Redox Microscopy: A sensitive Method to quantify Production and Degradation of H_2O_2 from single human Monocytes

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Hydrogen peroxide (H_2O_2) oxidizes intracellular target molecules, thereby controlling cellular signaling. However, quantification and sensitivity to determine production and degradation of H_2O_2 from single cells are limited. Using an electrochemical setup (redox microscope) and applying different voltammetric techniques, with a bare disk platinum ultramicroelectrode (10 µm; vs Ag/AgCl), very low [H_2O_2] could be resolved at plasma membrane production sites of single cells: 2 nmol/l (square wave voltammetry), 50 nmol/l (cyclic and linear scan voltammetry) and 500 nmol/l (chronoamperometry, CA). Although offering the lowest sensitivity for H_2O_2 CA measurements are unbeatable for long-term determinations with high temporal resolution (≥ 1 Hz). From single human monocytes, average H_2O_2 production was 1.5 nmol/l/s over 60 minutes following stimulation with the phorbolester TPA. During the initial phase (25 min) rate was 3.4 nmol/l/s (n=23). Considering quantitatively the concomitant H_2O_2 degradation by the same cell, net production rates reached 9.0 nmol/l/s. Single cell measurements were validated in human monocyte populations by electron spin resonance spectroscopy and an adapted fluorescence-based Amplex® UltraRed assay. In summary, physiologically relevant low nanomolar [H_2O_2] can be spatially and temporally resolved direct at the H_2O_2 production sites of single cells.