Korean J Physiol Pharmacol
Vol 4: 361−367, October, 2000

Amperozide Decreases Cocaine-Induced Increase in Behavior and Immediate Early Gene Expression in the Dorsal Striatum

Eun Sang Choe and Jong Yeon Kim[1]

Department of Anatomy and Cell Biology, East Carolina University School of Medicine, Greenville, NC 27858, U.S.A.; [1]Department of Physiology, Yeungnam University College of Medicine, Taegu 705−717, Korea

Cocaine functions as indirect dopamine and serotonin (5-hydroxytryptamine, 5HT) agonists and induces genomic and behavioral alterations in the striatum. Previously we demonstrated that ritanserin, a 5HT2/1C receptor antagonist, is not responsible for cocaine-induced behavioral alterations and zif268 mRNA gene expression in the striatum (see the previous paper in this issue). In this study, it was hypothesized that dopamine and 5HT2/1C receptors are required for cocaine-induced behavioral alterations and c-fos and zif268 mRNA expression. This hypothesis was addressed by infusing amperozide which antagonizes both 5HT2/1C and dopamine receptors and was analyzed using the quantitative in situ hybridization histochemistry in vivo. Systemic injection of amperozide (5 mg/kg, s.c.) significantly blocked increase in behavior, c-fos and zif268 mRNA expression induced by 15 mg/kg cocaine, i.p., in the dorsal striatum. These data suggest that dopamine and 5HT2/1C receptors are necessary for cocaine-induced behavioral alterations and immediate early gene expression in the dorsal striatum.

Key Words: Amperozide, Cocaine, Behavioral alteration, c-fos, zif268, In situ hybridization, Striatum

INTRODUCTION

The dorsal striatum is the focus of studies for neurochemical interactions underlying the effects of drugs of abuse because of its integrative role within basal ganglia circuits. Cocaine inhibits the presynaptic uptake of dopamine and 5HT in the central nervous system (Bhat & Baraban, 1993). The inhibition of monoamine uptake on the dopaminergic and serotonergic systems suggests that cocaine functions as an indirect dopamine and 5HT agonist (Cooper et al, 1995) and produces genomic and behavioral alterations. Cocaine as a blocker of the presynaptic transporters contributes to induce dopamine signaling via complicated intracellular mechanisms, which regulates immediate early gene expression, such as zif/268 and c-fos mRNAs (Daunais & McGinty, 1994, 1995, 1996; Moratalla et al, 1994).

Corresponding to: Jong Yeon Kim, Department of Physiology, Yeungnam University College of Medicine, Taegu 705-717, Korea. (Tel) 82-53-620-4330, (Fax) 82-53-651-3651, (E-mail) jykim@medical.yeungnam.ac.kr

Fos, a member of the Fos/Jun leucine zipper family, and Zif/268, a member of the zinc-finger family (Lau & Nathans, 1987), are widely studied transcription factors which have been demonstrated to mediate stimulus-induced long-term adaptive changes in cellular physiology (Sheng & Greenberg, 1990; Morgan & Curran, 1991; Nestler et al, 1993). These factors bind to regulatory regions of the target genes in a sequence-specific manner and orchestrate alterations in gene expression underlying stimulus-induced neuronal plasticity (Rhodes & Klug, 1993). Numerous in vivo studies showed that indirectly acting dopamine agonists, such as cocaine, amphetamine, or methamphetamine produced dramatic increase in c-fos and zif/268 mRNAs in rat forebrain.

Previously, we demonstrated that ritanserin, a 5HT2/1C receptor antagonist, did not block cocaine-induced behavioral alterations and zif268 mRNA expression in the striatum (Choe & Kim, see the previous paper in this issue). These data suggest that 5HT2/1C receptors are not responsible for cocaine-induced behavioral alterations and zif268 mRNA gene expression. In this study, therefore, we hypothesized that

5HT2/1C and dopamine receptors are required for cocaine-induced behavioral alterations and expression of immediate early genes, such as c-fos and zif268 mRNAs in the striatum. This hypothesis was addressed by infusing amperozide, which antagonizes both 5HT2/1C and dopamine receptors, prior to cocaine using the quantitative in situ hybridization histochemistry in vivo.

METHODS

Animals

Adult male Wistar rats (250~270 g) were obtained from Charles River Laboratories, Raleigh, NC. Rats were individually housed during all experimental treatment in the Department of Comparative Medicine, The Brody School of Medicine at East Carolina University. Food and water were provided ad libitum and the rats were maintained on a 12 hr light/dark cycle. All efforts were made to minimize animal suffering, to reduce the number of animals used, and to utilize alternatives to in vivo techniques. All the animal use procedures were approved by the University Animal Care and Use Committee, and were accomplished in accordance with the provisions of the NIH "Guide for the Care and Use of Laboratory Animals".

Experimental design

Rats were randomly divided into four groups: saline +saline, n=5; saline+15 mg/kg cocaine HCl (NIDA, calculated as the salt, diluted in sterile saline, pH 7.4), n=5; 5 mg/kg amperozide (RBI)+saline, n=5; 5 mg/kg amperozide+15 mg/kg cocaine, n=5. The first solutions were injected subcutaneously (s.c.) and the second solutions were injected intraperitoneally (i.p.) 30 min after the first injection. Rats were observed and behaviorally rated 5 min before the first injection and every 5 min for 55 min after the second injection using the modified nine-point rating scale from Ellinwood & Balster, 1974: 1) asleep and inactive, 2) normal activities, grooming, 3) increased activity, 4) hyperactive running with jerky movements, 5) slow patterned repetitive exploration, 6) fast patterned repetitive exploration with hyperactivity, 7) stereotypy (repetitive sniffing/rearing in one location to the exclusion of other activities), 8) continuous gnawing, sniffing or licking, 9) dyskinesia and seizure. The trained raters were blind to the drug treatments. The rats were anesthetized with Equithesin (5 ml/kg, i.p.) 55 min after the second injection and decapitated. The brains were removed from the skull and immediately frozen in isopentane at $-40^{\circ}C$ until they were sectioned for in situ hybridization histochemistry.

In situ hybridization histochemistry

Quantitative in situ hybridization histochemistry measuring c-fos and zif268 mRNAs was performed as described in Wang & McGinty, 1997. Briefly, 12 mm sections throughout the striatum were cut in a cryostat and thaw-mounted onto gelatin/chromalum-coated slides. The sections were fixed, defatted and pretreated before hybridization using a synthetic cDNA oligonucleotide probe of 40 base pairs directed against c-fos or zif/268 (Oncogene Research Products). The probes were labeled at the 3' end using a-^{35}S deoxyadenosine triphosphate (1,075 Ci/mmol, New England Nuclear) and terminal deoxynucleotidal transferase (Boehringer Mannheim) to a specific activity of $7-12\times10^5$ cpm/ml. Pretreated slides were incubated with 1×10^6 cpm/ml/25 ml hybridization buffer/section overnight (20 hr) at $37^{\circ}C$ in a humid environment. The slides were washed, dehydrated and exposed, along with ^{14}C standards (American Radiolabeled Chemicals), to Kodak X-OMAT film for 10 days. After film development, the slides were dipped in emulsion (Kodak NTB-3, IBI), exposed for three weeks, then developed with Dektol and fixed with Kodak fixer. Selected slides were lightly counterstained for Nissl substance with 0.1% Thionin. Quantitation of the mRNA hybridization signals on X-ray films was performed using NIH Image software. The ^{14}C standards were measured, plotted against known dpm/mg, and converted to ^{35}S equivalents to generate a calibration curve. After correcting for nonuniform illumination and subtracting film background by density slicing, the hybridization signals in the dorsal striatum were measured in each animal using a circular field with constant size of 168×215 pixels (Fig. 1). All measurements were made at a point corresponding to 1.2 mm rostral to Bregma (Swanson, 1992) from the right three adjacent coronal sections of each animal matched across animals for their rostral/caudal level. Quantitative changes were expressed as the number of labeled pixels per area (area), the mean density of tissue in dpm/mg, and the

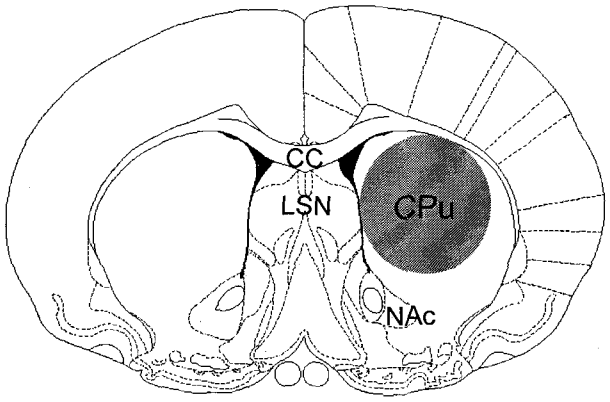

Fig. 1. Diagram of a coronal section depicting areas measured by imaging in rat forebrain. Circled region (CPu) indicates the area in which hybridization signals of c-fos or zif268 mRNA was measured by quantitative image analysis. CPu, Caudate-Putamen; NAc, Nucleus Accumbens; CC, Corpus Callosum; LSN, Lateral Septal Nuclei.

integrated density which is the product of area times mean density (Wang & McGinty, 1997).

Statistics

Behavioral data were analyzed by calculating the area under the curve (AUC) for the rating values plotted against time. One-way ANOVA followed by a Tukey's HSD (honest significant difference) test in SAS (Cary, NC) was performed on the AUC values. Statistical significance in area, mean density, and integrated density between groups was determined by a nested two-way ANOVA followed by a Bonfferroni-Dunn comparison of groups. This test is more precise than Tukey's HSD test when comparing least square means. All data except AUC values were subjected to a log transformation in order to obtain homogeneity of data.

RESULTS

Amperozide blocked cocaine-induced behavioral alterations

Rats treated with saline/saline showed a minimal fluctuation of behavioral activity after injection. Rats treated with saline/cocaine displayed sharply increased behavioral alterations within 5 min and reached a peak at 10 min after the cocaine injection, and then

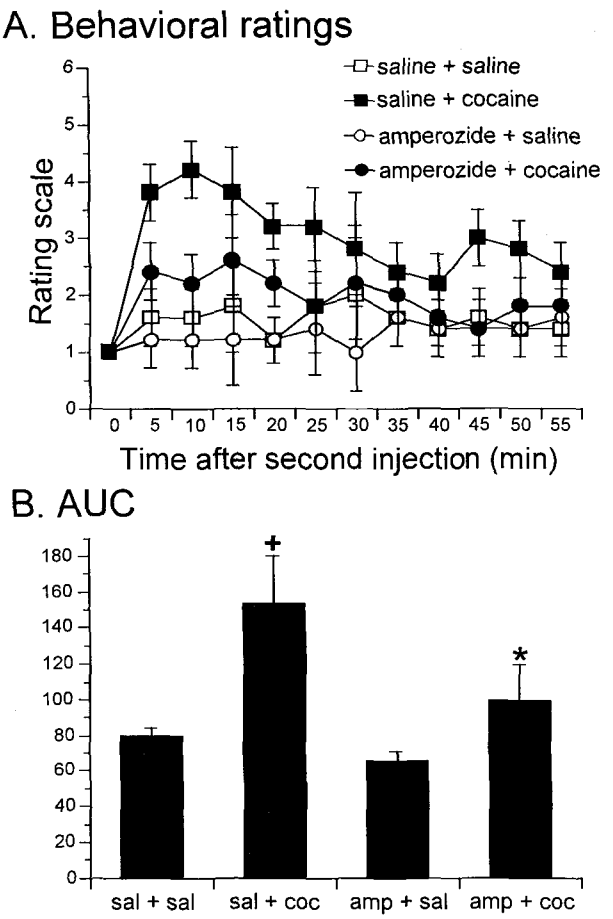

Fig. 2. Effects of amperozide on the behavioral alterations induced by cocaine in the rat (A). Behavioral alterations were measured 5 min before the first injection and every 5 min for 55 min after the second injection. The AUC values represented in B demonstrate that amperozide blocked cocaine-induced behavior. sal, saline; coc, cocaine; amp, amperozide. $^{\dagger}p$ and $^{*}p<0.05$ as compared with saline/saline and saline/cocaine, respectively.

gradually decreased their behavioral alterations along with the time course of the experiment. Quantitative analysis of the behavioral ratings (Fig. 2A) using overall AUC (Fig. 2B) revealed that saline/saline or amperozide/saline did not show statistical significance in generating behavioral alterations, whereas saline/cocaine significantly altered behavior as compared with the saline/saline control group. Pretreatment of amperozide significantly blocked increase in behavioral changes induced by cocaine as seen in Fig. 2B.

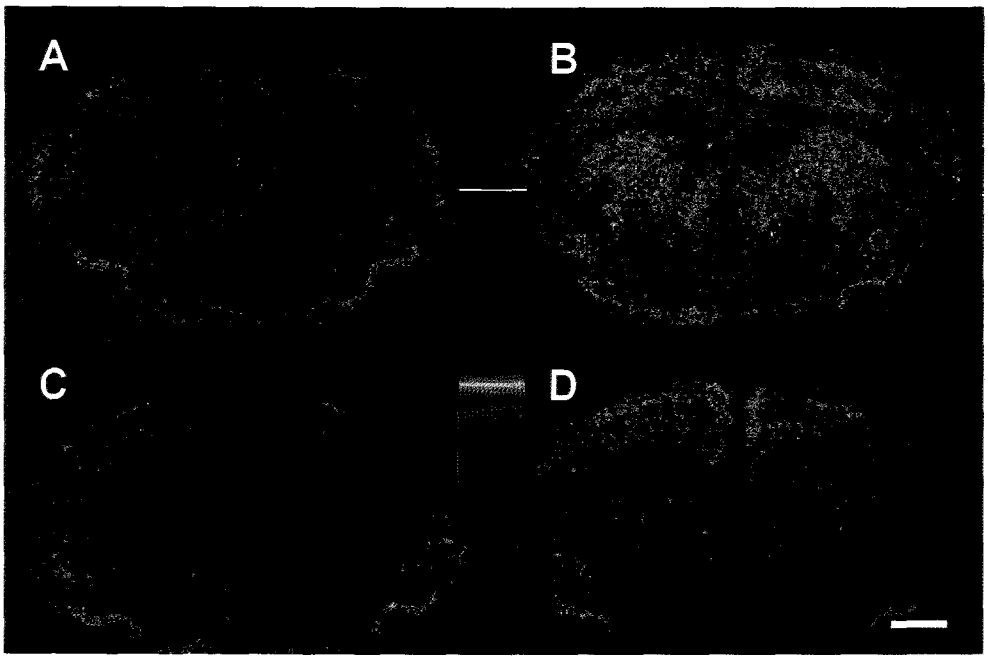

Fig. 3. Pseudo-color image from autoradiography illustrating the effect of amperozide on the constitutive and cocaine- induced expression of c-fos mRNA in the forebrain 55 min after a single injection of cocaine. A, saline/saline; B, saline/cocaine; C, amperozide/saline; D, amperozide/cocaine. In the color scale, red represents the strongest intensity of the marker. Bar represents 1 mm.

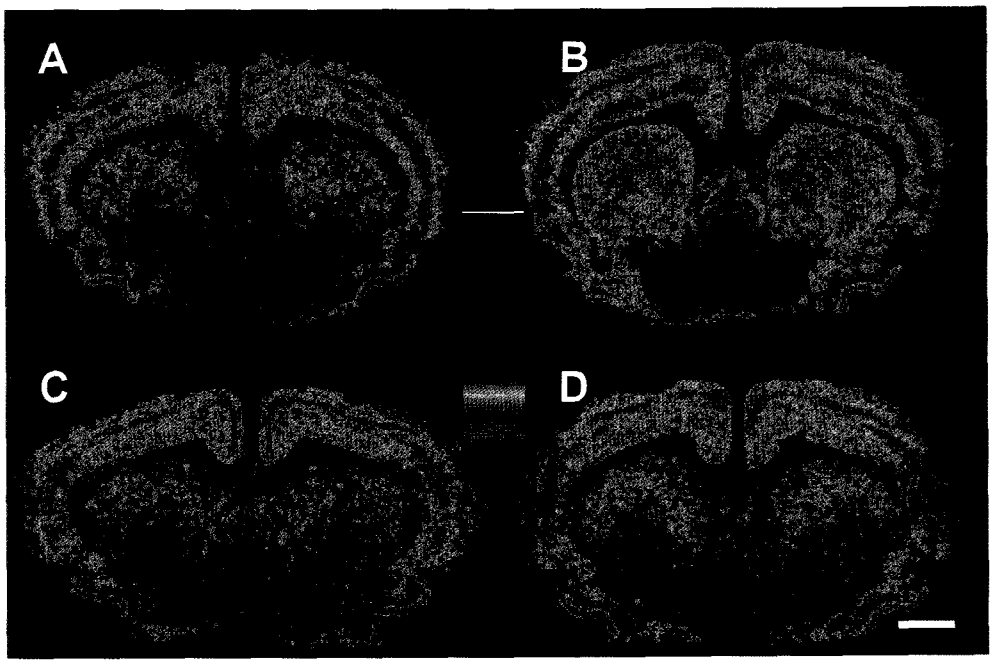

Fig. 4. Pseudo-color image from autoradiography illustrating the effect of amperozide on the constitutive and cocaine- induced expression of zif268 mRNA in the forebrain 55 min after a single injection of cocaine. A, saline/saline; B, saline/cocaine; C, amperozide/saline; D, amperozide/cocaine. In the color scale, red represents the strongest intensity of the marker. Bar represents 1 mm.

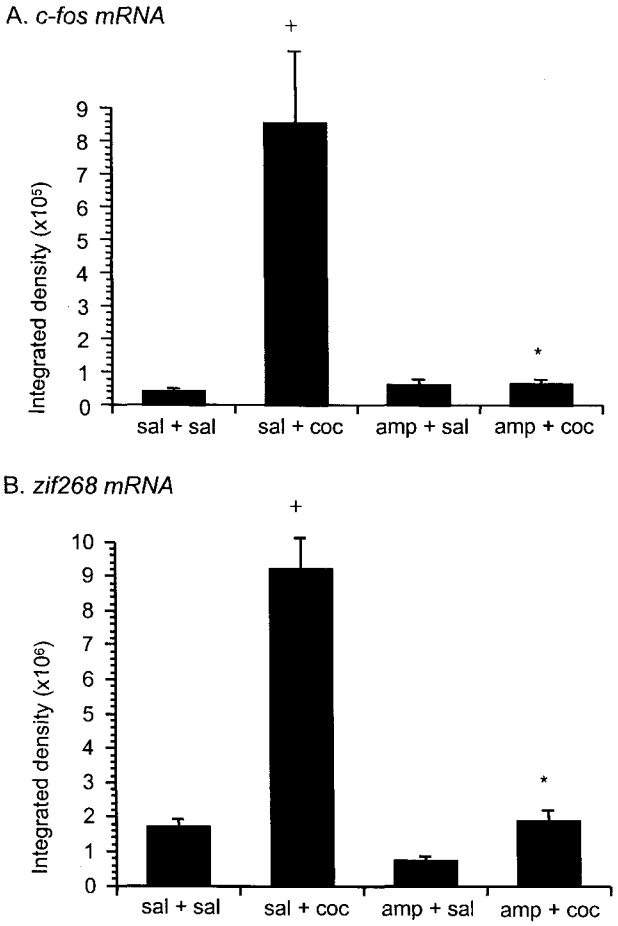

Fig. 5. Quantitative image analysis of the effect of amperozide on the integrated density values for cocaine-induced c-fos (A) and zif268 mRNA (B) expression in the dorsal striatum as illustrated in Fig. 1. Note that amperozide significantly blocked cocaine-induced c-fos and zif268 mRNA expression. sal, saline; coc, cocaine; amp, amperozide. $^{\dagger}p$ and $^{*}p<0.05$ as compared with saline/saline and saline/cocaine, respectively.

Amperozide blocked cocaine-induced c-fos and zif268 mRNA expression

Control sections from saline/saline or amperozide/saline showed low basal levels of c-fos mRNA throughout the sections (Fig. 3A, 3C). Cocaine increased the marker consistently in the dorsal striatum, lateral septal nuclei, and somatosensory cortices as compared to saline/saline controls (Fig. 3B). Pretreatment with amperozide decreased cocaine-induced c-fos mRNA expression in the dorsal striatum and lateral septal nuclei (Fig. 3D). Quantitative analysis revealed that amperozide infusion completely blocked cocaine-induced c-fos mRNA in the dorsal striatum (Fig. 5A). As compared to c-fos mRNA, the control sections from saline/saline or amperozide/saline infusion showed high basal levels of zif268 mRNA expression in the dorsal striatum and cortex (Fig. 4A, 4C). Treatment with saline/cocaine increased zif268 mRNA levels in the dorsal striatum (Fig. 4B). This increase was blocked by pre-infusion of amperozide (Fig. 4D). Quantitative analysis revealed that amperozide significantly blocked the cocaine-induced zif268 mRNA in the dorsal striatum (Fig. 5B).

DISCUSSION

In this study, amperozide decreased behavioral alterations and c-fos and zif268 mRNA expression induced by a single injection of cocaine in the dorsal striatum. Investigation of the pharmacological effects of amperozide revealed that behavioral changes and c-fos and zif268 mRNA gene expression induced by cocaine are closely related to 5HT2/1C and dopamine receptors in the dorsal striatum.

The hypothesis that 5HT2/1C receptors and dopamine receptors are required for cocaine-induced behavioral and immediate early gene expression was addressed by systemic administration of amperozide and cocaine. The present data shows that amperozide completely blocked cocaine-induced behavioral alterations in the dorsal striatum. Consistent with these data, Arolfo & McMillen (1999) demonstrated that amperozide prevented cocaine-induced conditioning of a place preference. Previous studies also demonstrated that amperozide attenuated cocaine-induced hyperlocomotion (Kimura et al, 1993), conditioned place preference (Jones & McMillen, 1999) as well as craving for cocaine (McMillen et al, 1993) in rats. These data suggest that amperozide influences the functional activity of both dopaminergic and serotonergic neurons by blocking dopamine and 5HT receptors in the mesolimbic system. This suggestion is supported by the finding that amperozide has affinity for 5HT2, D1 and D2 dopamine receptors in the forebrain regions (McMillen et al, 1993).

The present data also shows that amperozide completely blocked cocaine-induced c-fos and zif268 mRNA expression in the dorsal striatum. As a dopamine reuptake blocker, cocaine induced dramatic increase in c-fos and zif268 mRNA expression in the dorsal and ventral striatum of rat brain (Moratalla et al, 1992; Daunais & McGinty, 1994, 1995, 1996;

Ennulat et al, 1994). Bhat & Baraban (1993) demonstrated that transcription factor gene expression induced by cocaine appears to be mediated by pharmacologically defined D1 dopamine receptors. They also showed that the induction of immediate early gene expression in the striatum is due to the contribution of both dopaminergic and serotonergic systems. These data suggest that both systems are required for behavioral and genomic responsiveness by cocaine. The finding that immediate early gene expression including c-fos and zif268 mRNAs and behavior alterations induced by cocaine were decreased by amperozide suggests that the antagonism of dopamine and 5HT2/1C receptors is required for generating cocaine effects in the striatum. This conclusion would be supported indirectly by the finding that ritanserin, another 5HT2/1C receptor antagonist which acts on 5HT2/1C receptors, failed to block cocaine-induced behavioral alterations and zif268 mRNA expression in the striatum (Choe & Kim, 2000). Thus, both dopaminergic and serotonergic systems in the striatum would mediate behavioral changes and c-fos and zif268 mRNA expression induced by cocaine. However, the degree to which cocaine-induced behavior changes and gene expression may be D1- or D1 and D2 dopamine receptor-dependent in the striatum is unclear.

In conclusion, dopamine and 5HT2/1C receptors in the dorsal striatum may be involved in c-fos and zif268 mRNA expression as well as behavioral alterations induced by a single injection of cocaine. Further study would be required to determine how amperozide alters cocaine-induced behavior and gene expression. This information may contribute to the understanding of the neurochemical interactions underlying cocaine-mediated behavior and gene expression in the striatum in vivo.

ACKNOWLEDGMENTS

The authors would like to thank Dr. Jacqueline F. McGinty at the Department of Physiology and Neuroscience of Medical University of South Carolina for her generous support. The authors also would like to thank Denise C. Mayer at the Department of Anatomy and Cell Biology of East Carolina University School of Medicine for technical assistance and behavioral ratings.

REFERENCES

Arolfo MP, McMillen BA. Effects of amperozide and tiospirone, atypical antipsychotic 5-HT2 drugs, on food-reinforced behavior in rats. *Physiol Behav* 68: 93–98, 1999

Bhat RV, Baraban JM. Activation of transcription factor genes in striatum by cocaine: role of both serotonin and dopamine systems. *J Pharmacol Exp Ther* 267: 496–505, 1993

Choe ES, Kim JY. Ritanserin, a 5HT2/1C receptor antagonist, does not block cocaine-induced behavioral alterations and zif268 mRNA expression in the striatum of the rats. *Korean J Physiol Pharmacol* 4: 355–359, 2000

Cooper JR, Bloom FE, Roth RH. The biochemical basis of neuropharmacology. 5th ed. Oxford University Press, New York, NY, 1995

Daunais JB, McGinty JF. Acute and chronic cocaine administration differentially alters striatal opioid and nuclear transcription factor mRNAs. *Synapse* 18: 35–45, 1994

Daunais JB, McGinty JF. Cocaine binges differentially alter striatal preprodynorphin and *zif/268* mRNAs. *Mol Brain Res* 29: 201–210, 1995

Daunais JB, McGinty JF. The effects of D1 or D2 dopamine receptor blockade on *zif/268* and preprodynorphin gene expression in rat forebrain following a short-term cocaine binge. *Mol Brain Res* 35: 237–248, 1996

Ellinwood EH, Balster RL. Rating the behavioral effects of amphetamine. *Eur J Pharmacol* 28: 35–41, 1974

Ennulat DJ, Babb SM, Cohen BM. Persistent reduction of immediate early gene mRNA in rat forebrain following single or multiple doses of cocaine. *Mol Brain Res* 26: 106–112, 1994

Jones EA, McMillen BA. The cardiovascular effects of amperozide: interactions with cocaine. *Pharmacol Toxicol* 84: 53–58, 1999

Kimura K, Nomikos GG, Svensson H. Effects of amperozide on psychostimulant-induced hyperlocomotion and dopamine release in the nucleus accumbens. *Pharmacol Biochem Behav* 44: 27–36, 1993

Lau LF, Nathans D. Expression of a set of growth-related immediate early genes in BALB/c 3T3 cells: coordinate regulation with *c-fos* or *c-myc*. *Proc Natl Acad Sci USA* 84: 1182–1186, 1987

McMillen BA, Jones EA, Hill LJ, Williams HL, Bjork A, Myers RD. Amperozide, a 5-HT2 antagonist, attenuates craving for cocaine by rats. *Pharmacol Biochem Behav* 46: 125–129, 1993

Moratalla R, Robertson HA, Graybiel AM. Dynamic regulation of *NGFI-A* (*zif268, egr1*) gene expression in the striatum. *J Neurosci* 12: 2609–2622, 1992

Moratalla R, Elibol B, Graybiel AM. Acute and chronic psychostimulants induce different sets of immediate-

early genes in the striatum. *Soc Neurosci Abstr* 20: 990, 1994

Morgan JI, Curran T. Stimulus-transcription coupling in the nervous system: involvement of the inducible proto-oncogenes fos and jun. *A Rev Neurosci* 14: 421, 1991

Nestler EJ, Hope BT, Widnell KL. Drug addiction: model for the molecular basis of neural plasticity. *Neuron* 11: 995–1006, 1993

Rhodes D, Klug A. Zinc fingers. *Scientific American* 268: 56–65, 1993

Sheng M, Greenberg ME. The regulation and function of c-fos and other immediate early genes in the nervous system. *Neuron* 4: 477–485, 1990

Swanson LW. Brain maps: structure of rat brain. Elsevier, Amsterdam, 1992

Wang JQ, McGinty JF. Intrastriatal injection of a muscarinic receptor agonist and antagonist regulates striatal neuropeptide mRNA expression in normal and amphetamine-treated rats. *Brain Res* 748: 62–70, 1997