SELECTIVE INHIBITION OF CYCLOOXYGENASE-2 EXACERBATES METHAMPHETAMINE-INDUCED DOPAMINE DEPLETION IN THE STRIATUM IN RATS

X. ZHANG, F. DONG, G. E. MAYER, D. C. BRUCH, J. REN AND B. CULVER*
Division of Pharmaceutical Sciences, Graduate Neuroscience Program and Center for Cardiovascular Research and Alternative Medicine, University of Wyoming College of Health Sciences, Laramie, WY 82071-3375, USA

Abstract—Neuroinflammatory processes associated with induction of cyclooxygenase (COX) have been implicated in the deleterious events resulting in neurodegeneration. The present study was designed to investigate the impact of acute methamphetamine (MA) administration on COX expression and prostaglandin E2 (PGE2) production, and to evaluate the effect of selective COX-2 inhibition using celecoxib in MA-induced degeneration of dopaminergic terminal and cell apoptosis in the striatum. Male Sprague–Dawley rats were treated with either a neurotoxic regimen of methamphetamine hydrochloride (5 mg/kg, i.p., every 2 h for four injections) with or without celecoxib (7.5 mg/kg) or vehicle. COX-1 expression was not affected by MA, while both COX-2 protein expression and number of COX-2 positive cells in striatum were significantly reduced 24 h after MA treatment. However, after 72 h, a significant upregulation of COX-2 protein was detected. PGE2 production was correlated with altered COX-2 levels. NFκB (NFκxB), a key regulator of COX-2 expression, was activated 72 h after MA administration, and was accompanied by increased Ikappa B (IκB) phosphorylation. Animals receiving MA exhibited an increase in apoptotic cells and notable reductions in dopamine (DA) content (63.9%) in immunoreactivity of tyrosine hydroxylase (TH) and neuron specific microtubule-associated protein 2 (MAP2) in striatum. Administration of celecoxib exacerbated MA-induced DA depletion, and did not affect MA-induced MAP2 damage, apoptosis or proliferation of glial cells. Our findings suggest that COX-2 containing cells are targets of the damage during earlier stages of MA-related neurotoxicity, and that the selective inhibition of COX-2 enzyme is harmful rather than protective. The COX-2 induction appears during the recovery period, and NFκB activation may be an important mechanism. © 2007 IBRO. Published by Elsevier Ltd. All rights reserved.

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Methamphetamine (MA; N-methyl-o-phenylisopropylamine) is a powerful stimulant drug of abuse with potent addictive and neurotoxic properties. Repeated administration of MA causes persistent degeneration of the dopamine (DA) terminals as well as cell apoptosis in the striatum (Davidson et al., 2001; Cadet et al., 2003; Kita et al., 2003). During the past decade, a number of hypotheses have been proposed to elucidate the mechanism underlying MA-induced neurotoxicity. The leading theories include oxidative stress, N-methyl-D-aspartic acid (NMDA) receptor-mediated excitotoxicity, and intraneuronal DA redistribution from synaptic vesicles to cytoplasmic compartments produced by MA (Gluck et al., 2001; Cadet et al., 2003; Kita et al., 2003; Lau et al., 2000; Riddle et al., 2006). However, much more remains to be learned. Emerging evidence reveals microglial activation in response to MA intoxication, and suggests that neuroinflammation may play a role in MA-induced neurotoxicity (Thomas et al., 2004; LaVoie et al., 2004).

Cyclooxygenase (COX) is one of the major inflammatory mediators. It functions as the rate limiting enzyme in prostaglandin biosynthesis and immunohistochemistry studies have shown that COX is present in neurons as well as glial cells in the CNS (Consilvio et al., 2004). Two distinct isoforms of COX have been identified. COX-1 is constitutively expressed throughout the brain (Dubois et al., 1998; Katori and Majima, 2000). COX-2, initially characterized as an inducible enzyme that is expressed in response to pathological stimuli, cytokines and mitogens, is now known to be also present in the normal CNS (Hoffmann, 2000; Warner and Mitchell, 2004). Constitutive COX-2 has been associated with neurodevelopment and fundamental brain functions such as synaptic activity and memory consolidation (Katori and Majima, 2000; O’Banion, 1999; Warner and Mitchell, 2004). The expression of COX-2 is regulated directly by the nuclear transcription factor NFκB (NFκxB). An NFκxB binding site has been located in the COX-2 promoter region (Crofford et al., 2000). Induction of COX-2 has been linked with neurodegeneration, and there is an increased interest in using nonsteroidal anti-inflammatory drugs (NSAIDs) which inhibit COX as potent therapeutic agents to slow the progression of neurodegeneration (Etminan et al., 2003; Gasparini et al., 2004; Hoffmann, 2000; Mhatre et al., 2004; Sanchez-Pernaute et al., 2004). However, owing to the complex functions COX and PGs exert in both pathologic and physiologic conditions in the CNS, the role of COX and NSAIDs in neurodegenerative diseases is still highly controversial despite the intense research in the last decade. A few papers reported the possible implication for COX in DA ter-
minal degeneration caused by MA intoxication (Asanuma et al., 2003; Kita et al., 2000; Thomas and Kuhn, 2005). However, the results from different groups are greatly inconsistent, and the existing studies exclusively used mice as an experimental model. Since distinct species difference in MA metabolism has been reported, and rats’ MA metabolism better resembles that of humans, it is necessary to look into the aspect of COX-related inflammation in MA intoxication in the rat model (Caldwell et al., 1972; Yanagisawa et al., 1997). The current study will examine the influence of repeated MA administration on COX and prostanoid expression as well as NFκB pathway activation in the rat brain, and evaluate the effects of the selective COX-2 inhibition by celecoxib on MA-induced DA terminal degeneration and cell apoptosis in the striatum.

**EXPERIMENTAL PROCEDURES**

**Animals**

Experiments were conducted in adult male Sprague–Dawley rats (obtained from Charles River Laboratories International Inc., Wilmington, MA, USA) in accordance to the NIH Guide for the Care and Use of Laboratory Animals and were approved by the Animal Care Committee at University of Wyoming. All efforts were made to minimize animal suffering and reduce the number of animals used. The animals weighed between 300 and 400 g and were housed two rats per cage on a 12-h light/dark cycle with food and water available ad libitum. The ambient temperature within the treatment room was 22 °C and was monitored throughout all experiments.

**Acute toxic dose of MA administration and selective COX-2 inhibitor celecoxib treatment**

The rats were randomly divided into four groups with 10–15 animals in each group. Rats in the MA group were injected i.p. with MA hydrochloride (Sigma, St. Louis, MO, USA) at a dose of 5 mg/kg in four injections with a 2-h interval between each injection. Controls received four injections of saline on the same schedule as MA-treatment. Rats in the COX-2 inhibitor-treatment group received one 7.5 mg/kg i.p. dosage of celecoxib (TRC Biomedical Research Chemicals, Canada) 10 min after the first dose of MA. The celecoxib-only group was intended to control for possible effects of the COX-2 inhibitor. Rats in this group received saline and celecoxib (7.5 mg/kg i.p.; 10 min after the first injection of saline). Animals were killed by decapitation under deep anesthesia (sodium pentobarbital, 50 mg/kg, i.p.) at 24 h or 72 h following the treatment. Brains were quickly removed, and the striatum were carefully dissected and sampled according to different assay protocols.

**Western blot analysis of COX-1, COX-2, Ikappa B (IκB), phospho-IκB, tyrosine hydroxylase (TH), microtubule-associated protein 2 (MAP2) and glial fibrillary acidic protein (GFAP)**

Tissues samples from striatum were homogenized in a lysis buffer containing 20 mM Tris (pH 7.4), 150 mM NaCl, 1 mM EDTA, 1 mM EGTA, 1% Triton, 0.1% SDS and protease inhibitor cocktail. Samples were then sonicated for 15 s and centrifuged at 12,000×g for 20 min at 4 °C. The protein concentration was evaluated using Protein Assay Reagent (Bio-Rad, Hercules, CA, USA). Equal amounts (50 μg protein/lane) of protein and prestained molecular weight markers (Gibco-BRL, Gaithersburg, MD, USA) were separated on 10% SDS–polyacrylamide gels in a minigel apparatus (Mini-PROTEAN II, Bio-Rad) and transferred electrophoretically to polyvinylidene difluoride membranes. The membranes were incubated for 2 h in a blocking solution containing 5% skimmed milk in TBS, and were then washed briefly in TBS and incubated overnight at 4 °C with the appropriate dilution of antibody: anti-COX-1 (1:1000), anti-COX-2 (1:500), anti-IκB (1:1000), anti-phospho-IκB (1:1000), anti-TH (1:2000), anti-MAP2 (1:2000) and anti-GFAP (1:2000). COX-2 polyclonal antibody was purchased from Santa Cruz Biotechnology, Inc., Santa Cruz, CA, USA. COX-1 monoclonal antibody was obtained from Cayman Chemical, Ann Arbor, MI, USA. Anti-IκB and phospho-IκB polyclonal antibodies were from Cell Signaling Technology, Beverly, MA, USA. Anti-TH, anti-GFAP and anti-MAP2 monoclonal antibody were from Sigma. After washing to remove excess primary antibody binding, blots were incubated for 1 h with the appropriate horseradish peroxidase–conjugated secondary antibody (1:5000). Antibody binding was detected using enhanced chemiluminescence (Pierce, Rockford, IL, USA), and the film was scanned and the intensity of immunoblot bands was detected with a Bio-Rad Calibrated Densitometer (Model: GS-800) (Zhang et al., 2005).

**PGE2 enzyme immunoassay**

After homogenization on ice, the striatal tissue homogenates were centrifuged (30 min, 20,000 r.p.m. at 4 °C) and the supernatant was used for PGE2 measurement. Concentrations of PGE2 in striatal tissues were determined using a commercially available enzyme immunoassay ELISA kit (Assay Designs, Inc., Ann Arbor, MI, USA) according to the manufacturer’s instructions.

**DA measurement**

DA was measured using HPLC (Beckman, System Gold, Fullerton, CA, USA). Striatal samples were homogenized in 0.2 N perchloric acid. After centrifugation (20,000 r.p.m. for 20 min), the supernatant was filtered and injected onto a Adsorbosphere cat-echolamine 3 μm column (100 mm×4.6 mm; Alltech, Dearfield, IL, USA) connected to a Coulochem II electrochemical detector (Esa, Chelmsfevol, MA, USA). The mobile phase used for the analysis of DA consisted of 0.1 mM monochloroacetic acid, 2 mM disodium EDTA, and 25 mg/l octanesulfonic acid sodium salt, adjusted to pH 3.0. Flow rate was 1.5 ml/min.

**Immunohistochemical detection of COX-2, NFκB, TH, MAP2 and GFAP**

Immunofluorescence was performed on formalin-fixed paraffin embedded sections (8 μm). Coronal sections in the caudate-putamen of the striatum were taken from bregma 0.2±0.1 mm. Sections were first dewaxed and then subjected to antigen retrieval procedures. Sections were treated for 10 min in 3% hydrogen peroxide (Sigma) and blocked in 5% normal goat serum for 2 h prior to overnight incubation with the primary antibody. The primary antibodies utilized were anti-COX-2 (1:50), anti-NFκB p65 (1:50; Cell Signaling Technology), anti-TH (1:300), anti-MAP2 (1:300) or anti-GFAP (1:400). Sections were then washed with PBS followed by incubation with bio-conjugated secondary (Vector Laboratories, Burlingame, CA, USA), Alexa Fluor 488-conjugated or 594-conjugated secondary antibody (Molecular Probes, Eugene, OR, USA). Fluorescent images were captured at 400× magnification using an Olympus BX51 fluorescent microscope equipped with a digital camera.

**Apoptosis determine using terminal deoxynucleotidyl transferase-mediated biotinylated UTP nick end labeling (TUNEL) assay**

TUNEL was performed using an in situ cell death detection kit (Roche Applied Science, IN, USA) following the manufacturer’s instructions. In brief, after proteinase K digestion, sections were
incubated in the labeling solution containing terminal deoxynucleotidyltransferase, biotinylated-16-dUTP (20 \mu M; Boehringer Mannheim), and 1.5 \mu M cobalt chloride. The slides were observed using an Olympus BX51 fluorescent microscope equipped with digital camera, and captured fluorescent images were quantified using Microsuite software (Soft Imaging System, Lakewood, CO, USA).

Quantitation of immunohistochemical data

TUNEL positive cells, COX-2 positive cells, and NF\(\kappa B\) positive cells in each field were counted using the "touch count" function of the Microsuite software. Counterstaining was applied using nuclear dye 4',6-diamidino-2-phenylindole dihydrochloride (Dapi) (Invitrogen, Carlsbad, CA, USA), and the number of total striatal cells per field was obtained by counting the Dapi-stained nuclei. Three different sections were quantified in each animal. For each section, 10 fields within the caudate-putamen were analyzed bilaterally. Six rats were sampled per group.

Statistical analysis

All data are expressed as mean±S.E. Two group comparisons were evaluated by \(t\)-tests, and statistical differences among more than three groups were determined using a one-way ANOVA. A \(P\) value less than 0.05 was considered statistically significant.

RESULTS

Expression of COX-1 and COX-2 in brain following MA administration

The COX-1 protein was shown to be constitutively expressed in the striatum, and MA administration did not affect its expression (Fig. 1). A stable expression of COX-2 protein was also found in the control striatum. Surprisingly, the neurotoxic regimen of MA significantly decreased COX-2 expression 24 h after MA treatment in rat striatum. However, a significant upregulation of COX-2 protein (18.1%) was induced at 72 h after MA treatment (Fig. 1).

To verify these results and further elucidate the cellular origin of COX-2 expression, immunohistochemistry was performed. COX-2 positive cells were seen in both striatum and cerebral cortex. Double immunofluorescence further revealed that COX-2 was expressed in both MAP2 positive cells (neurons) and GFAP positive cells (glial cells) (Fig. 2A–D). Interestingly, the COX-2 positive neurons were found only in the cerebral cortex but not in the striatum,

Fig. 1. Influence of MA on COX-1 and COX-2 expression in rat striatum. Rats were treated with saline or a neurotoxic regimen of MA. Striatal COX-1 and COX-2 protein expression was measured by Western blot at 24 or 72 h after treatment. Protein expression of COX-1 was not affected by MA, while COX-2 levels were decreased at the 24-h time point (\(P<0.05\)) but significantly increased at the 72-h time point (\(P<0.05\)). The upper panel shows representative blots, and the bottom panel is a summary of the results. Data are mean±S.E. for four to six rats per group.

Fig. 2. Illustration of COX-2 immunolocalization in cortex and striatum following repeated MA administration. Photomicrographs in the upper panel (A–L) are representative fluorescence images and the bar graph (M) in the bottom panel shows summarized data. Data are mean±S.E. for six rats per group (** \(P<0.01\)). COX-2-positive cells (red) are seen in both striatum and cortex in control rats (A–D). These composite images show that COX-2 immunofluorescence co-localized with both MAP2 (green; A, E, I) and GFAP (green; C, G, K) in cortex but only co-localized with GFAP staining in striatum (B, F, J, D, H, L). The double positive cells are marked with arrows. There are a number of COX-2 positive cells that did not co-localize with either of the two markers. MA administration significantly reduced the number of COX-2 positive cells in both cortex and striatum at 24 h after the treatment (E–H, M). However, the total number of COX-2 positive cells recovered to normal levels 72 h after MA administration (I–L, M). Scale bar=20 \mu m. For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.

COX-2 expression 24 h after MA treatment in rat striatum. However, a significant upregulation of COX-2 protein (18.1%) was induced at 72 h after MA treatment (Fig. 1). To verify these results and further elucidate the cellular origin of COX-2 expression, immunohistochemistry was performed. COX-2 positive cells were seen in both striatum and cerebral cortex. Double immunofluorescence further revealed that COX-2 was expressed in both MAP2 positive cells (neurons) and GFAP positive cells (glial cells) (Fig. 2A–D). Interestingly, the COX-2 positive neurons were found only in the cerebral cortex but not in the striatum,
while non-neuronal COX-2 positive cells are located in both striatum and cerebral cortex. MA administration significantly reduced the number of COX-2 positive cells in both cortex and striatum at 24 h after the treatment (Fig. 2E–H, M). However, the total number of COX-2 positive cells recovered to the normal level 72 h after MA administration (I–L, M).

**MA stimulated NFκB signaling pathway activation**

To explore the regulatory mechanisms underlying COX-2 alteration following MA administration, NFκB, a key regulator of COX-2 expression was examined. As shown in Fig. 3, the immunofluorescence revealed a slight increase in numbers of NFκB p65 subunit positive nuclei in MA-treated rats at 24 h following injections, and a statistically significant increase was found at 72 h after the treatment. These results suggest an enhancement of NFκB nuclear translocation following MA administration. Phosphorylation and degradation of IκB are essential for NFκB translocation (Li et al., 1998). To find out if MA enhanced NFκB translocation via the IκB pathway, we examined the total and phosphorylated IκB. Results shown in Fig. 4 revealed that MA significantly elevated phospho-IκB levels although it had no effect on the expression of total IκB. These results suggest a role of IκB phosphorylation in MA-induced NFκB translocation.

**Effect of MA on the striatal PGE$_2$ levels and impact of a selective COX-2 inhibitor, celecoxib**

Measurements of PGE$_2$ revealed that the striatal levels were significantly decreased in rats 24 h after the MA treatment (Fig. 5). Administration of celecoxib along with MA diminished the PGE$_2$ levels even more than in rats administered MA alone ($P<0.05$). PGE$_2$ levels rose to the level of the control group at 72 h after the MA administration. As expected, celecoxib produced a significant inhibition of COX-2 activity in brain as evidenced by notably reduced PGE$_2$ levels in the celecoxib group.

**Celecoxib exacerbates MA-induced DA depletion**

Striatal DA concentration was markedly decreased (63.9%) at 24 h after MA administration and were killed 24 h or 72 h later as described under Experimental Procedures. Representative blots showing immunostaining with anti-IκB or anti-phospho-IκB antibodies in rat brain striatum. Means±S.E.M., $n=4$. A significant increase of phosphorylated IκB was found when compared with the control group ($P<0.05$).
levels were not affected by administration of celecoxib alone (Fig. 6).

Selective inhibition of COX-2 using celecoxib did not alleviate MA-induced neurotoxicity in the striatum of rats

Significantly reduced TH expression was found in the striatum in the MA-treated rats (Fig. 7), suggesting a notable damage by MA to dopaminergic axon terminals. TH expression was similar in rats given both celecoxib and MA and those given only MA, showing the COX-2 inhibitor did not decrease neurodegeneration. MAP2 is an important cytoskeletal protein in neurons, and is detected mainly in dendrites. Disturbed MAP2 expression has been associated with impairments of dendrites and synaptic signal transduction (Iwata et al., 2005). Thus, its immunoreactivity was used to signal damage to local neurons in the striatum. As shown in Fig. 8, the control and celecoxib only group showed similar levels of MAP2 protein, whereas a marked lesion effect on MAP2 expression was found in MA-treated animals. There was no difference in MAP2 levels between the MA+celecoxib group and MA only group. No significant difference in GFAP protein expression was observed among these groups (Fig. 9). MA caused a significant increase in TUNEL-positive cells in the striatum, and this increase was not significantly affected by co-administration of celecoxib (Fig. 10).

DISCUSSION

To the best of our knowledge, this is the first study to examine the effects of MA administration on the COX enzymes and PGE2 in rats. We also demonstrated for the first time that MA stimulated upregulation of phospho-1kB, suggesting a role of IxkB phosphorylation in MA-induced NFkB activation. Moreover, for the first time, the role of a COX-2 selective inhibitor celecoxib in MA-induced cell apoptosis in the striatum was determined. The current study revealed significantly diminished PGE2 production, decreased COX-2 protein expression and reduced COX-2 positive cell numbers in the striatum 24 h following acute repeated administration of MA. Although the number of COX-2 positive cells was recovered and COX-2 expression was even upregulated at 72 h after MA administration, our findings suggest that the COX-PG system per se is a target during the early stages of MA-induced acute toxicity. Increased nuclear translocation of NFkB was observed at 72 h following MA treatment, and these results indicate that NFkB activation may be a mechanism underlying the MA-induced COX-2 upregulation at this time point. Repeated administration of MA to rats induced DA depletion and led to dopaminergic terminal destruction, neuron damage, and cell apoptosis in striatum. Early COX-2-specific NSAID intervention using celecoxib failed to abate these neurotoxic effects induced by MA intoxication. In addition, celecoxib markedly exacerbated MA-induced DA depletion, which raises the possibility that COX-2 specific NSAIDs taken with MA could be more harmful during the
early stage of acute toxicity even though they may afford some protection in the later stages of MA toxicity. Studies on the association of COX with MA abuse are scarce at this time and the existing studies are restricted to the mouse model with conflicting results. Thomas and Kuhn (2005) reported that COX-2 induction can be induced as early as 3 h after acute toxic MA administration in C57 mice, but this induction was not accompanied by changes of PGE2 levels. Kita et al. (2000) observed the effects of MA administration on COX-2 expression in BALB/cAnNcrj mice at 2, 6, 24, and 72 h after acute toxic MA treatment. However, their results revealed that COX-2 protein expression was not significantly affected by MA until 72 h following MA administration. Our results demonstrate a significant reduction in striatal COX-2 expression and PGE2 production 24 h following MA administration and a later upregulation of COX-2 at 72 h after MA administration in rats. Additionally, immunohistochemistry showed that COX-2 positive cells were located in the striatum, and these cells are non-neural cells. Both astrocytes and microglia are major sources of brain prostaglandins, and they have been demonstrated to release larger amounts of prostaglandins than neurons (Tzeng et al., 2005). Thus our data may suggest an important role of glial cells in regulating COX/PGE2 signaling in the striatum. Prostaglandins produced by glial cells diffuse immediately after synthesis and affect the adjacent cells including neurons by activating specific membrane receptors. Prostaglandin receptors have been found in both neurons and glial cells (Tzeng et al., 2005). Moreover, COX-2 also has peroxidase activity, which can lead to the formation of reactive oxygen species (ROS) (Tzeng et al., 2005). Since COX-2 is located in the endoplasmic reticulum and nuclear membrane (inside cells), this enzyme is capable of contributing to the increased intracellular ROS associated with MA administration and account for the subsequent oxidative damage (Funk, 2001). Therefore, COX-2 positive cells may be vulnerable to MA-induced oxidative stress. The number of COX-2 positive cells was decreased by MA at the 24-h time point, but recovered to the normal level at the 72-h time point following MA treatment. These data reinforce our finding from the Western blot analysis, and suggest that MA-induced cell death might contribute to the decline of COX-2 level at the 24-h time point. We believe this is the first observation of COX-2 containing cells in the striatum.

Fig. 8. Effect of MA with or without celecoxib on MAP2 immunoreactivity in the striatum. Rats were treated with a neurotoxic regimen of MA and killed 24 h later. Brain sections were stained for MAP2 (A). Quantitation of MAP2 protein using Western blot is reported in B (representative blots) and C (a summary of the results). A significant decrease of MAP2 expression was observed in the MA group. The reduced MAP2 expression by MA did not change when celecoxib was co-administered with MA. No differences in MAP2 levels were noted between the control and celecoxib only groups. Data are means±S.E. of six rats per group. ** P<0.01 compared with controls. Scale bar=50 μm.

Fig. 9. Effect of MA and celecoxib on GFAP expression. Rats were treated with a neurotoxic regimen of MA and killed 24 h later. Astrocytes and other glial cells were stained using anti-GFAP antibody (A, green). Western blot analysis of GFAP protein in the striatum is shown in B (representative blots) and C (a summary of the results). No differences in GFAP expression were noted among the four groups. Data are means±S.E. of six rats per group. Scale bar=50 μm. For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.
min binding in different animals indicates the effect of MA may vary among species. The nuclear transcription factor NFκB is regarded as a central regulator of inflammatory and immune reactions. An NFκB binding site is located in the promoter region of the COX-2 gene, where it can function as a direct regulator in COX-2 expression (Consilvio et al., 2004). NFκB is composed of a p50/p65 heterodimer which is sequestered in the cytoplasm by its inhibitory proteins, the IκB, under unstimulated conditions. Phosphorylation of IκB can release NFκB to enter the nucleus through a ubiquitin–proteasome pathway, and initiate targeted transcription (Traenckner et al., 1995). In accordance with earlier reports (Asanuma and Cadet, 1998; Lee et al., 2001), our results also showed that MA injections can activate striatal NFκB translocation which might be involved in MA-induced COX-2 induction in the later stage of neurotoxicity. Furthermore, phosphorylated IκB was significantly upregulated 72 h following MA administration which is consistent with the time course of NFκB translocation. This finding indicates that phosphorylation of IκB might mediate MA-induced NFκB activation.

The role of COX-targeted inhibition in MA-induced neurotoxicity is also under hot debate. So far, all the published studies have been performed in mouse models, and the results are highly controversial. In the MA acute toxic dosing model, a number of NSAIDs have been evaluated including COX-1 selective inhibitors (sc-560, low dose aspirin), nonselective inhibitors of the COX-1/2 isoforms (indomethacin, ketoprofen and high dose aspirin), and COX-2 selective inhibitors (NS-398 and rofecoxib) (Asanuma et al., 2003, 2004; Thomas and Kuhn, 2005). The current study is the first one to assess the effect of celecoxib on MA caused neurotoxicity. Co-administration of MA (4 mg/kg, i.p.) indomethacin (5 mg/kg×4) was reported to decrease the MA-produced dopamine transporter (DAT) reduction while, in the same study, high dose aspirin (100 mg/kg×4) showed no effects on the repeated MA injection-induced reduction of striatal DAT positive signal (Asanuma et al., 2004). Another earlier study completed by these researchers showed that pre-treatment with ketoprofen, but not either low or high doses of aspirin, attenuated acute MA-induced reduction of DAT-positive signals and accumulation of microglial cells in the striatum (Asanuma et al., 2003). However, based on the DA level in the striatum 48 h after MA-treatment, Thomas and Kuhn (2005) concluded that none of the inhibitors they tested (sc-560, NS-398, rofecoxib and ketoprofen) protected mice from MA-induced neurotoxicity. In accordance with the later report, our results suggest that early COX-2-specific inhibition using celecoxib does not halt MA-induced striatal neurotoxicity. In the current study, MA caused a significant reduction of DA content, degeneration in dopaminergic axon terminals, serious damage to dendrites of local neurons, and increased cell apoptosis in the striatum. Celecoxib coadministration did not abate MA-induced damages but instead markedly exacerbated MA-induced DA depletion. Thus, our findings suggest that COX-2 specific NSAIDs taken with MA may be more harmful during the
early stage of acute toxicity. The effects of COX inhibition largely depend on the products of this enzyme, the PGs. PGs are a family of molecules involved in a wide array of physiological and pathological functions. The actions of PGs in CNS are varied and remain unclear. PGD\textsubscript{2} and PGE\textsubscript{2}, which are the major PGs in the brain of mammals, have been shown to powerfully induce secretion of nerve growth factor and brain-derived neurotropic factor (Toyomoto et al., 2004). In addition, protective effects of both PGD\textsubscript{2} and PGE\textsubscript{2} in CNS have been reported (Masuda et al., 1986; McCullough et al., 2004; Taniguchi et al., 2007). These results indicate that a reduction of PGs might be harmful, thus supporting our finding.

CONCLUSION

In conclusion, our findings suggest that rather than a one-way induction, the COX-related neuroinflammatory response may vary during MA-intoxication, depending on the stage of pathological progression. During the early stage of MA-intoxication, COX-2 containing cells were targets of the toxic effect and COX-2 protein levels were decreased. Thus COX-2 was unlikely to contribute to cell apoptosis and degeneration of the dopaminergic system in the rat striatum. Additionally, further inhibition of this enzyme by giving the selective COX-2 inhibitor celecoxib enhanced MA-induced PGE\textsubscript{2} reduction and aggravated DA depletion. This raises the possibility that co-intake of COX-2 selective NSAIDs and MA might be harmful in the early stage of MA toxicity.

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