

POSTER PRESENTATION - SHORT COMMUNICATION

Effect of iron on the activation of the MAPK/ERK pathway in PC12 neuroblastoma cells

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ABSTRACT

Recent evidence suggests that reactive oxygen species function as second messenger molecules in normal physiological processes. For example, activation of N-Methyl-D-Aspartate receptor results in the production of ROS, which appears to be critical for synaptic plasticity, one of the cellular mechanisms that underlie learning and memory. In this work, we studied the effect of iron in the activation of MAPK/ERK pathway and on Ca²⁺ signaling in neuronal PC12 cells. We found that iron-dependent generation of hydroxyl radicals is likely to modulate Ca²⁺ signaling through RyR calcium channel activation, which, in turn, activates the MAPK/ERK pathway. These findings underline the relevance of iron in normal neuronal function.

Key terms: NMDA receptor, calcium, iron, oxidative stress, MAPK/ERK, hippocampal slices, long-term potentiation

INTRODUCTION

In hippocampal synapses, a rise in intracellular postsynaptic calcium concentration is necessary in order to induce activation of MAPK/ERK pathway and changes in synaptic plasticity. This increase is initially produced by Ca²⁺ influx through NMDAR. Nevertheless, Ca²⁺ released from ryanodine-sensitive intracellular stores through Ca²⁺-induced Ca²⁺-release amplifies the initial Ca²⁺ entry signal triggering the activation of a number of signaling cascades, such as the MAPK/ERK pathway (Krapivinsky et al., 2003). Recent evidence suggests that reactive oxygen species (ROS) function as second messenger molecules in normal physiological processes. For example, activation of N-Methyl-D-Aspartate receptor (NMDAR) results in the production of ROS, which appears to be

critical for synaptic plasticity, one of the cellular mechanisms that underlie learning and memory (Bailey et al., 2004; Kahlert et al., 2005). In this work, we studied the effect of iron in the activation of MAPK/ERK pathway and on Ca²⁺ signaling in neuronal PC12 cells.

RESULTS AND DISCUSSION

Given that RyR calcium channels are activated by oxidation (Hidalgo et al., 2000; Donoso et al., 2000; Carrasco et al., 2004), and iron is a notorious ROS producer in neuronal cells (Núñez et al., 2004), we hypothesized that iron-induced ROS could modulate Ca²⁺ release from Ry-sensitive intracellular stores. To address this possibility, we used confocal imaging in the line-scan mode to detect local Ca²⁺ signals. When PC12 cells were treated with iron,

spark-like Ca^{2+} transients were triggered. This effect was blocked by Ry, a direct indication that the iron-induced Ca^{2+} sparks were mediated by the RYR calcium channel (Fig. 1).

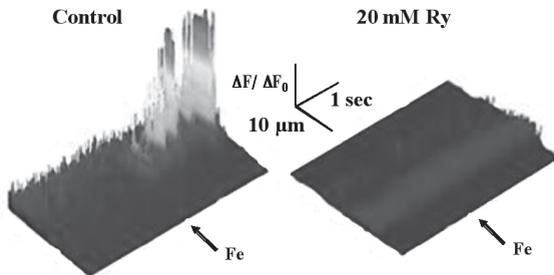


Figure 1. Iron-induced Ca^{2+} release. Control PC12 cells, or cells pre-treated with 20 μM Ry for 1 hour, were loaded with the Ca^{2+} indicator Fluo 3-AM and then challenged with Fe-NTA (1: 2.2, mol: mol). Confocal line-scan images were taken at a high temporal resolution (2 msec per line) in a LSM 510 confocal microscope equipped with a CO_2 - and temperature-controlled chamber. Arrows indicate the application of 20 μM Fe-NTA.

Since iron activated RyR calcium channels, it was of interest to test if iron could also activate the MAPK pathway. Indeed, iron enhanced ERK1/2 phosphorylation in PC12 cells, in a time- and concentration-dependent form (Fig. 2).

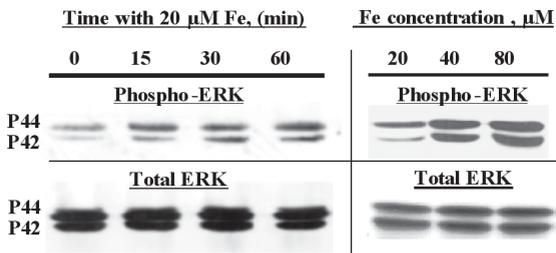


Figure 2. Effect of iron on the expression of total and phospho-ERK1/2. Quantitative Western blot analysis of ERK phosphorylation (upper sections) in PC12 cells exposed to 20 μM Fe-NTA during 1 hour (left sections) or to 20-80 mM Fe for 1 hour (right sections). The membranes were stripped and re-blotted with anti-total ERK (lower sections). P44 and P42 indicate the migration of the 44 KDa and 42 KDa sub-units.

Immunoblot assays with an antibody that recognizes both the phosphorylated and the unphosphorylated forms of ERK1/2 showed that the observed increases were not due to increased levels of ERK1/2 protein. Treatment of PC12 cells with the iron chelator deferoxamine or the hydroxyl radical scavenger mannitol attenuated NMDA-induced phosphorylation of ERKs (Muñoz et al., manuscript in preparation). Thus, iron is an effective activator of pre-existing ERK1/2.

In summary, these results suggest that iron-dependent generation of hydroxyl radicals is likely to modulate Ca^{2+} signaling through RyR calcium channel activation, which, in turn, activates the MAPK/ERK pathway. These findings underline the relevance of iron in normal neuronal function.

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