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Real-Time Detection of Reactive Oxygen Intermediates From Single Microglial Cells

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A growing body of evidence indicates that activation of microglia (macrophages resident in brain) aggravates the inflammatory process and thus can contribute significantly to the progression of various neurodegenerative diseases (1). As with other tissue-specific macrophages, microglia are thought to exert some of their cytotoxic effects through the production of reactive-oxygen-intermediates (ROI). For example, β -amyloid, an abundant component of amyloid (“senile”) plaques, was shown to induce the production of ROI by cultured microglia within 1–2 min (2). Any damage caused to surrounding tissue by microglial cells is mainly dependent upon the magnitude of the gradient of ROI that is generated on the surface of the cell’s membrane. Therefore, quantification at a high spatial and temporal resolution of ROI distribution in the microenvironment of an activated microglial cell is important for the assessment of neurotoxicity.

The enzyme responsible for the generation of ROI in an oxidative burst in microglial cells is NADPH oxidase, which transfers an electron from a single cytoplasmic NADPH molecule to an oxygen molecule, producing superoxide anion (O_2^-). O_2^- and its dismutation product, hydrogen peroxide (H_2O_2), diffuse away from the microglial cells and have the potential to oxidize cellular components in neighboring cells, including proteins, lipids, and DNA (3). However, H_2O_2 is a much more stable product than O_2^- and therefore can be used as a reliable indicator for detection of an oxidative burst.

The self-referencing technique has the capacity to detect, with high spatial and temporal resolution, concentration gradients of specific molecules surrounding single cells (4, 5). In the current investigation, we used an H_2O_2 -sensitive microprobe as a sensor of ROI production by microglia cells. To test the feasibility of the self-referencing technique for the detection of ROI from single microglial cells, we activated the NADPH oxidase machinery with phorbol-12-myristate-13-acetate (PMA), a potent activator of this enzyme (6).

H_2O_2 microsensors were prepared as described previously for oxygen sensors, but with slight modification (7). Briefly, 25- μ m diameter platinum (Pt) wires were immersed in an aqueous solution of 4 M KCN and 1 M NaOH and then etched down to \sim 2 μ m diameter by the application of square waves (amplitude, 4.0 V; period, 4 ms). The etched Pt wires were inserted into pulled glass capillaries, insulated with optical adhesive, and then coated with 10% cellulose acetate. The total tip diameter of the sensor was about 3 μ m. For all measurements, the sensor was polarized at +0.60 V against a Ag/AgCl reference electrode; its sensitivity was 0.85 ± 0.12 pA/ μ M (mean \pm SD, $n = 4$). Although the sensor can potentially detect other ROI beside

H_2O_2 (such as nitric oxide and O_2^-), H_2O_2 was probably the major component of the concentration gradient, considering its longer half-life time and the composition of the media (<100 μ M L-arginine).

Purified microglia were isolated from rat brains, as described elsewhere (8), and were plated into 35-mm diameter culture dishes at a density of 3000 cells/ml to allow a distribution of single cells (one cell where no other cells can be detected within a range of \sim 200 μ m).

Figure 1A demonstrates the experimental protocol that we used to detect H_2O_2 production in a single microglial cell. In the presence of culture medium only, no significant H_2O_2 efflux was detected in the close vicinity of the cell (Fig. 1A, trace (a), 5, 15 μ m). However, 10 min after adding PMA (final concentration of 130 nM), a measurement from the same location detected an H_2O_2 efflux of 0.46 pmol/cm²/s. The magnitude of the H_2O_2 efflux was inversely related to the distance from cell surface (traces (c–g) in Fig. 1A) and was nearly zero when the microprobe was moved 40 μ m from the cell surface (trace (g) in Fig. 1A). Similar results to those shown in Figure 1 were obtained in 80% of the isolated microglial cells (12 out of 15) when PMA was added to the solution; in the remaining 20% no H_2O_2 was detected. The range of peak flux was 0.22 pmol/cm²/s (SD \pm 0.17) and the average threshold detection distance was 22.4 μ m (SD \pm 4.2) ($n = 12$). The average latency for response was 4.3 min and in all the responding cells the time period of detectable gradient exceeded 30 min.

To ensure that the signal detected by the probe originated from the production of ROI, catalase, an enzyme that hydrolyzes H_2O_2 , was added to the bath. Catalase significantly attenuated the H_2O_2 efflux regardless of the distance from the cell surface (Fig. 1A: compare (h) with (i) and Fig. 1B: compare closed with open circles).

The present study demonstrates that a self-referencing microsensor can detect H_2O_2 changes in the nano-molar range near the surface of a single microglial cell. Though numerous assays are available to measure an oxidative burst within macrophages, the self-referencing technique is unique in providing the ability to measure the microenvironment around a single cell or cluster of cells in real-time and with high spatial and temporal resolution. Because microglial cells can enhance neurotoxicity of the surrounding tissue, this assay may be useful for quantifying the potential contribution of endogenous microglial-induced activators in neurodegenerative diseases.

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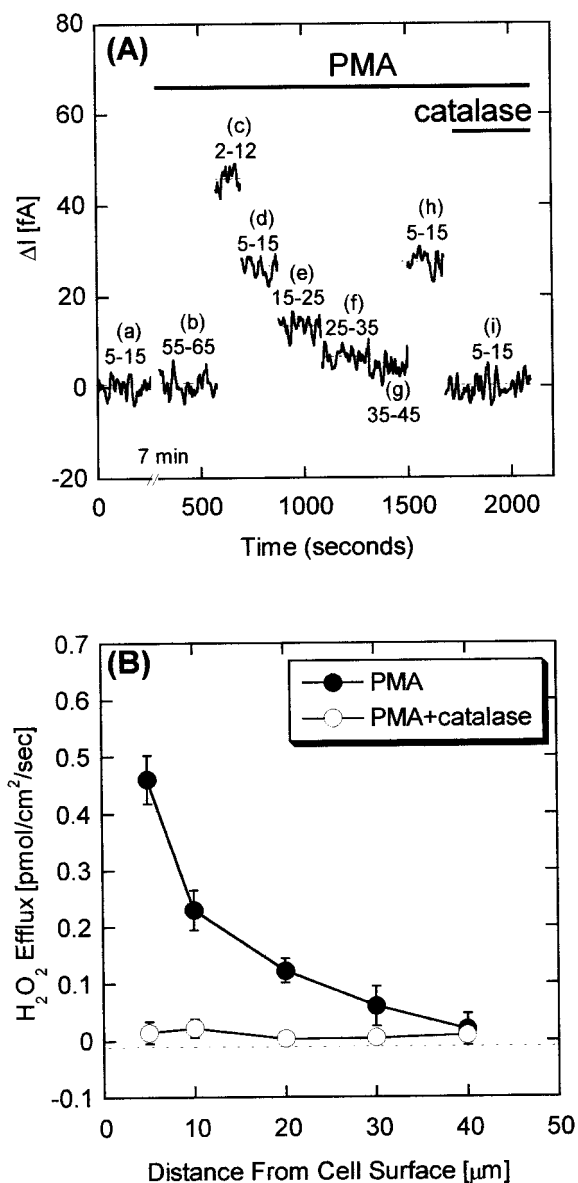


Figure 1. Self-referencing H_2O_2 measurement of a single microglial cell. (A) The difference in current values detected by the microelectrode when self-referencing at different distances from the cell surface. In all traces, the excursion (distance of the probe in the self-referencing format) was $10 \mu\text{m}$, and the number on the top of each trace represents the two positions of the microsensor in μm . Note that addition of PMA (upper solid horizontal line) induced a significant elevation in H_2O_2 efflux [(a) vs. (c–g)] and that in the presence of catalase (0.19 mg/ml) the H_2O_2 efflux was abolished [(i) vs. (h)]. Trace (b) is a “background” measure taken $60 \mu\text{m}$ away from the cell. (B) The relationships between H_2O_2 efflux and the average distance from a membrane surface in the presence of PMA; data from the same cell shown in (A). Note that application of catalase abolishes the H_2O_2 efflux.

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