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Cytochrome *c* oxidase response to changes in cerebral oxygen delivery in the adult brain shows higher brain-specificity than haemoglobin ☆

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ABSTRACT

The redox state of cerebral mitochondrial cytochrome *c* oxidase monitored with near-infrared spectroscopy (Δ[oxCCO]) is a signal with strong potential as a non-invasive, bedside biomarker of cerebral metabolic status. We hypothesised that the higher mitochondrial density of brain compared to skin and skull would lead to evidence of brain-specificity of the Δ[oxCCO] signal when measured with a multi-distance near-infrared spectroscopy (NIRS) system. Measurements of Δ[oxCCO] as well as of concentration changes in oxygenated (Δ[HbO_2]) and deoxygenated haemoglobin (Δ[HHb]) were taken at multiple source-detector distances during systemic hypoxia and hypocapnia (decrease in cerebral oxygen delivery), and hyperoxia and hypercapnia (increase in cerebral oxygen delivery) from 15 adult healthy volunteers. Increasing source-detector spacing is associated with increasing light penetration depth and thus higher sensitivity to cerebral changes. An increase in Δ[oxCCO] was observed during the challenges that increased cerebral oxygen delivery and the opposite was observed when cerebral oxygen delivery decreased. A consistent pattern of statistically significant increasing amplitude of the Δ[oxCCO] response with increasing light penetration depth was observed in all four challenges, a behaviour that was distinctly different from that of the haemoglobin chromophores, which did not show this statistically significant depth gradient. This depth-dependence of the Δ[oxCCO] signal corroborates the notion of higher concentrations of CCO being present in cerebral tissue compared to extracranial components and highlights the value of NIRS-derived Δ[oxCCO] as a brain-specific signal of cerebral metabolism, superior in this aspect to haemoglobin.

Introduction

Transcranial near-infrared spectroscopy (NIRS) provides a measure of cerebral oxygen delivery by monitoring concentration changes in oxygenated (Δ[HbO_2]) and deoxygenated haemoglobin (Δ[HHb]), non-invasively. A third spectral signal is present (Tisdall et al., 2008a), consistent with the features of mitochondrial cytochrome *c* oxidase (CCO) (Jöbsis, 1977). CCO is the terminal electron acceptor in the mitochondrial respiratory chain and, being responsible for over 95% of oxygen metabolism, it is instrumental in aerobic ATP synthesis (Richter and Ludwig, 2003). Since in the short term the total concentration of CCO remains constant, concentration changes of oxidised cytochrome *c* oxidase monitored with NIRS (Δ[oxCCO]) represent changes in the CCO redox state, which reflects the balance between cerebral energy supply and demand (Smith, 2011). Thus, Δ[oxCCO] is an appealing target for the bedside assessment of regional cerebral metabolic status and oxygen utilisation, and provides information complementary to Δ[HbO_2] and Δ[HHb], which only reflect intravascular oxygenation. The information is also complementary to cerebral oximetry, which delivers a measure of absolute cerebral haemoglobin oxygen saturation based on the technique of spatially resolved spectroscopy (SRS). Regardless of the high sensitivity and specificity of cerebral oximetry to intracerebral changes (Al-Rawi et al., 2001), it is still affected by extracerebral changes (Davie and Grocott, 2012) and so far it has not been successful in providing a robust clinical marker of sufficient cerebral oxygen delivery and tissue status (Boas and Franceschini, 2011; Ghosh et al., 2012).

Despite its potential as a non-invasive bedside marker of cerebral cellular oxygen metabolism, there is still debate about the use of Δ[oxCCO] outside research clinical settings, primarily due to technical complexities associated with this measurement in the adult brain, in the presence of significantly higher concentrations of haemoglobin. The possible interference of changes in optical scattering with the NIRS recordings and the insufficient chromophore separation by the algorithm used to convert optical density into concentration changes

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are the most notable challenges (Cooper and Springett, 1997; Cooper et al., 1999). A hybrid optical spectrometer (pHOS) and accompanying algorithm designed to address the above issues and provide robust Δ[oxCCO] data, have recently been developed by our group (Kolyva et al., 2012). In addition, the pHOS has the capacity for measurements at multiple source-detector distances (and therefore at multiple depths), a technological advance that enables for the first time multi-distance Δ[oxCCO] recordings in vivo in adults. These recordings may contribute considerably to the interpretation of the Δ[oxCCO] signal, by determining if there is a distance/depth-dependent response of Δ[oxCCO] in the adult head, an expectation based on the higher mitochondrial density of the brain compared to tissues with lower metabolic rates, such as skin and skull (Kakihana et al., 2008; Smith and Elwell, 2009; Tisdall et al., 2007).

The aim of the present study was to investigate the multi-depth response of Δ[oxCCO] to global changes in cerebral oxygen delivery secondary to systemic hypoxia, hyperoxia, hypocapnia and hypercapnia in the healthy adult brain. To enable the more thorough monitoring of the physiological mechanisms activated in the brain during oxygen delivery manipulation, cerebral blood flow velocity and absolute tissue oxygen saturation were studied simultaneously with $\Delta[HbO_2]$, Δ[HHb] and Δ[oxCCO]. We hypothesised that Δ[oxCCO] would show an incremental response with increasing source-detector separation, mirroring potential differences in the extra- and intracranial distribution of this chromophore, and reinforcing confidence in the use of this signal as a brain-specific biomarker of cerebral metabolic status.

Methods

This study was approved by the local ethics committee and all subjects provided written informed consent.

Monitoring

NIRS monitoring

A hybrid spectroscopy device (pHOS) described in more detail elsewhere, was used to obtain multi-distance near-infrared measurements (Kolyva et al., 2012; Tachtsidis et al., 2010). The pHOS combines frequency domain (FD) and broadband (BB) components and can measure light absorption and scattering at discrete wavelengths (690, 750, 790 and 850 nm), together with broadband light attenuation in the range 504–1068 nm. Each of the two pHOS optodes incorporates an FD channel (source-detector spacing 3.0 and 3.5 cm) and BB channel (source-detector spacing 2.0, 2.5, 3.0 and 3.5 cm). One sampling cycle of the pHOS lasts 3.2 s, and BB and FD measurements are made sequentially. A single optode was placed on the forehead in the mid-pupillary line, high enough to avoid the frontal sinuses.

Other physiological monitoring

Other monitoring included beat-to-beat pulse oximetry (Oxypleth, NovaMetrix, MA), continuous non-invasive arterial blood pressure (PortaPres, Finapres Medical Systems, The Netherlands), electrocardiography (IntelliVue MP50, Philips Healthcare, The Netherlands), capnography (CO_2SMO, NovaMetrix) and inspired/expired oxygen partial pressure (IntelliVue Anaesthetic Gas Module, Philips Healthcare). Transcranial Doppler (TCD) ultrasonography was used to measure middle cerebral artery flow velocity ipsilateral to the pHOS optode, as a surrogate of cerebral blood flow (DWL Doppler Box, Compumedics, Germany).

Protocols

Cerebral oxygen delivery was manipulated through four separate systemic physiological challenges: hypoxia, hyperoxia, hypocapnia and hypercapnia.

Each study protocol was commenced with 5 min of baseline recording (air inhalation). Subsequently, hyperoxia was instigated by increasing the inspired fraction of oxygen (FiO_2) to >90%. Analogously, hypercapnia was induced by the addition of 6% CO_2 to the inspired gas mix, targeting an increase of ~2 kPa in end-tidal partial pressure of CO_2 ($EtCO_2$). Hypocapnia was achieved by instructing the subjects to hyperventilate; verbal feedback was provided to them for guidance, in order to reach and maintain a ~2 kPa reduction in $EtCO_2$. Each of these manipulations lasted 5 min ('*challenge*' period) (Figs. 1B–D). The protocols were then concluded with a further 5-minute period of baseline recording. In the hyperoxia/hypercapnia protocols, this second period of baseline commenced after the return of end-tidal O_2/CO_2 partial pressures to their pre-challenge levels, once inhalation of room air had been resumed. In the hypocapnia protocol, the second period of baseline recording directly followed the cessation of hyperventilation.

The hypoxia protocol differed from the others to account for physiological variability in the speed of haemoglobin desaturation during inhalation of a hypoxic gas mix. After the initial 5-minute period of baseline recording, hypoxaemia was induced through the delivery of a hypoxic gas mix: the initial FiO_2 of 8% was titrated to achieve a reduction in arterial oxygen saturation (SpO_2) to 80% ('*induction*' period). Once this was accomplished, SpO_2 was sustained at 80% for 5 min ('*plateau*' period), before returning the inspired gas to room air (Fig. 1A). After return of end-tidal O_2 back to baseline levels, the recording was continued for a further 5-minute period of baseline recording.

For hypoxaemia, hyperoxia and hypercapnia, a sequential gas delivery circuit was used to maintain a constant $EtCO_2$ despite changes in minute volume (Slessarev et al., 2007). For hypocapnia, the subjects were instructed to hyperventilate through a mouthpiece incorporating the sensors for capnography and tidal oximetry.

Data analysis

NIRS algorithms

Differential spectroscopy. Data analysis was performed in MATLAB (version R2010b, MathWorks, Natick, MA). Based on the modified Beer–Lambert law, $\Delta[HbO_2]$, Δ[HHb]) and Δ[oxCCO] were determined from the measured changes in broadband light attenuation using the UCL*n* algorithm, a least-squares fitting technique (Kolyva et al., 2012; Matcher et al., 1995). The wavelength range used for the fitting was 780–900 nm and the wavelength dependence of differential pathlength factor (DPF) (Essenpreis et al., 1993), demonstrated as a decrease in DPF with increasing wavelength, was incorporated in the calculations, which were carried out individually for the four detectors. DPF was assumed to be equal to 6.26 for the two detectors proximal to the BB light source (source-detector spacing 2.0 and 2.5 cm) (Duncan et al., 1995) and was calculated for the two distal detectors (source-detector spacing 3.0 and 3.5 cm) using an initial FD measurement at 790 nm (Fantini et al., 1999). Changes in total haemoglobin concentration were defined as $\Delta[HbT] = \Delta[HbO_2] + \Delta[HHb]$ and in haemoglobin difference as $\Delta[Hbdiff] = \Delta[HbO_2] - \Delta[HHb]$. The absorption ($\mu_a$) and reduced scattering (μ_s') coefficients were quantified from the FD measurements.

Spatially resolved spectroscopy. The tissue oxygenation index (TOI) was calculated using the SRS methodology, through which the slope of BB light attenuation within the range 740–900 nm was derived (Suzuki et al., 1999).

Linear regression was performed between the measured attenuations (A) of the four detectors and the corresponding source-detector distances (ρ) (assuming that the latter were the nominal distances described in the NIRS monitoring section), yielding an attenuation slope ($\partial A / \partial \rho$). At every sampling point, this calculation was repeated for every wavelength (λ) within the range 740–900 nm. This in turn enabled the derivation of a relative