Neuroscience 138 (2006) 1289–1298

EXTRACELLULAR SIGNAL-REGULATED MITOGEN-ACTIVATED PROTEIN KINASE INHIBITORS DECREASE AMPHETAMINE-INDUCED BEHAVIOR AND NEUROPEPTIDE GENE EXPRESSION IN THE STRIATUM

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Abstract—The aim of this study was to determine whether inhibition of the extracellular-regulated kinase signaling pathway decreases acute amphetamine-induced behavioral activity and neuropeptide gene expression in the rat striatum. Western blotting revealed that extracellular-regulated kinase1/2 phosphorylation was highly induced in the rat striatum 15 min after an acute amphetamine (2.5 mg/kg, i.p.) injection without altering the total amount of extracellular-regulated kinase protein. In a separate experiment, the systemic injection of SL327, a selective inhibitor of extracellular regulated kinase kinase that crosses the blood–brain barrier, 1 h prior to amphetamine administration decreased amphetamine-induced vertical and horizontal activity. Quantitative *in situ* hybridization histochemistry showed that SL327 abolished the high levels of preproenkephalin and preprodynorphin mRNA induced by amphetamine in the striatum with no alteration of their basal levels. In another set of experiments, the hyperlocomotor activity induced by amphetamine was reduced by pretreatment with intra-striatal infusion of U0126. U0126 also blocked the amphetamine-induced increases in phospho-extracellular-regulated kinase and preproenkephalin and preprodynorphin gene expression in the striatum. These data indicate that activation of the extracellular-regulated kinase cascade contributes to the behavioral effects and changes in striatal neuropeptide gene expression induced by acute amphetamine. © 2006 Published by Elsevier Ltd on behalf of IBRO.

Key words: psychostimulants, preproenkephalin, preprodynorphin, *in situ* hybridization.

Changes in gene expression induced by psychostimulants in discrete brain structures are thought to be crucial for triggering the neurobiological and behavioral responses underlying addictive processes (Nestler and Aghajanian, 1997; Berke and Hyman, 2000). By increasing the release of dopamine (DA) from dopaminergic terminals in the striatum (Zetterstrom et al., 1983; Sharp et al., 1987), an acute injection of amphetamine induces rapid and transient increases in expression of the transcription factors, c-*fos* and *zif/268*, and phosphorylation of cAMP response element binding protein (CREB) in the rat striatum (Graybiel et al., 1990; Nguyen et al., 1992; Moratalla et al., 1992). As a consequence, a delayed and prolonged increase in opioid peptide gene expression in striatal neurons occurs (Smith and McGinty, 1994; Wang et al., 1995; Wang and McGinty, 1995b; Zhou et al., 2004). The peptides, dynorphin and enkephalin, that are encoded by these genes modulate the release of DA and glutamate by activating local receptors in the striatum (Heijna et al., 1989; Wang and McGinty, 1995a; Gray et al., 1999; Rawls and McGinty, 2000). Alterations in IEG and opioid gene expression are thought to represent an initial step in the development of neuroplasticity underlying long-term profiles of drugs of abuse (Nestler et al., 1993; Nestler, 1997; Chiasson et al., 1997; Wang et al., 2003). However, the key intracellular signaling molecules that participate in regulating the alterations in opioid peptide gene expression by acute amphetamine remain unclear.

Several lines of evidence suggest an important role for intracellular signal transduction pathways in the mechanisms of neural plasticity in response to drugs of abuse (Nestler, 2001). The extracellular signal-regulated kinase (ERK)/mitogen-activated protein kinase (MAPK) pathway involves a complex intracellular signaling cascade that regulates various neurobiological effects necessary for synaptic remodeling and long-term changes in synaptic efficacy (Berhow et al., 1996; Mazzucchelli et al., 2002). ERK1 (44 kDa) and ERK 2 (42 kDa) are members of the MAPK family that are induced by psychostimulants. After cocaine or amphetamine-induced activation, ERK1/2 proteins are translocated to the nucleus, resulting in phosphorylation and activation of transcription factors such as CREB and Elk-1 in medium spiny neurons (Valjent et al., 2000, 2005; Choe et al., 2002; Zhang et al., 2004). Phospho-CREB regulates transcription of the genes encoding the neuropeptides, dynorphin and enkephalin (Konradi et al., 1994; Cole et al., 1995) which are also upregulated by acute and chronic psychostimulant administration in several species (Hurd and Herkenham, 1992; Hurd et al., 1992; Daunais et al., 1993; Steiner and Gerfen, 1993; Smith and McGinty, 1994; Fagergren et al., 2003; Zhang et al., 2004).

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Abbreviations: ACSF, artificial cerebrospinal fluid; AUC, area under the curve; CPu, caudate-putamen; CREB, cAMP response element binding protein; DA, dopamine; DMSO, dimethylsulfoxide; ERK, extracellular signal-regulated kinase; MAPK, mitogen-activated protein kinase; MEK, mitogen-activated protein kinase/extracellular signal-regulated kinase activating kinase; NAc, nucleus accumbens; PKA, protein kinase A; PPD, preprodynorphin; PPE, preproenkephalin; SDS, sodium dodecyl sulfate; TBST, Tris-buffered saline plus 0.1% Tween-20.

0306-4522/06$30.00+0.00 © 2006 Published by Elsevier Ltd on behalf of IBRO.
doi:10.1016/j.neuroscience.2005.12.024

Psychostimulants such as amphetamine, not only increase the release of DA but also increase dialysate levels of glutamate in the dorsal and ventral striatum (Xue et al., 1996; Gray et al., 1999; Rawls and McGinty, 2000). Valjent et al. (2005) reported that activation of ERK by cocaine and amphetamine in medium-sized striatal neurons was prevented by blocking both D1 DA receptors and NMDA receptors. These findings indicate that ERK activation in the striatum in response to cocaine or amphetamine requires convergent DA and glutamate signals.

The functional significance of ERK phosphorylation to psychostimulant effects has been demonstrated by antagonizing ERK activation with selective mitogen-activated protein kinase/extracellular signal-regulated kinase activating kinase (MEK) inhibitors. For example, SL327, a systemically active MEK inhibitor, decreased cocaine-induced increases in phospho-ERK and fos family proteins in the striatum of mice in a D1 DA receptor-dependent manner (Valjent et al., 2000; Zhang et al., 2004). Further, SL327 blocked cocaine-induced hyperlocomotion and amphetamine-induced conditioned place preference (Valjent et al., 2000) as well as MDMA-induced locomotion and *c-fos* expression (Salzmann et al., 2003). Intra-accumbens infusion of the non-blood–brain barrier permeable MEK inhibitor, U0126, decreased cocaine-induced phospho-ERK and conditioned place preference (Miller and Marshall, 2005). These data indicate that ERK activation plays an important role in striatal gene expression and its behavioral responses related to the development of addiction.

However, the involvement of the ERK signaling pathway in striatal neuropeptide gene expression in response to amphetamine has not been investigated. The aim of this study was to investigate whether the blockade of the ERK signaling pathway decreases acute amphetamine-induced behavioral activity and neuropeptide gene expression in the rat striatum. In the present study, the selective MEK inhibitors, SL327 (systemic injection) or U0126 (intrastriatal administration), were used to determine the role of the ERK cascade in regulating preproenkephalin (PPE) and preprodynorphin (PPD) gene expression in the striatum and behavioral effects induced by amphetamine.

EXPERIMENTAL PROCEDURES

Animals

Adult male Sprague–Dawley rats (250–275 g, Charles River, Raleigh, NC, USA) were housed two per cage in a controlled environment 3–7 days prior to the experiments. Food and water were provided *ad libitum* and rats were maintained on a 12-h light/dark cycle (lights on at 7:00 AM). All animal procedures used were in strict accordance with the NIH Guide for the Care and Use of Laboratory Animals and were approved by the Institutional Animal Care and Use Committee. All efforts were made to minimize animal suffering, to reduce the number of animals used and to utilize alternatives to *in vivo* techniques. Following surgery, the rats were housed individually and allowed at least 1 week to recover before the experiments.

Drug treatments and behavior

The first experiment was performed to confirm that amphetamine upregulates the phosphorylation of ERK1/2 by Western blot using our injection paradigm. Rats were randomly divided into two groups ($n=5$ per group) and injected with 2.5 mg/kg, i.p. amphetamine or saline. The dose of amphetamine was based on extensive studies in this laboratory that have investigated behavioral activity and regulation of opioid gene expression after 2.5 mg/kg, i.p. amphetamine (Wang et al., 1995; Wang and McGinty, 1995b, 1996; Zhou et al., 2004). The rats were deeply anesthetized with Equithesin (made from reagents described in Deacon and Rawlins, 1966; 10 ml/kg, i.p.) and decapitated 15 min following drug administration. We investigated the activation of ERK1/2 15 min after acute amphetamine injection because amphetamine-induced ERK activation in the striatum has been demonstrated to peak at this time point (Miller and Marshall, 2005; Valjent et al., 2005). Striatal tissues were removed immediately and stored at −80 °C. until they were homogenized for Western blotting (see below). The brains were extracted and the striatum was dissected for measurement of phospho-ERK and total ERK by Western blotting.

The second experiment was designed to detect whether systemic injection of a blood–brain barrier permeable MEK inhibitor suppresses amphetamine-induced behavioral activity and neuropeptide gene expression in rat striatum. Rats were randomly assigned to four different groups: DMSO+saline, DMSO+amphetamine, SL327+saline, or SL327+amphetamine ($n=5$–6/group). The day before the test day, the rats were habituated in photocell chambers (Accuscan Instruments, Inc., Columbus, OH, USA). Each box contained a series of 16 photocell beams measuring horizontal distance traveled and eight photocell beams measuring vertical activity. Beam breaks were continuously counted and recorded once every 5 min by a PC running VersaMax/Digiscan System Software (Accuscan Instruments, Inc.). On the test day, after 1 h habituation in the chamber, each rat was injected i.p. with DMSO or 50 mg/kg SL327. One hour later, each rat received an injection of saline or amphetamine (2.5 mg/kg, i.p.). Total distance traveled and vertical activity were recorded for 3 h after the second injection. Immediately after the test, all rats were anesthetized with Equithesin (10 mg/kg, i.p.) and decapitated. The brains were removed and frozen in isopentane at −40 °C and stored at −80 °C until they were sectioned for histological localization of cannulas and PPE and PPD gene expression by *in situ* hybridization.

In the third experiment, a non-blood–brain barrier-penetrating MEK inhibitor, U0126, was infused bilaterally into the striatum 30 min prior to amphetamine administration via injection needles inserted into chronically implanted guide cannulas (see below). Rats were randomly assigned to four different groups ($n=5$–6/group) and habituated to the photocell chambers as in experiment two. Prior to microinjection, the stylets were removed and 30-gauge stainless steel injection cannulas (13 mm in length) were inserted bilaterally to extend 3 mm below the end of the guides. A 1 μl volume of either vehicle (50% dimethylsulfoxide, DMSO) or 1 μg U0126 was infused bilaterally at a rate of 0.2 μl/min into the striatum of the freely moving animal. The dose of U0126 and the pretreatment time were chosen based on previous studies (Kelly et al., 2003; Schafe et al., 2000). After each infusion, the injector cannulas were left in place for an additional 2 min to reduce the risk of fluid backflow up the guide cannula. Thirty minutes later, 2.5 mg/kg amphetamine or saline was injected i.p. and the behavior of the rats was recorded as described in experiment 2.

In experiment 4, to determine whether the intra-striatal infusion of U0126 effectively blocked the amphetamine-induced phosphorylation of ERK1/2, the experimental design of the third experiment was repeated (intra-striatal U0126 infusion followed by i.p. amphetamine or saline) except that rats were anesthetized and decapitated 15 min after the i.p. injections, the striatum was dissected, and Western blotting of phospho-ERK1/2 was performed as in experiment 1.