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Phosphoproteomic analysis of the striatum from pleiotrophin knockout and midkine knockout mice treated with cocaine reveals regulation of oxidative stress-related proteins potentially underlying cocaine-induced neurotoxicity and neurodegeneration

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ABSTRACT

The neurotrophic factors pleiotrophin (PTN) and midkine (MK) are highly upregulated in different brain areas relevant to drug addiction after administrations of different drugs of abuse, including psychostimulants. We have previously demonstrated that PTN and MK modulate amphetamine-induced neurotoxicity and that PTN prevents cocaine-induced cytotoxicity in NG108-15 and PC12 cells. In an effort to dissect the different mechanisms of action triggered by PTN and MK to exert their protective roles against psychostimulant neurotoxicity, we have now used a proteomic approach to study protein phosphorylation, in which we combined phosphoprotein enrichment, by immobilized metal affinity chromatography (IMAC), with two-dimensional gel electrophoresis and mass spectrometry, in order to identify the phosphoproteins regulated in the striatum of PTN knockout, MK knockout and wild type mice treated with a single dose of cocaine (15 mg/kg, i.p.). We identified 7 differentially expressed phosphoproteins: 5′(3′)-deoxyribonucleotidase, endoplasmic reticulum resident protein 60 (ERP60), peroxiredoxin-6 (PRDX6), glutamate dehydrogenase 1 (GLUD1), aconitase and two subunits of hemoglobin. Most of these proteins are related to neurodegeneration processes and oxidative stress and their variations specially affect the PTN knockout mice, suggesting a protective role of endogenous PTN against cocaine-induced neural alterations. Further studies are needed to validate these proteins as possible targets against neural alterations induced by cocaine.

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1. Introduction

Pleiotrophin (PTN) and midkine (MK), two highly redundant in function cytokines (Herradón et al., 2005), are survival factors for dopaminergic neurons and capable to induce the differentiation of stem cells to dopaminergic neurons (Herradón and Ezquerra, 2009; Muramatsu, 2011). Pleiotrophin and MK are highly upregulated in different brain areas relevant to drug addiction such as hippocampus, striatum, cingulate cortex, fronto-parietal cortex and prefrontal cortex after administrations of different drugs like nicotine, amphetamine, cannabis, alcohol and morphine (Mailleux et al., 1994; Le Grevès, 2005; Ezquerra et al., 2008; Flatscher-Bader and Wilce, 2006, 2008). Regarding psychostimulants, we have recently demonstrated that MK and PTN regulate drug-induced addictive behavior as seen in Conditioned Place Preference (CPP) experiments designed to test drug conditioning effects. In those experiments it was found that PTN genetically deficient (PTN−/−) mice maintain amphetamine-seeking behavior longer than wild type (WT+/+) and MK genetically deficient (MK−/−) mice (Gramage et al., 2010a; Martín et al., 2013), whereas, MK−/− mice show a significant delay in the extinction of cocaine-induced CPP (Gramage et al., 2013b). The influence of PTN and MK on the effects of psychostimulants has been also extended to these drugs-induced neurotoxicity. For instance, it has also been described that PTN prevents cocaine-induced cytotoxicity in NG108-15 and PC12 cell cultures (Gramage et al., 2008; Herradón et al., 2009). Moreover, amphetamine induces a significantly enhanced astrocytosis in the

Abbreviations: NT5C, 5′(3′)-deoxyribonucleotidase; ERP29, endoplasmic reticulum resident protein 29; ERP60, endoplasmic reticulum resident protein 60; ERK, extracellular signalregulated kinase; GLUD1, glutamate dehydrogenase 1; HBB1, hemoglobin subunit beta-1; HBB2, hemoglobin subunit beta-2; IMAC, immobilized metal affinity chromatography; MALDI-TOF, matrix-assisted laser desorption/ionization time-of-flight; MK, midkine; MC, MK−/− cocaine; MS, MK−/− saline; PD, Parkinson's disease; PRDX6, peroxiredoxin-6; PTN, pleiotrophin; PC, PTN−/− cocaine; PS, PTN−/− saline; RPTP, receptor protein tyrosine phosphatase; 2D-PAGE, two-dimensional polyacrylamide gel electrophoresis; WT, wild type; WC, WT cocaine; WS, WT saline.

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striatum of PTN−/− and MK−/− mice and also a loss of striatal dopaminergic terminals in PTN−/− mice compared to WT mice (Gramage et al., 2010a, 2010b, 2011).

Some of the proteins involved in the mechanism of action of PTN and MK have been suggested to be key for the neuroprotective roles of these cytokines against psychostimulants-induced neurotoxicity (Gramage and Herradón, 2011). One of the common receptors for PTN and MK is the Receptor Protein Tyrosine Phosphatase (RPTP) β/ζ (Meng et al., 2000; Sakaguchi et al., 2003). PTN and MK bind RPTPβ/ζ and inactivate the intrinsic tyrosine phosphatase activity of RPTPβ/ζ leading to a rapid increase in the steady state levels of tyrosine phosphorylation of substrates of RPTPβ/ζ (see review by Herradón and Ezquerra, 2009), many of which have been found to be very important for PTN and MK neuroprotective effects (Gramage and Herradón, 2011). In an effort to further dissect the different mechanisms of action triggered by PTN and MK to exert these protective roles against psychostimulant neurotoxicity we have recently compared the striatal phosphoproteome of WT+/+, PTN−/− and MK−/− after a neurotoxic treatment with amphetamine (Gramage et al., 2013b). In that study, we identified 13 differentially expressed phosphoproteins that are judged to be relevant in the neuroprotective roles of PTN and MK (Gramage et al., 2013b). Interestingly, 4 of these phosphoproteins, annexin A7, COP9 signalosome subunit 5, aldehyde dehydrogenase family 1 member A1 and creatine kinase U-type, were known to be involved in Parkinson's disease (PD), a result of significant importance since PTN and MK have also been shown to limit PD progress in animal models (Taravini et al., 2011; Prediger et al., 2011; Gombash et al., 2012) and have been suggested to be among the important genetic factors possibly preventing the development of PD in methamphetamine abusers (Gramage and Herradón, 2011; Callaghan et al., 2012).

Cocaine is another psychostimulant whose abuse has been connected to PD. Cocaine has been shown to increase the levels of α-synuclein, the main component of Lewy bodies in PD patients, in dopaminergic neurons (Ziolkowska et al., 2005; Qin et al., 2005; Mash et al., 2013); and, recently, it has been confirmed the dose dependent toxicity of α-synuclein and its ability to induce neurodegeneration in dopaminergic neurons and other neuronal populations (Sánchez-Guajardo et al., 2013). In the present work we aimed to extend our knowledge on the molecular mechanisms triggered by PTN and MK to modulate psychostimulant neurotoxic and addictive effects by comparing the striatal phosphoproteome of WT+/+, PTN−/− and MK−/− mice, 24 h after treatment with a single dose of cocaine (15 mg/kg, i.p.). We chose this treatment because acute administrations of cocaine (5–20 mg/kg) are known to efficiently induce rewarding effects in rodents (Tzschentke, 2007) and the dose used in our studies has been proved to induce different addictive behaviors in the same mouse genotypes used in the present study (Gramage et al., 2013a). In addition, it is interesting to note that a single dose of cocaine within the same dose range has been shown to down-regulate mitochondrial genome and to increase the mitochondrial hydrogen peroxide generation together with a reduced functioning of the mitochondrial complex I in the striatum in response to cocaine (Dietrich et al., 2005). In a similar manner to studies recently performed by our group with amphetamine (Gramage et al., 2013b), we sought the identification of novel phosphoproteins differentially regulated in the striatum by cocaine administration depending on the presence of endogenous PTN or MK by employing a proteomic approach in which we combined immobilized metal affinity chromatography (IMAC) for phosphoprotein enrichment, two-dimensional polyacrylamide gel electrophoresis (2D-PAGE) for protein separation and matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry for protein identification.

2. Methods and experimental procedures

2.1. PTN and MK genetically deficient mice

PTN−/− and MK−/− mice were kindly provided by Dr. Thomas F. Deuel (The Scripps Research Institute, La Jolla, CA). PTN−/− mice were generated as previously described (Amet et al., 2001; Del Olmo et al., 2009). The PTN gene consists of five exons encoding an 18-kDa protein with a 32 amino acid signal peptide. The replacement targeting vector generated a PTN null allele (PTN 2-4neo) by deleting exons 2–4. MK−/− mice were generated as previously described by using a basic vector to target a part of exon 1, intron 1 and a part of exon 2 of MK (Ezquerra et al., 2005, 2006; Nakamura et al., 1998). Male PTN−/−, MK−/− and WT+/+ mice on a C57BL/6J background were used at 8–10 weeks of age (20–25 g). Animals were carefully distributed so the average of the animal's age was similar in every experimental group. The genotypes of PTN−/− mice were confirmed by polymerase chain reaction using as primers 5′-GAT TGA ACA AGA TGG ATT GC-3′ forward and 5′-CAT TTA GGC AAA CAG GAA GGA CG-3′ reverse to generate a cDNA of ∼0.7 kb detected in agarose gels from genomic DNA extracted from tails of PTN−/− and WT mice. The genotypes of the MK−/− mice were confirmed with the polymerase chain reaction using as primers 5′-ATC GGT TCC AAG TCC TCC CTC CGT C-3′ forward and 5′-CAC CTT CCT CAG TTG ACA AAG ACA AGC-3′ reverse to generate from genomic DNA extracted from tails of MK−/− and WT mice a cDNA of ∼0.7 kb.

All the animals used in this study were maintained in accordance with European Union Laboratory Animal Care Rules (86/609/ECC directive) and the protocols were approved by the Animal Research Committee of USP-CEU.

2.2. Cocaine treatment

WT+/+, PTN−/− and MK−/− mice (n = 8 per group) were administered with a single dose of 15 mg/kg (i.p.) of cocaine HCl (Alcaliber, Madrid, Spain) or saline (control, 10 mL/kg). The dose of cocaine was chosen because it was proved to be effective in the induction of cocaine-seeking behavior in place conditioning studies performed in our laboratory with the same mouse genotypes (Gramage et al., 2013b). In addition, a similar acute dose (20 mg/kg) increases the production of reactive oxygen species in the striatum of rats (Dietrich et al., 2005) which is relevant in terms of cocaine-induced neurotoxic effects.

The six experimental groups depending on genotype and treatment were WT+/+ saline (WS), WT+/+ cocaine (WC), PTN−/− saline (PS), PTN−/− cocaine (PC), MK−/− saline (MS), and MK−/− cocaine (MC). 24 h after administration of cocaine or saline, mice were sacrificed, their brains removed, the striatum dissected and preserved at −80 °C.

2.3. Phosphoprotein identification: proteomic analysis

Since we were interested in the identification of differentially phosphorylated proteins we used a proteomic approach, previously employed in our laboratory (Gramage et al., 2013a), in which we combined phosphoprotein enrichment, by immobilized metal affinity chromatography (IMAC), with two-dimensional polyacrylamide gel electrophoresis (2D-PAGE) and matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry. This approach has been previously used by others for nearly identical experimental purposes (Lee et al., 2010; Talvas et al., 2008). Finally, some of the differentially phosphorylated proteins detected by the proteomic techniques were tested in Western blots in individual samples.

2.3.1. Extraction and enrichment of phosphoproteins

For the extraction and enrichment of phosphoproteins we used the Pierce Phosphoprotein Enrichment Kit (Thermo Scientific, USA), which is based on a metal affinity chromatography (IMAC) and results in highly specific and efficient purification of phosphoprotein containing phosphotyrosine, phosphoserine and phosphothreonine residues (Nilsson et al., 2010). We followed the manufacturer's recommendations with slight modifications routinely used in our laboratory (Gramage et al., 2013a; Castillo et al., 2009). In brief, tissue samples (n = 8) of each experimental group were pooled and homogenized by sonication (30 s bursts) with an ultrasonic probe (Dr. Hielscher, Germany), in 1 mL of the Lysis/Binding/Wash Buffer with CHAPS (0.25%), provided by the kit, 10 μL of Halt Protease Inhibitor Single-Use Cocktail EDTA-Free (Thermo Scientific, USA), and 10 μL of Halt Phosphatase Inhibitor Cocktail (Thermo Scientific, USA). The samples were kept on ice to prevent over-heating during sonication. Then the samples were centrifuged (10,000 rpm, 20 min, 4 °C) and the supernatants collected. Supernatants, containing up to 4 mg of total protein, were then applied to a column of the kit that contained a proprietary enrichment gel and buffer, for phosphoprotein enrichment. Samples were then incubated in the column for 30 min at 4 °C and washed with the Lysis/Binding/Wash Buffer with CHAPS (0.25%) to remove unbound proteins. Bound proteins were eluted with five column washes of 1 mL of the elution buffer provided in the kit. The pooled elution fractions were placed into the concentrator columns of the kit and centrifuged (1000 rpm, 4 °C) for 60 min or until the sample volume was 150 μL, approximately. The concentrated phosphoprotein-enriched samples were desalted by acetone (J.T. Baker, USA) precipitation (80%, v/v, −20 °C, overnight). Precipitates were finally re-suspended to a final protein concentration of 0.27 μg/μL,

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