Neurosci Bull June 1, 2011, 27(3): 163–172. http://www.neurosci.cn
DOI: 10.1007/s12264-011-1007-7

·Original Article·

Effect of neuronal excitotoxicity on Munc18-1 distribution in nuclei of rat hippocampal neuron and primary cultured neuron

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Abstract: **Objective** Munc18-1 has an important role in neurotransmitter release, and controls every step in the exocytotic pathway in the central nervous system. In the present study, whether epileptic seizure causes a change of Munc18 localization in neuronal nuclei was analyzed. **Methods** Epilepsy models were established by injection of kainic acid (KA) solution into hippocampus of Sprague-Dawley (SD) rats or intraperitoneal injection of KA in Kunming mice. The hippocampal neurons were prepared from embryonic day 18 SD rats, and cultured in neurobasal medium, followed by treatment with glutamate for 3 h. Neuronal and glial nuclei of hippocampus were separated by sucrose density gradient centrifugation. The nucleus-enriched fractions were stained with 0.1% Cresyl Violet for morphological assay. Immunochemistry and immunoelectron microscopy with anti-Munc18-1 antibody were used to determine the nuclear localization of Munc18-1. Immunoblotting was used to detect the protein level of Munc18-1. **Results** The localization of Munc18-1 in nucleus of rat hippocampal neuron was confirmed by immunochemistry, immunoelectron microscopy, and immunoblotting detection of neuronal nucleus fraction. In animals receiving intrahippocampal or intraperitoneal injection of KA, immunostaining revealed that the expression of Munc18-1 decreased in pyramidal cell layer of CA regions, as well as in hilus and granular cell layer of dentate gyrus in hippocampus. Moreover, immunoblotting analysis showed that the expression level of Munc18-1 in nucleus fraction of hippocampus significantly decreased in KA-treated animals. The relationship between the change of Munc18-1 expression in neuronal nuclei and neuronal over-activation was also tested in primary cultured neurons. After treatment with 50 μmol/L glutamate acid for 3 h, Munc18-1 level was decreased in nucleus fraction and increased in cytoplasmic fraction of primary cultured neurons. **Conclusion** These results suggest that excitatory stimulation can induce the distribution change of Munc18-1 in neuron, which may subsequently modulate neuronal functions in brain.

Keywords: Munc18-1; nucleus; kainic acid; glutamate; hippocampus; primary cultured neurons

1 Introduction

Sec1-Munc18 (SM) proteins are essential proteins that regulate soluble N-ethylmaleimide-sensitive factor attachment protein receptor (SNARE) machineries in exocytosis, by interacting with specific syntaxins and modulating the formation of the SNARE complex. Munc18-1, also referred to as p67 and syntaxin-binding protein 1 (STXBP1), is a member of the SM family and plays an important role in neurotransmitter exocytosis[1-4]. Deletion of Munc18-1 causes loss of neurotransmitter secretion from synaptic vesicles[5]. Moreover, autoantibodies against Munc18-1

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Article ID: 1673-7067(2011)03-0163-10
Received date: 2011-02-22; Accepted date: 2011-05-01

have been found in patients suffering from Rasmussen's encephalitis[6,7], a rare disease with severe epileptic seizures. Recently, missense mutations have been found in the gene encoding STXBP1 in patients with early infantile epileptic encephalopathy[8]. These studies suggest that Munc18-1 plays an important role in the central nervous system, and the dysfunction of this protein may be involved in the occurrence of brain disorders.

Many studies have focused on the interactions between Munc18-1 and SNARE proteins in neurotransmitter exocytosis. However, current data imply that Munc18-1 has multiple functions. For instance, Munc18-1 in axon mediates membrane fusion events required for neurite elongation and branching[9,10], and regulates cytoskeleton via interaction with Cdk5 and cytoskeletal components[11]. Besides, the nuclear localization of Munc18-1 in neurons has been revealed recently[12]. Moveover, it has been demonstrated that Munc18-1 could tightly bind to double stranded DNA, suggesting that Munc18-1 may be involved in the regulation of gene expression[12]. However, so far, little has been known about the relationship between disorders of nervous system and the distribution of Munc18-1 in neuronal nuclei. On the basis of the studies mentioned above, the present study was conducted to investigate the effect of neuronal over-excitation on the distribution of Munc18-1 in nuclei in kainic acid (KA)-induced epileptic model and in primary cultured neurons treated by glutamate acid.

2 Materials and methods

2.1 Establishment of the epilepsy model In the present study, 2 epilepsy models were established in Sprague-Dawley (SD) rats and Kunming mice, respectively. Briefly, male SD rats (220–250 g) were anaesthetized with 10% chloral hydrate solution (360 mg/kg, i.p.). According to the Rat Atlas of Paxinos and Watson, a microsyringe was inserted into the right hippocampus (3.2 mm posterior, 1.8 mm lateral, and 4.0 mm deep to bregma) using a stereotaxical technique, followed by injection of 1 μg KA (dissolved in alkalized isotonic saline at a concentration 1 μg/μL) or saline. The male Kunming mice (18–22 g) received intraperitoneal injection of KA (10 mg/kg) solution. The behaviors of animals were observed for 3 h continuously following KA administration[13].

2.2 Primary culture of neurons Neurons were prepared from the hippocampi of embryonic day 18 SD rats, as previously described[14]. Briefly, the dissociated hippocampal neurons were plated onto poly-*L*-lysine-coated culture dishes or coverslips in Dulbecco's modified eagle medium (Invitrogen, CA, USA) supplemented with F12 nutrients (Invitrogen, CA, USA), 10% fetal calf serum, 100 U/mL penicillin and 100 txg/mL streptomycin. After 4–6 h, the media was replaced by Neurobasal medium (Invitrogen, CA, USA) supplement with 2% B27 (Invitrogen, CA, USA). After being cultured for 7 d, neurons were treated with 50 μmol/L glutamate or vehicle for 3 h.

2.3 Immunohistochemistry and immunocytochemistry Immunohistochemistry was carried out according to our previous descriptions[15]. Rabbit anti-Munc18-1 (N-terminal) polyclonal antibody (Sigma, MO, USA; 1:200), biotinylated secondary antibody (Santa Cruz, MA, USA; 1:200) and avidin-biotin-peroxidase (Santa Cruz, MA, USA) were used for slice staining. Negative controls which were performed by omitting primary antibody showed no positive staining. Fluoro-Jade B staining was performed to detect degenerating neurons as described by Schmued and Hopkins[16]. For immunocytochemistry, the neurons cultured on coverslips were rinsed gently in 0.01 mol/L PBS and fixed in ice cold 4% paraformaldehyde (PFA). After being blocked with 10% normal goat serum in PBS containing 0.2% Triton-X100, the coverslips were incubated with anti-Munc18-1 primary antibody (1:200) overnight at 4 °C, and then incubated with fluorescein isothiocyanate (FITC)-labeled anti-rabbit IgG (Beyotime Co., China) and incubated at 37 °C for 1 h. Cells were counter-stained with the nuclear dye Hoechst 33342 (Beyotime Co., China).

2.4 Immunoelectron microscopy Immunogold labeling was carried out according to the modified method[17]. Adult male SD rats were perfused transcardially with 4% PFA and 2.5% glutaraldehyde. The hippocampi were post-fixed for 6 h, and then cut into 50 μm-thick sections with a vibratome, followed by cryoprotection in 0.1 mol/L PB

(containing 25% sucrose and 10% glycerol) for 30 min. Subsequently, the sections were freeze-thawed with liquid nitrogen. Then, the sections were labeled by Munc18-1 antibody (1:200) and donkey anti-rabbit IgG antibody conjugated with 6-nm gold particles (Jackson, West Grove, PA, USA; 1:10). After being washed, the sections were postfixed with 1% glutaraldehyde for 10 min. Then silver enhancement was done in the dark with an HQ Silver Kit (Nanoprobes, Yaphank, NY, USA). The ultrathin sections were cut and examined with a transmission electron microscope (Philips CM120; Eindhoven, The Netherlands).

2.5 Isolation and fractionation of the rat hippocampal nuclei Nuclei from rat hippocampus were prepared according to the previous studies[12,18]. Briefly, hippocampi were homogenized in 15 volumes of 0.32 mol/L sucrose buffer (containing 3 mmol/L $MgCl_2$ and 0.1 mmol/L potassium phosphate, pH 6.4). Homogenate was filtered through nylon mesh filter and centrifuged for 10 min at 850 g. The crude nuclear pellet was washed with 0.32 mol/L sucrose buffer, and resuspended in 2.4 mol/L sucrose buffer, followed by centrifugation at 4 °C for 1 h at 75 000 g. The precipitated nuclear pellet (N1) was resuspended in 2.4 mol/L sucrose buffer, overlaid with 1.8 mol/L sucrose solution and spun at 85 000 g for 30 min at 4 °C to obtain neuronal (at interface, N2) and glial (in the pellet, N3) populations of nuclear preparations. The nuclear rich fractions were stained with 0.1% Cresyl Violet for morphological assay, and subjected to SDS-PAGE followed by Western blot analysis. Cytoplasmic and nuclear proteins of primary cultured neurons were isolated with Nuclear and Cytoplasmic Protein Extraction Kit (Beyotime Co., China) according to the manufacturer's instructions.

2.6 Western blot analysis For Western blotting, protein concentration was determined using a Micro-BCA Protein Assay Kit (Pierce, Rockford, IL, USA). Equal amounts of samples were separated in 12% SDS-PAGE, and transferred to PVDF membrane. Membranes were incubated with anti-Munc18-1 (Sigma, MO, USA), anti-β-actin (Sigma, MO, USA) or anti-Histone1 antibody (Sigma, MO, USA) overnight at 4 °C, followed by incubation with horseradish peroxidase-conjugated goat anti-rabbit IgG (Santa Cruz, MA, USA; 1:2 000). Proteins were visualized by DAB or ECL chemiluminescence kit (Santa Cruz, MA, USA).

2.7 Statistical analysis All data were expressed as mean±SEM and analyzed using Student's *t*-test. $P < 0.05$ was considered to be statistically significant.

3 Results

3.1 Munc18-1 was localized in the neuronal nucleus of hippocampus in normal adult rat brain Immunohistochemical analysis showed that Munc18-1 was widely distributed in brain, including cortex, hippocampus and striatum. Besides in neuronal soma, processes and punctate nerve terminal-like structures, Munc18-1 was obviously detected in nuclei (Fig. 1C). Moreover, immunoelectron microscopy analysis of hippocampus showed that Munc18-1 immunogold particles were distributed not only in neuronal nucleus but also on the membrane of nucleus (Fig. 1A, B). Cresyl Violet staining showed that the nuclei (N1) isolated from hippocampus were differentiated in morphology, suggesting that N1 fraction contained a mixed population of neuronal and glial nuclei (Fig. 1D). N2 fraction separated by ultra-centrifugation mainly contained larger nuclei with faint staining of nucleoplasm, indicating neuronal nuclei (Fig. 1D). Glial nuclei (N3) were smaller and dense (Fig. 1D). Western blot analysis showed that Munc18-1 was mainly distributed in the neuronal nuclei of N2 fraction (Fig. 1E).

3.2 The distribution pattern and expression of Munc18-1 in neurons of hippocampus were changed after epileptic seizures To observe the distribution of Munc18-1 after neuronal over-activation, immunohistochemical staining was performed in different epileptic animal models. In normal and vehicle-injected adult rat hippocampi, Munc18-1-immunopositive neurons were distributed in pyramidal cell layer, as well as in hilus and granular cell layer of dentate gyrus (DG) (Fig. 2A, upper), which contained intensively stained nuclei. After KA injection into hippocampus, rats exhibited epileptic seizures, which reached grade III at 3 h. At this time point, immunopositive signal was significantly decreased in hippocampus, including the pyramidal cell layer of CA1–3 regions, and hilus and granular cell layer

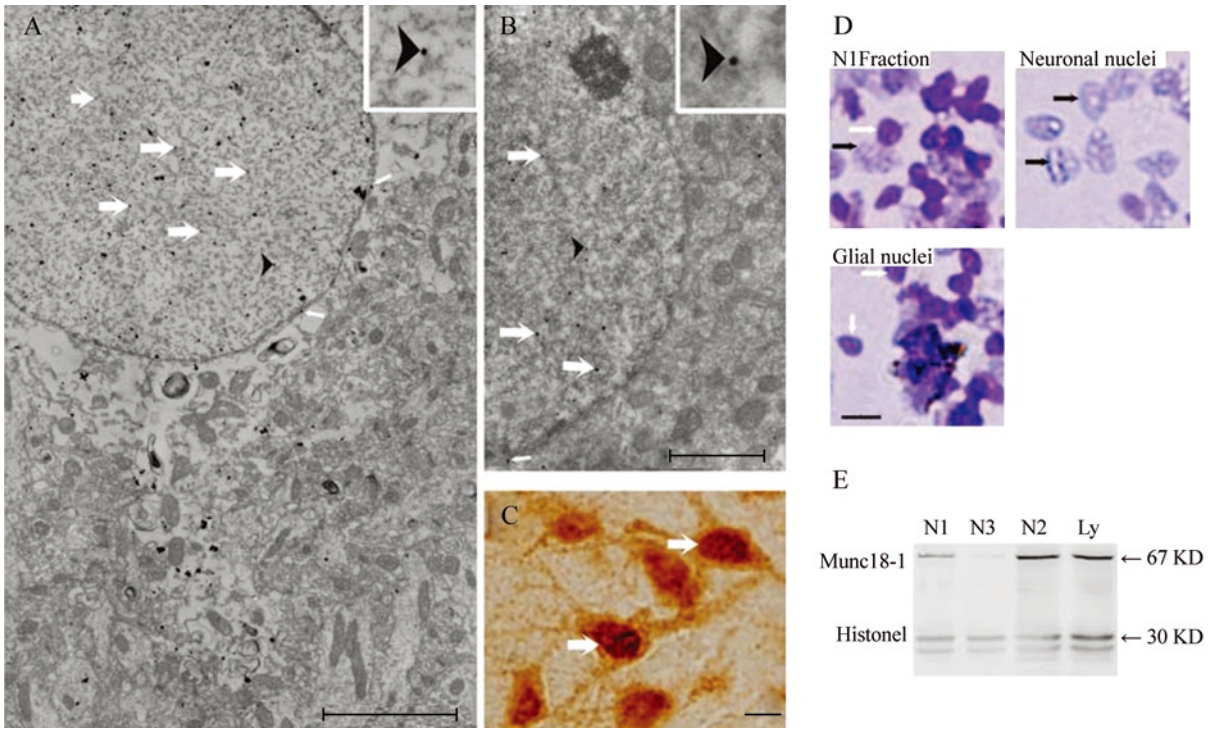

Fig. 1 Immunoelectron microscopy, immunohistochemical and biochemical analyses of Munc18-1 in neuronal nuclei of normal adult rat hippocampus. A and B: Immunoelectron microscopy showed that Munc18-1-immunopositive gold particles were localized in nucleus (large white arrows and black arrow heads) as well as on nuclear membrane (small white arrows) of neurons. The inserts were magnification of Munc18-1-immunopositive gold particles in nucleus. C: Munc18-1-immunopositive matter was distributed in neuronal soma and processes of pyramidal neurons of hippocampus, and was enriched in nuclei (white arrows). D: Cresyl Violet staining of glial and neuronal nuclei in nuclear fraction (N1) isolated from rat brain. Neuronal nuclei (N2) had pale stained nucleoplasm. Glial nuclei (N3) were dark and smaller. White arrows indicate glial nuclei, black arrows indicate neuronal nuclei. E: Western blot profile of Munc18-1 in total nuclear preparation (N1), neuronal nuclear fraction (N2), glial nuclear fraction (N3) and adult rat hippocampus lysate as control (Ly). Scale bar for A: 2 μm. Scale bar for B: 1 μm. Scale bar for C and D: 50 μm.

of DG (Fig. 2A, below). After KA injection, the neurons in hilus of rat hippocampus displayed obviously Munc18-immunopositive matter in cytoplasm (Fig. 2A, below, insert). However, some neurons with lower levels of Munc18-1 in nuclei did not show any increase of Munc18-1 in cytoplasm, such as neurons in CA1 and CA3.

In addition, a decrease of Munc18-1-immunopositive cells in hippocampus was also observed in mice at 3 h after intraperitoneal injection of KA (Fig. 2B), which showed epileptic symptoms ranked grade I or II seizure. To study the relationship between neuronal injury and the changes of Munc18-1 expression and distribution, Fluoro-Jade B staining was used here to visualize degenerating neurons in rats[16]. As shown in Fig. 2C, the administration of KA resulted in the appearance of many Fluoro-Jade B-stained neurons in hippocampus, especially in CA3 and hilus regions, but not in granular layer (Fig. 2C).

Western blot analysis showed that compared with that in the vehicle group, Munc18-1 expression level decreased in hippocampus of epileptic rats induced by KA (Fig. 3A). Munc18-1 in nuclear fraction also decreased significantly (Fig. 3B). Meanwhile, Munc18-1 level in cytoplasmic fraction of hippocampus increased in KA-administered rats (Fig. 3C).

3.3 The distribution pattern and expression of Munc18-1 in the primary cultured neurons were changed after glutamate acid treatment Considering the specific distribution of Munc18-1 in primary cultured neurons (Fig. 4A), cultured neurons were used to analyze Munc18-1 expression in nucleus after treatment with glutamate. As

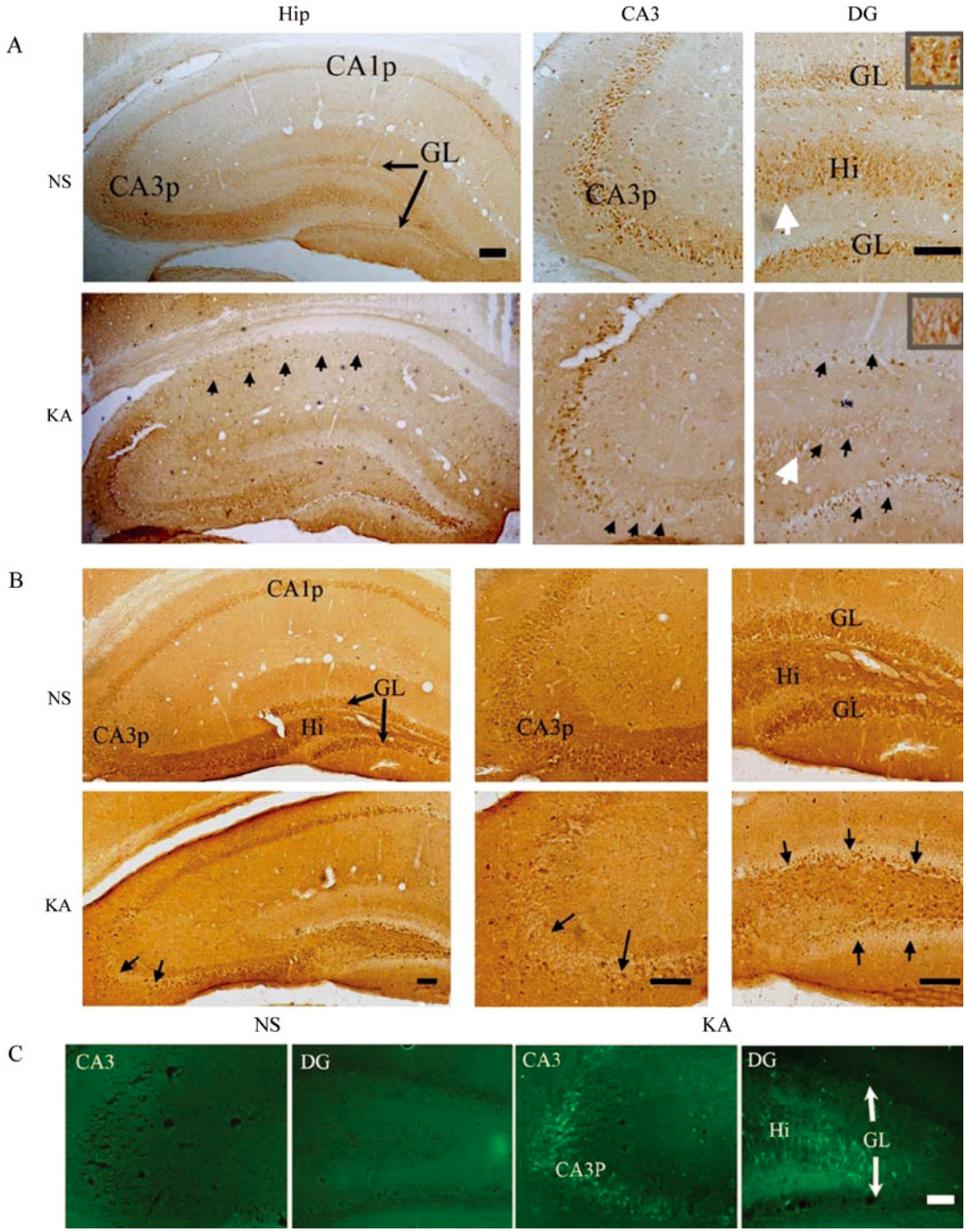

Fig. 2 **A: Kainic acid (KA)-induced changes of Munc18-1 distribution in rat hippocampus. Compared with those in rats receiving normal saline (NS) injection, Munc18-1-positive neurons in rat hippocampus receiving KA treatment significantly decreased in pyramidal cell layer, hilus and granular cell layer of dentate gyrus (DG). Short black arrows indicated the regions with a decrease of Munc18-1-positive neurons. In hilus and granular cell layer of DG of rat hippocampus with KA injection, Munc18-1 was mainly distributed in cytoplasm, as shown in inserted figures, which were magnifications of the white arrow-indicated regions. B: In hippocampus of mice receiving intraperitoneal injection of KA, Munc18-1 immunostaining decreased in pyramidal cell layer of CA3 regions, and granular cell layer of DG. C: The administration of KA resulted in many Fluoro-Jade B-stained neurons in hippocampus of rats, especially in CA3 and hilus regions, but without obvious injury in granular cell layer of DG. CA1p, stratum pyramidal of CA1; CA3p, stratum pyramidal of CA3; GL, granule cell layer; Hi, hilus. Scale bar for A and C: 200 μm. Scale bar for B: 100 μm.**

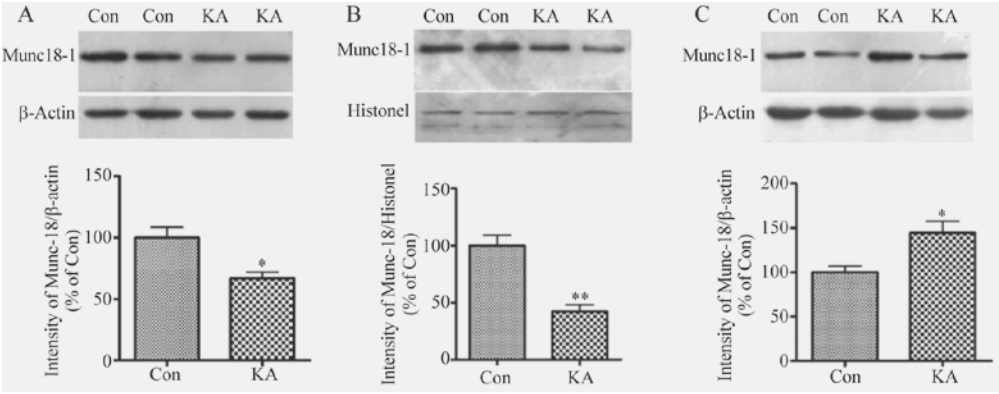

Fig. 3 Western blot analysis of Munc18-1 in hippocampus of rats subjected to intrahippocampal injection of kainic acid (KA) and saline as control (Con). Munc18-1 protein levels in the hippocampus (A) and in nuclear extracts (B) of rats receiving KA injection were significantly decreased, compared with the control levels, while Munc18-1 level in cytoplasmic extracts (C) from hippocampus was significantly increased after KA administration. *$P < 0.05$, **$P < 0.01$ *vs* control. $n = 6$ in each group, n indicated the number of animals.

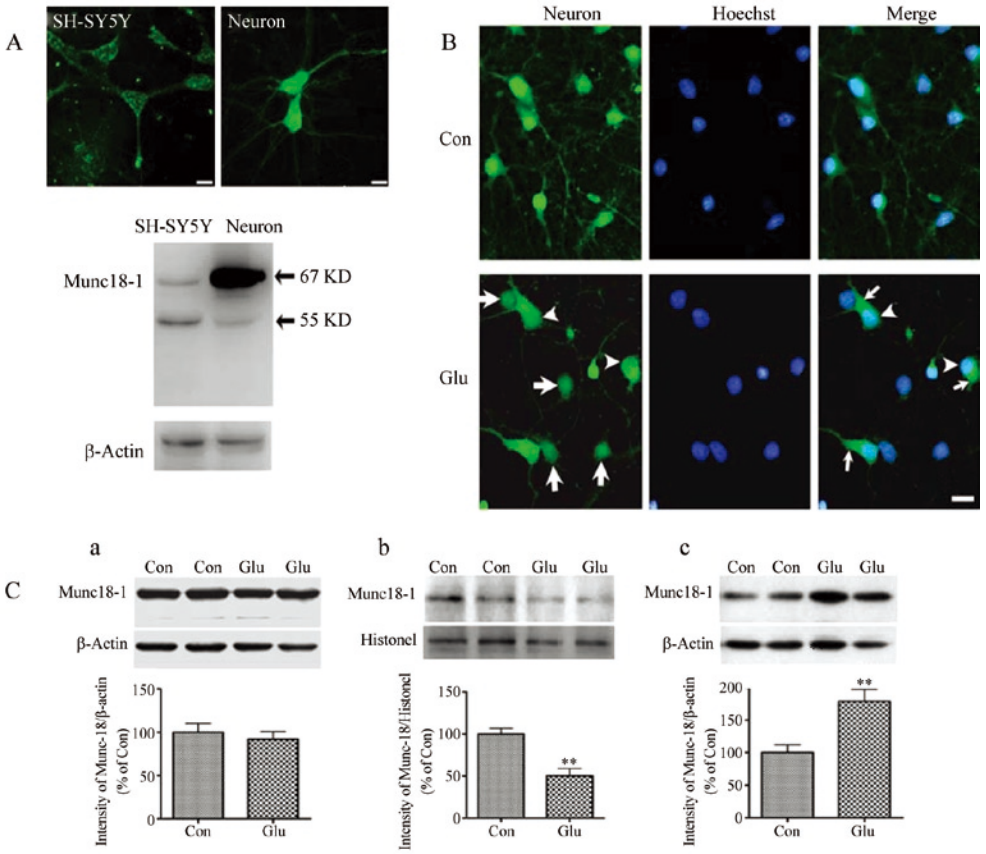

Fig. 4 The effect of glutamate on the distribution of Munc18-1 in primary cultured neurons. A: The distribution of Munc18-1 in primary cultured neuron and human SH-SY5Y neuroblastoma cell. Munc18-1 was enriched in nuclei of cultured neurons, but was not in SH-SY5Y cells. Western blot analysis showed that the protein level of Munc18-1 in SH-SY5Y cell was lower than that in cultured neuron, and the level of degraded fragment of Munc18-1 was higher than that in cultured neuron. B: After treatment with 50 μmol/L glutamate (Glu) for 3 h, Munc18-1 staining decreased (thick arrows) in some neurons, or increased in neuronal soma and their processes (thin arrows), compared with the vehicle treatment (Con) group. Besides, its nuclear-enriched distribution was lost. Arrow heads indicate less staining in nuclear region than other region of soma. C: Western blot analysis of Munc18-1 in cultured neurons subjected to Glu or vehicle (Con) treatment for 3 h. a: Munc18-1 in total protein of cultured neurons; b: Munc18-1 in nuclei of neurons; c: Munc18-1 in cytoplasm of neurons. $n = 6$, **$P < 0.01$ *vs* control. Scale bar: 50 μm.

shown in Fig. 4B, after exposure to 50 μmol/L glutamate for 3 h, Munc18-1 was relatively uniformly distributed in neuronal soma and processes in some neurons, and lost the nucleus-enriched phenomenon, while in some other neurons, Munc18-1 staining was decreased (Fig. 4B). Western blot analysis showed a tendency of decrease of Munc18-1 expression in neurons after glutamate treatment (50 μmol/L) for 3 h, with no statistical significance (Fig. 4Ca). Meanwhile, the protein level of Munc18-1 decreased obviously in the neuronal nuclei (Fig. 4Cb), and increased significantly in cytoplasm (Fig. 4Cc).

3.4 The distribution patterns of Munc18-1 in neuroblastma cell (SH-SY5Y) and cultured neuron were different Since Munc18-1 had been found to be located in the nuclei of PC12 cells[12], here protein levels of Munc18-1 in primary cultured neurons and neuroblastma cells (SH-SY5Y) were compared. As shown in Fig. 4A, the protein level of Munc18-1 in SH-SY5Y cell was lower than that in cultured neuron, while the level of degraded fragment of Munc18-1 was higher than that in cultured neuron. Immunofluorescent staining showed that Munc18-1 was not enriched in nuclei of SH-SY5Y cells. In contrast, Munc18-1 was distributed intensively in nuclei of cultured neurons (Fig. 4A, B).

4 Discussion

Munc18-1 plays important roles in controlling the exocytotic steps[2-5,19]. Moreover, accumulative data indicate that Munc18-1 has other multiple functions in neurons[10-12,20]. Of note, it has been recently reported that Munc18-1 is highly distributed in nuclei of neurons and interacts with DNA[12], implying a role of Munc18-1 in regulation of gene expression. Munc18-1 cDNA-derived amino acid sequence illustrates certain characteristics suggestive of potential cellular functions in neuronal nuclei. There are 2 clusters of basic amino acids at residues 20–31 and 457–467, which are characteristics of bipartite nuclear localization signal (NLS) sequence, and one cluster of hydrophobic amino acids at residues 405–414 in Munc18-1 representing the nuclear export signal (NES)[12]. In addition, we have also tested Munc18-1 amino acid sequence by Prediction of Protein Sorting Signals and Localization Sites in Amino Acid Sequences (PSORT) WWW Server. Surprisingly, the result of the *k*-nearest neighbor algorithm (*k*-NN) prediction indicated that Munc18-1 has a 69.6% possibility for nuclear, 13.0% for cytoplasmic, 8.7% for mitochondrial, 4.3% for vesicles of secretory system and 4.3% for endoplasmic reticulum localization. "NNCN" score of Reinhardt's method for cytoplasmic/nuclear discrimination predicted that Munc18-1 is a nuclear protein with 70.6% reliability. Therefore, Munc18-1 is considered as a nuclear protein. However, as we know, there is only one report about the localization of Munc18-1 in nucleus[12]. In this study, by using immunostaining, immunoelectron microscopy and western blot detection of neuronal nuclear extract, we confirmed that Munc18-1 is distributed in neuronal nuclei in brain and in primary cultured neurons. Moreover, we found that Munc18-1 is not enriched in nuclei of SY5Y neuroblastoma cells. However, another neuroblastama cell line PC12 cells show a nuclear enrichment of Munc18-1, either in differential or in undifferential states[12]. Therefore, the distribution of Munc18-1 in nucleus might be not readily related to differential states. Interestingly, immunoblotting showed a much higher level of full-length Munc18-1 in cultured neurons than in SY5Y cells, indicating the different mechanisms for controlling Munc18-1 expression.

Munc18-1 is widely expressed in different brain regions. In hippocampus, we observed that Munc18-1 was distributed in pyramidal neurons in CA regions, polymorph cells and granular cells in DG. Munc18-1 expression decreased dramatically in neuronal layers of hippocampus after KA administration. In KA-induced epileptic animals, not only the total expression level of Munc18-1, but also Munc18-1 level in nuclear fraction was decreased. For the rat model, the KA injection site was at hilus and granular cell layer of DG region. However, the dramatic change of Munc18-1 was also observed in the CA3 region. This may be related to the diffusion of KA. The CA3 region is a vulnerable region in the model with either intrahippocampal injection or systemic administration of KA[21], which might be due to a high level of KA-

binding sites in this region[22]. Meanwhile, Munc18-1 level was significantly increased in cytoplasm of some neurons especially those in hilus of DG region. In contrast, some neurons such as neurons in CA1 and CA3 of epileptic rats, displayed decreased Munc18-1 immunostaining in nuclei, without significant increase in cytoplasm. We consider that cytoplasmic Munc18-1 could be released[23] or degraded, and these processes might be differentially activated depending on the types of neurons, the affection of KA and epileptic seizure. These results indicate that the localization change of Munc18-1 in nuclei is related to epileptic seizure.

Excitatory amino acid receptors have been categorized as ionotropic glutamate (iGlu) receptors which mediate fast synaptic transmission through ligand-gated ion-channels and metabotropic glutamate (mGlu) receptors which are G-proteins coupled to second messenger systems and have been implicated in the modulation of synaptic transmission. To date, 3 main groups of iGlu receptors have been identified according to their selective agonists: N-methyl-*D*-aspartate (NMDA), (S)-2-amino-3-(3-hydroxy-5-methylisoxazol-4-yl) propanoic acid (AMPA) and kainite receptors. Glutamate itself activates all of these receptors, whereas KA selectively activates kainate receptors at low concentrations and AMPA receptors at higher concentrations. In the machinery of epilepsy, glutamate plays an important role in mediating neuronal over-activation and neuronal injury[24,25]. Therefore, here we tested the effect of glutamate on nuclear localization of Munc18-1 in primary cultured neuron. Results showed that Munc18-1 level increased in cytoplasm and decreased in nucleus of cultured neuron after glutamate treatment. Therefore, the distribution of Munc18-1 in nucleus could be affected by glutamate-mediated neuronal activation. However, complete elucidation of mechanisms underlying nuclear import and export signaling for Munc18-1 under neuronal activation needs further investigations. Since KA-induced decrease of Munc18-1 in nuclei was accompanied by neuronal degeneration in pyramidal neurons in CA regions and polymorph cells in hilus of DG, it needs to be considered that the elements related to neuronal injury might be involved in the distributional change of Munc18-1. However, after KA administration, we found no obvious Fluoro-Jade B signals for injured neurons in granular cell layer of DG where the change of Munc18-1 distribution also appeared. Therefore, the decrease of Munc18-1 in neuron might be not sufficient for determination of neuronal death.

Munc18-1 is enriched in nucleus of neuron, suggesting its role within the nucleus. Abnormal localization of Munc18-1 might cause dysregulation of neuron-specific gene expression, and subsequently mediates neuronal malfunction or neurodegeneration. On the other hand, excessive accumulation of Munc18-1 in cytoplasma might be also involved in the pathogenesis of neurodegeneration, because accumulation of Munc18-1 in neurofibrillary tangle-bearing neurons has been found in Alzheimer type dementia brains[26]. Munc18-1/p67 is an activator of Cdk5[9], and the elevated level of Munc18-1 in cytoplasma will activate Cdk5, resulting in phosphorylation of tau protein, the main component of neurofibrillary tangles in Alzheimer's disease.

Taken together, Munc18-1 is localized specifically to neuronal nuclei, and excitatory stimulation by KA or glutamate is able to induce the change of Munc18-1 level in neuronal nuclei. The nucleocytoplasmic shuttling mechanisms of Munc18-1 need further investigation, which may provide new insights into the pathogenesis of epilepsy.

Acknowledgements: This work was supported by grants from the National Natural Science Foundation of China (No. 81071017, 30470536, 90919004).

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神经元兴奋性毒性对 Munc18-1 蛋白在大鼠海马神经元和原代培养神经元胞核内分布的影响

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摘要：目的 Munc18-1在中枢神经系统递质释放过程中具有重要作用，控制着突触囊泡释放步骤的每一个环节。Munc18-1功能异常与癫痫发病相关。本文主要探讨癫痫是否会引起神经元胞核内Munc18-1定位的改变。**方法** 通过海马内注射海人藻酸建立Sprague-Dawley (SD)大鼠癫痫模型，腹腔注射海人藻酸建立昆明小鼠癫痫模型。分离胎龄18天SD大鼠海马神经元，用Neurobasal培养基培养7天后，用谷氨酸处理3 h。用蔗糖密度梯度离心法分离神经元和神经胶质细胞的细胞核组份，通过甲酚紫染色对上述富含细胞核的组份进行形态学鉴别。用免疫组化和免疫电镜分析法确定Munc18-1的细胞核定位。免疫印迹法检测不同细胞组分的Munc18-1蛋白的表达水平。**结果** 免疫组化、免疫电镜以及对神经元细胞核组份的免疫印迹证实了Munc18-1在海马神经元细胞核的分布定位。在海人藻酸诱导癫痫的动物海马内，免疫组化染色显示Munc18-1在海马CA区锥体细胞层、齿状回颗粒细胞层和门区的多型细胞表达减少。同时，免疫印迹分析表明，Munc18-1在海马神经元胞核中的表达水平明显下降。免疫印迹检测显示，原代培养神经元经50 μmol/L谷氨酸处理3 h后，Munc18-1在神经元胞核中的分布减少，而胞浆组份中含量增加。免疫荧光的形态学检测也显示部分神经元明显失去了Munc18-1在细胞核聚集分布的特征。**结论** 兴奋性刺激能够使Munc18-1在神经元的表达分布发生改变，这种变化可能参与调节脑神经元的功能。

关键词：Munc18-1；细胞核；海人藻酸；谷氨酸；海马；原代培养神经元