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Research article

Brain-derived neurotrophic factor downregulates immunoglobulin heavy chain binding protein expression after repeated cocaine administration in the rat dorsal striatum

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HIGHLIGHTS

- BDNF decreased the cocaine-induced increases in BiP and pJNK immunoreactivity.
- TrkB antagonist reversed the BDNF-induced decreases in BiP and pJNK immunoreactivity.
- BDNF may contribute to the restoration of the ER functions by deactivating JNK.

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ABSTRACT

Brain-derived neurotrophic factor (BDNF) is a key molecule involved in the regulation of glutamatergic neurotransmission in response to chronic stimulation of psychostimulants. This study demonstrated that BDNF in the dorsal striatum regulates the endoplasmic reticulum (ER) stress response after repeated exposure to cocaine. The results showed that unilateral intracaudate infusion of BDNF (0.40, 0.75, or 1.50 μg/μL) decreased the repeated cocaine-induced increase in the expression of immunoglobulin heavy chain binding protein (BiP) sensing unfolded or misfolded proteins in a dose-dependent manner. Unilateral intracaudate infusion of BDNF (0.75 μg/μL) also decreased the phosphorylation of c-Jun N-terminal kinase (JNK), which had been initially elevated by seven consecutive daily intraperitoneal injections of cocaine (20 mg/kg/day). These decreases were reversed by unilateral intracaudate infusion of the specific tropomyosin receptor kinase B (TrkB) antagonist, cyclotraxin B (1 ng/μL). These findings suggest that BDNF regulates the unfolded protein response via TrkB-linked JNK inactivation in the dorsal striatum after repeated cocaine administration, thus contributing to the restoration of the ER functions.

1. Introduction

Cocaine blocks the reuptake of dopamine in its terminals and increases the concentrations of extracellular dopamine in the dorsal striatum [1,2]. This increase, in turn, upregulates glutamate concentrations through stimulation of thalamocorticostriatal projection neurons [3–6]. Glutamate overflow then leads to the endoplasmic reticulum (ER) stress through the disruption of Ca^{2+} homeostasis coupled with ionotropic- and metabotropic glutamate receptors (mGluRs) [7].

To restore the ER functions, the ER stress triggers a coordinated adaptive process called the unfolded protein response (UPR), which is part of the ER stress response, by repairing unfolded or misfolded proteins [8,9]. UPR is controlled by three branches of the stress sensor proteins, inositol requiring enzyme-1 α (IRE1α), activating transcription factor 6 (ATF6), and protein kinase R-like ER kinase (PERK), within the ER membrane [10–12]. The ER chaperone, immunoglobulin heavy chain binding protein (BiP), detects the unfolded or misfolded proteins and triggers UPR by dissociating the stress sensor proteins [13]. Thus, an alteration in BiP expression would be a useful marker for UPR in response to stressful stimuli such as drug exposure. Previous studies have shown that c-Jun N-terminal kinase (JNK), a member of the stress-activated protein kinase, is required to produce UPR. For example, stimulation of glutamate receptors after repeated cocaine exposure upregulates the

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expression of BiP via JNK phosphorylation in the dorsal striatum [14,15].

Brain-derived neurotrophic factor (BDNF) has been reported to be the key molecule for preventing neuronal toxicity caused by cocaine exposure and acts by stimulating its receptor, tropomyosin receptor kinase B (TrkB), located in the reward-related brain regions [16]. Emerging evidence shows that acute and chronic exposure to cocaine upregulates mRNA and BDNF protein expression via elevation of glutamate release in the striatum [17,18]. This increase is likely an adaptive process to protect against cell damage. For example, treatment of BDNF downregulates the expression of C/EBP homologous protein (CHOP), a key transcription factor in the ER stress-induced apoptotic cell death, in cultured cerebral neurons and human neuroblastoma SY-SY5Y cells [19,20]. BDNF induced by the endogenous neuromodulator, hydrogen sulfide, prevents the expression of CHOP and BiP in the rat hippocampus and PC12 cells [21]. Taken together, these findings suggest that BDNF could be a key factor in the regulation of UPR in response to stressful drug stimuli. Currently, little has been reported on psychostimulants impairing the ER functions and leading to UPR. Moreover, the contribution of BDNF to drug-induced UPR has not been elucidated. Herein, we demonstrate that BDNF regulates UPR to restore the ER functions in the rat dorsal striatum after repeated cocaine administration.

2. Materials and methods

Adult male Sprague-Dawley rats (250–300 g) were obtained from Hyo-Chang Science Co. (Daegu, Korea). Rats were housed in pairs in a controlled environment, and maintained on a 12 h light/dark cycle throughout all experimental treatments. Food and water were provided ad libitum. On the day of the experiment, injections were given in the home cage to minimize the stress level of the animals. All animal procedures were approved by the Institutional Animal Care and Use Committee and carried out in accordance with the provisions of the NIH Guide for the Care and Use of Laboratory Animals.

All drugs, except cocaine (Louvain-La-Neuve, Belgium), were purchased from Tocris Bioscience (Bristol, UK). All drug solutions were freshly prepared before the experiments. BDNF (0.40, 0.75, and 1.50 μg/μL) [22] and cyclotraxin B (1 ng/μL) [23], a TrkB-specific antagonist, were dissolved in artificial cerebrospinal 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). The same aCSF solution was used as the vehicle control for the given drug. All drug solutions were neutralized to pH 7.2–7.4 with 1 M NaOH, if necessary. Cocaine was dissolved in physiological saline (0.9% NaCl). Each rat received cocaine (20 mg/kg) once a day for seven consecutive days via intraperitoneal (i.p.) injection.

Surgery and infusion of drugs into the dorsal striatum were performed as previously described [24]. Rats were anesthetized with a mixture of Zoletil 50 (75 μL/kg) (Virbac Korea, Seoul, Korea) and Rompun (50 μL/kg) (Bayer Korea, Seoul, Korea) via i.p. injections. Rats were then fixed in a stereotaxic apparatus. Under aseptic conditions, a 23-gauge stainless steel guide cannula (0.29 mm inner diameter, 10 mm in length) was implanted unilaterally into the right hemisphere (1 mm anterior to the bregma, 2.5 mm right of the midline, and 2.5 mm below the surface of the skull) to minimize damage. The guide cannula was sealed with a stainless steel wire of the same length. Rats were then allowed to recover from surgery for 5 days prior to the experiment. On the day of the experiment, the inner steel wire was replaced with a 30-gauge stainless steel injection cannula (0.15 mm inner diameter, 12.5 mm in length) that protruded 2.5 mm beyond the guide cannula. Throughout the experiments, all drugs were infused unilaterally into the central part of the right dorsal striatum 5 min prior to the final injection of cocaine (20 mg/kg/day, i.p.) or saline in a volume of 1 μL, at a rate of 0.5 μL/min in freely moving rats. Progress of the injection was monitored by observing the movement of a small air bubble along the length of a precalibrated PE-10 tube inserted between the injection cannula and a 2.5 μL Hamilton microsyringe (Fisher Scientific, Pittsburgh, PA, USA). After completing the injection, the injector was left in place for an additional 5 min to reduce any possible backflow of solution along the injection tract. Timeline for experiments conducted in this study is represented in Fig. 1A. The possibility of gliosis by implantation of the guide cannula and the unilateral intracaudate drug infusions was determined by using Nissl staining (Fig. 1B).

Western immunoblotting was routinely performed, as previously described [25,26], and repeated more than three times. Briefly, rats were deeply anesthetized with a mixture of Zoletil 50 and Rompun, then decapitated 30 min after the final drug injection. Next, brains were removed, frozen in isopentane at −70 °C, and stored in a deep freezer until used. Brain sections were serially cut by using a cryostat (Leica Biosystems, Nussloch, Germany) at −20 °C, after which the injected right dorsal striatum was removed with a steel borer (2 mm inner diameter). All tissue samples were transferred to a homogenization buffer containing (mM) 10 Tris-HCl, pH 7.4, 5 NaF, 1 Na_3VO_4, 1 EDTA, and 1 EGTA, sonicated three times for 9 s each, and then incubated on ice for 1 h. After sonicating, the samples were centrifuged at 13,000 rpm for 30 min at 4 °C. The pellet, which primarily contained nuclei and large debris, was discarded, and the supernatant was centrifuged at 13,000 rpm for 30 min at 4 °C. The concentration of the solubilized proteins in the supernatant fraction was determined based on the Bradford method by using a Bio-Rad Protein Assay (Bio-Rad Laboratories, Hercules, CA, USA). The proteins in the supernatant were resolved by using 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis, after which the separated proteins were transferred to a nitrocellulose membrane. The membrane was then blocked with blocking buffer containing 5% skim milk in a mixture of Tris-buffered saline and Tween-20 (TBST), then washed three times for 10 min each with TBST. After washing, the membrane was probed with either a rabbit primary antiserum against phosphorylated (p)JNK (1:1000), JNK (1:1000) or BiP (1:2000) for 18 h at 4 °C on a shaker. The membrane was washed again then incubated with an appropriate rabbit secondary antiserum (KPL, Gaithersburg, MD, USA) under the same conditions as that for the primary antiserum for 1 h at room temperature. Immunoreactive protein bands were detected on X-ray films by using enhanced chemi-luminescence reagents (Ab Frontier, Seoul, Korea; ratio of reagents A to B = 1:500). The samples were probed for unphosphorylated proteins after stripping the same membrane that was confirmed to contain phosphorylated protein. The same membrane was also probed for β-tubulin (1:2000) to normalize the blots. Immunoreactive protein bands, which were visualized on the films, were semi-quantified by using an imaging digital camera and NIH Image 1.62 software as previously described [25]. All primary antibodies were purchased from Cell Signaling Technology (Danvers, MA, USA), except for the antiserum against β-tubulin (Santa Cruz Biotechnology, Inc., Dallas, TX, USA).

Rats were deeply anesthetized with a mixture of Zoletil 50 and Rompun and then transcardially perfused with 4% paraformaldehyde in phosphate-buffered saline (PBS, pH 7.2) at 4 °C. The brains were then removed and post-fixed in a solution of 10% sucrose in 4% paraformaldehyde for 2 h at 4 °C, after which they were placed in 20% sucrose in PBS and held at 4 °C overnight [25]. Using a freezing sliding microtome, 16 μm frozen sections were obtained. Three sections per brain were used for immunofluorescence staining. The sections were blocked with a blocking buffer containing 4% normal goat serum and 1% bovine serum albumin in PBS, then washed with