Neuroscience Letters 577 (2014) 117–122

Contents lists available at ScienceDirect

Neuroscience Letters

journal homepage: www.elsevier.com/locate/neulet

Cocaine challenge increases the expression of immunoglobulin heavy chain binding protein in the rat nucleus accumbens

In Soo Ryu, Eun Sang Choe *

Department of Biological Sciences, Pusan National University, 63-2 Pusandaehak-ro, Kumjeong-gu, Pusan 609-735, Republic of Korea

HIGHLIGHTS

- A renewed cocaine challenge increased BiP expression in the nucleus accumbens.
- Blockade of NMDA receptors decreased elevated BiP expression.
- Blockade of ryanodine-sensitive Ca^{2+} channels decreased elevated BiP expression.
- Inhibition of JNK decreased elevated BiP expression.

ARTICLE INFO

Article history:
Received 29 April 2014
Received in revised form 14 June 2014
Accepted 16 June 2014
Available online 23 June 2014

Keywords:
ER stress response
Ionotropic glutamate receptor
JNK
Psychostimulant
Sensitization
Ventral striatum

ABSTRACT

Repeated cocaine administration increases the endoplasmic reticulum (ER) stress response in the dorsal striatum. This study was performed to investigate that cocaine challenge after cocaine abstinence is associated with the ER stress response in the nucleus accumbens (NAc). The results demonstrated that a renewed cocaine challenge (20 mg/kg) after six days of withdrawal following seven daily systemic injections of cocaine (20 mg/kg), significantly increased the expression of the ER stress response protein, immunoglobulin heavy chain binding protein (BiP). The elevation of BiP expression was decreased by blocking *N*-methyl-D-aspartate (NMDA) receptors and ryanodine-sensitive Ca^{2+} channels. The inhibition of c-Jun N-terminal kinase (JNK) also attenuated the renewed cocaine challenge-induced increase in BiP expression. These findings suggest that JNK phosphorylation, via stimulation of NMDA receptors and ryanodine-sensitive Ca^{2+} channels evoked by cocaine challenge after cocaine abstinence, is necessary for the induction of the ER stress response in the NAc.

1. Introduction

The nucleus accumbens (NAc), a primary target of dopaminergic and glutamatergic projections from the ventral tegmental area and cortices, respectively, is responsible for drug-mediated reward [10]. Cocaine, an indirect dopamine agonist, increases extracellular dopamine concentrations by blocking dopamine reuptake into nerve terminals in the NAc. Accumulation of dopamine in the synaptic cleft increases glutamate release into the NAc via transsynaptic activation of forebrain reward circuits. This increase in glutamate concentrations in the NAc facilitates Ca^{2+} signaling cascades by the activation of glutamate receptors [10,15].

The endoplasmic reticulum (ER) is an intracellular organelle essential for multiple cellular processes, such as the synthesis, folding and maturation of proteins, and intracellular Ca^{2+} homeostasis [12]. A variety of stimuli, including exposure to drugs of abuse, disrupt Ca^{2+} homeostasis [11]. The extreme change in cytosolic Ca^{2+} concentrations leads to the accumulation of misfolded proteins, causing the ER stress response. To protect the cell from damage, the ER stress response facilitates unfolded protein response (UPR) signaling cascades through the ER stress-related proteins, such as inositol requiring enzyme-1a (Ire1a), protein kinase R-like ER kinase (PERK), and activating transcription factor 6 (ATF6) [16]. Immunoglobulin heavy chain binding protein (BiP), an ER stress sensor protein in the ER membrane, binds to the ER stress-related proteins under normal cellular conditions. However, under stressful conditions, the ER stress-related proteins activate the UPR signaling cascades, reducing the ER stress response via BiP overexpression [16]. Thus, BiP overexpression is considered a crucial marker for the ER stress response.

Previous studies have demonstrated that repeated cocaine administration upregulates the expression of mRNAs and proteins for the ER stress response in the NAc and the dorsal striatum, respectively [2,3,18,22]. Activation of Ca^{2+}-dependent

* Corresponding author. Tel.: +82 51 510 2272; fax: +82 51 581 2962.
E-mail address: eschoe@pusan.ac.kr (E.S. Choe).

http://dx.doi.org/10.1016/j.neulet.2014.06.031
0304-3940/

c-Jun N-terminal kinase (JNK) linked to dopamine D1 receptors and glutamate receptors leads to the ER stress response after repeated cocaine administration in a Ca^{2+}-dependent manner [8]. Cocaine withdrawal has shown remarkable decreases in the functions of Ca^{2+} channels in the NAc [9]. Taken together, these data suggest that re-exposure to cocaine after withdrawal followed by repeated cocaine regulates the ER stress response through dysfunctions in Ca^{2+} signaling. Therefore, this study examined whether cocaine challenge after cocaine abstinence evokes BiP overexpression in the NAc.

2. Materials and methods

Adult male Sprague-Dawley rats initially weighing approximately 200–250 g were purchased from Hyo Chang Science Co. (Daegu, Korea). Rats were housed in a temperature and humidity-controlled environment on a 12 h light–dark cycle during all experimental treatments. Water and food were available ad libitum. Rats were allowed to acclimate for a minimum of 5 days before conducting experiments. On the day of the experiments, all injections were given in the home cage and in a quiet room to minimize stress to the animals. All experiments were approved by the Institutional Animal Care and Use Committee and performed in accordance with the provisions of the NIH Guide for the Care and Use of Laboratory Animals.

The *N*-methyl-D-aspartate (NMDA) receptor antagonists, MK801 (2 nmol) and AP5 (2 nmol), the IP_3-sensitive Ca^{2+} channel blocker, xestospongin C (0.004 nmol), the ryanodine-sensitive Ca^{2+} channel blocker, dantrolene (20 nmol), the L-type voltage-operated Ca^{2+} channel blocker, nifedipine (60 nmol), the Na^+ channel blocker, tetradotoxin citrate (TTX) (1 pmol), and the JNK inhibitor, SP600125 (30 nmol), were dissolved in the minimum concentration of dimethylsulfoxide (DMSO) and then diluted with artificial cerebro-spinal fluid (aCSF) containing (mM) 123 NaCl, 0.86 $CaCl_2$, 3.0 KCl, 0.89 $MgCl_2$, 0.50 NaH_2PO_4, and 0.25 Na_2HPO_4 aerated with 95% O_2/5% CO_2 (pH 7.2–7.4) for working solutions. Solutions of all drugs were prepared at pH 7.2–7.4. DMSO mixed with aCSF was therefore used as the vehicle control for these agents. All drugs, except xestospongin C (Millipore Bioscience Research, Billerica, MA, USA), were purchased from Tocris Bioscience (Bristol, UK) and working solutions were always prepared fresh before starting each experiment. Cocaine (Balgopia, Louvain-La-Neuve, Belgium) was dissolved in physiological saline (0.9% NaCl). The doses or concentrations of the reagents were determined from previous studies [2,5,8]. Rats were received repeated intraperitoneal (i.p.) injections of saline or cocaine (20 mg/kg) for seven consecutive days. Rats were then given a drug-free period for six days. On the 14th day, rats were sacrificed for experiments after the final saline or cocaine challenge injection (20 mg/kg, i.p.) (Fig. 1A).

Surgery and intra-accumbal drug infusion were performed as previously described [6]. Rats were anesthetized with 8% chloral hydrate (6 ml/kg, i.p.) and unilaterally implanted with a 23 gauge stainless steel guide cannula (0.29 mm inner diameter, 15 mm in length) 1.6 mm anterior of the bregma, 1.2 mm to the right of the midline, and 7.5 mm below the surface of the skull in a stereotaxic apparatus. The guide cannula was sealed with a stainless steel wire stylet of the same length, and more than 5 days were allowed for rats to recover from surgery. On the day of experiment, the sealing inner steel wire stylet was replaced by a 30 gauge stainless steel injection cannula (0.15 mm inner diameter, 17.5 mm in length) that protruded 2.0 mm beyond the guide cannula. Drug solutions were infused through the injection cannula into the border of the core and the shell of the right NAc, in a total volume of 1 μl at a rate of 0.2 μl/min in freely moving rats as previously described [4]. The progress of the injection was monitored by observing the movement of a small air bubble through a length of precalibrated PE 10 tubing inserted between the injection cannula and a 2.5 μl Hamilton microsyringe (Fisher Scientific, Pittsburgh, PA, USA). After the injection was completed, the injection needle was held in place for an extra 5 min to reduce any possible backflow of the solution along the injection tract. The physical accuracy of the injection was verified by the reconstruction of microinjection placements (Fig. 1B). Nissl staining was performed to verify the possibility of gliosis caused by implantation of the guide cannula and the infusion of drugs dissolved in DMSO/aCSF (Fig. 1B).

Western immunoblot analysis was performed as previously described [6]. The concentration of solubilized proteins in the supernatant was measured based on the Bradford method using the Bio-Rad Protein Assay (Bio-Rad Laboratories, Hercules, CA, USA). The supernatants were loaded onto a 10% sodium dodecyl sulfate–polyacrylamide gel and separated proteins were transferred to a nitrocellulose membrane. The membrane was blocked with a blocking buffer containing 5% skim milk in a mixture of tris-buffered saline and tween-20 (TBST) and then washed three times with TBST for 10 min. The membrane was then probed with primary antiserum against BiP (Cell Signaling Technology Danvers, MA, USA, 1:2000) or β-tubulin (Santa Cruz Biotechnology, Inc, Santa Cruz, CA, USA, 1:1000) for 20 h at 4 °C on a rolling shaker. After washing three times with TBST for 10 min, the membrane was incubated with the appropriate secondary antiserum (KPL, Gaithersburg, MD, USA) for 1 h at room temperature on a rolling shaker. Membranes containing immunoreactive protein bands were developed using Westsave (AbFrontier, Seoul, Korea) on X-ray films. β-Tubulin was also reprobed on the same membrane for normalization of the immunoblots.

Immunohistochemistry was performed as previously described [6]. Perfused brains were serially cut in 30 μm sections using a sliding microtome with freezing plate. Three sections per brain were collected and processed for immunohistochemistry. Sections were washed with 1% Triton X-100 in phosphate buffered saline (PBS, pH 7.2) for 10 min. The sections were then preincubated in 0.6% H_2O_2 in the dark for 30 min, after washing three times in PBS for 10 min. The sections were incubated with normal goat serum (Vector Laboratories, Burlingame, CA, USA) to eliminate the possibility of non-specific cross reactions. Finally, the sections were incubated with BiP antiserum (Abcam, MA, USA) at 1:500 for 20 h at 4 °C on a rolling shaker. After washing three times with PBS for 10 min, the sections were incubated in goat anti-rabbit secondary antiserum (Vector Laboratories) for 1 h, followed by avidin–biotin–peroxidase reagents (Vector Laboratories) for 1 h at room temperature on a rolling shaker. Diaminobenzidine was used as the chromogen and $NiCl_2$ was added to enhance the reaction product. Finally, the sections were mounted onto gelatin-coated slides.

Immunoreactive protein bands visualized on X-ray films or BiP immunoreactivity on brain sections were semi-quantified with NIH image 1.62 software and a digital imaging camera as previously described [1,6,13]. Briefly, the background of X-ray films or brain sections was measured and saved as a "blank field" to correct for uneven illumination. The upper limit of the density slice option was set to eliminate any nonspecific background and this value was used to measure all images. The lower limit was set at the bottom of the lookup table scale. The immunoreactive protein bands were measured by using a rectangle that covered each individual band. Similarly, the area of BiP immunostaining on brain sections measured near the injection tract in the NAc was also semi-quantified.

The statistical significance between groups for the number of immunoreactive pixels per measured area following Western immunoblotting and immunohistochemistry was determined by one- or two-way ANOVA with repeated measurements followed by Tukey's post hoc test in GraphPad Prism 4 (GraphPad Software