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BRAIN RESEARCH

Research Report

Characteristics of dual specificity phosphatases mRNA regulation by 3,4-methylenedioxymethamphetamine acute treatment in mice striatum

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ABSTRACT

3,4-methylenedioxymethamphetamine (MDMA) is a popular recreational drug that has rewarding properties in rodents but little is known about its effects at the cellular and molecular levels. We have previously shown that the ERK pathway is important for the regulation in gene expression observed in mice striatum after acute treatment with MDMA. Interestingly, three dual specificity phosphatases were found among the genes modulated by MDMA acute treatment. In this study we investigated the signalling pathways leading to the up-regulation of these three mRNAs and the kinetics of their regulation. We found that the increase in Dusp14 mRNA depends on the activation of ERK and lasts longer than those of Dusp1 and Dusp5. The modulation of the three studied Dusps depends partially on the activation of D1 receptors but is independent of the activation of D2 receptors. These results suggest that at least two separate signalling cascades lead to the up-regulation of MAPK phosphatase mRNAs. The increase of Dusp1 and Dusp5 mRNAs is not controlled by ERK activation while that of Dusp14 is a direct negative-feedback mechanism of MDMA-induced ERK signalling. Both mechanisms converge to increase the expression levels of phosphatases able to inactivate ERK.

1. Introduction

MDMA (3-4-methylenedioxymethamphetamine) is the psychoactive compound of the widely abused drug ecstasy. The mechanism of action of MDMA is complex and not well-known, most of its effects can be attributed to the increase of dopamine and serotonin observed and the subsequent interaction of the neurotransmitters with their respective pre- and post-synaptic receptors (review in Colado et al., 2004). MDMA inhibits the serotonin and dopamine transporters but also displays a moderate affinity to a broad variety of receptors whose activation could be at the origin of certain effects of the drug (Battaglia et al., 1988). At the intracellular level we have shown, in mice, that ERK (Extracellular signal-Regulated Kinase) signaling is involved in several MDMA behavioral and transcriptional effects, by using a specific inhibitor of ERK activation, SL327 (Salzmann et al., 2003). ERK is an important regulator of neuronal functions, and is involved in various neurobiological events such as synaptic plasticity and memory (Sweatt, 2001). It has been suggested that ERK may play a

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Abbreviations: PCR, polymerase chain reaction; DMSO, dimethyl sulfoxide; MDMA, 3,4-methylenedioxymethamphetamine; ERK, extracellular signal-regulated kinase

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role in the rewarding properties of several drugs of abuse (Valjent et al., 2004). In addition, it has been shown that D-amphetamine- and cocaine-activated ERK pathway was restricted within a subpopulation of dynorphin and D1 receptor-positive striatal medium-sized spiny neurons in mouse (Valjent et al., 2005). Moreover, Acquas et al. (2007) have shown that activated ERK may represent a post-synaptic correlate of the stimulant effect of MDMA on D1-dependent dopamine transmission in the ventral striatum of rat. Furthermore, it has been shown that D1 receptors play a key role in the acute MDMA-induced hyperlocomotion and that the activation of the ERK pathway is partially under D1 receptor control (Benturquia et al., 2008).

Since MAPK signaling pathway is involved in numerous biological functions, dysregulation of this pathway can have widespread consequences on cellular functions. The specificity of the cellular effects induced by a stimulus depends on the magnitude and duration of the activation of the MAPKs (Ebisuya et al., 2005). One of the major negative regulatory mechanisms consists of the dephosphorylation of the activated protein by phosphatases. In mammalian cells, MAPK inactivation is controlled mainly by a family of phosphatases, the dual specificity (Thr/Tyr) phosphatases (Dusp) (Owens and Keyse, 2007). The Dusp family is subdivided into four groups (I–IV) according to their functional domains, mechanism of substrate recognition and enzymatic regulation (Farooq and Zhou, 2004).

Using oligonucleotide arrays, we identified several genes regulated by acute administration of MDMA within mice dorsal striatum (Marie-Claire et al., 2007, 2008; Salzmann et al., 2006). Three dual specificity phosphatases were found among the 27 genes modulated: *Dusp1*, 5 and 14. Since the role of these phosphatases is to regulate MAPK activation, it's interesting that they were up-regulated by the same drug that could activate ERK. Of the three Dusps regulated by MDMA, one (Dusp14) belongs to subgroup I and its modulation was partially dependent on ERK activation while the two others (Dusp1 and 5) which belong to subgroup II did not depend on this pathway for MDMA-induced up-regulation (Salzmann et al., 2006).

We therefore investigated the regulation of the three Dusp genes by MDMA using real time quantitative RT-PCR, in order to validate the modulation observed in the microarray study and the potential implication of the ERK pathway. A time course of the regulation of the Dusps in mice striatum after acute MDMA injection was performed. We also used D1 and D2 receptor antagonists (SCH23390 and eticlopride respectively) to study the involvement of these receptors in the MDMA-induced regulation of these phosphatases.

2. Results

2.1. *Effect of SL327 on the MDMA-induced up-regulation of Dusp1, Dusp5 and Dusp14*

In order to study the involvement of the ERK pathway in the modulation observed, we studied the impact of a pre-treatment with the ERK activation inhibitor SL327 on mRNA levels of the three regulated Dusps in mice striatum (Fig. 1). Dusp1 and Dusp5 mRNA levels increase were not affected by a pre-treatment with the ERK activation inhibitor. On the contrary, Dusp14 up-regulation was significantly reduced (36%) by a pre-treatment with SL327.

2.2. *Time course of dual specificity phosphatases regulation by MDMA*

In order to determine whether the differences observed with Dusp14 could be attributed to its subgroup belonging, we

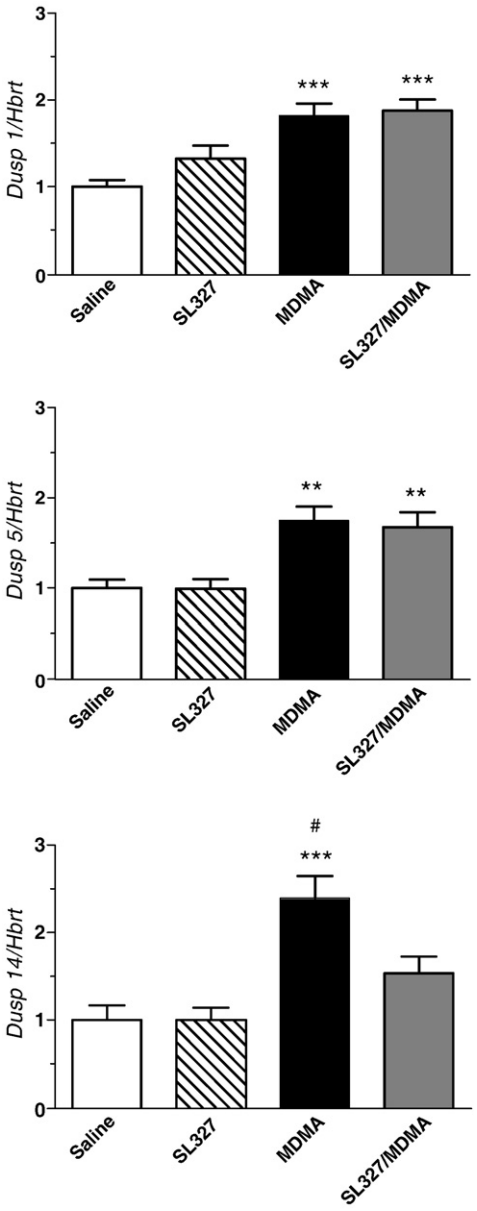

Fig. 1 – Real time quantitative PCR study of the effect of SL327 pre-treatment on the regulation of Dusp1, Dusp5 and Dusp14 in mice striatum. SL327 (50 mg/kg) was administered 1 h before MDMA (9 mg/kg) and mice were killed 2 h after MDMA injection. Results represent the means ± SEM (10–12 animals per group). Statistical analysis was done by ANOVA followed by Bonferroni test. $^{**}p<0.01$, $^{***}p<0.001$ as compared to the control group; $^{\#}p<0.05$ as compared to the SL327/MDMA group.