Increased Microglial Activation and Astrogliosis after Intranasal Administration of Kainic Acid in C57BL/6 Mice

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ABSTRACT: **Glutamate excitotoxicity plays a key role in inducing neuronal cell death in many neurological diseases. In mice, intranasal administration of kainic acid (KA), an analogue of the excitotoxin glutamate, results in hippocampal cell death and provides a well-characterized model for studies of human neurodegenerative diseases. In this study, we describe neurodegeneration and gliosis following intranasal administration of KA in C57BL/6 mice. By using Nissl's staining, neurodegeneration was found in area CA3 of hippocampus, and neuronal apoptosis was demonstrated by enhanced FAS(CD95/APO-1) expression detected by immunohistochemistry and Western blotting. Astrogliosis was exhibited by increased glial fibrillary acidic protein (GFAP) expression in the hippocampus and cortex. We also studied the profile of molecular expression on microglia in C57BL/6 mice. One and 3 days after KA administration, CD45, F4/80, CD86, MHCII, iNOS but not CD40 expression was enhanced or induced on microglia. In summary, KA administration results in an early microglial activation and a prolonged astrogliosis in C57BL/6 mice.** © 2004 Wiley Periodicals, Inc. J Neurobiol 62: 207–218, 2005

Keywords: **kainic acid; microglia; neurodegeneration; apoptosis; astrocyte**

INTRODUCTION

Neurotoxicity of excitatory amino acid is a contributing factor to acute neuronal damage, in traumatic brain injury and stroke, and in most chronic neurodegenerative diseases, such as Alzheimer's disease and multiple sclerosis (Beal, 1995; Dirnagl et al., 1999; Lee et al., 1999; Smith et al., 2000). In mice, administration of kainic acid (KA), an analogue of the excitotoxin glutamate, results in hippocampal cell

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death and seizures, and provides a well-characterized model for studies of human neurodegenerative diseases (Chen et al., 2002). In the course of KA-induced hippocampal injury, the neurons will interact with the surrounding structure, such as astrocytes and microglia. The activities of those glial cells have important influence on the survival and post-injury repair of hippocampal neurons.

Astrocytes are essential for coping with central nervous system (CNS) damage. Astrogliosis is induced by excitotoxicity and is considered a marker of neurotoxicity and strong neuronal activity (Steward et al., 1991; Norton et al., 1992; Torre et al., 1993). It is secondary to neuronal injury and believed to be mainly neuroprotective (Ridet et al., 1997; Ding et al., 2000).

Microglia, the main effector cell type of the innate immune system in the CNS, form about 10% of the total brain cells. Upon neuronal injury, microglia rapidly assume changes in morphology and expression of surface antigens and soluble molecules (Kreutzberg, 1995, 1996). Some studies suggest that activated microglia are neuroprotective in that microglia can release tumor necrosis factor- α (TNF-α) and neurotrophins (Bruce et al., 1996; Elkabes et al., 1996). However, evidence is also accumulating that activated microglia contribute to the neuropathological changes in several CNS diseases (McGeer et al., 1988; Itagaki et al., 1989; Matsumoto et al., 1992). Activation of microglia and consequent release of cytotoxic and/or proinflammatory factors are believed to contribute to neurodegenerative processes. Some *in vitro* studies showed that activation of microglia potentiates neurotoxicity in neuron-glia cultures treated with lipopolysaccharide (LPS), β-amyloid, glutamate, or N-methyl-D-aspartate (NMDA) (Dawson et al., 1994; Meda et al., 1995; Kim et al., 2000; Tikka and Koistinaho, 2001).

Most studies on microglia used *in vitro* cultures from newborn mice/rats, which may miss features that depend on other cell types or conditions only provided by a tissue environment. In this study, we used microglia enriched by Percoll gradient from adult mice and studied the pattern of several activation and destruction markers' expression after *in vivo* KA FAS(CD95/APO-1) treatment. In addition, application of Western blotting for detection of astrocyte marker, glial fibrillary acidic protein (GFAP) and apoptosis marker, FAS provided a way to quantify the extent of astrocyte activation and neuronal apoptosis, respectively, after KA treatment.

METHODS

Animals

A total of 64 (37 surviving out of 58 KA-treated and 27 water-treated) male C57BL/6 mice 5–6 weeks of age were used in this study. All mice were purchased from the Jackson Laboratory (Bar Harbor, ME) and housed on a 12-h light-dark schedule with water and food available ad libitum.

KA Administration

Mice were partially anesthetized with Isofluen (Abbott Laboratories Ltd., Kent, UK) and hold on their backs by hand. KA (Sigma-Aldrich, Stockholm, Sweden) dissolved in water (10 mg/1.3 mL) was slowly and gently dropped by micropipette into the noses of the mice at a dose of 45 mg KA per kilogram body weight as previously described (Chen et al., 2002). Age- and body weight-matched control mice received the same amount of water intranasally.

Histopathological Analysis

Five surviving KA-treated mice and 3 water-treated control mice were anesthetized with sodium pentobarbital and transcardially perfused with phosphate-buffered saline (PBS) 7 days after administration of KA. Brains were obtained, fixed in 4% buffered formaldehyde, and embedded in paraffin. Multiple coronal sections (7 μm slices) from -1.7 mm relative to bregma were prepared according to the information in Franklin's brain atlas (Franklin and Paxinos, 1997). Sections were stained by Nissl's method to evaluate the morphology of neurons.

Immunohistochemistry of Brain Sections

Eleven surviving KA-treated mice (4, 3, and 4 mice at 1, 3, and 5 days, respectively, after KA treatment) and 3 water-treated control mice were perfused with PBS followed by 4% buffered formaldehyde and the brains were kept in 10% sucrose until being frozen and cryosectioned at the thickness of 12 μm. Paraffin sections prepared as described for histopathological analysis were dewaxed and immersed in citric buffer (pH 6.0) and heated by microwave at 94°C for 9 min. After washes with water and Tris buffer, both frozen and paraffin-embedded sections were blocked by "protein block" (DAKO A/S, Glostrup, Denmark) at room temperature for 30 min. Subsequently, the frozen sections were exposed to rat anti-mouse F4/80 (1:100, Serotec, Oxford, UK) and the paraffin-embedded sections were exposed to rabbit antibodies to FAS (1:50, Santa Cruz Biotechnology, Santa Cruz, CA) and rabbit antibodies to GFAP (1:1600; DAKO), respectively. Sections were stained by using the avidin-biotin technique (Vectastain Elite Kit; Vector Labs, Burlingame, CA). Omission of primary antibodies served as negative controls. Peroxidase-substrate solution DAB

(Sigma-Aldrich, Stockholm, Sweden) was added until the desired intensity of color (yellow) developed.

Western Blotting

Seven days after KA treatment, three surviving KA-treated and three water-treated control mice in each of the two separate experiments were perfused with PBS and the hippocampi were dissected out and put into 1.5 mL microcentrifuge tubes with 200 μl IP buffer [1 mM PMSF, 0.1 M Tris-Cl (pH 8.0), 0.15 M NaCl, 5 mM EDTA and 1% Triton X-100]. After sonication for 1 s, 67 μl Laemmli buffer [248 mM Tris-Cl (pH 6.8), 40% glycerol and 8% SDS] was added. The samples were boiled at 100°C for 10 min and spun down at 12,000 rpm for 10 min. The supernatants were collected and stored at −70°C until use. Protein concentrations were quantified by using DC protein assay kit (Bio-Rad, Stockholm, Sweden). For protein separation, 100 μg of each sample was electrophoresed on a 12% polyacrylamide gel and transferred to a nitrocellulose membranes followed by staining with Ponceau S (Sigma-Aldrich) for loading control. The membrane was then blocked in 5% nonfat dry milk in PBS-Tween 20 for 1 h at room temperature with gentle agitation. After blocking, membranes were incubated with rabbit anti-mouse FAS (1:600, Santa Cruz Biotechnology) or rabbit anti-cow/mouse GFAP antibody (1:5000, DAKO) overnight at 4°C with gentle agitation. After extensive washes with PBS-Tween 20, membranes were incubated with peroxidase-conjugated donkey anti-rabbit secondary antibody (1:50,000, Jackson ImmunoResearch, West Grove, PA) for 1 h at room temperature. The membranes were rinsed again in PBS-Tween 20. ECL Western blotting detection reagents (Bio-Rad) were used for exposure according to the manufacture's instructions. Densitometric analysis was performed using program Scion Image (National Institute of Health, Bethesda, MD). The relative density of immunoreactive bands was indicated by gray value (mean gray value multiplied by the area of selected band).

Isolation of Microglia

One, 3, and 5 days after KA treatment, 15 (6, 3, and 6 mice at 1, 3, and 5 days post-treatment, respectively) surviving KA-treated mice and 15 water-treated control mice were sacrificed with an overdose of anesthetic and perfused with PBS. After the blood vessels were removed, the cerebral hemispheres were dissected and dissociated by pipetting. Following trypsinization at 37°C for 15 min, fetal bovine serum (10% final concentration) was added to inactivate trypsin activity. The tissues were then dissociated by repeated pipetting in KRB solution [120 mM NaCl, 5 mM KCl, 1.2 mM KH_2PO_4, 25 mM $NaHCO_3$, 14 mM D-glucose, 2.5 mM $MgSO_4$, 0.3% bovine serum albumin (BSA)] containing DNase I (Sigma-Aldrich). The cell suspensions were passed through a 70-μm-pore-size strainer and spun down. Cell pellets were re-suspended in 30% Percoll in PBS and centrifuged at 500*g* for 20 min. The pellets were suspended, passed through a 30-μm-pore-size strainer and collected for flow cytometry staining. To ascertain that the cells isolated were microglia, an aliquot of isolated cells from water-treated mice were suspended in 1% buffered formaldehyde and spread onto slides, followed by immunocytochemical staining with rat anti-mouse CD11b antibody (Serotec). Omission of primary antibody served as negative control.

Surface Immunofluorescence Staining and Flow Cytometric Analysis of Microglia

The microglia were washed with PBS containing 1% BSA (BSA/PBS) and stained with combinations of different cell surface antibodies for 30 min at 4°C. The antibodies were: FITC-labeled rat anti-mouse CD11b (Pharmingen, San Diego, CA), APC-labeled rat anti-mouse CD11b (Caltag, Burlingame, CA), RPE-labeled rat anti-mouse CD45 (Serotec), APC-labeled rat anti-mouse F4/80 (Caltag), RPE-labeled rat anti-mouse CD86 (Caltag), FITC-labeled mouse anti-mouse/rat MHCII (Serotec), and RPE-labeled rat anti-mouse CD40 (Caltag). For intracellular staining, cells were first incubated with surface antibodies, fixed with 2% buffered formaldehyde for 20 min at 4°C, and then permeabilized with 0.5% saponin in PBS/BSA. Antibodies used for intracellular staining were rabbit anti-mouse iNOS (primary antibody, Alpha Diagnostic, San Antonio, TX), and Cy5-labeled goat anti-rabbit IgG(H+L) (secondary antibody, Jackson ImmunoResearch). FITC-, RPE-, APC-labeled rat IgG and FITC-labeled mouse IgG (all from Serotec except APC-labeled rat IgG from Caltag) were used as negative control for surface staining. As the negative control for intracellular staining, rabbit IgG (Kamiya Biomedical Company, Seattle, WA) was used followed by the staining of Cy5-labeled goat anti-rabbit IgG(H+L). After washes with BSA/PBS, cells were analyzed by a four-color FACSCalibur flow cytometer using CellQuest software (both from Becton Dickinson, San Jose, CA).

Statistics

Differences between two groups were tested by Student's *t*-test. All tests were two-sided. Values are expressed as means ± S.E.M. and the level of significance was set at $p < 0.05$.

Ethics

The KA-induced excitotoxic model in mice was approved by the South Stockholm Research Animal Ethics Committee, Huddinge County Court, Stockholm, Sweden. All efforts were made to minimize the number of animals used and their suffering.

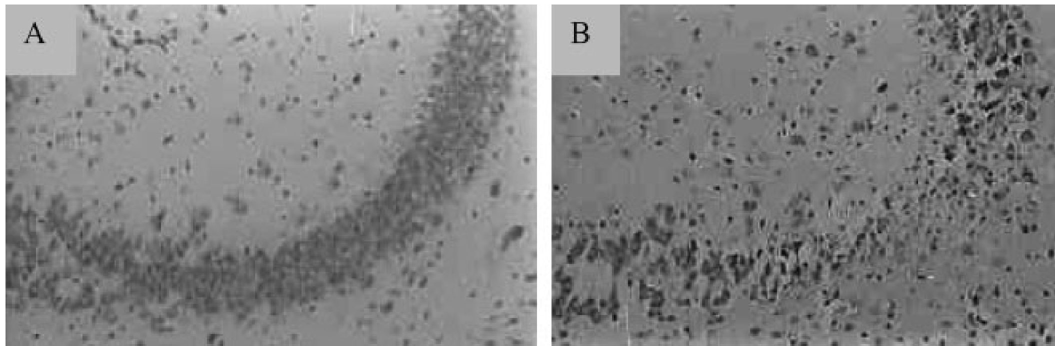


Figure 1 Seven days after administration of KA, brain sections were stained by Nissl's method to evaluate the morphology of neurons. (A) There was no pathological change observed in control mice treated with water. (B) KA induced a selective hippocampal neurodegeneration in area CA3.

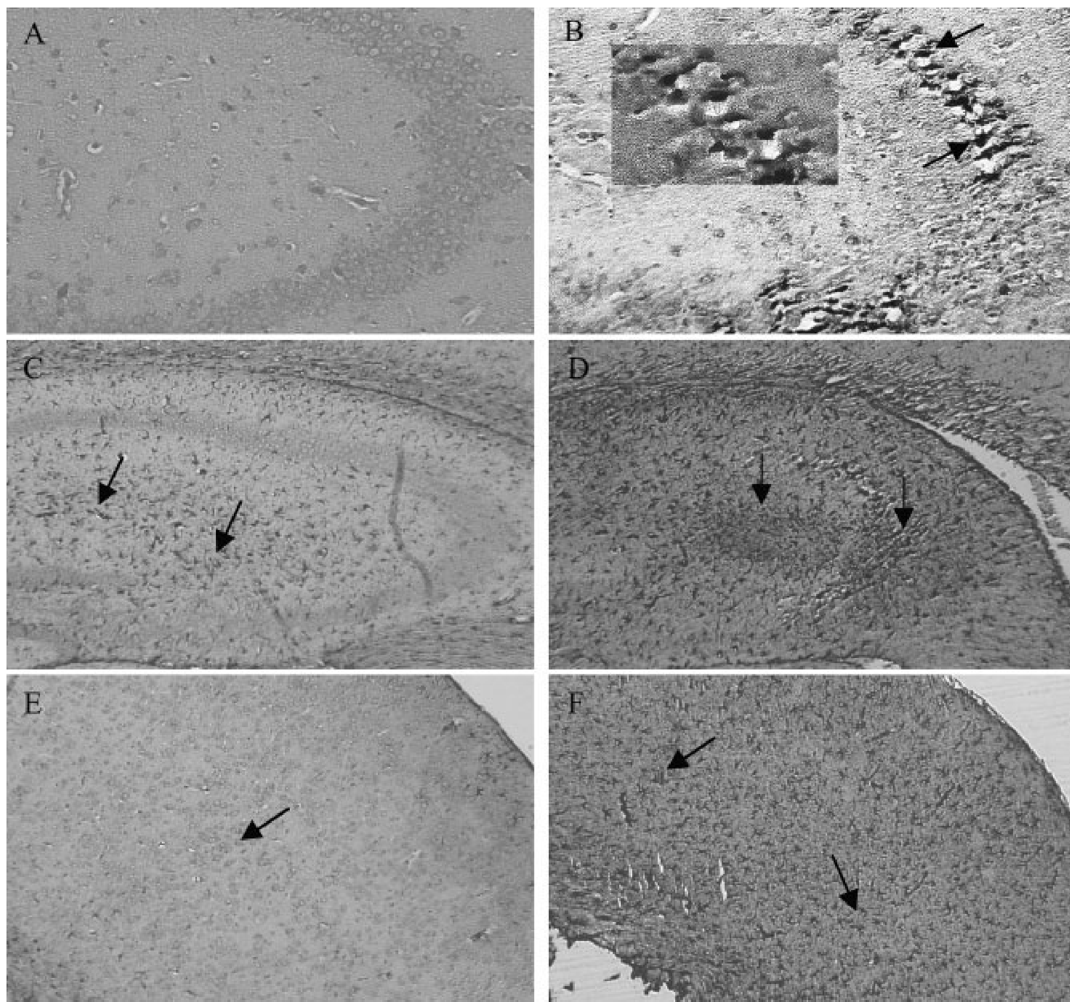
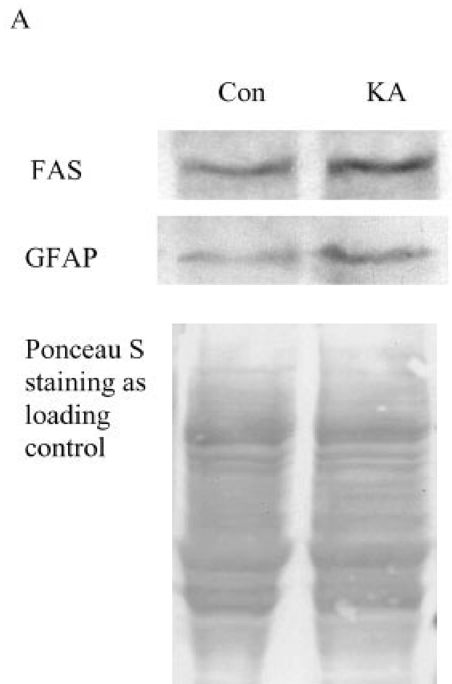


Figure 2 FAS and GFAP expression 7 days after water or KA treatment were detected with immunohistochemistry. (A) No positive staining of FAS was found on sections from water-treated mice; (B) strong positive staining of FAS was detected on sections from KA-treated mice with an inset of higher magnification; (C) weak staining of GFAP was found at the hippocampus from water-treated mice; (D) strong staining of GFAP was detected at the hippocampus from KA-treated mice; (E) weak staining of GFAP at the cortex from water-treated mice; (F) strong staining of GFAP at the cortex from KA-treated mice. Arrows indicate positive staining.



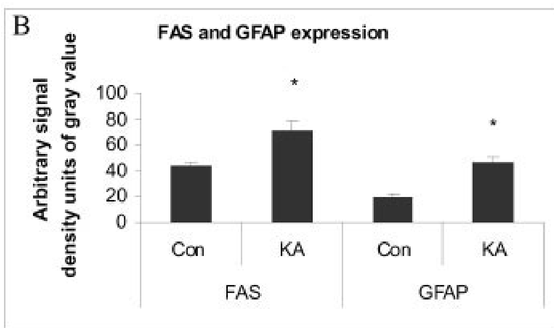


Figure 3 FAS and GFAP expression in the hippocampus 7 days after water or KA treatment was detected by Western blotting with Ponceau S staining of membranes used as loading control (A). Signal intensities were quantified by densitometric analysis and compared for each molecule before and after KA treatment by using Student's *t*-test (B). A significant increase in both FAS and GFAP expression was observed after KA treatment. The results are presented as mean values ± S.E.M. The data shown are one representative of three performed. Statistical evaluation compares values before and after KA treatment. $*p < 0.05$; Con, control mice; KA, KA-treated mice.

RESULTS

KA-Induced Clinical Signs

Control mice given water did not show any clinical change. In the mice treated with KA, catatonic and staring behavior started to appear within 15 min after treatment. Myoclonic twitching and often frequent rearing and falling followed. Within 20–30 min after KA administration, all the mice displayed continuous seizures, which lasted less than 5 h. Some serious cases died during this period. After the seizure activity ceased, mice assumed a hunched posture and were immobile for another few hours.

KA-Induced Hippocampal Neurodegeneration

Administration of KA is well-known to produce CA3 pyramidal cell death in mouse hippocampus. In control mice treated with water, there was no pathological sign observed [Fig. 1(A)]. Seven days after KA administration, a selective hippocampal neurodegeneration was found in area CA3 with the pyramidal neurons being pyknotic and/or ablated on both sides of the hippocampus in all the surviving mice [Fig. 1(B)].

Enhanced FAS and GFAP Expression after KA Treatment

FAS, a transmembrane glycoprotein and receptor for the FAS ligand, plays an important role in apoptosis and is considered as an apoptosis marker (Tan et al., 2001; Szodoray et al., 2003; McVicar et al., 2004). GFAP is a marker for astrocytes and its expression is correlated with the level of astrocyte activation (Bendotti et al., 2000; Ding et al., 2000). FAS and GFAP expression at the hippocampus 7 days after water or KA treatment was detected with immunohistochemistry and Western blotting. The cortex was also stained for GFAP by immunohistochemistry. No staining of FAS at the hippocampus and very weak staining of GFAP at both the hippocampus and cortex were found on sections from water-treated mice by immunohistochemistry [Fig. 2(A,C,E)]. However, after KA treatment, expression of both molecules was induced or upregulated at the hippocampus or cortex [Fig. 2(B,D,F)]. Signal intensities obtained by Western blotting were quantified by densitometric analysis and compared for each molecule before and after KA treatment by using Student's *t*-test. A significant in-

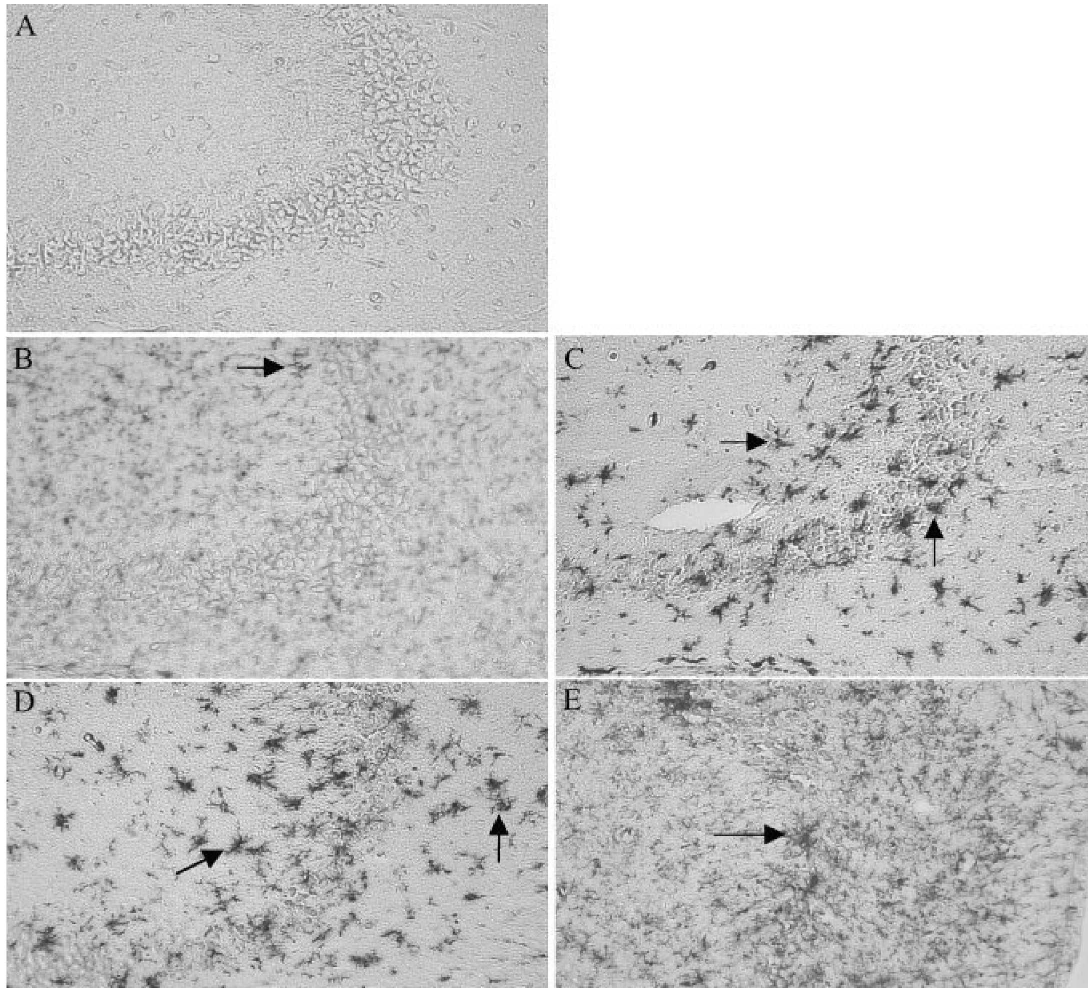


Figure 4 Microglial activation revealed by staining of F4/80 on the frozen sections from mice 1, 3, and 5 days after KA treatment. There was no staining on the negative control section with the omission of primary antibody (A) and a few stained microglia before KA treatment (B); microglia were activated 1 (C) and 3 (D) days after KA treatment but became less activated 5 days after treatment (E) in the lesioned CA3 area. Arrows indicate stained microglia.

crease in both FAS and GFAP expression was observed after KA treatment [Fig. 3(A,B)].

Activation of Microglia after KA Treatment

F4/80 can stain both activated and nonactivated microglia but its expression becomes increased after activation. Staining of F4/80 on frozen brain sections from mice sacrificed 1, 3, and 5 days after KA treatment revealed that microglia were activated 1 and 3 days but became less activated 5 days post-treatment in the lesioned CA3 area (Fig. 4). In addition, microglia were isolated from the mouse brains at the same three time points (1, 3, and 5 days after KA treatment) and subjected to flow cytometric analysis for detection of different molecules' expression. Intraparenchymal microglia were enriched by Percoll gradient from the perfused CNS of adult mice and identified as a homogeneous population as revealed in the light scatter analysis. This population of cells was gated as R1 [Fig. 5(A)]. Microglial cells ($CD11b^+$) which were gated as R2 [Fig. 5(B)] comprise 88 $\pm$ 2% of the gated cells in R1 and 38 $\pm$ 2% of the total cells. An aliquot of isolated cells from water-treated mice were suspended in 1% buffered formaldehyde and stained for CD11b, confirming that the isolated cells include microglia [Fig. 5(C)]. Negative control showed no positive staining [Fig. 5(D)]. CD45 is a differentiation marker between microglia and macro-

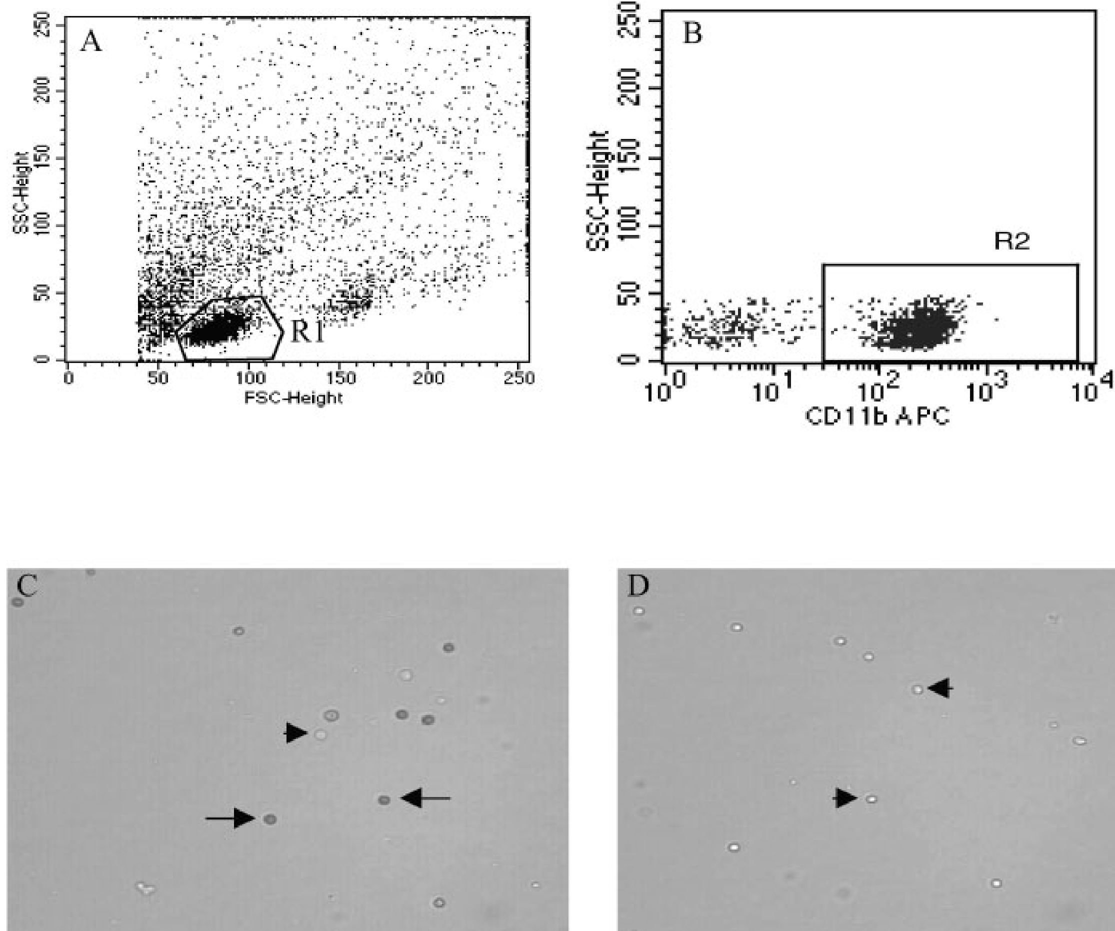


Figure 5 Light scatter analysis of the cells isolated from brain. Cell preparation was described under Methods. A homogeneous population was identified and gated [R1 (A)]. The CD11b$^+$ cells in R1 were regated as R2 for subsequent analysis (B). An aliquot of isolated cells from water-treated mice were suspended in 1% buffered formaldehyde, spread onto slides and stained for CD11b (C). Omission of primary antibody served as negative control (D). Long arrows indicate cells with positive staining and short arrows indicate negative staining.

phages (Aloisi et al., 2000) and it is also a negative regulator for microglial activation (Tan et al., 2000a,b). CD86, MHCII, and CD40 expression can represent the antigen presenting function of microglia. iNOS is the nitric oxide (NO) synthase and its expression can indicate the situation of NO secretion, which mediates many important signal cascades in the CNS. The cells were double stained with antibodies against CD11b and antibodies against one of the six molecules described above. The CD11b$^+$ cells in R2 were selected and the mean fluorescence intensities (MFI) of the six molecules expressed by the microglia 1 day after KA treatment are presented in Table 1 and Figure 6. As can be seen in Table 1, all molecules examined except CD40 showed enhanced intensities on the microglia 1 day after KA treatment. Three days after KA treatment, the situation of microglial activation was not changed, i.e., the expression of all the molecules except CD40 was still enhanced. Five days after KA treatment, the MFI of CD45, CD86, and

Table 1 MFI of Indicated Molecules on CD11b$^+$ Cells 1 Day after KA Treatment

	Con	KA
CD45	109 ± 1.76	144 ± 4.06*
F4/80	89.0 ± 3.22	132 ± 4.62*
CD86	8.79 ± 0.05	15.2 ± 1.26*
MHCII	5.15 ± 0.09	5.95 ± 0.04*
iNOS	178 ± 6.01	222 ± 5.29*
CD40	5.78 ± 0.17	5.96 ± 0.16

MFI, mean fluorescence intensity; Con, control mice; KA, KA-treated mice.

* $p < 0.05$, comparison between before and after KA treatment.

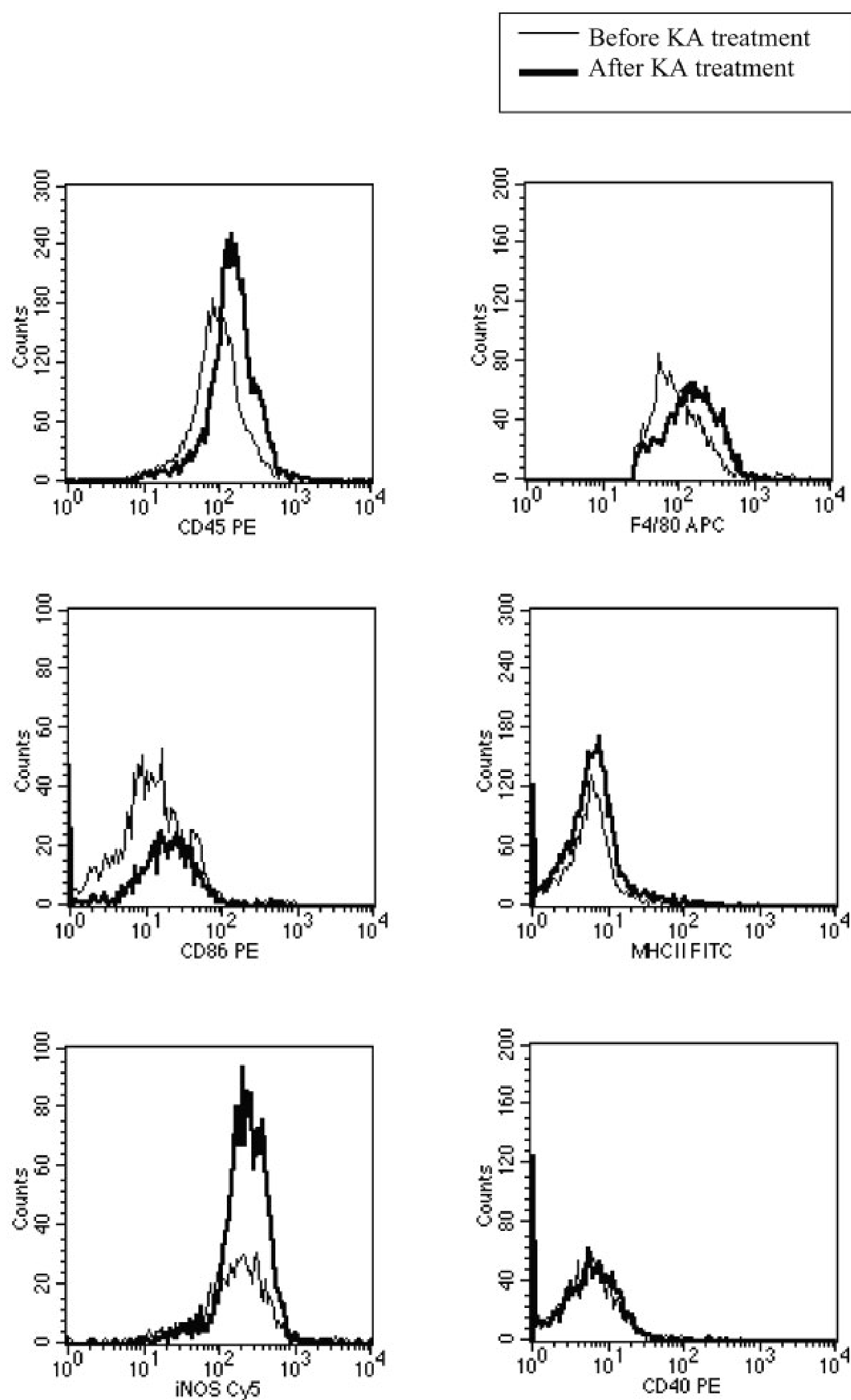


Figure 6

CD40 remained at the same level as 1 day after KA treatment while the MFI of all the other molecules dropped to the untreated levels.

DISCUSSION

In the present study, hippocampal neurodegeneration and gliosis were investigated in C57BL/6 mice taking advantage of the possibility of inducing excitotoxic neurodegeneration specifically in the hippocampus, but not other parts in the brain, by administration of KA via the intranasal route (Chen et al., 2002). Besides the morphological analysis by Nissl's staining, neuronal apoptosis and associated astrogliosis caused by KA treatment were confirmed by enhanced FAS and GFAP expression, respectively, as detected by immunohistochemistry and Western blotting. Following KA treatment, microglia were also activated and induction or upregulation of CD45, F4/80, CD86, MHCII, and iNOS was detected by flow cytometry.

Excitotoxic injury appears to be predominantly mediated by excessive influx of calcium into neurons through ionic channels. Several lines of evidences indicate that calcium entry into neurons plays a critical role in seizure genesis (Meyer, 1989; Speckmann et al., 1989; McNamara, 1992). Also, calcium entry into cells through L-channels can cause calcium overload and mitochondrial disruption that eventually leads to the release of mediators responsible for activation of apoptotic cascade and cell death (Cano-Abad et al., 2001). FAS, the receptor for FAS ligand, plays an important role in apoptosis and its induction correlates with neuronal apoptosis after KA treatment (Tan et al., 2001). Both FAS and FAS ligand can be expressed by neurons and astrocytes (Park et al., 1998; Bechmann et al., 1999; Choi et al., 1999). However, FAS induction in KA-induced exctitotoxic model is restricted in neurons but not glial cells (Tan et al., 2001). Although both necrosis and apoptosis take place in KA-induced hippocampal injury, the extent of apoptosis can partially reflect the level of neurodegeneration. Since our previous study has shown that, at 7 days after KA treatment, there are apoptotic cells in the lesioned CA3 area as detected by TdT-mediated dUTP nick end-labeling (Chen et al., 2002), we expected to see an increased expression of FAS at the same time point. We indeed found increased FAS expression after KA treatment in our present study, confirming that neuronal apoptosis has taken place. The use of FAS also provides a parameter for quantitative studies in which the hippocampal neurodegeneration needs to be quantified. Combined with pathological analysis, the level of FAS expression can give good estimations about the extent of neurodegeneration in the brain.

Another feature of the excitotoxic neurodegenerative process is astrogliosis (Chen et al., 2002). GFAP expression has been shown to steadily increase from 1 to 3 days up to 1 month after intrahippocampal or intraperitoneal injection of KA (Bendotti et al., 2000; Ding et al., 2000). Based on those studies, we thought there would be a good possibility to detect increased expression of GFAP by Western blotting at 7 days post-treatment; this proved to be the case in the present study. Combined with morphological observations by immunohistochemistry, detection of GFAP expression by Western blotting can provide a quantitative method for studies on astrocyte activation.

Here we focus on the contribution of microglia to the neurodegenerative process. Most evidence for microglial involvement in the pathologic process comes from studies employing pure or mixed primary cultures. Cultures are routinely prepared from perinatal tissue, a period when microglia are not yet at resting state. This may contribute to the alerted status of cultured microglia. Cell lines are very useful tools to study microglial activity, but they bear risk of deviated properties, including failure to produce the full spectrum of inducible factors (Stohwasser et al., 2000). In the present study, *ex vivo* preparation of microglia enriched by Percoll gradient from adult mice were used for flow cytometric analysis. Avoidance of *in vitro* culture may help to reflect a more real condition of microglia in brain. In addition, flow cytometric analysis provides an accurate methodology to quantify the levels of molecular expression on

Figure 6 (***Continued***) Profile of expression of CD45, F4/80, CD86, MHCII, iNOS, and CD40 on microglia freshly isolated from C57BL/6 mice before and 1 day after KA treatment. Cells were double-labeled with anti-CD11b and anti-CD45, anti-F4/80, anti-CD86, anti-MHCII, anti-iNOS, or anti-CD40. CD11b$^+$ cells in R2 were plotted in histogram to show the intensities of CD45, F4/80, CD86, MHCII, iNOS, and CD40 staining before and after KA treatment. KA-treated mice showed higher intensities of all molecules staining except of CD40. The data shown are one representative of three performed. Thin lines, control mice; thick lines, KA-treated mice.

microglia, which may not be easy in studies performed on brain sections.

Intensities of CD45 staining can be used to differentiate microglia and macrophage, with microglia having a moderate staining (MFI around 10^2) while macrophage having a high staining (MFI around 10^3) (Sedgwick et al., 1991; Aloisi et al., 2000). In the present study, the MFIs of CD45 staining before and after KA treatment were around 100 and 150, respectively, indicating that no macrophages infiltrated into the CNS either before or after KA treatment. Some studies show that CD45 is a negative regulator for microglial activation. Ligation to CD45 can markedly inhibit microglia activity via inhibition of p44/42 mitogen-activated protein kinase (MAPK) (Tan et al., 2000a,b). Enhancement of CD45 expression in the present study may reflect a mechanism for microglia to avoid over-stimulation. F4/80, a glycoprotein with homology to the G-protein linked 7-transmembrane hormone receptor family, is considered a marker for microglia (Lawson et al., 1993; Castano et al., 1996; O'Donnell et al., 2002). Enhancement of F4/80 expression indicates that microglia were activated after KA treatment. CD86, MHCII, and CD40 are related to co-stimulatory signaling and can represent the antigen presenting function of microglia. Induction of CD86 and MHCII may reflect the maturing of microglia as antigen presenting cells. However, the reason for the failure of CD40 induction is unclear. Microglial activation has been shown to be necessary, but not sufficient for excitotoxin-induced neurodegeneration in mouse hippocampus (Tsirka et al., 1997; Rogove and Tsirka, 1998). Activated microglia can potentiate excitotoxin-mediated neuronal death by secreting neurotoxic substances, such as NO (Colton and Gilbert, 1987; Piani et al., 1991, 1992; Chao et al., 1992, 1995; Chao and Hu, 1994; Espey et al., 1997). NO can react favorably with another intracellular constituent, superoxide, and produce peroxynitrite, which is considered to be an important factor in causing cellular damage (Lipton et al., 1993). In microglia, NO is produced by an inducible form of NO synthase, iNOS. Induction of iNOS results in high levels of release of NO for extended periods of time (Nathan and Xie, 1994). The induction of iNOS in the present study indicates an important role of microglia in the genesis of neurodegeneration. Five days after KA treatment, the expression of most molecules (F4/80, MHCII, and iNOS) dropped to untreated levels, indicating a short time window for microglial activation in the present neurodegeneration model induced by intranasal administration of KA.

Taken together, this study points to a differential kinetic pattern of activation and destruction marker expression on and in microglia associated with excitotoxin-induced neurodegeneration and astrogliosis in an intranasal KA administration model.

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