BICUCULLINE-INDUCED SEIZURES: A CHALLENGE FOR OPTICAL AND BIOCHEMICAL MODELING OF THE CYTOCHROME OXIDASE Cu\textsubscript{A} NIRS SIGNAL

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**Abstract:** The effect of seizures on brain blood flow and metabolism has been extensively studied. However, few studies have focused on mitochondria. We used near infrared spectroscopy (NIRS) to study hemoglobin and cytochrome oxidase changes during seizures, induced by the GABA antagonist bicuculline, in the adult rat. A broadband spectroscopy system was used with the optodes placed across the rat head. We focused on the initial seizures post-bicuculline addition during which oxyhemoglobin (HbO\textsubscript{2}) increased, deoxyhemoglobin (HHb) decreased and total hemoglobin (Hbtot) increased. The NIRS signal associated with the oxidised Cu\textsubscript{A} centre of mitochondrial cytochrome c oxidase (oxCCO) decreased. At the highest bicuculline doses (0.25 mg/animal) the maximum values recorded were: \(\Delta\text{HbO}_2 = +19 \pm 7 \text{ \mu M}\); \(\Delta\text{HHb} = -12 \pm 4 \text{ \mu M}\); \(\Delta\text{Hbtot} = +7 \pm 4 \text{ \mu M}\), \(\Delta\text{oxCCO} = - 1.7 \pm 0.3 \text{ \mu M}\). These results are broadly in line with other NIRS studies. However, previous measurements of NADH fluorescence indicate oxidation of the mitochondrial redox chain under these conditions. The changes induced by bicuculline provide an interesting challenge to the physics and biochemistry of using NIRS to study mitochondrial redox states in vivo and we explore the possible spectroscopic and/or biochemical meaning of these apparent anomalies.

1. **INTRODUCTION**

Since 1977\textsuperscript{1}, near infrared spectroscopy (NIRS) has been extensively used to study redox state changes in mitochondrial cytochrome c oxidase (CCO). In the wavelength range 700 – 900 nm these are dominated by a broad band centred at 830 nm and associated with the oxidised form of the binuclear Cu\textsubscript{A} redox centre\textsuperscript{2}. This centre is the initial electron acceptor from the substrate, reduced cytochrome c. Electron transfer

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to/from cytochrome c and Cuₐ is fast compared to enzyme turnover³ and it is likely that changes in the Cuₐ redox state reflect those in cytochrome c and vice versa.

There has been considerable controversy about the ability of NIRS techniques to detect changes in the redox state of CCO in vivo (oxCCO) in the presence of the generally larger concentration changes of hemoglobin. We have reviewed this field previously² and suggested the use of mitochondrial inhibitors (e.g. cyanide) to test algorithms claiming to be sensitive to changes in oxCCO⁴. However, it would also be useful to have reproducible tests that reported on activations of mitochondrial metabolism. We have looked at the increased mitochondrial oxygen consumption induced by the mitochondrial uncoupler, dinitrophenol⁵. In this paper we look at an alternative, more physiological, method to cause increases in cerebral mitochondrial metabolism, the use of the GABA antagonist, bicuculline; bicuculline is interesting in this regard as it causes changes in both cerebral biochemistry/physiology⁶ (increases in cerebral glucose utilisation, blood flow, nitric oxide production, oxygen consumption and ATP turnover) and cerebral optical properties (due to seizures and increases in blood volume). In this study we report on the changes in oxCCO NIRS in the adult rat brain following the initial addition of bicuculline and comment on the implications for our ability to detect, and importantly interpret, these changes in oxCCO.

2. METHODS

All the work described here was carried out in accordance with UK regulations for the use of animals in research. Male Wistar rats (n = 6) weighing 300 to 500 g were anaesthetised with urethane (ethyl carbonate 36% w/v solution, 0.5 ml/100 g body weight intra-peritoneal). Tracheostomy was performed and a femoral artery and vein cannulated. The skull was then exposed by an incision along the top of the head lengthwise, the scalp tissues reflected and the temporal muscles removed. Cautery sealed the exposed tissue to prevent blood loss. The head was immobilised in a stereotactic holder and a source and detector optical fibre bundle were positioned either side of the rat’s skull in contact with the parietal bones. A clear gel was used between the optical fibres and the skull to improve optical coupling. This enabled transillumination of approximately 1.4 cm width across the rat’s brain. Light was delivered to the source fibre from a broadband white light source and a cooled CCD detector was used to detect the transmitted NIR light between 700 and 900 nm⁷. Electroencephalographic (EEG) electrodes to monitor cerebral function were placed in burr holes made in the skull. In some studies the animal was paralysed with curare (intra-venously 0.2 ml of 1.5 mg/ml solution, additional doses given throughout when required). Anaesthesia was maintained throughout by giving 0.2 ml of 24% urethane intra-venously every 2 to 3 hours. Body temperature was maintained at 37 ± 1°C via a heated bed. Arterial blood pressure was monitored continuously by a transducer (Elcomatic model 750, U.K.) attached to the arterial catheter. The attenuation changes measured by the broadband spectroscopy system were converted into changes in HbO₂, HHb and oxCCO using an algorithm that allowed for a non-linear conversion of attenuation to extinction coefficient at each wavelength and at each time point⁸,⁹. Multilinear regression was then used to convert the modified data to changes in [HbO₂], [HHb] and [oxCCO]. The wavelength range used was between 700 and 900 nm.