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Endocytosis controls glutamate-induced nuclear accumulation of ERK

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ABSTRACT

Nuclear translocation of activated extracellular signal-regulated kinases (ERK) in neurons is critical for gene regulations underlying long-term neuronal adaptation and memory formation. However, it is unknown how activated ERK travel from the post-synaptic elements where their activation occurs, to the nucleus where they translocate to exert their transcriptional roles. In cultured neurons, we identified endocytosis as a prime event in glutamate-induced nuclear trafficking of ERK2. We show that glutamate triggers a rapid recruitment of ERK2 to a protein complex comprising markers of the clathrin-dependent endocytotic and AMPA/glutamate receptor subtype. Inhibition of endocytosis results in a neuritic withholding of activated ERK2 without modification of ERK2 activity. As a consequence, endocytosis blockade alters ERK-dependent nuclear events, such as mitogen and stressed-activated kinase-1 (MSK-1) activation, histone H3 phosphorylation and gene regulations. Our data provide the first evidence that the endocytic pathway controls ERK nuclear translocation and ERK-dependent gene regulations induced by glutamate.

Introduction

The extracellular signal-regulated kinase (ERK) pathway, a member of the evolutionarily conserved family of mitogen-activated protein kinase (MAPK) cascades, regulates a wide spectrum of functions ranging from growth and proliferation to differentiation and apoptosis (Schaeffer and Weber, 1999). In the central nervous system, *in vitro* and *in vivo* studies have shown the key role of ERK in the control of gene regulations required for neuronal plasticity and long-term memory (Sweatt, 2004; Valjent et al., 2001). At the cellular level, ERK are localized and activated in the cytoplasm (Ortiz et al., 1995), where they potentially phosphorylate multiple substrates. To mediate gene transcription and allow synaptic plasticity, activated ERK translocate to the nucleus where they play a pivotal role in both chromatin remodelling and transcription factor activation (Brami-Cherrier et al., 2007, 2005; Kauderer and Kandel, 2000; Sgambato et al., 1998). Therefore, further understanding the mechanisms involved in the nuclear translocation of activated ERK in neurons is a critical issue for determining their role in neuronal plasticity and memory formation.

Most of the studies dedicated to analyze the long-range propagation of kinases towards the nucleus in neurons were performed in the context of the retrograde axonal transport of signalling molecules induced by trophic factors (Howe and Mobley, 2004). These studies tackled how receptor tyrosine kinase (RTK)-dependent activation of the ERK pathway by neurotrophins could be elicited at the plasma membrane and subsequently transported towards the cell body. They showed that NGF-mediated ERK activation critically depends on TrkA receptors endocytosis. Furthermore, clathrin-coated vesicles (CCVs), originating from this endocytosis, were the source of signalling endosomes that transported the NGF signal from the axon terminal to the soma, and catalytically active ERK were associated with these CCVs (Howe et al., 2001). Within these vesicles, ERK interact with scaffolding proteins that prevent their dephosphorylation by restricting the access of MAPK phosphatases. Thus, retrograde trafficking allows an efficient propagation of activated ERK on the distance covering the axon terminal to the soma (Kholodenko, 2002).

The ERK pathway is activated by neurotrophins but also by neurotransmitters and neuromodulators (Valjent et al., 2001). Among these, the excitatory neurotransmitter glutamate plays a critical role in synaptic plasticity underlying long-term memory. Glutamate is a potent activator of the ERK cascade and ERK-induced gene regulation (Vanhoutte et al., 1999; Yang et al., 2004). This was well illustrated in striatal neurons, where ERK are activated by electrical stimulation of the glutamatergic cortico-striatal pathway *in vivo* (Sgambato et al.,

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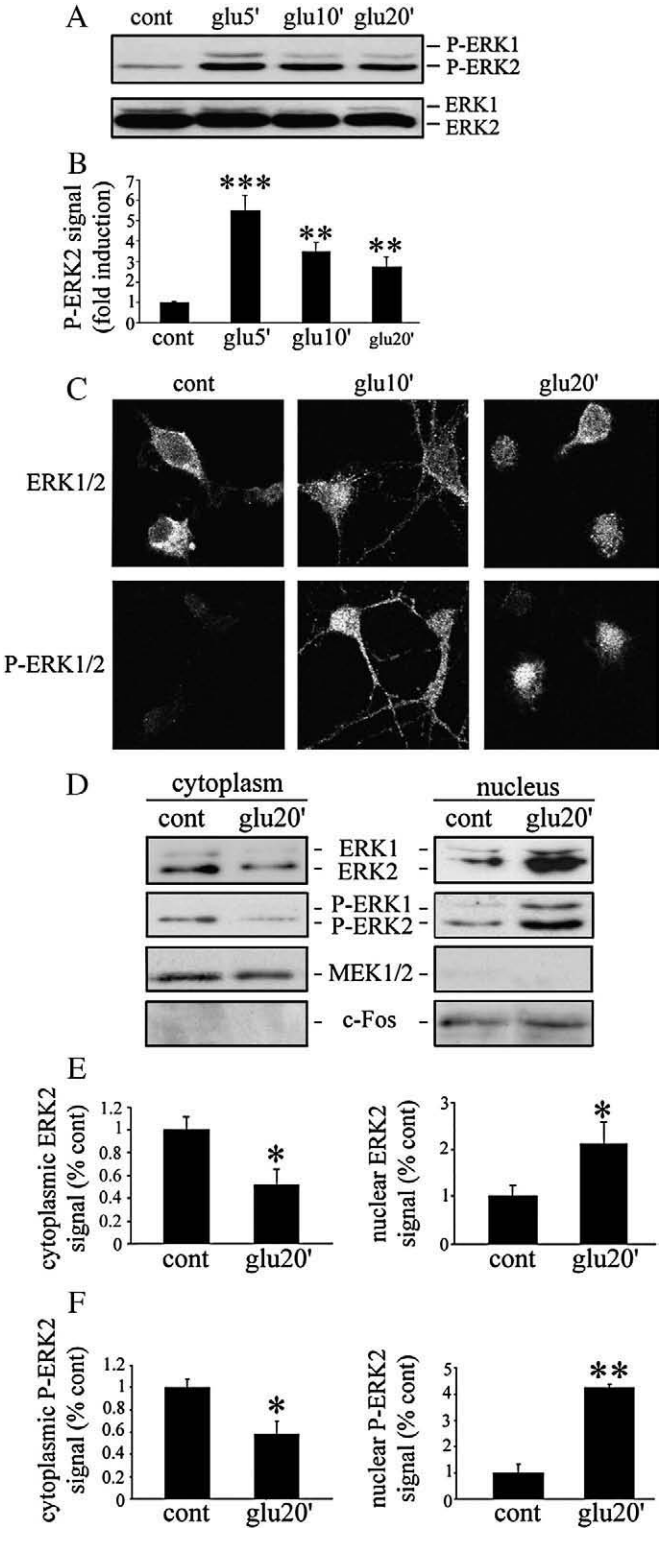
1998). In this model, activated ERK translocated from distal dendrites, where glutamatergic inputs impinge, to the nucleus where they controlled immediate early gene (IEG) expression via the phosphorylation of the transcription factors CREB and Elk-1.

In the present work, we wished to unravel the cellular mechanisms governing the nuclear translocation of ERK activated by glutamate in striatal neurons. Based on the mechanisms described for neurotrophin-induced retrograde transport, we hypothesized that dendrite-to-nucleus trafficking of activated ERK in response to glutamate might also involve endocytosis. We show that glutamate triggers the transient recruitment of ERK2, but not of their upstream activator MEK, to a molecular complex including proteins of the clathrin-dependent endocytotic pathway and glutamate receptors of the AMPA subtype (AMPA-R). As opposed to neurotrophic factor-mediated activation of ERK2, inhibition of clathrin-dependent endocytosis does not alter ERK2 activation in response to glutamate. This inhibition rather leads to a neuritic withholding of activated ERK2 and their cytoplasmic substrate, the transcription factor Elk-1. Subsequently, ERK-dependent nuclear events, such as chromatin remodelling or IEGs induction, are impaired. Our study thus provides the first clues that glutamate-mediated trafficking of ERK2 towards the nucleus is controlled by the endocytotic machinery.



Results

Glutamate-induced ERK2 activation and nuclear translocation in primary cultured neurons

To dissect out the molecular mechanisms responsible for ERK nuclear translocation, we used an *in vitro* model of glutamate-induced nuclear translocation of ERK in primary cultures of striatal neurons (Brami-Cherrier et al., 2007; Lavaur et al., 2007). Due to low expression levels of ERK1 proteins compared to ERK2 in cultured striatal neurons (see Fig. 1A), all the experiments performed in the present work were focused on the analysis of ERK2 activation and subcellular localization. A time course of glutamate application was performed and the profile of ERK2 activation was analyzed with a phospho-specific ERK antibody. Western blots showed a significant activation of ERK2 from 5 to 20 min of glutamate treatment, with a maximum at 5 min (Figs. 1A, B). Subcellular distribution of total and activated ERK proteins was then determined by confocal microscopy. Total ERK proteins were cytoplasmic in control conditions and progressively translocated to the nucleus (Fig. 1C, upper panel). After 20 min of glutamate treatment, ERK proteins were mainly nuclear. No phospho-ERK staining was detectable in basal conditions but a strong signal was observed in dendrites, soma and nucleus of neurons after 10 min of glutamate treatment. At 20 min, phosphorylated ERK were mainly localized in the nucleus and the soma (Fig. 1C, lower panel). These observations were confirmed by immunoblots performed from cytoplasmic and nuclear fractions, using MEK and c-Fos proteins as markers of these compartments, respectively (Fig. 1D). Quantifications revealed that a glutamate application of 20 min triggered a significant nuclear accumulation of ERK2, along with phosphorylated ERK2, concomitantly with a clearance from the cytoplasm (Figs. 1E, F).

Glutamate induces clathrin-dependent endocytosis of AMPA receptors as well as the recruitment of ERK2 to CCV markers in striatal neurons

In this *in vitro* model, we tested whether endocytosis of glutamate receptor (GluR) occurred. A first index of endocytosis is clathrin-coats assembly, characterized by interactions of β-adaptin with clathrin (Szymkiewicz et al., 2004). Using co-immunoprecipitation assays, we found a significant two-fold increase of β-adaptin/clathrin interaction

Fig. 1. Glutamate-induced ERK2 nuclear translocation in striatal neurons. (A) Neurons were incubated in the absence (cont) or presence of 100 μM glutamate for 5 (glu5′), 10 (glu10′) or 20 (glu20′) minutes and ERK2 phosphorylation was analyzed by immunoblot. The ERK immunoblot serves as a loading control. (B) Due to low levels of ERK1 and phosphorylated-ERK1, the fold induction of P-ERK2 signals was quantified ($n=3$; $^{**}p<0.01$; $^{***}p<0.001$ when compared to control). (C) Representative confocal sections of ERK1/2 (top panels) and phospho-ERK1/2 (bottom panels) immunolabeling in control conditions and after different delays of glutamate stimulation. (D) ERK2 and phospho-ERK1/2 immunoblots performed on cytoplasmic and nuclear fractions without or with glutamate applied for the indicated time. MEK1/2 and c-Fos immunoblots were used as cytoplasmic and nuclear markers, respectively. ERK2 (E) and phosphorylated ERK2 (F) signals were quantified in the cytoplasm (left) and the nucleus (right) in control and glutamate conditions ($n=3$; $^{*}p<0.05$; $^{**}p<0.01$ when compared to control). (F).