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Short communication

Electrochemical sensor for the detection of multiple reactive oxygen and nitrogen species from ageing central nervous system homogenates

A. Fagan-Murphy, L. Hachoumi, M.S. Yeoman, B.A Patel*

School of Pharmacy and Biomolecular Sciences, University of Brighton, Brighton, UK

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ABSTRACT

Reactive oxygen and nitrogen species (ROS/RNS) have been widely implicated in the ageing process and various approaches exist for monitoring these species in biological tissues. These approaches at present are limited to monitoring either a single pro-oxidant species or total pro-oxidant levels and therefore provide limited insight into the range of pro-oxidant species and their relative proportions in the ageing process. We have utilised a sensor that allows us to simultaneously monitor hydrogen peroxide, peroxynitrite, nitric oxide and nitrite. Using CNS homogenates from the pond snail, Lymnaea, we were able to show that levels of these ROS/RNS increased between young and old CNS homogenates and were different in various aged CNS regions.

The free radical theory of ageing is one of the most prominent theories to explain the ageing process (Harman, 1956). This theory postulates that ageing and age-related diseases arise as a result of cumulative changes and/or damage to cells and tissues inflicted by reactive species. Under normal physiological conditions, redox homeostasis establishes a dynamic balance between pro-oxidants and antioxidants whereby the production of reactive species are scavenged by antioxidants to minimise damage. However, with increasing age, studies have shown that the pro-oxidant-antioxidant balance is compromised with a shift to the former resulting in an increase in reactive species. This imbalance induces oxidative stress, a state where endogenous antioxidant defense systems are overwhelmed leading to oxidative damage. More recent advances of this theory have shown that while, in laboratory reared animals, oxidative damage does not appear to affect lifespan it certainly increases oxidative damage and can contribute to age-related pathology (Salmon et al., 2010). Others have suggested that ageing represents a decline in the plasticity of pathways that have evolved to enable cells, tissue and organism to survive periods of environmental stress and increase fitness early in life (Jones, 2015). What all these theories have in common is that age is associated with an increase in the production of reactive species and being able to monitor these species simultaneously in realtime will be vital to furthering our knowledge of the ageing process and age-related pathology. There are various reactive species formed in biological environments, with a range of chemical reactivities which in turn can cause different levels of cellular damage. Little is however known about how the levels of different reactive oxygen and nitrogen species (ROS/RNS) vary with time in cellular environments during ageing

Determination of ROS/RNS species is a highly challenging analytical problem due to the low concentrations and high chemical reactivity of these species. Various methods have been developed in an attempt to measure ROS/RNS, of which fluorescence and chemiluminescence measurements have been most suitable for biological measurements (Kielland et al., 2009; Wang et al., 2013). However, these approaches have limitations due to their lack of selectivity for specific ROS/RNS. Electroanalytical approaches offer similar sensitivity, spatial and temporal resolution as these imaging based approaches, but, importantly, are able to simultaneously detect multiple identified ROS/RNS species in a single measurement (Amatore et al., 2008, 2010).

Within this manuscript we demonstrate the fabrication and utilisation of a simple electrochemical sensor that offers the ability to simultaneously monitor the production of four ROS/RNS species. These are hydrogen peroxide (H_2O_2), peroxynitrite ($ONOO^-$), nitric oxide ($NO^\bullet$) and nitrite (NO_2^-). The developed sensor was fabricated using multiwall-carbon nanotube (MWCNT) composite electrodes and modified by coating it with platinum black (Supplementary Fig. 1) using a previously published technology to enable

* Corresponding author.
E-mail address: b.a.patel@brighton.ac.uk (B.A Patel).

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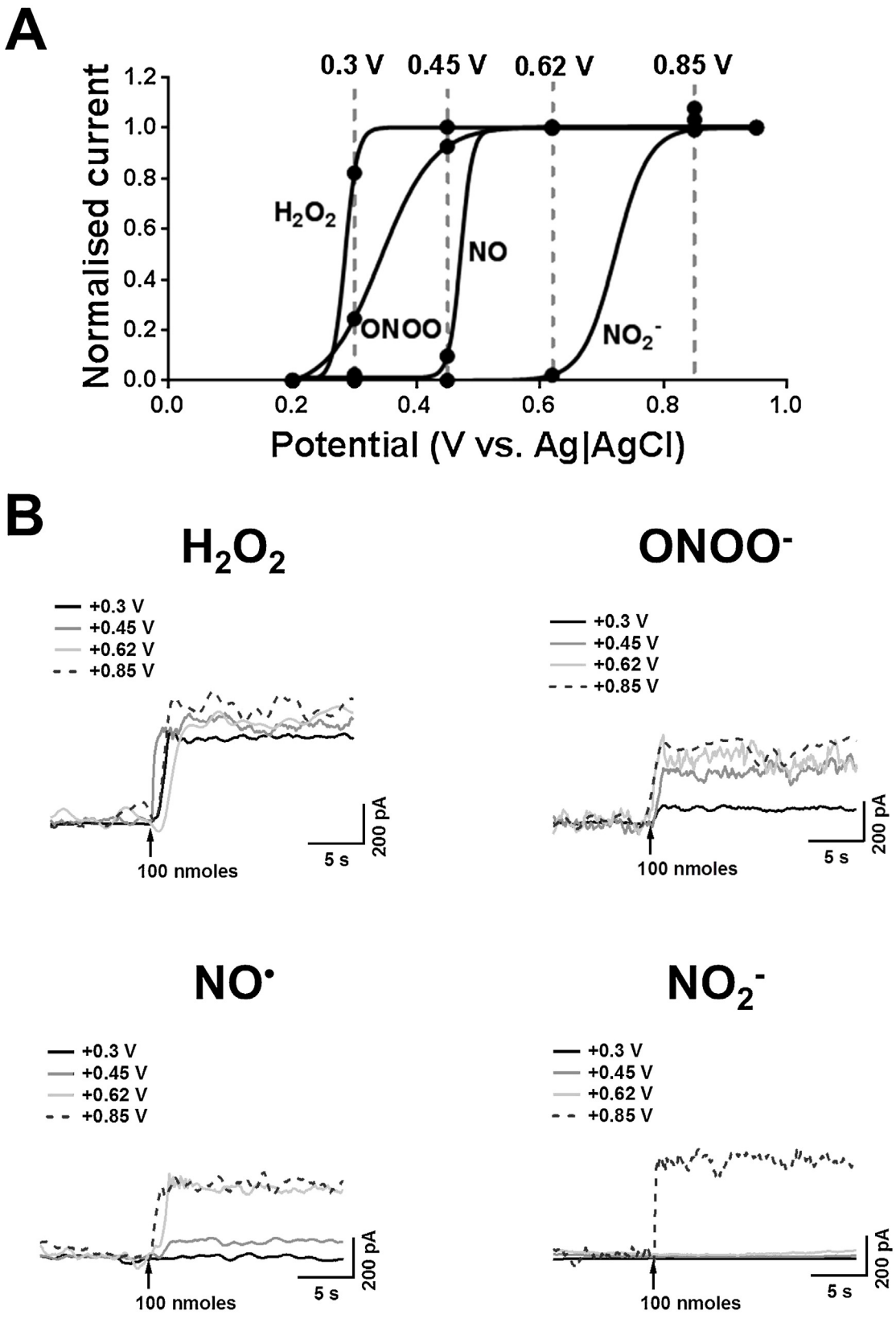

Fig. 1. Detection of ROS/RNS. (A) normalised voltammograms obtained at Pt-black MWCNT composite electrodes in 100 nmoles of H_2O_2, $ONOO^-$, $NO^\bullet$ and NO_2^- at varying potentials relative to a Ag|AgCl reference electrode. The dashed line indicates the potentials that were utilised for detection of the four different ROS/RNS. In order to obtain the current for each of the species, Eqs. (1)–(4) were applied. (B) Shows response at the chosen potentials from (A) for 100 nmoles of H_2O_2, $ONOO^-$, $NO^\bullet$ and NO_2^-. Measurements were carried out in stirred solution, where stable responses were observed that mapped well to the profile obtained in (A). For all measurements the pH was 7.4 for H_2O_2, $NO^\bullet$ and NO_2^- and pH 11.5 was utilised for recordings of $ONOO^-$.