ascher, Alberto Arregui, Carl Rovainen, and Thomas A. Woolsey. Increased brain capillaries in chronic hypoxia. J. Appl. Physiol. 86(4): 1211–1219, 1999.—The effect of chronic hypobaric hypoxia (28 days, 455 Torr) on the organization of brain vessels was studied in Balb/c mice. In comparison to age-matched controls kept at sea level, emulsion-perfused capillaries in hypoxic mice showed marked dilation in all brain areas studied. Capillary length per unit volume of tissue \( L_v \) was increased in the cerebellar granular layer, the cedate nucleus, the globus pallidus, the substantia nigra, the superior colliculus, and the dentate gyrus. There was a selective increase of \( L_v \) in the hippocampus (CA1 strata pyramidale and lacunosum and CA3 strata pyramidale and oriens) and in somatosensory cortex layers V and VI, motor cortex layers II, III, V, and VI, and auditory cortex layers II and III. An increase in capillary surface area per unit volume of tissue was also determined in several brain areas, including layer IV of somatosensory cortex, where \( L_v \) was not significantly increased. The \( O_2 \) diffusion conductance and \( P_{O_2} \) in the tissues were estimated with a mathematical model. The remodeling of capillary diameter and length during chronic hypoxia accounts for the significant increase of \( O_2 \) conductance to neural tissues. Also the estimated tissue \( P_{O_2} \) in chronic brain hypoxia is markedly increased in the cedate nucleus and the substantia nigra compared with acute hypoxia. These results suggest that formation of new capillaries is an important mechanism to restore the \( O_2 \) deficit in chronic brain hypoxia and that local rates of energy utilization may influence angiogenesis in different areas of the brain.

whisker barrels; capillary remodeling

OXYGEN DIFFUSION through brain capillaries is a critical step in the energy metabolism of neurons. \( P_{O_2} \) in the blood, the effective vascular surface of gas exchange, and its utilization by the brain are critical parameters involved in this process. When \( O_2 \) availability is reduced, as in acute mild-to-moderate hypoxia, an increase of blood flow helps restore the overall delivery of \( O_2 \) to the brain. However, if the hypoxic insult persists, polycythemia and brain angiogenesis develop (7, 11, 12, 22–25, 28), and this suggests that the initial compensatory mechanisms may not be sufficient to sustain a normal \( P_{O_2} \). Although several studies have shown that capillary networks were enlarged in chronic hypoxia, capillary length and surface areas have been quantitatively evaluated only in normoxic conditions (2). In addition, regional differences in brain energy metabolism suggest that marked variations in angiogenesis might develop (18). We hypothesized that changes in the capillary length and surface area per unit volume of tissue as well as a reduction of the intercapillary distances (ICD) might also reflect the heterogeneity of energy utilization among different layers and groups of neurons in the brain. The evaluation of these parameters is required to estimate the effectiveness of angiogenesis to restore \( P_{O_2} \) in small regions of the brain during chronic hypoxia. To answer these questions, we studied the vascular architecture of young adult mice exposed to chronic hypoxia for 4 wk and determined the changes induced in capillary length, surface area, internal diameters, and ICD of several brain regions. A mathematical model was used to estimate \( O_2 \) diffusion conductances (\( E_\nu \)) and tissue tensions.

METHODS

Animals

Seven 21-day-old male Balb/c mice were maintained in a hypobaric chamber at 455 Torr for 28 days, as described previously (4, 30). The animals remained in the chamber for 23 h/day; they descended to sea level for 1 h for cleaning and feeding. Littermate controls (n = 7) were housed at sea level outside the chamber in similar conditions (755 Torr). Food and water were provided ad libitum, and temperature was maintained at 20°C.

Tissue Processing

After 4 wk, control and experimental animals were deeply anesthetized with pentobarbital sodium (40 mg/kg ip) and perfused through the heart with use of a Masterflex pump at 1 ml/min, first with saline heparin (1 U/ml)-1 mM EDTA for 10 min, then with PLP (0.01 M sodium m-periodate, 0.075 M lysine, 2% paraformaldehyde, 0.073 M sodium phosphate) for 10 min, and finally with Kodak NTB 2 photographic emulsion (International Biotechnologies, New Haven, CT) (6) for 10 min. All the solutions were kept at 37°C during perfusion. The brains were carefully removed, placed in cold PLP-30% sucrose to sink, and stored at 4°C until processed. Serial coronal frozen sections were cut at 50 μm, mounted on chrome-alum-subbed slides, developed photographically to visualize the emulsion-filled vessels (5), stained with 1% thionin, dehydrated, cleared in xylol, and coverslipped.

Image Analysis

Images of the capillaries were obtained by video microscopy (37) and digitized with a capture card (Scion, Frederick, MD) in a Macintosh IIx computer (Apple, Cupertino, CA). The software Image 1.31 (developed by Dr. Wayne Rasband, National Institutes of Health, Bethesda, MD) was used to obtain measurements of the capillary length per unit volume of tissue \( (L_v) \), diameters, and the projected areas of the capillary plexuses. These measurements were performed in regions containing capillaries completely filled with the emulsion. The length of all the capillaries and their diameters within a selected area were drawn by hand on the computer screen, and the length was calculated by converting the pixels to micrometers according to calibrated scales that were digi-
Parameters Measured and Calculated

$L_v$ (mm/mm³), capillary surface area ($S_v$, mm²/mm³), and ICD (µm) were used to determine the capillary density. Capillary diameters were averaged for each brain region from 40 measurements perpendicular to the longitudinal axis of the vessels. $L_v$ was calculated from the total projected length of capillaries ($\Sigma L_v$) within the volume of the sections (volume = section thickness (T) × area (A)) by the equation $L_v = 4\Sigma L_v/\pi TA$ (29). Because $L_v$ varies greatly between adjacent layers in the cortex and other brain nuclei, we limited our measurements of $L_v$ within the anatomic boundaries of these regions. The correction factor $4/\pi$ assumes random orientation of capillaries in the tissue. $S_v$ was estimated from the projected areas of capillaries ($\Sigma A_v$). $\Sigma A_v$ was measured in pixels by using a threshold that filled all the capillaries with the program Image 1.31. $S_v$ was calculated as follows: $S_v = 4\Sigma A_v/TA$ (29). To evaluate changes in the ICD, the diameter of the Krogh cylinder (2R) can be used as an estimation of this parameter. $R$, the Krogh radius, was estimated as follows: $R = 1/\sqrt{\pi L_v}$.

Model

A model to estimate $E_c$ using morphological parameters was derived. In this particular study, $E_c$ is estimated from the measurements of $L_v$ and capillary radius (r) with a modified version of Sharan et al. (32) that gives $E_c$ per unit volume of neural tissue ($V_n$). It is also assumed that the tissue consists of homogeneous cylindrical compartments that surround capillaries that are involved in the exchange process. The utilization of $O_2$ by the tissues (M) is considered to be homogeneous within the cylinder. In this study we assume a value of $M$ for brain metabolism of $0.8 \times 10^{-3}$ ml $O_2$·g⁻¹·s⁻¹ (32). Although $P_O_2$ is likely to be heterogeneous along the capillaries, in this study we consider the capillary pressure similar to the end-capillary pressure. For derivation of equations for $E_c$ and tissue $P_O_2$ (PtO₂) see the APPENDIX.

Statistics

Indexes of capillary density, the internal diameters, and diffusion conductances were compared for different brain regions between the chronic hypoxic and control groups by use of the Student’s independent t-test, and differences were accepted as significant when $P < 0.05$.

RESULTS

Similar to previous studies, hypoxic mice had lower body and brain weights. However, although the hypoxic brains were grossly similar to controls, after tissue processing all the hypoxic animals showed an increase of 25% of cortical volume compared with controls. The striatum, thalamus, and mesencephalic nuclei as well as the cerebellum did not show a change in volume.

Histology

Capillaries in different brain regions of chronic hypoxic and control animals were filled with photographic emulsion. The cerebral and cerebellar cortices as well as the deeper nuclei of hypoxic mice showed altered capillary networks. The cerebellar folia of hypoxic mice showed a dramatic increase in the number of capillaries in the granular layer (Fig. 1, A and B). Compared with controls, hypoxic capillaries appeared elongated and more tortuous and dilated. Changes similar to those observed in the granular layer were also evident in the corpus striatum and thalamic and mesencephalic nuclei. The increase in capillary length was associated with a reduction of the ICD, as shown for capillaries of the substantia nigra (Fig. 1, C and D). The capillary plexuses in the hippocampal formation were also remodeled with chronic hypoxia. As shown in Fig. 2, elongated capillaries were visualized in most layers of areas CA1 and CA3 and the dentate gyrus.

All the layers of motor cortex showed enlarged capillaries in chronic hypoxia (Fig. 3). These capillaries were more tortuous and dilated than those of the control group. Capillaries in the somatosensory cortex of hypoxic mice were denser, with larger diameters (Fig. 4).

Morphometry

$L_v$ and ICD. Capillary lengths and the estimated ICDs are shown in Table 1 and were consistent with histological changes in chronic hypoxia. In the hypoxic group the granular layer of the cerebellum had the greatest absolute increase in $L_v$, but $L_v$ did not increase in the molecular layer. Other regions with significantly increased $L_v$ were the superior colliculus (1.67-fold), substantia nigra (1.66-fold), caudate nucleus (1.63-fold), globus pallidus (1.32-fold), and subiculum (1.58-fold). In the hippocampus, longer capillaries were detected in CA1 strata pyramidal (1.33-fold) and lacunosum (1.54-fold), CA3 strata oriens (1.47-fold) and pyramidale (1.28-fold), and dentate gyrus strata moleculare (1.34-fold) and granulosum (1.23-fold).

The neocortex of hypoxic mice showed a wider range of changes in $L_v$ between different areas and their layers. The $L_v$ increase was uniform between layers II, III, V, and VI in the motor cortex, but in the somatosensory and auditory cortices more restricted responses were
observed in different layers. $L_v$ in the somatosensory cortex did not change significantly in layers II, III, and IV but increased in layers V and VI. In contrast to the control group, where $L_v$ in layer IV was greater than in layers V and VI, there were no differences in $L_v$ between the layers in the hypoxic group. In layer IV of the auditory cortex, as in the somatosensory cortex, $L_v$ did not change with chronic hypoxia. However, $L_v$ increased in layers II and III of the auditory cortex. The visual cortex did not change $L_v$ in the chronic hypoxic group, despite the marked increase observed in the superior colliculus.

The ICDs calculated for both groups were inversely related to $L_v$. Significant reductions in the ICD were found in the superior colliculus (0.76-fold), substantia nigra (0.77-fold), subiculum (0.79-fold), caudate nucleus (0.85-fold), and globus pallidus (0.89-fold). In the granular layer of the cerebellum, the ICD was decreased only by 0.86-fold. In the neocortex, modest reductions in ICD were statistically significant in layers V and VI of the motor (0.82-fold) and somatosensory (0.88-fold) areas. More modest reductions of ICD were found in several layers of the hippocampus.

$S_v$ and internal diameters. To examine the contribution of other variables to capillary remodeling in chronic hypoxia, $S_v$ and the average capillary diameter ($D$) were measured in selected nuclei and areas of the hippocampus and neocortex. $S_v$ from digitized images are shown in Table 2. In the regions and layers studied in the hypoxic animals, $S_v$ increased 1.21- to 3.03-fold...
Table 1. Effects of chronic hypobaric hypoxia on regional brain capillary length, density, and intercapillary distance

<table>
<thead>
<tr>
<th>Brain Region</th>
<th>Control</th>
<th>Chronic Hypoxia</th>
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<tbody>
<tr>
<td></td>
<td>$L_v$ mm/mm$^3$</td>
<td>$ICD, \mu m$</td>
</tr>
<tr>
<td>Cerebellum</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Granular layer</td>
<td>1,302 ± 236</td>
<td>31.56 ± 2.70</td>
</tr>
<tr>
<td>Molecular layer</td>
<td>1,088 ± 192</td>
<td>34.56 ± 3.12</td>
</tr>
<tr>
<td>Substantia nigra</td>
<td>532 ± 123</td>
<td>49.66 ± 6.22</td>
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<tr>
<td>Superior colliculus</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stratum superficiale</td>
<td>567 ± 176</td>
<td>48.62 ± 8.00</td>
</tr>
<tr>
<td>Stratum profundum</td>
<td>513 ± 99</td>
<td>51.02 ± 6.44</td>
</tr>
<tr>
<td>Subiculum</td>
<td>434 ± 60</td>
<td>54.66 ± 3.46</td>
</tr>
<tr>
<td>Caudate nucleus</td>
<td>485 ± 95</td>
<td>51.84 ± 5.52</td>
</tr>
<tr>
<td>Globus pallidus</td>
<td>552 ± 37</td>
<td>47.62 ± 1.60</td>
</tr>
<tr>
<td>Auditory cortex</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Layers II and III</td>
<td>696 ± 109</td>
<td>43.10 ± 3.64</td>
</tr>
<tr>
<td>Layer IV</td>
<td>1,041 ± 167</td>
<td>35.20 ± 2.60</td>
</tr>
<tr>
<td>Layers V and VI</td>
<td>854 ± 148</td>
<td>38.94 ± 3.36</td>
</tr>
<tr>
<td>Motor cortex</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Layers II and III</td>
<td>893 ± 228</td>
<td>38.42 ± 5.06</td>
</tr>
<tr>
<td>Layers V and VI</td>
<td>854 ± 148</td>
<td>38.94 ± 3.36</td>
</tr>
<tr>
<td>Somatosensory cortex</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Layers II and III</td>
<td>1,103 ± 166</td>
<td>32.42 ± 2.70</td>
</tr>
<tr>
<td>Layer IV</td>
<td>1,237 ± 55</td>
<td>32.10 ± 0.72</td>
</tr>
<tr>
<td>Layers V and VI</td>
<td>866 ± 110</td>
<td>38.50 ± 2.44</td>
</tr>
<tr>
<td>Visual cortex</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Layers II and III</td>
<td>883 ± 169</td>
<td>38.36 ± 3.54</td>
</tr>
<tr>
<td>Layer IV</td>
<td>754 ± 99</td>
<td>41.28 ± 2.58</td>
</tr>
<tr>
<td>Layers V and VI</td>
<td>711 ± 52</td>
<td>42.38 ± 1.62</td>
</tr>
<tr>
<td>Hippocampus</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CA1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stratum oriens</td>
<td>619 ± 43</td>
<td>46.04 ± 1.96</td>
</tr>
<tr>
<td>Stratum pyramidale</td>
<td>592 ± 62</td>
<td>47.06 ± 2.30</td>
</tr>
<tr>
<td>Stratum radiatum</td>
<td>491 ± 94</td>
<td>52.40 ± 7.02</td>
</tr>
<tr>
<td>Stratum lacunosum</td>
<td>605 ± 134</td>
<td>47.22 ± 5.38</td>
</tr>
<tr>
<td>CA3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stratum lucidum</td>
<td>521 ± 131</td>
<td>51.60 ± 6.94</td>
</tr>
<tr>
<td>Stratum pyramidale</td>
<td>669 ± 108</td>
<td>44.64 ± 3.50</td>
</tr>
<tr>
<td>Stratum oriens</td>
<td>496 ± 109</td>
<td>52.00 ± 6.12</td>
</tr>
<tr>
<td>Dentate gyrus</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stratum moleculare</td>
<td>616 ± 78</td>
<td>46.32 ± 2.40</td>
</tr>
<tr>
<td>Stratum granulosum</td>
<td>578 ± 79</td>
<td>48.02 ± 2.82</td>
</tr>
</tbody>
</table>

Values are means ± SD; $n$, number of mice. $L_v$, capillary length per unit volume; $ICD$, intercapillary distance. *Significantly different from Control by Student's 2-tailed $t$-test, $P < 0.05$.

Compared with the control group, Layers II, III, and IV of the somatosensory cortex, which did not show a significant increase in $L_v$ after chronic hypoxia, still increased the total surface of the capillary bed (Fig. 5). $S_v$ also increased in all other layers in the somatosen-sory and motor cortices as well as nuclei of the basal ganglia, cerebellum, and hippocampus.

A change in $S_v$ depends on the capillary length and capillary diameter. In general, hypoxic capillaries were more dilated than controls, except for those in the hippocampal formation. In the hippocampus, we found a statistically significant increase of internal capillary diameters only in the stratum oriens of CA1. In somatosensory layer IV, $S_v$ increase is attributable to capillary dilation, since the $L_v$ is not increased.

$E_c$ and $\text{PtiO}_2$. $E_c$ estimates in different brain regions and layers of the neocortex and hippocampus are presented in Fig. 6. Except for somatosensory layers II and III, a marked increase of $E_c$ is observed in all areas studied in the hypoxic group compared with the controls. $E_c$ is a function of capillary $L_v$ and diameter. In layer IV of the somatosensory cortex, the increase in $E_c$ is explained only by capillary dilation. In contrast, the modest increase in several layers of CA1 and CA3 is only secondary to an increase in $L_v$.

The contributions of these different anatomic parameters to the estimation of $\text{PtiO}_2$ with Krogh’s model were evaluated and are shown in Fig. 7. Overall, an increase in $L_v$ (or decrease in ICD) brought the $\text{PtiO}_2$ values closer to the venous PO$_2$. In the caudate nucleus and the substantia nigra, these changes account for a 7- and 6-Torr increase in $\text{PtiO}_2$ compared with acute hypoxia, respectively. However, in other brain areas, such as the cerebellar granular layer and cortical layers, the increase is more modest.

**DISCUSSION**

The balance between the energy requirements and the effective capillary surface area of a brain region is poorly understood. In conditions of normal PO$_2$, different brain regions have shown a remarkable plasticity.
to adapt to a local change of energy demands. Regions with high neuronal activity such as layers IV and V of the somatosensory cortex (barrel field) (40) contain capillary networks with a high density of microvessels and shorter ICD. Our results confirm and extend previous studies (7, 11, 12, 22–25, 28) showing an increase of capillary networks in the brain of rodents exposed to chronic hypoxia.

We have measured several morphometric parameters (L_v, S_v, and ICD). Our data show a remarkable range of L_v among different cortical layers in normoxic and chronic hypoxic mice, probably reflecting the different metabolic activities of their neurons. In this regard, somatosensory layer IV neurons, which are very active and contain high levels of cytochrome c oxidase activity (15, 39), are surrounded by densely packed capillary networks.

**Figure 5.** Dilated and tortuous capillaries in layers II and III (A) and IV (C) of somatosensory cortex of chronic hypoxic mice compared with normoxic controls (B and D, respectively). Scale bar, 20 μm.
A question that must be addressed is whether these capillaries represent new vessels or opening of preexisting ones. With use of a double-staining technique (10), it has been shown that perfused and morphologically existing capillary networks in different brain regions of awake normocapnic rats are the same. Also an elevation of brain blood flow secondary to hypercapnia is not associated with capillary recruitment (9), and the increase in blood flow of several brain areas in acute hypoxia is mediated by an increase of flow velocity and microvessel dilation (3). Additional evidence of brain angiogenesis induced by chronic hypobaric hypoxia was recently demonstrated in rats (12), in which early hypertrophy of brain microvessels was followed by hyperplasia. These data are consistent with the concept that reserve capillaries are absent in the brain and that the vessels measured contain a subpopulation of capillaries induced by hypoxia.

Dilation of brain capillaries is another marker of capillary remodeling in the brains of chronic hypoxic mice. This effect of chronic hypoxia was initially described in adult rats by Mercker and Schneider (23), and it is not restricted to areas where \( L_v \) was increased but also was prominent in somatosensory layer IV, an area in which \( L_v \) did not change. Capillary dilation by itself is a potential mechanism to increase blood flow. In the setting of chronic hypoxia and blood hyperviscosity, capillary dilation would not only help accommodate the increased blood flow but also reduce the resistance due to hyperviscosity (17). According to Poiseuille’s law, the flow of a fluid in a vessel decreases linearly with an increase of its viscosity, but it increases as the fourth power of its internal radius. In this regard, it has been determined that the optimal cardiovascular response to chronic hypoxia requires a hematocrit close to normal values (34, 38) and that the development of polycythemia and hyperviscosity as part of the acclimatization to high altitude in humans and other mammals may be detrimental for blood flow (26, 27).

The initial adaptive response to acute hypoxia is an increase of the respiratory rate and a preferential redistribution of blood flow to the brain and heart (8, 14). In acute hypoxia, blood flow increases in various brain regions (35), but as hyperventilation persists, a reduced arterial CO2 blunts the initial increase in blood flow (31, 33) and the vasodilatory response of cerebral arterioles is partially attenuated. In chronic hypoxia, total capillary volume is increased, and, as a result, an elevation of brain blood flow could also be expected. However, in humans native to high altitude (Puno, Peru, 4,000 m) a reduction of blood flow has been shown in several brain areas (16). The higher prevalence of migraine and polycythemia in human populations of the high altitudes (1) suggests that other disturbances of vascular autoregulatory mechanisms in the brain may influence capillary blood flow. It is possible that...
brain capillary dilation in chronic hypoxia is an adaptive mechanism that begins in the initial steps of the hypoxic insult and persists as polycythemia develops. In this setting, dynamic changes in local blood flow that would accompany a transitory increase in tissue metabolism must be critical to sustain an appropriate PtO2 proportionate to O2 consumption. Also, it is possible that a failure of the autoregulatory properties of the cerebral vasculature may contribute to the pathophysiology of migraine in natives of high altitudes.

It is important to consider how the rearrangement of capillary plexuses in the brain might affect O2 diffusion and its partial pressure in the tissues, but both variables are difficult to measure directly in the brain. Tenney and Ou (36) predicted increased O2 diffusion into tissues on the basis of the diffusion of CO from subcutaneous pockets implanted in chronic hypoxic rats. Our estimates of Ec for different brain regions also show that an increased diffusion of O2 is expected secondary to the increased Sa in chronic hypoxia. However, the modest increase of Ec in several layers of the hippocampus suggests that this area must be at a particularly higher risk of chronic hypoxia.

The morphological changes described account for an increased Ec in chronic hypoxia, but the gradient of O2 diffusion is also dependent on capillary PO2 and PtO2. When PtO2 is estimated using Eq. 2 (Fig. 7), even with an elevated Ec (increases in Lv and r), the effective pressure of O2 in the tissues is limited by the venous PO2. Nevertheless, changes in microvasculature lead to an average increase in PtO2 of 3 Torr compared with acute hypoxia. Moreover, the estimates of PtO2 depicted in Fig. 7 suggest that, depending on the initial position on the PtO2-Lv curve, some brain regions may show greater increases in O2 as a result of increasing Lv than do others. For example, the increase in Ec in the caudate nucleus and substantia nigra, two areas with initially low Lv, accounts for a greater increase in PtO2 than in the cerebellar granular layer, in which Lv also increases substantially (7.92 and 1.65 Torr in the caudate nucleus and cerebellar granule cell layer, respectively). In contrast, in somatosensory layer IV, a region in which hypoxia does not induce the formation of new capillaries, there is only a small increase in PtO2 (1.25 Torr) related to diameter increases. This suggests than in normal conditions some well-vascularized areas of the brain already have a maximal density of capillaries that is not changed by the hypoxic stimulus. More studies are needed to determine the nature of the factors that may limit angiogenesis in chronic hypoxia.

The persistence of brain hypoxia, despite the development of polycythemia and the remodeling of capillary plexuses, leads one to look outside these parameters for other adaptive mechanisms. According to Kety's equation for Krogh's model (19), an additional increase in PtO2 is predicted when M decreases. In this regard, metabolically intact mitochondria isolated from the cerebral cortex of chronic hypoxic mice showed a decreased O2 consumption in the presence of ADP (state 3 of respiration) compared with mitochondria from normoxic controls (4). Also the activities of complex I-NADH dehydrogenase and cytochrome c oxidase of the respiratory chain are decreased in the brain after exposure to prolonged environmental hypoxia (4, 21), showing that a reduced expression of these enzymes contributes to the decrease in O2 consumption by brain mitochondria. The increase in the metabolic rate for glucose (13) and the density of glucose transporter in isolated microvessels of rats exposed to hypobaric hypoxia (11) suggest that brain energy metabolism in chronic hypoxia is more dependent on anaerobic glycolysis. A possible effect of reduced brain mitochondrial O2 consumption in chronic hypoxia would be an increase in PtO2 values closer to the levels calculated for normoxic conditions. Thus it is possible that a reduction of O2 utilization coupled with increased O2 diffusion secondary to angiogenesis may help restore PtO2 and adapt the brain to low blood PO2. However, more accurate determinations of local O2 utilization are needed to compare energy metabolism and capillary architecture in the brain.

APPENDIX

To estimate the mean PtO2, we derived an equation from the Krogh-Erlang formula modified by Kety (19, 20)

\[
\text{PtO}_2 = \frac{\text{PcO}_2 + \left( \frac{M}{8D_i \alpha_0 \pi L_v} \right) \left[ 3R^2 - r^2 - \left( \frac{4R^4}{R^2 - r^2} \right) \log R/r \right]}{1 - \pi L_v}
\]

In this model, R is replaced by \( \frac{1}{\sqrt{\pi L_v}} \)

\[
\text{PtO}_2 = \frac{\text{PcO}_2 + \left( \frac{M}{8D_i \alpha_0 \pi L_v} \right) \left[ 3 - \pi L_v r^2 - \left( \frac{4}{1 - \pi L_v r^2} \right) \log \left( \frac{1}{r} \sqrt{\frac{1}{\pi L_v}} \right) \right]}{1 - \pi L_v}
\]

In this model, \( D_i \) is the O2 diffusion coefficient \( (1.5 \times 10^{-5} \text{ cm}^2/\text{s}) \) and \( \alpha_0 \) is the O2 solubility in the tissue \( (3 \times 10^{-3} \text{ ml·ml}^{-1} \cdot \text{Torr}^{-1}) \). R is the radius of the Krogh's cylinder, and r is the capillary radius. PcO2 is taken as the end-capillary PO2 with values of 40 Torr at sea level and 30 Torr in the chronic hypoxic group; these values are similar to those estimated in chronic hypoxic rats by Tenney and Ou (36). M is the O2 consumption per unit volume of Krogh cylinder (not including the intracapillary volume). It is important to note that the Krogh-Erlang equation is based on the assumption that O2 diffusion is adequate to supply the metabolic need of O2 throughout the cylinder.

We slightly modify the model of Sharan et al. (32) by taking quantities per unit neural tissue volume instead of per whole brain. The amount of O2 delivered from the capillary compartment per unit volume of Krogh cylinder \((J_c)\) is

\[
J_c = E_c (\text{PcO}_2 - \text{PtO}_2)
\]

where \( E_c \) is the diffusion conductance per unit volume of Krogh cylinder and \((\text{PcO}_2 - \text{PtO}_2)\) is the average PO2 differential between the capillary and the tissue. In addition, \( J_c \) can be expressed as

\[
J_c = M
\]
From Eqs. 2–4, $E_c$ can be expressed as

$$E_c = \frac{M}{(P_{cO_2} - P_{tO_2})} = \frac{8\pi L D_{w} \alpha_q (1 - \pi L r^2)}{4 \log_{r} \left( \frac{1}{r \sqrt{\pi} L_w} \right) - (3 - \pi L r^2) (1 - \pi L r^2)}$$

in ml $O_2 \cdot s^{-1} \cdot ml^{-1} \cdot Torr^{-1}$.

We are grateful to Dr. Carlos Monge for advice during the preparation of the manuscript, Manuel Panta for excellent technical support, and Kathryn Diekmann for help in the preparation of the manuscript.

This study was supported by National Institute of Neurological Disorders and Stroke Grants NS-17763 and NS-28781, the McDonnell Center for Studies of Higher Brain Function, an award from the Kiwanis International, and Consejo Nacional de Ciencia y Technologia (Mexico). Address for reprint requests: T. A. Woolsey, Dept. of Neurological Surgery, Campus Box 8057, Washington University School of Medicine, St. Louis, MO 63110 (E-mail: woolseyt@medicine.wustl.edu). Received 24 December 1997; accepted in final form 18 November 1998.

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