Oral *Uncaria Rhynchophylla* (UR) Reduces Kainic Acid-Induced Epileptic Seizures and Neuronal Death accompanied by Attenuating Glial Cell Proliferation and S100B Proteins in Rats.

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Running title: UR reduced KA-induced epileptic seizures by Glial cells and S100B proteins

Number of text pages: 36

Figures number: 5

1. Introduction

Temporal lobe epilepsy (TLE) is a common clinical syndrome that is due to abnormal discharges in the brain and especially in the hippocampus. Kainic acid (KA) is a potent neuroexcitatory and neurotoxic agonist of the KA subtype of glutamate receptors, which have been shown to contribute to epilepsy. Recent studies have suggested that injection of KA can induce TLE in both rats and mice (Dudek et al., 2010; Kim et al., 2010; Tauck and Nadler, 1985). The epilepsy symptoms induced by intraperitoneal KA injection are similar to temporal lobe seizures in humans especially in the hippocampus (Raedt et al., 2009; Antonussi et al., 2009; Rao et al., 2006). Glutamate is the major excitatory neurotransmitter in the mammalian central nervous system (CNS), which is released from presynaptic terminals and binds to glutamate receptors including KA receptors (Barnes and Henley, 1992; Hollmann and Heinemann, 1994). A balanced neuronal network is important for mammalian brain homeostasis and losing of this balance in the brain's network is usually associated with neurological and neurodegenerative diseases such as epilepsy (Talathi et al., 2009), Parkinson's (Llinas et al., 1999), Huntington's disease (Rubenstein and Merzenich, 2003), and schizophrenia (Wassef et al., 2003). Epilepsy is often presented in neurodegenerative diseases where patients suffer from recurrent seizures and it is usually associated with an imbalance of excitatory and inhibitory neurons in the CNS (Brenner, 2004). Dramatic increases of excitatory or decreases in inhibitory neurotransmission are often reported as

causes of epilepsy.

S100 proteins are low-molecular weight proteins that have calcium-binding properties and were first isolated from brain in 1965 (Moore, 1965; Donato, 1999). There are over 19 types of S100 proteins that can be further subtyped into S100A and S100B (Isobe and Okuyama, 1981). These proteins are expressed in a variety of tissues and influence protein phosphorylation, cytoskeleton assembly and disassembly, cell differentiation and proliferation, and intracellular calcium homeostasis (Donato, 1999). The S100B proteins are highly expressed in the CNS and non-neuronal cells including melanocytes, chondrocytes, and adipocytes (Rickmann and Wolff, 1995; Ichikawa et al., 1997; Donato, 1999). S100B is reported to be secreted from cells (Shashoua et al., 1984) to enhance neurite outgrowth (Winningham-Major et al., 1989) and stimulates astrocyte proliferation in vitro (Selinfreund et al., 1991). Age-related increases in astrocytes have been observed in the cortex and hippocampi, as indicated by the expression of the astrocyte specific marker, glial fibrillary acidic protein (GFAP). S100B can also increase intracellular free calcium concentrations (Barger and Van Eldik, 1992). Increases of S100B by astrocytes from neuronal damage or dysfunction usually result from chronic epilepsy (Griffin et al., 1995).

The γ-aminobutyric acid (GABA) receptors are major inhibitory neurotransmitters in the mammalian CNS that can be divided into the three following subtypes: $GABA_A$, $GABA_B$, and $GABA_C$. The $GABA_A$ receptor is the major mediator of fast inhibitory synaptic

transmission in the brain and has been usually reported to contribute to animal models of epilepsy (Kapur and Macdonald, 1997; Kohling et al., 2000; Cohen et al., 2003). Compounds leading to the activation of $GABA_A$ receptors are often used for synaptic inhibition and have been developed for several anti-epileptic drugs such as benzodiazepines, gabapentan, barbiturates and neurosteroids (Sieghart and Sperk, 2002; Kang and Macdonald, 2009). Therefore, direct injection of GABA agonists into the epileptogenic area is considered to be the most effective method to treat epilepsy (Schramm and Clusmann, 2008).

Transient receptor potential vanilloid subtype 1 (TRPV1) was recently classified as the capsaicin receptor, which is the burning element in peppers. TRPV1 has been shown to be in the hippocampus and is considered an important factor for maintaining the expression of long-term potentiation (LTP) (Marsch et al., 2007). TRPV1 is also expressed in bladder epithelia, and mice lacking TRPV1 have a reduced response to bladder filling (Birder et al., 2002). Recent studies have reported that TRPV1 is a novel anti-epileptogenic target (Fu et al., 2009). Activation of TRPV1 can also increase excitatory circuit activity in the dentate gyrus of mice with TLE, which implies that TRPV1 participates in the epileptic process (Bhaskaran and Smith, 2010; Fu et al., 2009).

In Traditional Chinese Medicine (TCM), *Uncaria rhynchophylla* (UR) is usually used to decrease hyperfunction of the liver, dizziness, and epilepsy. Several studies have suggested that UR has an anti-convulsive effect in KA-induced epileptic seizures in rats (Heieh et al.,

1999; Tang et al., 2010). The alkaloid components of UR including *rhynchophylline*, *isorhynchophylline*, and *isocorynoxeine* are reported to protect neurons from glutamate-induced cell death in cerebellar granule cells (Shimada et al., 1999). In addition, UR can reduce apoptosis and plays as a role in neuronal protection (Tang et al., 2010), which may be mediated via the inhibition of c-Jun N-terminal kinase phosphorylation and nuclear factor-κB activity in KA-treated rats (Hsieh et al., 2009).

To identify the curative effects and detail mechanisms of UR on KA-induced epileptic seizures, we examined whether UR could attenuate KA-induced hippocampal neuron firing. UR is known for its anti-convulsant role and anti-epileptic actions through inhibition of abnormal neural discharges and apoptosis. Here, we used immunohistochemistry, western blotting, and electrophysiology to evaluate the role of UR in KA-induced epileptic seizures. In this study, we compared the expression of GFAP, S100B protein, $GABA_A$, and TRPV1 in control, KA-induced, and UR-treated groups. Overall, oral UR decreased the firing of hippocampal CA1 neurons through attenuating GFAP, S100B protein levels but not $GABA_A$ and TRPV1 receptors.

2. Material and methods

2.1 Animals

Male Sprague-Dawley (SD) rats weighing 200-300 g were used in this study. Rats were

fasted overnight with free access to water. Usage of these animals was approved by the Institute Animal Care and Use Committee of China Medical University and followed the Guide for the Use of Laboratory Animals (National Academy Press).

2.2 Extraction of UR

The UR [Rubiaceae, Uncaria rhynchophylla (Miq.) Jacks.] in the present study was purchased from China, and authenticated by Chiu-Lin Tsai (director, division of Traditional Medicine Pharmacy, China Medical University Hospital). The UR was extracted by the Koda Pharmaceutical Company (Taoyuan, Taiwan). The voucher specimen was kept in the neuroscience laboratory room of China Medical University. Eight kilograms of crude UR was extracted with 64 kilograms of 70% alcohol by boiling for 35 min. These extracts were filtered, freeze-dried, and then stored in a drier box. The total yield was 566.63 g (7.08%). The freeze-dried extracts of UR were qualified by a high performance liquid chromatography (HPLC) system (interface D-700, Pump L-7100, UV-Vis Detector L-7420; Hitachi Instruments Service Co. Ltd., Ibaraki-ken, Japan) using *rhynchophylline* (Matsuura Yakugyo Co. Ltd., Japan) as a standard from the Koda Pharmaceutical Company. Each gram of freeze-dried extract contained 1.81 mg of pure alkaloid component of UR. The dose response for this compound was reported in our previous study (Hesih et al., 1999); hence, we used this effective dose for all experiments in this study.

2.3 Establishment of epileptic seizure model

A total of 30 SD rats were used for these experiments. Four days prior to the electroencephalogram (EEG) and electromyogram (EMG) recordings, all rats underwent stereotactic surgery with chloral hydrate (400 mg/kg, i.p.) anesthesia. The scalp was then incised from the midline and the skull was exposed. Stainless steel screws electrodes were implanted on the dura over the bilateral sensorimotor cortices to serve as recording electrodes. A reference electrode was placed in the frontal sinus. Bipolar electrical wires were placed on the neck muscles for EMG recordings. Electrodes were connected to an EEG and EMG-monitoring machine (MPlOOWSW, BIOPAC System, Inc., CA, USA). The epileptic seizures were confirmed by behavior observation (including wet dog shakes, paw tremors and facial myoclonia under a freely moving and conscious state), and epileptiform discharges on EEG recordings.

The rats were randomly divided into three experimental groups including electrophysiological studies (9 rats total with 3 rats in each group), immunohistochemistry staining (IHC) of NeuN and GFAP (9 rats total with 3 rats in each group), and western blot analysis of S100B proteins, $GABA_A$, and TRPV1 receptors (12 rats total with 4 rats per group) after KA-induced epileptic seizures.

Each experiment was divided into the three following groups: 1) the control group with phosphate buffer solution (PBS) i.p. only without KA; 2) the KA group with KA at 12 mg/kg i. p. only ; 3) the UR group receiving oral UR at 1 g/kg 5 days/week continuously for 2 weeks

starting the next day after KA injection. All the rats were sacrificed ON the 14th day after KA injection and the brains were removed for electrophysiological, IHC and western blot studies.

2.4 Electrophysiology

Adult male SD rats were anesthetized with isoflurane and decapitated. The brains were quickly removed and placed in ice-cold artificial CSF (ACSF) containing the following (mM): 119 NaCl, 2.5 KCl, 26.2 $NaHCO_3$, 1 NaH_2PO_4, 1.3 $MgSO_4$, 2.5 $CaCl_2$, and 11 glucose (the pH was adjusted to 7.4 by gassing with 5% CO_2–95% O_2). Transverse hippocampal slices (450 μm thick) were cut with a vibrating tissue slicer (Campden Instruments, Loughborough, UK) and transferred to an interface-type holding chamber at room temperature (25°C). The slices were allowed to recover for at least 90 min and then were transferred to an immersion-type recording chamber and perfused at 2 ml/min with ACSF. For extracellular field potential recording, a glass pipette filled with 3 M NaCl was positioned in the stratum pyramidal of the CA1 neuronal layer and the population spikes (PSs) were recorded. Bipolar stainless steel stimulating electrodes (Frederick Haer Company, Bowdoinham, ME) were placed in the striatum radiatum to stimulate Schaffer collateral (SC) branches. PS activity was recorded by applying a short-duration voltage pulse (~1 msec) at the determined intensity every 30 sec. All signals were filtered at 2 kHz using a low-pass Bessel filter provided with the amplifier and digitized at 5 kHz using CED micro 1401 interface running Signal software (Cambridge Electronic Design, Cambridge, UK). The average size of the PS was used for

statistical comparisons. All data are presented as the mean ± standard error. Statistical significance was tested using the Mann–Whitney U test. A p value < 0.05 was considered statistically significant.

2.5 Immunohistochemistry staining

The animals were deeply anesthetized with chloral hydrate (400 mg/kg, i.p.), and perfused with normal saline via the cardiac vascular system followed by 4% paraformaldehyde (Merck, Frankfurt, Germany) in 0.1 M phosphate buffer saline (PBS, pH = 7.4). The brains were removed and put in the same fixative overnight at 4°C. After a brief wash with PBS, the brains were transferred to 30% sucrose in 0.01 M PB for cryoprotection and then coronal sections containing the hippocampal area were cut to 20 μm in thickness using a cryo-sectioning technique. The sections were then preincubated (2 hours, 25°C) with 10% horse serum and 0.3% Triton X-100 in PBS to avoid non-specific binding. Sections were then incubated overnight at 4°C with a mixture of rat monoclonal antibody against GFAP (1:200; Oncogene, USA) and NeuN (1:1000; Chemicon, USA), 0.1% horse serum, and 0.1% Triton X-100 in PBS. Sections were subsequently incubated (2 hours, 25°C) with biotinylated-conjugated secondary antibody (1:200 diluted; Vector, Burlingame, CA 94010, USA), followed by incubation with avidin-horseradish peroxidase complex (ABC-Elite, Vector), and finally were visualized with 3,3’-diaminobenzidine as the chromogen. Sections were washed with PBS between incubation steps 3 times for 10 minutes each time.

2.6 Western blot analysis

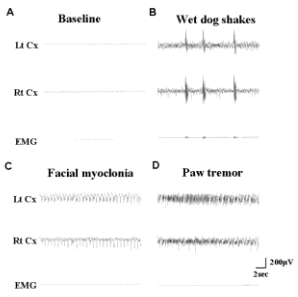
Bilateral hippocampi were immediately excised to extract protein. Total protein was prepared by homogenizing hippocampi in lysis buffer containing 20 mmol/L imidazole-HCl (pH 6.8), 100 mmol/L KCl, 2 mmol/L MgCl2, 20 mmol/L EGTA (pH 7.0), 300 mmol/L sucrose, 1 mmol/L NaF, 1 mmol/L sodium vanadate, 1 mmol/L sodium molybdate, 0.2% Triton X-100 and a proteinase inhibitor cocktail for 1 hour at 4°C. Proteins were extracted (30μg per sample assessed by BCA protein assay) and subjected to 7.5% to 10% SDS-Tris glycine gel electrophoresis and transferred to a nitrocellulose membrane. The membrane was blocked with 5% nonfat milk in TBST buffer (10 mmol/L Tris [pH 7.5], 100 mmol/L NaCl, 0.1% Tween 20), incubated with primary antibody in TBST with bovine serum albumin, and incubated for 1 hour at room temperature. Peroxidase-conjugated secondary antibody (1:500) was used as the secondary antibody. The membrane was developed with the ECL-Plus protein detection kit.

3. Results

3.1 The effects of UR on KA-induced seizures through EEG

Since epilepsy is a common clinical disease resulting from overactivity of networks in the brain, we tried to induce an in vivo animal model of epilepsy in SD rats. Here, epileptic seizures were successfully induced in a total of 30 SD rats after KA injection (12 mg/kg, i.p.).

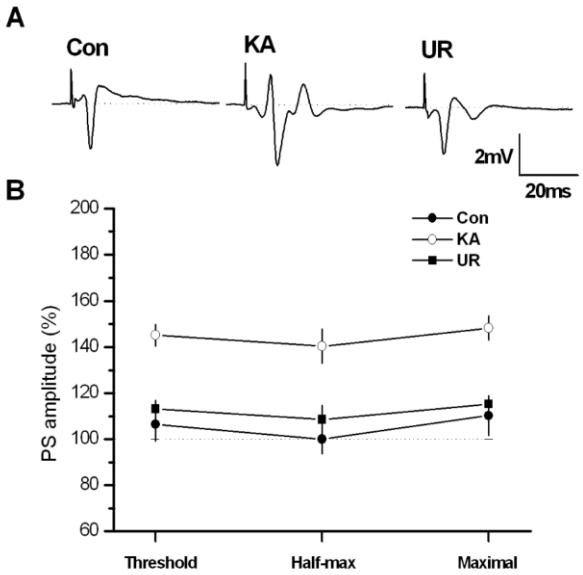
There were three major types of seizures with their own characteristic electrophysiological activity. Limbic motor signs such as wet dog shakes, paw tremor and facial myoclonia were all recorded. Baseline conditions are displayed in Figure 1A. Wet dog shakes were indicated by intermittent polyspike-like EEG activity (Figure 1B). Facial myoclonia was defined by characteristic continuous sharp EEG activity (Figure 1C). Paw tremor was considered to be characterized by continuous spike EEG activity (Figure 1D). With these successful criteria, we used this model to investigate the effect of UR on epilepsy for all of our experiments.



3.2 The effect of UR on KA induced epilepsy in hippocampal CA1 pyramidal neurons

Since electrical epileptiform discharge events often require signals from CA3, we stimulated SC branches to evoke firing of CA1 pyramidal neurons. Therefore, we first evoked PSs in CA1 areas by stimulating at SC. In brain slices taken from control animals, we readily induced PSs in CA1 pyramidal neurons with a maximal mean amplitude of approximately 2.2±0.3 mV (Figure 2A, left panel, n=8). To determine whether i.p. injection of KA successfully increased PSs in hippocampal CA1 areas, we recorded PSs in slices from KA-induced animals. Importantly, the amplitude of PSs were dramatically larger in KA treated animals than in control slices (4.1±0.4 mV, Figure 2A, middle panel, n=8).

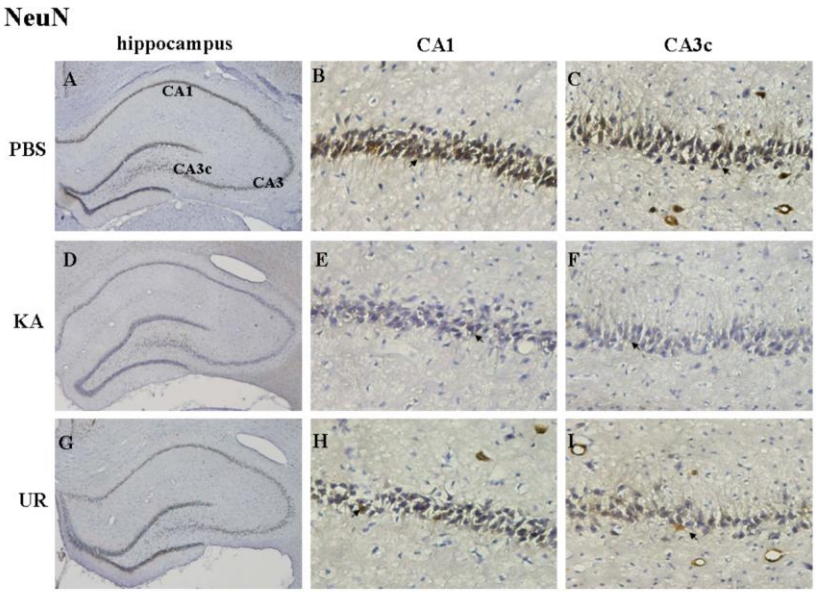
Furthermore, to exam the role of UR in KA-induced epilepsy, we induced PSs in slices from UR-treated animals. Interestingly, the PSs induced in UR groups were significantly reduced in amplitude (2.1±0.3 mV, Figure 2A, right panel, n=8). The relationship between PS amplitude and stimulations is shown in Figure 2B. Accordingly, we suggest that oral UR can successfully rescue this KA-induced epilepsy phenotype in hippocampal CA1 pyramidal neurons.



3.3 Oral UR prevents hippocampal neuronal death after KA-induced epilepsy

Since KA is often reported to induce TLE in animal models, we wanted to determine if oral UR could prevent hippocampal neurons form death as determined by the neuronal marker NeuN. Notably, we found that PBS injection did not significantly cause hippocampal neurons death (Figure 3A), especially in CA1 (Figure 3B, 322 ± 23.3 neurons/field) and CA3 areas (Figure 3C, 61.3 ± 4.7 neurons/field). Following injection of KA, our results showed

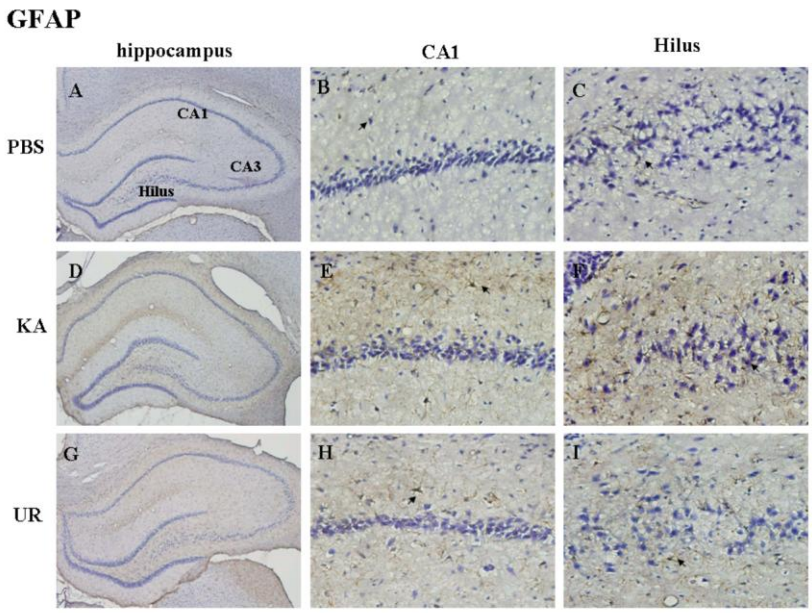
that NeuN immunostaining was significantly reduced (Figure 3D). Importantly, KA could successfully induce neuronal death both in CA1 (Figure 3E, 34 ± 4.6 neurons/field) and CA3c cell layers (Figure 3F, 16.3 ± 1.9 neurons/field). Accordingly, we wanted to test if oral UR could reduce neuronal death from i.p. KA injection. Upon oral UR treatment, hippocampal neurons became resistant to cell death from KA injection (Figure 3G), which mainly occurred in CA1 (Figure 3H, 191.7 ± 48.6 neurons/field) and CA3 cell layers (Figure 3I, 37.3 ± 7.9 neurons/field). These results show that KA injection can successfully induce hippocampal neuronal death and this phenomenon can be decreased by oral UR.



3.4 Oral UR reduces hippocampal glial cell proliferation in KA induced epilepsy

Many studies have reported that i.p. injection of KA can successfully induce epilepsy and induce glial cell overexpression and interactions with neurons simultaneously (Nadkarni and Jung, 2005). We next investigated if oral UR could decrease the overexpression of glial

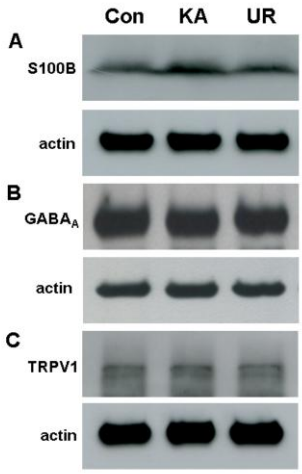
cells in hippocampal neurons from KA injection. Here we demonstrated that PBS injection, which served as the negative control, did not cause significant proliferation of glial cells with GFAP immunohistochemistry staining (Figure 4A) especially in CA1 (Figure 4B, 28 ± 2 glial cells/field) and the hilus (Figure 4C, 57.3 ± 5.5 glial cells/field). Consistent with these findings, animals that received KA injection showed increased GFAP expression (Figure 4D). These phenomena were clearly observed in CA1 (Figure 4E, 158.3 ± 14.6 glial cells/field) and hilar areas (Figure 4F, 139.7 ± 12.4 glial cells/field), suggesting that KA-induced epilepsy was accompanied by glial cell proliferation. Subsequently, we used UR to exam its role in modulation of KA-induced glial cell proliferation. These results demonstrate that oral UR can attenuate glial cell proliferation in the hippocampus (Figure 4G) especially in CA1 (Figure 4H, 60.3 ± 4.1 glial cells/field) and hilar areas (Figure 4I, 67.3 ± 2.6 glial cells/field). These results suggest that KA can induce glial cell proliferation and that oral application of UR can reverse this phenomenon.



3.5 Oral UR attenuates hippocampal epileptic discharges through S100B protein but not by $GABA_A$ and TRPV1 receptors

Since we demonstrated that oral UR could successfully reduce neuronal death and glial cell proliferation, we further wanted to test if UR could decrease the glial cell-associated protein, S100B. Many studies have reported that S100B proteins are critical for the development of epilepsy, but the relationship between UR and S100B is unclear. Our results showed that KA injection could significantly increase the expression of S100B proteins (120.7 ± 2.55 %, compared with control group) and that this phenomena could be reversed with oral UR (Figure 5A, 107.6 ± 4.7 % compared with control group). Furthermore, we also examined $GABA_A$ receptor levels because they also have been reported to be involved in epilepsy. Our results suggested that $GABA_A$ receptors were not altered during epilepsy (92.9 ± 5.17 %, compared with control group) or in the UR group (Figure 5B, 96.2 ± 3.61 %,

compared with the control group). Similar results were also observed for TRPV1 receptors (Figure 5C). These results suggest that oral UR may reduce the effects of epilepsy in animals through regulating S100B proteins but not $GABA_A$ and TRPV1 receptors.



4. Discussion

In this study, we first established and animal model of an epileptic syndrome after i.p. injection of KA. There were three major types of seizures that were recorded such as wet dog shakes, paw tremors and facial myoclonia. These results implied that we could successfully induce seizures for further investigation of the protective effects of UR. We then used extracellular recording techniques to record PSs from hippocampal CA1 pyramidal neurons to investigate the temporal dynamics of evoked electrical activity in an animal model of TLE. Our results demonstrated that PSs (utilized as physiological features representing the integrated synaptic activity generated by synchronous firing of populations of neurons) were up-regulated with KA injection and further attenuated by oral UR. These phenomena included neuronal protection and decreased GFAP expression. Furthermore, oral UR

decreased S100B expression, which in turn down-regulated glial cell proliferation to attenuate neuronal death. These changes were $GABA_A$ and TRPV1 independent since these receptors were not altered during the epileptic process.

Recent studies have suggested that expression of apoptosis associated genes including p53 and bax were significantly increased in a quinolinic acid-induced lesion area. Also, NMDA-induced neuronal cell death and apoptosis associated genes were increased in hippocampal slices (Hughes et al., 1996). The alkaloid profile of UR is mainly constituted by five components: *rhynchophylline*, *isorhynchophylline*, *corynoxeine*, *hirsutine*, and *hirsuteine*. *Rhynchophylline* is an oxindole alkaloid that not only can protect rat neuronal cells from NMDA-induced neurotoxicity (Shimada et al., 1999), but can also inhibit Ca^{2+}-activated channels and NMDA responses in oocytes (Kang et al., 2002).

UR belongs to a family of Chinese medicinal herbs that have sedative and anticonvulsive effects and have been used to treat epilepsy. Recent studies have reported that UR can protect hippocampal neurons from NMDA-induced neuronal cell death. This phenomenon was shown to be mediated by the alkaloid fraction of UR. When cultured hippocampal neurons are pretreated with the alkaloid fraction of UR, neuronal death and apoptotic associated genes including c-jun, bax, and p53 were attenuated (Lee et al., 2003; Shan et al., 1997). Our previous studies have suggested that UR can decrease KA-induced lipid peroxide levels in vitro and behavior symptoms such as wet dog shakes (WDS), paw

tremor (PT), and facial myoclonia (FM). These results are consistent with findings that UR actually has anticonvulsive and free radical scavenging activities (Hsieh et al., 1999). In this study, we showed that UR has a neuroprotective role against KA-induced epilepsy, and the mechanisms included regulation of GFAP and S100B proteins but not $GABA_A$ and TRPV1 receptors.

It has been well studied that brain trauma can produce temporal lobe damage, which in turn leads to epilepsy, and then induced hippocampal sclerosis and temporal neocortical damage (Rathore et al., 2009; Kapur et al., 1994). However, the mechanisms underlying hippocampal neuronal damage after trauma in humans with TLE are still unclear. Many studies have reported significant neuronal loss in the hippocampus including CA3, and the hilus of the dentate gyrus (DG) (Houser, 1990; Kim et al., 2010). Clearly, it is important to attempt to control epilepsy by decreasing neuronal death from necrosis and apoptosis. In animal models of brain injury, the injury of the hippocampus is mainly expressed in the hilar area of the DG and CA3 but rarely in CA1 areas (Coulter et al., 1996; Golarai et al., 2001). In this study, we found that oral UR can successfully prevent hippocampal neuron death, especially in CA1 and CA3 areas. At the same time, oral UR can also down-regulate the over-expression of glial cells with GFAP immunostaining. These results suggest that oral UR is a potential candidate for the clinical therapy of epilepsy with mechanisms that are through increased neuron survival and attenuate glial cell proliferation.

The expression of S100B proteins is often increased in chronic epilepsy (Griffin et al., 1995), Alzheimer's disease (Chaves et al., 2010), and head trauma (Morochovic et al., 2009). The overexpression of S100B protein is highly dependent on environmental factors including harmful stimuli. The overexpression of S100B protein during the epileptic process (especially in chronic epilepsy) suggests a principle role of S100B (Sakatani et al., 2007; de Oliveira et al., 2008). In the current study, we showed that S100B protein was increased with KA-induced epilepsy and this phenomenon was reversed by oral UR. This is important since S100B proteins can increase free calcium concentrations through activation of phospholipase C and IP_3 generation in the CNS (Barger and Van Eldik, 1992). Here, we suggest that oral UR for 2 weeks can serve a protective role in animals recovering from KA-induced epilepsy through attenuation of S100B proteins.

The GABA system has a major inhibitory effect on the CNS, and the loss of GABAergic inhibitory transmission often accounts for TLE and in vitro models of epilepsy. For example, pretreatment of hippocampal neurons with GABA receptor antagonist CTZ results in a dramatic decrease in miniature inhibitory postsynaptic currents (mIPSCs) and GABA-mediated currents (Qi et al., 2006). At the same time, injection of KA can mimic this phenomenon by activation of ionotropic glutamate receptors with a large Ca^{2+} influx to cause epilepsy and subsequent neurotoxicity. KA-induced epilepsy has been shown to result in an increased NMDA-dependent excitatory current and PSs, and decreased GABA-mediated

currents (Williams et al., 1993Kang et al., 2004). Importantly, synaptically localized $GABA_A$ receptors were unchanged as indicated by the immunoreactivity of postsynaptic $GABA_A$ receptors (Qi et al., 2006). In this study, $GABA_A$ receptor quantities were similar, which was determined by western blotting among controls, KA-induced and UR-treated groups. These results suggest that KA-induced epilepsy may have been mediated through attenuation of GABAergic currents but not total $GABA_A$ receptors. Accordingly, regulation of tonic inhibition plays a more important role in epilepsy rather than a general alteration of $GABA_A$ receptors (Qi et al., 2006).

Recent studies have reported that TRPV1 receptors are significantly increased in the dentate gyrus of mice with TLE compared with control mice. These studies suggest that TRPV1 agonists can increase neuronal excitability in the dentate gyrus of mice with TLE. This is an important factor to examine because novel anticonvulsant therapies are being based on TRPV1 receptor modulation (Bhaskaran and Smith, 2010). Our results showed that TRPV1 receptors were not altered in either KA-injection or UR-pretreated groups. We suggest that this result was likely due to the whole hippocampus being used for western blot analysis in our study, which may have caused dilution of the relevant fraction.

5. Conclusions

In summary, we suggest that oral UR can successfully decrease neuronal death (as

determined by NeuN immunostaining) and epileptiform discharges in hippocampal CA1 pyramidal neurons. The PSs were increased by KA but were attenuated in the UR-treated groups. Oral UR can protect neuronal death from KA-induced epilepsy by decreasing GFAP and S100B proteins but not $GABA_A$ and TRPV1 receptors.

Glossary

UR, *Uncaria Rhynchophylla*; TCM, Traditional Chinese Medicine; KA, kainic acid; CNS, central nervous system; GFAP, glial fibrillary acidic protein; GABA, γ-aminobutyric acid; TRPV1, Transient receptor potential vanilloid subtype 1; HPLC, high performance liquid chromatography; EEG, electroencephalogram; EMG, electromyogram; IHC, immunohistochemistry staining; PBS, phosphate buffer solution; ACSF, artificial cerebrospinal fluid; PS, population spikes. TLE, temporal lobe epilepsy. Schaffer collateral (SC)

Acknowledgments

This study was supported by grant NSC 97-2320-B-039-011-MY3 from the National Science Council, Taiwan, R. O. C. and was also supported in part by the Taiwanese Department of Health, Clinical Trials and Research Center of Excellence (DOH99-TD-B-111-004).

Conflict of interest:

The authors confirm that there are no known conflicts of interest associated with this publication.

Disclosure Statement for Authors: There was no significant financial support for this work that could have influenced its outcome.

Role of the funding source:

None.

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Figure captions

Figure 1. The alteration of electroencephalographic (EEG) signals in KA-injected animals. Baseline EEG activity in the sensorimotor cortex was characterized by 6-8 Hz activity in rats when awake (A). KA-induced temporal lobe seizures, including wet dog shakes (WDS) with intermittent polyspike-like activity (B), facial myoclonia with continuous sharp waves (C) and paw tremor (PT) with continuous spike activity (D). Each type of seizure had its own characteristic EEG activity. Lt Cx= EEG recording of the left sensorimotor cortex; Rt Cx=

EEG recording of the right sensorimotor cortex; EMG= EMG recording of the neck muscle.

Figure 2. Oral UR decreased epileptiform discharges recorded in the hippocampal CA1 area. (A). Classical population spikes (PSs) evoked in PBS, KA and UR groups. (B). The PSs were significantly larger in the KA group (open circle) than control (solid circle) both at threshold, half-maximal and maximal stimulations. The increase of PSs with KA injection was reversed with oral UR treatement (solid square).

Figure 3. Immunohistochemistry staining of HE and NeuN in hippocampal slices from PBS, KA and UR pretreated groups. HE (blue) and NeuN (brown) immunostaining in whole hippocampus (A), CA1 (B) and CA3c (C) areas in PBS group. HE (blue) and NeuN (brown) immunostaining in whole hippocampus (D), CA1 (E) and CA3c (F) areas in KA group. HE (blue) and NeuN (brown) immunostaining in whole hippocampus (G), CA1 (H) and CA3c (I) areas in UR group. The left panel was imaged at 40X magnification while the middle and right panel were at 400X magnification.

Figure 4. Immunohistochemistry staining of HE and GFAP in hippocampal slices from PBS, KA and UR pretreated groups. HE (blue) and GFAP (brown) immunostaining in whole hippocampus (A), CA1 (B) and hilus (C) areas in PBS group. HE (blue) and GFAP (brown)

immunostaining in whole hippocampus (D), CA1 (E) and hilus (F) areas in KA group. HE (blue) and GFAP (brown) immunostaining in whole hippocampus (G), CA1 (H) and hilus (I) areas in UR group. The left panel was imaged at 40X magnification while the middle and right panel were at 400X magnification.

Figure 5. Western blot analysis of S100B protein, $GABA_A$ and TRPV1 receptors in hippocampi from PBS, KA and UR pretreated groups. (A) S100B proteins in PBS, KA and UR groups. (B). $GABA_A$ receptors in PBS, KA and UR groups. (C). TRPV1 receptors in PBS, KA and UR groups.

Figure 1

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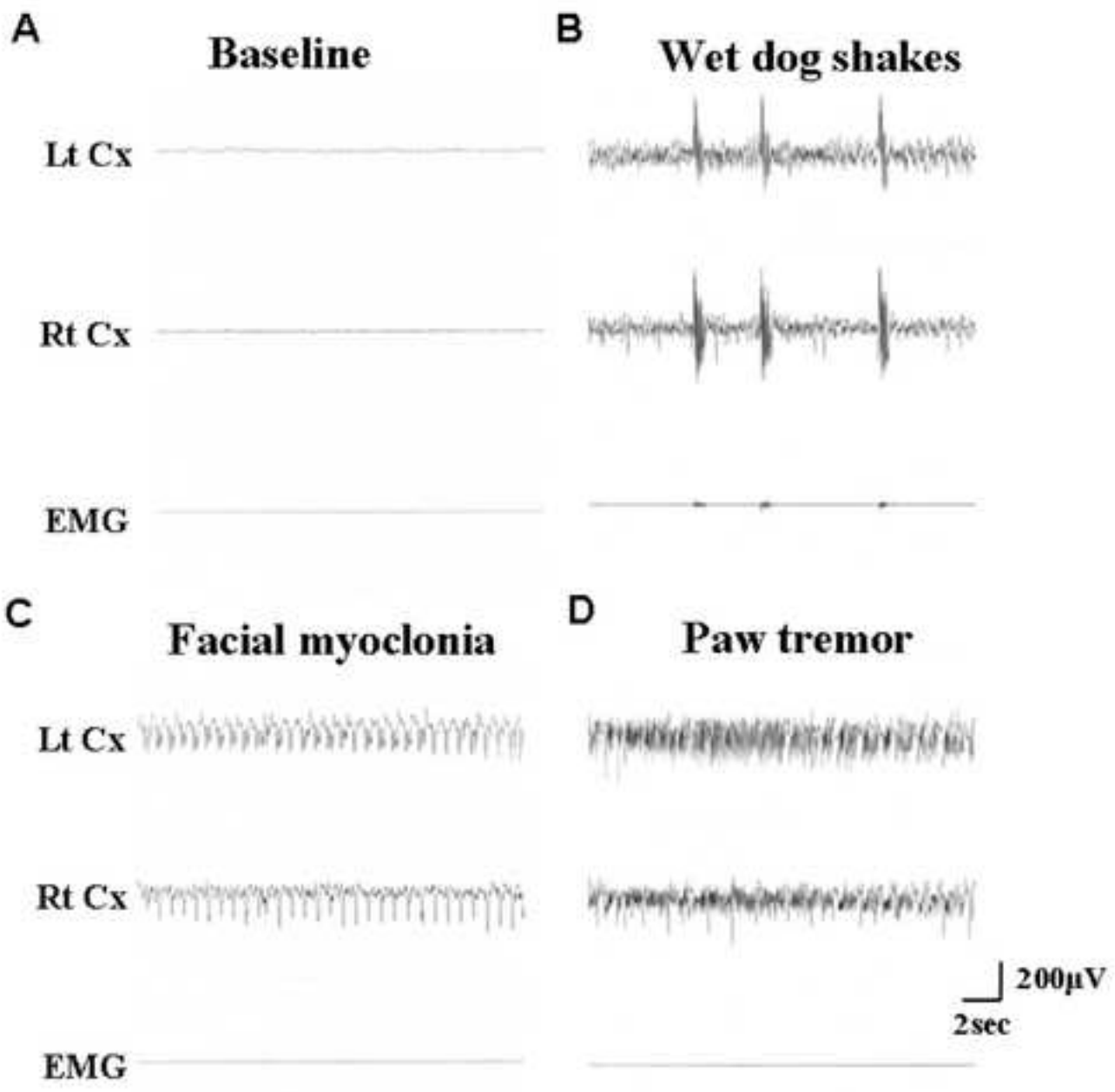

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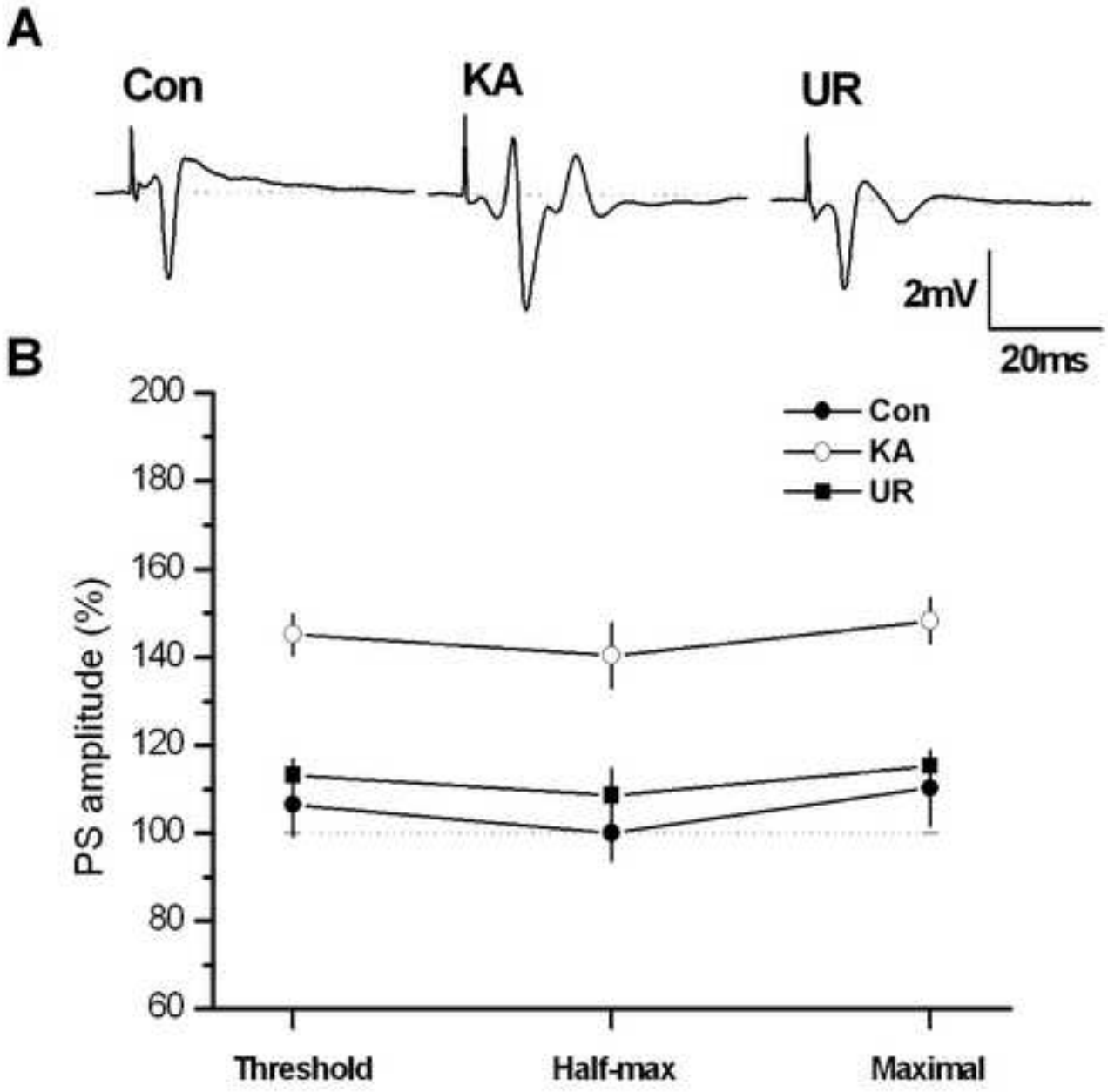

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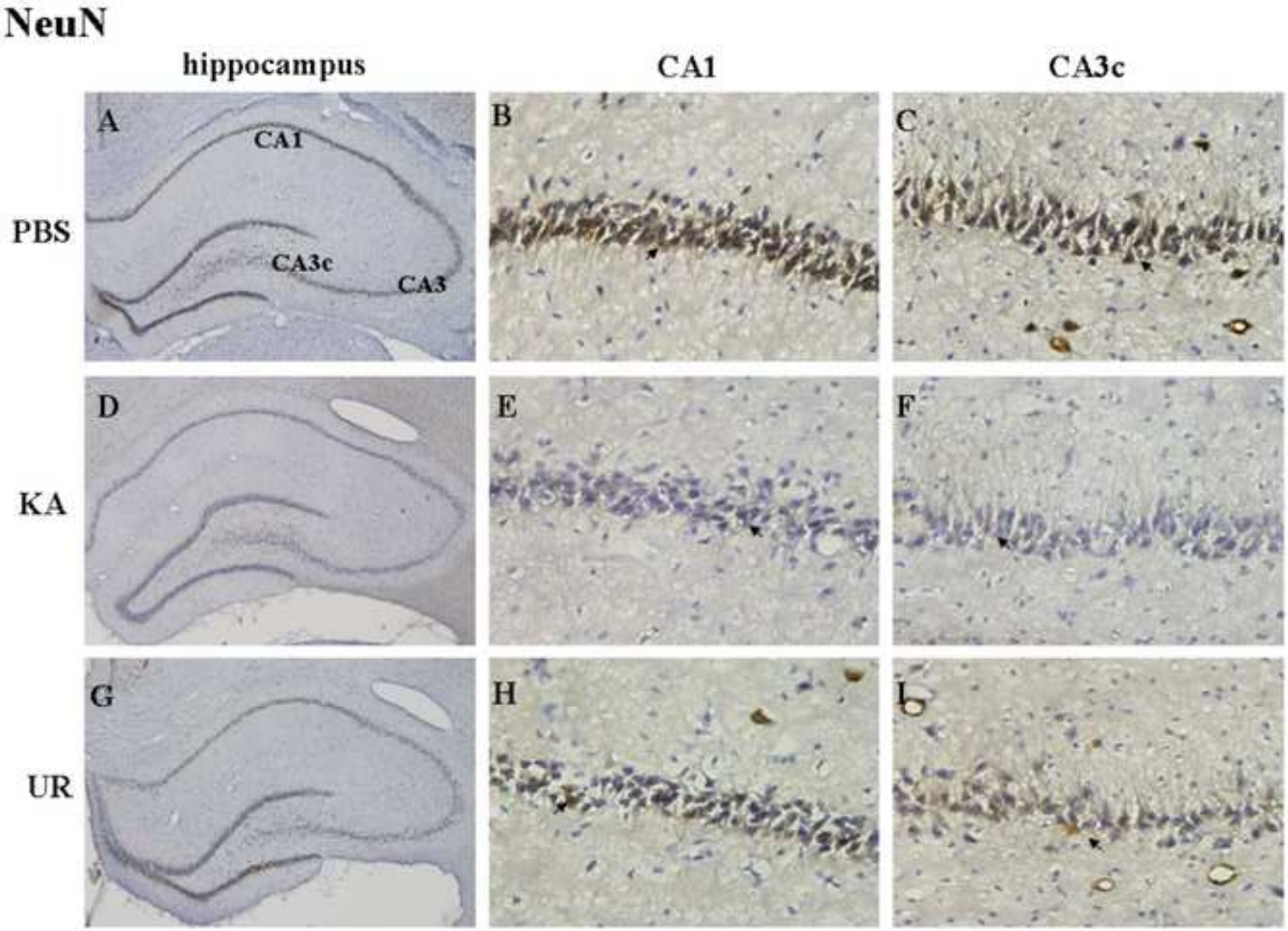

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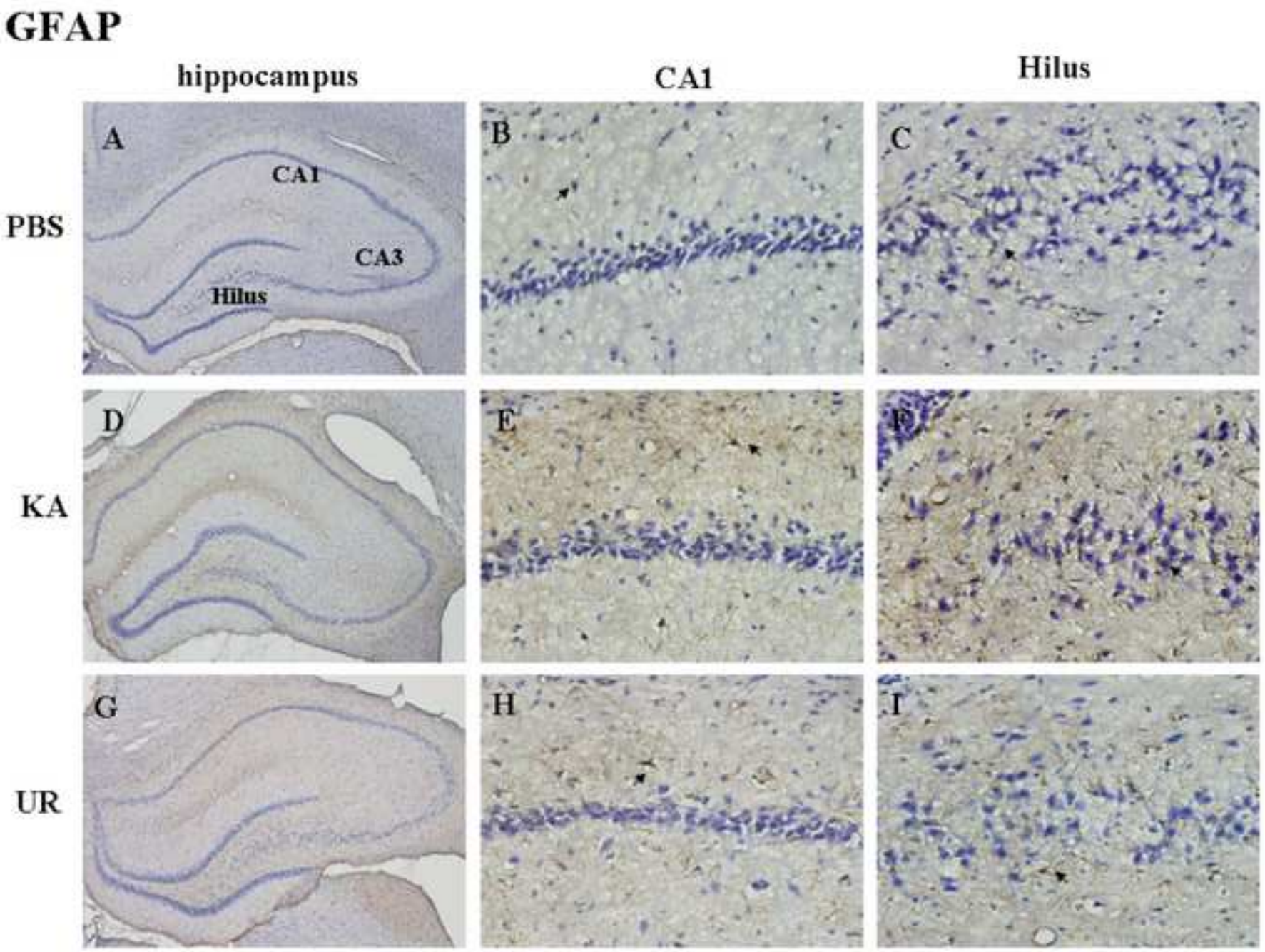

Figure 5

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