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Short Communication

High intrinsic oxidative stress may underlie selective vulnerability of the hippocampal CA1 region

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Abstract

Oxidative stress (OS) causes extensive cell death in the CA1 but not the CA3 region of the hippocampus. We found that the CA1 region of hippocampus explants, cultured under normal conditions, had significantly higher superoxide levels and expressed both anti-oxidant genes and genes related to the generation of reactive oxygen species at significantly higher levels than the CA3. These observations were indicative of high intrinsic OS in CA1.

Theme: Disorders of the nervous system
Topic: Neurotoxicity

Keywords: Selective vulnerability; Oxidative stress; Hippocampus; CA1; CA3; *Nrf2*

Selective neuronal population vulnerability is widely observed in various neurodegenerative diseases. For example, neurons in the cerebral cortex, hippocampus and amygdala are most vulnerable in Alzheimer's disease (AD) [18,44], dopaminergic neurons in the substantia nigra undergo selective degeneration in Parkinson's disease (PD) [10] and cortical, brain stem and spinal motor neurons degenerate in amyotrophic lateral sclerosis (ALS) [40]. Within the human and rodent hippocampus, neurons of the CA1 region are more vulnerable to cell death following episodes of global cerebral ischemia [36,42] and suffer greater degrees of degeneration in the hippocampus of AD patients [35] and in patients suffering from epileptic seizures [28] than neurons of the CA3 region. Although selective vulnerability is common to many neurodegenerative processes, little is known about the underlying mechanisms for such cellular selectivity. Following an examination of differential gene expression in CA1 and CA3 neurons of the human hippocampus in a recent study, it was concluded that "selective vulnerability and selective resistance in hippocampal subregions are unrelated to intrinsic differences in the expression of those genes associated with cell death, apoptotic regulation, cell growth and cell maintenance" [46].

Abbreviations: AD, Alzheimer's disease; ALS, Amyotrophic lateral sclerosis; ARE, Anti-oxidant response element; DHE, dihydroethidine; DQ, Duroquinone; γ-ECS, glutamate–cysteine ligase catalytic subunit; *GST1*, microsomal glutathione S-transferase 1; *GST12-12*, glutathione S-transferase, theta 2; *MAPK*, mitogen-activated protein kinase; *Nfl*, nuclear factor I/X; NFκB, nuclear factor kappa B; *Nqo1*, NAD(P)H:quinone oxidoreductase 1; *Nrf2*, Nuclear factor E2-related factor 2; OS, Oxidative stress; PD, Parkinson's disease; PI, Propidium iodide; ROS, Reactive oxygen species; *Txnrd1/2*, thioredoxin reductase 1/2; *UGT1A6*, UDP glycosyltransferase 1 family, polypeptide A6

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In the studies performed to date, there has been no mention of differential gene expression between CA1 and CA3 subfields that might cause oxidative stress (OS) as a result of increased generation of either reactive oxygen (ROS) or reactive nitrogen species (RNS), nor of genes that might enhance anti-oxidant defenses. Based on a large amount of experimental data, a consensus has emerged on the importance of OS in the neurodegeneration that is seen in various disease states [2], including disease states such as AD [52], PD [12,19,48], ALS [7] and brain ischemia [14]. Recent evidence also indicates that ROS may function as both intra- and *trans*-cellular signaling molecules in the central nervous system, especially for neurons of the hippocampus [11,13,21,45]. Thus, it might be expected that differential levels of enzymes involved in both generation and scavenging of these reactive species would be present in cells of different brain regions. Under normal conditions, cellular redox homeostasis would be maintained and cells would function normally. However, when the balance between free radical generation and scavenging is compromised, cells would experience increased OS that may lead to cell death. The hypothesis explored in the present study was that CA1 neurons may be more vulnerable to damage induced by ROS than neurons of the CA3 subfield of the hippocampus because the CA1 neurons and surrounding cells are generating greater amounts of ROS, even under normal conditions, possibly for purposes of intra- or extracellular signaling.

In order to assess the relationship between selective vulnerability and anti-oxidant defenses or OS sensitivity, an organotypic hippocampal slice culture was employed as the model for differential sensitivity of CA1 as compared with CA3 neurons [51]. It was previously reported that in this model, exposure of the explant cultures to an exogenously introduced agent that causes OS leads to extensive cell loss in the CA1 region, while the adjoining CA3 region is mostly spared [51]. We first confirmed this selective vulnerability of the hippocampal CA1 cells. Briefly, hippocampal slices (300 μm in thickness) were obtained from three10-day-old male Sprague–Dawley rat pups, all from the same litter, and were cultured for 7 days on BD Falcon Cell Culture Inserts (0.4 μm, BD Biosciences). On day 7, the culture medium was changed to serum-free medium as described [51] and the slices were maintained in this medium for 24 h prior to the application of duroquinone (DQ) dissolved in dimethyl sulfoxide (final concentration 100 μM). DQ undergoes redox cycling to generate superoxide and induce OS in cells [24]. After a 3-h treatment, the medium was removed, replaced with fresh medium and the pattern of cell death in the CA1 and CA3 regions examined using propidium iodide (PI) staining (24 h after DQ treatment). It was previously reported that 64% of CA1 neurons die but only 6% of CA3 neurons [51]. The greater vulnerability of CA1 neurons to OS was confirmed in the present study as shown in Fig. 1. We also subjected the organotypic cultures to a 1-h treatment with 30 μM $FeSO_4$, a treatment that induces OS inside cells by releasing H_2O_2. Treatment with $FeSO_4$ also induced greater amounts of cell death within 24 h in the CA1 area as compared with the CA3 (data not shown). The results with DQ and $FeSO_4$-induced cell death were consistent with a greater sensitivity of CA1 neurons to intracellular generation of ROS.

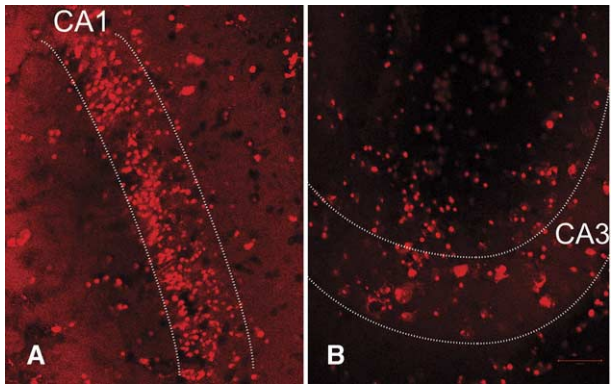

Fig. 1. Selective vulnerability of the hippocampal CA1 region (A) to oxidative stress as compared with that of the CA3 (B). Organotypic hippocampal slice cultures were subjected to exogenous oxidative stress elicited by treatment for 3 h with DQ (100 μM). Extensive cell death was observed within 24 h in the CA1 area, while the adjoining CA3 area (B) was mostly spared. Cell death was detected through labeling with PI.

To determine whether cells in the CA1 region produced higher levels of ROS than those in CA3, we employed a specific fluorescence indicator of superoxide formation, dihydroethidine (DHE) [4], and measured the relative levels of fluorescence in each region (Fig. 2). Although conversion of DHE to the fluorescent ethidium product by neuronal populations of both CA1 and CA3 had been observed previously and the kinetics of such conversion measured [4], possible quantitative differences between CA1 and CA3 were not reported. In this study, we found that the mean fluorescence intensity in the CA1 region of six explant cultures was significantly higher ($P = 0.002$, paired t test) than that of the CA3 region (Fig. 2). The DHE measurements of superoxide levels in cells of the CA1 region shown in Fig. 2 also fit the observation that mitochondria (the main source of superoxide generation in cells) isolated from the CA1 region generate more ROS than those from the CA3 [29].

Subsequently, microarray analysis was performed on total RNA extracted from microdissected CA1 and CA3 cell regions in order to compare gene expression patterns between these two subfields under normal conditions. The two regions were microdissected from organotypic hippocampus cultures maintained in the absence of any stimuli that might induce OS. Brief fixation and staining using the HistoGene™ LCM Staining Kit (Arcturus) were used to visualize the CA1 and CA3 cell regions. Total RNA was immediately isolated from the microdissected samples using Qiagen RNeasy® Mini Kit. The Rat Expression 230A Oligonucleotide GeneChips from Affymetrix were used for the microarray analyses. Since the amount of starting total