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Regulation of Immediate Early Gene Expression by Glutamate Receptor Activation in C6 Rat Glioma Cells

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We have studied the effects of excitatory amino acids on the expression of the c-fos and c-jun mRNA in rat C6 glioma cells. The glutamate, N-methyl-D-aspartate (NMDA), and kainic acid (KA) increased c-fos mRNA level in a concentration-dependent manner. However, they did not affect c-jun mRNA level. In addition, forskolin and phorbol 12-myristate 13-acetate (PMA) increased c-fos mRNA level. Furthermore, PMA increased c-jun mRNA level whereas forskolin downregulated c-jun mRNA level. The glutamate, NMDA and KA, at a concentration of 0.25 mM, did not affect the basal c-fos and c-jun mRNA levels, and also did not affect forskolin- and PMA-induced responses. Furthermore, both forskolin and PMA itself increased the phosphorylation of ERK (extracellular signal regulated kinase) and CREB (cyclicAMP responsible element binding protein) proteins. The KA, NMDA, and glutamate did not affect forskolin-induced increase of ERK and CREB phosphorylation. The KA decreased PMA-induced increase of phosphorylation of ERK and CREB proteins, whereas glutamate and NMDA did not affect the phosphorylation of ERK and CREB proteins induced by PMA. These findings suggest that, in C6 glioma cells, c-fos mRNA induction induced by EAAs is not mediated by phosphorylation of ERK and CREB proteins.

Key Words: Kainic acid, Glutamic acid, N-methylaspartate, fos genes, jun protooncogene, Glioma

INTRODUCTION

Excitatory amino acids (EAAs) have been divided into three major subtypes on the basis of electrophysiological studies performed *in vivo* and *in vitro* on neural tissues; the kainic acid (KA) receptor, the quisqualic acid receptor and N-methyl-D-aspartate (NMDA) receptor (Collingridge & Lester, 1989; Young & Fagg, 1990). Activation of NMDA, AMPA (alpha-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid), KA and metabotropic receptor subtypes by glutamate, the most ubiquitous cerebral neurotransmitter, leads to an increase in the levels of free intracellular calcium (Coyle & Puttfarcken, 1993). Glutamate release is closely associated with serious neurological disorders such as epilepsy, stroke, hypoxia, and brain trauma (Coyle & Puttfarcken, 1993; Kalda et al, 1998). In addition to its important role as a neurotransmitter, glutamate at high level is excitotoxic to neurons. Glutamate also increases DNA binding of the redox-regulated transcription factors, NF-*k*B and AP-1, in human neuroblastoma cells (Griffiths et al, 1997; Lezoualc'h et al, 1998), and increases the expression of the immediate early gene, c-fos, in murine neuronal cells (Griffiths et al, 1997). A rapid and transient elevation of mRNA levels for c-fos was observed after addition of glutamate to primary cultured astrocytes. Furthermore, the level of AP-1 DNA binding activity, as measured by the electrophoretic mobility shift assay, was also increased after addition of glutamate to cultured astrocytes (Condorelli et al, 1993). In several studies in neurons, MAPKs and CREB were activated by excitatory amino acids (Kurino et al, 1995; DeCoster et al, 1998; Vanhoutte et al, 1999). However, the roles of mitogen activating protein

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kinase (MAPK) and cyclic AMP responsible element binding protein (CREB) in the regulation of immediate early genes by excitatory amino acids in glial cells have not been well characterized.

The present studies were then designed to examine the possible role of EAAs in the regulation of c-fos and c-jun mRNA expression, and the phosphorylation of ERK and CREB proteins in C6 rat glioma cells. Furthermore, the possible modulatory role of excitatory amino acids in the regulation of c-fos and c-jun mRNA expression induced by PKA (forskolin) and PKC (PMA) activators was examined.

METHODS

Cell culture and drug treatment

C6 rat glioma cells were plated on 25 cm^2 culture flasks (Corning, NY, USA) containing growth media; Dulbecco's modified eagle's medium (DMEM) (Gibco, NY, USA) containing 10% heat inactivated Fetal bovine serum (FBS) (Gibco, NY, USA), 2.2 g/l sodium bicarbonate, 0.6% (w/v) D-glucose, and 20 mg/ml gentamicin. The cultures were incubated at 37°C in 5% CO_2 for 3 days. The cells were incubated with serum free culture medium for 24 h prior to the incubation with drugs. Drugs used for the treatment were phorbol 12-myristate 13-acetate (PMA) (RBI, Natick, MA), forskolin (Sigma, St Louis, MO), kainic acid (Sigma, St Louis, MO), glutamate (Sigma, St Louis, MO), and *N*-methyl-D-aspartate (RBI, Natick, MA).

Preparation of DIG-labeled cRNA probes

The cRNA probes for *c-fos* (Curran et al, 1987), *c-jun* (Kitabayahi et al, 1990), and *cyclophilin* (Danielson et al, 1988) were synthesized *in vitro* from linearized expression pGEM-4Z vector, which contained SP6 or T7 viral promoter. One microgram of linearized plasmid was mixed with RNA labeling mixture (Boehringer Mannheim, Mannheim, Germany) that containing ATP, CTP, GTP and DIG-labeled-UTP, and transcription buffer, and SP6 or T7 RNA polymerase. After incubation at 37°C for 2 h, the mixture was co-incubated with DNase I (RNase free) at 37°C for 15 min, precipitated in 100% ethanol containing lithium chloride at 70°Ç for 30 min, and washed with 70% chilled ethanol.

Isolation of total RNA

Total cellular RNA was extracted from rat C6 glioma cells using a rapid guanidine thiocyanate-water saturated phenol/chloroform extraction and subsequent precipitation with acidic sodium acetate (Chomczynski & Sacchi, 1987). Total cellular RNA in the aqueous phase was precipitated with cold isopropyl alcohol. Isolated RNA samples were subjected to spectrophotometric analysis at 260 nm.

Non-isotope northern blot hybridization analysis

Ten micrograms total RNA were denatured and electrophoresed on 1% agarose-formaldehyde gels (Kopchik et al, 1981) and transferred to nylon hybond-N hybridization membrane sheets (Amersham, Buckinghamshire, England). After UV cross-linking, the membranes were prehybridized at 68°C for at least 1 hr in prehybridization buffer (5 X SSC, 50% formamide, 0.02% SDS, 0.1% sodium N-lauroyl sarcosine, and 2% blocking reagent). The DIG-labeled *c-fos* and *c-jun* probes were added to prehybridization buffer containing 50% formamide. The membranes were incubated overnight at 68°C in a shaking water bath, and washed twice for 15 min per wash in 2 X wash solution (2 X SSC, 0.1% SDS) at room temperature. Then, the membranes were washed twice for 15 min per wash in 0.1 X washing solution (0.1 X SSC, 0.1% SDS). After equilibrating the membranes in buffer I (100 mM maleic acid [pH 7.5], 150 mM NaCl) for 1 min, the membranes were gently agitated in buffer II (1% blocking reagent in buffer I) for 30-60 min. The membranes were hybridized with the diluted anti-DIG-alkaline phosphatase [1 : 10,000 (75 mU/ml)] in buffer II for 30 min. After washing the membrane twice for 15 min in 0.3% Tween 20 in buffer I, the membranes were equilibrated in buffer III (100 mM Tris-HCl [pH 9.5], 100 mM NaCl, 50 mM $MgCl_2$) for 2 min. Diluted CSPD® (Boehringer Mannheim, Mannheim, Germany) (1 : 100 diluted in buffer III) was spread over the surface of membrane. After incubation of membrane at 37°C for 15~20 min, the membranes were exposed to Hyperfilm-ECL (Amersham, Buckinghamshire, England) for detection of the chemilluminescent signal. For rehybridization, membranes were washed for 20 min at room temperature in sterile millipore water, then the membranes were washed for overnight at 65°C in 50 mM Tris-HCl (pH 8.0), 50% dimethylformamide and 1% SDS to remove the hy-

bridized probe and rehybridized to DIG-labeled rat *cyclophilin* cRNA probe, a gene encoding peptidyl-prolyl cis-trans isomerase, which is constitutively expressed in most mammalian tissues with the exception of skeletal muscle (Takahashi et al, 1989).

Total cellular protein extraction and western blot analysis

After incubation in the presence or absence of different stimuli (forskolin, PMA, cycloheximide, and dexamethasone), cells were washed two times with cold Tris buffered saline (TBS; 20 mM Trizma base and 137 mM NaCl, pH 7.5). Immediately after washing, cells were lysed with SDS lysis buffer (62.5 mM Trizma base, 2% w/v SDS, 10% glycerol) containing 0.1 mM Na_3VO_4, 3 mg/ml aprotonin, and 20 mM NaF. After brief sonication to shear DNA and reduce viscosity, the concentration of protein was determined with the detergent compatible protein assay reagent (Bio-Rad Laboratories, CA, USA) using bovine serum albumin as the standard. After adding with dithiothreitol (5 mM) and bromophenol blue (0.1% w/v), the proteins were boiled, separated by electrophoresis in 10~12% polyacrylamide gels, and transferred onto a polyvinylidene difluoride membrane. The membranes were immunoblotted with antibodies against phospho-CREB and phospho-ERK1/2 (New England Biolabs, Bevery, MA) and visualized with ECL-plus solution (Amersham, Buckinghamshire, England).

RESULTS

The effect of EAAs on the expression of c-fos and c-jun mRNA

C-fos and c-jun mRNA levels in rat C6 glioma cells were examined after the treatment with EAAs, such as KA, glutamate, and NMDA. As shown in Fig. 1, c-fos mRNA level was increased by KA, NMDA, and glutamate in a concentration-dependent manner 1 h after drug treatment. Maximal increase of c-fos mRNA level by all EAAs was detected at a highest concentration (1 mM). In contrast to the results with c-fos, KA, NMDA, and glutamate did not affect c-jun mRNA level (Fig. 1).

The effects of c-fos and c-jun mRNA expression by EAAs on PKA and PKC acivated C6 glioma cells

To determine if EAAs can modulate the c-fos and c-jun mRNA expression induced by activation of PKA and PKC pathways in C6 rat glioma cells, lower

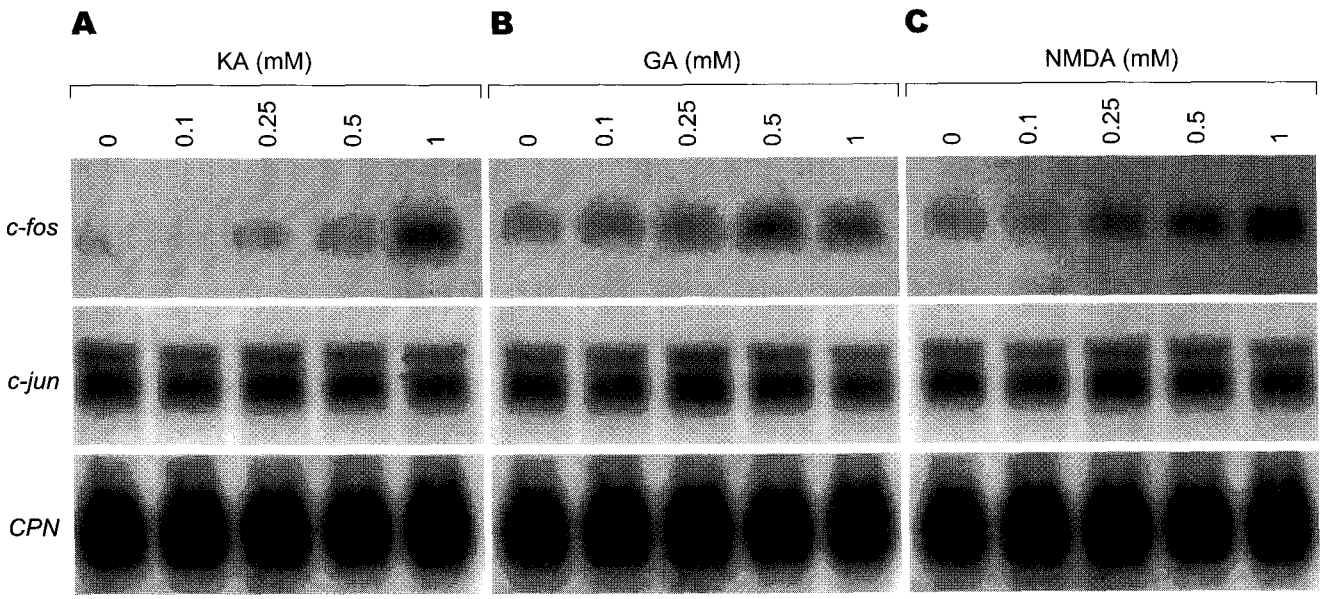

Fig. 1. The concentration-dependent effects of excitatory amino acids on c-fos and c-jun mRNA expression in C6 glioma cells. At 1 h after (A) kainic acid (KA); (B) glutamate (GA); (C) N-methyl-D-aspartate (NMDA) treatment, the levels of c-fos amd c-jun mRNA were examined using Northern blot analysis. The unregulated mRNA level of cyclophilin (CPN) was used for internal loading control in Northern blot anaysis.

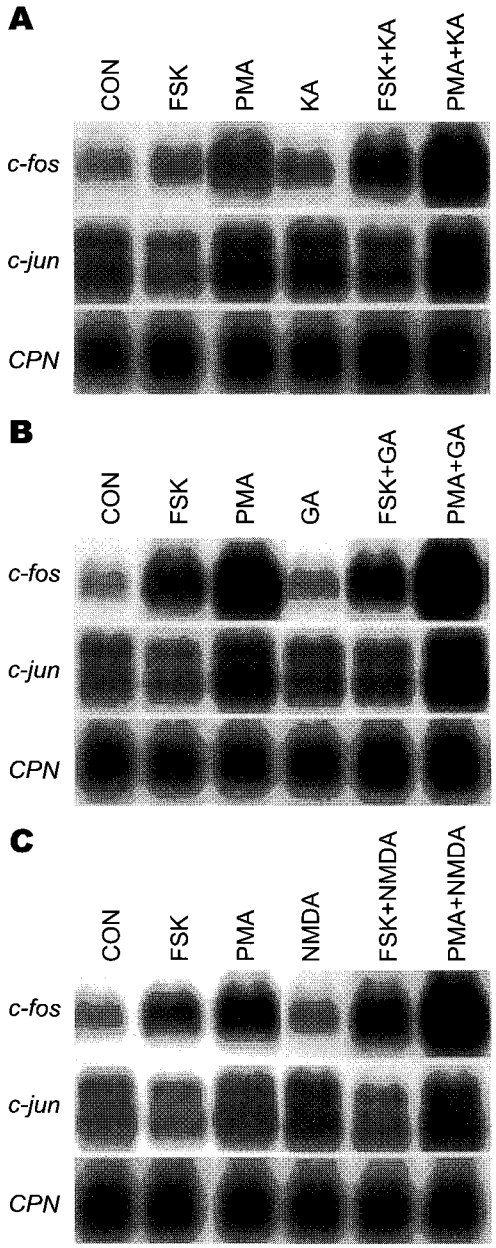

Fig. 2. The effects of excitatory amino acids on c-fos and c-jun mRNA levels in the PKA and PKC stimulated C6 glioma cells. At 1 h after (A) kainic acid (KA; 0.25 mM); (B) glutamate (GA; 0.25 mM); (C) N-methyl-D-aspartate (NMDA; 0.25 mM) treatment, the levels of c-fos and c-jun mRNA were examined using Northern blot analysis. Forskolin (FSK; 3 mM) and phorbol 12-myristate 13-acetate (PMA; 1 mM) were pretreated 30 min prior to EAAs treatment. The unregulated mRNA level of cyclophilin (CPN) was used for loading control in Northern blot anaysis.

concentration of EAAs was cotreated with forskolin (FSK) or PMA. The PKA activator forskolin alone increased c-fos mRNA level whereas c-jun mRNA

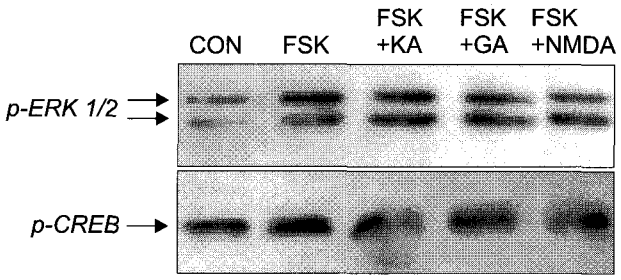

Fig. 3. The effects of EAAs on the phosphorylation of ERK 1/2 and CREB in the PKA activated C6 glioma cells. At 1 h after EAAs (KA, GA, and NMDA; 0.25 mM) treatment, the phosphorylation of ERK 1/2 (42/44 kDa) and CREB (43 kDa) was examined using the western blot analysis. Forskolin (FSK; 3 mM) was pretreated 30 min prior to EAAs treatment. Antibodies against phospho-ERK 1/2 and phospho-CREB were used at a 1 : 1000 dilution.

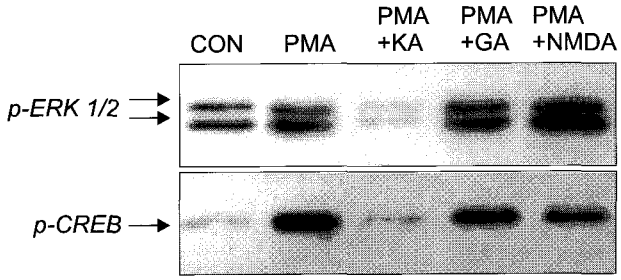

Fig. 4. The effects of EAAs on the phosphorylation of ERK 1/2 and CREB in the PKC activated C6 glioma cells. At 1 h after EAAs (KA, GA, and NMDA; 0.25 mM) treatment, the phosphorylation of ERK 1/2 (42/44 kDa) and CREB (43 kDa) was examined using the western blot analysis. Phorbol 12-myristate 13-acetate (PMA; 1 mM) was pretreated 30 min prior to EAAs treatment. Antibodies against phospho-ERK 1/2 and phospho-CREB were used at a 1 : 1000 dilution.

level was down-regulated (Fig. 2). However, PKC activator PMA alone increased both c-fos and c-jun mRNA levels (Fig. 2). The glutamate, NMDA, and KA, at a concentration of 0.25 mM, did not affect the basal c-fos and c-jun mRNA levels, and did not affect forskolin- and PMA-induced c-fos and c-jun mRNA expressions (Fig. 2).

The effects of phosphorylation of ERK 1/2 and CREB proteins by EAAs on PKA and PKC activated C6 glioma cells

To examine the possible role of ERK or CREB

proteins in the regulation of immediate early gene expression, western blot analysis using antibodies against phospho-ERK 1/2 (p44/p42 MAPK) and phospho-CREB (43 kDa) proteins were performed. Both forskolin and PMA increased the phosphorylation of ERK and CREB (Figs. 3 and 4). However, phospho-ERK and phospho-CREB levels were not affected by EAAs in a dose-dependent manner (data not shown). The KA, NMDA, and glutamate did not affect forskolin-induced increase of ERK and CREB phosphorylation (Fig. 3). Furthermore, KA decreased PMA-induced increase of phospho-ERK and phospho-CREB, whereas glutamate and NMDA did not affect the phosphorylation of ERK and CREB induced by PMA (Fig. 4).

DISCUSSION

In the present study, we tried to characterize the effect of EAAs on the regulation of c-fos and c-jun immediate early genes (IEGs) in C6 rat glioma cells, and found that EAAs (KA, glutamate, and NMDA) increased c-fos, but not c-jun, mRNA level in a concentration-dependent manner. This result suggests that both NMDA and non-NMDA receptors are involved in induction of c-fos mRNA level by EAAs in C6 glioma cells. In addition, in primary cultured astrocytes, a rapid and transient elevation of mRNA level for c-fos was observed after addition of glutamate (Condorelli et al, 1993). Furthermore, glutamate and KA strongly induced c-fos mRNA in oligodendrocyte (Liu & Almazan, 1995). However, earlier study by McNaughton & Hunt (1992) has demonstrated that NMDA receptor activation did not affect c-fos mRNA expression in primary cultured astrocytes. Based on these findings, it is suggested that the role of NMDA or non-NMDA receptors in the regulation of c-fos mRNA expression appears to be different in several types of glial cells.

We found in the present study that, in C6 rat glioma cells, c-jun mRNA level is not altered by EAAs. In contrast to our results in c-jun mRNA expression by EAAs, previous studies have reported that EAAs cause the elevation of c-jun mRNA regulation in primary astroglial culture (McNaughton & Hunt, 1992; Vaccarino et al, 1992; Condorelli et al, 1993). Additionally, in neuronal cells, a number of studies have reported that c-jun mRNA level is upregulated by KA, glutamate, or NMDA (Szekely et al, 1989; Condorelli et al, 1994; Bading et al, 1995; Griffiths et al, 1997; Cheung et al, 1998). Thus, it is suggested that the role of NMDA or non-NMDA receptors for the regulation of c-jun mRNA expression appears to be different in glioma cells, primary cultured astrocytes, and neuronal cells.

In the present study, we found that forskolin increased c-fos mRNA level whereas c-jun mRNA level was down-regulated by forskolin. In addition, PMA increased both c-fos and c-jun mRNA levels. Recently, we have reported that forskolin induced down-regulation of c-jun mRNA expression through a PKA, L-type calcium channels, calmodulin and Ca^{2+}/calmodulin-dependent protein kinase II (Lee et al, 1999). The cellular action of EAAs is primarily attributed to the Ca^{2+} in flux (Bading et al, 1995; Rodriguez et al, 2000; Skradski & White, 2000). Thus, it is possibly expected that EAAs may modulate c-fos expression induced by PKA or PKC activator. However, in the present study, we found that glutamate, NMDA, and KA, at a concentration of 0.25 mM, which did not affect the basal c-fos and c-jun mRNA levels, did not affect forskolin- and PMA-induced c-fos and c-jun mRNA expression. These findings suggest that no modulatory role of EAAs for the regulation of immediate early genes expression induced by PKA and PKC activation in C6 rat glioma cells.

To examine the involvement of phosphorylation of ERK 1/2 and CREB proteins in EAAs-induced c-fos mRNA expression, effects of EAAs, forskolin, and PMA on the phosphorylation of ERK 1/2 and CREB proteins were examined. The western blot analysis revealed that phosphorylation of ERK and CREB proteins was not affected by EAAs at all concentrations (0.1 ~ 1 mM) tested (data not shown). In contrast to our finding, several studies have reported that the phosphorylation of ERK 1/2 or CREB proteins in primary cultured hippocampal neurons (Kurino et al, 1995; DeCoster et al, 1998), cultured striatal neurons (Schwarzschild et al, 1999), oligodendroglial cells (Liu et al, 1999), and auditory neurons (Zirpel et al, 2000) were increased. In addition, we found in the present study that forskolin or PMA itself increases the phosphorylation of ERK 1/2 and CREB proteins. These results are in line with previous studies that the phosphorylation of ERK 1/2 and CREB proteins increased by PKA or PKC activation in astrocytes and glioma cells (Won et al, 1998; Abe & Saito, 2000; Won & Suh, 2000). All KA, NMDA, and glutamate did not affect the phosphorylation of

ERK 1/2 and CREB proteins induced by forskolin. Furthermore, NMDA and glutamate did not affect the phosphorylation of ERK 1/2 and CREB proteins induced by PMA. However, KA decreased the phosphorylation of ERK 1/2 and CREB proteins induced by PKC activation. Although, the underlying mechanism involved in this finding is currently not well understood, our findings suggest that c-fos up-regulation induced by glutamate, NMDA, and KA may not be mediated by the phosphorylation of ERK 1/2 or CREB protein.

In summary, in C6 rat glioma cells, c-fos mRNA expression was increased by KA, NMDA, and glutamate whereas c-jun mRNA level was not affected by EAAs. The elevation of c-fos mRNA level induced by KA, NMDA, and glutamate is not mediated by the phosphorylation of ERK and CREB proteins.

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REFERENCES

Abe K, Saito H. The p44/42 mitogen-activated protein kinase cascade is involved in the induction and maintenance of astrocyte stellation mediated by protein kinase C. *Neurosci Res* 36: 251−257, 2000

Bading H, Segal MM, Sucher NJ, Dudek H, Lipton SA, Greenberg ME. N-methyl-D-aspartate receptors are critical for mediating the effects of glutamate on intracellular calcium concentration and immediate early gene expression in cultured hippocampal neurons. *Neuroscience* 64: 653−664, 1995

Cheung NS, Carroll FY, Larm JA, Beart PM, Giardina SF. Kainate-induced apoptosis correlates with c-jun activation in cultured cerebellar granule cells. *J Neurosci Res* 52: 69−82, 1998

Chomczynski P, Sacchi N. Single-step method of RNA isolation by acid guanidium thiocyanate-phenol-chloroform extraction. *Anal Biochem* 162: 156−159, 1987

Collingridge GL, Lester RA. Excitatory amino acid receptors in the vertebrate central nervous system. *Pharmacol Rev* 41: 143−210, 1989

Condorelli DF, Dell'Albani P, Amico C, Kaczmarek L, Nicoletti F, Lukasiuk K, Stella AM. Induction of primary response genes by excitatory amino acid receptor agonists in primary astroglial cultures. *J Neurochem* 60: 877−885, 1993

Condorelli DF, Dell'Albani P, Amico C, Lukasiuk K, Kaczmarek L, Giuffrida-Stella AM. Glutamate receptor-driven activation of transcription factors in primary neuronal cultures. *Neurochem Res* 19: 489−499, 1994

Coyle JT, Puttfarcken P. Oxidative stress, glutamate, and neurodegenerative disorders. *Science* 262: 689−695, 1993

Curran T, Gordon MB, Rubino KL, Sambucetti LC. Isolation and characterization of c-fos (rat) cDNA and analysis of post-translational modification in vitro. *Oncogene* 2: 79−84, 1987

Danielson PE, Forss-Petter S, Brow MA, Calavetta L, Douglas J, Milne RJ, Sutchliffe JG. p1B15: a cDNA clone of the rat mRNA encoding cyclophilin. *DNA* 7: 261−267, 1988

DeCoster MA, Mukherjee PK, Davis RJ, Bazan NG. Platelet-activating factor is a downstream messenger of kainate-induced activation of mitogen-activated protein kinases in primary hippocampal neurons. *J Neurosci Res* 53: 297−303, 1998

Griffiths R, Malcolm C, Ritchie L, Frandsen A, Schousboe A, Scott M, Rumsby P, Meredith C. Association of c-fos mRNA expression and excitotoxicity in primary cultures of mouse neocortical and cerebellar neurons. *J Neurosci Res* 48: 533−542, 1997

Kalda A, Eriste E, Vassiljev V, Zharkovsky A. Medium transitory oxygen-glucose deprivation induced both apoptosis and necrosis in cerebellar granule cells. *Neurosci Lett* 240: 21−24, 1998

Kitabayahi I, Saka F, Gachelin G, Yokoyama K. Nucleotide sequence of rat c-jun proto-oncogene. *Nucleic Acids Res* 18: 3400, 1990

Kopchik TT, Cullen R, Stacey DW. Rapid analysis of small nucleic acid samples by gel electrophoresis. *Anal Biochem* 115: 419−423, 1981

Kurino M, Fukunaga K, Ushio Y, Miyamoto E. Activation of mitogen-activated protein kinase in cultured rat hippocampal neurons by stimulation of glutamate receptors. *J Neurochem* 65: 1282−1289, 1995

Lee JK, Choi MR, Song DK, Huh SO, Kim YH, Suh HW. Activation of adenylate cyclase results in down-regulation of c-jun mRNA expression in rat C6 glioma cells. *Neurosci Lett* 276: 53−56, 1999

Lezoualc'h F, Sparapani M, Behl C. N-acetyl-serotonin (normelatonin) and melatonin protect neurons against oxidative challenges and suppress the activity of the transcription factor NF-kappaB. *J Pineal Res* 24: 168−178, 1998

Liu HN, Almazan G. Glutamate induces c-fos proto-oncogene expression and inhibits proliferation in ligodendrocyte progenitors: receptor characterization. *Eur J Neurosci* 7: 2355−2363, 1995

Liu HN, Larocca JN, Almazan G. Molecular pathways mediating activation by kainate of mitogen-activated protein kinase in oligodendrocyte progenitors. *Brain Res Mol Brain Res* 66: 50−61, 1999

McNaughton LA, Hunt SP. Regulation of gene expression in astrocytes by excitatory amino acids. *Brain Res Mol Brain Res* 16: 261−266, 1992

Rodriguez MJ, Bernal F, Andres N, Malpesa Y, Mahy N. Excitatory amino acids and neurodegeneration: a hypothetical role of calcium precipitation. *Int J Dev Neurosci* 18: 299−307, 2000

Schwarzschild MA, Cole RL, Meyers MA, Hyman SE. Contrasting calcium dependencies of SAPK and ERK activations by glutamate in cultured striatal neurons. *J Neurochem* 72: 2248−2255, 1999

Skradski S, White HS. Topiramate blocks kainate-evoked cobalt influx into cultured neurons. *Epilepsia* 41: S45−S47, 2000

Szekely AM, Barbaccia ML, Alho H, Costa E. In primary cultures of cerebellar granule cells the activation of N-methyl-D-aspartate-sensitive glutamate receptors induces c-fos mRNA expression. *Mol Pharmacol* 35: 401−408, 1989

Takahashi N, Hayano T, Suzuki M. Peptidyl-prolyl cis-trans isomerase is the cyclosporin A-binding protein cyclophilin. *Nature* 337: 473−475, 1989

Vaccarino FM, Hayward MD, Nestler EJ, Duman RS, Tallman JF. Differential induction of immediate early genes by excitatory amino acid receptor types in primary cultures of cortical and striatal neurons. *Brain Res Mol Brain Res* 12: 233−241, 1992

Vanhoutte P, Barnier JV, Guibert B, Pages C, Besson MJ, Hipskind RA, Caboche J. Glutamate induces phosphorylation of Elk-1 and CREB, along with c-fos activation, via an extracellular signal-regulated kinase-dependent pathway in brain slices. *Mol Cell Biol* 19: 136−146, 1999

Won JS, Song DK, Kim YH, Huh SO, Suh HW. The stimulation of rat astrocytes with phorbol-12-myristate-13-acetate increases the proenkephalin mRNA: involvement of proto-oncogenes. *Mol Brain Res* 54: 288−297, 1998

Won J, Suh H. The differential molecular mechanisms underlying proenkephalin mRNA expression induced by forskolin and phorbol-12-myristic-13-acetate in primary cultured astrocytes. *Brain Res Mol Brain Res* 84: 41−51, 2000

Young AB, Fagg GE. Excitatory amino acid receptors in the brain: membrane binding and receptor autoradiographic approaches. *Trends Pharmacol Sci* 11: 126−133, 1990

Zirpel L, Janowiak MA, Veltri CA, Parks TN. AMPA receptor-mediated, calcium-dependent CREB phosphorylation in a subpopulation of auditory neurons surviving activity deprivation. *J Neurosci* 20: 6267−6275, 2000