Oxygen dependency and precision of cytochrome oxidase signal from full spectral NIRS of the piglet brain

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Springett, R., J. Newman, M. Cope, and D. T. Delpy. Oxygen dependency and precision of cytochrome oxidase signal from full spectral NIRS of the piglet brain. Am J Physiol Heart Circ Physiol 279: H2202–H2209, 2000.—Oxidation changes of the copper A (CuA) center of cytochrome oxidase in the brain were measured during brief anoxic swings at both normocapnia and hypercapnia (arterial \( P_{\text{CO}_2} \approx 55 \text{ mmHg} \)). Hypercapnia increased total hemoglobin from 37.5 ± 9.1 to 50.8 ± 12.9 \( \mu \text{mol} / \text{l} \) (means ± SD; \( n = 7 \)), increased mean cerebral saturation (SmcO\(_2\)) from 65 ± 4 to 77 ± 3%, and oxidized CuA by 0.43 ± 0.23 \( \mu \text{mol} / \text{l} \). During the onset of anoxia, there were no significant changes in the CuA oxidation state until SmcO\(_2\) had fallen to 43 ± 5 and 21 ± 6% at normocapnia and hypercapnia, respectively, and the maximum reduction during anoxia was not significantly different at hypercapnia (1.49 ± 0.40 \( \mu \text{mol} / \text{l} \)) compared with normocapnia (1.53 ± 0.44 \( \mu \text{mol} / \text{l} \)). Residuals of the least squares fitting algorithm used to convert near-infrared spectra to concentrations are presented and shown to be small compared with the component of attenuation attributed to the CuA signal. From these observations, we conclude that there is minimal interference between the hemoglobin and CuA signals in this model, the CuA oxidation state is independent of cerebral oxygenation at normocapnia, and the oxidation after hypercapnia is not the result of increased cerebral oxygenation.

Changes in the redox state of the electron transport chain can, in theory, be measured using optical techniques: the mitochondrial NADH/NAD\(^+\) redox couple can be measured using NADH fluorescence, the copper A (CuA) center of cytochrome oxidase can be measured using near-infrared (NIR) spectroscopy (NIRS), and the heme a center of cytochrome oxidase can be measured using visible surface reflectance spectroscopy. All these techniques reveal an oxidation of the electron transport chain during hypercapnia [see Gyulai et al. (8) for NADH fluorescence, see Edwards et al. (5) for NIRS, and see Kreisman et al. (16) for visible reflectance spectroscopy], and, in general, it has been concluded that this oxidation is the result of increased oxygen delivery and increased \( P_{\text{O}_2} \) at the mitochondrial level, and the effects of the perturbation of carbohydrate metabolism are not discussed (although see Ref. 10). However, this conclusion is based on the observation that these methods show a continuous change in the redox state of the electron transport chain from hyperoxia to mild hypoxemia [see Gyulai et al. (7) for NADH fluorescence, see Kreisman et al. (16) for visible spectroscopy, and see Hampson et al. (9) for NIRS], although there are exceptions (4, 11, 24).

All optical techniques are susceptible to interference by oxyhemoglobin (HbO\(_2\)) and deoxyhemoglobin (Hb), which do show continuous changes between hyperoxia and hypoxia and are also present in much greater concentrations than cytochrome oxidase or NADH. In general, NADH fluorescence spectroscopy and visible heme a absorption spectroscopy are usually implemented as two wavelength methods (although see Ref. 15), where one wavelength measures the change in the oxidation state of redox component and the other attempts to correct for changes in total hemoglobin. However, both these methods cannot also correct for changes in hemoglobin saturation, which are substantial during hypoxemia.

To obtain the most accurate measurement of the redox state of CuA, a full spectral charge-coupled device (CCD)-based NIR system was used, which has been shown by modeling to be more robust in separating the cytochrome oxidase component than two, four,

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or six wavelength techniques (21). An added advantage of the use of a full spectral system is that the optical path length can be measured from the second differential of the water absorption features (20) so that changes in hemoglobin concentration and CuA redox state can be quantified in units of concentration (μmol/l). In addition, absolute deoxyhemoglobin concentration (aHb) can be obtained from the second differential technique (19), and absolute oxyhemoglobin concentration (aHbO2) can be calculated by assuming that cerebral HbO2 falls to zero during brief anoxia. Mean cerebral saturation (SmcO2) can then be calculated from aHbO2 and aHb.

This study was designed to show that the oxidation of the CuA signal observed during hypercapnia accurately reflects oxidation changes of cytochrome oxidase and to show that this oxidation is not the result of the increase in oxygen delivery and PO2 associated with hypercapnia. This was achieved by performing brief anoxias at normocapnia and hypercapnia and determining the relationship between the CuA oxidation state and SmcO2 during the onset of anoxia using NIRS. We have previously compared CuA oxidation changes with hypercapnia preperfluorocarbon and postperfluorocarbon exchange (24) and found that the oxidation is still present when hematocrit is reduced by 80%. This study uses an improved NIR system with greater sensitivity and higher temporal and spectra resolution to study uses an improved NIR system with greater sensitivity and higher temporal and spectra resolution to verify the CuA changes in the normal hematocrit piglet's head with a glass optic fiber bundle (3.3-mm diameter) pressed 1 cm square) pressed 1 cm posterior to the eyes. The optodes were positioned ~35 mm apart symmetrically about the midline 1 cm posterior to the eyes. The optodes were surrounded by NIR-opaque sponges (~1 cm square) pressed firmly against the head, and the cranium painted with NIR-opaque paint. The use of the sponges and paint ensured that light emerging near the transmit optodes did not reenter the head near the receive optode and interfere with the attenuation measurement.

Changes in concentration of oxyhemoglobin (ΔHbO2), deoxyhemoglobin (ΔHb), and CuA oxidation state (ΔCuA) were obtained by performing a least-squares fit of

$$\Delta \phi_i(\lambda) = \sum_i \Delta C_i \alpha_i(\lambda), \quad (1)$$

to the measured change in attenuation spectra (Δph) between 780 and 900 nm, where i represents the chromophores HbO2, Hb, and CuA; ΔCi is the product of path length and change in chromophore concentration, and αi is the chromophore in vitro specific absorption spectra (21), which has been corrected for the wavelength dependence of path length. The optical path length at the 840-nm water absorption feature was obtained by fitting the second differential of the attenuation spectra to the second differential of water and Hb absorption spectra between 800 and 880 nm, and the path length at 840 nm was converted to units of centimeters by assuming an average cerebral water content of 85%. Changes in HbO2, Hb, and CuA (ΔCi of Eq. 1) were converted to units of micromoles per liter using the 840-nm water path length derived from baseline spectra. The path length at 740 nm and aHb were obtained by fitting the second differential of the attenuation spectra to the second differential of water and Hb absorption spectra between 700 and 800 nm. Total hemoglobin (HbT) is the sum of HbO2, Hb, and can be used to calculate the cerebral blood volume (CBV) if the cerebral hematocrit is known.

Spectral data were collected every 5 s throughout the study, and heart rate, blood pressure, arterial saturation, and rectal temperature were logged simultaneously. The gas mixture fed to the piglets was controlled using a computerized gas blender (6) and was switched synchronously with the spectral data collection. However, slight differences in ventilation parameters led to slightly different time delays between gas changes and the observed changes in the NIR parameters. To time normalize the data sets, the time normalization point was chosen as the first data point at which ΔHbO2 fell below zero during the onset of anoxia.

At the end of surgery and once the piglet had been positioned in the stereotaxic frame, the isoflurane was reduced to 1.5–1.8%, and the piglet allowed to stabilize over a period of at least 1 h, during which NIRS spectra were recorded. The FiO2 and inspired carbon dioxide fraction (FiCO2) were switched independently, and the balance of the gas fraction was always nitrogen. The FiO2 was maintained at 0.4 throughout the experiment except during periods of anoxia. The FiCO2 was maintained at 0.0 except during hypercapnia.

The protocol started with 5 min of recorded baseline followed by 90 s of anoxia and then reoxygenation. A period of 20 min was allowed for the hemodynamic state to stabilize, and moderate hypercapnia was then induced by increasing FiCO2 (see below). Once the hemodynamic signals were sta-
Table 1. Physiological parameters before the anoxia at normocapnia and hypercapnia

<table>
<thead>
<tr>
<th>Physiological Parameter</th>
<th>Normocapnia</th>
<th>Hypercapnia</th>
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<tbody>
<tr>
<td>PaCO₂, mmHg</td>
<td>33.5 ± 4.9</td>
<td>54.3 ± 5.5</td>
</tr>
<tr>
<td>pH</td>
<td>7.543 ± 0.032</td>
<td>7.345 ± 0.025</td>
</tr>
<tr>
<td>PaO₂, mmHg</td>
<td>184 ± 20</td>
<td>155 ± 14</td>
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<tr>
<td>SæO₂, %</td>
<td>98.6 ± 0.8</td>
<td>97.9 ± 1.1</td>
</tr>
<tr>
<td>MABP, mmHg</td>
<td>47.7 ± 4.5</td>
<td>52.0 ± 6.4</td>
</tr>
<tr>
<td>Heart rate, beats/min</td>
<td>151 ± 14</td>
<td>161 ± 22</td>
</tr>
<tr>
<td>TR, °C</td>
<td>38.5 ± 0.13</td>
<td>38.4 ± 0.2</td>
</tr>
<tr>
<td>Glucose, mM</td>
<td>5.65 ± 0.89</td>
<td>5.27 ± 0.55</td>
</tr>
<tr>
<td>Lactate, mM</td>
<td>2.15 ± 0.92</td>
<td>1.92 ± 0.94</td>
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</table>

All values are means ± SD; n = 7 piglets. Glucose and lactate refer to their respective plasma concentrations; pH, the partial pressure of arterial carbon dioxide (PaCO₂), the partial pressure of arterial oxygen (PaO₂), and arterial oxygen saturation (SæO₂) refer to arterial blood; TR is rectal temperature; and MABP is mean arterial blood pressure.

At normocapnia, a second anoxia of 120 s was initiated followed by reoxygenation. From 0.05 from 4 min before the anoxia until 5 min after reoxygenation. Blood samples for gas analysis were collected immediately before the anoxias.

To stabilize the arterial PCo₂ in the shortest possible time, FCO₂ was switched to 0.10 for 4 min and then reduced to 0.05 for 4 min before the anoxia because it has been shown previously (29) that equilibrium is reached more rapidly in the off phase than the on phase. A period of 90 s of anoxia at normocapnia and 120 s at hypercapnia was chosen after a pilot study indicated that 120 s of anoxia could produce profound bradycardia at normocapnia and the CuA signal did not fully reduce in 90 s at hypercapnia.

Analysis of variance (ANOVA) was used to compare repeated measurements, and all data are presented as means ± SD (n = 7 animals). The NIRS data were collected at regular time intervals (5 s), and the means ± SD were calculated over the data from the seven piglets on a point-by-point basis. The significance of changes was determined using a paired Student’s t-test; the criterion for significance was P < 0.05.

RESULTS

Typical root-mean-square noise values on the baseline were 0.2, 0.1, and 0.03 μmol/l for the ΔHbO₂, ΔHb, and ΔCuA signals, respectively.

Physiological parameters obtained at baseline, normocapnia, and hypercapnia before the anoxia are presented in Table 1. Baseline normocapnic parameters were within normal ranges, and, although arterial pH was slightly alkalotic, this is normal for the newborn piglet. Hypercapnia resulted in a significant increase in arterial PCO₂ of 20.9 ± 5.6 mmHg and a significant decrease in pH of 0.198 ± 0.32, as would be expected. Heart rate, rectal temperature, and arterial glucose and lactate concentrations did not significantly differ between normocapnia and hypercapnia. Hematocrit was 30.6 ± 4.6%.

Figure 1 shows the pooled data for ΔHbO₂, ΔHb, ΔCuA, and the change in HbT during an anoxic swing at normocapnia (left) and hypercapnia (right). Time = 0 on Fig. 1 represents the first data point at which there is a substantial fall in ΔHbO₂ from the respective baseline. The time-normalization point at normocapnia (see METHODS) is at 0 s, whereas the time-normalization point at hypercapnia is at 35 s because the baseline ΔHbO₂ at hypercapnia is above zero.

At normocapnia (Fig. 1 (left)), the reduction in arterial saturation leads to a cerebral desaturation starting at 0 s and resulting in a fall in HbO₂ and an
increase in Hb, initially with no change in HbT. During the desaturation, PtO₂ is expected to fall but it is not until 25 s after the onset of anoxia that the first significant reduction of the Cuₐ signal and an increase in HbO₂. Arterial saturation rapidly returns to normal levels but, at the elevated CBF, leads to an increase in HbO₂ and a decrease in Hb over baseline values. HbT reaches a maximum at −145 s, and HbT, HbO₂, and Hb then return toward baseline values over the subsequent 6 min but do not return completely to baseline until 10 min after the onset of anoxia.

During the reoxygenation phase, Cuₐ rapidly reoxidizes and reaches baseline values before HbO₂ returns to baseline. During the hyperemic period postreoxygenation, Cuₐ oxidizes by 0.26 ± 0.12 μmol/l (P < 0.01) above baseline and then returns to baseline over the same time period as HbO₂.

Heart rate and mean arterial blood pressure increased during the anoxic swing (data not shown) and reached a maximum during the reoxygenation period and then returned to baseline over approximately the subsequent 10 min.

During mild hypercapnia, the cerebral metabolic rate of oxygen (CMR O₂) is expected to remain constant (26), but there is an increase in both CBF and CBV. The increase in CBF is predominantly due to dilation of the pial arteries, whereas the increase in CBV is mainly due to venous distension. At constant CMR O₂ and CBF, an increase in the venous volume would be expected to increase HbO₂ and Hb in the ratio of the average venous saturation. At constant CMR O₂ and CBV, an increase in CBF would increase HbO₂ and decrease Hb in equal magnitude. The net effect when CMR O₂ is constant but both CBF and CBV increase would be an increase in HbO₂ and either a smaller increase or a small decrease in Hb, depending on the relative compliance of the venous side. The observed changes, which are shown in the baseline period of Fig. 1 (right) and presented in Table 2, consist of a large increase in HbT and HbO₂ and a small decrease in Hb. In this study, the Cuₐ oxidation resulting from the hypercapnia was 0.43 ± 0.23 μmol/l (P < 0.005).

Figure 1 (right) shows the pooled NIR parameters during an anoxic swing at mild hypercapnia. Qualitatively, the changes are similar to those at normocapnia, but the delay between the fall in HbO₂ and the reduction in Cuₐ is greater.

Figure 2 (left) compares the measured attenuation changes with the residuals of the least squares fitting for a typical piglet at five different time points. The residuals are the difference between the measured attenuation change (Δfₜₐₖ) and the attenuation change due to the chromophore concentration changes obtained by the least squares algorithm, that is Δfₜₐₖ − Δf₀, where Δf₀ is defined in Eq. 1. The residuals give an indication of the quality of the fit but cannot be used to calculate quantitative errors in the calculated concentration changes of the chromophotes; for a perfect fit, they would show noise centered around zero. Figure 2 (right) compare the residuals with the Cuₐ component of Eq. 1 at the same time points as Fig. 2 (left) but on an expanded scale. The Cuₐ component of Eq. 1 is ΔCₐx(λ), where i refers to Cuₐ and represents the component of the change in measured attenuation change that the fitting algorithm attributes to changes in the redox state of Cuₐ. The differential path length measured from the 840-nm water feature for this piglet was 16.0 cm.

The time points of Fig. 2 are the following: a, during the anoxia at normocapnia immediately before there is a substantial change in the Cuₐ signal; b, just before reoxygenation at normocapnia; c, from the hypercapnia baseline period; d, during the anoxia at hypercapnia immediately before there is a substantial change in the Cuₐ signal; and e, just before reoxygenation at hypercapnia. These time points are marked on Fig. 1 as a−e, respectively.

In all cases except where there is no change in the Cuₐ signal from baseline, the residuals are small compared with the attenuation change due to the change in redox state of Cuₐ.

Figures 3 and 4 show the change in Cuₐ plotted against ΔHbO₂ and SmcO₂, respectively, during the

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<th>Table 2. NIRs parameters at baseline, at the depth of the anoxia, and during the hyperaemia at normocapnia and hypercapnia</th>
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<tr>
<td>Parameters</td>
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<tr>
<td></td>
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<tr>
<td>ΔHbO₂</td>
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<tr>
<td>ΔHb</td>
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<td>ΔHbT</td>
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<td>aHbO₂</td>
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<td>aHbT</td>
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<tr>
<td>ΔCuₐ</td>
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<td>SMCO₂</td>
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</table>

All values are means ± SD, n = 7 piglets. Hemoglobin and the changes in copper A (Cuₐ) oxidation state are measured in micromoles per liter, and the mean cerebral oxygen saturation (SmcO₂) is measured in percent. ΔHbO₂, ΔHb, and ΔHbT: changes in oxyhemoglobin, deoxyhemoglobin, and total hemoglobin, respectively; aHbO₂, aHb, and aHbT: absolute concentrations of oxyhemoglobin, deoxyhemoglobin, and total hemoglobin, respectively. NIRs, near-infrared spectroscopy.
onset period of anoxia at normocapnia and hypercapnia. During normocapnia, the first significant reduction in CuA occurs when HbO2 has dropped by 28.0 ± 2.4 μmol/l from a baseline of 24.6 ± 6.8 μmol/l and SmcO2 had fallen to 43.2 ± 5.4%. With hypercapnia, at the beginning of the anoxia, there is a small but significant reduction in CuA from 0.44 ± 0.26 to 0.39 ± 0.17 μmol/l (P < 0.05) when HbO2 falls from 14.4 ± 6.7 to 11.0 ± 4.1 μmol/l, but there is then no significant change in the CuA redox state until HbO2 falls to 19.6 ± 4.3 μmol/l. The change in the CuA signal between HbO2 of 11.0 ± 4.1 and −13.8 ± 3.0 μmol/l when SmcO2 falls from 77.4 ± 3.1 to 21.3 ± 6.3% is −0.04 ± 0.11 μmol/l.

At the first significant reduction of CuA, HbT has increased by 0.96 ± 0.67 μmol/l at normocapnia and by 1.2 ± 0.7 μmol/l at hypercapnia compared with the respective baseline. Both of these changes are significant (P < 0.01) but small compared with the normocapnia-to-hypercapnia change in HbT of 10.6 ± 3.6 μmol/l. Therefore, although the cerebral vasculature has responded to the hypoxemia, this response is small compared with that induced by hypercapnia.

DISCUSSION

The CuA center lies on the cytosolic side of cytochrome oxidase close to the cytochrome c binding site and far from the binuclear where molecular oxygen is reduced to water (14, 27). In general, the redox state of cytochrome c and heme a are measured by visible absorption spectroscopy in mitochondrial studies because they provide substantial attenuation at visible wavelengths at the concentration and optical path lengths typically used in these studies compared with the low attenuation of the NIR band of CuA. When the oxidation state of CuA and cytochrome c have been measured simultaneously, it has been found that the CuA center senses the same membrane potential as cytochrome c (25) and is in redox equilibrium with cytochrome c during coupled turnover (23). Therefore, the changes in the oxidation state of CuA from this in vivo model can be directly compared with those of cytochrome c from in vitro mitochondrial models.

At high oxygen tensions, the redox state of cytochrome c and, therefore, the CuA center is independent of oxygen tension and determined by the metabolic...
state and the activity of the dehydrogenases of the tricarboxylic acid cycle (2). As oxygen tension is reduced to zero, all the components of the electron chain become reduced, and the mitochondrial oxygen tension ($P_{mO_2}$) at which oxidation changes are first observed in cytochrome c is between 0.6 and 20 mmHg, depending on metabolic state (30).

Direct measurement with microelectrodes has shown considerable heterogeneity in cortical $PtO_2$, with values ranging from arterial $P_O_2$ down to essentially zero (17) and with $\sim 2\%$ of sites having a $PtO_2$ of $<5$ mmHg (18). Therefore, even at normoxia, there is likely to be a population of mitochondria in which the electron transport chain redox state is oxygen dependent. However, if this population is very small, then small increases or decreases in $S_{mC_2}$ will have a negligible effect on the size of this population and a negligible effect on the mean $CuA$ oxidation state. However, it is difficult to determine the size of this population, given that the microelectrodes measure $PtO_2$ and not $PmO_2$, the presence of an oxygen gradient between mitochondrion and capillary (3), and the uncertainty in the critical $PmO_2$ in vivo.

Optical techniques have the potential to measure changes in the redox state of the electron transport chain directly, but great care must be taken to accurately separate the redox signal from the hemoglobin signals. NADH fluorescence is often assumed to be a more robust method than absorption spectroscopy because the reduction state of NADH is proportional to the intensity of the fluorescence signal. However, both the excitation light and the fluorescence light will be attenuated by the hemoglobin in the field of view, and this attenuation is a complex nonlinear function of hemoglobin concentration and saturation (13) so, in a scattering medium like tissue, a simple two or three wavelength ratiometric measurement cannot fully eliminate these effects.

The cytochrome oxidase signal from NIRS and visible absorption spectroscopy represents a small component of the overall attenuation change with anoxia or hypercapnia. For example, the attenuation change caused by the $CuA$ oxidation seen during the hypercapnia represents typically 6% of the total attenuation change, the majority being hemoglobin (see Fig. 2C). This makes the cytochrome oxidase signal sensitive to cross talk, that is, changes in the concentration of chromophores that result in the attenuation changes not fully accounted for by the chromophore absorption spectra used in the least squares fitting. Cross talk would be manifest as changes in the cytochrome oxidase signal that track one, or a combination, of the hemoglobin signal(s). Other confounding effects would be changes in the scattering coefficient of the tissue or the movement of the tissue with respect to the optodes.

To show that cross talk is minimal in this system, it is necessary to show that $Hb$ and $HbO_2$ (or any two noncollinear combinations) can be varied without affecting the $CuA$ signal. However, in general, it is not known a priori whether a particular procedure that varies the hemoglobin concentration will affect the $CuA$ redox state. For instance, although at normocapnia there was no significant reduction in the $CuA$ signal during the onset of anoxia until there was a substantial fall in $S_{mC_2}$, it is possible that the $CuA$ redox state was changing during this period and that the $CuA$ signal was stable as a result of cross talk with hemoglobin. However, at normocapnia, $HbO_2$ fell to $5.3 \pm 1.6 \mu mol/l$ with no significant change in the $CuA$ signal, whereas, at hypercapnia, $HbO_2$ to fell from $12.6 \pm 5.3$ to $-13.8 \pm 3.0 \mu mol/l$ with no significant change in the $CuA$ signal ($P > 0.1$). The observation that the cerebral saturation can be changed with no change in the $CuA$ signal and that the point at which the $CuA$ signal begins to reduce can be altered with a physiological

![Fig. 3. Changes in the $CuA$ redox state plotted against $\Delta HbO_2$ at both normocapnia (N) and hypercapnia (H) during the onset of anoxia. The results are shown as means ± SD ($n = 7$ piglets).](image)

![Fig. 4. Changes in the $CuA$ redox state plotted against mean cerebral saturation ($S_{mC_2}$) $HbO_2$ at both normocapnia and hypercapnia during the onset of anoxia. The results are shown as means ± SD ($n = 7$ piglets).](image)
maneuver would suggest that the cytochrome signal is robust with respect to HbO₂ and Hb under these conditions where HbT was constant.

Hypercapnia at normoxia lead to an increase in HbT of 12.6 ± 6.2 μmol/l and an oxidation in the CuA signal of 0.43 ± 0.27 μmol/l. If this oxidation were the result of cross talk with HbT, then this result should remain when the CuA center is expected to be fully reduced during the anoxic swings but where the increase in HbT is still present. However, the reduction of CuA at normocapnia was to −1.53 ± 0.44 μmol/l and the reduction at hypercapnia was to −1.49 ± 0.40 μmol/l; the difference being 0.039 ± 0.086 μmol/l and not significant (P > 0.25). The observation that HbO₂ fell to the same value during the anoxia at normocapnia and hypercapnia [−24.0 ± 6.4 and −24.5 ± 6.8 μmol/l, respectively; the difference is not significant (P > 0.25)] is evidence that the hemoglobin was fully desaturated during the anoxia. Thus the CuA signal is robust with respect to changes in HbT.

That the CuA signal is robust under both changes in HbT and hemoglobin saturation would suggest that the CuA signal from this system using this algorithm accurately reflects the redox state of the CuA center in this model and under these conditions. This conclusion is further confirmed by the observation that the residuals of the fitting algorithm are small compared with the attenuation changes due to the CuA redox changes.

If it is assumed that the CuA center becomes fully reduced during the anoxia and fully oxidized in the hyperemic period after anoxia at hypercapnia, then the total concentration of redox-active cytochrome oxidase in the piglet brain is 2.21 ± 0.16 μmol/l, and the baseline oxidation is 67.3 ± 18.8% oxidized. This concentration for the newborn piglet is in general agreement with the results of biochemical analysis of the developing rat brain, which shows an increase in the concentration of cytochrome oxidase from 1.3 μmol/l at birth to 5.8 μmol/l in the adult (1), and further emphasizes the fact that the concentration of cytochrome oxidase in the newborn piglet is small compared with the concentration of hemoglobin, which is between 40 and 50 μmol/l depending on arterial PCO₂ (see Table 2).

During the onset of anoxia at normocapnia, the first observed change in the CuA signal occurred when SmcO₂ had fallen from a baseline value of 65 ± 4 to 43 ± 5% when the reduction was 0.08 ± 0.04 μmol/l or a fall of 3.6% of the total cytochrome oxidase. This would suggest that the oxygen tension at the great majority of mitochondria is above the value at which their redox state becomes oxygen dependent, and it is not until there is a substantial fall in oxygen tension that a sufficiently large population of mitochondria have an oxygen tension sufficiently low to affect the measured CuA oxidation state. The shift of the critical SmcO₂ to lower values seen during hypercapnia is probably the result of two effects of hypercapnia. First, the Bohr effect at reduced arterial and venous pH will increase the oxygen tension at a given hemoglobin saturation, and it is the difference in oxygen tension between vasculature and mitochondria that drives the diffusion of oxygen. Second, the microcirculation is more heterogeneous at normocapnia than at hypercapnia (12), and this would tend to broaden the response of CuA to SmcO₂ and shift the critical SmcO₂ to higher values at normocapnia compared with hypercapnia.

If the CuA redox state is independent of oxygen tension at normoxia, then increasing oxygen tension by increasing SmcO₂ should not produce the oxidation observed during hypercapnia. Further confirmation that the oxidation observed with hypercapnia is not a response to increased oxygen tension is the observation that, under these conditions, SmcO₂ can be lowered to normocapnia levels and below during the onset of anoxia without a reduction of the CuA signal back to the normocapnia baseline.

Therefore, in conclusion, the results presented here are consistent with microelectrode measurements of PO₂ and a low critical PmO₂ value as measured in isolated mitochondria: at normoxia and normocapnia, the PO₂ at the majority of mitochondria is well above a value that would limit oxygen consumption. The oxidation observed during hypercapnia is not consistent with the oxidation of an oxygen-limited population. In light of this, it is likely that the oxidation observed during hypercapnia is the result of the perturbation in carbohydrate metabolism or other effects of the reduced intracellular pH (10).

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REFERENCES


