Journal of Biochemistry and Molecular Biology, Vol. 40, No. 4, July 2007, pp. 475-485

JOURNAL OF Biochemistry and Molecular Biology

Gene Expression Profiling of Rewarding Effect in Methamphetamine Treated Bax-deficient Mouse

Na Kyung Ryu[1,#], Moon Hee Yang[1,#], Min Seok Jung[2], Jeong Ok Jeon[1], Kee Won Kim[2,*] and Jong Hoon Park[1,*]

[1]Department of Biological Science, Sookmyung Women's University, Seoul, Republic of Korea

[2]Departments of Pharmacology and Neurology, Institute for Medical Sciences, Chonbuk National University Medical School, Chonju, Chonbuk, Republic of Korea

Received 9 January 2007, Accepted 23 January 2007

Methamphetamine is an illicit drug that is often abused and can cause neuropsychiatric and neurotoxic damage. Repeated administration of psychostimulants such as methamphetamine induces a behavioral sensitization. According to a previous study, Bax was involved in neurotoxicity by methamphetamine, but the function of Bax in rewarding effect has not yet been elucidated. Therefore, we have studied the function of Bax in a rewarding effect model. In the present study, we treated chronic methamphetamine exposure in a Bax-deficient mouse model and examined behavioral change using a conditioned place preference (CPP) test. The CPP score in Bax knockout mice was decreased compared to that of wild-type mice. Therefore, we screened for Bax-related genes that are involved in rewarding effect using microarray technology. In order to confirm microarray data, we applied the RT-PCR method to observe relative changes of Bcl2, a pro-apoptotic family gene. As a result, using our experiment microarray, we selected genes that were associated with Bax in microarray data, and eventually selected the Tgfbr2 gene. Expression of the Tgfbr2 gene was decreased by methamphetamine in Bax knockout mice, and the gene was overexpressed in Bax wild-type mice. Additionally, we confirmed that Creb, FosB, and c-Fos were related to rewarding effect and Bax using immunohistochemistry.

Keywords: Bax, Brain, Conditioned place preference, Methamphetamine, Microarray,

Introduction

Methamphetamine is a neurotoxic, illicit drug of abuse that can cause neuropsychiatric and neurotoxic damage in the brain regions of animals (Genc *et al.*, 2003).

Methamphetamine has been associated with the respective up- and down-regulation of anti-apoptotic protein and pro-apoptotic protein regulators of the Bcl2 family genes (Deng *et al.*, 2002). Bcl2 family proteins are functionally categorized into death-inhibiting or death-inducing members (Jayanthi *et al.*, 2001). Bax is a pro-apoptotic protein of the Bcl2 family, and it is expressed as a 21-kDa protein that is capable of combining Bcl2 proteins to form a heterogeneous dimer with Bcl2 and Bclx (Sorenson *et al.*, 2004; Duan *et al.*, 2005). The Bax protein, a Bcl2-related homologue protein that promotes apoptosis (Amirghofran *et al.*, 2005), increase the apoptotic susceptibility of cells in brain organ (Vereide *et al.*, 2005). Recent studies provide confirmation of a role for Bcl2 family genes in methamphetamine-induced neuronal cell death (Cadet *et al.*, 1997; Jayanthi *et al.*, 2001).

In unstimulated cells, Bax resides in the cytosol, but a cell death signal such as caspase activation causes translocation of Bax to the mitochondria, where it integrates in the membrane via its transmembrane domain and mediates and amplifies membrane dysfunctions (Goping *et al.*, 1998). However, the function of Bax was not that sees yet concretely and proceeds because have gouged neurotoxicity by methamphetamine. Pro-apoptotic members of the Bcl2 family are essential for the initiation of those pathways to apoptosis that are regulated by their pro-survival relatives (Puthalakath and Strasser, 2002). Within the pro-apoptotic Bcl2 family, Bid and Bad possess the minimal death domain, BH3, and the phosphorylation of Bad connects proximal survival signals to the Bcl2 family (Chao and Korsmeyer, 1998). Bid has also been shown to bind to Bax.

Methamphetamine affects the DNA-binding activity of specific redox-sensitive transcription factors in the mouse

[#]These authors are equally contributed

*To whom correspondence should be addressed.
Tel: 82-2-710-9414; Fax: 82-2-2077-7257
E-mail: parkjh@sookmyung.ac.kr

brain (Asnuma *et al.*, 2000; Lee *et al.*, 2002). Transcription factors are known to act as gene expression regulators, possibly linking extracellular stimuli to long-term modifications at the neuronal level (Perisco *et al.*, 1995). Transcription factors such as Creb, FosB, and c-Fos are thought to play a major role in the rewarding properties of many drugs of abuse (Walters *et al.*, 2005). In the case of the rat brain, regional expression of transcription factor genes has suggested potential correlations between those changes and behavioral novelty responses (Perisco *et al.*, 1995).

The aim of this study was to determine the effect that the Bax gene has on the mouse brain and to confirm the function of Bax in relation to methamphetamine. We investigated Bax function in rewarding effect by methamphetamine. In addition, we confirmed the existence of such effects by searching Bax WT and KO mice that were treated methamphetamine or saline, and assessed any differences observed following treatment. We treated mice with a treatment to mimic chronic methamphetamine use. These experiments suggested that the expression of Bax increases in the normal brain with chronic exposure to methamphetamine, and we attempted to recognize what influence methamphetamine exerts on Bax expression. We know through our CPP test that methamphetamine affected behavior. The CPP response is a behavior that developed through the association of reinforcing effects of drugs within the context in which animals have previously obtained positive reinforcing effects (Hoffman, 1989).

And we do action change that appears by treating methamphetamine in mouse so that digitize and appeared by CPP score. In animal models, repeated administration of psychostimulants such as methamphetamine induces a phenomenon known as behavioral sensitization, a progressive enhancement of the psychomotor activating and rewarding effects of the drug (Narita *et al.*, 2005).

In this study, we wished to utilize Bax KO mice to closely examine the exact function of Bax in methamphetamine neurotoxicity. After we observed that Bax gene increases in rat that methamphetamine is treated equally, we again considered the relationship between methamphetamine and Bax. Also, observed whether methamphetamine other gene plain that recognize gene plain that become regulation that becomes up, down by compares KO mice with processed WT mouse and does microarray and is some to Bax gene about region in striatum among this brain region plain is effect. In the present study, expression of transcription factors such as Creb, FosB, and c-Fos protein were found to be up-regulated by treatment with methamphetamine in Bax WT brain. Transcription factor, Creb shows up-regulation and resultant activation. In contrast, induction of another transcription factor, termed delta FosB, exerts the opposite effect and may contribute to sensitized responses to drug exposure (Netsler, 2001). c-Fos and many other Fos family members show rapid and transient induction in specific brain regions in response to many types of acute perturbations, including repeated administration of drugs of abuse (Netsler, 1999).

Materials and Methods

Animals and drug treatment. Nine male and seven female mice were used in this study. Two of nine male mice and five of seven female mice were Bax knockout mice. Each mouse weighed approximately 50 g at the beginning of the experiments, and the mice were housed in groups of four (wild/knockout) in a temperature-controlled room. They were maintained on a 12-h light/dark cycle (light on 8 : 00 a.m. to 8 : 00 p.m.) with laboratory mouse chow and water available ad libitum. METH hydrochloride (Dainihonn Pharmaceutical Co.) was dissolved in saline (SAL). Rapamycin (Calbiochem Novaviochem Co.) was dissolved in 1% dimethyl sulfoxide in SAL.

Conditioned place preference. Place conditioning studies were conducted using an apparatus consisting of a shuttle box (W 30 cm_D 60 cm_H 30 cm) that was made of an acrylic resin board and divided into two equal-sized compartments. One compartment was white with a textured floor, and the other was black with a smooth floor; these were considered to be equally inviting compartments. All sessions were conducted under conditions of dim illumination (40 lux lamp). The place conditioning schedule consisted of three phases (pre-conditioning test, conditioning, and post-conditioning test).

Pre-conditioning test: For two successive days before the conditioning test, the pre-conditioning test was performed as follows: the partition separating the two compartments was raised to 12 cm above the floor, and a neutral platform was inserted along the seam separating the compartments. Mice that had not been treated with either drugs or SAL were then placed on the platform. The time spent in each compartment during a 900-s session was then recorded automatically using an infrared beam sensor (KN-80; Natsume Seisakusyo Co., Ltd.).

Conditioning test: After the pre-conditioning test, conditioning sessions [one session/day_2 (days) for METH: one session/ day_2 (days) for SAL] were performed once daily for four successive days as follows: immediately after intraperitoneal (i.p.) injection of METH (0.5 mg/kg), these animals were placed in one compartment, the compartment opposite the side of the box that animals had spent the most time in during the pre-conditioning test, for 50 min. On alternate days, the animals that received vehicle were placed in the other compartment for 50 min.

Post-conditioning test: A day after these conditioning sessions, each animal was placed in the test apparatus without any confinements, and then the relative amount of time spent in each compartment was measured. In the post-conditioning test, the animal had the opportunity to move freely between the different compartments.

RNA preparation and hybridization. After the CPP test, we immediately sacrificed the mice. We dissected each region of the brain: the mid brain, cerebellum, cerebrum, hippocampus, and striatum. Total RNA was prepared by using the Qiagen Lipid kit as directed by the manufacturer. We utilized the UniSet Mouse I Expression Bioarray (Amersham) containing 9028 genes probes; this array contains a broad range of genes derived from publicly available, well-annotated mRNA sequences. The CodeLink array is unique in that it is capable of detecting minimal differences in gene

expression, as low as 1.3-fold with 95% confidence. Ten micrograms of total RNA were amplified, labeled with cRNA produced using the kit available from Amersham, and designed for use with the CodeLink® arrays. Biotin-labeled cRNA was hybridized to the array overnight in a shaking incubator at 37°C, and excess target was washed away using a series of SSC washes. The array was stained by treatment with streptavidin-Alexaflour 647 (Molecular Probes), the excess was washed away, and the array was scanned at an excitation wavelength of 632 nm using an Axon Instruments GenePix scanner. The resulting image was quantified, and the intensity of each spot was divided by the median spot intensity to provide a scaled and comparable number across multiple arrays. Bacterial spots provided both positive and negative controls.

Semi-quantitative RT-PCR. Total RNA from brain tissue was extracted using Qiazol (Qiagen) as directed by the RNeasy Lipid Tissue Mini kit (cat. No. 74804). Single-stranded cDNAs were synthesized by incubating total RNA (5 μg) with AML-V (200 U), oligo (dT) 12-18 primer (100 nM), dNTPs (1 mM), and RNase inhibitor (40 U) at 42°C for 1hr in a final volume of 25 μl. The reaction was terminated by incubation at 70°C for 15 min. The PCR primers were specifically designed for the individual rat or mouse sequences with the "Primer 3 Software". The initial amount of mRNA and reaction conditions were optimized to obtain linearity for 18 s rRNA in control cells. RT-PCR cycling conditions for 18 s rRNA were as follows: 10 min at 95°C, 25 cycles of 1min at 94°C, 1 min at 57°C, 1 min at 72°C, and 10 min at 72°C. The RT-PCR products were separated on a 1% agarose gel, stained with ethidium bromide, and visualized. We performed further semi-quantification of the mRNA of Bax, Bid, Bad, Tgfbr2, Creb, FosB, and c-Fos genes in the striatum using an RT-PCR array. Images of PCR products were captured by the Bio Imaging Analyzer Las-3000, and intensities were analyzed with Kodak Multi-Gauge V2.3 software.

Immmunohistochemistry. Unstained 10-mm sections were cut from the frozen samples using standard methods. Put slides to 3'DW during 5 minutes. Endogenous peroxidase was blocked with 3% hydrogen peroxide (LAB Vision, AHP40305) for 10 min, and the samples were then rinsed in 3'DW. Blocking of biotin was performed using the CAS blocking solution (Zymed, 00-8120) for 1 h at room temperature (RT). Slides were incubated with the primary antibody overnight at 40°C; antibodies included anti-Bax (1 : 500), anti-Tgfbr2 (1 : 500), anti-FosB (1 : 250), anti-Creb (1 : 250), and c-Fos (1 : 250). After washing with PBST three times for 10 min, slides were incubated with the secondary antibody (DAKO, K0690) for 30 min at RT. After washing with PBST three times for 10 min, slides were incubated with the streptavidin-HRP (DAKO, K0690) for 30 min at RT. After washing with PBS twice for 10 min, slides were applied to AEC (Zymed, 87-8153) for 10 minutes. Slides were washed with 1X PBS, Counterstaining was finally carried out with hematoxylin (LAB VISION, TA-125-MH), and coverslips were attached using aqueous mounting medium (LAB VISION, TA-125-UMX).

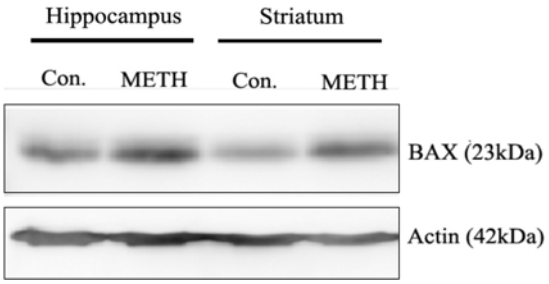

Fig. 1. Western blot analysis of the BAX protein in hippocampus and striatum tissue of methamphetamine-treated normal rat. We analyzed Bax protein expression in two rat brain regions-hippocampus and striatum after repeat methamphetamine treatment. Actin was used positive control. Con.: saline treated normal rat brain; METH: repeated methaemphtamine treated normal rat brain.

Results

Expression of Bax increased by methamphetamine treatment. Bax gene is well-known pro-apoptotic gene. As a previous study, Bax expression increased by single treatment of methamphetamine (Jayanthi *et al.*, 2001). For comparing Bax expression alteration between single treatment and repeat treatment, we investigated Bax expression in rat brain after repeat treatment of methamphetamine. As shown Fig. 1, Bax expression increased in striatum and hippocampus. Therefore for investigation of Bax function in repeat methamphetamine treatment, we used Bax deficient mouse obtained from Dr. Woong Sun. We applied the RT-PCR method to observe changes in Bcl2 pro-apoptotic family genes such as Bax by methamphetamine in Bax KO mice. As a constitutive control, 18s rRNA amplification was used. We were able to observe a trend with which similar increases were with Bax KO and Bax WT mice in the case of treatment with methamphetamine. The Bad and Bid genes are pro-apoptotic genes in the striatum that were observed to be the most deeply connected to medicinal poisoning among all of the regions of the brain, but their expression does not appear to change much in Bax KO mice, even following treatment with methamphetamine (Fig. 2).

Bax gene influenced rewarding effect by methamphetamine treatment. We expected that when methamphetamine was used to treat to Bax WT and Bax KO mice, similar results would be observed for both groups. As shown in Fig. 1A, we confirmed that the CPP score is clearly increased in rats. For screening of addiction-related genes, we divided the mice into four groups: saline treatment of Bax WT, methamphetamine treatment of Bax WT, saline treatment of Bax KO, methamphetamine treatment of Bax KO. We observed a larger increase in mice given methamphetamine treatment than in

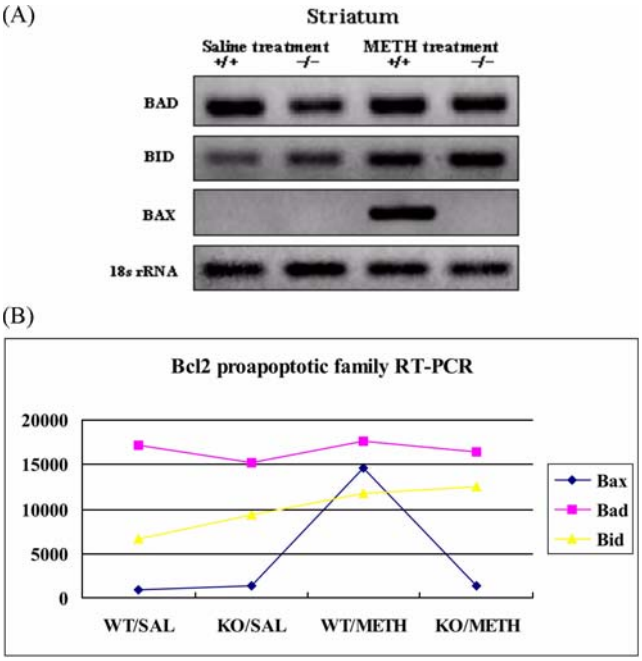

Fig. 2. Verification of several pro-apoptotic genes expression in Bax deficient mouse brain using RT PCR. We analyzed in striatum after repeated methamphetamine treatment. (A) RT PCR analysis of several pro-apoptotic genes including BAX. (B) Quantification graph of RT PCR. 18sRNA was used as internal control. The RNA expression levels were quantities by Multiguage program (Fujifilm Worldwide, Japan).

those given saline, and also noted increased CPP scores in Bax KO mice than in Bax WT mice. This suggests a potential relationship between the Bax gene and drug poisoning phenomena as well as neurotoxic effects of drugs (Fig 3).

We investigated the expression of an occasional regulator of Creb transcription in samples obtained from methamphetamine-treated mice (Fig. 4A), and observed that FosB and c-Fos increase in Bax WT mice that were treated with methamphetamine (Fig. 4C). Increases in Creb and FosB expression were observed in methamphetamine-treated samples among saline WT, saline KO, methamphetamine WT, and methamphetamine KO mice (Fig. 4B). Observed changes in behavior were associated with rewarding effect as well as the Bax gene. Immunohistochemistry result patterns for Creb, FosB, and c-Fos are similar to the CPP test pattern of Bax.

Gene expression profiling using microarray. As a result of these analyses, we obtained information regarding the up- and down-regulation of genes connected with Bax. We compared the patterns of gene expression between the brains of the methamphetamine-treated Bax KO and WT mice using a microarray in 10K mouse oligo chips. We used mRNA that was confirmed by semi-quantitative RT-PCR. We analyzed chip data using the global normalization method and found relevant genes using the single-slide method plot which yield information regarding the up- and down-regulation of genes

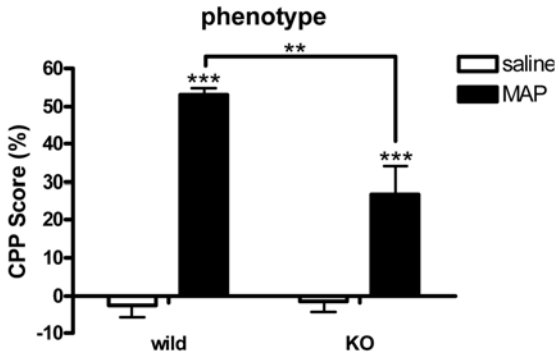

Fig. 3. Measurement of rewarding effect using the CPP test. Methamphetamine induced CPP level in Bax deficient mouse. Open column, saline-treated mouse; closed column, Mathemaphetamine-treated mouse. *Asterisk* denotes a significant difference from the saline paired compartment, $p < 0.05$ t values (mean ± SEM).

connected with Bax. We found 76 genes that were up-regulated and 52 genes that were down-regulated in the methamphetamine-treated Bax WT mice (Table 1, 2).

The Confirmation of the differential gene expression by RT-PCR. We observed an increase in Bax gene in protein levels upon treatment with methamphetamine using immunohistochemistry (data not shown). The association indicated by the CPP score was the highest in Bax WT mice treated with methamphetamine. We think that caused behavior change as Bax is concerned to rewarding effect. Our study indicated that the Tgfbr2 gene is not affected by methamphetamine. The Tgfbr2 gene has been shown to have a relationship with Bax gene irrespective of methamphetamine treatment. From the results of immunohistochemical analysis, we observed that the Bax WT showed higher expression than the Bax KO. Therefore, the Tgfbr2 gene was determined to be related the Bax gene (Fig. 5).

Discussion

Rewarding effect is a chronic disease characterized by neurotic drug use despite the severe negative consequences associated with it. Repeated exposure to drugs of abuse results in molecular adaptations in neuronal signaling pathways, which eventually becomes apparent in the complex behavioral alterations that differentiate addiction (Ron and Jord, 2005). Methamphetamine is a psychostimulant drug that produces both acute psychomotor stimulation and long-lasting behavioral effects, including addiction and psychosis (Zeng *et al.*, 2004). The main findings of this report indicated that methamphetamine induced changes in Bax gene expression and behavior.

After we first confirmed the existence of this phenomenon in the rat brain, we performed an additional experiment. We treated rats with methamphetamine and observed that the CPP score increased. We also observed through Western blotting

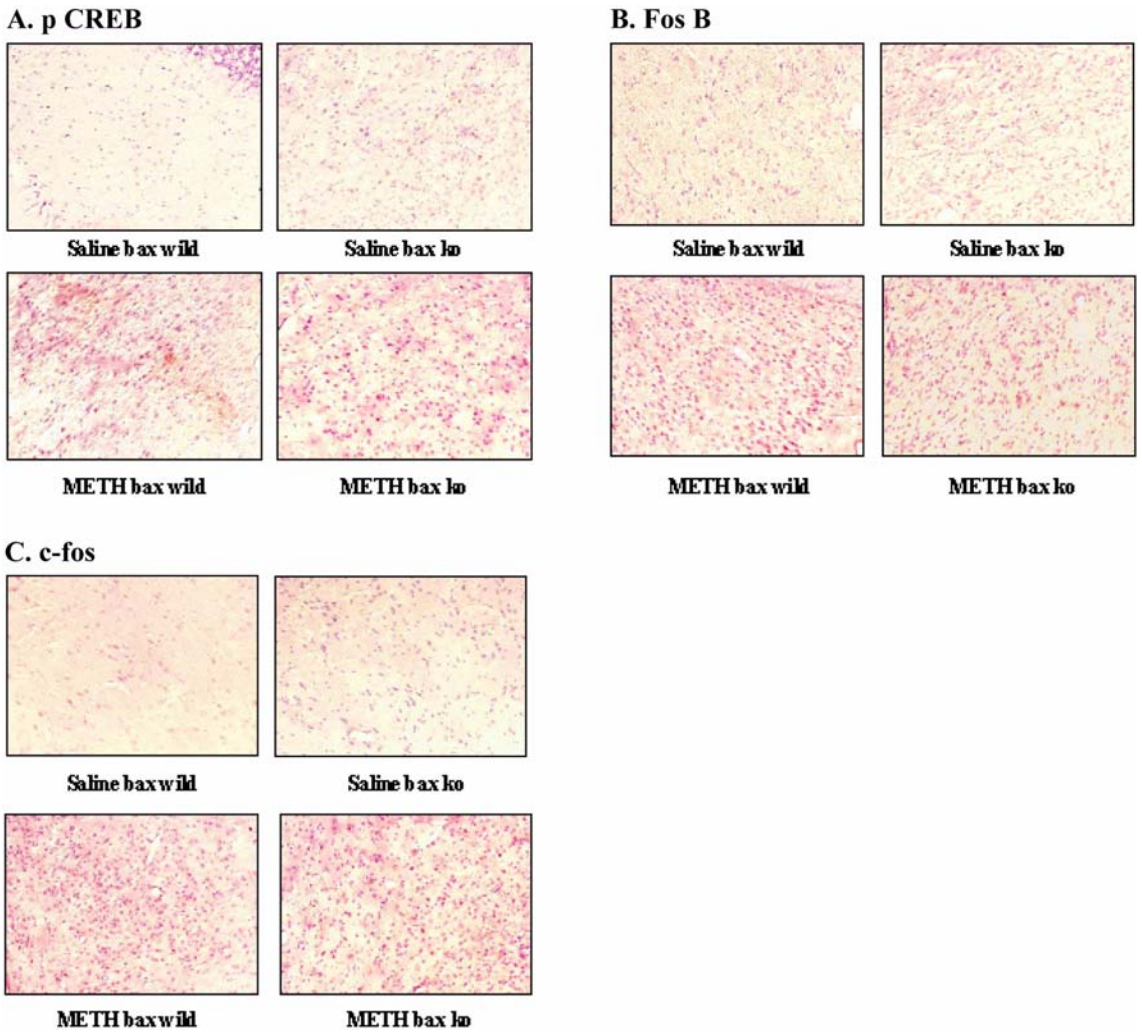

Fig. 4. Expression analysis of well-known genes related rewarding effect using immunohistochemistry in repeated methamphetamine treated Bax deficient mouse. A. stained with pCREB antibody (×200). B. Stained with Fos B antibody (×200). C. Stained with C-fos antibody (×200).

that Bax gene expression increases in the brains of rats treated with methamphetamine, and this was also found to be true in the mouse brain, as determined by immunohistochemistry. This result indicates that the Bax gene affects rewarding effect. Bax KO mice that we are coincided in purpose of two experiments that do genotyping preferentially and certainly became KO confirm and measured this about action of mouse change after methamphetamine processing using conditioned place preference's method. This allows us to conclude that methamphetamine affects the mouse brain region. A major difference was not observed in CPP scores according to sex. It is well-known that the Bax gene exerts an effect on the mouse brain, and several papers have shown that the Bax gene is involved in rewarding effect.

We know that the striatum is affected by rewarding effect within the mouse brain. Using RT-PCR, we investigate changes in Bax gene expression following treatment with methamphetamine. Methamphetamine-induced neurotoxicity is reduced in Bcl2 over-expressing neural cells (Kita and Nakashima, 2002). Additionally, within the Bcl2 family, was shown phenomenon increasing remarkably in WT that methamphetamine is treated likewise in occasion of pro-apoptotic protein Bad and Bid such as Bax and weak signal could observe that is seen in methamphetamine KO. This provides further evidence that increases in gene expression compared to the initial state following the processing of methamphetamine occurs in a similar manner in pro-apoptotic proteins such as Bad, Bid, and Bax. We also observed that genes become are up- and down-regulated in connection with Bax gene expression when comparing Bax KO and Bax WT mice following treatment with methamphetamine. we will find genes connected with Bax that come out through cDNA microarray and search relation continuously with methamphetamine. Repetitive tracts within the coding regions of Tgfbr2 and Bax are frequently mutated in mismatch repair-deficient tumors and have been implicated in tumor progression (Bacon *et al.*, 2001). In the

Table 1. Bax knockout up-regulation genes (52 genes)

Accession No.	Up regulated genes	Fold charge
	ATP binding	
NM_011361	Serum/glucocorticoid regulated kinase	2.40
NM_011666	Ubiquitin-activating enzyme E1C	2.60
	calcium ion binding	
NM_009113	S100 calcium binding protein A13	2.06
NM_009129	Secretogranin II	2.46
NM_010471	Hippocalcin	2.68
NM_009115	S100 protein, beta polypeptide, neural	2.10
	cytoskeletal protein binding	
NM_013813	RIKEN cDNA 2410021H03 gene	2.00
	DNA binding	
NM_020558	Nuclear DNA binding protein	2.02
	glutathione transferase activity	
NM_008185	Glutathione S-transferase, theta 1	2.04
	glycine amidinotransferase activity	
NM_025961	Glycine amidinotransferase (L-arginine:glycine amidinotransferase)	3.50
	growth factor activity	
NM_010784	Midkine	2.62
	GTP binding	
NM_008997	RAB11B, member RAS oncogene family	2.32
NM_009005	RAB7, member RAS oncogene family	2.04
NM_009861	Cell division cycle 42 homolog (S. cerevisiae)	2.48
X03688	Eukaryotic translation elongation factor 1 alpha 1	2.20
NM_019665	RIKEN cDNA A930013N22 gene	2.06
	GTPase activity	
NM_007487	ADP-ribosylation factor-like 4	2.02
	hydrolase activity	
NM_013737	Phospholipase A2, group VII (platelet-activating factor acetylhydrolase, plasma)	2.26
AK011529	Eukaryotic translation initiation factor 4A1	2.02
NM_021330	Acid phosphatase 1, soluble	2.02
NM_007610	Caspase 2	2.10
NM_007509	ATPase, H+ transporting, V1 subunit B, isoform 2	2.68
	lyase activity	
NM_008254	3-hydroxy-3-methylglutaryl-Coenzyme A lyase	2.16
	motor activity	
AK005789	Dynein, cytoplasmic, light chain 2B	2.00
	nuclease activity	
NM_008287	Heat-responsive protein 12	3.32
	oxidoreductase activity	
NM_019657	Hydroxysteroid (17-beta) dehydrogenase 12	2.22
NM_026612	NADH dehydrogenase (ubiquinone) 1 beta subcomplex, 2	2.10
NM_007861	Dihydrolipoamide dehydrogenase	2.22
AK010065	Isocitrate dehydrogenase 3 (NAD+) alpha	2.66
	pre-mRNA splicing factor activity	
NM_009225	Small nuclear ribonucleoprotein B	2.04
	protein binding	
NM_022985	Zinc finger, A20 domain containing 3	2.48
NM_007657	CD9 antigen	2.02
NM_011119	Proliferation-associated 2G4	2.16
	protein phosphatase inhibitor activity	
NM_026731	Protein phosphatase 1, regulatory (inhibitor) subunit 14A	2.42

Table 1. Continued

Accession No.	Up regulated genes	Fold charge
	protein transporter activity	
AK002825	Synaptobrevin like 1	2.80
	protein tyrosine phosphatase activity	
NM_011200	Protein tyrosine phosphatase 4a1	2.12
	receptor binding	
NM_009312	Tachykinin 2	2.30
	stearoyl-CoA 9-desaturase activity	
NM_025797	Cytochrome b-5	2.16
	structural constituent of myelin sheath	
NM_008614	Myelin-associated oligodendrocytic basic protein	2.20
NM_025553	Mitochondrial ribosomal protein L11	2.08
NM_026467	Ribosomal protein S27-like	2.36
NM_011569	Tektin 1	2.12
	transcription factor activity	
NM_009537	YY1 transcription factor	2.28
	translation initiation factor activity	
U54563	Eukaryotic translation initiation factor 3, subunit 6	2.20
	tRNA nucleotidyltransferase activity	
AK010662	TRNA nucleotidyl transferase, CCA-adding, 1	2.08
	trypsin activity	
NM_011177	Protease, serine, 18	2.12
	tumor necrosis factor receptor binding	
NM_010735	Lymphotoxin A	2.22
	unfolded protein binding	
X53584	Heat shock protein 1 (chaperonin)	2.84
	voltage-gated potassium channel activity	
NM_026214	Potassium channel tetramerisation domain containing 4	2.30
	zinc ion binding	
NM_008505	LIM domain only 2	2.26
NM_010087	Dystrobrevin alpha	2.46
NM_019712	Ring-box 1	2.02

Table 2. Bax knockout down-regulation genes (76 genes)

Accession No.	Down regulation genes	Fold charge
	ATP binding	
NM_010716	Ligase III, DNA, ATP-dependent	-2.38
NM_008444	Kinesin family member 3B	-2.10
NM_011076	ATP-binding cassette, sub-family B (MDR/TAP), member 1A	-3.48
NM_030238	Dynein, cytoplasmic, heavy chain 1	-2.00
AK012664	Vaccinia related kinase 2	-2.36
NM_013686	T-complex protein 1	-2.14
	calcium channel regulator activity	
AJ006803	Neurexin II	-2.18
	calcium ion binding	
NM_013650	S100 calcium binding protein A8 (calgranulin A)	-9.34
NM_016760	Clathrin, light polypeptide (Lca)	-4.16
NM_013879	Calcium binding protein 1	-.2.72
NM_009038	Recoverin	-2.12
	cation channel activity	
AK017560	Potassium large conductance calcium-activated channel, subfamily M, alpha member 1	-2.72

Table 2. Continued

Accession No.	Down regulation genes	Fold charge
	chemokine activity	
NM_011339	Chemokine (C-X-C motif) ligand 15	-2.08
	cysteine protease inhibitor activity	
NM_008694	Neutrophilic granule protein	-4.52
	DNA binding	
NM_013685	Transcription factor 4	-2.00
NM_010056	Distal-less homeobox 5	-2.22
NM_008037	Fos-like antigen 2	-3.68
NM_025364	RIKEN cDNA 1110005A23 gene	-3.58
NM_009322	T-box brain gene 1	-2.36
NM_023472	Ankyrin repeat, family A (RFXANK-like), 2	-2.04
	galactoside 2-alpha-L-fucosyltransferase activity	
NM_019934	Secretory blood group 1	-2.00
	G-protein coupled receptor activity	
NM_053118	G protein-coupled receptor, family C, group 5, member D	-2.54
	GTP binding	
NM_011579	T-cell specific GTPase	-2.26
	guanyl-nucleotide exchange factor activity	
AK010755	RIKEN cDNA C330023D02 gene	-2.18
	hormone activity	
Z31361	Vasoactive intestinal polypeptide	-3.68
	hydrolase activity	
NM_007840	DEAD (Asp-Glu-Ala-Asp) box polypeptide 5	-3.76
NM_009645	Activation-induced cytidine deaminase	-2.12
NM_013590	P lysozyme structural	-3.46
Z23056	Protein tyrosine phosphatase, receptor type, B	-2.06
NM_011131	Polymerase (DNA directed), delta 1, catalytic subunit	-2.70
	insulin-like growth factor binding	
NM_008048	Insulin-like growth factor binding protein 7	-2.02
	kinase activity	
AK004757	Serine/threonine kinase 11 interacting protein	-2.48
NM_025741	RIKEN cDNA 4931412G03 gene	-2.1
	lyase activity	
NM_009799	Carbonic anhydrase 1	-2.04
	metal ion transporter activity	
AK003191	Solute carrier family 39 (metal ion transporter), member 13	-2.00
	NOT store-operated calcium channel activity	
NM_011644	Transient receptor potential cation channel, subfamily C, member 2	-2.04
	nuclease activity	
NM_019957	Deoxyribonuclease II beta	-2.46
NM_020583	Interferon-stimulated protein	-2.14
	nucleic acid binding	
NM_009577	Zinc finger protein interacting with K protein 1	-2.88
NM_009212	Immunoglobulin mu binding protein 2	-2.34
	oxidoreductase activity	
AK010185	RIKEN cDNA 2310075M15 gene	-2.3
NM_009656	Aldehyde dehydrogenase 2, mitochondrial	-2.52
NM_010343	Glutathione peroxidase 5	-2.56
	peptidoglycan receptor activity	
NM_009402	Peptidoglycan recognition protein 1	-2.08

Table 2. Continued

Accession No.	Down regulation genes	Fold charge
	protein binding	
NM_053126	Protocadherin beta 1	-2.04
NM_008164	Rhophilin, Rho GTPase binding protein 1	-2.36
NM_008507	Linker of T-cell receptor pathways	-2.00
NM_023585	Ubiquitin-conjugating enzyme E2 variant 2	-3.20
	protein phosphatase type 2A activity	
AK004686	Protein phosphatase 1, catalytic subunit, beta isoform	-2.04
	protein-tyrosine kinase activity	
NM_009371	Transforming growth factor, beta receptor II	-2.02
	receptor activity	
NM_007407	Adenylate cyclase activating polypeptide 1 receptor 1	-2.46
NM_008990	Poliovirus receptor-related 2	-2.04
NM_008330	Olfactory receptor 56	-2.28
NM_030553	Olfactory receptor 160	-2.02
	reduced folate carrier activity	
NM_031196	Solute carrier family 19 (sodium/hydrogen exchanger), member 1	-2.40
	rhodopsin-like receptor activity	
NM_010045	Duffy blood group	-3.10
	RNA binding	
NM_009080	Ribosomal protein L26	-2.42
	single-stranded RNA binding	
NM_021311	Piwi like homolog 1 (Drosophila)	-2.20
	structural constituent of cytoskeleton	
NM_009265	Small proline-rich protein 1B	-2.70
	structural constituent of ribosome	
NM_009082	Ribosomal protein L29	-3.20
NM_018853	Ribosomal protein, large, P1	-2.94
	transcription coactivator activity	
NM_008679	Nuclear receptor coactivator 3	-2.60
	transcription factor activity	
NM_008390	Interferon regulatory factor 1	-2.02
	transcriptional repressor activity	
NM_007528	B-cell CLL/lymphoma 6, member B	-4.38
	transferase activity, transferring glycosyl groups	
NM_008595	Manic fringe homolog (Drosophila)	-2.32
NM_021888	Queuine tRNA-ribosyltransferase 1	-2.30
	transferase activity	
NM_023850	Carbohydrate (keratan sulfate Gal-6) sulfotransferase 1	-2.38
AK017329	RIKEN cDNA 5430420P03 gene	-2.14
AK004787	N-deacetylase/N-sulfotransferase (heparan glucosaminyl) 1	-3.28
NM_009050	Ret proto-oncogene	-2.36
	transporter activity	
M23384	Solute carrier family 2 (facilitated glucose transporter), member 1	-2.20
NM_009197	Coiled-coil-helix-coiled-coil-helix domain containing 2	-2.26
	unfolded protein binding	
NM_025384	DnaJ (Hsp40) homolog, subfamily D, member 1	-2.94
	zinc ion binding	
NM_010712	LIM homeobox protein 4	-2.20
NM_007496	AT motif binding factor 1	-2.08
NM_009545	Ring finger protein 110	-2.12

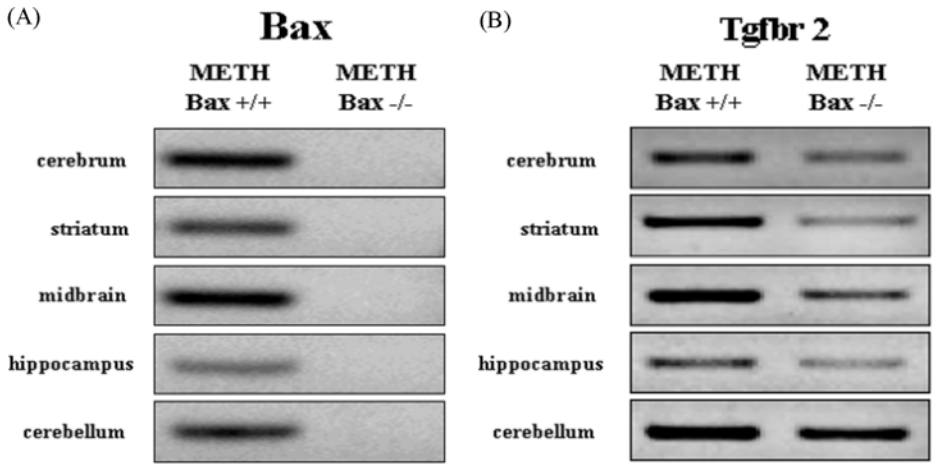

Fig. 5. Confirmation of Tgfbr2 expression using RT PCR. We analyzed Bax and Tgfbr2 gene expression in Bax deficient mouse each brain region after repeated methamphetamine treatment. A. Bax expression in each major brain region. B. Tgfbr2 expression in each major brain region.

present study, we analyzed the Tgfbr2 and Bax genes to further clarify the relationships between inactivation of the two genes and genomic instability in sporadic colorectal cancers. Tgfbr2 and Bax gene mutations contribute to tumor progression through the mutator phenotype pathway (Fernández-Peraltaa *et al.*, 2005).

Additionally, Bax gene and transcription regulators (Creb, FosB, c-Fos) affect rewarding effect in the mouse brain. We observed increase in CPP scores in Bax WT mice treated with methamphetamine. The transcription factor cAMP-responsive element-binding protein (Creb) has been shown to regulate rewarding effect and emotional behavior (Valverde *et al.*, 2004). Accordingly, the results of our Creb immunohistochemical experiment indicated a similar pattern with the CPP score of Bax upon exposure to methamphetamine, and Creb RT-PCR data is also demonstrated an impact of methamphetamine and Bax gene expression. FosB also showed a similar pattern to that of Creb. Immunohistochemical analysis of FosB and c-Fos expression revealed significant interactions with chronic methamphetamine treatment (Okabe *et al.*, 2005). Therefore, via an upside experiment methamphetamine influence on the transcription factors. As expected, Creb, FosB, and c-Fos were increased in samples following methamphetamine treatment. Also, because these genes were of concern in relation to apoptosis by Bax, we believe that expression would be higher in Bax WT mice, but the up-regulation of the Bax gene is likely to occur independently of the increased experssion of the c-Fos gene. The above results indicate that the expressions of Creb, FosB, and c-Fos suffer due to the impacts of methamphetamine. The early growth response (protein 1) and nerve growth factor-induced protein B (NGFI-B) were upregulated at 1 and 3 h after treatment with methamphetamine, as described previously. The expressions of novel genes, RL/IF-1 (coding for lkBa chain) and serum/glucocorticoid-regulated serine/threonine protein kinase (SGK), were also increased throughout the striatum.

Finally, we determined that methamphetamine affects mouse behavior or Bax and transcription factors related to drug use, and also found that the Bax gene influences transcription factors in mice following treatment with methamphetamine.

Acknowledgments This work was supported by the Korea Science and Engineering Foundation (KOSEF) through the National Research Lab. Program funded by MOST (M10500000101-06J0000-10110), the SRC/ERC program of MOST/KOSEF (Research Center for Women's Diseases), and a grant from the Neurobiology Research Program and Brain Research Center from the Korea MOST (M103KV010008 05K2201 00830). We are grateful to Dr. Woong Sun (Korea Univ.) for providing Bax deficient mouse.

References

Amirghofran, Z., Monabati, A. and Gholijani, N. (2005) Apoptosis in prostate cancer: Bax correlation with stage. *Int. J. Urol.* **12**, 340-345.

Asanuma, M., Hayashi, T., Ordonez, S. V., Ogawa, N. and Cadet, J. L. (2000) Direct interactions of methamphetamine with the nucleus. *Brain Res. Mol. Brain Res.* **80**, 237-243.

Bacon, A. L., Farrington, S. M. and Dunlop, M. G (2001) Mutation frequency in coding and non-coding repeat sequences in mismatch repair deficient cells derived from normal human tissue. *Oncogene* **20**, 7464-7471.

Cadet, J. L., Ordonez, S. V. and Ordonez, J. V. (1997) Methamphetamine induces apoptosis in immortalized neural cells: protection by the proto-oncogene, bcl-2. *Synapse* **25**, 176-184.

Chao, D. T. and Korsmeyer, S. J. (1998) Bcl2 FAMILY: Regulators of cell death. *Annu. Rev. Immunol.* **16**, 395-419.

Deng, X., Cai, N. S., McCoy, M. T., Chen, W., Trush, M. and Cadet, J. L. (2002) Methamphetamine induces apoptosis in an immortalized rat striatal cell line by activating the mitochondrial cell death pathway. *Neuropharmcology* **42**, 837-845.

Duan, X. X., Ou, J. S., Li, Y., Su, J. J., Ou, C., Yang, C., Yue, H. F. and Ban, K. C. (2005) Dynamic expression of apoptosis-related genes during development of laboratory hepatocellular carcinoma and its relation to apoptosis. *World J. Gastroenterol.* **11**, 4740-4744.

Fernández-Peraltaa, A. M., Nejdaa, N., Oliartb, S., Medinac, V., Azcoitad M. M. and González-Aguileraa, J. J. (2005) Significance of mutations in TGFBR2 and Bax in neoplastic progression and patient outcome in sporadic colorectal tumors with high-frequency microsatellite instability. *Cancer Genet. Cytogenet.* **157**, 18-24.

Genc, K., Genc, S., Kizildag, S., Sonmez, U., Yilmaz, O., Tugyan, K., Ergur, B., Sonmez, A. and Buldan, Z. (2003) Methamphetamine induces oligodendroglial cell death *in vitro*. *Brain Res.* **982**, 125-130.

Goping, I. S., Gross, A., Lavoie, J. N., Nguyen, M., Jemmerson, R., Roth, K., Korsmeyer, S. J. and Shore, G. C. (1998) Regulated Targeting of Bax to Mitochondria. *J. Cell Biol.* **143**, 207-215.

Hoffman, D. C. (1989) The use of place conditioning in studying the neuropharmacology of drug reinforcement. *Brain Res. Bull.* **23**, 373-387.

Jayanthi, S., Deng, X., Bordelon, M., McCoy, M. T. and Cadet, J. L. (2001) Methamphetamine causes differential regulation of pro-death and anti-death Bcl-2 genes in the mouse neocortex. *FASEB J.* **15**, 1745-1752.

Kita, T. and Nakashima, T. (2002) A recent trend in methamphetamine-induced neurotoxicity. *Nihon Shinkei Seishin Yakurigaku Zasshi*. **22**, 35-47.

Lee, Y. W., Son, K. W., Flora, G., Hennig, B., Nath, A. and Toborek, M. (2002) Methamphetamine activates DNA binding of specific redox-responsive transcription factors in mouse brain. *J. Neurosci. Res.* **70**, 82-89.

Narita, M., Akai, H., Kita, T., Nagumo, Y., Narita, M., Sunagawa, N., Hara, C., Hasebe, K., Nagase and H. and Suzuki, T. (2005) Involvement of mitogen-stimulated p70-s6 kinase in the development of sensitization to the methamphetamine induced rewarding effect in rats. *Neuroscience* **132**, 553-560.

Nestler, E. J., Kelz, M. B. and Chen, J. (1999) DeltaFosB: a molecular mediator of long-term neural and behavioral plasticity. *J. Brain Res.* **835**, 10-17.

Nestler, E. J. (2001) Molecular neurobiology of addiction. *Am J Addict.* **10**, 201-217.

Okabe, C., Takeshim, H. and Murphy, N. P. (2005) Methamphetamine sensitization in nociceptin receptor KO mice: locomotor and c-Fos expression. *Eur. J. Pharmacol.* **507**, 57-67.

Persico, A. M., Schindler, C.W., Zaczek, R., Brannock, M. T. and Uhl, G. R. (1995) Brain transcription factor gene expression, neurotransmitter levels, and novelty response behaviors: alterations during rat amphetamine withdrawal and following chronic injection stress. *Synapse* **19**, 212-227.

Puthalakath, H. and Strasser, A. (2002) Keeping killers on a tight leash: transcriptional and posttranslational control of the pro-apoptotic activity of BH3-only proteins. *Cell Death Differ.* **9**, 505-512.

Ron, D. and Jurd, R. (2005) The “ups and downs” of signaling cascades in addiction. *Sci STKE*. **2005**, 14.

Sorenson, C. M. (2004) Bcl-2 family members and disease. *Biochim. Biophys. Acta* **1644**, 169-177.

Valverde, O., Mantamadiotis, T., Torrecilla M., Ugedo L., Pineda J., Bleckmann, S. Gass P., Kretz, O., Mitchell, J. M., Schütz, G. and Maldonado, R. (2004) Modulation of anxiety-like behavior and morphine dependence in creb-deficient mice. *Neuropsychopharmacol.* **29**, 1122-1133.

Vereide A. B., Kaino, T., Sager, G. and Orbo, A. (2005) Bcl-2, Bax, and apoptosis in endometrial hyperplasia after high dose gestagen therapy: A comparison of responses in patients treated with intrauterine levonorgestrel and systemic medroxyprogesterone. *Gynecol. Oncol.* **97**, 740-750.

Walters, C. L., Cleck, J. N. and Kuo, Y. C. (2005) Opioid receptor and Creb activation are required for nicotine reward. *Neuron* **46**, 933-943.

Zhao, R. J., Woo, R. S., Jeong, M. S., Shin, B. S., Kim, D. G. and Kim, K. W. (2003) Orphanin FQ/nociceptin blocks methamphetamine place preference in rats. *Neuroreport* **14**, 2383-2385.

Zeng, N., Nakajima, A., He, J., Nitta, A., Yamada, K. and Nabeshima, T. (2004) Fos expression associated with the discriminative stimulus effects of methamphetamine in rats *Ann. N.Y. Acad. Sci.* **1025**, 236-241.