COCAINE INCREASES ENDOPLASMIC RETICULUM STRESS PROTEIN EXPRESSION IN STRIATAL NEURONS

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Abstract—Cocaine administration upregulates the levels of extracellular glutamate and dopamine in the striatum. Activation of the receptors alters calcium homeostasis in striatal neurons leading to the expression of the endoplasmic reticulum (ER) stress proteins. It was therefore hypothesized that cocaine upregulates the expression of the ER stress proteins, immunoglobulin heavy chain binding protein (BiP), and peripherin via glutamate and dopamine receptor activation. A novel glutamate microsensor and Western immunoblot analyses were mainly performed to test the hypothesis in the rat dorsal striatum. The results showed that i.p. injection of repeated cocaine (20 mg/kg) for nine consecutive days significantly increased extracellular glutamate levels while acute cocaine injection did not. However, the immunoreactivities (IR) of the ER stress proteins in the dorsal striatum were significantly increased by either acute or repeated cocaine injections as compared with saline controls. Intrastriatal injection (i.s.) of the selective group I metabotropic glutamate receptor (mGluR) antagonist N-phenyl-7-(hydroxyimino)cyclopropa[b]chromen-1a-carboxamide (PHCCC; 25 nmol) or the mGluR5 subtype antagonist 2-methyl-6-(phenylethynyl)pyridine hydrochloride (MPEP; 2 and 25 nmol) significantly decreased repeated cocaine-induced increases in the IR of the ER stress proteins in the injected dorsal striatum. Similarly, the selective D1 antagonist dizocilpine/(S,S,10R)-(+)5-methyl-10,11-dihydro-5H-ibenzox[a,d]cyclohepten-5,10-imine maleate (MK801; 2 nmol, i.s.) decreased acute or repeated cocaine-induced increases in the IR of the ER stress proteins in the dorsal striatum. These data suggest that cocaine upregulates expression of the ER stress proteins in striatal neurons via a mechanism involving activation of glutamate and dopamine receptors. © 2006 IBRO. Published by Elsevier Ltd. All rights reserved.

Key words: drugs of abuse, BiP, Ire1α, peripherin, glutamate, microsensor.

Cocaine administration is known to upregulate the levels of extracellular glutamate and dopamine in the striatum. Activation of the receptors by cocaine disrupts calcium homeostasis that may express the endoplasmic reticulum (ER) stress proteins in the striatum. Since ER stress has been considered to be one of the most important routes that are involved in neuronal cell apoptosis (Cadet et al., 2003; Jayanthi et al., 2004), it is interesting to understand putative mechanisms underlying cocaine-mediated ER stress protein expression in the striatum.

ER is the site of synthesis, folding and assembly of secretory proteins, which is controlled in part by immunoglobulin heavy chain binding protein (BiP), an ER Hsp70 family member. BiP, the first identified component of the ER quality control apparatus, was found by virtue of its association with the unassembled, non-transported heavy chains produced in pre-B cell lines (Haas and Wabl, 1983). Disturbance of normal cellular physiology can affect the biosynthesis of proteins in the ER, resulting in the accumulation of unfolded proteins. Cells under stress activate a signaling cascade termed the unfolded protein response (UPR) to prevent the formation of insoluble protein aggregates (Kaufman, 1999). Prolonged UPR activation leads to apoptotic cell death (Patil and Walter, 2001). The UPR pathway transmits information about protein folding status in the ER lumen to the cytoplasm and the nucleus. Three ER-transmembrane proteins have been identified as the transducers of the UPR: Ire1α, peripherin and ATF6. Ire1 and peripherin have cytoplasmic serine/threonine kinase domains. In case of Ire1 RNase domain is also present in the cytoplasmic domain. Under the ER stress the luminal domains are homodimerized and subsequently autoprophosphorylated in the cytoplasmic domains (Cox et al., 1993; Mori et al., 1993; Harding et al., 1999).

The present study, therefore, was performed to test the hypothesis that cocaine upregulates expression of the ER stress proteins in the striatum since cocaine increases levels of glutamate (Hotsenpiller and Wolf, 2003; McFarland et al., 2003; Smith et al., 1995) and dopamine

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(Jocham et al., 2006; Izawa et al., 2006), which further regulates calcium homeostasis. A novel electrochemical glutamate microbiosensor and Western blotting analyses were mainly applied to test the hypothesis in the dorsal striatum.

**Experimental Procedures**

**Animals**

Adult male Sprague–Dawley rats (200–250 g) were obtained from Hyo-Chang Science Co. (Daegu, Korea). Rats were individually housed in a controlled environment during all experimental treatments. Food and water were provided *ad libitum* and rats were maintained on a 12-h light/dark cycle. On the day of the experiment injection was made in a quiet room to minimize stress. All animal use procedures were approved by the Institutional Animal Care and Use Committee and were accomplished in accordance with the provisions of the NIH “Guide for the Care and Use of Laboratory Animals.”

**Experimental design**

Five separate experiments were conducted in this study. The first experiment was designed to investigate whether acute or repeated cocaine alters extracellular glutamate release using a glutamate microbiosensor developed in our research group (see below). Rats were randomly divided into four groups (*n*=3–4 per group). Each rat received i.p. injection of acute saline, acute cocaine, repeated saline or repeated cocaine (20 mg/kg). For repeated cocaine, rats received cocaine (20 mg/kg, i.p.) once daily for nine consecutive days. The second experiment was designed to investigate whether acute or repeated cocaine induces expression of the ER stress proteins using Western blot analysis. Rats were randomly divided into four groups (*n*=4–5 per group). Each rat received i.p. injection of acute saline, acute cocaine, repeated saline or repeated cocaine. Parallel to the sensor experiment, each rat received i.p. injection of cocaine once daily for nine consecutive days for repeated cocaine. The third and fourth experiments were designed to investigate whether group I metabotropic glutamate receptors (mGlURs) regulate expression of the ER stress proteins by repeated cocaine using Western blot analysis. Rats were randomly divided into four groups (*n*=4–5 per group) for each experiment. The selective group 1 mGlUR antagonist N-phenyl-2,3,4,5-tetrahydro-1H-pyridine-3-carboxamide (PHCCC; 25 nmol) (Tocris Cookson, Ballwin, MO, USA) or the selective mGlUR5 subtype antagonist 2-methyl-6-(phenylethynyl)pyridine hydrochloride (MPEP; 2 and 25 nmol) (Tocris Cookson) was infused into the center of the dorsal striatum 0.5 (BiP), 1 (perk) or 4 (i:10a) h after each or final cocaine injection (i.p.) depending on the maximum induction of the proteins as revealed by the second experiment. The fifth experiment was designed to investigate whether D1 or N-methyl-D-aspartate (NMDA) receptor activation by acute or repeated cocaine regulates expression of the ER stress proteins using Western blot analysis. Rats were randomly divided into four groups (*n*=4–5 per group) for acute or repeated experiment. The selective D1 receptor antagonist (R)-(+)-7-chloro-8-hydroxy-3-methyl-1-phenyl-2,3,4,5-tetrahydro-1H-3-benzazepine hydrochloride (SCH23390; 0.1 mg/kg, i.p.) (Tocris Cookson) or the selective NMDA antagonist dizocilpine(55,10R)-(+)-5-methyl-10,11-dihydro-5H-ibenzol[a]dicyclohepten-5,10-imine maleate (MK801; 2 nmol) (Tocris Cookson) was infused into the center of the dorsal striatum 0.5 (BiP), 1 (perk) or 4 (i:10a) h after each or final cocaine injection (i.p.) as conducted by the third and fourth experiments. The doses of antagonists were determined from our previous studies (Wang and McGinty, 1995; Choe and Wang, 2001, 2002; Choe et al., 2005). Throughout the experiment, cocaine (Belgopia, Louvain-La-Neuve, Belgium) was dissolved in physiological saline (0.9% sodium chloride).

**Preparation of a glutamate microbiosensor**

The glutamate microbiosensor was prepared as described previously (Rahman et al., 2005). Briefly, a micropipette was made by pulling a glass capillary (inner diameter 1.1–1.2 mm, length 75 mm) (Chase Scientific Glass Inc., Rockwood, TN, USA) with a microelectrode puller. The tip of the micropipette was broken to the desired inner diameter (150 μm) with a diamond cutter. A Pt-wire (Pt 99.99%, 100 μm in diameter) (Johnson Matthey Inc., West Chester, PA, USA) of about 2 mm long was connected with a copper wire for electrical contact using electroconductive silver paste (Dotte Electroconductive, Type D-500) (Fujikura Kasei Co., Tokyo, Japan). This copper wire connected Pt electrode was introduced into the micropipette and advanced until it protruded about 0.2–0.3 mm from hollow opening of the micropipette. Then, the tip of the micropipette was heated gently in a butane flame until the glass collapsed and was sealed the Pt electrode. The other end of the micropipette was fixed using epoxy resin. The total length of the Pt microelectrode was 40 mm. The Pt microelectrode was coated with a conductive polymer (CP) through the electropolymerization of 5,2-terthiophene-3-containing carboxylic acid containing monomer (Lee and Shim, 2001) by cycling the potential three times at a fast scan rate. The CP-coated Pt microelectrode was then immersed in a 0.1 M phosphate buffer solution (pH=7.0) containing 10 mM 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide to activate the carboxylic acid groups of the CP. Then, glutamate oxidase (GluOx, EC. 1.4.3.11, 10.8 units mg−1) (Sigma-Aldrich, St. Louis, MO, USA) and ascorbate oxidase (AsOx, EC. 1.10.3.3, 100 units mg−1) (Sigma-Aldrich) were co-immobilized onto the CP layer through the formation of covalent bonds between the carboxylic acid groups of the CP and the amine groups of the GluOx and AsOx.

The working mechanism of glutamate microbiosensor is shown in Fig. 1. The GluOx/CP modified Pt microelectrode was used as a working electrode for the detection of glutamate. Amperometric responses were recorded using a Potentiotstat (KST-P2, Kosentech, Busan, Korea) with two electrode configurations: a glutamate microbiosensor and a micro-Ag/AgCl (in saturated KCl) electrode as working and reference/counterelectrodes, respectively. The experiments were carried out by applying a potential of +0.40 V at GluOx/CP/Pt microbiosensor to oxidize the H2O2 generated from the enzymatic reaction. All the microbiosensors were calibrated before and after measurements.

**Surgery and glutamate microbiosensor detection**

On the day of the experiment, rats were anesthetized with 8% chloral hydrate (6 ml/kg, i.p.) and placed in a Stoelting stereotaxic apparatus. Under aseptic conditions, the enzyme-coated electrode and the reference electrode of glutamate microbiosensor were implanted at the coordinates of 1 mm anterior to bregma, 2.5 mm right to midline and 4 mm below the surface of the skull. The sensor tip was inserted unilaterally into the central part of the right dorsal striatum. The output of electrodes was connected to the electrochemical detector, Potentiotstat. The electrochemical signal can be read and stored by the workstation. The measurement of glutamate levels in the dorsal striatum can be performed immediately after implantation of the microbiosensor electrodes. Before the measurement, one sensor among the same batch was calibrated with a series of glutamate standard solutions. In this study, 30 min time point was selected based on the study delivered from time course after repeated cocaine injections in which glutamate levels at 30 min were significantly increased as compared with acute and saline injections (Rahman et al., 2005).
Intrastriatal drug infusion

Rats were anesthetized with 8% chloral hydrate (6 ml/kg, i.p.) and placed in a Stoelting stereotaxic apparatus. Under aseptic conditions, a 23-gauge stainless steel guide cannula (inner diameter: 0.29 mm, 10 mm in length) was implanted at the coordinates of 1 mm anterior to bregma, 2.5 mm right to midline and 4 mm below the surface of skull. The guide cannula was sealed with a stainless steel wire of the same length. Rats were allowed 3 days’ recovery from surgery. On the day of the experiment, the inner steel wire was replaced by a 30-gauge stainless steel injection cannula (inner diameter: 0.15 mm) with a length of 12.5 mm that protruded 2.5 mm beyond the guide cannula. Through the injection cannula, PHCCC, MPEP or MK801 was infused unilaterally into the central part of the right dorsal striatum in a volume of 0.92 μl at a rate of 0.2 μl/min in freely moving rats. Progress of injection was monitored by observing 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 (Hamilton, Reno, NV, USA). After completion of injection, the injector was left in place for an additional 5 min to reduce any possible backflow of the solution along with the injection tract. All injections were made in the home cage.

Western blotting

Rats were deeply anesthetized with 8% chloral hydrate (6 ml/kg, i.p.) and decapitated at 0.5, 1, 2 or 4 h after final injection. Brains were removed, frozen in isopentane at –70 °C and stored in a deep freezer. Sections were serially cut in a cryostat and injected right dorsal striatum was removed with a steel borer (inner diameter: 2 mm). All tissue samples were lysed in sodium dodecyl sulfate (SDS) sample buffer for 5 min at 95 °C. The samples were then sonicated for 30 s on ice and centrifuged for 10 min at 12,000×g with an Eppendorf tabletop centrifuge (Eppendorf, Hamburg, Germany). The supernatant was resolved using 10% SDS–polyacrylamide gel electrophoresis (SDS-PAGE) and the separated proteins were transferred to a nitrocellulose membrane. The membrane was blocked with blocking buffer (5% skim milk in TBST). The membrane was probed with each primary antiserum against BiP (1:1000), Ire1α (1:1000), perk (1:1000) or actin (1:1000) overnight at 4 °C on a shaker. BiP antiserum was kindly provided by Dr. L. M. Hendershot (Department of Tumor Cell Biology, St. Jude Children’s Research Hospital, Memphis, TN, USA). Perk and Ire1α antisera were purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA), and actin antiserum was purchased from Sigma-Aldrich. The membrane was then incubated with appropriate secondary antisera for 1 h. Immunoreactive proteins were detected by enhanced chemiluminescence reagents (ECL; Amersham Pharmacia Biotech, Piscataway, NJ, USA) on X-ray films.

Immunohistochemistry

Parallel to the Western blot analysis, rats were deeply anesthetized with 8% chloral hydrate (6 ml/kg, i.p.), and transcardially perfused with 4% paraformaldehyde at 4 °C. Brains were removed and post-fixed in 10% sucrose/4% paraformaldehyde for 2 h at 4 °C and then placed in 20% sucrose/phosphate-buffered saline (PBS) at 4 °C overnight. Using a cryostat microtome, 30 μm frozen sections were cut. Two sections per BiP antisera per brain were collected at striatal levels and processed for immunohistochemistry with the methods as described previously (Choe et al., 2004). Briefly, sections were incubated with BiP antiserum for 20 h at 4 °C on a shaker. Sections were then incubated in goat anti-rabbit secondary antisera (Vector Laboratories, Burlingame, CA, USA) for 1 h, followed by avidin–biotin–peroxidase reagents (Elite Vectastain kit, Vector Laboratories) for 1 h at room temperature. Diaminobenzidine was used as the chromogen and NiCl2 was added to enhance reaction product.

Quantitation of immunoreactivity (IR)

Immunoreactive protein bands on films were semi-quantified using an imaging digital camera and NIH Image 1.62 software. Briefly, film background was measured and saved as a “blank field” to correct uneven illumination. The upper limit of the density slice option was set to eliminate any background, and this value was used to measure all images. The lower limit was set at the

Fig. 1. Schematic diagram illustrating a glutamate microbiosensor system and its working model for the detection of altered extracellular glutamate levels evoked by saline or cocaine administration. Dorsal striatum caudate-putamen (CPu) was removed to prepare for Western blot analysis, which was performed at the same time points measured with the microbiosensor.
bottom of the LUT scale. The immunoreactive protein bands were measured using a rectangle covered the individual band.

Statistics

Statistical significance on the glutamate levels or the number of immunoreactive pixels per measured area between groups was determined using a one-way ANOVA on ranked data followed by a Tukey’s HSD (honestly significant difference) test in SAS (Cary, NC, USA). Statistically significant level was taken as $P < 0.05$.

RESULTS

Repeated cocaine, but not acute, increased glutamate responses in the dorsal striatum

A glutamate microbiosensor was applied to investigate whether acute or repeated cocaine elevates extracellular glutamate levels in the striatum. Fig. 2A showed current-time plots of glutamate responses obtained upon the addition of varying amounts of standard glutamate solutions in the 0.1 M phosphate buffer solution at pH 7.2. The linearity of the calibration curve for the current vs. glutamate concentration was qualified ($r = 0.99$) as presented in Fig. 2B. As shown in Fig. 2C, amperometric response produced at 30 min after repeated cocaine is significantly increased as compared with both saline and acute cocaine injections. Glutamate concentrations in acute saline or repeated saline, acute cocaine and repeated cocaine were determined to be 0.3, 0.35 and 1.0 $\mu$M, respectively from the calibration plot after in vivo measurement (Fig. 2B). Semiquantitative analysis shown in Fig. 2D confirmed that rats treated with daily cocaine injection for 9 days significantly increased the response in the dorsal striatum. * $P < 0.05$ as compared with repeated saline and acute cocaine groups.
Fig. 3. Western immunoblot analysis for the effect of cocaine on BiP (A), Ire1α (B), perk (C) and actin (D) IR in striatal neurons at different time points. Semi-quantitative analysis confirms that both acute and repeated cocaine injections caused increases in the IR of BiP, Ire1α and perk in striatal neurons (n=3–4 per group). L1, saline; L2, 0.5 h; L3, 1 h; L4, 2 h; L5, 4 h. * P<0.05 as compared with saline groups. Immunohistochemical localization of BiP IR (E) induced by 30 min after the final injection of either acute or repeated cocaine was enhanced in the cytoplasmic compartments in striatal neurons arrow as compared with saline (a, saline; b, cocaine). Inset c was magnified from the neurons around arrow in b. Scale bar=100 μm.
stress proteins in the dorsal striatum. The changes of BiP, Ire1α and perk IR in the dorsal striatum were monitored at 0.5, 1, 2 and 4 h after both acute and repeated cocaine injections. The results showed that acute or repeated cocaine significantly increased the IR of the proteins as compared with actin controls. The BiP IR (Fig. 3A) was significantly increased at 1 h and was prolonged up to 2 h after acute cocaine injection, whereas it was increased at 0.5 h and reached a peak at 1 h after repeated cocaine injections. BiP IR induced by acute or repeated cocaine was enhanced in the cytoplasmic compartments of striatal neurons as compared with saline injection (Fig. 3E). Similarly, perk IR (Fig. 3C) was also significantly increased at 0.5 h and was prolonged up to 2 h after acute cocaine injection, whereas it was observed to be prolonged up to 1 h by repeated cocaine. However, Ire1α IR (Fig. 3B) was significantly increased at 2 and 4 h after acute and repeated cocaine injections, respectively. Control actin IR was not altered at all time points as shown in Fig. 3D.

**PHCCC or MPEP decreased repeated cocaine-stimulated BiP, Ire1α and perk IR in the dorsal striatum**

Since repeated cocaine increased glutamate responses and expression of the ER stress proteins in the dorsal striatum, the group I mGluR antagonist PHCCC was
infused intrastrially to determine whether group I mGluRs regulate BiP, Ire1α and perk induction by repeated cocaine. Like saline or vehicle, PHCCC infusion alone did not alter the basal levels of BiP, Ire1α and perk IR (data not shown). As shown in Fig. 4, repeated cocaine injections significantly increased IR of the ER stress proteins as compared with the controls. In the presence of PHCCC, repeated cocaine induced much less BiP (Fig. 4A), Ire1α (Fig. 4B) and perk (Fig. 4C) IR. Semi-quantitation confirmed that repeated cocaine increased IR of all the ER stress proteins in the dorsal striatum, which was blocked by pretreatment with PHCCC.

A separate study was carried out to evaluate the importance of mGluR5 in the regulation of the ER stress protein expression by repeated cocaine. Like saline or vehicle, MPEP alone at the two doses (2 and 25 nmol, intrastriatal injection (i.s.)) had no significant effects on the basal levels of BiP, Ire1α and perk IR in the dorsal striatum (data not shown). Pretreatment of MPEP at a lower dose (2 nmol) attenuated BiP, Ire1α and perk IR by repeated cocaine in the dorsal striatum. Similarly, MPEP at a higher dose (25 nmol) also significantly attenuated the IR of all three ER stress proteins induced by repeated cocaine (Fig. 4).

**Fig. 5.** The effect of the D1 dopamine antagonist SCH23390 (0.1 mg/kg) or the NMDA antagonist MK801 (2 nmol) on acute or repeated cocaine-induced BiP (A), Ire1α (B) and perk (C) IR in striatal neurons. Semi-quantitative analysis confirms that pretreatment of either SCH23390 or MK801 significantly decreased acute or repeated cocaine induction of the IR of ER stress proteins in striatal neurons (n=3–4 per group). L1, saline; L2, cocaine; L3, SCH23390+cocaine; L4, MK801+cocaine. *P<0.05 as compared with saline groups; **P<0.05 as compared with acute or repeated cocaine groups at 0.5 (A, BiP; E, Actin), 1 (C, perk) and 4 (B, Ire1α) h groups.
SCH23390 or MK801 decreased acute or repeated cocaine-stimulated BiP, Ire1α and perk IR in the dorsal striatum

Since cocaine increases dopamine and glutamate levels and the ER stress protein expression in the dorsal striatum, the D1 antagonist SCH23390 (0.1 mg/kg, i.p.) or the NMDA antagonist MK801 (2 nmol, i.s.) was infused to determine the involvement of dopamine or NMDA receptors in the regulation of BiP, Ire1α and perk induction by either acute or repeated cocaine. The results demonstrated that SCH23390 or MK801 significantly decreased acute or repeated cocaine induction of the IR of the ER stress proteins in the dorsal striatum (Fig. 5), whereas SCH23390 or MK801 had no significant effects on the basal levels of BiP, Ire1α and perk IR in the dorsal striatum (data not shown).

DISCUSSION

The present data demonstrated that cocaine has the capability to induce expression of the ER stress proteins and glutamate and dopamine receptors participate in the mediation of the expression of those proteins induced by acute or repeated cocaine in the dorsal striatum.

Repeated cocaine-regulated glutamate release in the dorsal striatum

In this study, the glutamate response was significantly increased in the dorsal striatum by i.p. injection of repeated, but not by acute cocaine. The altered glutamate concentration by repeated cocaine was determined to be threefold greater than that of acute cocaine, suggesting an increase in extracellular glutamate release in the dorsal striatum by repeated cocaine injections. Our data are consistent with previous studies from our laboratory and other brain regions such as the nucleus accumbens and the ventral tegmental area in which acute cocaine did not alter glutamate release, while repeated cocaine produced marked glutamate release (Rahman et al., 2005; Hotsenpiller and Wolf, 2003; McFarland et al., 2003). Cocaine at 30 mg/kg increased glutamate levels over saline controls in the nucleus accumbens (Smith et al., 1995), suggesting that glutamate release is most likely due to the high dose of cocaine. However, we could not exclude the possibility that extracellular glutamate levels can be altered by acute cocaine because our data were determined only at 30 min time point.

Although the results from this study and others indicate a strong stimulation of glutamate release in the striatum in response to cocaine stimulation, precise mechanism(s) underlying this event are poorly understood. It has been suggested that changes in the physiological activity of several intracellular signaling proteins such as activator of G protein signaling 3 (AGS3) and cystine-glutamate exchanger may account for the facilitation of striatal glutamate release after cocaine administration (Kalivias et al., 2003). In addition, amphetamine, another indirect dopamine receptor agonist, increased glutamate release in the striatum (Nash and Yamamoto, 1993; Del Arco et al., 1999) and this effect was thought to be mediated through trans-synaptic circuits in the basal ganglia (Rawls and McGinty, 2000). Nevertheless, data from this study showed a stimulatory effect of cocaine on glutamate release. These data were obtained through a glutamate microbiosensor and are consistent with the results from previous reports using different methods. Thus, the glutamate microbiosensor could be an effective tool for monitoring the real-time changes in extracellular glutamate levels in response to stimulant drug exposure.

Regulation of the ER stress proteins by cocaine in the dorsal striatum

The present data showed that both acute and repeated cocaine significantly increased induction of the ER stress proteins such as BiP, Ire1α and perk at several different time points, suggesting that cocaine upregulates ER stress protein expression in the dorsal striatum. The ER is an important organelle that participates in cellular homeostasis by regulating calcium signaling cascades and protein folding (Ernack and Davies, 2002). The disruption of intracellular homeostasis or oxidative stress can cause ER stress in primary neuronal cell cultures and other cell lines (McCullough et al., 2001; Paschen and Frandsen, 2001). Recent studies demonstrated that the ER stress pathway plays an important role in neuronal apoptosis (Sanders and Wride, 1995; Hale et al., 1996; Vaux and Strasser, 1996). However, there has been almost no report to illuminate the effect of cocaine uptake on the expression of the ER stress proteins in the brain. Nassogne and coworkers (1997) reported that methamphetamine increased the expression of BiP that is considered to be associated with UPR and participates in the ER-induced apoptosis (Paschen and Frandsen, 2001). The increase in BiP levels might serve a protective function because BiP overexpression protects cells against apoptotic insults (Morris et al., 1997). Ire1α is also an ER-associated protein and is believed to initiate UPR that is responsible for protein folding in the ER under stressed conditions (Miyoshi et al., 2000). Under the conditions of the ER stress UPR is downregulated and thus apoptotic process in neurons is enhanced (Katawama et al., 1999).

Our results also showed a significant increase in BiP and Ire1α IR in the dorsal striatum, suggesting the ER stress can be induced by cocaine administration. A UPR can be also identified by activation of the ER-resident transmembrane protein kinase called perk, which leads to the hyper-phosphorylation of elf2 alpha, upregulation of GPR78, increased amounts of GADD153/CHOP, and cleavage of procaspase-12 in immortalized astrocytes, C1 cells, and primary cultured astrocytes (Liu et al., 2004). It can be conceivable that cocaine-induced ER stress increases perk expression, which further induces neuronal apoptosis in the striatum. These findings suggest that cocaine is closely related to the induction of the ER stress proteins and contributes to neuronal cell apoptosis in the striatum. Furthermore, our data also indicate that pretreatment of the group I mGluR antagonist PHCCC, the
mGluR5 subtype antagonist MPEP or the NMDA antagonist MK801 significantly attenuated induction of the ER stress proteins by repeated cocaine. It is likely that increased levels of glutamate by repeated cocaine activate striatal neurons expressing glutamate receptors including group I mGluRs and NMDA. Thus, activation of glutamate receptors in striatal neurons by repeated cocaine plays a critical role in the upregulation of the ER stress gene expression. Parallel with these data, infusion of the selective D1 antagonist SCH2330 significantly decreased acute or repeated cocaine induction of the IR of the ER stress proteins in the dorsal striatum, suggesting that activation of dopamine receptors in striatal neurons by cocaine also plays an important role in the upregulation of the ER stress protein expression probably by interacting with NMDA (Choe and McGinty, 2000). Therefore interaction of glutamate and dopamine receptors alters NMDA-mediated intracellular calcium homeostasis leading to the ER stress protein expression in the striatum, although mechanisms involving the precise intracellular routes to express the ER stress proteins from the receptors remain to be investigated.

CONCLUSION

In conclusion, this study investigated the involvement of glutamate and dopamine receptors in the regulation of ER stress proteins in response to cocaine administration in the rat dorsal striatum. The results demonstrated that repeated cocaine, but not acute, augmented extracellular glutamate release in the dorsal striatum. However, both acute and repeated cocaine significantly increased expression of the ER stress proteins via activation of dopamine and glutamate receptors. These data suggest that stimulation of glutamate and dopamine receptors by cocaine possesses capability to express the ER stress proteins in the dorsal striatum.

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