Glutamate enhances the surface distribution and release of Munc18 in cerebral cortical neurons

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Abstract: Objective Munc18 is considered as an intracellular protein that plays an important role in exocytosis of neurotransmitters. Previous studies have demonstrated the presence of autoantibodies against Munc18 in a subgroup of Rasmussen's encephalitis patients. However, the machinery of Munc18 autoimmunity is still elusive. The present study was aimed to investigate Munc18 release from primary cultured neurons, Munc18 distribution on the outer plasma membrane of neurons, and the neurotoxicity of Munc18 antibody. **Methods** The cerebral cortical neurons from embryonic day 17 Sprague-Dawley rats were prepared and cultured in neurobasal medium. The proteins in culture medium were precipitated with 10% trichloroacetic acid, and analyzed by immunoblotting. The proteins on neuronal surface were biotinylated with EZ-Link-sulfo-NHS-LC-Biotin, and collected with avidin-conjugated agarose beads followed by immunoblotting analysis. For cell surface immunofluorescent staining, the living neurons were labeled with anti-Munc18 antibody at 4 °C. Neuronal injury was assessed by lactate dehydrogenase(LDH) release. **Results** Munc18 was detected in culture medium by immunoblotting analysis. After treatment with 50 μmol/L glutamate for 1 h, Munc18 content in medium was increased. Meanwhile, β-actin and syntaxin1 were not detected in culture medium, and LDH release was not significantly increased. Moreover, glutamate enhanced Munc18 distribution on outer plasma membrane. Living neuron staining also demonstrated the localization of Munc18 on neuronal surface after glutamate treatment, especially at contacting regions between neurons. Glutamate-induced increase of surface Munc18 distribution was suppressed by NMDA receptor antagonist MK801, but not by AMPA receptor antagonist NBQX. Moreover, compared with c-Fos antibody, Munc18 antibody could induce neuronal injury, when culture medium contained the components of serum. **Conclusion** A portion of Munc18 can be released from neurons or distributed on neuronal surface, which can be enhanced by glutamate treatment via activation of NMDA receptors. Besides, Munc18 antibody-induced neuronal injury depends on the serum components.

Keywords: Munc18; neuron; release; cell surface; glutamate

1 Introduction

Synaptic vesicle exocytosis is mediated by the neuronal

soluble N-ethylmaleimide-sensitive factor attachment protein receptor (SNARE) proteins synaptobrevin/vesicle-associated membrane protein (VAMP) on the vesicles and synaptosomal-associated protein 25 (SNAP-25) and syntaxin-1A/1B on the plasma membrane. The Sec1/Munc18-like (SM) protein Munc18-1, together with SNARE proteins syntaxin-1, SNAP-25, and synaptobrevin/VAMP, constitutes the core fusion

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machinery of synaptic vesicle exocytosis^[1].

The SM proteins are essential for regulating SNARE machineries by interacting with specific syntaxins and modulating the formation of SNARE complex^[2]. The SM protein Munc18-1, which was originally identified as a syntaxin1-binding protein^[3,4], mediates synaptic vesicle exocytosis^[5,6]. Current evidence suggests that Munc18-1 acts via interactions with syntaxin1 and the other SNARE proteins in distinct modes, and influences all the steps that lead to exocytosis, including vesicle recruitment, tethering, docking, priming, and membrane fusion^[7].

Munc18-1, a 67-kD presynaptic intracellular protein, attaches intracellularly to inner plasma membrane by interaction with syntaxin1^[8]. However, Sharma *et al.* demonstrate that Munc18-1 is also localized to the nucleus of neurons of adult rat brain and PC12 cells^[9]. Meanwhile, Yang has found the autoimmunity to Munc18 in Rasmussen's encephalitis (RE)^[10], suggesting an out-surface distribution pattern of Munc18. Indeed, previous observation has offered cues of out-surface distribution. For instance, Sec1p, a member of the SM family in *Saccharomyces cerevisiae*, is broadly distributed as patches throughout the plasma membrane, and concentrates in the bud neck of newly budded cells. In the bud neck, double labeling of Sec1p and plasma membrane SNARE Sso1p showed that a portion of Sec1p was obviously located outside of Sso1p on membrane, although it was not pinpointed by the authors^[11]. These findings raise the possibility that Munc18-1 may be released from the neurons and localized extracellularly.

The present study was designed to find the evidence for the extracellular localization of Munc18 in glutamate-treated cerebral cortical neurons.

2 Materials and methods

2.1 Primary culture and treatment of cerebral cortical neurons Cerebral cortical neurons were prepared from embryonic day 17 Sprague-Dawley rats as described previously^[12,13]. To prepare conditioned medium (CM), cells were rinsed twice and cultured with fresh neurobasal medium. *L*-Glutamate was then added into the medium to different final concentrations (50-200 μ mol/L) at different time points, and media were all

collected at 6 h^[12], to ensure the 0.5-h, 1-h, 3-h, or 6-h incubation. In other groups, cells were pretreated with MK801 (Sigma) or NBQX (Sigma) for 5 min, followed by glutamate treatment. CM was collected and centrifuged to remove loose cells and debris, and the supernatant was collected for further Munc18 analysis. Adherent cells were used for cell surface biotinylation, Western blot and immunofluorescent staining.

2.2 Treatment of CM After cell treatment, the proteins in the CM were precipitated with 10% trichloroacetic acid (TAC). Samples were centrifuged at 20 000 g and pellets were washed twice with ice-cold methanol. The pellets were resuspended in radioimmunoprecipitation assay (RIPA) buffer consisting of 1% Triton X-100, 1% sodium deoxycholate, 0.1% SDS, 150 mmol/L NaCl, 25 mmol/L Tris (pH 7.4), and 1 mmol/L EDTA supplemented with protease inhibitor cocktail (Roche). Then Western blot was conducted.

2.3 Biotin-labeled cell surface proteins After treatment with glutamate, cells were washed 3 times with ice-cold Dulbecco's phosphate-buffered saline (PBS), and cell surface proteins were biotinylated with 0.5 mg/mL EZ-Link-sulfo-NHS-LC-Biotin (Pierce) in PBS for 30 min at 4 °C. Then the solution was removed and cells were incubated with PBS supplemented with 20 mmol/L glycine for 5 min, to terminate the biotinylation reaction. After being washed twice with ice-cold PBS, cells were lysed by RIPA buffer, followed by centrifugation at 5 000 g for 5 min (4 °C). Then the supernatant was collected and incubated with avidin-conjugated agarose beads (Sigma) for 2 h at 4 °C. The proteins (cell surface proteins) captured by avidin-coupled beads were analyzed by western blot with anti-Munc18 antibody. In addition, the levels of Munc18 and β -actin in aliquoted cell lysates (total proteins) were also determined.

2.4 Live cell surface staining Surface Munc18 immunostaining in cortical neurons was performed as described previously^[14]. Briefly, neurons were gently washed 3 times with ice-cold PBS and blocked with 10% fetal bovine serum (FBS) prepared in PBS for 30 min at 4 °C. After that, neurons were incubated with anti-Munc18 antibody for 1 h at 4 °C, washed twice with ice-cold PBS and incubated with Texas Red (TR)- or FITC-conjugated anti-rabbit secondary antibody for 1 h at

4 °C. After being washed, cells were examined with a laser scanning confocal microscope (Leica TCS SP2) or a fluorescent microscope. All the procedures were conducted at 4 °C to prevent internalization of cell surface-localized protein^[15,16].

2.5 Filtration of serum Rabbit serum (200 µL) was diluted in PBS (v:v, 1:9), loaded to the concentrator filter unit (Amicon Centricon) (30 000 MW cutoff) and centrifuged at 5 000 rpm until the volume was reduced to less than 400 µL. This procedure was repeated 4 times to remove small molecules in serum such as glutamate^[17]. After the last centrifugation, the PBS was replaced with the fresh neurobasal medium. The final centrifugation continued until the original serum volume of less than 200 µL was achieved, and neurobasal medium was added to reach a volume of 200 µL. Filtered serum was kept on ice. It was diluted in 9 volumes of neurobasal immediately before use.

2.6 Western blot analysis Protein concentration in samples was determined by Micro-BCA protein assay (Pierce). Samples were resolved by SDS-PAGE and transferred onto polyvinylidene difluoride (PVDF) membranes. The membranes were incubated with rabbit anti-Munc18 antibody (1:2 000; Sigma) that recognizes a N-terminal fragment of Munc18, mouse anti-Munc18 antibody (1:800; BD Transduction Laboratories), mouse anti-transferrin receptor (TfR) antibody (1:2 000; Zymed Laboratories), mouse anti-syntaxin1 antibody (1:1 000; Santa Cruz biotechnology) or anti-β-actin antibody (1:2 000; Sigma). After being washed, the membranes were incubated with horseradish peroxidase-conjugated secondary antibodies. Proteins were visualized with ECL system, and the protein levels were analyzed using Leica Qwin (version 2.2) software. For CM proteins, the intensity values of immunoreactive bands were normalized to the protein amount of cultured cells in corresponding dishes. For cell surface proteins and total proteins, the band intensities of Munc18 were normalized to those of TfR and β-actin, respectively.

2.7 Lactate dehydrogenase (LDH) release assay Neurotoxicity of glutamate and Munc18 antibody was evaluated by analyzing LDH content in culture medium using a cytotoxicity detection kit (Roche) according to the manufacturer's

instructions. Results were expressed as the percentage to the normal control. The neurotoxicity of rabbit anti-Munc18 antibody (1:500, Sigma) was compared with rabbit anti-c-Fos antibody (1:500, Sigma) with or without 10% filtered rabbit sera.

2.8 Statistical analysis Data were presented as mean±SD and analyzed by Student's *t*-test. *P* < 0.05 was considered to be statistically different.

3 Results

3.1 Effect of glutamate on Munc18 release from cultured neurons

A previous study has indicated that treatment of cerebral cortical neurons with a high concentration of glutamate (1 mmol/L) could cause neurodegeneration and cytoskeletal protein release^[12]. In this study, we first detected the release of Munc18 from cortical neurons in the absence or presence of glutamate at a relatively lower concentration. As shown in Fig. 1A, Munc18 was barely detected in fresh neurobasal culture medium. Meanwhile, a weak band at approximately 67 kD was detected in CM from the control cerebral cortical neurons without glutamate treatment. Treatment with 50 µmol/L glutamate induced an acute increase in the level of Munc18 in CM, followed by a decrease. The increase was the most significant at 1 h (Fig. 1A). Meanwhile, β-actin could not be detected in CM from the glutamate-treated neurons. Moreover, an increase in Munc18 level in CM after treatment with glutamate was also detected with an antibody against C-terminal of Munc18 (Fig. 1B). However, syntaxin1, a Munc18-binding protein involved in exocytosis, was not detected in CM after glutamate treatment. To determine whether glutamate induced the increase of Munc18 release in a concentration-dependent manner, the cerebral cortical neurons were treated with glutamate at different concentrations (50, 100 and 200 µmol/L). As shown in Fig. 1C, after 1-h glutamate treatment, Munc18 release into CM was concentration-dependently increased. Meanwhile, the LDH analysis revealed no significant increase of LDH release after 1-h glutamate (50-200 µmol/L) treatment (Fig. 1D), which excluded the possibility that the release of Munc18 was neuronal injury-dependent.

3.2 Effect of glutamate on outer-surface distribution of

Fig. 1 Glutamate-enhanced Munc18 release from primary cultured cortical neurons. **A:** To examine the effects of glutamate on Munc18 release, glutamate was added at different time points into culture medium to a final concentration of 50 $\mu\text{mol/L}$, and media from different cultures were all collected at 6 h, to ensure 0.5-h, 1-h, 3-h, or 6-h glutamate treatment. Treatment with 50 $\mu\text{mol/L}$ glutamate induced an acute increase in the level of Munc18 in CM, which was significant at 1 h, followed by a decrease. The release of β -actin was not detected. FM: fresh medium. **B:** The release of Munc18 after glutamate treatment was also detected by a mouse monoclonal antibody. The exocytosis-related protein Syntaxin1 was not detected in CM. Cell lysate served as a positive control. **C:** Munc18 release was analyzed with Munc18 antibody after treatment with or without different concentrations of glutamate for 1 h ($n = 3$). **D:** There was no significant increase of LDH release after 1-h glutamate (50–200 $\mu\text{mol/L}$) treatment ($n = 6$). Data were from 3 independent experiments. ** $P < 0.01$ vs samples without glutamate treatment.

Munc18 To examine whether glutamate altered the outer-surface distribution of Munc18, the living neurons were immunostained with antibodies against Munc18. Treatment with 50 $\mu\text{mol/L}$ glutamate for 1 h obviously enhanced Munc18 distribution on the surface of some neurons. It was noted that on neuronal surface, Munc18 was mainly located at the regions of neuronal contact sites or at converging sites of neurites (Fig. 2A). Moreover, cell surface biotinylation assay was performed to further investigate the effect of glutamate on outer-surface distribution of Munc18. Cell surface proteins were biotinylated and isolated with avidin beads. Immunoblotting analysis revealed that glutamate treatment (50 $\mu\text{mol/L}$ or 100 $\mu\text{mol/L}$) for 1 h increased the level of cell surface Munc18 (Fig. 2B). Meanwhile, the total Munc18 level of neurons was not significantly changed after treatment with 50 $\mu\text{mol/L}$ glutamate, although it was decreased after 100

$\mu\text{mol/L}$ glutamate treatment (Fig. 2B).

3.3 NMDA receptor activity increases outer-surface distribution of Munc18 in primary cultured cerebral cortical neurons

To examine whether the observed glutamate-evoked increase in the level of surface Munc18 was due to the stimulation of NMDA- or AMPA-type glutamate receptors, the cerebral cortical neurons were treated with glutamate in the absence or presence of the non-competitive antagonist of NMDA receptor MK-801 or the competitive antagonist of AMPA receptor NBQX. As shown in Fig. 3, MK-801 prevented the glutamate-mediated increase in surface distribution of Munc18 (Fig. 3A), while NBQX had no noticeable effect on surface distribution of Munc18 (Fig. 3B). These results indicated that glutamate could enhance the outer-surface distribution of Munc18, probably through stimulation of NMDA-type glutamate receptors.

Fig. 2 Glutamate-enhanced Munc18 distribution on neuronal surface. **A:** Primary cultured neurons were treated with 50 $\mu\text{mol/L}$ glutamate for 1 h and the living neurons were immunostained with Munc18 antibody at 4 $^{\circ}\text{C}$ without fixing by paraformaldehyde. Arrows in a, b, and c indicated that Munc18 was distributed on the site where a neurite contacts with a cell body, and arrows in d, e, and f indicated that Munc18 was distributed on converging sites between neurites. Arrow heads in g indicated a calyx synapse where Munc18 was distributed extracellularly after treatment with glutamate. Scale bar: 20 μm . **B:** Cell lysates and biotinylated cell surface fractions from control and glutamate-treated neurons were analyzed by Western blotting with antibodies against Munc18, β -actin and TfR. Results showed that glutamate induced an increase of surface level of Munc18 ($n=3$). * $P < 0.05$, ** $P < 0.01$ vs control group.

Fig. 3 The effect of glutamate receptor antagonist on glutamate-induced enhancement of Munc18 distribution on neuronal surface. Neurons were treated with glutamate in the absence or presence of (A) MK-801, the non-competitive antagonist of NMDA receptor, or (B) NBQX, the competitive antagonist of AMPA receptor. Protein levels of Munc18 and TfR in biotinylated cell surface fractions were assayed by western blotting. Results showed that MK-801, but not NBQX, inhibited glutamate-induced enhancement in Munc18 distribution on neuronal surface ($n = 3$). ** $P < 0.01$ vs samples without glutamate treatment, # $P < 0.05$ vs samples treated with 50 $\mu\text{mol/L}$ glutamate only.

Fig. 4 Effect of Munc18 antibody on LDH release of neurons. Munc18 antibody induced an increase in LDH release, compared with c-Fos antibody, in the presence of serum components ($n=6$). Data were from 3 independent experiments. # $P > 0.05$, ** $P < 0.01$.

3.4 Munc18 antibody could induce neuronal injury Since a portion of Munc18 was located in the outer-surface of cerebral cortical neurons, and glutamate could enhance surface distribution of Munc18 in cortical neurons, further experiments were conducted to determine whether Munc18 antibody could induce cell injury. To study the neurotoxicity of Munc18 antibody that had been found in a subgroup of RE patients^[18], LDH release was examined after treatment with Munc18 antibody for 12 h. The commercially available antibodies contain substances for reserving the activity of antibody. However, some of these substances might be toxic for neurons. Therefore, the possible toxicity of rabbit anti-Munc18 antibody was compared with that of the rabbit anti-c-Fos antibody from Sigma. As shown in Fig. 4, 12-h treatment with Munc18 antibody did not significantly induce LDH release, compared with the control antibody against c-Fos protein. However, when culture medium was supplemented with 10% filtered rabbit serum, Munc18 antibody treatment resulted in an increase of LDH release compared with c-Fos antibody. Of note, for the study of neuronal autoimmunity, filtered serum is better, because serum contains many small molecules including glutamate^[17]. These results indicate that Munc18 antibody-induced neuronal injury might be related to the outer-surface distribution of Munc18, and depended on serum components such as complement elements.

4 Discussion

Recent findings suggest that Munc18 not only controls the synaptic vesicle secretion cascade via interaction with syntaxin1 or with the assembled SNARE complex^[8,19], but also participates in the regulation of calcium channels and gene transcription^[9,20]. Although Munc18 is considered as an intracellular protein, the present study shows that it could be released from neurons and distributed on neuronal outer surface. Besides, despite the fact that neurological insults are diverse in nature, there are common mechanisms of cell injury, and glutamate toxicity plays an integral role in a variety of neurodegenerative diseases and in neurotrauma^[21]. Previous studies have found that many proteins are released after neuronal injury^[12,22]. Treatment with 1 mmol/L glutamate for 5 h induces release of several proteins, such as β -actin, from neurons^[12]. In this study, treatment with 50-200 μ mol/L glutamate for 1 h was enough for Munc18 release enhancement, while β -actin release was not detected. Since glutamate application at a low concentration for a short time could not induce significant apoptotic phenomena^[23] or LDH release (Fig. 1D), the enhanced release of Munc18 might be an early response to neuronal injury. A portion of Munc18 is linked to the inner-side of plasma membrane via binding with membrane proteins. When neurons are injured, Munc18 may be carried to outer-side of plasma membrane or extracellular space, a process somewhat like that of Annexin-V. Under normal conditions, Annexin-V binds to phosphatidylserine in inner leaflet of the plasma membrane and forms a membrane-bound two-dimensional crystal lattice, while during apoptosis, Annexin-V is exposed to extracellular surface through externalization of phosphatidylserine to the outer leaflet of the plasma membrane^[24]. Also, Munc18 release might be related to the overactivation of exocytotic process caused by glutamate treatment, although we did not detect the release of exocytosis-related protein syntaxin1, a Munc18-binding protein^[8]. We suppose that the released Munc18 probably induces the production of autoantibody under pathological conditions or in susceptible individuals, such as a subgroup of RE cases.

It has been shown that Munc18 attaches to intracellular

plasma membrane through interaction with syntaxin1^[8], assembled SNARE complexes^[19] and other proteins such as CaV₂^[20]. In this study, we observed surface distribution of Munc18 and Munc18 release from cerebral cortical neurons (Fig. 2), which could be enhanced by glutamate. Although this is a new finding in neuronal cells, previous observation has actually offered cues of outer-surface distribution. For instance, Sec1p, a member of the SM family in *Saccharomyces cerevisiae*, is broadly distributed as patches throughout the plasma membrane and concentrated in the bud neck of newly budded cells. In the bud neck, double-labeling of Sec1p and plasma membrane SNARE Sso1p shows that a portion of Sec1p is obviously located outside of Sso1p on membrane, although it was not pinpointed by the authors^[11]. Interestingly, the present study revealed that the surface Munc18 was also mainly located on contact sites between neurons (Fig. 2). The function of extracellular Munc18 and its binding molecules on outer-surface plasma membrane need to be further studied.

In this study, the NMDA receptor antagonist MK-801 significantly suppressed glutamate-induced enhancement of cell surface distribution of Munc18, indicating that glutamate receptors are involved in this effect. Surprisingly, the competitive AMPA receptor antagonist NBQX failed to suppress glutamate-induced changes of Munc18 surface distribution. NBQX has been shown to protect neurons that are selectively vulnerable to focal or global ischemic episodes in which a machinery of glutamate excitotoxicity is involved^[25-27]. In experiments showing a neuroprotective role of NBQX, the drug was delivered either immediately or within hours after reperfusion. However, results show that efficacy of NBQX would decline significantly when administration is delayed, and MK-801, but not NBQX, provides protection against the transneuronal degeneration of substantia nigra pars reticulata following a lesion of the striatum by ibotenic acid^[28]. Thus, here neurons were preincubated with NBQX for 5 min. However, it was still ineffective for glutamate-induced changes of surface distribution of Munc18. The AMPA receptor may be not involved in glutamate-induced ectopic distribution of Munc18. Besides, since in brain, there are many mGluRs on neurons also mediating glutamate effects,

it still remains to be determined whether glutamate induces the abnormal distribution of Munc18 via mGluR activation.

The functional abnormality of Munc18 caused by mutation is related to epilepsy^[29]. Whether and how Munc18 antibody in a subgroup of RE mediates epilepsy symptom are still obscure. One hypothesis suggests that Munc18 antibodies probably enter neurons and affect the functions of Munc18^[10,18]. Here we show that the existence of Munc18 on neuronal surface can be enhanced by glutamate treatment, and Munc18 antibody can induce neuronal injury in the presence of filtered serum containing the molecules >30 kD, including the immunoreactive components. Therefore, we propose that the Munc18 antibody-induced neuronal injury depends on the serum components, such as complement elements, and that neuronal surface Munc18 might offer the target antigen for Munc18 autoimmunity in a subgroup of RE. In fact, GluR3 antibody also exists in RE, and GluR3 autoantibody-decorating neurons would trigger complement-mediated neuronal damage that contributes to the pathogenesis of RE^[30].

In conclusion, the present study provides evidence that a portion of Munc18 could be released and distributed on neuronal surface, and glutamate treatment increases Munc18 release and ectopic surface distribution. MK801 inhibits glutamate-induced increase of Munc18 surface distribution, implying the involvement of NMDA receptor activation. Munc18 release from neurons raises the possibility of autoimmunity triggering, and Munc18 on neuronal surface may provide a target for Munc18 autoantibody. However, how Munc18 is released and distributed on neuronal surface, as well as whether ectopically localized Munc18 participates in neurotransmission requires further studies.

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谷氨酸促进大脑皮层神经元 Munc18 的释放和细胞表面的分布

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摘要: **目的** Munc18 被普遍认为存在于神经元细胞内, 参与神经递质的释放。虽然已有研究表明在 Rasmussen's 脑炎病人中有 Munc18 自身抗体的产生, 但对于 Munc18 的自身免疫机制目前还不清楚。本研究旨在观察 Munc18 是否可由神经元释放及是否部分分布于神经元细胞表面, 并对 Munc18 抗体是否能诱导神经元损伤进行研究。**方法** 分离胎龄 17 天 Sprague-Dawley 胎鼠大脑皮层神经元, 用 Neurobasal 培养基培养。培养液中的蛋白经三氯乙酸沉淀后进行免疫印迹检测, 细胞表面蛋白则用 EZ-Link-sulfo-NHS-LC-Biotin 进行生物素标记, 细胞裂解后用结合有亲和素的琼脂糖珠获取生物素标记的蛋白, 随后进行免疫印迹检测。在 4 °C 进行活细胞免疫荧光染色, 观察神经元细胞表面 Munc18 的分布。用乳酸脱氢酶(lactate dehydrogenase, LDH)释放指标检测神经元损伤。**结果** 用免疫印迹法可在神经元培养的条件培养液中检测到 Munc18 蛋白。用谷氨酸(50 $\mu\text{mol/L}$)处理神经元 1 h 后, 培养液中 Munc18 含量显著增加, 同时培养液中未检测到 β -actin 和 syntaxin1, LDH 释放也未明显增加。此外, 谷氨酸处理能增强神经元表面的 Munc18 分布。活细胞免疫荧光染色检测到在谷氨酸处理后, 神经元表面分布有 Munc18, 主要位于神经突起与另一神经元接触的部位以及不同神经元的神经末梢交汇部位。谷氨酸诱导的神经元表面 Munc18 分布增加可被 NMDA 受体拮抗剂 MK801 抑制, 而 AMPA 受体拮抗剂未有明显的抑制作用。LDH 检测显示, 在培养液含有血清成份的情况下, 与对照的 c-Fos 抗体相比, Munc18 抗体可明显诱导神经元损伤。**结论** 一部分 Munc18 可由神经元释出或在神经细胞表面分布。谷氨酸可通过兴奋 NMDA 受体促进 Munc18 的释放和在神经细胞表面的分布。并且, Munc18 抗体诱导的神经元损伤依赖于血清中的因子。

关键词: Munc18; 神经元; 释放; 细胞表面; 谷氨酸