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Characterisation of carbon paste electrodes for real-time amperometric monitoring of brain tissue oxygen

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ABSTRACT

Tissue O_2 can be monitored using a variety of electrochemical techniques and electrodes. *In vitro* and *in vivo* characterisation studies for O_2 reduction at carbon paste electrodes (CPEs) using constant potential amperometry (CPA) are presented. Cyclic voltammetry indicated that an applied potential of −650 mV is required for O_2 reduction at CPEs. High sensitivity (−1.49 ± 0.01 nA/μM), low detection limit (*ca.* 0.1 μM) and good linear response characteristics ($R^2 > 0.99$) were observed in calibration experiments performed at this potential. There was also no effect of pH, temperature, and ion changes, and no dependence upon flow/fluid convection (stirring). Several compounds (e.g. dopamine and its metabolites) present in brain extracellular fluid were tested at physiological concentrations and shown not to interfere with the CPA O_2 signal. *In vivo* experiments confirmed a sub-second response time observed *in vitro* and demonstrated long-term stability extending over twelve weeks, with minimal O_2 consumption (*ca.* 1 nmol/h). These results indicate that CPEs operating amperometrically at a constant potential of −650 mV (*vs.* SCE) can be used reliably to continuously monitor brain extracellular tissue O_2.
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1. Introduction

Brain cells are critically dependent on a continuous supply of O_2 for their normal energy metabolism with the brain consuming approximately 20% of the total O_2 used by the body at any given time. The tissue concentration is determined by the balance between blood supply and local utilization. A decrease in supply, or a large increase in consumption without adequate compensation, can seriously compromise brain function due to the low reserve of dissolved O_2 present in the tissue; the distribution of concentrations reported ranges from 40 μM to 80 μM (Nair et al., 1987; McCreery et al., 1990; Kayama et al., 1991; Murr et al., 1994; Zauner et al., 1995) depending on the depth of measurement (Baumgärtl et al., 1989), and the heterogeneity of the tissue (Murr et al., 1994; Lüebbers and Baumgäertl, 1997).

Oxygen levels in the brain can be measured in several ways: indirectly, using non-invasive near-infrared spectroscopy (Matsumoto et al., 1996; Rasmussen et al., 2007); globally, using fibre-optic catheters to monitor jugular venous O_2 saturation (SjO_2) (Coplin et al., 1998; Gopinath et al., 1999; Howard et al., 1999); and locally, using Clark-type electrode technology to directly monitor O_2 partial pressure (pO_2) (Clark et al., 1958; Thompson et al., 2003; Piilgaard and Lauritzen, 2009). The latter operates by measuring the electrochemical reduction of O_2 and can be used in freely moving animals. It also offers significant spatial (∼10 μm) and temporal (millisecond) advantages compared to other techniques.

Since the pioneering 'brain polarography' research carried out by Clark and colleagues (Clark et al., 1953; Thompson et al., 2003) over 50 years ago a wide variety of electrodes (sensors) have been used. These can essentially be divided into two main groups: noble metal electrodes, such as Pt (Clark et al., 1958; Travis and Clark, 1965; Thompson et al., 2003; Offenhauser et al., 2005) and Au (Cooper, 1963; Holmström et al., 1998; El-Deab and Ohsaka, 2003); and carbon-based electrodes, such as glassy carbon (Clark and Clark, 1964), carbon fibre (Zimmerman and Wightman, 1991; Zimmerman et al., 1992; Venton et al., 2003), carbon epoxy (Bazzu et al., 2009), and carbon paste (CPE) (Lowry et al., 1996, 1997; Bolger and Lowry, 2005). While carbon electrodes tend to be more labour intensive in terms of their construction, they have the advantage that they are less prone to surface poisoning and as such do not require the use of protecting membranes which are a characteristic of metal-based O_2 electrodes. Of the three types used, glassy

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carbon can be restricted by its large size (>1 mm) (Jin et al., 2005), while with carbon fibre electrodes their small dimension (typically 10 μm) means that the concentration of O_2 observed can vary depending on the orientation of the electrode relative to the blood vessels and metabolically active sites, and on the depth of penetration into the tissue (Baumgärtl et al., 1989). The latter is particularly important when using cylinder electrodes. Since the dimension (typically 100–200 μm) (Justice, 1987) of carbon epoxy and CPEs is greater than the scale of a capillary zone (*ca.* 70 μm) (Silver, 1965), an average tissue O_2 level is detected, but this must be balanced against the increased tissue damaged caused by the larger electrode (Duff and O'Neill, 1994; Khan and Michael, 2003). While microdialysis probes of similar dimensions have been reported to alter dopamine levels (Khan and Michael, 2003) there is no evidence to suggest that this is the case for an electrochemically detected freely diffusing gaseous species such as O_2.

We have previously reported preliminary data demonstrating that CPEs can be used to cathodically monitor real-time changes in brain tissue O_2 during neuronal activation (physiological stimulation) in freely moving rodents using both differential pulse (Lowry et al., 1996; Bolger and Lowry, 2005) and constant potential (Lowry et al., 1997) amperometry (DPA and CPA). The increases observed with DPA were also found to correlate with increases in regional cerebral blood flow measured using the H_2 clearance technique (Lowry et al., 1997). While we have subsequently published detailed characterisation results for DPA O_2 monitoring at CPEs (Lowry et al., 1996) we now present the results of comprehensive characterisation studies for CPA, a technique which has several advantages compared to pulsed methods, including simple instrumentation and experimental design (i.e. it does not require optimized pulse sequences), and continuous real-time recording with high sensitivity and low background (baseline) noise. Such studies are important in establishing the properties confirming suitability for *in vivo* monitoring and include testing sensitivity, selectivity, consumption/depletion and stability (Phillips and Wightman, 2003).

2. Materials and methods

2.1. Chemicals and solutions

The NaCl (SigmaUltra), NaH_2PO_4 (Sigma, A.C.S. reagent), NaOH (SigmaUltra), KCl (SigmaUltra), $CaCl_2$ (SigmaUltra), and $MgCl_2$ (SigmaUltra) were used as supplied (Sigma–Aldrich Ireland Ltd). Compounds used in the interference study were: L-ascorbic acid (AA; A.C.S. reagent, Sigma), dehydroascorbic acid (DHAA; Aldrich), uric acid (UA; sodium salt, Sigma), glutathione (oxidised disodium salt, Aldrich), dopamine (DA; hydrochloride, Sigma), 3,4-dihydroxyphenylacetic acid (DOPAC; Sigma), homovanilic acid (HVA; Fluka Biochemika), 5-hydroxytryptamine (5-HT; hydrochloride, Sigma), 5-hydroxyindole-3-acetic acid (5-HIAA; Fluka Biochemika), L-tryptophan (99%, Aldrich), L-cysteine (>98%, Sigma), L-tyrosine (99%, Aldrich). Stock standard solutions of all compounds were prepared from the supplied chemicals at the beginning of each experiment to avoid problems associated with gradual decomposition.

Unless otherwise stated *in vitro* experiments were carried out in phosphate buffer saline (PBS) solution, pH 7.4 (0.15 M NaCl, 0.04 M NaH_2PO_4 and 0.04 M NaOH). In pH studies the buffer pH was adjusted to between 6.5 and 8.0 using solutions of NaH_2PO_4 and NaOH. Experiments investigating the effects of ion changes (Ca^{2+} and Mg^{2+}) on O_2 sensitivity were performed in artificial cerebrospinal fluid (aCSF): 147 mM NaCl; 4 mM KCl; 1.2 mM $CaCl_2$; and 1 mM $MgCl_2$ (Deboer et al., 1990). All solutions were prepared using deoxygenated doubly distilled deionised water and stored at 4 °C when not in use.

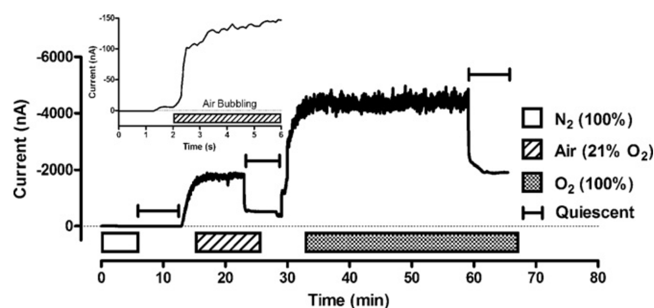

Fig. 1. Typical *in vitro* current–time response for an O_2 calibration (0–1200 μM; N_2, air and O_2-saturation) at a carbon paste electrode (CPE) carried out using constant potential amperometry (CPA) at −650 mV (*vs.* SCE) in PBS, pH 7.4. *Inset*: typical example of the effect of changing the O_2 concentration (bubbling air) on the CPE response characteristics for O_2 reduction. CPE background currents subtracted.

2.2. Working electrode preparation

Carbon paste was prepared by thoroughly mixing 0.71 g of graphite powder (1–2 μm, Aldrich) with 250 μL of silicone oil (high temperature, Aldrich) (O'Neill et al., 1982). CPEs (8 T, 200-μm bare diameter, 256-μm coated diameter) were either made in-house from Teflon-coated silver wire (Advent Research Materials, Suffolk, UK) as reported previously (Lowry et al., 1997) or supplied by Blue Box Sensors Ltd (Dublin, Ireland). When not in use all electrodes were stored in PBS at 4 °C.

2.3. Instrumentation and software

All electrochemical techniques (constant potential amperometry (CPA) and cyclic voltammetry) were carried out using a low-noise potentiostat (Biostat IV, ACM Instruments, Cumbria, UK). Data acquisition was performed with a notebook PC, a PowerLab interface system (ADInstruments Ltd., Oxford, UK) and LabChart for Windows software (ADInstruments Ltd.).

All data are presented as mean ± standard error (SEM), with n = number of electrodes. All analysis was performed using Microsoft Excel 2007 and the commercial packages Prism (version 5.01) and InStat (GraphPad Software Inc., CA, USA). The statistical significance of differences observed was calculated using Student's t-tests (two-tailed paired or unpaired observations where appropriate) or one-way ANOVA (Kruskal–Wallis test with Dunn's post test). Values of $P < 0.05$ were considered to indicate statistical significance.

2.4. Experiments in vitro

Experiments *in vitro* were performed in a standard three-electrode glass electrochemical cell containing 15 mL PBS at room temperature unless otherwise stated. A saturated calomel electrode (SCE) was used as the reference electrode, and a Pt wire served as the auxiliary electrode. CPEs were allowed settle under the influence of the applied CPA potential (−650 mV or 0 mV *vs.* SCE) until the non-faradaic current had reached a stable baseline level—typically 30 min.

To attain effective deaeration, the PBS solution was vigorously purged with O_2-free N_2 (BOC Ireland, average O_2 content 2 ppm, maximum O_2 content 5 ppm) for at least 30 min before recording began. In calibration experiments involving 0–1200 μM solution O_2 either N_2, atmospheric air (from a RENA air pump) or pure O_2 (compressed gas) was bubbled through the PBS for a similar period and the appropriate gaseous atmosphere then maintained over the cell solution during quiescent recording (see Fig. 1A). The concentrations of solution O_2 were taken as 0 μM (N_2-saturated), 240 μM

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