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BRAIN RESEARCH

Research Report

Effect of oxidative preconditioning on neural progenitor cells

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ARTICLE INFO

Article history:
Accepted 5 August 2008
Available online 18 August 2008

Keywords:
Neural progenitor cell
Differentiation
Chain migration
Preconditioning
Survival

ABSTRACT

Neural progenitor cells (NPCs) have drawn attention because they offer possible treatment for neurodegenerative disorders in the form of regenerative therapy or transplantation. NPCs adapt and change in response to the cues in the pathological environment. We assessed the effect of pre-exposure to non-cytotoxic levels of oxidative stress, a common pathogenic factor in a number of neurological disorders, on the cell viability and neurosphere morphology of NPCs derived from the periventricular zone of mice brain. Neural progenitor cell viability and neurosphere morphology (neurosphere number, size and chain migration) were assessed in response to cytotoxic levels of oxidative stress in the presence or absence of preconditioning with non-cytotoxic doses of hydrogen peroxide (H_2O_2). Preconditioning with non-cytotoxic levels of H_2O_2 provided significant protection against subsequent exposure to lethal doses of H_2O_2. Precon-ditioning also modulated alteration in the neurosphere morphology in response to oxidative stress. Oxidative stress increased chain migration and neurosphere size while decreasing neurosphere numbers, specially in the cultures that were preconditioned with higher doses of H_2O_2. Non-cytotoxic exposure to oxidative stress can evoke endogenous cytoprotection in NPCs. Redox signaling plays a role in other cellular functions of NPCs, namely the chain migration of NPCs from neurospheres, perhaps as a result of its effect on cell differentiation.

1. Introduction

In addition to neural stem cells with long-term self-renewal and multipotency, there also exists a range of progenitor cells that have more limited capacities of growth and differentiation (Temple 2001). There is growing evidence that these progenitors have the ability to produce new neurons and glia in the adult central nervous system (CNS). Adult progenitors are known to be abundant in the periventricular areas, but recent studies have revealed their presence in various other CNS regions as well (Palmer et al. 1999; Yamamoto et al. 2001). Neural progenitor cells (NPCs) have become a subject of intense research because they provide a possible therapeutic strategy for neural regeneration and transplantation.

The progenitor cells respond to the cues in the pathological environment. It has been shown that the proliferation and/or differentiation rate of the progenitor cells is affected by a number of environmental factors. Some increase the rate of proliferation and neurogenesis (Kempermann et al. 1997; Nakagawa et al. 2002), whereas others decrease these rates (Liu et al. 1998; Eisch et al. 2000). For example, ischemia has been shown to stimulate the proliferation of neural progenitors (Liu et al. 1998; Jin et al. 2001).

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0006-8993/$ – see front matter © 2008 Published by Elsevier B.V.
doi:10.1016/j.brainres.2008.08.025

Oxidative stress plays an important role in a number of neuropathological conditions, most significantly in neurodegenerative disorders (Sharma 2007). Many of these disorders are potentially amenable to neural transplantation therapy. NPCs are sensitive to excessive levels of reactive oxygen species. The effects of non-cytotoxic oxidative stress on NPCs' function are not well known, although relevant for NPC pathobiology. The aim of this study was to characterize the effect of pre-exposure to non-cytotoxic doses of oxidative stress on neural progenitor cell functions, in particular cell viability and cell morphology (an indicator of differentiation). A greater understanding of the biology of NPCs will provide important information relevant to neural transplantation and regeneration therapy.

2. Results

Cells were harvested from subventricular zone (SVZ) that contains NPCs. Immunostaining of the cryosections of brain region from where the NPCs were harvested confirmed the presence of Nestin immunoreactive cells (Fig. 1A). The cultured NPCs fluoresced green. The NPCs formed neurospheres in culture and, as described in the Experimental procedures section, consisted of a cluster of undifferentiated cells in the center with differentiated cells in the periphery and in the migratory chains (Figs. 1E–I). The green fluorescence facilitated morphometric analysis of the photomicrographs as we could easily apply a threshold to these images to demarcate neurospheres (Fig. 1G) from the migrating cells (Fig. 1F). With the result, neurosphere size and numbers as well as the migrating cells could be quantified within the same photomicrograph (Fig. 1E). This allowed us to directly compare three morphometric parameters, neurosphere size, neurosphere numbers and the number of migrating cells within the same photomicrograph.

The NPC nature of cultured cells was confirmed by immunocytochemical labeling for nestin (Fig. 1B) and when cultured in serum containing media (differentiation media) these cells showed evidence of differentiation typical of NPCs' by becoming positive for GFAP and O1 (Figs. 1C, D). Markers of differentiation were not present in serum free media.

2.1. *Protective effect of preconditioning*

The optimum cytotoxic dose for inducing cell death in neurospheres was determined to be 50 μM H_2O_2. Preconditioning the neurospheres with low, non-lethal doses of H_2O_2 (0.5–5 μM) protected the cells from the cytotoxic effects of higher doses (50 μM; Fig. 2). Results obtained by WST-1 assay (Fig. 2A) were confirmed by assessing cell viability using PI staining and FACS analysis (Fig. 2B). Results obtained by both these methods were comparable. The protective effect of H_2O_2 preconditioning was dose-dependent with significant protection observed even at the lowest dose (0.05 μM) used in our experiments. Controls were kept for each preconditioning treatment to eliminate the possibility of erroneous results due to factors already prevalent during preconditioning. There was a dose-dependant protective effect of preconditioning (Fig. 2). The protective effect increased to 0.1 μM H_2O_2. Increased vulnerability to cell death was observed with preconditioning at concentrations higher than 0.1 μM. However, WST-1 analysis in cells preconditioned with 5 μM H_2O_2 showed abnormally high viability (more than 100%; see Discussion).

These results were confirmed by microscopic examination, using the presence of pyknotic nuclei and PI staining as indicators of cell death (data not shown).

2.2. *Effect of preconditioning on NPC culture morphology (neurosphere number, size and migration)*

Our results show that the H_2O_2 treatment had measurable effects on the morphology of the cultures. Oxidative stress with 50 μM H_2O_2 caused increased chain migration of cells in control and preconditioned cells, especially in cultures that were preconditioned with 5 μM H_2O_2 (Figs. 3A and B; Figs. 4A and B). Concentration of H_2O_2 used as preconditioning doses did not have any significant effect on neurosphere numbers or size in lower doses (0.1 and 0.5 μM), but in higher doses (5 μM) the number of neurospheres decreased whereas the size increased. Addition of 50 μM decreased the neurosphere numbers and increased the size in control as well as preconditioned cultures in a dose-dependent manner (Figs. 3C and D).

3. Discussion

Results presented here provide the first evidence that non-cytotoxic oxidative stress produced by exposure to hydrogen peroxide can have protective effects on NPC viability. The protective effect of preconditioning increased in a dose-dependent manner up to a certain limit, after which it declined possibly due to exhaustion of endogenous cytoprotective capacity of cells. The protective effect of ischemic preconditioning has been studied in retinal neurons (Lin and Roth 1999), but our results show similar effects for oxidative preconditioning.

There are elaborate antioxidant defense mechanisms in the cells that keep reactive oxidant species in check. Most cells have enzymatic cellular defense against H_2O_2 including catalase and glutathione peroxidase (Simonian and Coyle 1996). When the cells are challenged with small doses of H_2O_2, the defenses are upregulated, and with the result, cells are more resilient to subsequent exposure to cytotoxic doses (Valen et al. 1998; Lee and Um 1999; Tang et al. 2006; Sharma and Netland 2007). However, under the conditions of stress, the antioxidant cellular defense system becomes depleted (Conklin 2000). Abundance of free radicals can also exhaust these mechanisms and render cells susceptible to lipid peroxidation as well as DNA and protein damage (Berlett and Stadtman 1997; Cadet et al. 2001). For example, it has been shown that H_2O_2 overload causes exhaustion of reduced glutathione (GSH) (Su et al. 1999; Spector et al. 2002; Hammerschmidt and Wahn 2004). In other injuries involving ROS generation, such as irradiation, pronounced increase in ROS leads to GSH exhaustion (Chen et al. 2003).

Our results also show the effect of oxidative stress on other cellular functions. Cells migrated out of the neurospheres in response to 50 μM exposure, especially the ones that were preconditioned with 5 μM (>200% increase). Cells migrating from the neurospheres (that are clusters of undifferentiated NPCs) in chains have been associated with cell differentiation