Introduction

Parkinson’s disease is a progressive degenerative disorder that destroys dopamine neurons of the substantia nigra[1]. Research efforts have long attempted to elucidate the mechanisms of this disease. Studies have pointed to a myriad of contributing factors ranging from genetic mutations to exposure to environmental toxins[2]. While these suspected causes seem to be very different, they have been shown to cause cellular changes in metabolism that increase production of reactive oxygen species (ROS)[3]. H$_2$O$_2$ is considered to be an indicator molecule alerting to the presence of more damaging and reactive ROS[4]. H$_2$O$_2$, although less reactive than traditional ROS, is better able to penetrate cellular membranes and is an important neuromodulator [4]. Endogenous H$_2$O$_2$ generation due to mitochondrial dysfunction elicited by rotenone, or by inhibition of GSH peroxidase (MCS), can lead to K$_{ATP}$ channel opening and suppression of axonal DA release[5]. Research to date has been unable to quantitatively monitor sub-second levels of H$_2$O$_2$ in an intact brain and assess its effects on basal levels of DA. This study seeks to enable quantification of endogenous hydrogen peroxide (H$_2$O$_2$) fluctuations in living brain tissue under basal conditions and in response to oxidative stress using fast-scan cyclic voltammetry (FSCV) at bare carbon-fiber microelectrodes. In addition, we wish to assess the effect of acute increases in H2O2 production on both stimulated dopamine release and basal levels of DA.

Methods

Stereotaxic surgery was performed on male Sprague-Dawley rats. An electrode was lowered into the caudate putamen (+1.2 mm A/P, +2.0 mm M/L, -4.5-6.0 mm D/V), through a custom made micromanipulator and allowed to equilibrate for 30 minutes prior to recording. An injection cannula fixed to the micromanipulator guide cannula was placed 50-100 microns from the working electrode. A reference electrode Ag/AgCl was placed superficially in the contralateral cortex. Electrochemical measurements were made every 100 msec by applying a triangular waveform (+0.1V to +1.4V, 400 V/s). Experiments consisted of 10 min of baseline collection (pre-infusion), 10-15 min of recording during and after drug or saline microinfusion, and an electrical stimulation of the nerve terminals ($n = 4$). Drugs (Sigma-Aldrich) were unilaterally microinfused at a rate of 0.5ul/min for 60 s with a syringe pump via an infusion cannulae (33 gauge) inserted into the implanted guide, at a distance of 50-100 microns from the working electrode in the caudate putamen.
Results and Discussion
The DA neurons of the nigrostriatal dopamine pathway have terminals in the caudate putamen. They are particularly vulnerable to oxidative stress created by the generation of endogenous ROS, and the motor symptoms of PD are linked to the degeneration and anatomical loss of these neurons. A 10uM bolus injection of H2O2 in a flow cell apparatus (Figure 1 B left) shows a single oxidation peak at ~1.2V. In vivo, H2O2 fluctuations are detected in a spatially heterogeneous manner in CPU. Representative color plots containing background-subtracted cyclic voltammograms for H2O2 oxidation in basal conditions are shown in Figure 1B right. Basal H2O2 fluctuations were observed to be intermittent, sporadic and ~5uM (Figure 1B right). The color plots in Figure 2 demonstrate that as the H2O2 signal increases by ~100nM after a 100mM MCS microinfusion. Basal DA levels (without electrical stimulation) were also observed to decrease by ~100-150nM upon microinjection of 100mM MCS. Our data is the first to show that pharmacological increases in basal H2O2 levels in an intact brain attenuate basal DA levels. Our data support previously reported findings suggesting that increased H2O2 (measured by fluorescence) attenuates stimulated DA release in brain slices.

Figure 1. H2O2 is generated at a robust striatal recording site in basal conditions (right) compared with voltammetric data collected in vitro (left). A: Triangular potential waveform. B: Color plots each containing 150 background-subtracted voltammograms. The ordinate is potential applied to the carbon-fiber electrode, the abscissa is time, and the current (nA) is depicted in false color. C: Concentration vs. time traces extracted from the data at 1.2V, the peak oxidation potential for H2O2. The current is converted to concentration upon electrode calibration. Center C: The cyclic voltammogram for an H2O2 transient collected in vivo is presented as a black line, and that collected in vitro is presented as a red line for comparison (correlation =0.91).
Figure 2. H2O2 (left) and DA (right) voltammetric data generated at a striatal recording site after local microinfusion of MCS. (A) Averaged color plots (n=4) collected 4 minutes after microinfusion. (B) Individual cyclic voltammograms extracted from the averaged data. (C) Concentration vs. time traces extracted from the data at 1.2 V, the peak oxidation potential for H2O2; and 0.7 V, the peak oxidation potential for DA.

References
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