Cortical oxidative metabolism under conditions of ischemia, hypoxia, and asphyxia in the rabbit

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The purpose of this investigation was to compare the effects of hypoxia, asphyxia, and ischemia on brain cortical oxidative metabolism. This study was carried out using 14 New Zealand White rabbits. The effects of episodic stress were measured simultaneously on brain functional metabolism by monitoring cortical oxygen tension (brain pO2), cortical cerebral blood flow (cCBF), cortical blood volume, and mitochondrial oxidative metabolism. During hypoxia (when the fraction of inspired O2 (FiO2) was reduced to 10%) and asphyxia (induced by turning the respirator off), there was a decrease of brain pO2 but an increase of cCBF and blood volume. Similarly, there was a reduction of cortical oxidative metabolism. In post-asphyxic conditions, an overshoot of brain pO2 and post-asphyxic oxidation of cytochrome (Cyt.) aa3 were usually shown. Under ischemic conditions (induced by sudden severe hypotension plus bilateral common carotid occlusion), cCBF and blood volume were decreased. There was also a decrease of brain pO2 and a reduction of Cyt. aa3 following ischemia. These techniques are applicable in intraoperative monitoring of patients.

Key Words • cortical metabolism • ischemia • asphyxia • hypoxia • oxygen tension • cytochrome aa3

It is known that transient ischemic attacks (TIA's) can be caused by a hemodynamic effect. Hemodynamic TIA's are thought to be due to background factors consisting of a subthreshold flow-limiting lesion of the arteries leading to the brain, causing some degree of ischemia. Triggering factors include a transient drop in perfusion pressure due to decreased cardiac output. These factors together produce a critical reduction in cerebral blood flow (CBF) to the point that brain oxygen tension (brain pO2) is lowered to a threshold level for TIA's. However, hypoxia (low PaO2) and asphyxia (low PaO2 plus high PaCO2) are also known to produce a similarly decreased brain pO2 and to cause clinical symptoms.

To investigate these mechanisms, we used a hemodynamic ischemic model consisting of sudden severe hypotension plus transient bilateral common carotid occlusion, and observed the effects on brain cortical oxidative metabolism. In addition, we compared the effects of hypoxia and asphyxia.

Materials and Methods

Protocol

Fourteen adult New Zealand White rabbits of both sexes were used in this investigation. Each rabbit weighed between 3 and 5 kg. After a preliminary dose of ketamine, a tracheostomy was performed. All animals were anesthetized throughout the studies with nitrous oxide and oxygen in a 3:1 mixture. Rectal temperature was maintained between 36° and 38°C. The animals were ventilated by respirator, and were immobilized with gallamine triethiodide (Flaxedil). Changes in gas mixtures were made by altering the input to the respirator. The femoral artery was cannulated to monitor the systemic blood pressure and sample the blood for arterial pH, PaO2, and PaCO2. The mean values were: arterial pH 7.34 ± 0.02, PaO2 103.1 ± 5.5 mm Hg, and PaCO2 32.1 ± 1.2 mm Hg (Table 1). The control blood pressure was 92.0 ± 8.6 mm Hg.

The femoral vein was cannulated for administering additional Flaxedil and saline, and for injections of trimethaphan camyslate (Arfonad) or phenylephrine hydrochloride (Neo-Synephrine). Silk ligatures were placed loosely around both common carotid arteries for temporary occlusion. The head of the animal was fixed in a stereotaxic frame for performing bilateral carotid ligations. Hypoxia was induced by reducing the fraction of inspired oxygen (FiO2) from 25% to 10% for 2 minutes, while continuously ventilating the rabbit at the same
FIG. 1. Response of brain cortical oxygen tension (bPO2), cytochrome (Cyt) aa3, cortical cerebral blood flow (cCBF), and cortical blood volume (B1 Vol) during hypoxia. FiO2 = fraction of inspired oxygen; Red. = reduction; F.S. = percent of the full-scale optical signal.

rate and tidal volume. Asphyxia was produced by turning off the respirator and allowing no respiratory exchange in the animal for a 2-minute period. Ischemic insults were caused by briefly inducing hypotension (usually a decrease in mean blood pressure of 30 mm Hg) with an injection of Arfonad, and then temporarily occluding both common carotid arteries.

Monitoring Techniques

Cortical Oxygen Tension. Cortical oxygen tension was continuously recorded by the polarographic method, as modified by Yonekura and Austin (unpublished data), using a 25-μ platinum electrode and 250-μ silver-silver chloride indifferent electrode. The oxygen microelectrode used in these experiments and the techniques were essentially the same as those described by Silver.23,24 The electronic circuitry for the polarographic measurement of brain pO2 was provided by a Schema Versatae Model 400 polarographic current amplifier.*

At another cortical site, cCBF was monitored continuously by a thermal diffusion flow probe for dynamic quantitative measurement. The probe consisted of a Peltier stack with an L-shaped gold plate and thermocouple attached to the back of a portion of the gold plate. This technique has been described previously by Brawley7 and Carter and Atkinson.8,9 Carter and Erspamer10 reported that the thermal gradient between the thermocouple in microvolts was highly correlated with cortical blood flow as determined by xenon-133 clearance. This thermal diffusion technique was used to monitor cortical blood flow under conditions of ischemia, asphyxia, and hypoxia.

Cortical Mitochondrial Oxidative Metabolism. Cortical mitochondrial oxidative metabolism was monitored by two noninvasive optical methods, based on the original work of Chance, et al.11,12 and Jobis, et al.15,16 The first method involved the ratio of reduced cytochrome (Cyt.) aa3 to oxidized Cyt. aa3, as recorded continuously by a dual-beam dual-wavelength reflectance spectrophotometer, modified by Jobis, et al.15,16 This measurement is based on the observation that Cyt. aa3 in its reduced form absorbs light at a specific wavelength (605 nm). As a comparison, to rule out hemoglobin absorption, the cortex was also illuminated with light at 590 nm, a so-called “equibestic point” where the relative amounts of reduced and oxygenated hemoglobin are the same as at 605 nm. The spectrophotometric difference tracing was calibrated as a percentage change of total light intensity. The relative blood volume was estimated from the change in total amount of hemoglobin, monitored at the equibestic wavelength (590 nm).15

The second method of measurement involved the redox level of mitochondrial flavoprotein, monitored by a real-time flying-spot fluorometer designed by Chance, et al.11,12 Flavoprotein in oxidized form becomes fluorescent at 540 nm when excited by light at 442 nm. Flavoprotein excitation is obtained with a 10-mW helium-cadmium laser.† A rectangular region of

* Schema Versatae Model 400 polarographic current amplifier manufactured by Tegal Scientific Inc., Berkeley, California.

† Liconix Model 902 helium-cadmium laser manufactured by Liconix, Mountain View, California.
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**TABLE 2**

<table>
<thead>
<tr>
<th>Oxygen Deficiency</th>
<th>Brain $pO_2$</th>
<th>cCBF</th>
<th>CBV</th>
<th>Cytochrome aa&lt;sub&gt;3&lt;/sub&gt;</th>
<th>Flavoprotein</th>
</tr>
</thead>
<tbody>
<tr>
<td>hypoxia</td>
<td>decrease</td>
<td>increase</td>
<td>slight increase</td>
<td>reduction</td>
<td>reduction</td>
</tr>
<tr>
<td>asphyxia</td>
<td>decrease</td>
<td>large increase</td>
<td>slight increase</td>
<td>reduction</td>
<td>reduction</td>
</tr>
<tr>
<td>ischemia</td>
<td>decrease</td>
<td>decrease</td>
<td>decrease</td>
<td>reduction</td>
<td>reduction</td>
</tr>
</tbody>
</table>

* Brain $pO_2$ = cortical oxygen tension; cCBF = cortical cerebral blood flow; CBV = cerebral blood volume.

Cortex was swept over an area of approximately $2 \times 4$ mm with a 0.1-mm spot. The flying-spot fluorometer has the advantage of providing a percentage change in oxidized heterogeneity of cortex.

**Results**

We observed cortical oxidative metabolism under conditions of ischemia, hypoxia, and asphyxia from the same cortical sites in all subjects. The results are summarized in Table 2. The mean values of brain $pO_2$ under normoxic steady conditions, was $30.6 \pm 4.3$ mm Hg from 42 measurements of these 14 cortical sites.

**Hypoxia**

Arterial $pO_2$ was decreased by reducing the FiO<sub>2</sub> from 25% to 10% for 2 minutes while continuing to ventilate the rabbit at the same rate and tidal volume. There was no significant difference in the PaCO<sub>2</sub> and pH between blood samples drawn during ventilation at a 25% FiO<sub>2</sub> and at a 10% FiO<sub>2</sub> (Table 1), but there was a relationship between arterial $pO_2$ and FiO<sub>2</sub>.

During a short period of hypoxia, the brain $pO_2$ initially decreased gradually and then leveled out for a short period of time. On return to normoxia from hypoxia, the brain $pO_2$ values at each of the 14 sites did not increase above the control value (Fig. 1). During hypoxia, brain $pO_2$ decreased an average 7.1 mm Hg from the initial value of $32.2 \pm 7.6$ mm Hg at the 14 sites (Table 3).

There was an increase of blood flow and blood volume following the period of decreased brain $pO_2$. The average increase in cCBF was 47.7% from the resting value of $24.3 \pm 3.2$ ml/100 gm/min. On the other hand, there was a reduction of Cyt. aa<sub>3</sub>, which paralleled the time course of decreased brain $pO_2$ during hypoxia (Fig. 1). It was also shown that there was a shift toward reduction of the other member of the mitochondrial respiratory chain, flavoprotein.

**Asphyxia**

Asphyxia was induced by turning off the respirator for 2 minutes, and was accompanied by a large decrease in PaO<sub>2</sub> and an increase in PaCO<sub>2</sub> compared with normal levels (Table 1). The brain $pO_2$ decreased abruptly after the respirator was turned off (Fig. 2), for an average decrease of 7.3 mm Hg from the initial value of $31.4 \pm 7.4$ mm Hg in the 14 sites. Comparison of brain $pO_2$ values recorded at the same 14 sites during hypoxia and asphyxia showed no significant differences (Table 3). With the onset of asphyxia, there was an immediate increase in the level of reduced Cyt. aa<sub>3</sub> (Fig. 2 upper). Also the fluorescence histogram of flavoprotein shifted to a lower intensity, indicating that it was reduced (Fig. 2 lower). On the other hand, cCBF was remarkably increased and there was a slight increase in blood volume during asphyxia, when the cortex was in good condition (Fig. 2 upper). After the respirator was restarted, there was a further increase in cCBF (Fig. 2 upper). The average increase in cCBF was 100% above the resting value of $24.4 \pm 3.0$ ml/100 gm/min, which was a greater increase than that found in hypoxia (Table 4). There was usually an overshoot of brain $pO_2$ after restarting the respirator and also a post-asphyxic oxidation of Cyt. aa<sub>3</sub> (Fig. 2 upper). The increased brain $pO_2$ then returned to its initial value, and paralleled the normalization of cCBF and redox level of Cyt. aa<sub>3</sub> (Fig. 2 upper).

**Ischemia**

When the blood pressure was abruptly reduced by Arfonad to a value of 30 mm Hg, on the average, the cCBF and blood volume were also decreased. Similarly, brain $pO_2$ was decreased, and a reduction of Cyt. aa<sub>3</sub> was observed during hypotension (Fig. 3). Subsequent bilateral common carotid occlusion, carried out while maintaining this severe hypotension, resulted in only slight additional changes of brain $pO_2$, Cyt. aa<sub>3</sub> and flavoprotein (Fig. 3). During ischemia, brain $pO_2$ decreased an average of 3.9 mm Hg from the resting.
value of 28.1 ± 7.7 mm Hg in the 14 sites (Table 3). The average decrease in cCBF was 27.5% from the resting value (26.0 ± 3.5 ml/100 gm/min, Table 4).

After the ischemic stress, blood pO2, and cCBF showed a slight tendency to return toward baseline. As soon as the blood pressure was raised by injecting 15% Neo-Synephrine, cCBF and brain pO2 increased dramatically, coupled with the oxidation of Cyt. aa3 (Fig. 3).

**Discussion**

Since the reports of polarographic measurement of oxygen tension in cortex by Davies and Brink,13 numerous reports have been published on oxygen tension in the brain cortex measured by microelectrodes.20,23,25 Silver26 and Bicher and Knisely6 reported that the cortical pO2 at a specific microarea is remarkably constant, and can be changed only through major alterations in blood supply or the composition of respiratory gases. In general, a correlation was shown between PaCO2 and the inspiratory O2 concentration.19

In these experiments, the brain pO2 during a short period of hypoxia initially decreased gradually, and then leveled out for a short period of time (Fig. 1). There was an increase of blood flow and blood volume following the period of decreased cortical oxygen tension (Fig. 1). These changes suggest an autoregulation mechanism to maintain a constant brain cortical oxygen environment in the intact brain. Bicher2 introduced the concept of maintaining a constant brain-cell oxygen microenvironment, which he called “oxygen autoregulatory mechanisms.” He used four criteria to identify the oxygen autoregulation mechanism in brain tissue following a short period of anoxia: 1) a short period of reoxygenation; 2) an increase in CBF; 3) an overshoot; and 4) electrical silence paralleling the period of tissue pO2 depression. In our studies, we observed either no overshoot or only a small one. The difference between our experimental procedure and Bicher’s protocol is that we exposed our animals to a 10% FiO2, whereas he obtained hypoxia by nitrogen respiration.

There were shifts toward reduction of members of the mitochondrial respiratory chains (Cyt. aa3 and flavoprotein) similar to the changes of brain pO2. However, Schutz, et al.,22 have shown that there was no change in isolated mitochondrial function after 37 minutes of severe systemic hypoxic normotension in the rabbit. This suggests that there is a marked difference in the mitochondrial respiration in isolated mitochondria and in the in vivo preparation. Rosenthal, et al.,21 already showed that increasing inspired oxygen levels produced an oxidation of Cyt. aa3, while there was a sharp reduction in response to lowering the FiO2. Our data confirm their results.

The time course of brain pO2 during and after asphyxia showed three phases: 1) a decrease; 2) a leveling off; and 3) a return to the initial pressure, accompanied by an overshoot. The leveling off phase could be ex-

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**TABLE 4**

Comparison of average changes at the same cortical sites of cCBF during oxygen deficiency

<table>
<thead>
<tr>
<th>Oxygen Deficiency</th>
<th>Resting Values of CBF (ml/100 gm/min)</th>
<th>Change in CBF (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>hypoxia</td>
<td>24.3 ± 3.2</td>
<td>+43.8 ± 14.6</td>
</tr>
<tr>
<td>asphyxia</td>
<td>24.4 ± 3.0</td>
<td>+100.0 ± 26.2</td>
</tr>
<tr>
<td>ischemia</td>
<td>26.0 ± 3.5</td>
<td>−27.5 ± 5.8</td>
</tr>
</tbody>
</table>

* cCBF = cortical cerebral blood flow.
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plained by decreased oxygen utilization, with reduced oxygen supply. After the respirator was restarted, there was a noticeable overshoot of brain pO2 paralleling the increased systemic blood pressure and further increased cCBF (Fig. 2). This is best explained by tissue hyperemia from high tissue carbon dioxide. The increased blood pressure and cCBF, coupled with the delayed return of the vasodilated cerebral vessels to their original state, rapidly resaturates the tissue with oxygen and leads to an overshoot of brain pO2.

The efficiency of oxygen supply and utilization can be tested by measuring the redox state of the mitochondrial respiratory chain. The time course of redox level of Cyt. aa3 under conditions of asphyxia showed a change parallel to the brain pO2. This would support the idea that mitochondrial respiration is the main consumer of oxygen, and that reduction of Cyt. aa3, is associated with a change of oxygen utilization, as shown by Austin, et al."^2,3

The drop in blood pressure produced by Arfonad followed by the brief bilateral carotid occlusion during ischemia was accompanied by a decrease in brain pO2, cCBF, and Cyt. aa3. Before autoregulation occurred, the abrupt drop in the mean blood pressure produced a drop in perfusion pressure and caused a decrease in CBF. The reduction in blood flow decreased oxygen delivery to the cortex. In our ischemic model (hypotension and bilateral carotid occlusion in the rabbit), there were no overshoots of brain pO2 and postocclusion oxidation of Cyt. aa3 after releasing the occluding ligatures. We attribute this finding to the still low perfusion pressure and a lack of development of collateral circulation. This suggested that circulatory perfusion has an important role in oxygen autoregulation mechanisms under severe conditions of decreased oxygen delivery. Eklöf and Siesjö"^4 reported that energy and ratios of nicotinamide adenine dinucleotide in its reduced to its oxidized form (NADH:NAD+ ratios) were grossly impaired at cerebral venous pO2 values of 32 mm Hg, but there was also a reduction of the CBF to 45% of normal, induced by carotid ligation and hypotension. The data we have presented suggest that members of the cortical mitochondrial respiratory chains may be more sensitive to changes in tissue oxygen tension and may be in need of a much higher concentration of oxygen in order to be fully oxidized.

On the other hand, it may be that fewer mitochondria are being oxidized when cortical blood flow is reduced, or under conditions of reduced PaO2, due to simple hypoxia or asphyxia. None of these mechanisms can at present be excluded. The increase of relative oxidation of members of the electron transport chain under conditions of increased cortical pO2 suggests that there may be mitochondria in a borderline region of hypoxia that only respond under conditions that we regard as hypoxic.

This study is important for the further understanding of human cortical oxidative metabolism by noninvasive monitoring. Austin, et al."^1,2,4 have reported that preliminary results of noninvasive monitoring in the operating room showed not only a significant increase in cCBF following superficial temporal artery-middle cerebral artery anastomosis, but an associated increase of brain oxygen availability and also an increase in cortical oxidative metabolism. The changes of cortical pO2 and cortical oxidative metabolism in the rabbit, cat, and human cortex therefore appear to vary in rather similar fashion in response to return to reoxygenation.

References


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