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Glutamate promotes NF-κB pathway in primary astrocytes: Protective effects of IRFI 016, a synthetic vitamin E analogue

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Abstract

Oxidative stress has been implicated in several neurodegenerative diseases affecting both neuronal and glial cells. The aim of this study was to investigate the involvement of reactive oxygen species in glutamate-evoked activation of NF-κB in primary astrocytes. A prolonged exposure to glutamate (24 h) caused a depletion of intracellular glutathione that, in astroglial cells, has been considered a biochemical change typical of early astrocyte dysfunction, leading to cell alterations occurring in the gliosis. These effects were initiated by AMPA/KA receptor activation and almost completely blocked by anti-oxidants. Indeed, we provide evidence that the incubation of primary astrocytes with a hydrophilic derivative of tocopherol, such as IRFI 016, was useful to reduce glutamate-induced oxidative effects. This agent also reduced in a dose-dependent manner the nuclear translocation of both p50 and p65 subunits of NF-κB. Altogether, these data confirm that GSH content plays a pivotal role to determine oxidative response to glutamate injury in primary astrocyte cultures and that NF-κB pathway is involved in this response. Furthermore, the positive effects obtained by IRFI 016 to prevent nuclear translocation of NF-κB may suggest new pharmacological strategies for antioxidant therapy and neuroprotection.

Keywords: Glutamate; ROS production; Primary astrocytes; NF-κB; Glutathione; IRFI 016

Introduction

Several findings demonstrated that both ionotropic and metabotropic glutamate receptors are present on glial cells in vivo at a similar density to that found in neurons (Steinhauser and Gallo, 1996). Thus, glutamate released from neurons can activate glutamate receptors on glia leading to regulation of synaptic transmission (Chen et al., 2000; Gallo and Ghiani, 2000).

Previous observations in vitro revealed that glutamate exposure may modulate astrocyte functions, including increase in intracellular calcium concentration (Glaum et al., 1990), changes in astrocyte volume (Hansson and Ronnback, 1995), oxygen consumption, and accumulation of reactive oxygen species (ROS) (Mawatari et al., 1996). However, in addition to its effects characterizing neural performance, glutamate may also act triggering biochemical alterations in astrocytes, with deleterious consequences for neuron–glia interactions.

The response to glutamate in cultured astrocytes remains poorly understood. However, it has been reported that alteration in glutamate transporters leads to ROS over-production, which might play a crucial role in cascade of

Abbreviations: DCF, 2′-7′-dichlorofluorescein; GSH/GSSG, reduced/oxidized glutathione; GYKI 52466, 1-[4-aminophenyl]-4-methyl-7,8-methylenedioxy-5H-2,3-benzodiazepine; LDH, lactate dehydrogenase; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide; ROS, reactive oxygen species.

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events triggering astrocyte cell damage (Had-Aissouni et al., 2002) and contributing to the pathogenesis of neurodegenerative diseases (Chen et al., 2000). In addition, these effects have been implicated in normal aging and may also be a common mechanism underlying various forms of cell death, including necrosis, apoptosis, and excitotoxicity (Takuma et al., 2004).

Although these observations could help in understanding the role of oxidative stress on neuron–glia system, very little is known about the target genes which are transcriptionally activated by moderate levels of ROS in astroglial cells. However, it is well known that ROS production leads to the activation of nuclear transcription factor kappa B (NF-κB), which, in turn, promotes the activation of several genes usually involved in biochemical pathways activated in cell response to stimulation by cytokines or stress (O'Neill and Kaltschmidt, 1997). On the other hand, [there is a substantial evidence that a number of] ROS scavengers may be useful as therapies to prevent cell death in various neurological disorders. These include antioxidants such as melatonin, vitamin E, glutathione, and spin trap agents (Reiter, 1998; Sandhu et al., 2003; Shults et al., 2002).

In the light of these observations, synthetic hydrophilic derivatives of tocopherol appear useful in reducing oxidative effects in various animal models (Campo et al., 2001; Iuliano et al., 1999; Marini et al., 2004).

These compounds, quenching free radicals and blocking the chain-breaking reaction, may act as modulators of the inflammatory cascade triggered by oxidative stress (Altavilla et al., 2003).

Therefore, the present study was aimed to first evaluate the action of a synthetic hydrophilic derivative of tocopherol, such as IRFI 016, to counteract glutamate-induced alterations in primary astrocytes. We also investigated the involvement of the transcription factor NF-κB in astrocyte response.

Materials and methods

Materials

Cell culture medium and sera were from Invitrogen (Milano, Italy). Aprotinin, leupeptin, pepstatin, glutathione reduced (GSH) ethyl ester, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT), and other chemicals of analytical grade were obtained from Sigma (Milano, Italy). 1-[4-aminophenyl]-4-methyl-7,8-methylenedioxy-5 H-2,3-benzo-diazepine (GYKI 52466) was from Research Biochemicals Inc. (Natick, MA, USA). IRFI 016 was generously supplied by Biomedica Foscama Research Centre, Ferentino, (FR), Italy. Mouse monoclonal antibodies for supershift of p50 and p65 subunits (*sc*-8414X, *sc*-8008X) of NF-κB were from Santa Cruz Biotechnology (Milano, Italy). NF-κB oligonucleotide probe was synthesized by MWG Biotech (Firenze, Italy). Biotin 3′ End DNA Labeling kit, LightShift Chemiluminescent EMSA kit and SuperSignal West Pico Chemiluminescent Substrate System were from Pierce Biotechnology (Milano, Italy). Kodak X-ray film was from Kodak (Milano, Italy).

Cell cultures

Primary cultures of astrocytes were prepared from newborn albino rat brains (1 to 2-day-old Wistar strain) and checked for purity as previously described (Campisi et al., 2003).

All efforts were made to minimize both the suffering and number of animal used. All experiments conformed to guidelines of the Ethical Committee of University of Messina, Italy.

Glutamate treatment

Astrocytes at 14 days in vitro (DIV) were exposed to glutamate (10–500 μM) for 24 h, in the presence or absence of a selective antagonist of AMPA/KA receptors, GYKI 52466 (100 μM).

To prevent or reduce glutamate effects on oxidative stress, cell cultures were also pre-incubated either with the antioxidants IRFI 016 (20–80 μM) or GSH ethyl ester (50–100 μM), prior to glutamate addition. Four replicates were performed for each sample.

Cytotoxicity assessment

Astrocyte survival analysis was performed by MTT reduction assay to evaluate mitochondrial activity, and lactate dehydrogenase (LDH) release measurement to assess necrotic cell death as described by Chen et al. (2000).

Glutathione measurement

Oxidative stress in glutamate-treated cultures, in presence or absence of antioxidants, was evaluated by a colorimetric determination of GSH intracellular content. Briefly, cells were scraped off and lysed in 50 μM sodium phosphate buffer, pH 7.4. Protein concentration in cell extracts was determined by Bradford assay (Bio-Rad, Richmond, CA, USA). Then, total glutathione intracellular content (GSH+GSSG) was chemically determined by a colorimetric assay, as described by Chen et al. (2000).

Evaluation of intracellular ROS production

To evaluate production of ROS, astrocyte cultures were treated with 2′-7′-dichlorofluorescein (DCF) diacetate for 20 min, rinsed with serum-free medium, and intracellular peroxide levels were measured for individual cells in