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Mechanisms and modulation of neural cell damage induced by oxidative stress

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Abstract

Oxidative stress has been linked to several neurodegenerative disorders characterized by neuronal death. Apoptosis and necrosis are the two major forms of cell death that have been described in the nervous system, and stimuli inducing oxidative stress can cause both types of death, depending on the intensity and the duration of the insult. In the present article, we report on a series of studies from our laboratory describing the intracellular pathways activated by oxidative stress in differentiated neurons, such as cerebellar granule cells, and neural stem cells. Using *in vitro*/*ex vivo* experimental models, we have investigated whether the susceptibility to injuries can be affected by the occurrence of potential insults taking place during development. We have found that prenatal exposure to high levels of glucocorticoids renders neural cells, including stem cells, more sensitive to oxidative stress damage. Similar effects were seen after *in utero* exposure to methylmercury. The analysis of behavior has proven to be a sensitive tool to detect mild alterations induced by early stimuli that increase susceptibility to oxidative stress. Our findings contribute to the understanding of how early events may have long-term consequences by modifying intracellular processes that predispose the affected cells to dysfunction, which can be unmasked or worsen by subsequent exposure to further injuries.

Keywords: Apoptosis; Oxidative stress; Methylmercury; Glucocorticoids; Cerebellar granule cells; Neural stem cells; Behavioral analysis

1. Introduction

Oxidative stress, defined as a disturbance in the prooxidant–antioxidant balance, has been implicated in a variety of physiological and pathological processes of the nervous system like aging, ischemia, and neurodegenerative conditions including Parkinson's, Alzheimer's, Huntington's disease and amyotrophic lateral sclerosis [1–4]. The generation of reactive oxygen species (ROS) such as superoxide anion, hydrogen peroxide, singlet oxygen, hydroxyl radical, and peroxy radical, can arise from toxic insults or normal metabolic processes as the mitochondrial electron transport chain. Under normal physiological conditions, enzymes like superoxide dismutases, SOD1 and SOD2, glutathione peroxidase and catalase prevent formation/accumulation of oxygen metabolites. However, overproduction of oxidants and/or dysfunction of endogenous antioxidant defences may occur and result in oxidative stress-induced injury with damage to all the major classes of biological macromolecules, such as nucleic acids, proteins, lipids and carbohydrates.

The central nervous system is particularly vulnerable to oxidative stress due to its high rate of metabolism, the disproportionately low levels of oxidative defence mechanisms, a high content of easily oxidized substrates, such as membrane polyunsaturated lipids, and chemical reactions, including dopamine oxidation and energy metabolism that generate ROS. The increased and unopposed ROS production can lead to neurotoxicity that results in neural damage and eventually cell death.

2. Oxidative stress induces apoptosis

The vulnerability of a cell to an insult is influenced by a number of factors including the proliferative status, the intracellular defense systems, and the expression of proteins that inhibit or promote the cell death process. The intensity and the duration of exposure to a toxic stimulus, including oxidative stress, determine whether a cell undergo apoptosis, an active cell death process requiring energy, or necrosis, a passive form

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of death [5]. In the nervous system, as in other organs, these two forms of cell death are characterized by various morphological and biochemical criteria. Morphologically, apoptosis is associated with cell shrinkage, nuclear and cytoplasmic condensation, externalization of phospahtidylserine (PS) at the plasma membrane level, and formation of apoptotic bodies. Release of mitochondrial factors [6], including cytochrome *c* and apoptosis inducing factor (AIF), activation of specific proteases such as caspases and calpains [7], degradation of chromosomal DNA into large (300, 50 kb) and small (180 bp) fragments by endonucleases are the major biochemical features of apoptotic cell death. The plasma membrane integrity that is maintained in apoptotic cells, and the exposure of PS, which promotes the engulfment process by phagocytic cells, prevent the leakage of cytosolic components into the extracellular space and the subsequent inflammatory reaction. In contrast to apoptosis, necrosis is characterized by loss of membrane integrity, cellular swelling, damage to the organelles and cell lysis that may lead to tissue inflammation.

We have shown that cerebellar granule cells (CGC) exposed to mild oxidative stress induced by hydrogen peroxide undergo cell death with morphological and biochemical changes, such as nuclear condensation, Tunel positivity, exposure of PS, and formation of high molecular weight DNA fragments typical of apoptosis. Caspases do not play a major role in this model as shown by the absence of caspase activity and the lack of protective effects of the pan-caspase inhibitor zVAD-fmk [8,9]. Thus, Caspase-activated DNase (CAD) are not responsible for DNA cleavage induced by oxidative stress in CGC, whereas translocation of AIF from the mitochondria to the nucleus seems to be a critical event leading to chromatin condensation and DNA degradation [10]. One of the factors that can initiate AIF release is the increase of intracellular Ca^{2+} level [11]. Moreover, it has been demonstrated that free radical overproduction may inhibit Ca^{2+}-ATPases, which then leads to altered regulation of Ca^{2+} levels [12]. The occurrence of an increase in intracellular Ca^{2+} provides an explanation for the activation of the Ca^{2+}-dependent proteases calpains that we have observed after oxidative stress [13].

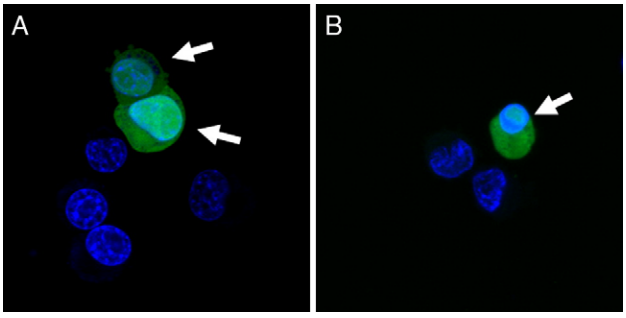

Fig. 1. ROS production in C17.2 cells exposed to 30 μM DMNQ for 9 h (**A**), or 0.5 μM MeHg for 18 h (**B**). Non-fluorescent cell permeable 5-(and-6)-carboxy-2′,7′-dichlorodihydro-fluorescein diacetate (Molecular Probes) is added to living cells after ending the toxic challenges and incubated for 30 min at 37 °C. Subsequently, cells are stained with the cell-permeant nucleic acid marker Hoechst 33342 (blue fluorescence) and analysed by confocal microscopy. Under conditions of oxidative stress, ROS oxidize the reduced fluorescein compound, which then emits bright green fluorescence (arrows).

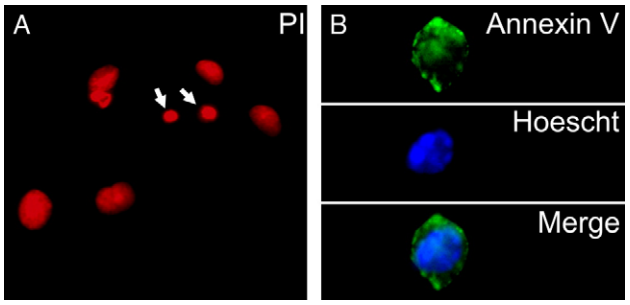

Fig. 2. (**A**) Primary cultures of rat cortical neural stem cells exposed to 0,05 μM MeHg for 24 h. Cells were fixed and stained with propidium iodide (PI) to visualize the DNA. Apoptotic cells showed typical condensed nuclei (arrows). (**B**) Cells from the neural stem cell line C17.2 were exposed to 30 μM DMNQ for 12 h. Non-fixed cells were triple-stained with Annexin-V (An-V), Hoechst 33342 (Hoe) and PI. The panel shows the same apoptotic cell with exposure of PS on the outer membrane, detected by An-V binding, and condensed nucleus, visualized with Hoe that can penetrate intact cell membranes. Apoptotic NSC were not stained by the impermeant dye PI (data not shown), unless fixed.

We obtained similar results by exposing CGC to methylmercury (MeHg), a neurotoxic environmental pollutant known to induce oxidative stress and increase intracellular Ca^{2+} [10,13]. As predicted, cells could be rescued by adding antioxidant compounds 30 min before or after MeHg exposure [8,13]. Moreover, antioxidants prevented the activation of calpain, suggesting that in these cells ROS formation is upstream the activation of the degradation pathway regulated by the increase in intracellular Ca^{2+} [13].

We have also studied the effects of oxidative stress on neural stem cells using primary cultures from embryos or adult rats, and the C17.2 cell line. These cell types have the intrinsic mitochondria-mediated cell death pathway active, whereas the extrinsic receptor-mediated pathway is not operative [14,15]. We induced oxidative stress by exposing cells to 2,3-dimethoxy-1,4-naphthoquinone (DMNQ) or MeHg [15,16]. Both compounds caused production of intracellular ROS, as visualized by confocal microscopy using a fluorescent ROS indicator (Fig. 1).

Exposed cells exhibited typical apoptotic morphology with nuclear condensation (Fig. 2A), PS exposure (Fig. 2B), and release of cytochrome *c* from the mitochondria with subsequent caspase activation [15,16].

In addition to caspases, the Ca^{2+}-dependent protease calpain was also activated in MeHg-exposed cells [16], indicating that multiple intracellular pathways can be operative in parallel during NSC apoptotic cell death. The schematic drawing in Fig. 3 summarizes the cascade of intracellular events occurring in NSC after exposure to MeHg that activates both the caspase- and the calpain-mediated pathway. Contrary to differentiated neuronal cells, MeHg-exposed NSC undergoing apoptosis activate different execution pathways where also caspases play a critical role.

3. Early events modify the susceptibility of neural cells to oxidative stress

There is increasing evidence that prenatal events may predispose to diseases later in life [17], a hypothesis that certainly