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Antioxidant responses of cortex neurons to iron loading

PABLA AGUIRRE[1], NATALIA MENA[1], VICTORIA TAPIA[1], ALEJANDRO ROJAS[1], MIGUEL ARREDONDO[2] and MARCO TULIO NÚÑEZ[1]

[1] Biology Department and Cell Dynamics and Biotechnology Research Center, Faculty of Sciences, Universidad de Chile, and [2] Nutrition and Food Technology Institute, Universidad de Chile.

ABSTRACT

Brain cells have a highly active oxidative metabolism, yet they contain only low to moderate superoxide dismutase and catalase activities. Thus, their antioxidant defenses rely mainly on cellular reduced glutathione levels. In this work, in cortical neurons we characterized viability and changes in reduced and oxidized glutathione levels in response to a protocol of iron accumulation. We found that massive death occurred after 2 days in culture with 10 μM Fe. Surviving cells developed an adaptative response that included increased synthesis of GSH and the maintenance of a glutathione-based reduction potential. These results highlight the fundamental role of glutathione homeostasis in the antioxidant response and provide novel insights into the adaptative mechanisms of neurons subjected to progressive iron loads.

Key terms: glutathione, iron, redox response, cortical neurons, cell death

INTRODUCTION

Iron is an essential element for brain functions such as myelin layer formation and neurotransmitters synthesis (Beard, 2003; Ortiz et al., 2004), thus neurons must strive to maintain adequate supplements of iron. However, reactive iron is one of the most significant pro-oxidant factors in neurons. Neuronal iron homeostasis is far from perfect, since neurons accumulate iron with time despite deleterious iron-induced oxidative stress (Zecca et al., 2001). Several neurodegenerative diseases have been linked to iron overload (Sayre et al., 2000). Hence, it is becoming increasingly important to elucidate neurons' defense mechanisms against iron accumulation.

Brain cells have a highly active oxidative metabolism, yet they contain only low to moderate superoxide dismutase and catalase activities. Their antioxidant defenses rely mainly on cellular reduced glutathione (GSH) levels (Cooper, 1997). Indeed, GSH is a crucial component of the long-term adaptative system against oxidative stress. In this work, in cortical neurons we characterized the changes in GSH and oxidized GSH (GSSG) levels in response to a protocol of iron accumulation.

RESULTS AND DISCUSSION

Neuronal iron and viability. To assay for iron toxicity, rat cortical neurons obtained from E18.5 rat embryos were cultured for 2 days with 1.5, 3, 5, 7.5, 10 or 20 μM iron in the culture media. Cell viability was determined by the MTT method. We observed that 10 μM Fe in the media killed 60-70% of the cells in culture. The remaining cells became resistant to this iron concentration for up to ten days in culture, the last time tested. Since increasing iron in the culture media results in increased intracellular iron and increase oxidative stress in SHSY5Y cells (Aguirre et al., 2005), most probably iron-induced cortical neurons death through a mechanism involving oxidative stress.

Corresponding author: Marco T. Núñez, Department of Biology, Faculty of Sciences, Universidad de Chile, Las Palmeras 3425, Ñuñoa, Santiago, Chile, Tel.: (56-2) 978-7360, Fax: (56-2) 271-2983, E-mail: mnunez@uchile.cl

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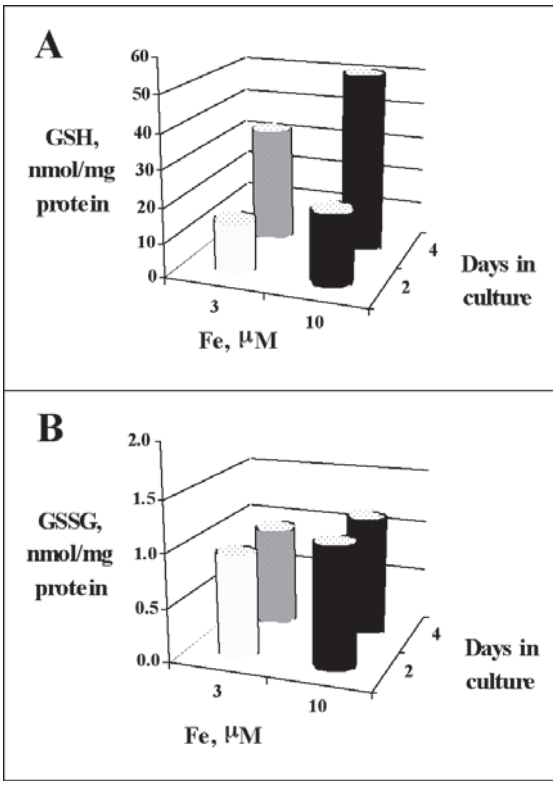

Figure 1. GSH and GSSG were determined in cortex neurons after 2 or 4 days in culture with either 3 or 10 μM iron added to the standard culture medium. GSH and GSSG were quantified determining the initial rate of formation of a yellow colored product generated by the GSH-mediated reduction of dithionitrobenzoic acid as described previously (Núñez et al., 2004). Color development was followed in a Tecan Sunrise (Grödig/Salzburg, Austria) microplate reader. Experimental points were tested in triplicates. GSH and GSSG were expressed as nmol per mg of cell protein. Show is one of three similar experiments.

GSH levels. GSH was measured in neurons treated with 3 or 10 μM Fe for 2 or 4 days. Cortex neurons remarkably increased their levels of GSH as a function of time of exposure, whereas changes in iron concentration produced non-significant changes ($p < 0.05$; $n = 3$) in GSH content (Fig. 1). The levels of GSH increased both with increasing time and increasing iron concentrations. The calculated GSH/GSSG half-cell reduction potential (Núñez et al., 2004) was kept relatively constant, with a minimal value of -311.2 ± 4.2 mVolt at 3 μM Fe, 2 days of culture, and a maximal value of -339.2 ± 3.6 mVolt at 10 μM Fe, 4 days in culture (mean ± SEM; n = 3). Hence, it is likely that surviving neurons were able to adapt to iron-induced oxidative stress by increasing GSH synthesis, thus controlling their reductive environment.

In summary, we found that massive death of cortical neurons occurred after 2 days in culture with 10 μM Fe. Surviving cells developed an adaptative response that included increased synthesis of GSH and the maintenance of a GSH-based reduction potential.

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