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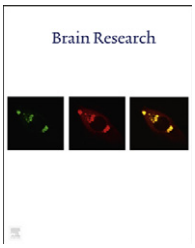

Research Report

Amphetamine increases phosphorylation of MAPK/ERK at synaptic sites in the rat striatum and medial prefrontal cortex

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ARTICLE INFO

Article history:
Accepted 20 November 2012
Available online 29 November 2012

Keywords:
Striatum
Basal ganglia
Dopamine
Stimulant
Postsynaptic density
Extrasynaptic membrane
Addiction

ABSTRACT

Mitogen-activated protein kinases (MAPKs) play a central role in cell signaling. Extracellular signal-regulated kinase (ERK) is a prototypic subclass of MAPKs and is densely expressed in postmitotic neurons of adult mammalian brains. Active ERK translocates into the nucleus to regulate gene expression. Additionally, ERK is visualized in neuronal peripheries, such as distal synaptic structures. While nuclear ERK is a known sensitive target of psychostimulants, little is known about the responsiveness of synaptic ERK to stimulants. In this study, we focused on ERK at synaptic versus extrasynaptic sites and investigated its responses to the psychostimulant amphetamine in the adult rat striatum and medial prefrontal cortex (mPFC) *in vivo*. We used a pre-validated biochemical fractionation procedure to isolate synapse- and extrasynapse-enriched membranes. We found that two common ERK isoforms (ERK1 and ERK2) were concentrated more in extrasynaptic fractions than in synaptic fractions in striatal and cortical neurons under normal conditions. At synaptic sites, ERK2 was noticeably more abundant than ERK1. Acute injection of amphetamine induced an increase in ERK2 phosphorylation in the synaptic fraction of striatal neurons, while the drug did not alter extrasynaptic ERK2 phosphorylation. Similar results were observed in the mPFC. In both synaptic and extrasynaptic compartments, total ERK1/2 proteins remained stable in response to amphetamine. Our data establish the subsynaptic distribution pattern of MAPK/ERK in striatal and cortical neurons. Moreover, the synaptic pool of ERK2 in these neurons can be selectively activated by amphetamine.

1. Introduction

Mitogen-activated protein kinases (MAPKs) are a family of serine/threonine protein kinases. These kinases are densely expressed in postmitotic neurons of adult mammalian brains and play a central role in cell signaling (Nozaki et al., 2001). Extracellular signal-regulated kinase (ERK) is the first subfamily of MAPKs (Pearson et al., 2001; Volmat and Pouyssegur, 2001). Like all other MAPKs, ERK is activated through a module cascade involving initial activation of small GTPases

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http://dx.doi.org/10.1016/j.brainres.2012.11.038

(Ras or Rac) and subsequent three-tiered protein kinase systems. As a kinase highly sensitive to diverse extracellular stimuli, ERK is vigorously involved in activity-dependent signaling transduction and synaptic plasticity in the central nervous system (reviewed in Sweatt, 2004; Thomas and Huganir, 2004; Wang et al., 2007).

As a prototype of MAPKs, ERK has been extensively investigated in its distribution and function in neurons. Traditionally, ERK, once activated, translocates into the nucleus, where it activates a discrete set of transcription factors and thus regulates gene expression (Treisman, 1996). While this is true for ERK in the cell body, a sub-pool of ERK also notably resides in neuronal peripheries, including postsynaptic dendritic spines (Boggio et al., 2007; Casar et al., 2009; Ortiz et al., 1995). In the postsynaptic density (PSD), ERK2 coexisted with all MAPK cascade components (Suzuki et al., 1995; 1999). After activation, immunostaining of phosphorylated ERK (pERK) was visualized in synaptic structures, in addition to nuclear envelopes, in hippocampal and visual cortical neurons (Boggio et al., 2007; Sindreu et al., 2007). Thus, ERK is reasoned to function at both nuclear and synaptic sites.

ERK has been well documented to be a sensitive target of addictive drugs and plays a pivotal role in neural adaptations and drug addiction (Wang et al., 2007). Among addictive drugs that readily activate ERK in the reward circuit *in vivo* are psychostimulants (cocaine and amphetamines). Acute injection of cocaine markedly increased phosphorylation of ERK in the striatum (Jenab et al., 2005; Valjent et al., 2000; 2005; 2006; Zhang et al., 2004). Similarly, acute amphetamine (AMPH) increased ERK phosphorylation in the striatum (Choe et al., 2002; Choe and Wang, 2002; Valjent et al., 2004; 2005; 2006). The psychostimulant-induced ERK phosphorylation requires activation of dopamine D1 receptors and group I metabotropic glutamate receptors (Choe et al., 2002; Choe and Wang, 2002; Valjent et al., 2000). Together, ERK in striatal neurons is activated in response to stimulants and is believed to participate in neuroadaptations related to the long-lasting addictive properties of drugs of abuse. However, to date, the response of ERK to stimulants was primarily detected and analyzed in the nuclear compartment. Little is known about the distribution and responsivity of nonnuclear ERK, such as the ERK at synaptic sites, to stimulants.

In this study, we carried out a series of experiments in adult rats to examine the subsynaptic distribution pattern of the two common ERK isoforms (ERK1 and ERK2) in the striatum and medial prefrontal cortex (mPFC), two prime projection sites in the mesocorticolimbic dopamine system which is actively involved in mediating AMPH effects. Subsequently, we assessed the response of synaptic ERK1/2 to AMPH. Using a pre-validated subsynaptic fractionation method, synaptic and extrasynaptic ERK1/2 were isolated from the striatum and mPFC. The effect of an acute injection of AMPH on phosphorylation of ERK1/2 in those defined pools was analyzed.

2. Results

2.1. *Enrichment of synaptic and extrasynaptic proteins*

We first evaluated the efficiency of our fractionation method in enriching synaptic and extrasynaptic proteins from the rat striatum. To this end, we separated synaptic and extrasynaptic membranes. The latter include all membranes except synaptic membranes. We then assayed the relative abundance of proteins known to be preferentially expressed in either membrane fraction. The *N*-methyl-D-aspartate (NMDA) glutamate receptor subunit NR2B, PSD-95, and Ca^{2+}/calmodulin-dependent protein kinase II α/β (CaMKIIα/β) are knowingly concentrated at synaptic

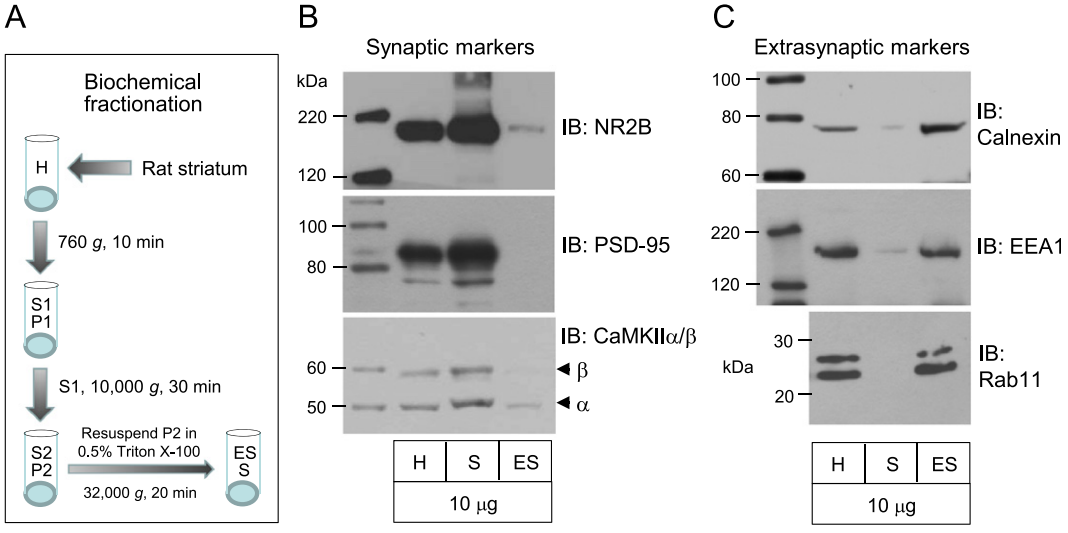

Fig. 1 – Distribution of synaptic and extrasynaptic markers in synaptic and extrasynaptic membranes. (A) Schematic illustration of Triton X-100-based fractionation procedures for enriching synaptic and extrasynaptic proteins from the rat striatum. (B) Representative immunoblots showing expression of synaptic markers (NR2B, PSD-95, and CaMKIIα/β) in synaptic and extrasynaptic membranes. (C) Representative immunoblots showing expression of extrasynaptic markers (calnexin, EEA1, and Rab11) in synaptic and extrasynaptic membranes. Note that synaptic and extrasynaptic markers are present predominantly in their respective fractions. Total proteins from homogenates (H) and enriched proteins from synaptic membranes (S) and extrasynaptic membranes (ES) were loaded at the same amount (10 μg per lane).