

Expression of AMPA receptor subunits in hippocampus after status convulsion

Yue Hu · Li Jiang · Hengsheng Chen · XiaoPing Zhang

Received: 14 December 2011 / Accepted: 26 March 2012 / Published online: 15 April 2012
© Springer-Verlag 2012

Abstract

Objective The expression of 2-amino-3-(5-methyl-3-oxo-1, 2-oxazol-4-yl) propanoic acid receptor (AMPA) subunits in the hippocampus of naive immature and adult rats (IRs, ARs) was investigated after status convulsion (SC).

Methods Seizures were induced in IRs and ARs with intraperitoneal injections of lithium and pilocarpine. Rats were killed at four time points (3 h, 1 day, 3 days, and 7 days) after SC. The proportion of apoptotic cells was quantified by Annexin V-FITC apoptosis detection. The location and type of apoptotic cells were assessed by using terminal deoxynucleotidyl

transferase-mediated dUTP nick end-labeling staining. Immunoblotting techniques were used to demonstrate changes in AMPAR subunit expression.

Results Severe seizures induced neuronal apoptosis in the hippocampus. The proportion of apoptotic cells in IRs was consistently lower than that in ARs after SC. The expressions of four AMPAR subunits in IRs were consistently lower than those in ARs before and after SC. SC for 1 h inhibited the expression of glutamate receptors (GluR1–4) in the hippocampus of IRs and ARs and altered the subunit composition of AMPARs. GluR2 was the predominant AMPAR subunit in the hippocampus of normal ARs, while the GluR2/3 subunits were predominantly expressed 7 days after SC. GluR3/4 subunits were mainly expressed in the hippocampus of normal IRs, which had the lowest levels of GluR2.

Conclusions Immature brain was more resistant to seizure-induced neural damage. The time course of reduction and recovery differed for each subunit and was dependent on developmental stage. The increased expression of GluR2 could confer early but transient protection in the immature brain after SC.

Y. Hu · L. Jiang (✉)

Department of Neurology, Children's Hospital,
Chongqing University of Medical Sciences,
No.136 Zhongshan 2nd Road, Yu Zhong District,
Chongqing 400014, China
e-mail: jiangli19640718@163.com

Y. Hu

e-mail: huyue915@163.com

Y. Hu · L. Jiang · H. Chen · X. Zhang

Ministry of Education Key Laboratory
of Child Development and Disorders,
Chongqing, China

H. Chen

e-mail: chenhsheng@sina.com

X. Zhang

e-mail: tuotai@163.com

Y. Hu · L. Jiang · H. Chen · X. Zhang

Key Laboratory of Pediatrics in Chongqing, CSTC2009CA5002,
Chongqing, China

Y. Hu · L. Jiang · H. Chen · X. Zhang

Chongqing International Science and Technology Cooperation
Center for Child Development and Disorders,
Chongqing, China

Keywords Status convulsion · Hippocampus · AMPA (2-amino-3-(5-methyl-3-oxo-1 · 2-oxazol-4-yl) propanoic acid) receptor · Glutamate receptor (GluR) subunits

Introduction

Laboratory models of prolonged seizures and status epilepticus in developing animals have demonstrated an age-dependent propensity for brain injury [1, 2]. Children are more vulnerable to seizures and SC; however, adults are at a higher risk than children of late seizures and disability [3, 4]. The mechanism that protects the developing brain when it is highly susceptible to seizures remains unknown, but

epileptogenesis in an immature brain is influenced by multiple changes that occur during development, such as the inhibitory and excitatory systems [5]. Glutamate is the major excitatory amino acid transmitter in the central nervous system and exerts its action through receptors that function as ion channels, such as α -amino-3-hydroxy-5-methyl-4-isoxazole propanoic acid receptors (AMPA receptors), *N*-methyl-D-aspartate receptors (NMDARs), and kainate receptors, as well as through signaling cascades via metabotropic receptors [6]. NMDARs are thought to be relatively stable in the membrane [7], while the number of AMPARs at synapses is modified by rapid activity-dependent insertion or removal of receptors during several forms of synaptic plasticity [8].

AMPA receptors are hetero-oligomeric proteins made of glutamate receptor subunits, GluR1–4 [6], which are responsible for primary depolarization in glutamate-mediated neurotransmission and play key roles in synaptic plasticity. Long-lasting and activity-dependent changes in synaptic strength (long-term potentiation (LTP), or long-term depression (LTD)) are associated with changes in the phosphorylation and cellular distribution of AMPARs and are thought to underlie learning and memory formation [9]. The deregulation of AMPAR activity is also involved in pathology [10]. In this paper, we investigated the dynamic changes in apoptosis and expression of AMPAR subunits in the hippocampus of immature and adult rats after status convulsion (SC) and clarified the possible roles of AMPARs in epileptogenesis.

Materials and methods

Animal models and groups

Thirty healthy adult (postnatal day 60 [P60]) and 30 immature (postnatal day 20 [P20]) Wistar rats (ARs and IRs) were provided from the animal center of Chongqing University of Medical Sciences. The genders of the rat were random. Experimental Animal Care Guidelines were followed, which were in compliance with the National Animal Ethical Policies. The SC animal model was adopted when SC is evoked by lithium and pilocarpine (Sigma Company, St. Louis, MO, USA). Seizures were induced in IRs and ARs individually with lithium (3 mEq/kg) followed by pilocarpine injected intraperitoneally (40 mg/kg) 18–20 h later. According to Smialowski's six category classification of seizures [11], rats with IV or V class seizures of 1-h duration were admitted. Fifteen minutes after the onset of the seizure, rats were injected intraperitoneally with atropine (1 mg/kg, He Feng Tragacanth Company, Shanghai, China). After 1 h (1 h SC), the seizures were terminated with an intraperitoneal injection of chloral hydrate (400 mg/kg, He Feng Tragacanth Company, Shanghai, China). Rats were killed at four time points (3 h, 1 day, 3 days, and 7 days) after the end of the SC ($n=5$). In

addition, normal (pre-SC) controls and experimental controls were established, which rats were injected intraperitoneally with lithium, atropine, and chloral hydrate, but without pilocarpine ($n=5$).

Annexin V-FITC apoptosis detection

The right-sided hippocampus was dissected and used to make the cell suspensions ($n=5$). The protocol followed the instruction manual of the Annexin V-FITC apoptosis assay kit (Biosea Biotechnology Co., Ltd., Beijing, China). Approximately 1×10^6 cells were resuspended in 100 μ L of reaction buffer, to which 10 μ L of Annexin V-FITC and 5 μ L of propidium iodide (PI) were then added. The cells were incubated in the dark for 20 min at room temperature. After which, 300 μ L of reaction buffer was added. The solution was then ready for analysis by flow cytometry. The histogram of apoptotic cells was made up of four quadrants. The lower left quadrant represents normal cells (Annexin V-/PI-). The lower right quadrant represents early apoptotic cells (Annexin V+/PI-). The upper left quadrant represents cells injured during cell collection (Annexin V-/PI+). The upper right quadrant represents late apoptotic and necrotic cells (Annexin V+/PI+). The proportion of early apoptotic cells were quantified in the lower right quadrant by counting 10,000 cells.

Terminal deoxynucleotidyl transferase-mediated dUTP nick end-labeling staining

Each rat brain was dissected and divided into consecutive frozen coronal sections (20 μ m thick, from the optic chiasm to the posterior horn of lateral ventricle). The location and type of apoptotic cells was assessed by using terminal deoxynucleotidyl transferase-mediated dUTP nick end-labeling (TUNEL) staining on brain sections in situ. This kit labels DNA strand breaks that are generated during apoptosis, and so differentiates between apoptosis and necrosis. The TUNEL labeling protocol followed the instruction manual of the TUNEL apoptosis assay kit (Boster Company, Wuhan, China). The frozen slides were fixed in 4 % paraformaldehyde/0.01M PBS (pH 7.0–7.6) at room temperature for 30–60 min, and then washed in 0.01 M PBS three times. Next, the slides were incubated for 1 min at 37°C in a working solution of 20 μ g/ml proteinase K. The slides were rinsed with PBS, and the area around the sample was dried. The slides were then incubated with 50 μ l of the TUNEL reaction mixture containing terminal deoxynucleotidyl transferase for 1–2 h in a dark, humidified atmosphere at 37°C. The slides were then rinsed three times with PBS. The slides were incubated with 50 μ l of anti-digoxin antibody (diluted 1:100) for 30 min at room temperature in the dark, and then washed in 0.01M PBS

three times. The slides were incubated with 50 μ l of HRP-labeled streptavidin (diluted 1:100) for 30 min at room temperature in the dark, and then washed four times in 0.01M PBS. The slides were stained with DAB and counterstained with hematoxylin, dehydrated in graded ethanol, cleared in xylene, and mounted.

Western blot

The left-sided hippocampus was dissected ($n=5$) and sonicated in radioimmunoprecipitation buffer [containing 50 mM Tris-HCl, pH 7.4, 10 mM EDTA, 100 μ M leupeptin, 1 mM pepstatin, 10 μ g/ml aprotinin, 10 μ g/ml bacitracin, 100 μ M phenylmethylsulfonyl fluoride, 1 mM sodium orthovanadate and 1 % Nonidet P40]. The tissue sample was homogenized and centrifuged at 4°C, 14,000 \times g for 15 min. The protein concentration (μ g/ml) in tissue homogenate was determined using a bicinchoninic acid (BCA) protein assay kit (Generay Biotech Co., Ltd., Shanghai, China). Proteins in the sample were separated by SDS-PAGE by running the sample through 9 % SDS-polyacrylamide gels (sample 40 μ g, marker glyceraldehyde-3-phosphate dehydrogenase (GAPDH) 10 μ l). The proteins were then transferred to polyvinylidene fluoride membranes (Immobilon-P, Millipore) and incubated with antibodies against glutamate receptor 1 (GluR1), GluR2, GluR3, or GluR4 (Cell Signaling, USA) before being diluted in 5 % non-fat dry milk and 1 % BSA for 2 h at room temperature. The membranes were then washed extensively in 0.01M PBS-Tween 20 and incubated for 1 h at room temperature with HRP-conjugated secondary antibodies (Promega, Madison, WI) and developed using an enhanced chemiluminescence method. The final dilution of antibodies was between 1:1,000 and 1:2,000. The optical densities of immunoreactive bands were quantified by densitometry using Labworks 4.6 software (EC3 Imaging System, UVP Inc. USA). The proteins of GluR1–GluR4 were semi-quantified by measuring the GluR1–4/GAPDH gray-scale ratio ($n=5$).

Statistical analysis

All data are presented as the mean \pm SEM. Statistical analysis was performed with a one-way analysis of variance (ANOVA) test with Bonferroni's corrections and a two-tailed independent-samples t test, using SPSS 13.0 for Windows (SPSS Inc., Chicago, Illinois, USA). The level of statistical significance was defined as a P value of less than 0.05.

Results

Dynamic changes in apoptotic cells following SC

Class IV or V seizures occurred in all the experimental rats after the intraperitoneal injection of lithium and pilocarpine. Compared to the time point before SC, the proportion of apoptotic cells in the hippocampus of IRs and ARs had clearly increased at 3 h after the end of the SC ($P<0.01$) with a maximal induction at 1 day (IRs 1.36 ± 0.14 %, ARs 1.73 ± 0.25 %). The apoptotic process continued for at least 7 days (IRs 0.35 ± 0.11 %, ARs 0.56 ± 0.15 %). In IRs, the proportion of apoptotic cells was lower than in ARs at each of the different time points following SC (see Table 1, Figs. 1 and 2). There was a significant difference between the two age groups at 1 day and 7 days after SC.

We detected the location and type of apoptotic cells by TUNEL staining. Few TUNEL-positive cells were observed in the normal control hemisphere (Fig. 3a, b). A marked increase in TUNEL-positive cells was observed 1 day after SC (Fig. 3c, d). Higher magnification (scale bar, 50 μ m) demonstrated TUNEL-positive staining in the neuronal nuclei, predominantly in the CA1 and CA3 regions of the hippocampus (Fig. 3).

Dynamic changes in AMPAR subunits following SC

We found no significant difference between the normal and experimental control groups of the same age when comparing the same AMPAR subunits. Severe seizures inhibited

Table 1 The proportion of early apoptotic cells at different time points after 1 h of status convulsion in immature and adult rat hippocampi (% , $n=5$)

	Immature group	Adult group	t	P
Normal control group	0.1 \pm 0.06	0.15 \pm 0.10	2.18	0.06
Experimental control group	0.14 \pm 0.04	0.21 \pm 0.06	2.12	0.07
3 h after SC	0.65 \pm 0.04**	0.71 \pm 0.08*	1.31	0.23
1 day after SC	1.36 \pm 0.14**	1.73 \pm 0.25**, ****	2.92	0.02
3 days after SC	1.11 \pm 0.23**	1.33 \pm 0.24**	1.50	0.17
7 days after SC	0.35 \pm 0.11**	0.56 \pm 0.15**,***	2.57	0.03

* $p<0.05$; ** $p<0.01$ (compared with the normal control group at the same age); *** $p<0.05$; **** $p<0.01$ (compared with the immature group at the same time point)

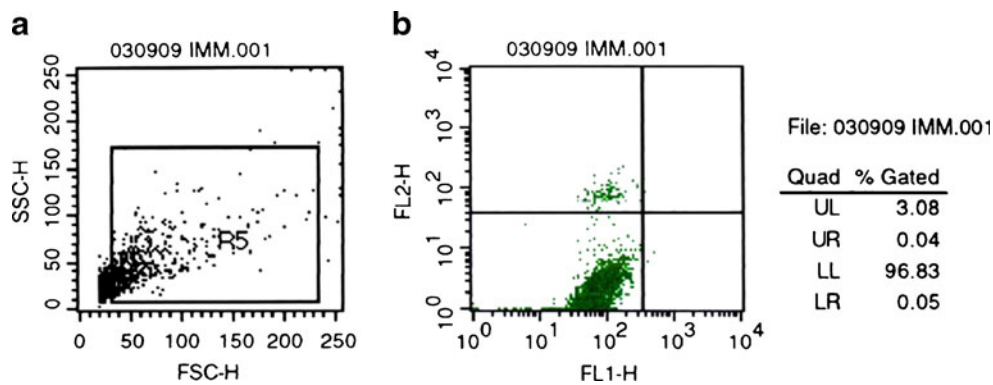


Fig. 1 Apoptotic cells stained by Annexin V-PI in the normal AR group. The proportion of early apoptotic cells was quantified in the lower right (LR) quadrant by counting 10,000 cells. **a** The cells selected for analysis, where the abscissa indicates the cell size (forward scatter), and the ordinate indicates the cell composition (side scatter). **b** The histogram of cells stained by Annexin V-PI, where the abscissa

indicates the fluorescence intensity of FITC-labeled Annexin V, and the ordinate indicates the fluorescence intensity of PI. The histogram of apoptotic cells was made up of four quadrants. The lower right (LR) quadrant represents the proportion of early apoptotic cells (Annexin V +/PI-). **b** The proportion of early apoptotic cells was 0.04 %. Five samples were used from each group

GluR1–4 expression in the hippocampus of IRs. GluR1, -3, and -4 expression levels were markedly lower than those of normal IRs at 3 h after SC ($P < 0.01$), while GluR2 expression decreased sharply at 3 days after SC. These reductions lasted for at least 7 days (Table 2, Fig. 4).

The level of AMPARs in ARs also showed a tendency to be downregulated after SC. The expressions of GluR1–4 in ARs were obviously lower at 3 h after SC ($P < 0.01$). The low levels of expression were maintained at least until 1 day after SC, after which, they began to increase gradually. Levels of GluR4 and GluR1/3 returned to normal at 3 days and 7 days after SC, respectively ($P > 0.05$). The level of GluR2 was consistently lower than that in normal ARs following SC ($P < 0.01$). Expression levels of all four AMPAR subunits in normal IRs were lower than those in normal ARs ($P < 0.01$). The age-dependent difference persisted for at least 7 days after SC ($P < 0.05$).

Dynamic changes in AMPAR subunit composition following SC

Table 3 shows that GluR2 ($47.3 \pm 1.9\%$, $P < 0.01$) predominated over other AMPAR subunits in normal ARs. The

subunit composition of AMPARs changed at 3 days after SC, where GluR2/3 subunits predominated until 7 days after SC.

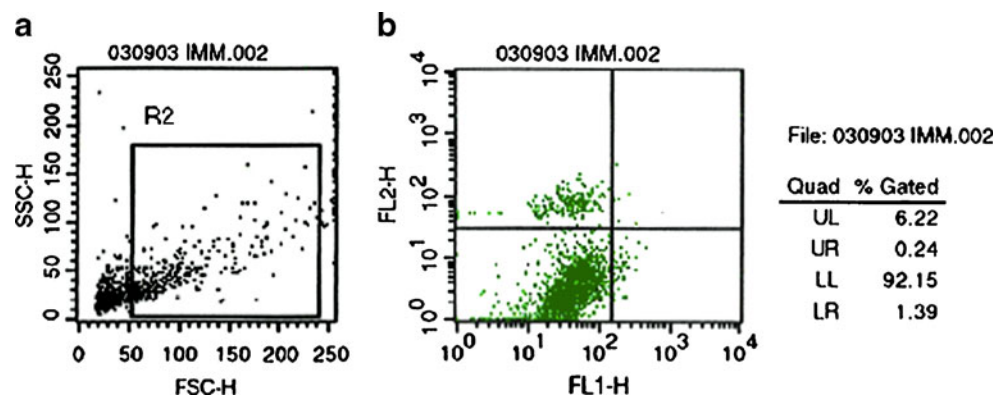
GluR3/4 subunits were mainly expressed in the hippocampus of normal IRs, while levels of GluR2 were at their lowest. The subunit composition of AMPARs was altered rapidly by seizures at 3 h after SC. The main composition of AMPARs changed from GluR3/4 subunits to GluR2/3 subunits. However, there was no significant difference in subunit composition between the normal and experimental IRs at 7 days after SC.

Discussion

Severe seizures could induce neuronal apoptosis in the hippocampus

An immature brain is more prone to seizures than a mature brain but with increased resistance to seizure-induced neural damage. This age-dependency could be due to differing amounts of hippocampal neuronal damage produced by

Fig. 2 Apoptotic cells stained by Annexin V-PI in the AR group at 3 days after SC. (b) SC-induced consistent neuronal apoptosis in the hippocampus. The lower right quadrant indicates that the proportion of early apoptotic cells was 1.39 %. Five samples were used from each group



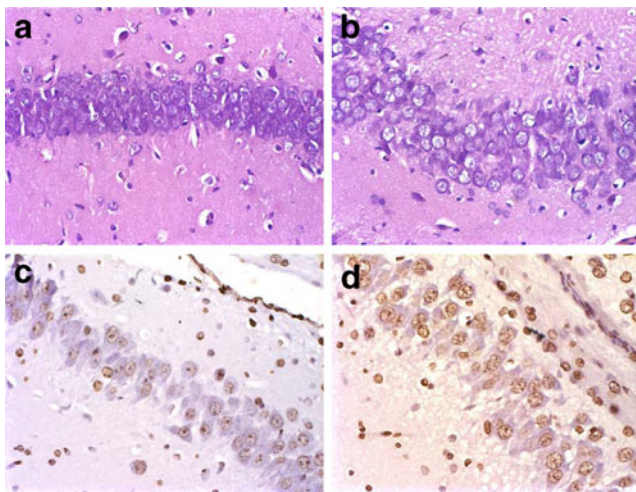


Fig. 3 Brain sections were immunostained for apoptosis by the TUNEL assay. **(a, b)** Almost no TUNEL-positive neurons were detected in the CA1 regions of normal immature **(a)** and adult **(b)** rats. **c, d** Representative photographs show an increase of TUNEL-positive neurons in the CA1 regions of immature **(c)** and adult **(d)** rats at 1 day after SC. (Scale bar=50 μm)

seizures at different ages [1–5]. To determine if there was an early developmental resistance to seizure-induced hippocampal damage, we compared the effects of pilocarpine-induced SC on the hippocampus of immature (20-day-old) and adult rats. We confirmed that SC could induce a time-dependent apoptosis process. However, the proportion of apoptotic cells in IRs was consistently lower than that in ARs following SC. Age was the main factor that influenced neuronal apoptosis, which prompted us to examine the principal underlying reason for the relative tolerance to brain damage of the immature brain. A better understanding of the pathophysiological mechanisms underlying age-specific changes to the brain induced by SC will lead to

the development of novel and effective strategies to ameliorate the deleterious consequences of prolonged seizures.

Subunit composition of AMPARs in the normal hippocampus

AMPARs are the principal molecular units for fast excitatory synaptic transmission in the central nervous system and are composed of four subunits, GluR1–GluR4. These subunits combine in tetramers, different stoichiometries, which determine channel function and trafficking to synapses. High expression of GluR2 favors the formation of Ca²⁺ impermeable (heteromeric) AMPARs, as this subunit contains a motif that blocks Ca²⁺ conductance. The heteromeric/homomeric complex of GluR1/-3/-4 subunits are Ca²⁺ permeable, which presumably increases sensitivity to the excitatory (depolarizing) effects of glutamate [6], the so-called GluR2 hypothesis.

The expression of AMPAR subunits is differentially regulated during development [12]. In the adult hippocampus, two species of AMPARs appear to predominate: receptors made of GluR1 and GluR2 or those composed of GluR3 and GluR2 [13]. The immature hippocampus, as well as other mature brain regions, expresses GluR4, which also complexes with GluR2 to form a receptor [14]. It is unclear what roles the age-related differences in AMPAR subunit expressions play in the reorganization of the brain during development.

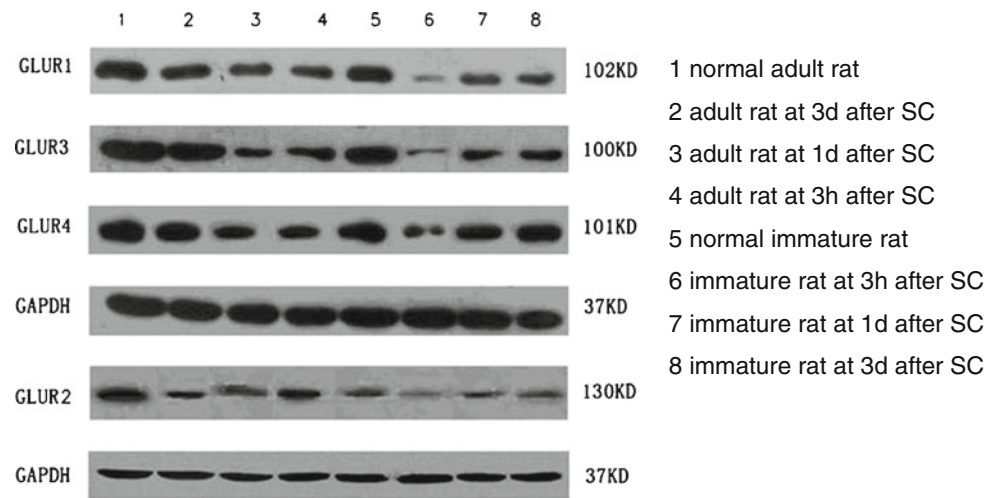
We revealed that the expression levels of four AMPAR subunits in normal IRs were lower than those in normal ARs (*P*<0.01). During the early stages of brain development, GluR3/4 subunits were primarily expressed, while GluR2 levels were at their lowest. Later in development, GluR4 expression in the hippocampus decreased while GluR2

Table 2 The content of AMPA receptor subunits at different time points after 1 h of status convulsion in immature and adult rat hippocampi (GluR1–4/GAPDH gray-scale ratio, *n*=5)

	Normal control group	Experimental control group	3 h after SC	1 day after SC	3 days after SC	7 days after SC
Immature group						
GluR1	0.62±0.12	0.60±0.10	0.10±0.05**	0.23±0.06**	0.24±0.07**	0.33±0.05**
GluR2	0.25±0.04	0.27±0.06	0.24±0.08	0.22±0.03	0.19±0.04*	0.19±0.03*
GluR3	0.97±0.11	0.94±0.13	0.13±0.04**	0.35±0.07**	0.57±0.05**	0.77±0.11*
GluR4	0.81±0.07	0.85±0.08	0.27±0.08**	0.52±0.11**	0.64±0.12*	0.70±0.07*
Adult group						
GluR1	0.83±0.10***	0.80±0.09***	0.28±0.10**,***	0.28±0.11**	0.55±0.11**,****	0.73±0.14****
GluR2	2.75±0.11****	2.74±0.13****	0.87±0.11**,****	0.97±0.23**,****	1.26±0.27**,****	1.38±0.30**,****
GluR3	1.27±0.12****	1.21±0.14****	0.28±0.07**,****	0.43±0.12**	1.07±0.11*,****	1.23±0.11****
GluR4	0.96±0.12***	0.90±0.10***	0.36±0.08**	0.39±0.09**	0.80±0.10	0.88±0.11***

* *p*<0.05; ** *p*<0.01 (compared with the same subunit of normal control group at the same age); *** *p*<0.05; **** *p*<0.01 (compared with the same subunit of immature group at the same time point)

Fig. 4 Western blot of the AMPAR subunits at different time points after SC in immature and adult rat hippocampi. The expressions of four AMPAR subunits in IRs were consistently lower than those in ARs before or after SC ($p < 0.05$ vs. control, ANOVA). SC could inhibit the expression levels of GluR1–4 in the hippocampi of IRs and ARs and altered the subunit composition of AMPARs. Five samples were used from each group



expression increased. GluR2 was the dominant AMPAR subunit in the hippocampus of normal ARs. The regulation of Ca^{2+} -permeable AMPARs [15] may be involved in the induction of synaptic plasticity, neuronal development, and neurological disease. Immature brain deficiency of GluR2 can make the brain more vulnerable to glutamate-mediated excitotoxic damage, which is possibly associated with the age-dependent difference in expression and subunit composition of AMPARs found in this study.

SC could inhibit AMPAR subunit expression in the hippocampus

Epileptogenesis in an immature brain is influenced by inhibitory and excitatory systems, the ionic microenvironment, and the degree of myelination. AMPARs therefore play a critical role in epileptogenesis. Grooms et al. [16] found a marked decrease in GluR2 expression 12–16 h after SC. Other studies [17–19] also found decreased levels of GluR-2 or GluR-1. In contrast, Condorelli et al. [20] did not observe a change in GluR-2 expression after SC, while Rakhade et al. [20] showed that seizures could induce a rapid increase in the phosphorylation of GluR1 and GluR2.

We then examined whether seizures had an effect on the expression of AMPARs in hippocampus. One hour of SC could inhibit GluR1–4 subunit expression levels in the hippocampus of IRs and ARs. The dynamic changes in apoptosis and AMPAR subunit compositions following SC were similar between the different ages. The severity of neuronal apoptosis and AMPAR expression levels in IRs were always lower than that in ARs.

The potential roles of reduced AMPARs in epileptogenesis are unknown. Increased excitability is temporally associated with a rapid increase in GluR1 and GluR2 expression after seizures [21]. The post-seizure administration of AMPAR antagonists [22] attenuates AMPAR potentiation and phosphorylation, prevents the concurrent increase during in vivo seizure susceptibility, and can be a more effective treatment for status epilepticus [22, 23]. We also demonstrated that the immature brains were more resistant to seizure-induced damage, and this was accompanied by the reduced expression of AMPARs. These studies indicate that the inhibition of AMPARs can prevent long-term increases in seizure susceptibility and seizure-induced neuronal injury. The hippocampus, especially during immature stages, has the ability to activate the endogenous anti-

Table 3 Subunit composition of AMPARs at different time points after 1 h of status convulsion in immature and adult rat hippocampi (%; $n=5$)

	Normal control group	3 h after SC	1 day after SC	3 days after SC	7 days after SC
Immature group					
GluR1	23.4±4.5	13.5±6.8	17.4±4.5	14.6±4.3	16.6±2.5
GluR2	9.4±1.5**	32.4±10.8**	16.7±2.3	11.6±2.4	9.5±1.5**
GluR3	36.6±4.2**	17.6±5.4	26.5±0.05**	34.8±3.0**	38.7±5.5**
GluR4	30.6±2.6*	36.5±10.8**	39.4±8.3**	39±7.3**	35.2±3.5**
Adult group					
GluR1	14.3±1.7***	15.6±5.6***	13.5±5.3***	14.9±3.0***	17.3±3.3***
GluR2	47.3±1.9	48.7±6.1	46.9±11.1	34.2±7.3	32.7±7.1
GluR3	21.9±4.5***	15.6±3.9***	20.8±5.8***	29.1±3.0	29.1±2.6
GluR4	16.5±4.5***	20.1±4.5***	18.8±4.3***	21.8±2.7***	20.9±2.6***

* $p < 0.05$; ** $p < 0.01$ (compared with the GluR1 submit of immature group at the same time point); *** $p < 0.01$ (compared with the GluR2 submit of adult group at the same time point)

epileptic mechanism to maintain the balance between excitation and inhibition after SC, and consequently reduce brain damage.

Age-dependent difference in AMPAR subunit composition after SC

The glutamate-mediated transmission efficiency of synaptic AMPARs depends not only on the number of synaptic AMPARs but also on the subunit composition and subunit modifications introduced by alternative splicing. Increasing evidence suggests [24] that the phosphorylation of AMPARs at many excitatory synapses, and their insertion or removal from the synapse, underlies the changes in synaptic strength associated with LTP or LTD, respectively. The GluR1 regulatory mechanism is dominant in the expression of LTP [23], while LTD is expressed by the removal of synaptic GluR2 [25–27].

With regards to the effects of seizures on the composition of AMPAR subunits, our research found that the dominant subunit composition of AMPARs in ARs was altered at 3 days after SC, switching from GluR2 to GluR2/3 as the dominant subunit. The reduction in GluR2 might serve as a “molecular switch”, leading to the formation of Ca²⁺-permeable AMPA receptors and enhancing the toxicity of endogenous glutamate following a neurological insult. LTD might be induced via removal of GluR2 subunits postsynaptically, decreasing the efficacy of synapse transmission. In the IR group, the subunit composition of AMPARs was altered rapidly by seizure at 3 h after SC. The composition of AMPARs changed from predominantly GluR3/4 subunits to predominantly GluR2/3 subunits. The increased expression of GluR2 could inhibit excessive calcium influx and protect neurons in the hippocampus from excitotoxic cell death, conferring early but transient protection in the immature brain after SC. The composition of subunits was consistent with that of normal IRs at 7 days after SC. The age-related changes of receptor properties may therefore participate in different functional consequences of SC.

Conclusion

We demonstrated that SC could induce neuronal apoptosis, and the immature brain was more resistant to seizure-induced damage. Further studies confirmed that SC could also inhibit AMPAR subunit expression, altering the subunit composition of AMPARs in the hippocampus. The rate of neuronal apoptosis and the expression levels of the four AMPAR subunits in IRs were both consistently lower than those in ARs after SC. These findings suggested that the inhibition of certain AMPAR subunits could prevent seizure-induced neuronal injury. The normal immature brain

deficiency of GluR2 could make the brain more vulnerable to glutamate-mediated excitotoxic damage; however, the increased subunit composition of GluR2 in IRs after SC appears to provide an early but transient protective mechanism to the immature brain. Controlling and regulating the expression of AMPARs is not only important in protecting neurons, but also underlies synaptic connections and synapse plasticity. The manipulation of these subunits could provide a new target for the treatment of brain damage induced by SC.

Acknowledgments This work was supported by grants from the National Natural Science Foundation of China (No. 81071056) and Natural Science Foundation Project of CQ CSTC (2009BB5265).

Conflict of interests The authors declare that they have no conflict of interests.

References

1. Scantlebury MH, Heida JG, Hasson HJ, Velísková J, Velisek L, Galanopoulou AS et al (2007) Age-dependent consequences of status epilepticus: animal models. *Epilepsia* 48(Suppl 2):75–82
2. Rajasekaran K, Zanelli SA, Goodkin HP (2010) Lessons from the laboratory: the pathophysiology, and consequences of status epilepticus. *Semin Pediatr Neurol* 17:136–43
3. Cowan LD (2002) The epidemiology of the epilepsies in children. *Ment Retard Dev Disabil Res Rev* 8:171–81
4. Ben-Ari Y, Holmes GL (2006) Effects of seizures on developmental processes in the immature brain. *Lancet Neurol* 5:1055–63
5. North Carolina, Joan R Coates (2005). Seizures in young dogs and cats: pathophysiology and diagnosis. *Compend Contin Educ Pract Vet* 7:447–460
6. Santos SD, Carvalho AL, Caldeira MV, Duarte CB (2009) Regulation of AMPA receptors and synaptic plasticity. *Neuroscience* 158:105–25
7. Ehlers MD (2000) Reinsertion or degradation of AMPA receptors determined by activity-dependent endocytic sorting. *Neuron* 28:511–25
8. Ju W, Morishita W, Tsui J, Gaietta G, Deerinck TJ, Adams SR et al (2004) Activity-dependent regulation of dendritic synthesis and trafficking of AMPA receptors. *Nat Neurosci* 7:244–53
9. Whitlock JR, Heynen AJ, Shuler MG (2006) Learning induces long-term potentiation in the hippocampus. *Science* 313:1093–97
10. Kwak S, Weiss JH (2006) Calcium-permeable AMPA channels in neurodegenerative disease and ischemia. *Curr Opin Neurobiol* 16:281–87
11. Piehl RG, Smialowski A, Gotwo T (1984) Development and persistence of kindled seizure after repeated injections of pentylenetetrazol in rats and guinea pigs. *Epilepsia* 25:506–10
12. Talos DM, Follett PL, Folkert RD, Fishman RE, Trachtenberg FL, Volpe JJ et al (2006) Developmental regulation of α -amino-3-hydroxy-5-methyl-4-isoxazole-propionic acid receptor subunit expression in forebrain and relationship to regional susceptibility to hypoxic/ischemic injury. II. Human cerebral white matter and cortex. *J Comp Neurol* 497:61–77
13. Wenthold RJ, Petralia RS, Blahos J II, Niedzielski AS (1996) Evidence for multiple AMPA receptor complexes in hippocampal CA1/CA2 neurons. *J Neurosci* 16:1982–89
14. Elias GM, Funke L, Stein V, Grant SG, Brecht DS, Nicoll RA (2006) Synapse-specific and developmentally regulated targeting

- of AMPA receptors by a family of MAGUK scaffolding proteins. *Neuron* 52:307–20
15. Friedman K (2006) Calcium: a role for neuroprotection and sustained adaptation. *Mol Interv* 6:315–29
 16. Grooms SY, Opitz T, Bennett MV, Zukin RS (2000) Status epilepticus decreases glutamate receptor 2 mRNA and protein expression in hippocampal pyramidal cells before neuronal death. *Proc Natl Acad Sci* 28:3631–6
 17. Irier HA, Shaw R, Lau A, Feng Y, Dingledine R (2009) Translational regulation of GluR2 mRNAs in rat hippocampus by alternative 3' untranslated regions. *J Neurochem* 109:584–94
 18. Solomonia R, Mikautadze E, Nozadze M, Kuchiashvili N, Lep-sveridze E, Kiguradze T (2010) Myo-inositol treatment prevents biochemical changes triggered by kainate-induced status epilepticus. *Neurosci Lett* 468:277–81
 19. Sanchez RM, Koh S, Rio C, Wang C, Lamperti ED, Sharma D et al (2001) Decreased glutamate receptor 2 expression and enhanced epileptogenesis in immature rat hippocampus after perinatal hypoxia-induced seizures. *J Neurosci* 21:8154–63
 20. Condorelli DF, Belluardo N, Mudò G, Dell'Albani P, Jiang X, Giuffrida-Stella AM (1994) Changes in gene expression of AMPA-selective glutamate receptor subunits induced by status epilepticus in rat brain. *Neurochem Int* 25:367–76
 21. Rakhade SN, Zhou C, Aujla PK, Fishman R, Sucher NJ, Jensen FE (2008) Early alterations of AMPA receptors mediate synaptic potentiation induced by neonatal seizures. *J Neurosci* 28:7979–90
 22. Fritsch B, Stott JJ, Joelle Donofrio J, Rogawski MA (2010) Treatment of early and late kainic acid-induced status epilepticus with the noncompetitive AMPA receptor antagonist GYKI 52466. *Epilepsia* 51:108–17
 23. Yamashita H, Ohno K, Amada Y, Inami H, Shishikura J, Sakamoto S et al (2004) Effect of YM928, a novel AMPA receptor antagonist, on seizures in EL mice and kainate-induced seizures in rats. *Naunyn Schmiedebergs Arch Pharmacol* 370:99–105
 24. Shi S, Hayashi Y, Esteban JA, Malinow R (2006) Subunit-specific rules governing AMPA receptor trafficking to synapses in hippocampal pyramidal neurons. *Cell* 105:331–43
 25. Fox CJ, Russell K, Titterness AK, Wang YT, Christie BR (2007) Tyrosine phosphorylation of the GluR2 subunit is required for long-term depression of synaptic efficacy in young animals in vivo. *Hippocampus* 7:600–5
 26. Steinberg JP, Takamiya K, Shen Y, Xia J, Rubio ME, Yu S et al (2006) Targeted in vivo mutations of the AMPA receptor subunit GluR2 and its interacting protein PICK1 eliminate cerebellar long-term depression. *Neuron* 49:845–60
 27. Zhou Q, Homma KJ (2004) Shrinkage of dendritic spines associated with long-term depression of hippocampal synapses. *Neuron* 44:749–57