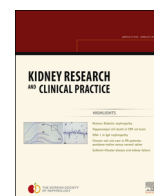




ELSEVIER

Kidney Research and Clinical Practice

journal homepage: <http://www.krcp-ksn.com>
Contents lists available at ScienceDirect

Original Article

Chronic renal failure induces cell death in rat hippocampal CA1 via upregulation of α CaMKII/NR2A synaptic complex and phosphorylated GluR1-containing AMPA receptor cascadesJong Wan Kim^{1,2}, Gyoung Yim Ha³, Yong Wook Jung^{1,2,*}¹ Section of Neuroscience Research, Medical Institute of Dongguk University, Gyeongju, Korea² Department of Anatomy, College of Medicine, Dongguk University, Gyeongju, Korea³ Department of Laboratory Medicine, College of Medicine, Dongguk University, Gyeongju, Korea

A B S T R A C T

Article history:

Received 14 March 2014

Received in revised form

4 July 2014

Accepted 20 July 2014

Available online 5 September 2014

Keywords:

Alpha-amino-3-hydroxy-5-methylisoxazole-4-propionic acid receptor

Chronic renal failure

Hippocampus

Isoform of calcium/calmodulin-dependent protein kinase II

N-methyl-D-aspartate receptor

Background: N-methyl-D-aspartate (NMDA) and alpha-amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA) receptors bound to postsynaptic density-95 (PSD-95) and α isoform of calcium/calmodulin-dependent protein kinase II (α CaMKII) is fundamentally involved in the regulation of working memory. The aim of present study was to investigate the alterations of NMDA and AMPA receptors responsible for hippocampal synaptic dysfunction and selective neuronal cell death after chronic renal failure (CRF) which may be associated with impairment of working memory.

Methods: Altered interactions between NMDA and AMPA receptors and PSD-95 and α CaMKII were analyzed in the cornu ammonis (CA) 1 and CA3/dentate gyrus (DG) subfields of the uremic rat hippocampi using the immunoblotting and immunoprecipitation methods.

Results: Uremia induced by CRF produced necrotic cell death and decreased neuronal nucleoli protein levels in the hippocampal CA1 subfield, but not in the CA3/DG subfields. The CA1 subfields of CRF rats exhibited significant decreases and increases, respectively, in the expressions of PSD-95/NR2B and α CaMKII/NR2A synaptic complex. Moreover, increased phosphorylation of glutamate receptor type 1 (GluR1) AMPA receptor at ser831 was observed in the CA1 subfield after CRF.

Conclusion: These hippocampal CA1 neuronal vulnerability may be responsible for memory dysfunction after CRF as mediated by an increase in NR2A-containing NMDA receptors bound to α CaMKII and subsequent activation of GluR1-containing AMPA receptors caused by the phosphorylation of GluR1 at ser831.

© 2014. The Korean Society of Nephrology. Published by Elsevier. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

* Corresponding author. Department of Anatomy, College of Medicine, Dongguk University, 123, Dongdae-ro, Gyeongju, 780-714, Korea.

E-mail address: jungyw@dongguk.ac.kr (YW Jung).

Introduction

In science, cognition is the mental processing that includes the attention of working memory, comprehending and producing language, calculating, reasoning, problem solving, and decision making [1]. The hippocampus belongs to the limbic system and plays important roles in the consolidation of spatial working memory. Moreover, hippocampal volume can be used to detect cognitive dysfunction and identify elderly people at risk of Alzheimer's disease [2]. However, the selective vulnerability of hippocampal neurons in chronic renal failure (CRF) remains speculative even though the hippocampus seems particularly susceptible to a variety of uremic toxins and metabolites [3]. Clinical studies have demonstrated a high risk for dementia and cognitive impairment in patients with chronic kidney disease (CKD) and those undergoing hemodialysis [4,5]. Even in patients with neurologically asymptomatic chronic renal disease, impaired cognitive processing can be disclosed by event-related potentials [4]. In animal studies, evidence indicates that uremia impairs synaptic transmission in the rat hippocampus, and that this results in increased glutamate release presynaptically, which in turn, impairs spontaneous synaptic activity [6]. These observations suggest that marked elevations in uremic toxin levels functionally perturb synaptic function of the hippocampus and leads to neuronal cell death and subsequent cognitive dysfunction.

N-methyl-D-aspartate (NMDA) receptors mediate long-term potentiation which is a candidate mechanism for memory [7], and play critical roles in synaptic dysfunction and neuronal cell death [8]. In the hippocampus, the NMDA receptors are composed of NR1 and NR2 subunits (NR2A, NR2B), which cluster with scaffolding proteins, such as, postsynaptic density-95 (PSD-95) and α isoform of calcium/calmodulin-dependent protein kinase II (α CaMKII) [9]. Previous studies have reported that the dynamic but independent regulations of the NR2A and NR2B subunits by α CaMKII and PSD-95, respectively, are modulated by competitive or antagonistic interactions between these two scaffolding proteins [10]. Furthermore, it has been shown that different levels of NR2A and NR2B at synapses differentially regulate the surface expression of glutamate receptor type 1 (GluR1)-containing 2-amino-3-(5-methyl-3-oxo-1,2-oxazol-4-yl) propanoic acid (AMPA) receptor via the Ras-activated extracellular signal-regulated kinase (ERK) pathway, which is essential for the modification of synaptic strength [11].

AMPA receptor is an ionotropic glutamate receptor that functions in fast synaptic transmission and plasticity [12]. At the excitatory synapses of central neurons, AMPA receptors are organized into multiprotein signaling complexes within the postsynaptic density [12]. In a previous study, it was demonstrated that protein kinase G regulates AMPA receptor trafficking by modulating GluR1-ser845 phosphorylation in hippocampal slices [13], and in another, it was shown that the α CaMKII-mediated Ser831 phosphorylation of GluR1 is associated with AMPA receptor expression and potentiation in the postsynaptic membrane [14]. However, diminished α CaMKII levels result in the detachment of GluR1 subunit-containing AMPA receptors from cell surfaces and in the attenuation of AMPA receptor currents [15].

Accordingly, alterations in the levels of postsynaptic proteins in the hippocampus can significantly affect cognitive impairment through the synaptic dysfunction and

subsequent neuronal vulnerability, and thus could contribute importantly to uremic encephalopathy in CRF. In this study, changes in the protein expressions of postsynaptic proteins and their interactions in the hippocampi of CRF rats were examined.

Methods

Experimental animals

Studies were performed using adult male Sprague–Dawley rats weighing 290 ± 8 g. Animals were maintained on a standard rodent diet with free access to water. The experimental procedures used were reviewed and approved by the Animal Care and Use Committee of Dongguk University, Gyeongju, Korea. Animal care and use were also in accord with the guidelines issued by the National Institute of Health (Bethesda, MD, USA).

Induction of CRF by surgical reduction

Experimental CRF was induced by excising approximately two-thirds of the left kidney and performing right total nephrectomy [16]. The protocols used in this study are depicted in Fig. 1. Rats were anesthetized with 3% isoflurane in a mixture of oxygen/nitrous oxide (30:70) and maintained on 1.5% isoflurane. During surgery, a heating pad was used to maintain a rectal temperature of 37–38°C. The left kidney was exposed via a left flank incision and gently dissected free from the adrenal gland. Approximately two-thirds of the left kidney, including the upper and lower poles, was then excised. One week after initial surgery, again under isoflurane anesthesia, right kidneys were excised (after release from the adrenal gland) and removed through a right flank incision. Some rats were subjected to an identical sham operation, but kidneys were left intact. Sham-operated rats and 5/6 nephrectomized CRF rats were monitored for 7 weeks after surgery for establishing the long-term effects of chronic kidney disease on the brain. Cornu ammonis (CA) 1 and CA3/ dentate gyrus (DG) tissue sections of hippocampus were separated under the surgical microscopy and then rapidly frozen at -80°C until required for protein assays.

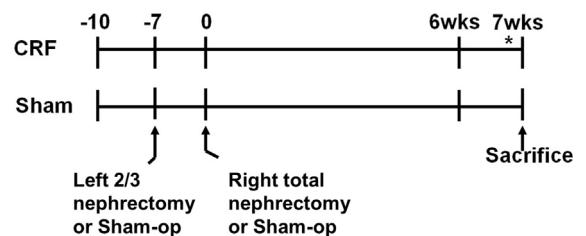


Figure 1. Diagram of the excision remnant kidney model. Experimental chronic renal failure (CRF) was induced by two-stage excision of about two-thirds of the left kidney and right total nephrectomy. The left kidney was exposed using a left flank incision and gently dissected free from the adrenal gland; approximately two-thirds of the left kidney including the upper and lower pole was excised. One week later, the right kidney was removed via a right flank incision after dissecting it free from the adrenal gland. Rats were maintained in metabolic cages on the days marked with an asterisk. As controls, some rats were subjected to a sham operation identical to that described above but without kidney tissue removal.

Plasma and urine analyses

Twenty four-hour urine samples were collected in metabolic cages on the last day of the experiment. Blood samples were taken from abdominal aortas under isoflurane-anesthesia and heparinized (10 IU/mL of blood). Plasma was immediately separated by centrifugation ($2,000 \times g$ for 3 minutes at 4°C). Urine and plasma samples were stored in a freezer (-80°C) until required for chemical analyses. Hematocrit, plasma creatinine, and urinary creatinine clearance were measured using conventional methods.

Cresyl violet staining

Sham-operated control rats ($n=3$) and 5/6 nephrectomized CRF rats ($n=3$) were anesthetized and the brain was fixed by a transcardiac perfusion of 4% paraformaldehyde in phosphate buffered saline (PBS) after removal of blood from the cerebral vasculature by perfusion of physiologic saline. The brain was removed and postfixed in the same fixative for 12 hours. Perfusion fixed brain tissues were paraffin-embedded and serial coronal sections ($5 \mu\text{m}$ thickness) were obtained at the level of dorsal third ventricle (bregma -4.16 mm). Paraffin wax was removed by xylene overnight at room temperature and the sections were rehydrated with ethanol (99%, 96%, and 70%). After washing in distilled water, the sections were stained with cresyl violet for 30 minutes at room temperature. The sections were then treated successively with ethanol (50%, 70%, 95%, and 100%) and differentiator (glacial acetic acid and 95% ethanol).

Hippocampal homogenate preparation

For protein extraction, each hippocampal CA1 and CA3/DG subfield tissue sections were homogenized in ice-cold homogenizing buffer [50mM HEPES, 50mM NaF, 20mM sodium

pyrophosphate (NaPPi), 1mM EDTA, 8.5mM leupeptin, and 1mM phenylmethylsulfonyl fluoride (PMSF); pH 7.4; $n=16$: sham operated control rats ($n=8$) and CRF rats ($n=8$)]. Protein contents were measured using the Bio-Rad protein assay kit (Bio-Rad Laboratories, Hercules, CA, USA).

Semiquantitative immunoblotting

The levels of proteins in hippocampal samples were determined by immunoblotting under conditions of protein linearity. After sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE), proteins were transferred to nitrocellulose membranes in a buffer solution containing 50mM tris-base, 380mM glycine, and 20% methanol. Membranes were then blocked with 5% milk in PBS-T (80mM Na_2HPO_4 , 20mM NaH_2PO_4 , 100mM NaCl, 0.1% Tween 20; pH 7.5) for 1 hour and incubated overnight at 4°C with mouse antineuronal nuclei (NeuN; 1:1,000; Chemicon, Temecula, CA, USA), rabbit antiPSD-95 (1:1,000), mouse anti-CaMKII (1:2,000), or mouse anti-p-Ser831 and 845 GluR1 (1:1,000; Santa Cruz Biotechnology, Texas, USA). Sites of antibody-antigen reaction were visualized using horseradish peroxidase (HRP)-conjugated secondary antibodies (P447 or P448, diluted 1:3,000; DAKO, Glostrup, Denmark) and an enhanced chemiluminescence system (ECL, Amersham Pharmacia Biotech, Little Chalfont, UK), and then exposed to photographic film (Hyperfilm ECL, RPN3103K, Amersham Pharmacia Biotech). Immunoblot signals developed using the ECL system were quantified using Scion Image software (version 1.59; Bethesda, Maryland, USA).

Immunoprecipitation

Hippocampal CA1 homogenates (600 μg) were diluted four-fold with 50mM HEPES buffer (10% glycerol, 150mM NaCl, 0.5% NP-40, 10mM Tris-HCl, 1.0mM EDTA, 1.0mM PMSF 1.0% Triton

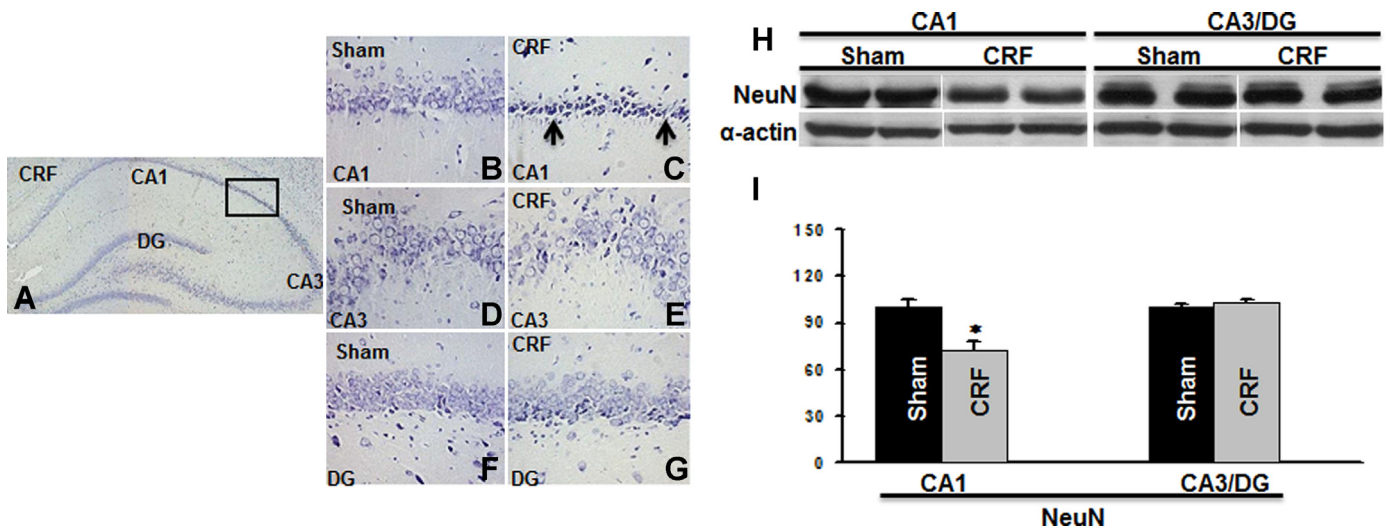


Figure 2. Neuronal cell death in the hippocampal CA1 subfield after CRF was determined by cresyl violet staining and immunoblotting. (A) Different subpopulations of hippocampal cells were chosen to characterize the cellular changes under CRF (X4). Rectangle indicates the necrotic subfield in the hippocampus. (B, D, F) There were no necrotic cells in the sham-operated rat hippocampus. (C) Cells in the CA1 subfield undergo typical necrotic cell death. (E, G) In contrast, the cells in the CA3/dentate gyrus region were more resistant to necrotic cell death (X20). Control $n=3$, CRF $n=3$. (H) Immunoblotting for neuronal nuclei (NeuN) protein revealed a major strong band at 32 kDa in sham-operated controls. α -actin was used as an internal control to confirm equal protein loading. (I) Immunoblotting analyses showed that the expressions of NeuN in the CA1 subfield were significantly decreased after CRF. In contrast, levels of NeuN in the CA3 and DG subfields were unchanged after CRF. Results are means \pm SEMs. Control $n=8$, CRF $n=8$. * $P < 0.01$. CA1, cornu ammonis 1; CA3, cornu ammonis 3; CRF, chronic renal failure; DG, dentate gyrus.

X-100, and 1.0% sodium deoxycholate; pH 7.4), and then affinity-purified rabbit anti-PSD-95 (Upstate Biotechnology, Lake Placid NY, USA) and α CaMKII antibodies (Affinity Bioreagents, Golden Colorado, USA; both 1:50) were added. After incubating mixtures for 4 hours at 4°C, protein A-agarose slurry (1:1) was added and incubated for a further 2 hours. Samples were then centrifuged at $100,000 \times g$ and the pellets collected were washed three times with HEPES buffer. Bound proteins were eluted by adding four volumes of SDS gel electrophoresis sample buffer, and then boiled for 7 minutes. Finally samples were centrifuged at $100,000 \times g$ for 2 minutes and the supernatants so obtained were applied to SDS-gels and electrotransferred onto nitrocellulose membrane for immunoblotting with anti-NR2A antibody or anti-NR2B antibody (1: 1,000; Upstate Biotechnology, Lake Placid NY, USA).

Data analysis

Values are presented as mean \pm standard error of mean (SEM). Groups were compared using one-way analysis of variance (ANOVA) followed by Bonferroni's post hoc test. The statistical differences were considered significant if the *P* values less than 0.05 or 0.01.

Results

Establishment of CRF induced by 5/6 nephrectomy

Seven weeks after 5/6 nephrectomy, CRF rats lost \sim 9% of body weight as compared with the sham-operated controls. Diminished renal function was demonstrated by significant increases in plasma creatinine levels (8.75 ± 0.47 in CRF vs. 1.13 ± 0.55 mg/dL in sham-operated controls; $P < 0.05$) and urine outputs (61.4 ± 10.9 in CRF vs. 45.9 ± 3.1 mL/kg/d in sham-operated controls; $P < 0.05$). Urinary creatinine clearance was also significantly decreased (4.45 ± 0.32 in CRF vs. 7.32 ± 0.83 L/kg/d in sham-operated controls; $P < 0.05$). In addition, CRF rats had lower hematocrit levels (37.4 ± 0.6 in CRF vs. $42.3 \pm 0.6\%$ in sham-operated controls; $P < 0.05$).

Neuronal cell death in the CA1 subfield of uremic hippocampi

Cresyl violet staining demonstrated that cells in the medial CA1 subfield of hippocampus (Fig. 2A, rectangle) underwent typical necrotic cell changes, such as dark in color, irregular shape, cork-screw processes, and expansion of perineuronal space (Fig. 2C, arrows). By contrast, cells in the CA3 subfield and DG region were more resistant and less necrotic cell death was observed (Fig. 2E and G). Semiquantitative immunoblotting for neuronal nuclei (NeuN) protein revealed a major strong band at 32 kDa in sham-operated controls (α -actin was used as internal loading control; Fig. 2H). Immunoblotting demonstrated that the expressions of NeuN in CA1 subfield homogenates were significantly reduced by CRF (to $72 \pm 6.3\%$ vs. sham-operated controls, $n=8$, $P < 0.01$). By contrast, levels of NeuN in CA3 and DG subfields were not changed by CRF (Fig. 2I).

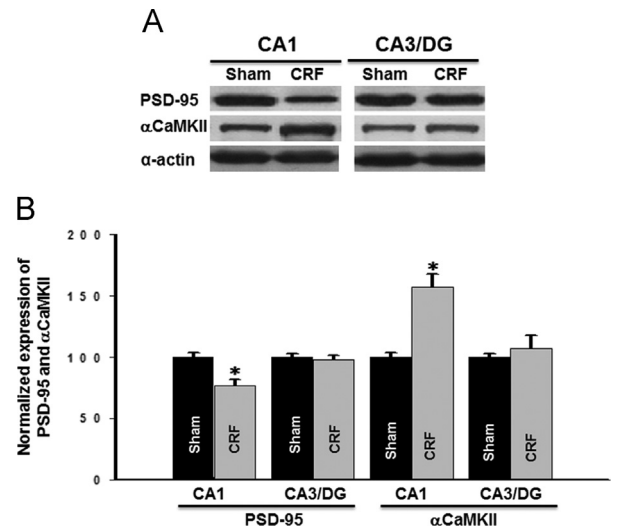


Figure 3. Normalized expressions of postsynaptic density-95 (PSD-95) and of a isoform of calcium/calmodulin-dependent protein kinase II (α CaMKII) in rats hippocampi after CRF. (A) Immunoblot with anti-PSD-95 and α CaMKII antibodies revealed 95 and 50 kDa bands, respectively, in sham-operated and CRF rats. (B) In CA1 subfields, densitometric analyses revealed that CRF significantly decreased PSD-95 expression but significantly increased α CaMKII expression. The expressions of PSD-95 and α CaMKII after CRF were unchanged in the CA3/DG subfields. Results are means \pm SEMs. Control $n=8$, CRF $n=8$. * $P < 0.01$. CA1, cornu ammonis 1; CA3, cornu ammonis 3; CRF, chronic renal failure; DG, dentate gyrus; SEM, standard error of mean.

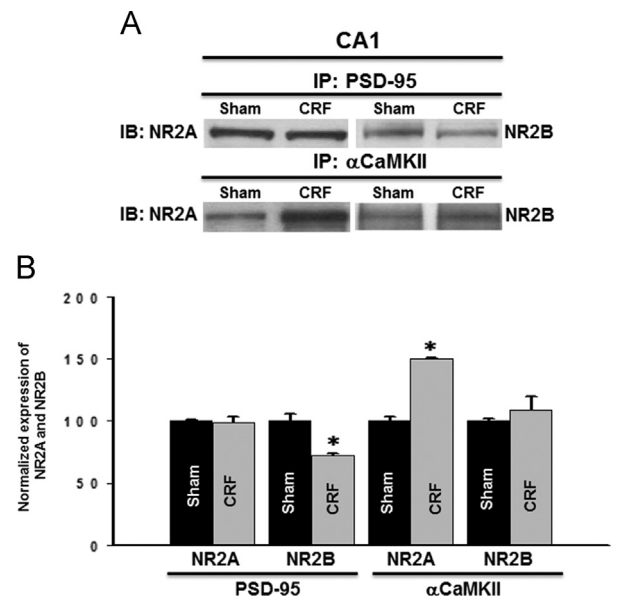


Figure 4. CRF induced changes in bindings between PSD-95 or α CaMKII and NMDA receptor subunits (NR2A and NR2B) in the rat hippocampal CA1 subfield. (A) Homogenate samples from sham-operated and CRF rats were immunoprecipitated with anti-PSD-95 and anti- α CaMKII antibodies, respectively. Immunoprecipitates were analyzed by immunoblotting with anti-NR2A or anti-NR2B antibodies. (B) Quantitative analysis of immunoblots showed the amount of NR2B that co-precipitated with PSD-95 was lower, but that the amount of NR2A that co-precipitated with α CaMKII was higher after CRF. Results are means \pm SEMs. Control $n=8$, CRF $n=8$. * $P < 0.01$. CA1, cornu ammonis 1; CRF, chronic renal failure; NMDA, N-methyl-D-aspartate; PSD-95, postsynaptic density-95; α CaMKII, α isoform of calcium/calmodulin-dependent protein kinase II; SEM, standard error of mean.

Protein expressions of PSD-95 and α CaMKII in uremic hippocampi

It was examined whether levels of PSD-95 and α CaMKII were altered in the CA1 and CA3/DG subfields by CRF. Semi-quantitative immunoblotting demonstrated that CRF induced significant changes in the levels of both proteins in the CA1 subfield but not in the CA3/DG subfields. In the hippocampi of sham-operated control rats and CRF rats, immunoblotting for PSD-95 and α CaMKII revealed major strong bands at 95 kDa and 50 kDa, respectively (Fig. 3A). In the CA1 subfield, PSD-95 expression was significantly lower in CRF rats than in sham-operated controls (to $77 \pm 4.7\%$ of sham-operated controls, $P < 0.01$, $n=8$). By contrast, α CaMKII expression in the CA1 subfield was significantly higher in CRF rats (to $157 \pm 10.3\%$ of sham-operated controls, $P < 0.01$, $n=8$, Fig. 3B). Immunoblot analysis of the CA3/DG subfields did not reveal any changes in the expressions of PSD-95 or α CaMKII (Fig. 3A and B). α -Actin was used as internal control to confirm equal protein loadings.

Bindings between the PSD-95/ α CaMKII and the subunits of NMDA receptors in the CA1 subfield of uremic hippocampus

Because the regulatory subunits (NR2A and NR2B) of NMDA receptors dynamically bind to α CaMKII [17] and PSD-95 [10], respectively, changes in the expressions of PSD-95 and α CaMKII raised the possibility that synaptic complexes between the PSD-95/ α CaMKII and the regulatory subunits (NR2B and NR2A, respectively) of NMDA receptors were disrupted by CRF. As indicated in Fig. 4A, the amount of NR2B that coprecipitated with PSD-95 was lower in CRF rats (to $72 \pm 1.7\%$ of that of sham-operated controls, $P < 0.01$, $n=8$; Fig. 4B). However, no change in the amount of NR2A that coprecipitated PSD-95 was detected after CRF. Protein homogenates were also immunoprecipitated

with anti- α CaMKII (Fig. 4A), and it was found that binding between α CaMKII and NR2A was significantly enhanced by CRF (to $150 \pm 0.9\%$ of that of sham-operated controls, $P < 0.01$, $n=8$; Fig. 4B). However, no significant changes were observed in the amount of α CaMKII that coprecipitated with NR2B after CRF.

Phosphorylations of GluR1 at ser831 and ser845 in the CA1 subfield

To determine whether CRF induces the phosphorylations of GluR1-ser831 and GluR1-ser845 in the uremic CA1 subfield, samples of sham-operated control and CRF CA1 tissues were immunoblotted with antiGluR1-ser831 and antiGluR1-ser845. In sham-operated control rats and CRF rats, immunoblotting for GluR1-ser831 and GluR1-ser845 revealed major strong bands at 132 kDa and 100 kDa, respectively (Fig. 5A). Furthermore, the expression of GluR1-ser831 in the uremic CA1 subfield was higher than in sham-operated controls ($129 \pm 3.5\%$ of sham-operated controls, $P < 0.01$, $n=8$). However, the expression of GluR1-ser845 showed no significant change (Fig. 5B).

Discussion

Several studies have suggested that the frequency of cognitive disturbance is higher than that previously suspected and can be detected even in moderate chronic renal insufficiency [4,18]. In the present study, CRF was induced in rats by 5/6 nephrectomy and animals were then monitored for 7 weeks. In 2013, Fujisaki et al [19] followed rats for 4 weeks or 8 weeks to establish the long-term effects of uremia and found that serum creatinine and blood urea nitrogen concentrations in 5/6 nephrectomy mice were significantly higher than in control mice. However, they reported that no significant difference in all parameters between CKD-4W and CKD-8W. Hence, we have chosen the duration of follow-up to 7 weeks after surgery.

Uremia induced by CRF in the present study produced necrotic cell death and decreased neuronal marker protein (NueN) levels in the hippocampal CA1 subfield, but not in the CA3/DG subfields, indicating that CA1 is more vulnerable to neuronal cell death in CRF than CA3/DG. This proposition is generally in accord with observations of ischemic injury-induced changes in postsynaptic density, during which CA1 neurons are more prone to injury than CA3 or DG neurons [20]. Kesner et al [21] emphasized that CA1 subfield involved in spatial working memory in pattern separation, with CA3 subfield being more important in spatial working memory in pattern completion and in pattern association. Compromised renal function was found to present model CRF, in terms of increased plasma creatinine levels and decreased creatinine clearance. Similar to guanidine, guanidinosuccinic acid, and methylguanidine, creatinine is a guanidine compound and is thus considered a candidate uremic toxin. During uremia, the accumulation of neurotoxic metabolites may be important in the pathogenesis of uremic encephalopathy [22,23]. Brain guanidinosuccinic acid and creatinine concentrations, corresponding with intraperitoneal doses that induce clonic convulsions in mice, are similar to the concentrations found in the brains of uremic patients [23]. Therefore, increased levels of creatinine in plasma is a candidate factor responsible for the cell death of hippocampal CA1 subfield which may be closely

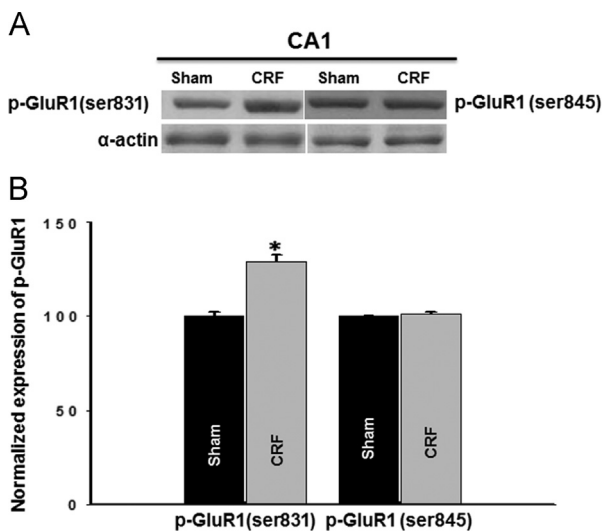


Figure 5. Determination of the phosphorylations of GluR1 at ser831 or ser845 in the hippocampal CA1 subfield after CRF. (A) Samples of sham-operated control and CRF CA1 tissues were immunoblotted with anti-GluR1-ser831 or anti-GluR1-ser845. Immunoblotting for GluR1-ser831 and GluR1-ser845 revealed major strong bands at 132 kDa and 100 kDa, respectively. (B) Densitometric analyses revealed that CRF significantly increased in p-GluR1 (ser831), but not p-GluR1 (ser845), in the CA1 subfield. Results are means \pm SEMs. Control $n=8$, CRF $n=8$. * $P < 0.01$. CA1, cornu ammonis 1; CRF, chronic renal failure; GluR1, glutamate receptor type 1; SEM, standard error of mean.

associated with dysfunction of spatial working memory in pattern separation. These toxins have been reported to increase the activation of NMDA receptors and to block GABA_A receptor ionophores [3]. The activation of NMDA receptors elicits Ca²⁺ influx, potentially causing calcium-mediated neurotoxicity. Immunoprecipitation and immunoblotting in the present study demonstrated that CRF was associated with significant changes in the expressions of postsynaptic protein complexes. First, a selective decrease in the expression of PSD-95/NR2B synaptic complex in the uremic CA1 subfield was found. Wang et al [24] showed that PDZ1 domain of PSD-95 protects neurons from cerebral ischemia. However, in a rat model, preconditioning ischemia was found to attenuate transient hippocampal ischemic damage by inducing an increase of PSD-95 [25]. In addition, previous studies have shown that upregulation of NR2A and NR2B after chronic ethanol exposure is likely to be an adaptive response to prolonged inhibition of channel activity by ethanol because of no corresponding change in the amount of PSD-95 [26,27]. Therefore, PSD-95 downregulation by CRF may reduce the targeting of NR2B to synapses and may be related to the synaptic dysfunction. Secondly, it was found that NR2A/αCaMKII synaptic complex was upregulated in the uremic CA1 subfield. αCaMKII, which is essential for memory and learning acquisition, abundantly expressed in the hippocampus and is known to be involved in the regulation of the functional subunit composition of NMDA receptors in the postsynaptic zone [28,29]. Moreover, the regulation of αCaMKII is dependent on many factors, including PSD-95 [30]. Previous studies have suggested that NR2A is a key NMDA receptor subunit with respect to the modulation of αCaMKII binding to NMDA channel [23,24]. The upregulation of αCaMKII in the CA1 subfield suggests that NR2A-containing NMDA receptors may be increased in the synapse after CRF. Thus, our results suggest that activation of NMDA receptor resulted from CRF is closely associated with an alteration of the NR2 subunits of NMDA receptors at synapses.

Recently, Kim et al [31] reported that NMDA receptor activation has differential effects on the surface delivery of GluR1, and that these effects depend on the NR2A/NR2B subunit ratios of NMDA receptors at synapses. In the hippocampus, AMPA receptors are mainly composed of GluR1/GluR2 or GluR2/GluR3 dimers with a minority of GluR1 homomers [32]. Recently, it was demonstrated that the surface expression of GluR1 in mature neurons is supported by NR2A-containing NMDA receptors, but inhibited by NR2B-containing NMDA receptors [33]. Other studies have also indicated that in mature neurons, NR2B is associated with the inhibition of (rather than the activation of) the Ras-ERK signaling pathway, which drives GluR1 to synapses [31,34]. Thus, our results suggest that increases in NR2A-containing NMDA receptors after CRF induce Ras-ERK pathway activation and subsequent increase GluR1 delivery to synapses.

In a recent study, it was implied that the ser831 phosphorylation of GluR1 might partly underlie synaptic modification [35]. Furthermore, accumulating evidence suggests that GluR1 phosphorylation at ser831 is closely related to αCaMKII/NR2A synaptic complex during long-term potentiation (LTP) [36].

αCaMKII translocated to the postsynaptic density, formed sites to anchor GluR1, and phosphorylated GluR1 after being activated by Ca²⁺ influx through NMDA receptor during LTP [36]. Thus, the observed increase in GluR1-ser831 phosphorylation, but not in GluR1-ser845 phosphorylation, in the uremic CA1 subfield suggests that activated αCaMKII translocated

to the synaptic membranes and then activated GluR1-containing AMPA receptors via GluR1-ser831 phosphorylation. Furthermore, as elevated levels of activated GluR1-containing AMPA receptors directly affect Na⁺ and Ca²⁺ influx, and hence, increased Na⁺ and Ca²⁺ influxes through activated GluR1-containing AMPA receptors may play a role in the neuronal vulnerability of the CA1 subfield. Our results suggest that the activation of GluR1-containing AMPA receptors at synapses, caused by the upregulation of αCaMKII/NR2A synaptic complex, is directly responsible for neurotoxicity in CA1 neurons rather than NMDA-induced neurotoxicity.

Taken together, our findings show that uremia after CRF can induce neuronal cell death of hippocampal CA1 subfield which may result in cognitive impairment in the uremic rat brain. Upregulation of αCaMKII/NR2A synaptic complex and subsequent augmentation of the phosphorylated GluR1-containing AMPA receptor at synapses can be suggested as the hypothetical mechanism of hippocampal CA1 cell death.

Conflict of interest

The authors declare no conflict of interest.

References

- [1] Sternberg RJ, Sternberg K: Cognitive psychology. 6th edition. Belmont, CA: Wadsworth, Cengage Learning; 2009
- [2] Wolf H, Grunwald M, Kruggel F: Hippocampal volume discriminates between normal cognition; questionable and mild dementia in the elderly. *Neurobiol Aging* 22:177–186, 2001
- [3] De Deyn PP, Vanholder R, Eloit S, Glorieux G: Guanidino compounds as uremic (neuro) toxins. *Semin Dial* 22:340–345, 2009
- [4] Kurella M, Chertow GM, Fried LF: Chronic kidney disease and cognitive impairment in the elderly: the health, aging, and body composition study. *J Am Soc Nephrol* 16:2127–2133, 2005
- [5] Murray AM, Tupper DE, Knopman DS: Cognitive impairment in hemodialysis patients is common. *Neurology* 67:216–223, 2006
- [6] D'Hooge R, Pei YQ, De Deyn PP: N-methyl-D-aspartate receptors contribute to guanidinosuccinate-induced convulsions in mice. *Neurosci Lett* 157:123–126, 1993
- [7] Harris EW, Ganong AH, Cotman CW: Long-term potentiation in the hippocampus involves activation of N-methyl-D-aspartate receptors. *Brain Res* 323:132–137, 1984
- [8] Sheng M, Kim MJ: Postsynaptic signaling and plasticity mechanisms. *Science* 298:776–780, 2002
- [9] Kennedy MB: Signal-processing machines at the postsynaptic density. *Science* 290:750–754, 2000
- [10] Park CS, Elgersma Y, Grant SG, Morrison JH: alpha-Isoform of calcium-calmodulin-dependent protein kinase II and postsynaptic density protein 95 differentially regulate synaptic expression of NR2A- and NR2B-containing N-methyl-D-aspartate receptors in hippocampus. *Neuroscience* 151:43–155, 2008
- [11] Kim MJ, Dunah AW, Wang YT, Sheng M: Differential roles of NR2A- and NR2B-containing NMDA receptors in Ras-ERK signaling and AMPA receptor trafficking. *Neuron* 46:745–760, 2005
- [12] Nakagawa T: The biochemistry, ultrastructure, and subunit assembly mechanism of AMPA receptors. *Mol Neurobiol* 42: 161–184, 2010
- [13] Serulle Y, Zhang S, Ninan I, Puzzo D, McCarthy M, Khatri L, Arancio O, Ziff EB: A GluR1-cGKII interaction regulates AMPA receptor trafficking. *Neuron* 56:670–688, 2007
- [14] Lüscher C, Xia H, Beattie EC, Carroll RC, von Zastrow M, Malenka RC, Nicoll RA: Role of AMPA receptor cycling in synaptic transmission and plasticity. *Neuron* 24:649–658, 1999

- [15] Hayashi Y, Shi SH, Esteban JA, Piccini A, Poncer JC, Malinow R: Driving AMPA receptors into synapses by LTP and CaMKII: requirement for GluR1 and PDZ domain interaction. *Science* 287:2262–2267, 2000
- [16] Kwon TH, Frøkiaer J, Knepper MA, Nielsen S: Reduced AQP1, -2, and -3 levels in kidneys of rats with CRF induced by surgical reduction in renal mass. *Am J Physiol* 275:724–741, 1998
- [17] Takagi N, Logan R, Teves L, Wallace MC, Gurd JW: Altered interaction between PSD-95 and the NMDA receptor following transient global ischemia. *J Neurochem* 74:169–178, 2000
- [18] Hailpern SM, Melamed ML, Cohen HW: Moderate chronic kidney disease and cognitive function in adults 20 to 59 years of age: Third National Health and Nutrition Examination Survey (NHANES III). *J Am Soc Nephrol* 18:2205–2213, 2007
- [19] Fujisaki K, Tsuruya K, Yamato M, Toyonaga J, Noguchi H, Nakano T, Taniguchi M, Tokumoto M, Hirakata H, Kitazono T: Cerebral oxidative stress induces spatial working memory dysfunction in uremic mice: neuroprotective effect of tempol. *Nephrol Dial Transplant* 29:529–538, 2014
- [20] Martone ME, Jones YZ, Young SJ, Ellisman MH, Zivin JA, Hu BR: Modification of postsynaptic densities after transient cerebral ischemia: a quantitative and three-dimensional ultrastructural study. *J Neurosci* 19:1988–1997, 1999
- [21] Kesner RP, Gilbert PE, Wallenstein GV: Testing neural network models of memory with behavioral experiments. *Curr Opin Neurobiol* 10:260–265, 2000
- [22] Brouns R, De Deyn PP: Neurological complications in renal failure: a review. *Clin Neurol Neurosurg* 107:1–16, 2004
- [23] De Deyn PP, D'Hooge R, Van Bogaert PP: Endogenous guanidine compounds as uremic neurotoxins. *Kidney Int Suppl* 78:77–83, 2001
- [24] Wang WW, Hu SQ, Li C, Zhou C, Qi SH, Zhang GY: Transduced PDZ1 domain of PSD-95 decreases Src phosphorylation and increases nNOS (Ser847) phosphorylation contributing to neuroprotection after cerebral ischemia. *Brain Res* 1328:162–170, 2010
- [25] Li C, Han D, Zhang F, Zhou C, Yu HM, Zhang GY: Preconditioning ischemia attenuates increased neurexin-neuroigin1-PSD-95 interaction after transient cerebral ischemia in rat hippocampus. *Neurosci Lett* 426:192–197, 2007
- [26] Chandler LJ, Norwood D, Sutton G: Chronic ethanol upregulates NMDA and AMPA, but kainite receptor subunit proteins in rat primary cortical cultures. *Alcohol Clin Exp Res* 23:363–370, 1999
- [27] Follesa P, Ticku MK: Chronic ethanol-mediated up-regulation of the N-methyl-D-aspartate receptor polypeptide subunits in mouse cortical neurons in culture. *J Biol Chem* 271:13297–13299, 1996
- [28] Lisman J, Schulman H, Cline H: The molecular basis of CaMKII function in synaptic and behavioral memory. *Nat Rev Neurosci* 3:175–190, 2002
- [29] Barria A, Malinow R: NMDA receptor subunit composition controls synaptic plasticity by regulating binding to CaMKII. *Neuron* 48:289–291, 2005
- [30] Weeber EJ, Jiang YH, Elgersma Y, Varga AW, Carrasquillo Y, Brown SE, Christian JM, Mirnikjoo B, Silva A, Beaudet AL, Sweatt JD: Derangements of hippocampal calcium/calmodulin-dependent protein kinase II in a mouse model for Angelman mental retardation syndrome. *J Neurosci* 23:2634–2644, 2003
- [31] Kim JH, Lee HK, Takamiya K, Haganir RL: The role of synaptic GTPase-activating protein in neuronal development and synaptic plasticity. *J Neurosci* 23:1119–1124, 2003
- [32] Wenthold RJ, Petralia RS, Blahos II J, Niedzielski AS: Evidence for multiple AMPA receptor complexes in hippocampal CA1/CA2 neurons. *J Neurosci* 16:1982–1989, 1996
- [33] Malinow R, Malenka RC: AMPA receptor trafficking and synaptic plasticity. *Annu Rev Neurosci* 25:103–126, 1999
- [34] Zhu JJ, Qin Y, Zhao M, Van Aelst L, Malinow R: Ras and Rap control AMPA receptor trafficking during synaptic plasticity. *Cell* 110:443–455, 2002
- [35] Song I, Haganir RL: Regulation of AMPA receptors during synaptic plasticity. *Trends Neurosci* 25:578–588, 2002
- [36] Lisman JE, Zhabotinsky AM: A model of synaptic memory: a CaMKII/PP1 switch that potentiates transmission by organizing an AMPA receptor anchoring assembly. *Neuron* 31:191–201, 2001