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Methamphetamine and MDMA (3,4-methylenedioxymethamphetamine) Induce Apoptosis in Both Human Serotonergic and Dopaminergic Cell Lines

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Abstract – Methamphetamine (METH) and 3,4-methylenedioxymethamphetamine (MDMA) have become popular recreational drugs of abuse in many countries. Although the neurotoxic damage caused by METH and MDMA is characterized by degeneration of the dopaminergic and serotonergic systems in brain, the molecular and cellular mechanisms remain to be clarified. Therefore, the purposes of this study were to confirm the capability of METH and MDMA to induce apoptosis and to clarify the action of its molecular mechanism by using serotonergic JAR cells and dopaminergic SK-N-SH cells. METH and MDMA were dose-dependently cytotoxic to human serotonergic JAR cells and dopaminergic SK-N-SH cells. The morphological change of apoptosis was found in Giemsa staining and TUNEL and further verified in DNA fragmentation analysis. Immunoblotting analysis revealed proteolytic cleavage of caspase-3 and -9 and change of bcl-2 and bax proteins. These results suggest that METH and MDMA may induce caspase-dependent apoptosis via the mitochondrial cell death pathway and METH and MDMA-induced neurotoxicity may happen to broadly and independently of both dopaminergic and serotonergic systems.

Key words □ METH, MDMA, neurotoxicity, apoptosis, dopaminergic and serotonergic cells

Methamphetamine (METH) is a psychostimulant and has become popular recreational drug of abuse in many countries. The neurotoxic damage caused by METH is characterized by degeneration of the dopaminergic and serotonergic systems in striatum and hippocampus (Kleven and Seiden, 1992; Baldwin *et al.*, 1993). Many studies have suggested the mechanism of METH neurotoxicity, such as dopamine release and subsequent enzymatic oxidation, dopamine auto-oxidation and mitochondrial disruption (Fleckenstein *et al.*, 2000; Davidson *et al.*, 2001). METH increases extracellular 5-HT levels in the brain, predominantly by releasing 5-HT from presynaptic terminals and by blocking uptake of 5-HT (Berger *et al.*, 1992; Kuczenski *et al.*, 1995). Although METH induces neurotoxicity by direct production of free radicals or triggering a mitochondria-dependent apoptosis *in vitro* and *in vivo* (Hirata et al., 1996; Lemaster et al., 1999), it is still needed to investigate the type and process of cell death in both dopaminergic and serotonergic cells.

3,4-Methylenedioxymethamphetamine (MDMA) is one of derivatives of amphetamine and used as a recreational drug or party drug. The neurotoxicity of MDMA is increasingly reported although it has been considered relatively safe (Leshner, 2001; Johnston *et al.*, 2000). MDMA degenerates dopaminergic and serotonergic system in brain (O'Shea *et al.*, 1998; Stone *et al.*, 1988; Ricaurte *et al.*, 1985). The mechanism of MDMA neurotoxicity has been proposed as serotonin release and promotion of oxidative stress, and apoptosis (Sprague *et al.*, 1998; Simantov *et al.*, 1997). However, the molecular and cellular mechanisms remain to be clarified.

Both METH and MDMA are well known abuse drugs and have serious side effects such as disruption of dopamine neurons, anxiety and hostility. We chose these chemicals to study the mechanisms and screening methods of cell death by toxic substances. In the present study, we examined whether the neurotoxicity of METH and MDMA is related to apoptosis and then which types of caspases and proteins are involved in the apoptosis by using in vitro model of immortalized neuronal cells, JAR and SK-N-SH. JAR cells were derived from a trophoblastic tumor of the human placenta and commercially available from ATCC.

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METHODS AND MATERIALS

Materials

METH, MDMA, MTT, 4,6-diamidino-2-phenylindole (DAPI), agarose, ethidium bromide and DMSO were purchased from Sigma Chemical Co.(MO, USA). Cytotoxicity detection kit (LDH) and TUNEL assy kit were obtained from Roche Diagnostics Corp. (Indianapolis, IN, USA). RPMI1640, fetal bovine serum, HEPES, DMEM, penicillin/streptomycin, and trypsin/EDTA were purchased from Gibco BRL (Gaithersburg, MD. USA). Acrylamide, Tris, SDS, ammonium persulfate, and TEMED were obtained from Sigma Chemical Co.(MO, USA). Transfer membrane was obtained from Bio-Rad (Hercules, CA, USA) and skim milk was purchased from Difco (Sparks, MD, USA). Nuclease P1, DNase I and alkaline phosphatase from *E. coli* were obtained from Sigma Chemical Co.(MO, USA).

Cell culture

JAR cell line, human serotonergic cells, was obtained from ATCC (American Type Culture Collection, Cat. #, HTB-144). JAR cells were maintained in RPMI 1640 with 10% FBS plus penicillin/streptomycin, 10 mM HEPES, 1.0 mM sodium pyruvate, 4.5 g/L glucose, and 1.5 g/L sodium bicarbonate. SK-N-SH cell line, human dopaminergic cells, was obtained from KCLB (Korean Cell Line Bank, KCLB30011). SK-N-SH cells were maintained in DMEM with 10% FBS plus penicillin/streptomycin, 10 mM HEPES, 1.0 mM sodium pyruvate, 4.5 g/L glucose, and 1.5 g/L sodium bicarbonate.

Determination of MTT cell viability

The MTT assay was used to assess viability by a procedure modified by Hansen *et al*[22]. This assay measures the ability of dehydrogenases in the intact mitochondria of living cells to convert MTT to its colored formazan product. Briefly, 2×10^4 cells of JAR and SK-N-SH were plated in 96-well plate with 200 μL per well, and various concentrations of METH and MDMA were added to attached cells. Following the addition of 50 μL of MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide) at a concentration of 2.0 mg/ml, the plate was incubated in dark for 2 h at 37°C to allow for color development. The colored reaction product was solubilized in extraction buffer containing dodecylsulfate and N,N-dimethyl formamide, pH 4.7. Absorbance of formazan production was measured at 540 nm using a Emax plate reader (Molecular Devices, Sunnyvale, CA). Viability was calculated by the formula As/Ao × 100, where As and Ao, represent the absorbance of treated and untreated cells, respectively.

Determination of LDH activity

Cytotoxicity was determined by measuring the release of lactate dehydrogenase (LDH) in the culture medium. The LDH activity is determined in an enzymatic test. In the first step NAD^+ is reduced to $NADH/H^+$ by the LDH-catalyzed conversion of lactate to pyruvate. In the second step the catalyst (diaphorase) transfers H/H^+ from $NADH/H^+$ to the tetrazolium salt INT (2-(4-iodophenyl)-3-(4-nitrophenyl)-5-phenyltetrazolium chloride) which is reduced to formazan. An increase in the amount of dead or plasma membrane-damaged cells results in an increase of the LDH enzyme activity in the culture supernatant. Briefly, 2×10^4 cells were plated in 96-well plate with 200 μL per well, and various concentrations of METH and MDMA were added to attached cells. After incubation of 24 h or 48 h, the medium was collected to determine the LDH activity. By using LDH assay kit (Roche Diagnostics Corp., Indianapolis, IN, USA), the amount of formazan formed for 30 min was determined at 490 nm.

Gel analysis of DNA fragmentation

The JAR and SK-N-SH cells of 3×10^6 were plated in 100 mm^2 dish with 10 mL culture medium, and 1.5 mM or 3.0 mM of METH and MDMA was added to attached cells. After 18 h, the cells were incubated for overnight in 0.5 mL of lysis buffer (0.6% SDS, 10 mM EDTA, pH 7.5) and at 4°C and centrifuged 10,000 × g for 20 min at 4°C. The supernatant was incubated with 3 μL of RNase A (10 μg/ml) for 30 min at 37°C and then added with 500 μL of phenol/chroloform/isoamylalchol (Sigma, USA). The mixture was centrifuged 10,000 × g for 20 min at 4°C. After additional purification with phenol/chroloform/isoamylalchol, the supernatant was added with 500 μL of chloroform and then centrifuged 10,000 × g for 20 min. Purified DNA was precipitated with isopropanol (50% (v/v)) at -20°C overnight. Fragmented DNA was collected by centrifugation at 10,000 × g for 20 min at 4°C, and the pellet was suspended in 70% ethanol and centrifuged at 10,000 × g for 20 min at 4°C. Then the pellet was dried under reduced pressure and resuspended in DW(distilled water). After electrophoresis on a 1.2% (w/v) agarose gel, DNA was visualized by ethidium bromide staining.

Giemsa staining of apoptotic nuclei

Briefly, 3×10^6 cells were plated in 100 mm^2 dish with 10

mL culture medium, and harvested at 37°C for 24 h in 5% CO_2 incubator (Nuaire, Plymouth, MN, USA). After removing the medium, 1.5 mM or 3.0 mM of METH and MDMA was added to attached cells and incubated for 18 h. The cell was collected and centrifuged at 500 × g for 5 min at 4°C. The cell was resuspended with cold 75 mM KCl and centrifuged at 500 × g for 5 min at 4°C. The pellet was suspended with 5 mL of fixative, methanol:acetic acid (3:1). Then the cell was remounted to single cell on slide glass by Pasteur pipet. After drying for about 30 min, the cell was dyed with 4% Giemsa solution for 25 min. After washing with tapwater, the apoptotic nuclei was examined by using microscope (×400).

DAPI (4', 6-Diamino-2-phenylindole) staining

1×10^6 cells/4 mL were plated in 6 well plate coated with poly-L-lycine and incubated for 24 h. After removing the medium, the new medium of 4 mL containing 1.5 mM or 3.0 mM of METH and MDMA was added and incubated for 18 h. Then, the cells were fixed by 4% paraformaldehyde (in PBS, pH 7.4) for 20 min. After washing out the cells with PBT (0.02 % tween in PBS) for 3 times, we stained the cells with DAPI (10 μg/mL). Rewashed out by PBT solution, the cells were covered with cover glass and the apoptotic body was examined by using fluorescent microscope (×100).

TdT-mediated dUTP-biotin nick end-labeling (TUNEL) assay

Cleavage of genomic DNA during apoptosis may yield double-stranded, low molecular weight DNA fragments as well as single strand breaks (nick) in high molecular weight DNA. 1×10^5 cells were plated in 8-chamber slide with 500 μL culture medium, and 1.5 mM or 3.0 mM of METH and MDMA was added to attached cells. After 18 h, the apoptotic cell was fixed with 4% paraformaldehyde in PBS (pH 7.4) for 3 days. By using detection kit (Roche Diagnostics Corp., Indianapolis, IN, USA), labeling of DNA strand breaks by TUNEL reaction was determined with DAB. The labeling was detected by using microscope (× 100).

Western blot analysis

The condition of cell culture and MDMA treatment was same above. The harvested cell was suspended in 5 volumes of lysis buffer (10 mM HEPES, 1.5 mM $MgCl_2$, 10 mM KCl, 1 mM DTT, 0.5% Np-40). The lysate was centrifuged at 10,000 × g for 20 min at 4°C and the supernatant proteins were determined. The protein was separated by 12% polyacrylamide gels containing 0.1% SDS to detect caspase 3, caspase 9, bax, bcl-2. For Western blot analysis, the proteins were electrotransferred to a nitrocellulose paper and the blot was soaked in 5% skim milk dissolved in TTBS (20 mM Tris-base, pH 7.5, 154 mM NaCl, containing 0.5% Tween 20) for 3 h to decrease nonspecific binding. The membrane was washed with TTBS and reacted for 4 h with specific antibodies against the designated proteins. The membrane was washed three times with TTBS, and was incubated with horseradish peroxidase-conjugated IgG. The blot was detected by reacting with ECL reagents (Amersham Pharmacia Biotech, England) followed by exposure to an X-ray film. Monoclonal antibody was used as the primary antibody and the antibodies were purchase from Santa Cruz Biotech. (CA, USA).

Statistical Analysis

ANOVA was determined by using SPSS. Differences from control values in all cases were assessed statistically using Duncun's multiple-range test. The level of statistical significance was set at $P < 0.05$.

RESULTS

Both METH and MDMA induced cell death in JAR and SK-N-SH cells

A concentration-dependent effect of METH and MDMA on cell viability by using MTT assay was apparent as well in both cell lines; treatment with 0.188-3.0 mM of METH and MDMA for 48 h decreased cell viability by 41.5-71.8% and 7.0-81.5% (Fig. 1) in JAR cells, respectively; treatment with 0.188-6.0 mM of METH and MDMA for 48 h decreased cell viability by 9.1-95.5% and 29.1-96.0% (Fig. 3) in SK-N-SH cells, respectively. In LDH assay for cell viability, Fig. 2 and Fig. 4 showed METH- and MDMA-induced concentration-dependent cytotoxicity to JAR and SK-N-SH cells.

Both METH and MDMA induced apoptosis in cell death of JAR and SK-N-SH cells

In attempt to elucidate the mode of METH and MDMA, the purified DNA of untreated, METH-treated, MDMA-treated JAR or SK-N-SH cells was isolated and analyzed on agarose gel. Fig. 5 showed that METH produced intranucleosomal DNA fragmentation in JAR cells, which appeared as a characteristic DNA ladder. Fig. 6 also showed intranucleosomal DNA fragmentation in SK-N-SH cells treated with METH and MDMA. Giemsa and DAPI staining explained a typical DNA

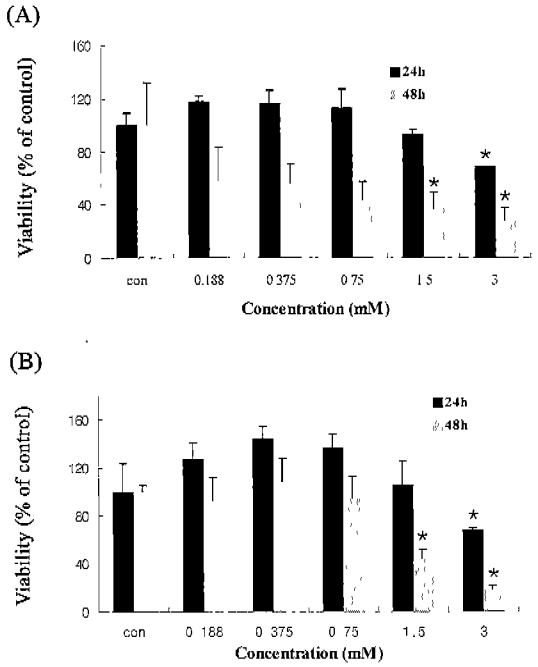

Fig. 1. Cytotoxicity of METH (A) and MDMA (B) in JAR cells by using mitochodrial dehydrogenase activity cleavage of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltrazolium bromide (MTT). Cells were treated with various METH and MDMA concentration for 24 or 48 h. Data are expressed as mean ± S.D. from experiments replicated 5 times. Asterisks (*) indicate a significant difference from control cells. $P < 0.05$

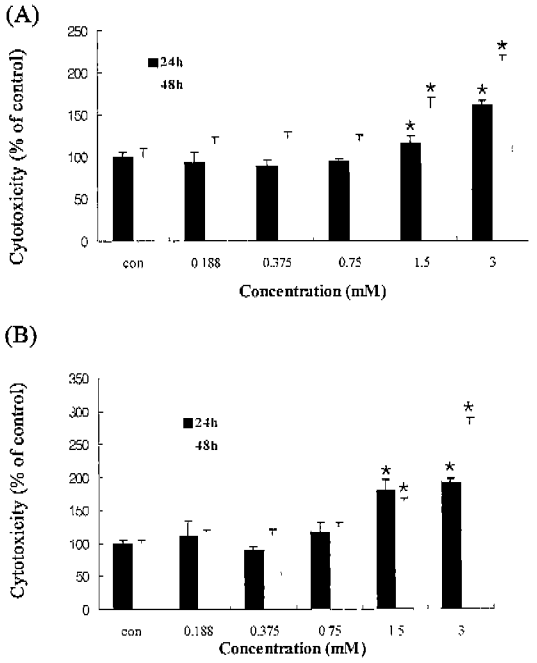

Fig. 2. Cytotoxicity of METH (A) and MDMA (B) in JAR cells by using lactate dehydrogenase (LDH) activity. Cells were treated with various METH and MDMA concentration for 24 or 48 h. Data are expressed as mean ± S.D. from experiments replicated 5 times. Asterisks (*) indicate a significant difference from control cells. $P < 0.05$.

fragmentation and apoptotic morphology in METH and MDMA treated JAR and SK-N-SH cells (Fig. 7, Fig. 8, Fig. 9, Fig. 10). As shown in Fig. 11 and Fig. 12, apoptotic nuclei was identified according to the characteristic strand breaks in the DNA. TUNEL assay revealed that METH and MDMA dose-dependently increased apoptotic nuclei in JAR and SK-N-SH cells. These results verified the DNA fragmentation and apoptotic morphology.

The apoptosis of METH and MDMA in JAR and SK-N-SH cells may be dependent on mitochondria mediated caspase

METH and MDMA activated caspase-3 in JAR and SK-N-SH cells (Fig. 13 and Fig. 14). Fig. 13 showed that procaspase-3 were detected protein bands with molecular masses of 31 kDa and the expression decreased with concentration-dependence of METH and MDMA in JAR cells. In addition, activated cleaved forms of caspase-3 (17 kDa) were detected in METH- and MDMA-treated JAR cells by immunoblot analysis (Fig. 13). Fig. 14 showed that the expression of procaspase-3 also decreased with concentration-dependence of METH and

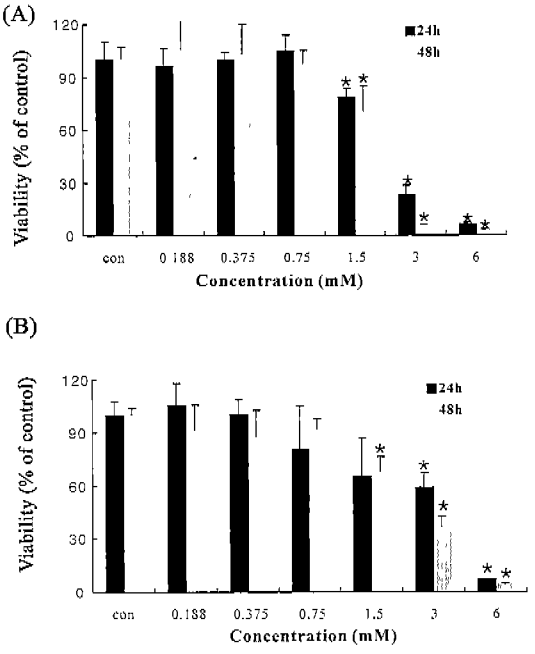

Fig. 3. Cytotoxicity of METH (A) and MDMA (B) in SK-N-SH cells by using mitochodrial dehydrogenase activity cleavage of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltrazolium bromide (MTT). Cells were treated with various METH and MDMA concentration for 24 or 48 h. Data are expressed as mean ± S.D. from experiments replicated 5 times. Asterisks (*) indicate a significant difference from control cells. $P < 0.05$

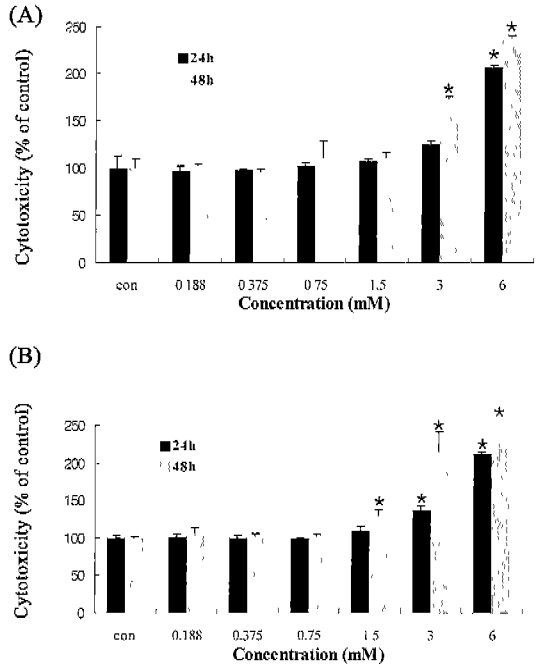

Fig. 4. Cytotoxicity of METH (A) and MDMA (B) in SK-N-SH cells by using lactate dehydrogenase (LDH) activity. Cells were treated with various METH and MDMA concentration for 24 or 48 h. Data are expressed as mean ± S.D. from experiments replicated 5 times. Asterisks (*) indicate a significant difference from control cells. $P < 0.05$

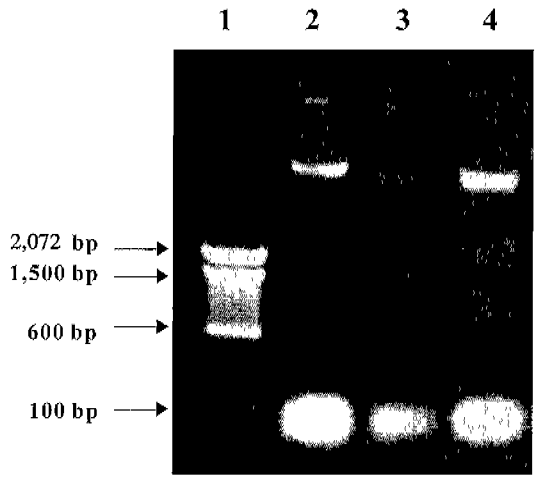

Fig. 5. METH-induced DNA fragmentation. DNA was extracted from JAR cells untreated (lane 2) or treated for 18 hrs with 1.5 mM (lane 3) and 3.0 mM (lane 4) of METH. 100 Bp marker was used (lane 1).

MDMA in SK-N-SH cells as like that in JAR cells. Although we could not find the cleaved forms of caspase-3 (17 kDa), the tendency of decrease in procaspase-3 showed that in SK-N-SH cells the active form of caspase-3 also might be involved in

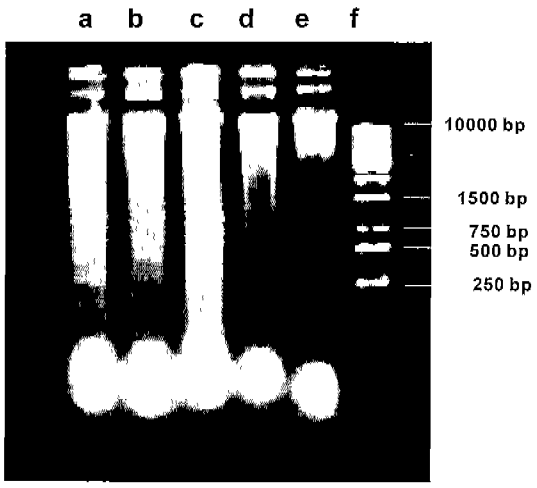

Fig. 6. METH- and MDMA-induced DNA fragmentation. DNA was extracted from SK-N-SH cells untreated (lane e) or treated for 18 hrs with 3.0 mM METH (lane a), 1.5 mM METH (lane b), 3.0 mM MDMA (lane c), and 1.5 mM MDMA (lane d). 250 Bp marker was used (lane f).

apoptosis. METH and MDMA also activated caspase-9 in both JAR and SK-N-SH cells (Fig. 15 and Fig. 16). Fig. 11 showed that activated cleaved forms of caspase-9 (35 kDa) were detected in METH- and MDMA-treated JAR cells by immunoblot analysis. Fig. 16 also showed that activated cleaved forms of caspase-9 (35 kDa) were detected in METH- and MDMA-treated SK-N-SH cells. Protein levels of bax were analyzed by Western blot after treating JAR and SK-N-SH cells for 18 h with METH (1.5 and 3.0 mM) and MDMA (1.5 and 3.0 mM). Bax was induced in both METH and MDMA treated cells (Fig. 17 and Fig. 18). The anti-apoptotic protein bcl-2 was decreased by METH and MDMA treatment both in JAR and SK-N-SH cells (Fig. 19 and Fig. 20).

DISCUSSION

METH, a widely abused drug, has long term neuropsychiatric effects and produces serious mental and physical problem (Woolverton *et al.*,1989). The neurotoxic effect of METH was extensively analyzed in verious animals and human (Lieberman *et al.*, 1990; Volkow *et al.*, 2001). Administration of high doses of METH results in long lasting dopamine or serotonin (5-HT) depletions in rodents and other species (Wagner et al., 1980). It has been proposed that METH-induced dopamine release is responsible for the METH-induced neurotoxicity; the excess dopamine is thought to encourage formation of oxygen radicals by auto-oxidation or enzymatic reaction of dopamine

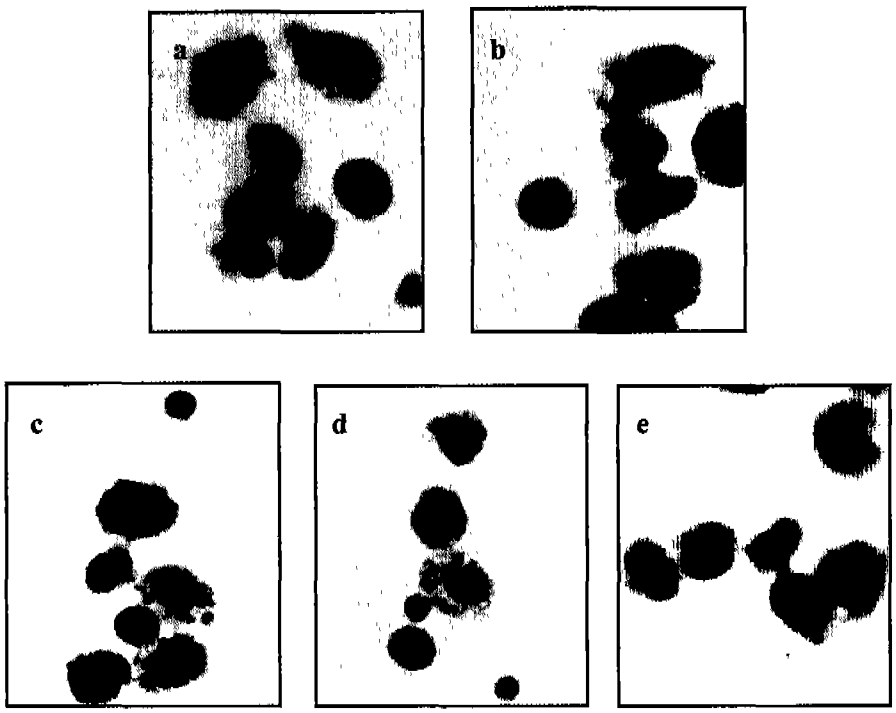

Fig. 7. Giemsa staining of JAR cells treated with METH and MDMA. a, control cells; b, MDMA 1.5 mM; c, MDMA 3 mM; d, METH 1.5 mM; e, METH 3 mM. The cells were treated for 18 hrs. (magnification; ×200)

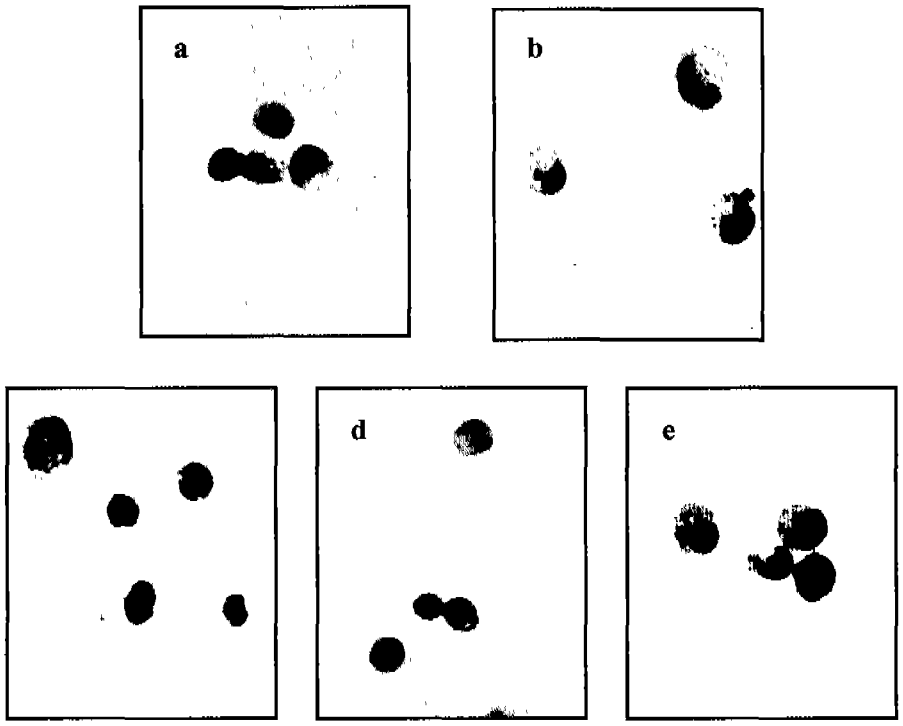

Fig. 8. Giemsa staining of SK-N-SH cells treated with METH and MDMA. a, control cells; b, MDMA 1.5 mM; c, MDMA 3 mM; d, METH 1.5 mM; c, METH 3 mM. The cells were treated for 18 hrs. (magnification; ×200)

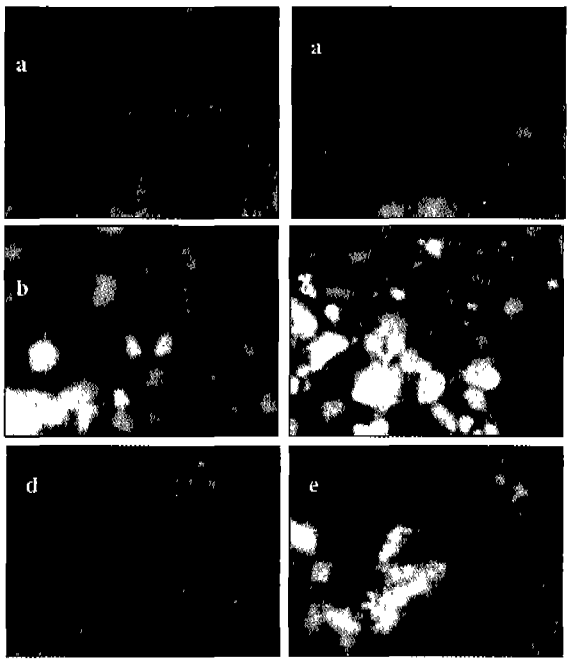

Fig. 9. DAPI staining of JAR cells treated with METH and MDMA. a, Control cells; b, MDMA 1.5 mM; c, MDMA 3 mM; d, METH 1.5 mM; e, METH 3 mM. The cells were treated for 18 hrs. (magnification; ×200)

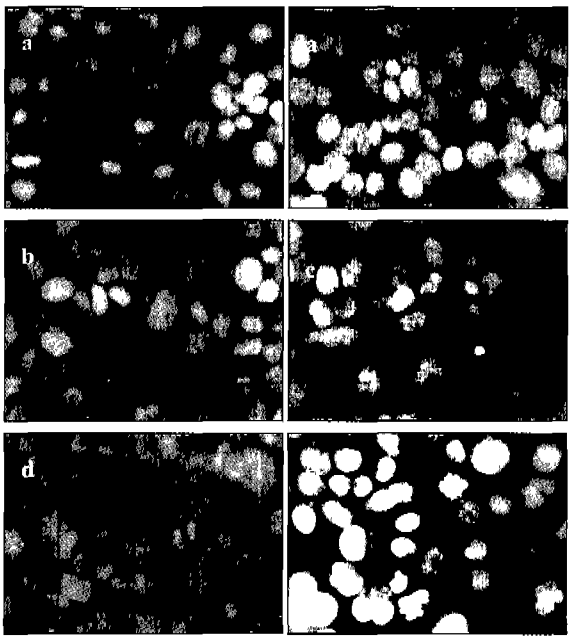

Fig. 10. DAPI staining of SK-N-SH cells treated with METH and MDMA. a, Control cells; b, MDMA 1.5 mM; c, MDMA 3 mM; d, METH 1.5 mM; e, METH 3 mM. The cells were treated for 18 hrs. (magnification; ×200)

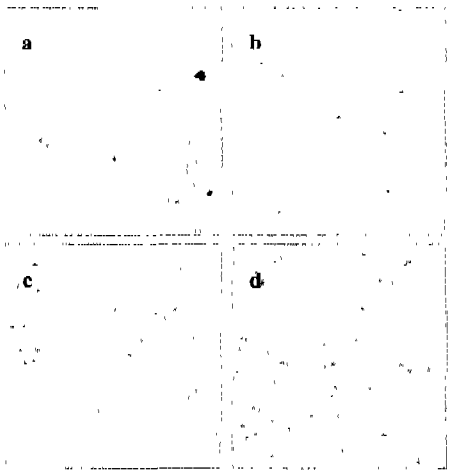

Fig. 11. Labeling of DNA strand breaks by TUNEL reaction in JAR cells. JAR cells were cultured on chamber slides at approximately 1× 10^5/slide and untreated (b) or treated for 18 hrs with 1.5 mM METH (c) or 3.0 mM METH (d). (a) shows background negative control. Magnification, ×200.

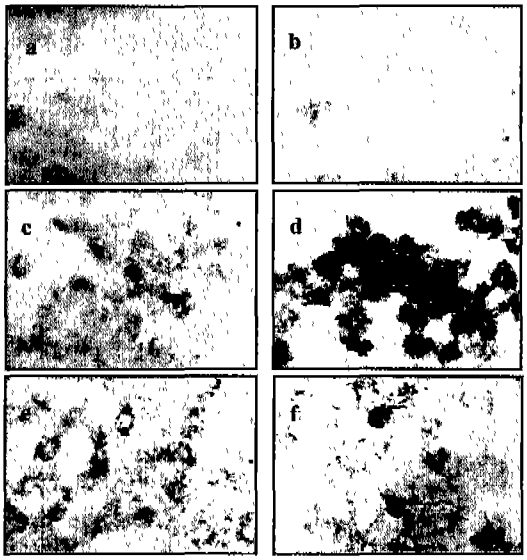

Fig. 12. Labeling of DNA strand breaks by TUNEL reaction in SK-N-SH cells. SK-N-SH cells were cultured on chamber slides at approximately 1× 10^5/slide and untreated (b) or treated for 18 hrs with 1.5 mM MDMA (c), 3.0 mM MDMA (d), 1.5 mM METH (e), and 3.0 mM METH (f). (a) shows background negative control. Magnification, ×200.

(Seiden and Vosmer, 1984; De Vito and Wagner, 1989; Giovanni *et al.*, 1995). However, there have been reported about the other process of METH-induced neurotoxicity in a manner independent of dopamine. Jayanthi *et al.* (Jayanthi *et al.*, 2001) showed that injections of toxic doses of METH to mice increased the pro-death Bcl-2 family genes, Bad, Bax, and Bid, and also decreased the anti-death gene, Bcl-2 and Bcl-X_L. Thiriet *et al.* (Thiriet *et al.*, 2001) reported that c-myc and $_L$-

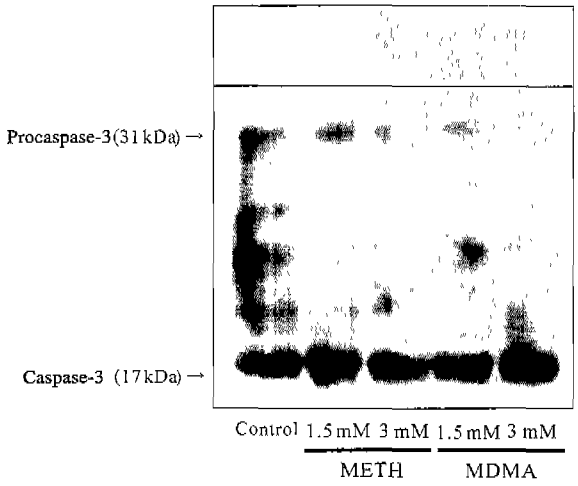

Fig. 13. Activation of caspase-3 in JAR cells treated with METH (1.5 and 3 mM) and MDMA (1.5 and 3 mM) for 18 hrs. a, Control cells; b, MDMA 1.5 mM; c, MDMA 3 mM; d, METH 1.5 mM; e, METH 3 mM.

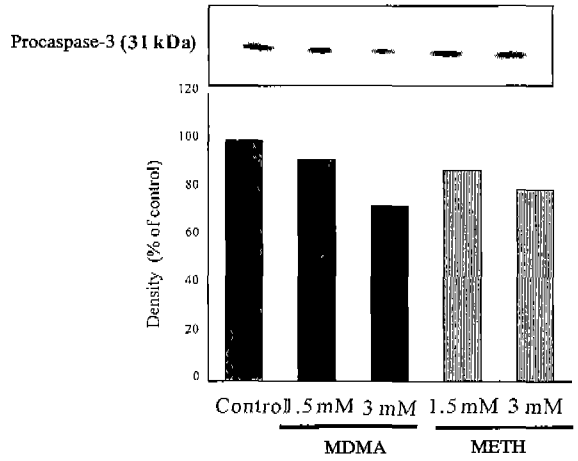

Fig. 14. Activation of caspase-3 in SK-N-SH cells treated with MDMA (1.5 mM, 3 mM) and METH (1.5 mM, 3 mM) for 18 hrs. a, Control cells; b, MDMA 1.5 mM; c, MDMA 3 mM; d, METH 1.5 mM; e, METH 3 mM.

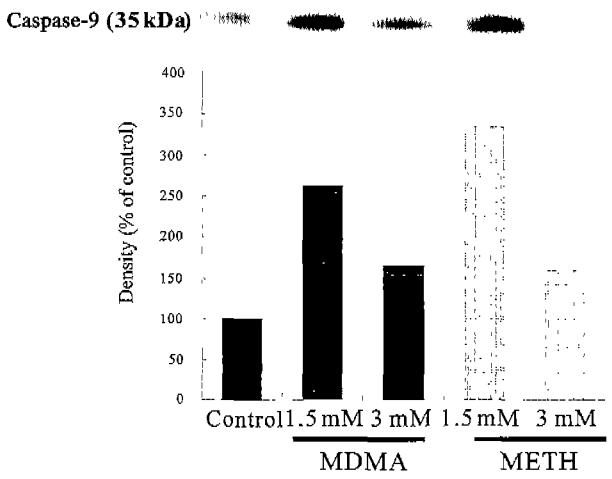

Fig. 15. Expression of caspase-9 in JAR cells treated with METH (1.5 and 3 mM) and MDMA (1.5 and 3 mM) for 18 hrs. a, Control cells; b, MDMA 1.5 mM; c, MDMA 3 mM; d, METH 1.5 mM; e, METH 3 mM.

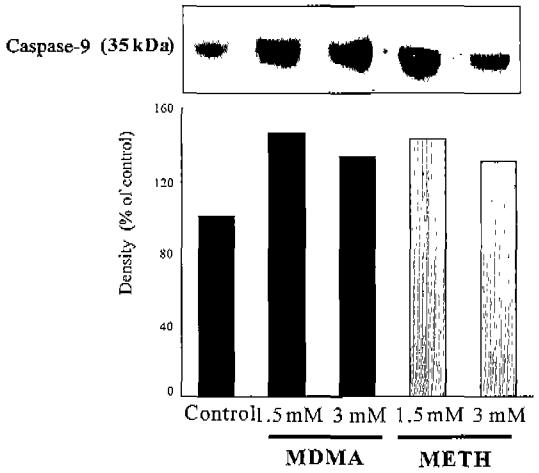

Fig. 16. Expression of caspase-9 in SK-N-SH cells treated with MDMA (1.5 mM, 3 mM) and METH (1.5 mM, 3 mM) for 18 hrs. a, Control cells; b, MDMA 1.5 mM; c, MDMA 3 mM; d, METH 1.5 mM; e, METH 3 mM.

myc were up-regulated by METH at both mRNA and protein levels. Imam et al. (Imam *et al.*, 2001) also proposed that METH might cause its neurotoxic effects via the production of free radicals and secondary perturbations in the expression of genes known to be involved in apoptosis and cell death machinery.

In this study, human serotonergic JAR and dopaminergic SK-N-SH cell line were used to reveal the cell death mechanism of METH. METH induced cell toxicity in both JAR and SK-N-SH cells concentration-dependently. The morphology of METH-treated cells showed apoptotic bodies in cytotoxic concentration for 18 h (Figs. 7, 8, 9, and 10). The TUNEL assay (Fig. 11 and 12) and intranucleosomal DNA fragmentation (Fig. 5 and 6) showed METH-induced cell death was via aoptosis process. Casapse-3 and caspase-9 were activated in METH-treated JAR and SK-N-SH cells. In this study, we confirmed the induction of cleaved activated forms of caspase-3 and caspase-9. This result showed the apoptosis was involved in caspase-3 and caspase-9 activation. Bax was induced and bcl-2 was decreased in METH-treated cells. These results were like the pattern of those in vivo studies (Jayanthi *et al.*, 2001). These findings suggest that METH induce apoptotic processes

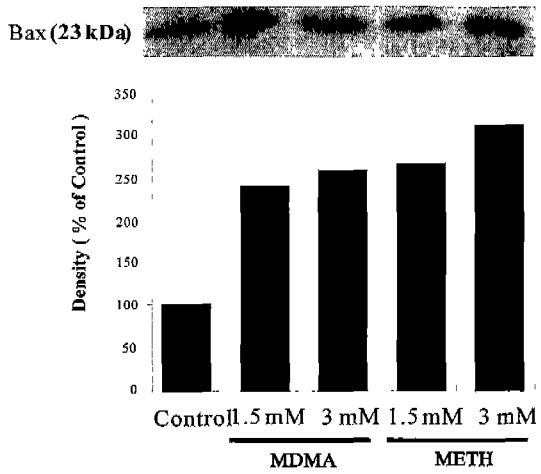

Fig. 17. Expression of bax in JAR cells treated with MDMA (1.5 mM, 3 mM) and METH (1.5 mM, 3 mM) for 18 hrs. a, Control cells; b, MDMA 1.5 mM; c, MDMA 3 mM; d, METH 1.5 mM; e, METH 3 mM.

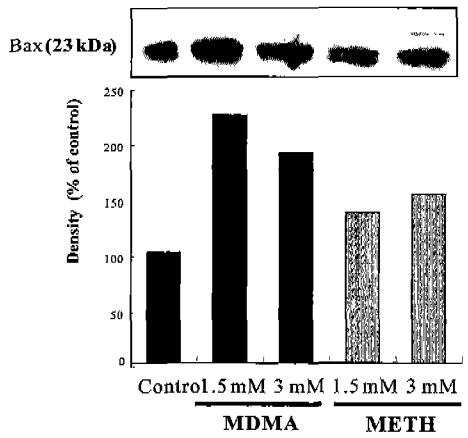

Fig. 18. Expression of bax in SK-N-SH cells treated with MDMA (1.5 mM, 3 mM) and METH (1.5 mM, 3 mM) for 18 hrs. a, Control cells; b, MDMA 1.5 mM; c, MDMA 3 mM; d, METH 1.5 mM; e, METH 3 mM.

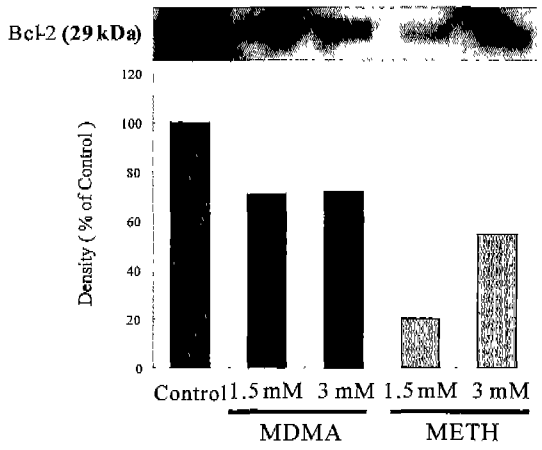

Fig. 19. Down regulation of bcl-2 in JAR cells treated with MDMA (1.5 mM, 3 mM) and METH (1.5 mM, 3 mM) for 18 hrs. a, Control cells; b, MDMA 1.5 mM; c, MDMA 3 mM; d, METH 1.5 mM; e, METH 3 mM.

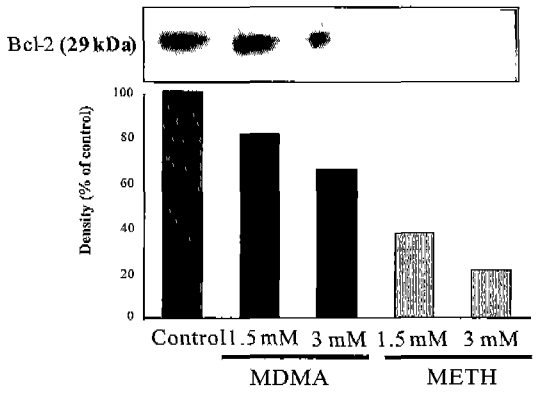

Fig. 20. Down regulation of bcl-2 in SK-N-SH cells treated with MDMA (1.5 mM, 3 mM) and METH (1.5 mM, 3 mM) for 18 hrs. a, Control cells; b, MDMA 1.5 mM; c, MDMA 3 mM; d, METH 1.5 mM; e, METH 3 mM.

in not only dopaminergic cells but also serotonergic cells. Moreover, the apoptosis may be involved in the pathway of mitochondria mediated caspase cascade.

MDMA, a widely abused recreational drug, has long term neuropsychiatric effects (Keenan *et al.*, 1993). The neurotoxic effect of MDMA was extensively analyzed in various animals (Ricaurte *et al.*, 1988; Insel *et al.*, 1989). In this study, human serotonergic and dopaminergic cell line were used to reveal the cell death mechanism of MDMA. MDMA induced cell toxicity, intranucleosomal DNA fragmentation, casapse-3 and caspase-9 activation, and bax and bcl-2 expression in SK-N-SH cells. These findings suggest that MDMA induce apoptotic processes in not only serotonergic cells but also dopaminergic cells. Moreover, the apoptosis may be involved in the pathway of mitochondria mediated caspase cascade.

METH and MDMA showed similar effects on both human serotonergic and dopaminergic cell line in current study. Both compounds are amphetamine analogs. Although there are different pharmacological effects between METH and MDMA, the apoptotic effects in neurons were similar.

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