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Role of γ-aminobutyric acid B ($GABA_B$) receptors in the regulation of kainic acid-induced cell death in mouse hippocampus

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Abbreviations: CNS, central nervous system; DG, dentate gyrus; 5-AV, 5-aminovaleric acid; $GABA_B$, γ-aminobutyric acid B; GFAP, glial fibrillary acidic protein; i.c.v., intra cerebroventricle; IR, immunoreactivity; KA, kainic acid; OX-42, complement receptor type 3; p-CaMK II, Ca^{2+}/calmodulin-dependent protein kinase II; p-ERK, phosphorylated extracellular signal-regulated kinase

Abstract

Kainic acid (KA) is well-known as an excitatory, neurotoxic substance. In mice, KA administered intracerebroventricularly (i.c.v.) lead to morphological damage of hippocampus expecially concentrated on the CA3 pyramidal neurons. In the present study, the possible role of γ-aminobutyric acid B ($GABA_B$) receptors in hippocampal cell death induced by KA (0.1 g) administered i.c.v. was examined. 5-Aminovaleric acid (5-AV; $GABA_B$ receptors antagonist, 20 g) reduced KA-induced CA3 pyramidal cell death. KA increased the phosphorylated extracellular signal-regulated kinase (p-ERK) and Ca^{2+}/calmodulin-dependent protein kinase II (p-CaMK II) immunoreactivities (IRs) 30 min after KA treatment, and c-Fos, c-Jun IR 2 h, and glial fibrillary acidic protein (GFAP), complement receptor type 3 (OX-42) IR 1 day in hippocampal area in KA-injected mice. 5-AV attenuated KA-induced p-CaMK II, GFAP and OX-42 IR in the hippocampal CA3 region. These results suggest that p-CaMK II may play as an important regulator on hippocampal cell death induced by KA administered i.c.v. in mice. Activated astrocytes, which was presented by GFAP IR, and activated microglia, which was presented by the OX-42 IR, may be a good indicator for measuring the cell death in hippocampal regions by KA excitotoxicity. Furthermore, it showed that $GABA_B$ receptors appear to be involved in hippocampal CA3 pyramidal cell death induced by KA administered i.c.v. in mice.

Keywords: γ-aminobutyric acid; 5-aminovaleric acid; astrocytes; GABA; hippocampus; kainic acid; microglia; receptors

Introduction

Kainic acid (KA), the analog of the excitatory amino acid L-glutamate, upon binding to non-NMDA glutamate receptors, causes depolarization of neurons followed by severe status epilepticus, neurodegeneration, plasticity, memory loss, neuronal cell death (Kaminska *et al.,* 1997; Izquierdo *et al.,* 2000; Zagulska-Szymczak *et al.,* 2001). The systemic or intracerebroventriculary (i.c.v.) KA injection produces a pyramidal cell death in CA1 or CA3 region of hippocampus. For example, delayed neuronal death is seen in the rat hippocampus CA1 and CA3 by KA administered intraperitoneally in the cresyl violet staining and TUNEL assay (Baik *et al.,* 1999). Neuronal loss is found in the CA1 and CA3 subfields of the rat hippocampus following by i.c.v. KA administration (Roe *et al.,* 1998; Matsuoka *et al.,* 1999). KA, administered i.c.v., induced the lesion of CA3 pyramidal neurons in mice (Ferraguti *et al.,* 2001; Lee *et al.,* 2002; Lee *et al.,* 2003).

The phosphorylated extracellular signal-regulated kinase (p-ERK) is increased by systemic administration of KA in the rat hippocampus (Mielke *et al.,* 1999; Jeon *et al.,* 2000). Seizures induce the sprouting of mossy fibers in the CA3 region of rat hippocampus (Ben-Ari and Cossart, 2000). We have also demonstrated p-ERK immunoreactivity (IR) is increased in the mouse hippocampus by KA administered i.c.v. (Lee *et al.,* 2002). The activation of ERK protein has been reported to be important in determining whether a cell survives or undergoes apoptosis (Xia *et al.,* 1995; Chung *et al.,* 2003), and also in activating transcription factors (Treisman, 1996).

Ca^{2+}/calmodulin-dependent protein kinase II (CaMK II) regulates a number of cellular functions in response to an increased intracellular calcium. CaMK II is widely distributed, but is predominantly ex-

pressed in the brain. It is involved in the regulation of neuronal functions such as neurotransmitter synthesis, neurotransmitter release, long-term potentiation, and the formation of spatial learning (Hanson and Schulman, 1992). The CaMK-related peptide and CaMK VI RNA are increased in the rat hippocampus by KA administration (Vreugdenhil *et al.,* 1999). A specific inhibitor of CaMK II attenuates the seizure-induced expression of brain-derived neurotrophic factor (BDNF) mRNA in the adult rat brain and shows a neuroprotection against NMDA- and hypoxia/hypoglycemia-induced cell death (Hajimohammadreza *et al.,* 1995; Murray *et al.,* 1998).

KA leads to the induction of several types of proto-oncogene products, such as Jun and Fos proteins, which serve as the third messengers in the hippocampus (Kaminska *et al.,* 1994; Won *et al.,* 1997). Administration of KA at a convulsant dose induces c-Fos, Fra and Jun in rat hippocampus and entorhinal cortex (Le Gal La Salle, 1988; Popovici *et al.,* 1990; Pennypacker *et al.,* 1993). The increases of c-Fos and c-Jun induced by KA might be a marker in seizure activity, excitotoxicity or the activation of target genes (Sperk *et al.,* 1983; Kitayama *et al.,* 1999; Won *et al.,* 2000).

Glial fibrillary acidic protein (GFAP) is the cell specific intermediate filament protein in astrocytes. Reactive astrocytes are characterized by expression of GFAP, hypertrophy and proliferation (Eddleston and Mucke, 1993; Ridet *et al.,* 1997). After brain injury, astrocytes undergo a number of cellular synthesis and release of a variety of growth factors and immunomodulatory cytokines (Hatten *et al.,* 1991; Schwartz *et al.,* 1993; Ridet *et al.,* 1997). Reactive astrocytes perform a variety of adaptive functions in the central nervous system (CNS), including maintenance of ion homeostasis, provision of energy substrate to neurons uptake of neurotransmitters. Reactive astrocytes are detected in mice i.c.v. administered KA (Ferraguti *et al.,* 2001; Cho *et al.,* 2002).

The activation of microglia can be detected using an activated microglial marker, complement receptor type 3 (OX-42). It is known that KA applied to CA3 region of hippocampus causes selective pyramidal cell death and activates microglial cells (Olney and de Gubareff, 1978; Crepel *et al.,* 1989). This is implicated that phagocytosis of degenerated neuronal elements raises the last step in the microglial activation (Kreutzberg, 1996; Streit *et al.,* 1999).

GABA mediates its fast action via ionotropic chloride selective $GABA_A$ and $GABA_C$ receptors. The early component of GABA inhibition is mediated by $GABA_A$ receptors (Macdonald and Olsen, 1994). The slower inhibitory actions of GABA are mediated by $GABA_B$ receptors, which are G-protein coupled receptors associated with the activation of a wide variety of intracellular effector systems (Mott and Lewis, 1994; Couve *et al.,* 2000). Both $GABA_A$ and $GABA_B$ receptors cause hyperpolarization of neuronal membranes and inhibition of neuronal excitability, but their mechanisms differ. $GABA_B$ receptors involve an efflux of K^+ rather than an influx of Cl^-, as in the case of $GABA_A$ and $GABA_C$ receptors (Costa, 1998).

However, the role of $GABA_B$ receptors in the regulation of cell death induced by KA administered i.c.v. in mice has not been characterized. Furthermore, the cellular mechanism of $GABA_B$ receptors involved in KA-induced neurotoxicity has not been well elucidated. Thus, the effects of $GABA_B$ receptors antagonist on KA-induced hippocampal cell death and expression of several intracellular molecules such as p-ERK, p-CaMK II, c-Fos, c-Jun, GFAP and OX-42 were examined in the present study.

Materials and Methods

These experiments were approved by the University of Hallym animal care and use committee. All procedures were conducted in accordance with the 'guide for care and use of laboratory animals' published by the National Institutes of Health. All efforts were made to minimize animal suffering and to reduce the number of animals used.

Materials

Male ICR mice weighing 26-30 g at the beginning of experiments (Myung-Jin, Inc., Seoul, Korea) were used in all the experiments. The mice were housed 5 per cage in a room maintained at 22 $\pm$ 1°C with an alternating 12 hour light-dark cycles. Food and water were available ad libitum. KA and 5-aminovaleric acid (5-AV; Sigma-Aldrich Co.) were prepared in PBS as vehicle.

The i.c.v. injection of drugs

The pretreatment of 5-AV (20 g/5 l) was performed 10 min before the KA (0.1 g/5 l) treatment. The i.c.v. administrations of 5-AV and KA were performed following the procedure established by Laursen and Belknap (1986). Briefly, each mouse was injected at bregma with a 50 l Hamilton microsyringe fitted with a 26-gauge needle that was inserted to a depth of 2.4 mm.

Cresyl violet staining method

Animals were sacrificed for the brain sample by

perfusion at the 0.5, 2 or 24 h after KA administration. All perfusion procedures worked in the fume hood. For perfusion, all mice were first deeply anesthetized with sodium pentobarbital (100 mg/kg, i.p., Hanlim Pharm. Co., Seoul, Korea) and perfused intracardially with physiological saline followed with ice-cold phosphate-buffered 4% paraformaldehyde (pH 7.4). Whole brain was removed from the skull and postfixed in the same fixative for 4 h at 4°C. Then the brains were cryoprotected in 30% sucrose for 24 h at 4°C and sectioned coronally (45 m) on a freezing microtome and collected in cryoprotectant for storage at -20°C until processed. Preparing sections were rinsed 3 × 10 min in PBS to remove cryoprotectant. Sections were mounted on microscope slides (Fisher) and dried on air. The slides mounted brain sections were soaked in cresyl violet working solution (0.02% in buffer solution; 0.2% sodium acetate, 0.3% acetic acid) for 2 min. Then, the sections were dehydrated through graded ethanols, cleared in histoclear, and coverslipped using Permount (Fisher).

Statistics

Statistical analysis was performed by ANOVA with post-Bonferroni's multiple comparison test using GraphPad Prism software version 3.02 for Windows (GraphPad Software, San Diego, CA). *P* values less than 0.05 were considered to indicate statistical

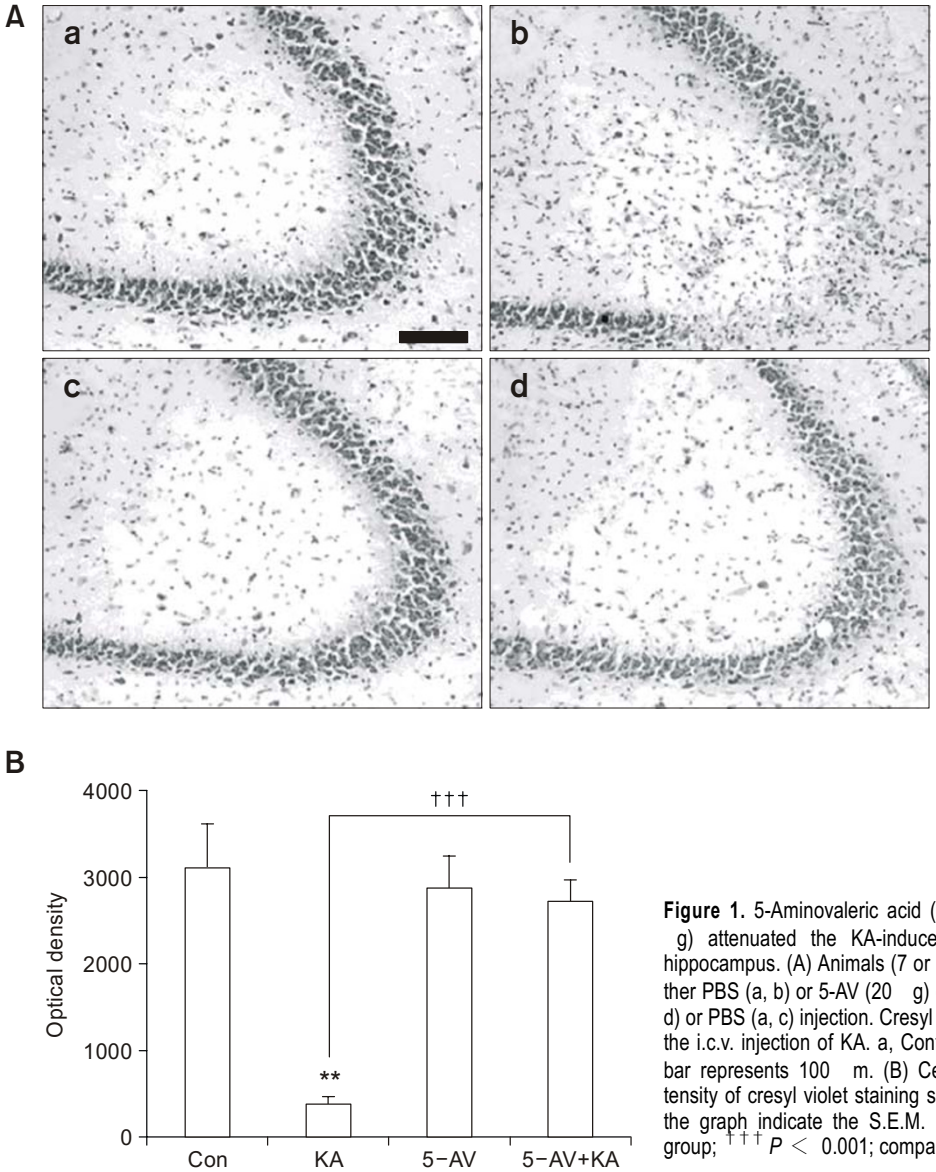

Figure 1. 5-Aminovaleric acid (5-AV; GABA$_B$ receptors antagonist, 20 g) attenuated the KA-induced cell death in CA3 region of the hippocampus. (A) Animals (7 or 10 mice/group) were pretreated with either PBS (a, b) or 5-AV (20 g) (c, d) for 10 min prior to KA (0.1 g) (b, d) or PBS (a, c) injection. Cresyl violet staining was performed 24 h after the i.c.v. injection of KA. a, Control; b, KA; c, 5-AV; d, 5-AV+KA. Scale bar represents 100 m. (B) Cell death was determined from the intensity of cresyl violet staining shown in (A). The vertical bars (*CA3*) in the graph indicate the S.E.M. (**$P < 0.01$; compared to the control group; †††$P < 0.001$; compared to the KA group).

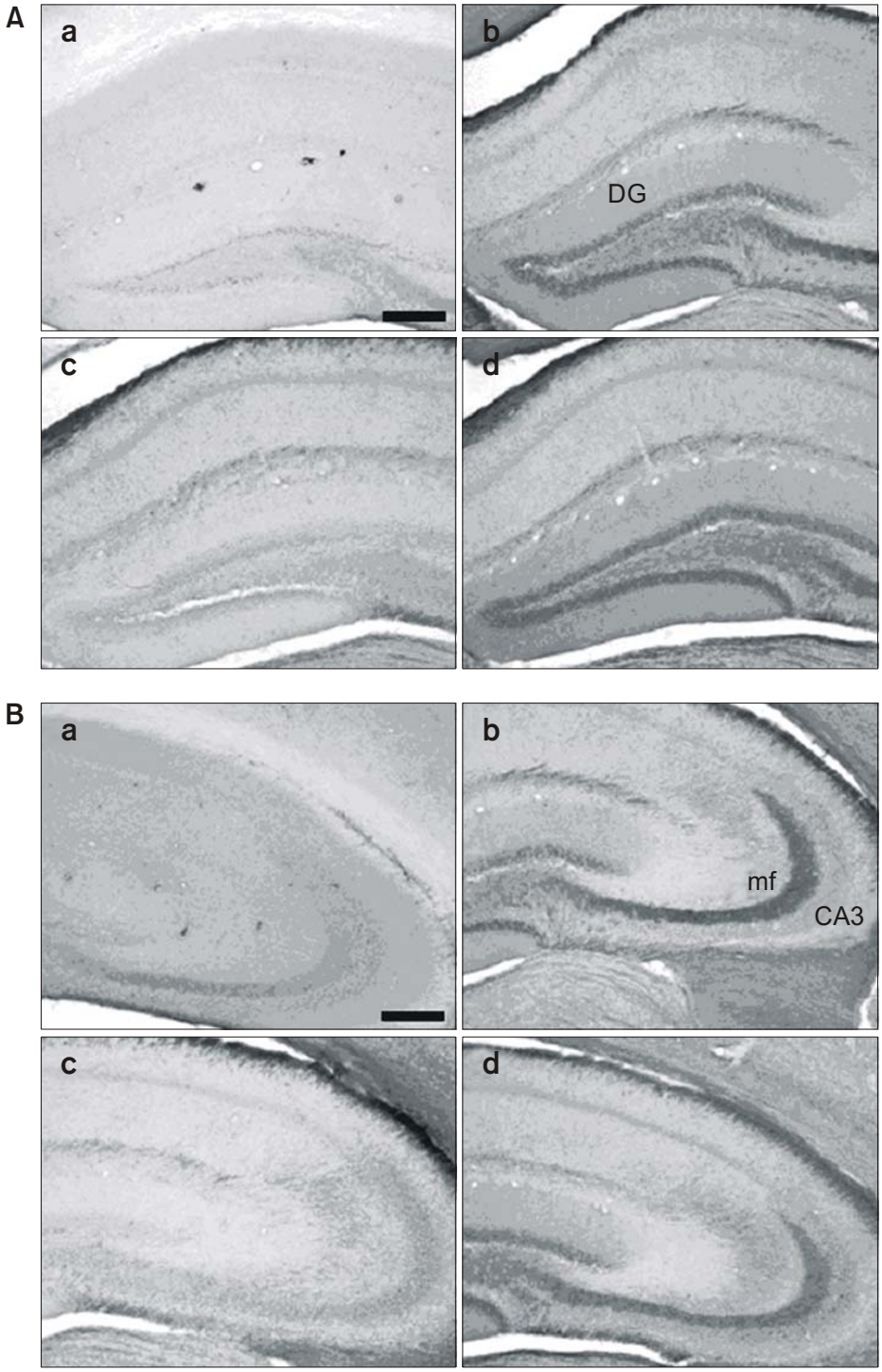

Figure 2. 5-AV does not affect KA-induced phosphorylation of ERK (p-ERK) in the dentate gyrus (DG) and mossy fibers. The p-ERK immunoreactivity (IR) in the hippocampus was examined at 30 min after i.c.v. injection of KA. Animals (7 or 10 mice/group) were pretreated with either PBS (a, b) or 5-AV (c, d) for 10 min prior to KA (b, d) or PBS (a, c) injection. And then, p-ERK IR in the DG (A) and mossy fiber (B) was examined. Antibody against p-ERK was used at a 1:200 dilution for immunohistochemistry. a, Control; b, KA; c, 5-AV; d, 5-AV+KA. Scale bars represent 200 m (A, B).

significance. All values were expressed as the mean ± S.E.M. We performed these experiments using 7 or 10 mice per every group.

Immunohistochemistry

Above brain samples were used for immunohistochemistry. Floating sections, involved hippocampal area, were processed according to a published method (Baker and Farbman, 1993). Sections were rinsed 3 times for 10 min each in PBS to remove

cryoprotectant, pre-incubated for 30 min in PBS with 1% bovine serum albumin and 0.2% Triton X-100 and incubated overnight with antibodies against p-ERK (1:200; Cell Signaling Technology), p-CaMK II (1:5,000; Santacruz), c-Fos (1:15,000; Santacruz), c-Jun (1:1,000; Santacruz), GFAP (1:50,000; Sigma) and OX-42 (1:75,000; Accurate Chemical). On the following day, free-floating sections were incubated for 1 h with biotinylated secondary antibody obtained from Vector Laboratories. After incubation with the Vector Elite ABC kit, antigens were detected with 3,3-diaminobenzidine tetrahydrochloride (DAB) as the chromogen. Sections were mounted, air-dried and dehydrated through graded ethanols, cleared in histoclear, and coverslipped using Permount (Fisher).

Results

Effect of 5-Aminovaleric acid (5-AV) on KA-induced cell death in CA3 region of the hippocampus

We have examined the cell death in hippocampus using the cresyl violet staining. The cresyl violet staining was performed with brains perfused at 1 day after i.c.v. KA administration. As shown in Figure 1b, in our morphological study, the neuronal death induced by i.c.v. administered KA (0.1 g, i.c.v.) in hippocampus was conspicuously concentrated on the CA3 pyramidal neurons. The pretreatment with 5-AV (20 g, i.c.v.), $GABA_B$ receptors antagonist, showed a protective effect significantly against the hippocampal cell death induced by i.c.v. administered KA.

Effect of 5-AV on KA-induced phosphorylation of ERK in the DG and mossy fibers

The immunohistochemical study was performed with brains perfused at 30 minutes after i.c.v. KA administration. KA (0.1 g, i.c.v.) dramatically increased the phosphorylation of ERK (p-ERK) IR in the DG and mossy fibers at 30 min after the KA treatment (Figure 2Ab, Bb). The p-ERK IR was not seen in other regions. Pretreatment with 5-AV (20 g, i.c.v.), $GABA_B$ receptors antagonist, showed no effect on KA-induced increase of p-ERK in the DG and mossy fibers (Figure 2Ad, Bd).

Effect of 5-AV on KA-induced phosphorylation of CaMK II in the CA3 region of hippocampus

KA (0.1 g) dramatically increased the p-CaMK II in the CA3 region of hippocampus at 30 min after the KA treatment (Figure 3B). There were not significant changes of p-CaMK II IR in other regions (data not shown). Pretreatment with 5-AV (20 g, i.c.v.), $GABA_B$ receptors antagonist, attenuated the KA-induced p-CaMK II IR in the CA3 region of hippocampus (Figure 3D).

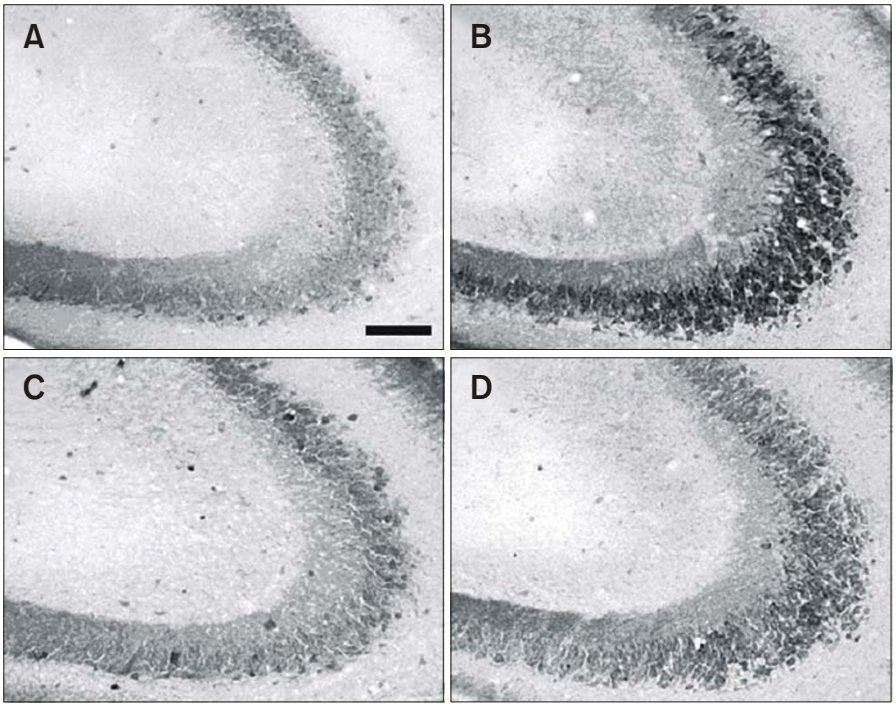

Figure 3. 5-AV attenuated KA-induced expression of p-CaMK II in the CA3 region of hippocampus. The p-CaMK II IR in the hippocampus was examined at 30 min after i.c.v. injection of KA. Animals (7 or 10 mice/group) were pretreated with either PBS (A, B) or 5-AV (C, D) 10 min prior KA (B, D) or PBS (A, C) injection. p-CaMK II IR in the hippocampus was examined. Antibody against p-CaMK II was used at a 1:5,000 dilution for immunohistochemistry. A, Control; B, KA; C, 5-AV; D, 5-AV+KA. Scale bar represents 100 m.

Effect of 5-AV on KA-induced c-Fos IR in the hippocampus

The immunohistochemical study was performed with brains perfused 2 h after KA treatment. KA (0.1 g, i.c.v.) dramatically increased the c-Fos IR in hippocampus at 2 h after the KA treatment (Figure 4Ab, Bb). Pretreatment with 5-AV (20 g, i.c.v.), $GABA_B$ receptor antagonist, showed no effect of KA-induced c-Fos in the hippocampus (Figure 4Ad, Bd).

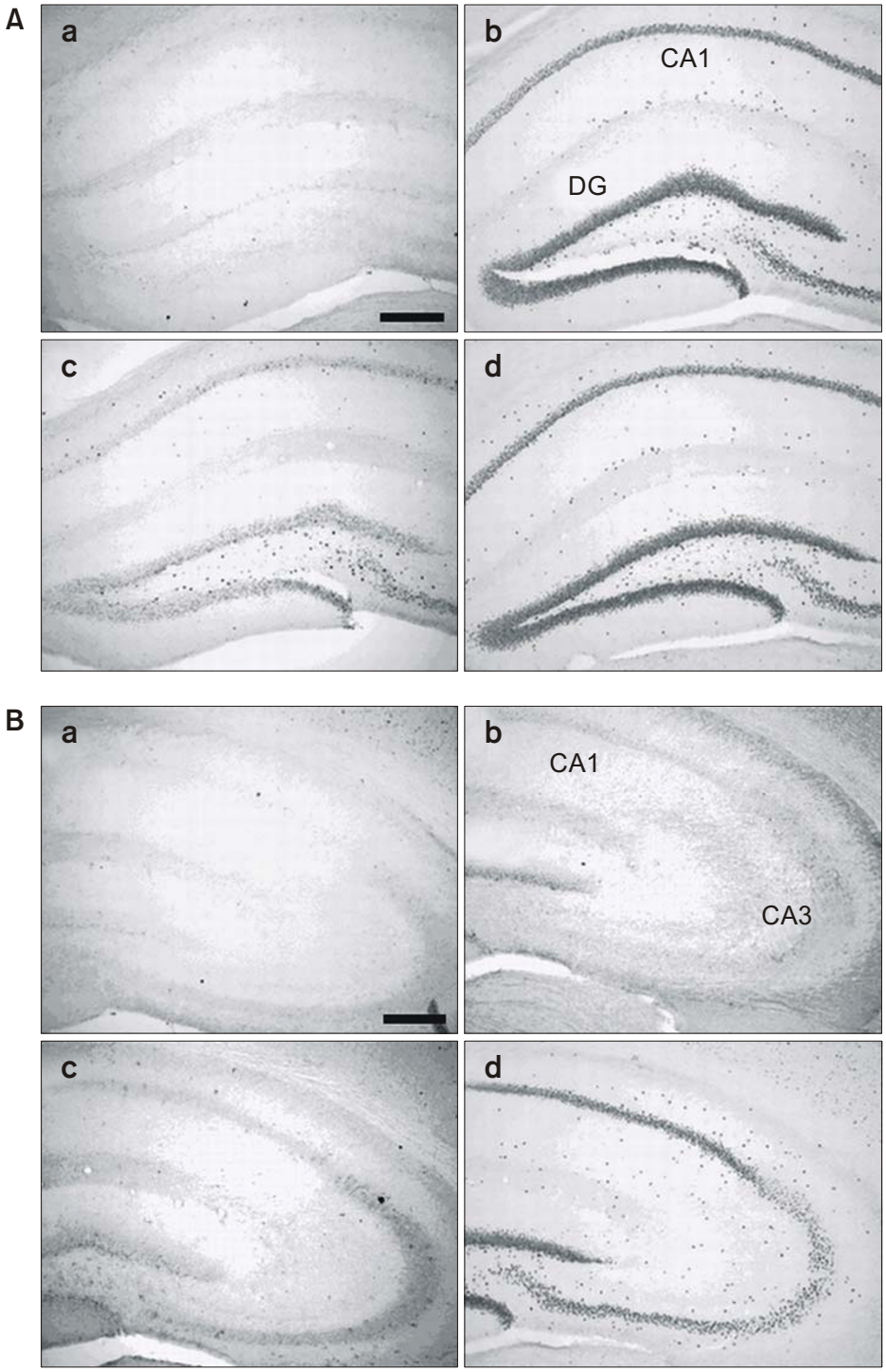

Figure 4. 5-AV does not affect KA-induced expression of c-Fos in the hippocampus. The c-Fos IR in the hippocampus was examined at 2 h after i.c.v. injection of KA. Animals (7 or 10 mice / group) were pretreated with either PBS (Aa, Ab, Ba and Bb) or 5-AV (Ac, Ad, Bc and Bd) for 10 min prior to KA (Ab, Ad, Bb and Bd) or PBS (Aa, Ac, Ba and Bc) injection. And then, c-Fos IR in the CA1, dentate gyrus and CA1-CA4 (A, B) was examined 2 h after i.c.v. injection of KA. Antibody against c-Fos was used at a 1:15,000 dilution for immunohistochemistry. A, CA1-2; B, CA3-4; a, Control; b, KA; c, 5-AV; d, 5-AV+KA. Scale bars represent 200 m (A, B).

Effect of 5-AV on KA-induced c-Jun IR in the dentate gyrus (DG) of hippocampus

KA (0.1 g, i.c.v.) increased the c-Jun IR in DG of hippocampus at 2 h after the KA treatment (Figure 5Ab, Bb). Pretreatment with 5-AV (20 g, i.c.v.), $GABA_B$ receptor antagonist, showed no effect of KA-induced c-Jun in the DG of hippocampus (Figure 5Ad, Bd).

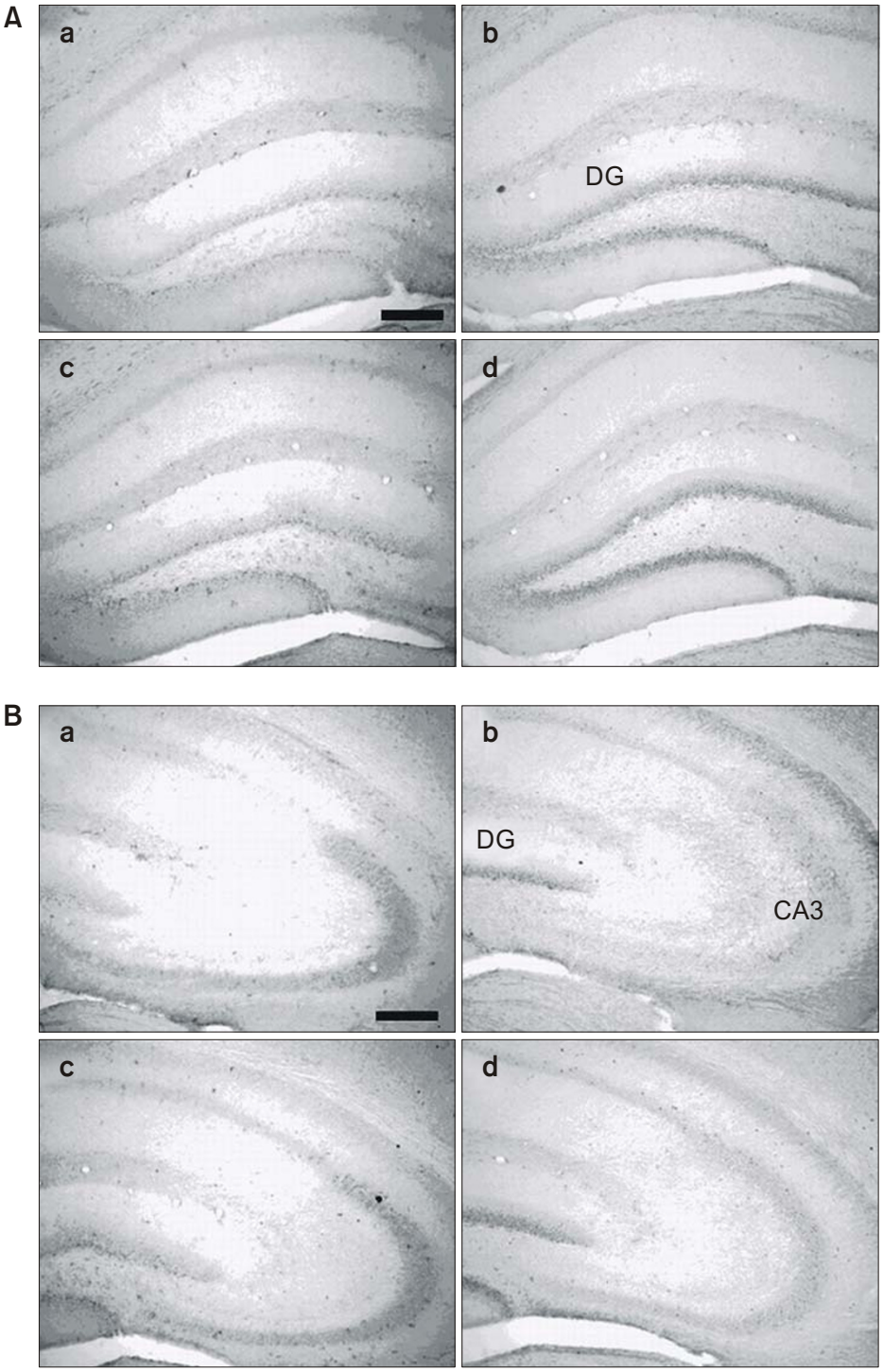

Figure 5. 5-AV did not affect KA-induced expression of c-Jun in the hippocampus. The c-Jun IR in the hippocampus was examined at 2 h after i.c.v. injection of KA. Animals (7 or 10 mice / group) were pretreated with either PBS (Aa, Ab, Ba and Bb) or 5-AV (Ac, Ad, Bc and Bd) for 10 min prior to KA (Ab, Ad, Bb and Bd) or PBS (Aa, Ac, Ba and Bc) injection. And then, c-Jun IR in the CA1, dentate gyrus and CA1-CA4 (A, B) was examined 2 h after i.c.v. injection of KA. Antibody against c-Jun was used at a 1:1,000 dilution for immunohistochemistry. A: CA1-2; B: CA3-4; a: Control; b: KA; c: 5-AV; d: 5-AV+KA. Scale bars represent 200 m (A, B).

Effect of 5-AV on KA-induced GFAP IR in the CA3 region of hippocampus

The immunohistochemical study was performed with brains perfused at 1 day after i.c.v. KA administration. KA (0.1 g) dramatically increased the GFAP IR in the CA3 region of hippocampus at 1 day after the KA treatment (Figure 6Ab, Bb). There were not significant changes of GFAP IR in other regions (data not shown). The 5-AV attenuated the KA-induced GFAP IR in the CA3 region of hippocampus (Figure 6Ad, Bd). Figure A represents the CA3

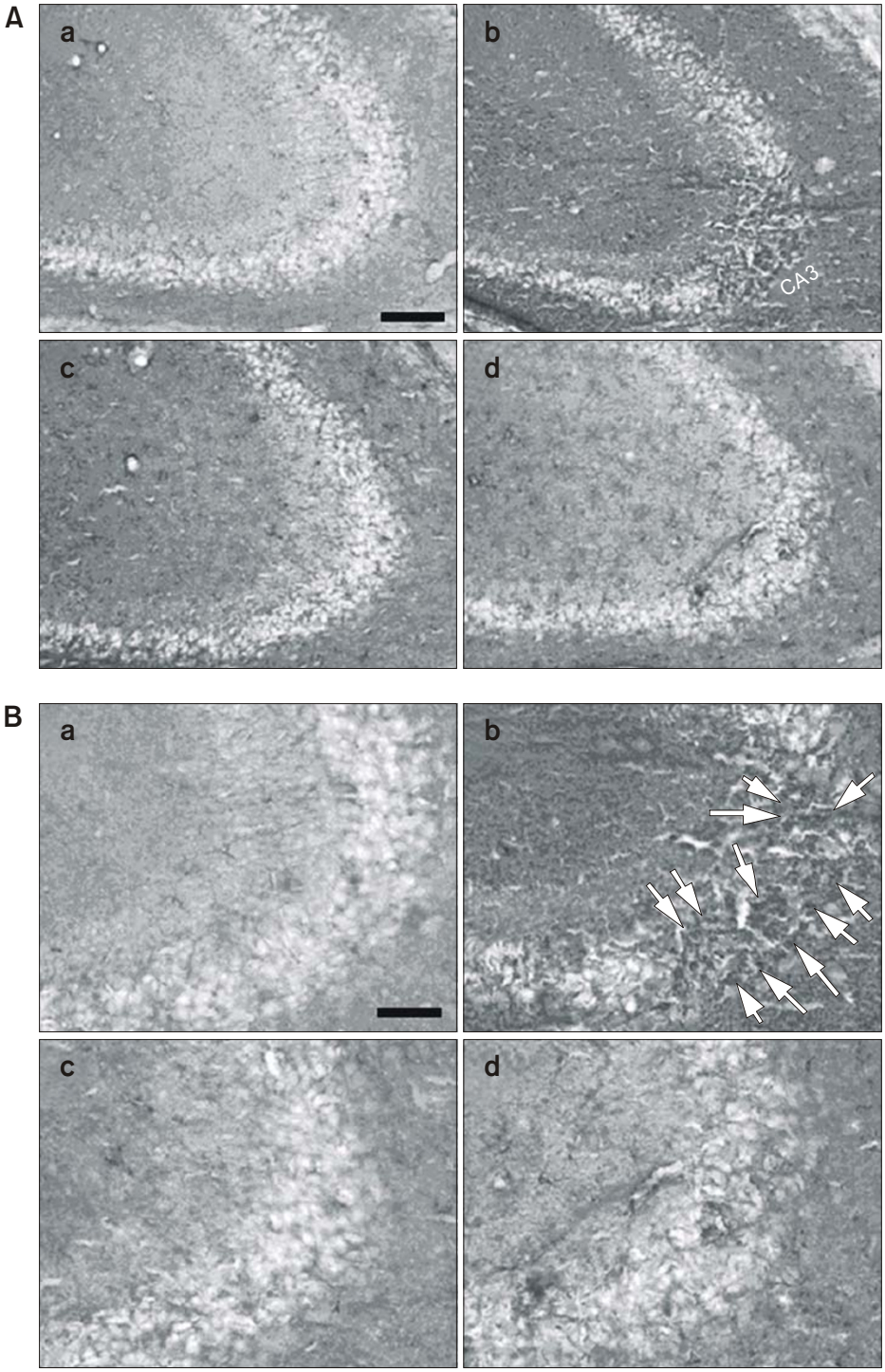

Figure 6. 5-AV attenuated KA-induced expression of GFAP in the CA3 region of hippocampus. A, CA3 region of hippocampus; B, higher magnification of the CA3 region. a, Control; b, KA; c, 5-AV; d, 5-AV+KA. The GFAP IR in the hippocampus was examined at 1 day after i.c.v. injection of KA. Antibody against GFAP was used at a 1:50,000 dilution for immunohistochemistry. The white arrows indicate GFAP-positive cells. Scale bars represent 100 (A) and 50 (B) m.

region of hippocampus, and figure B represents the higher magnification of CA3 region. White arrows indicate the GFAP-positive cells.

Effect of 5-AV on KA-induced OX-42 IR in the CA3 region of hippocampus

KA (0.1 g) dramatically increased the OX-42 IR in the CA3 region of hippocampus at 1 day after the KA treatment (Figure 7Ab, Bb). We observed the basal expressions of OX-42 IRs in other regions (data not

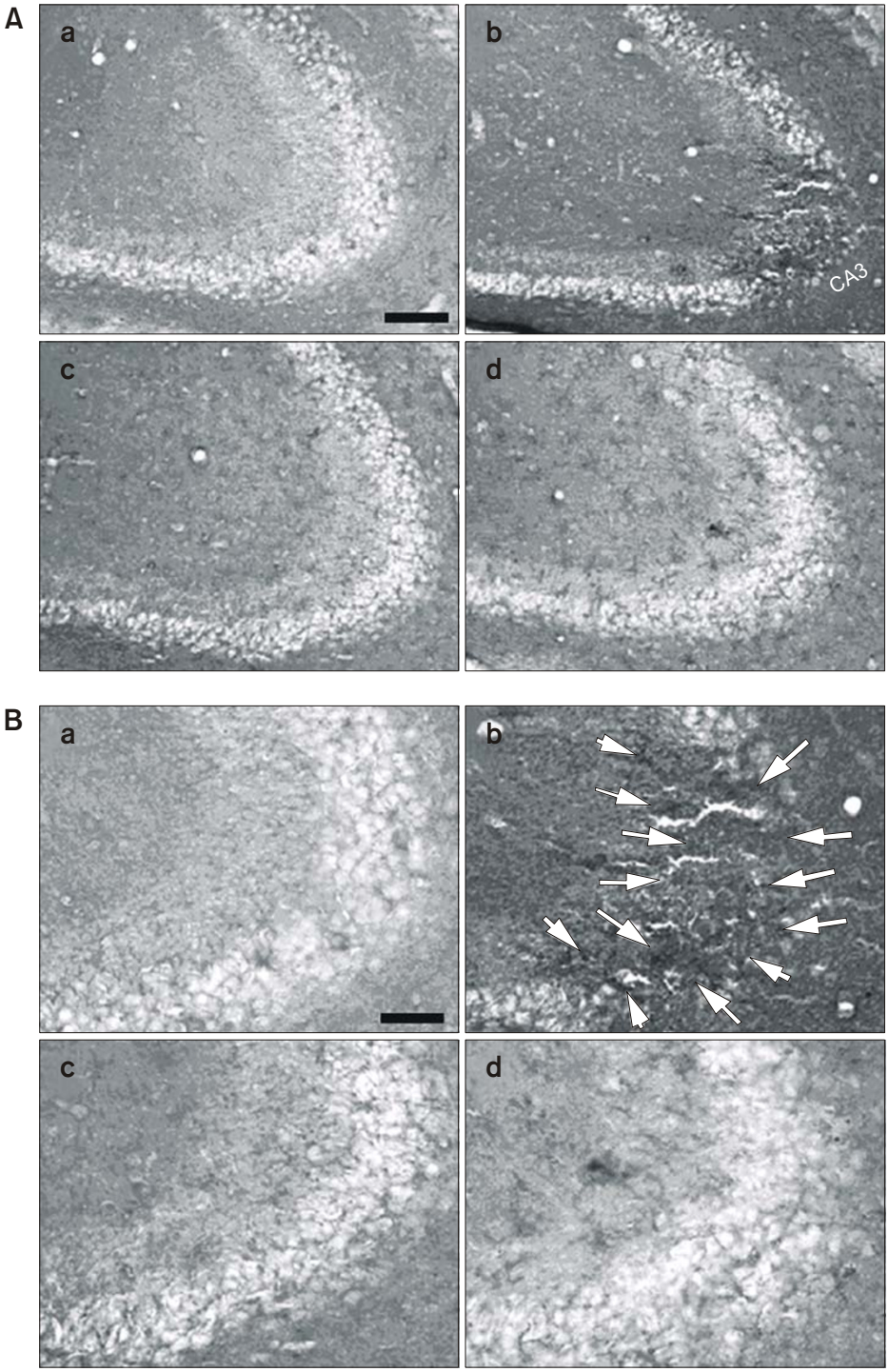

Figure 7. 5-AV attenuated KA-induced expression of OX-42 in the CA3 region of hippocampus. A, CA3 region of hippocampus; B, higher magnification of the CA3 region. a, Control; b, KA; c, 5-AV; d, 5-AV+KA. The OX-42 IR in the hippocampus was examined at 1 day after i.c.v. injection of KA. Antibody against OX-42 was used at a 1:75,000 dilution for immunohistochemistry. The white arrows indicate OX-42-positive cells. Scale bars represent 100 (A) and 50 (B) m.

shown). The 5-AV attenuated the KA-induced Ox-42 IR in the CA3 region of hippocampus (Figure 7Ad, Bd). White arrows indicate the OX-42-positive cells.

Discussion

Although activation of $GABA_B$ receptors is generally known to be inhibitory in action, $GABA_B$ agonists have been reported to possess both proconvulsive (Mott *et al.,* 1989) and anticonvulsive (Ault and Nadler, 1983; Morrisett *et al.,* 1993) activity in animals. $GABA_B$ receptor antagonists are effective in suppressing absence seizures in almost all rodent models tested (Hosford *et al.,* 1992; Marescaux *et al.,* 1992; Snead, 1992; Vergnes *et al.,* 1997; Hu *et al.,* 2000). More recent evidence suggests $GABA_B$ antagonists may have some efficacy as novel neuroprotectants (e.g. (Heese *et al.,* 2000)). G-protein coupled $GABA_B$ receptors are associated with the activation of a wide variety of intracellular effector systems (Mott and Lewis, 1994; Couve *et al.,* 2000). Immunocytochemical studies have revealed that $GABA_B$ receptors are distributed throughout the striatum, hippocampus, DG, hypothalamus, tectum, cerebellum, brainstem and spinal cord of rat (Fritschy *et al.,* 1999; Margeta-Mitrovic *et al.,* 1999; Calver *et al.,* 2000). In the present study, we found that i.c.v. administered KA-induced cell death was concentrated on CA3 region of hippocampus. Furthermore, 5-AV ($GABA_B$ receptors antagonist) attenuated KA-induced neuronal cell death in CA3 region of the hippocampus, suggesting that $GABA_B$ receptors appear to be involved in hippocampal cell death induced by KA.

KA increased the phosphorylation of ERK protein in DG and mossy fibers at 30 min after i.c.v. injection. Several studies reported that the p-ERK was increased by systemic administration of KA in the rat hippocampus (Mielke *et al.,* 1999; Jeon *et al.,* 2000). Recent studies showed that seizures induced the sprouting of mossy fibers in the rat hippocampus CA3 region (e.g. (Ben-Ari and Cossart, 2000)). We have recently reported that p-ERK IR is increased in the mouse hippocampus by KA-toxicity (Lee *et al.,* 2002). We found in the present study that 5-AV did not attenuate KA-induced p-ERK IR in the DG and mossy fibers. Mossy fibers project to the CA3 region of the hippocampus where they make large and complex synaptic contacts with the spines of pyramidal cells (Reid and Bliss, 2000). Postsynaptic KA receptors containing glutamate receptor subunits located on CA3 mossy fiber synapses are known to be key players for the generation of seizures by KA (Ben-Ari and Cossart, 2000). Although the phosphorylation of ERK was increased in the mossy fiber by KA administered i.c.v., p-ERK may not be involved in $GABA_B$ receptors-mediated CA3 pyramidal cell death by KA.

CaMK II is most prominent in the forebrain, cerebellum and hippocampus, and also CaMK II is predominantly expressed in neurons rather than glial cells (Erondu and Kennedy, 1985; Braun and Schulman, 1995). We found in the present study that the phosphorylation of CaMK II localized only in CA3 region of the hippocampus was selectively increased by KA administered i.c.v. in mice. 5-AV attenuated the increased p-CaMK II IR induced by KA administered i.c.v. in the hippocampal CA3 region, indicating that $GABA_B$ receptors are involved in p-CaMK II expression in the hippocampus by i.c.v. administered KA. KA can activate the AMPA receptor and AMPA channel may admit calcium influx. The CaMK-related peptide and CaMK VI RNA were increased in the rat hippocampus by KA (Vreugdenhil *et al.,* 1999). Although the exact functions of p-CaMK II in the hippocampus has not been well elucidated, several reports have demonstrated that the phosphorylation of CaMK II in the hippocampal region may play an important role in the regulation of neurotoxicity (Ouyang *et al.,* 1997; Shamloo *et al.,* 2000). Especially, our recent study has shown that KA administered i.c.v. caused the neuronal cell death in CA3 hippocampal region (Lee *et al.,* 2002; Lee *et al.,* 2003), and suggested that the increases of the phosphorylation of CaMK II may play important roles in the regulation of $GABA_B$ receptors-mediated hippocampal neuronal cell death induced by i.c.v. administered KA.

KA leads to the induction of several types of proto-oncogene products, such as Jun and Fos proteins, which serve as the third messengers in the hippocampus (Kaminska *et al.,* 1994; Won *et al.,* 1997). Our data shows that KA leads to the induction of c-Jun and c-Fos proteins in the DG. Especially, KA leads to the induction of c-Fos proteins in DG, CA1, CA2 and CA4 regions of hippocampus. 5-AV did not attenuate KA-induced c-Fos and c-Jun IR in the hippocampus. The increases of c-Fos and c-Jun induced by KA might be a marker in seizure activity, excitotoxicity or the activation of target genes (Sperk *et al.,* 1983; Kitayama *et al.,* 1999; Won *et al.,* 2000). Zhang *et al.* suggest that c-Fos is a genetic regulator for cellular mechanisms controlling neuronal excitability and survival (Zhang *et al.,* 2002). The findings observed in the present study suggest that c-Fos and c-Jun protein may not play as important messengers for $GABA_B$ receptors-mediated CA3 pyramidal cell death induced by KA.

Reactive astrocytes are characterized by expression of GFAP, hypertrophy and proliferation (Eddleston and Mucke, 1993; Ridet *et al.,* 1997). After

brain injury, astrocytes undergo a number of cellular synthesis and release of a variety of growth factors and immunomodulatory cytokines (Hatten *et al.,* 1991; Schwartz *et al.,* 1993; Ridet *et al.,* 1997). Reactive astrocytes perform a variety of adaptive functions in the CNS, including maintenance of ion homeostasis, provision of energy substrate to neurons uptake of neurotransmitters. Reactive astrocytes were detected in mice i.c.v. administered with KA (Ferraguti *et al.,* 2001; Cho *et al.,* 2002). Our data showed the appearance of reactive astrocytes in the CA3 region of hippocampus at 1 day after i.c.v. KA treatment. This suggests that KA-induced reactive astrocytes are involved in protective effects of lesions in the CA3 region of hippocampus and the protective effect of 5-AV, $GABA_B$ receptors antagonist, against the KA-toxicity decreases the number of reactive astrocytes. Thus, the magnitude of reactive astrocytes may be a good indicator for measuring the cell death in CNS regions.

The activation of microglia was detected by immunostaining with a monoclonal antibody against OX-42. KA applied to CA3 region of hippocampus causes selective pyramidal cell death and activates microglial cells (Olney and de Gubareff, 1978; Crepel *et al.,* 1989). Phagocytosis of degenerated neuronal elements raised the last step in the microglial activation (Kreutzberg, 1996; Streit *et al.,* 1999). In the present study, we found that OX-42 expression was increased around CA3 region, in which the pyramidal neuronal cell death occurs by i.c.v. administered KA. KA-induced hippocampal cell death was happened by apoptosis (Baik *et al.,* 1999). Furthermore, 5-AV attenuated the activation of OX-42 expression induced by KA, suggesting that the protective effect of 5-AV against KA-induced cell death may be responsible for the reduction of microglia expression. Thus, the magnitude of OX-42 expression may be a good indicator for measuring the cell death in CNS regions.

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