ORIGINAL PAPER

Neurotoxicity of sodium salicylate to the spiral ganglion neurons: GABA$_A$ receptor regulates NMDA receptor by Fyn-dependent phosphorylation

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Abstract

The purpose of this study was to observe the regulatory effects of GABA$_A$ (γ-aminobutyric acid A) receptor on the *N*-methyl-D-aspartate (NMDA) receptor during excitotoxicity in spiral ganglion neurons in the rat cochlea induced by sodium salicylate (SS). Western blot illustrated SS decreased the expression of NMDA receptor 2B subunit (NR2B) surface protein through affecting GABA$_A$ receptor, but the total protein content did not significantly change. Y1472 and S1480 are important phosphorylation sites in NR2B, SS downregulated the Fyn-dependent phosphorylation of Y1472 in a manner not related to the CK2 (Casein Kinase 2) dependent phosphorylation of S1480, thus regulating the surface distribution and internalization of NMDA receptor through GABA$_A$ receptor. These results suggest that the modified pattern of dynamic balance between excitation and inhibition by coactivation of the GABA$_A$ receptor can attenuate the excitatory NMDA receptor under the action of SS, via inhibiting the Fyn-dependent phosphorylation of Y1472.

Keywords Sodium salicylate · Spiral ganglion neurons · GABA$_A$R · NMDAR · Neurotoxicity

Abbreviations

Bic	Bicuculline
GABA$_A$	Receptor agonist
CK2	Casein Kinase 2
GABA$_A$R	γ-Aminobutyric acid A receptor
Mus	Muscimol, GABA$_A$ receptor antagonist
NMDAR	*N*-Methyl-D-aspartate receptor
NR2B	NMDA receptor 2B subunit
PP2	4-Amino-5-(4-chlorophenyl)-7-(*t*-butyl) pyrazolo[3,4-d] pyrimidine
RQ	Relative quantity
SGNs	Spiral ganglion neurons
SS	Sodium salicylate

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Introduction

There are many causes of deafness, but ototoxic drugs are the most common cause of acquired deafness. Sodium salicylate, namely, the active ingredient in aspirin, is one of the most widely used anti-inflammatory analgesic drugs; however, the use of salicylic acid drugs is often accompanied by ototoxicity (Wei et al. 2010; Chen et al. 2013), which has serious effects on the quality of patients' life who use these drugs long term and limits the further use of such drugs in clinical applications. Therefore, it is an increasingly urgent need to reduce the side effects of deafness associated with the use of salicylic acid drugs in clinical practice.

Studies have confirmed that salicylic acid drugs have direct effects on all parts of the auditory nervous system, from the outer hair cells to the auditory cortex (Wang et al. 2006; Caperton and Thompson 2011), and that these effects are mostly reversible (de Almeida-Silva et al. 2011). Both NMDAR and GABA$_A$R are directly involved in the neurotoxic effects of sodium salicylate on the auditory system (Xu et al. 2005; Wang et al. 2006; Zou and Shang 2012; Sahley et al. 2013; Yao et al. 2015). Recently, the topic of receptor interactions has attracted increasing attention from researchers. A direct crosstalk between postsynaptic NMDAR and GABA$_A$R contributes to the excitatory/inhibitory balance

in the nervous system, which restricts each other and jointly regulate neuron activity. For example, in inferior colliculus (Cong et al. 2011), hippocampus (Chen and Wong 1995) and cerebellum (Robello et al. 1997), it is found that the activation of NMDAR could inhibit the current of GABA$_A$R and that this inhibitory effect was regulated by Ca^{2+} in relation to CaMKII dependence. Other studies have shown that Systemic application of GABAergic agents reverses or prevents the hypermetabolic state (Kurumaji and McCulloch 1989), the excessive release of neurotransmitter (Giovannini et al. 1994), and the neurotoxic action of NMDAR (Ohkuma et al. 1994; Zhang et al. 2007; Oliveira-Pinto et al. 2015). These studies suggest that GABA$_A$R's regulation of NMDAR acts to compensate for and buffer the excessive excitation of neurons. However, studies on the regulatory effect of GABA$_A$R on NMDAR in the peripheral auditory nervous system, such as in SGNs, or on the ototoxicity induced by sodium salicylate have rarely been reported so far.

The SGNs are the first targets for the ototoxicity of salicylic acid drugs (Chen et al. 2010), which has been widely described. Our previous study confirmed that sodium salicylate could affect the expression of NMDAR and GABA$_A$R in SGNs (Tang et al. 2014; Yao et al. 2015). Therefore, we speculate that GABA$_A$R plays an important role in the regulation of NMDAR in sodium salicylate-induced ototoxicity. To test this hypothesis, this study used western blot to explore the underlying mechanism of ototoxicity.

Materials and methods

Primary culture of spiral ganglion neurons (SGNs)

The experimental animals were provided by the Experimental Animal Center of Guangxi Medical University. The animal experimental program was approved by the Animal Ethics Committee of Guangxi Medical University. The primary culture method for SGNs was the same as that previously reported by this study group (Chen et al. 2016). Briefly, this experiment used 3-day-old Sprague–Dawley rats. The cochlea were removed under an upright microscope (Olympus, Japan). The helical ligament was separated with filament forceps and a microdissection needle, the basement membrane was removed, and only the cochlear axial spiral tube containing the spiral ganglion was retained. The tissue containing the SGNs was shredded and incubated in a solution containing 0.25% trypsin (Gibco, USA) at 37 °C for 10 min, and then the digestion was terminated. Following centrifugation at 1000 r/min for 5 min and the removal of supernatant, the pellet was resuspended and gently triturated. Dissociated SGNs was plated onto poly-D-lysine-coated 35-mm culture dishes with DMEM/F12 containing 10% fetal bovine serum (Gibco, USA), and all cultures were maintained in

a humidified CO_2 (5%) incubator (Thermo, USA) for 48 h at 37 °C.

Treatment of the cultures

After 48 h of cell culture, drug intervention was carried out with normal culture medium or culture medium (all contained 10 µM GABA and 10 µM NMDA) with one of the following treatments: SS (0.5, 5 mM and 50 mM SS), GABA$_A$R agonist Mus (10, 100 and 1000 µM) and GABA (0.1, 1 and 10 mM), GABA$_A$R antagonist Bic (20, 100 and 500 µM) and Gabazine (10, 100 and 1000 µM), 10 µM PP2, 0.5 mM SS+10 µM Mus, 0.5 mM SS+20 µM Bic, 5 mM SS+10 µM Mus, or 5 mM SS+20 µM Bic. The above drugs were purchased from Sigma, USA. The treatments were terminated 30 min later.

Western blot

Total protein was obtained after 30 min of incubation with a lysate buffer including a protease inhibitor, a phosphatase inhibitor and radio immunoprecipitation assay. Extraction of surface protein was performed according to the instructions of the Cell Fractionation Kit (Cell Signaling Technology, USA). The brief steps are as follows. 400 µl of cell suspension was added into a 1.5 ml tube, centrifuged for 5 min at 500 (g) at 4°C, aspirated the supernatant and resuspended pellet in 500 µl of Cytoplasm Isolation Buffer. The mixture was vortexed for 5 s, incubated on ice for 5 min, centrifuged for 5 min at $500(g)$ and resuspended pellet in 500 µl of Membrane Isolation Buffer. Then the mixture was vortexed for 15 s, incubated on ice for 5 min and centrifuged for 5 min at $8000(g)$. Save the supernatant that is the surface protein. The protein concentration was determined with the Protein Assay Kit (Thermo, USA). The buffer supernatant containing 40 µg of protein was added to an SDS (sodium dodecyl sulfate)-polyacrylamide gel for electrophoretic separation. The protein was transferred to a polyvinylidene fluoride membrane with a stable 100 mA current, the membrane was cut according to the molecular weight markers (Thermo, USA), and then the membrane containing the target protein was blocked with 5% fat-free milk at room temperature for 1.5 h. The following antibodies were used: anti-NMDAR2B antibody (rabbit, 1:1000, Abcam, UK), anti-Fyn antibody (rabbit, 1:1000, Abcam, UK), anti-NMDAR2B (phospho-Y1472) antibody (rabbit, 1:1000, Millipore, USA), anti-Casein Kinase 2 beta antibody (rabbit, 1:1000, Abcam, UK), and anti-NMDAR2B (phospho-S1480) antibody (rabbit, 1:1000, Abcam, UK). The polyvinylidene fluoride membrane was incubated at 4 °C overnight and was then incubated with goat anti-rabbit fluorescence secondary antibody (1:500, Earthox, USA)

for 1.5 h at room temperature. After washing with tris buffered saline tween, the membrane was scanned using an infrared scanning device (Odyssey, USA). The band intensities (gray values) were measured with ImageJ software, and the β-3 tubulin protein was used as the internal reference. Protein relative expression level = (target band grayscale/reference band grayscale)/(control group grayscale/reference band grayscale) × 100%.

Statistical analysis

Statistical analysis involved the use of SPSS version 20.0 (SPSS Inc., USA). All experimental data are shown as the mean ± standard error of the mean. The significant differences among three or more groups were examined using one-way analysis of variance (ANOVA) with LSD or SNK tests if the ANOVA indicated a significant difference. A P value < 0.05 was considered to indicate a statistically significance.

Results

Sodium salicylate (SS) and activated GABA$_A$R down-regulated the expression of the NMDA receptor 2B subunit (NR2B) surface protein

To verify the effect of SS on NMDAR, SGNs were treated with SS (0.5, 5 and 50 mM) for 30 min, which significantly reduced the expression of NR2B surface protein by 13.49, 36.04 and 37.58% ($F = 20.214$, $P = 0.000 < 0.05$), as compared to the control group. The effects of SS was in a concentration-dependent manner. But there is no significance between 5 mM SS group and 50 mM SS group ($P = 0.795 > 0.05$) (Fig. 1), that is to say the saturated concentration of SS is 5 mM. In addition, SGNs were treated with Mus (GABA$_A$R agonist) (10, 100 and 1000 μM) to activate GABA$_A$R, which also decreased the NR2B surface protein expression levels by 8.44, 35.90 and 39.87% ($F = 423.826$, $P = 0.000 < 0.05$), as compared to the control group. After treatment of GABA (0.1, 1 and 10 mM), the expression of NR2B surface protein was
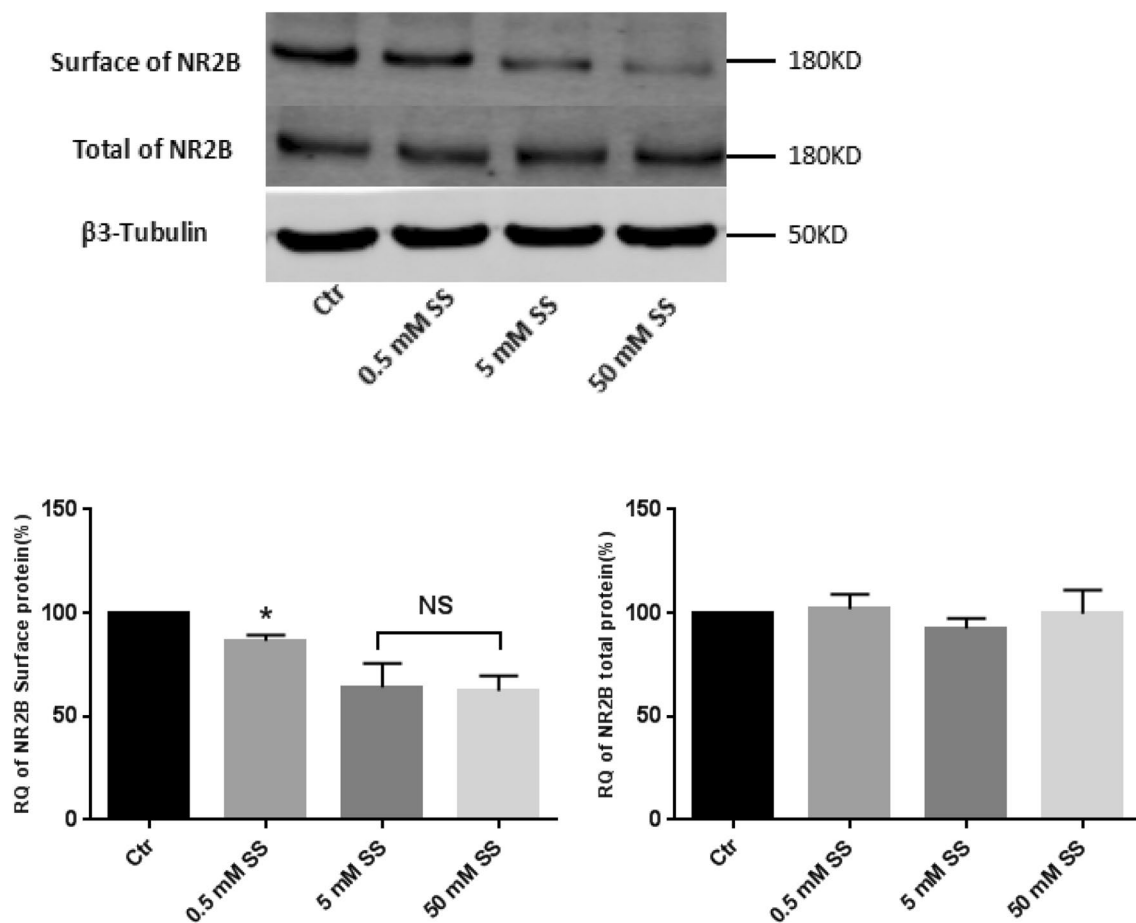

Fig. 1 Different concentrations of SS (0.5 mM, 5 mM and 50 mM) were applied to cultured SGNs for 30 min before cell lysis; surface protein and total protein of NR2B were analyzed by Western blot. *P < 0.05, compared with control group ($n = 3$ for all groups, $\bar{x} \pm s$)

reduced by 41.62, 67.56 and 74.64% ($F = 155.733$, $P = 0.000 < 0.05$), as compared to the control group. There is also no significance between 100 μM Mus group and 1000 μM Mus group ($P = 0.829 > 0.05$) or between 1 mM GABA group and 10 mM GABA group ($P = 0.102 > 0.05$) (Fig. 2), and the saturated concentration of Mus and GABA were 0.1, 1 mM, respectively. However, all the treatments had no significant change in total protein (SS group: $F = 1.006$, $P = 0.439 > 0.05$; Mus group: $F = 0.706$, $P = 0.575 > 0.05$; GABA group: $F = 0.231$, $P = 0.872 > 0.05$). The results suggested that SS or GABA$_A$R activation promoted NMDAR internalization.

GABA$_A$R inhibition up-regulated the expression of the NMDA receptor 2B subunit (NR2B) surface protein

To further confirm the effect of GABA$_A$R on NMDAR, we observed the effect of different concentrations of GABA$_A$R antagonist (Bic and Gabazine) on the surface protein of NR2B, on the premise of adding a small dose of GABA (10 μM) to the medium. Obviously, after treatment of Bic (20, 100, 500 μM) for 30 min, the expression of NR2B surface protein was increased by 15.73, 59.00 and 61.90%, respectively ($F = 33.530$, $P = 0.000 < 0.05$), as compared to the control group. Similarly, after application of Gabazine (10, 100 and 1000 μM), the expression levels of NR2B surface protein was increased by 30.63, 79.54 and 81.26% ($F = 42.147$, $P = 0.000 < 0.05$), as compared to the control group. However, there is also no significance between 100 μM Bic group and 500 μM Bic group ($P = 0.712 > 0.05$) or between 100 μM Gabazine group and 1000 μM Gabazine group ($P = 0.847 > 0.05$) in NR2B surface protein expression. What's more, there was no significant change in total protein after the treatment of GABA$_A$R inhibitors for all groups (Bic group: $F = 0.858$, $P = 0.501 > 0.05$; Gabazine group: $F = 0.549$, $P = 0.663 > 0.05$) (Fig. 3). The results suggest that attenuating GABA$_A$R inhibition may promote excitotoxic damage by increasing the function of NMDAR. Similar studies have also found that in the spinal dorsal horn nerve, anterior cingulate cortical neurons, application of Bic can cause hyperalgesia and also induce the delivery of NMDAR to the synapse (Baba et al. 2003; Buesa et al. 2006; Torsney and MacDermott 2006); electrophysiological records also show that attenuation of GABAergic inhibition can induce a new NMDAR-mediated synaptic current in the dorsal horn of the spinal cord (Wang et al. 2005).

Sodium salicylate (SS) decreased NMDA receptor 2B subunit (NR2B) surface protein expression through GABA$_A$R, and the effects of SS and GABA$_A$R modulation operated by the same mechanism

To verify the effect of the combination of SS and GABA$_A$R on NMDAR, we added SS with Mus or Bic to observe the

effect on the surface protein of NR2B. Compared with using Mus alone, the NR2B surface protein expression in the 0.5 mM SS+Mus group and 5 mM SS+Mus group was decreased by 30.82% ($F = 30.160$, $P = 0.000 < 0.05$) and 55.33% ($P = 0.000 < 0.05$), respectively. In contrast, compared with the Bic group, the expression of the NR2B surface protein in the 0.5 mM SS+Bic group and the 5 mM SS+Bic group was decreased by 14.23% ($P = 0.035 < 0.05$) and 29.72% ($P = 0.000 < 0.05$), respectively. However, there was no significant change in total protein expression between the treatment group and the control group ($F = 1.111$, $P = 0.401 > 0.05$) (Fig. 4).

The above experiments proved that SS and GABA$_A$R activation had a superposition effect. To further confirm the effect of SS or GABA$_A$R, SS and Mus with saturated concentration were selected to repeat part of the experiment. After 5 mM SS and 100 μM Mus treatment for 30 min, there was no statistical difference between 5 mM SS+100 μM Mus group and 5 mM SS group ($F = 22.016$, $P = 0.375 > 0.05$), nor was between 5 mM SS+100 μM Mus group and 100 μM Mus group ($P = 0.361 > 0.05$) (Fig. 5). That is to say, the effects of SS and GABA$_A$R modulation operated by the same mechanism.

Sodium salicylate (SS) down-regulated the Fyn-dependent phosphorylation of Y1472, but the downregulation was not related to the CK2 (Casein Kinase 2) dependent phosphorylation of S1480

To study the potential mechanism of SS-mediated NMDAR internalization, and verify how it regulates the effect of GABA$_A$R on NMDAR in SGNs, phosphorylation levels were determined to reveal the possible mechanisms. Mus down-regulated Fyn protein expression by 20.73% ($F = 34.688$, $P = 0.010 < 0.05$); conversely, Bic promoted Fyn protein expression by 29.07% ($P = 0.001 < 0.05$). Among the treatments, the combined effect of 0.5/5 mM SS and Mus was more significant than the effect of using Mus alone, decreasing by 17.48% ($P = 0.004 < 0.05$) and 48.65% ($P = 0.000 < 0.05$), respectively. The combined treatment of 0.5/5 mM SS and Bic reversed the effect of using Bic alone, decreasing by 20.91% ($P = 0.010 < 0.05$) and 33.06% ($P = 0.000 < 0.05$), respectively. On the other hand, Mus also down-regulated the expression of the P-Y1472 protein by 25.65% ($F = 30.821$, $P = 0.002 < 0.05$), and Bic upregulated the expression of the P-Y1472 protein by 38.48% ($P = 0.000 < 0.05$). Among the treatments, the combined effect of 0.5/5 mM SS and Mus was more significant than the effect of using Mus alone, decreasing by 25.82% ($P = 0.003 < 0.05$) and 29.95% ($P = 0.000 < 0.05$), respectively. The combination of 0.5 mM SS/5 mM SS and Bic reversed the effect of using Bic alone, decreasing by 25.43% ($P = 0.002 < 0.05$) and

Fig. 2 Different concentrations of Mus (10, 100 and 1000 μM) and GABA (0.1, 1 and 10 mM) were applied to cultured SGNs for 30 min before cell lysis; surface protein and total protein of NR2B were analyzed by Western blot. *$P < 0.05$, compared with control group ($n = 3$ for all groups, $\bar{x} \pm s$)
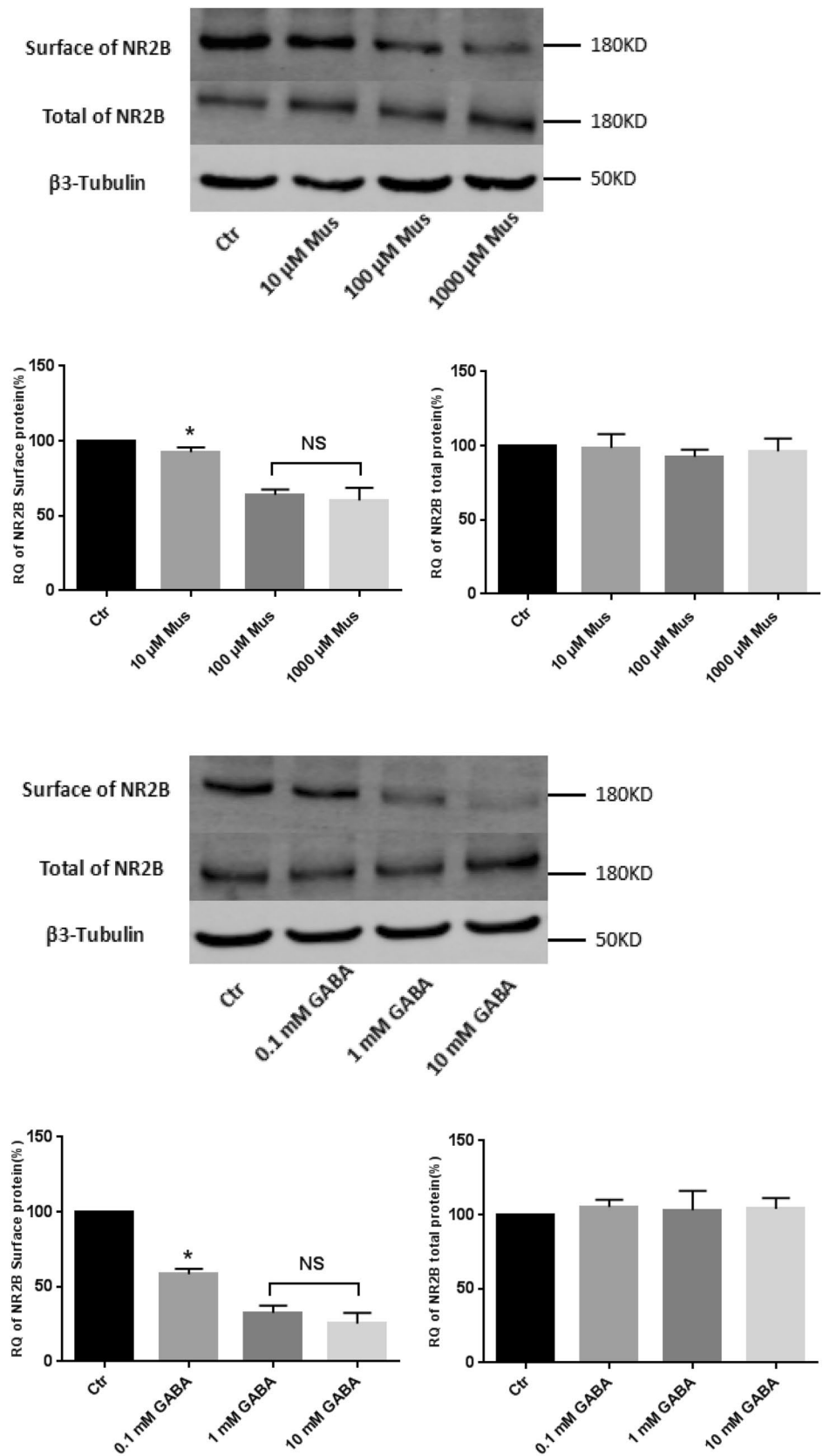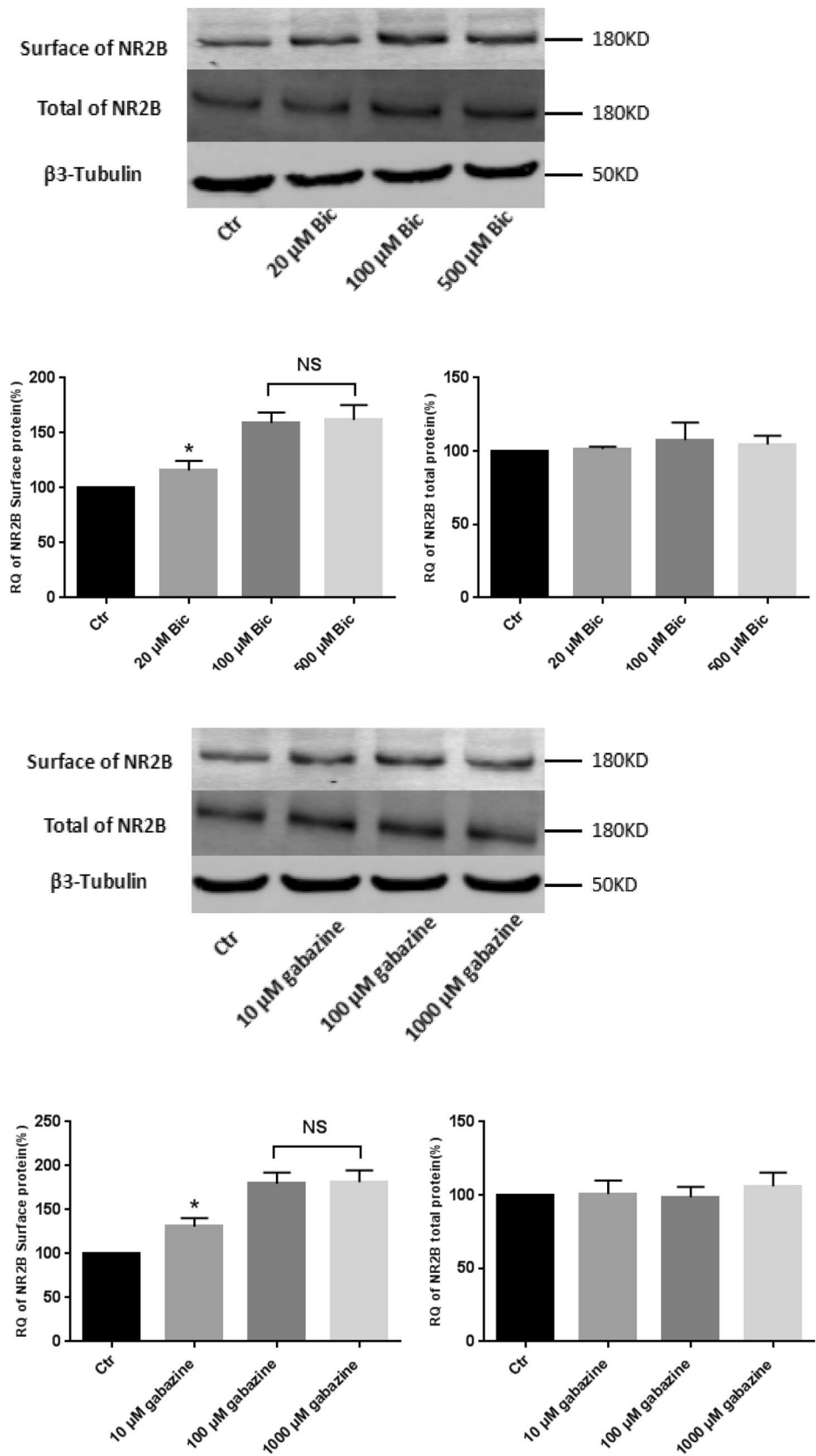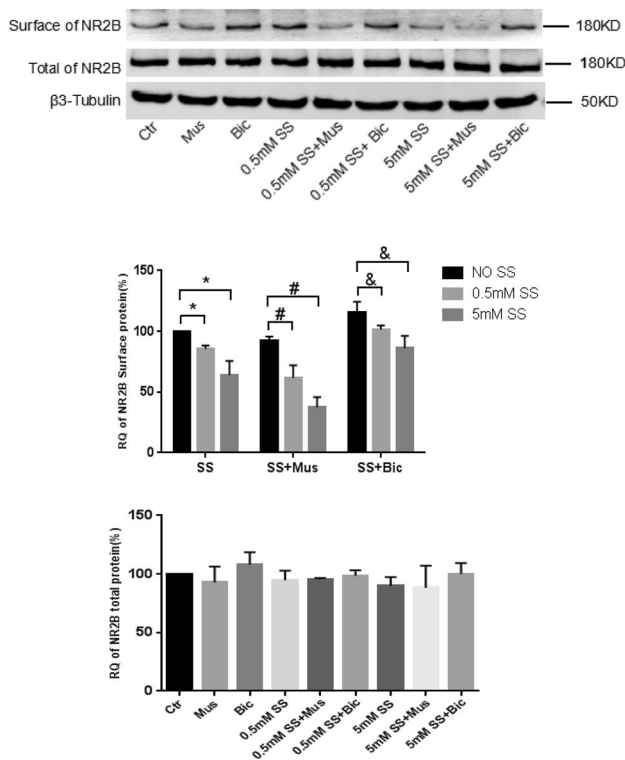
Fig. 3 Different concentrations of Bic (20, 100, 500 μM) and Gabazine (10, 100 and 1000 μM) were applied to cultured SGNs for 30 min before cell lysis; surface protein and total protein of NR2B were analyzed by Western blot. *$P < 0.05$, compared with control group ($n = 3$ for all groups, $\bar{x} \pm s$)

Fig. 4 Western blot analyzed surface protein and total protein of NR2B after 0.5 or 5 mM SS, 10 μM Mus and 20 μM Bic co-application for 30 min. *$P < 0.05$, compared with control group; #$P < 0.05$, compared with Mus group; &$P < 0.05$, compared with Bic group ($n = 3$ for all groups, $\bar{x} \pm s$)
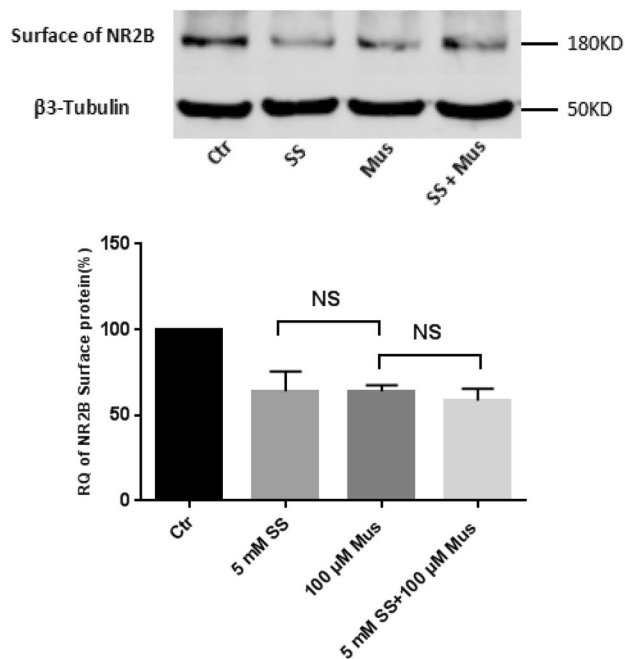

Fig. 5 Western blot analyzed surface protein of NR2B after 5 mM SS and 100 μM Mus co-application for 30 min. *$P < 0.05$, compared with control group ($n = 3$ for all groups, $\bar{x} \pm s$)

54.60% ($P = 0.000 < 0.05$), respectively (Fig. 6). However, under the influence of SS and GABA$_A$R activity, there was no significant change in CK2 protein expression between the treatment group and the control group ($F = 0.742$, $P = 0.655 > 0.05$), nor was there a change in P-S1480 protein expression ($F = 1.230$, $P = 0.337 > 0.05$) (Fig. 7). The results suggested that NMDAR internalization regulated by SS or GABA$_A$R is associated with Fyn and Y1472.

Fyn-dependent phosphorylation of Y1472 regulated NMDA receptor 2B subunit (NR2B) internalization

To further verify the correlation between NR2B internalization and Fyn-dependent phosphorylation of Y1472, PP2 (an inhibitor of Fyn) was added on the basis of SS, GABA$_A$R agonist and inhibitor. The results show that there was no significant change in NR2B surface protein expression among all groups ($F = 0.436$, $P = 0.884 > 0.05$), nor was P-Y1472 protein expression ($F = 0.340$, $P = 0.939 > 0.05$) (Fig. 8). Therefore, this further suggested that SS and GABA$_A$R regulate NMDAR internalization through Fyn-dependent phosphorylation of Y1472.
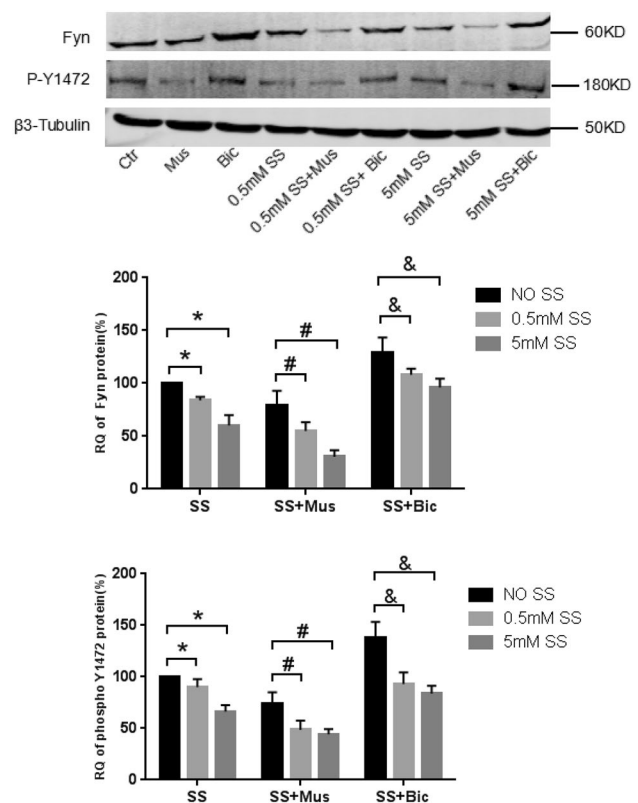

Fig. 6 Western blot analyzed protein of Fyn and P-Y1472 after 0.5 or 5 mM SS, 10 μM Mus and 20 μM Bic co-application for 30 min. *$P < 0.05$, compared with control group; #$P < 0.05$, compared with Mus group; &$P < 0.05$, compared with Bic group ($n = 3$ for all groups, $\bar{x} \pm s$)
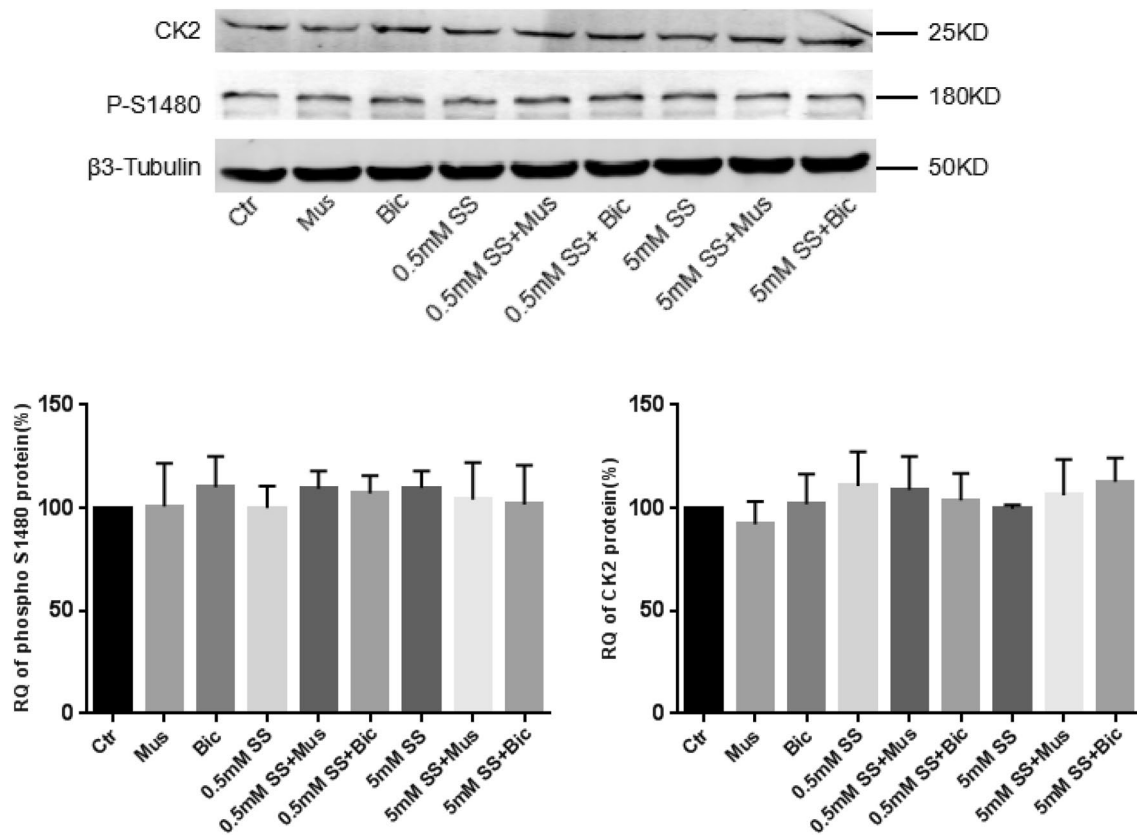
Fig. 7 Western blot analyzed protein of CK2 and P-S1480 after 0.5 or 5 mM SS, 10 μM Mus and 20 μM Bic co-application for 30 min. $*P <$ 0.05, compared with control group ($n = 3$ for all groups, $\bar{x} \pm s$)

Discussion

$GABA_AR$ and NMDAR are important inhibitory and excitatory receptors, respectively, in the central nervous system and are also highly expressed in the peripheral auditory system (Niedzielski and Wenthold 1995; Yao et al. 2015). It has been found that $GABA_AR$ reduces the response of NMDAR. For example, in inferior colliculus, $GABA_AR$ activation inhibits the current of NMDAR through the Ca^{2+}/CaMKII signaling pathway (Cong et al. 2011). The activated $GABA_AR$ inhibits the Ca^{2+} influx of postsynapse and causes the hyperpolarization of neuronal membrane (Ohkuma et al. 1994; Miura et al. 1997), which thereby may inhibit the function of NMDAR via attenuation of the increased interaction of Src, NR2A and scaffold protein PSD-95, consequently protecting neurons from apoptotic neuronal death (Zhang et al. 2007). $GABA_AR$ overactivation potentiates the effects of NMDA blockade in inducing locomotor hyperactivity during this period (Oliveira-Pinto et al. 2015). As for the SGNs, evidence implicates the $GABA_AR$ in mediating the inhibitory effect of GABA specifically. GABA-mediated (Cl^-) currents have been recorded from SGNs isolated from neonatal (E14-P5) mice (Lin et al. 2000), neonatal (P3) rat

(Malgrange et al. 1997). Recordings of afferent activity from high frequency regions of the intact guinea pig cochlea, show that GABA application decreases Glu- and ACh-induced increases in afferent spiking specifically through activation of $GABA_AR$ (Felix and Ehrenberger 1992; Arnold et al. 1998). However, cultured SGNs (P2) ECl is -18 mV (Zhang et al. 2015). Given this, it is probable that activating $GABA_AR$ will depolarize SGNs. In this study, we found that the activation of $GABA_AR$ in SGNs could downregulate the expression of NR2B surface protein without affecting total protein expression (Figs. 2, 3). But the exact mechanism for the protective action produced by $GABA_AR$ activation on the NMDAR, remains to be elucidated.

At present, considerable data have shown that $GABA_AR$ hypofunction and NMDAR hyperfunction are key events in ototoxicity induced by SS. On the one hand, salicylate increases the arachidonate content of the whole cochlea in vivo (Ruel et al. 2008), salicylate and arachidonate enable the Ca^{2+} influx and the neural excitatory effects of NMDA on SGNs (Sahley et al. 2013). On the other hand, SS inhibits $GABA_AR$-mediated postsynaptic currents, reducing synaptic transmission, and attenuates GABAergic inhibitory effects in rat hippocampal CA1 region (Xu et al. 2005;
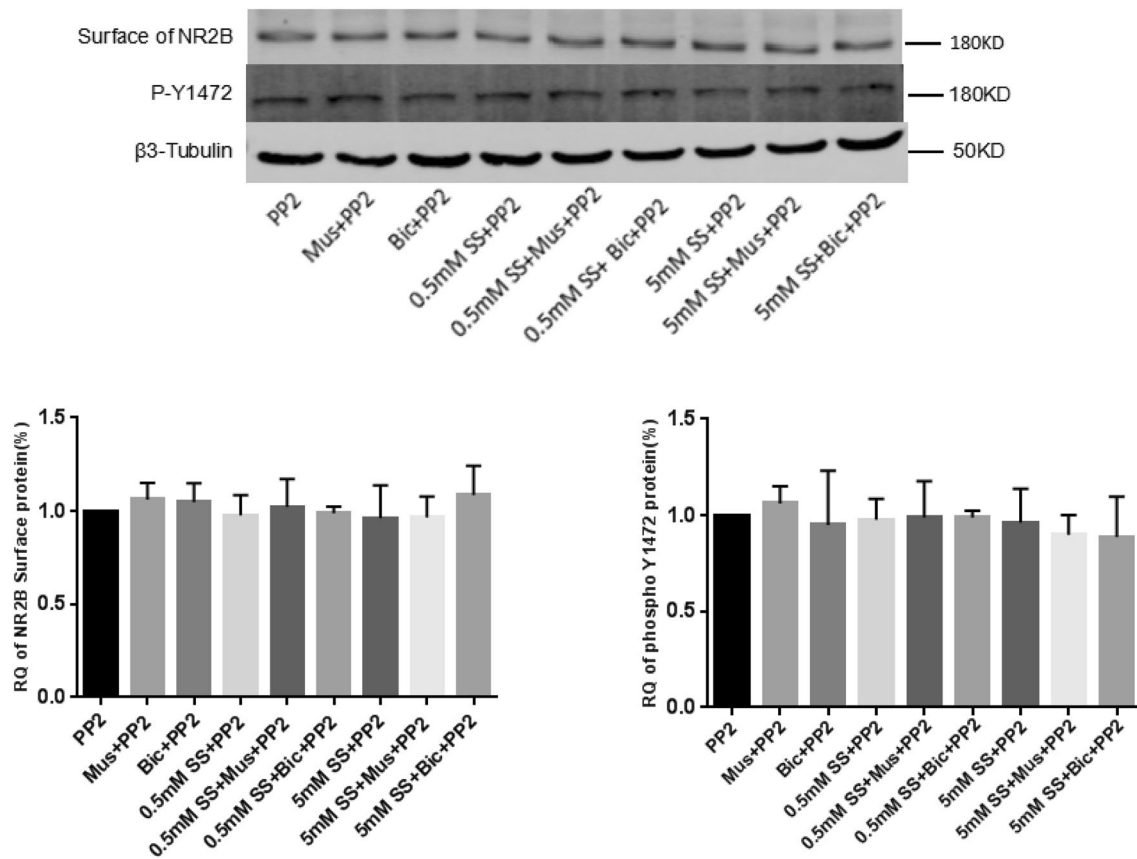
Fig. 8 Western blot analyzed protein of NR2B and P-Y1472 after 0.5 or 5 mM SS, 10 μM Mus and 20 μM Bic co-application for 30 min. *$P < 0.05$, compared with control group ($n = 3$ for all groups, $\bar{x} \pm s$)

Wang et al. 2006; Zou and Shang 2012). In our study, SS decreased NR2B surface protein expression through affecting GABA$_A$R, but the total protein did not significantly change (Fig. 4). Furthermore, there was no superposition of SS and GABA$_A$R activity at saturation concentration, which proved SS or GABA$_A$R activity is the same effect (Fig. 5). Previous studies suggested that activated NMDAR may be left from the cell membrane by internalization, thus avoiding reactivation. For instance, cortical neurons induce NMDAR internalization through the c-Jun phosphorylation pathway to alleviate the excitatory toxicity caused by excessive NMDA stimulation (Vaslin et al. 2007). Excitatory stimulation of hippocampal neurons in rats induces a reduction in the proportion of NR2B subunits and an elevation in the proportion of NR2A subunits (Bellone and Nicoll 2007). Neuronal suppression enhances NMDAR channel currents and increases NR2B subunits content (Lee et al. 2010). Therefore, we speculated that the imbalance between GABA$_A$R and NMDAR functional regulation through a receptor internalization mechanism mediates the excitotoxicity of SS.

In addition, this study showed that the application of 0.5/5 mM SS significantly decreased Fyn and P-Y1472 protein expression (Fig. 6), and PP2 could reverse this process (Fig. 8). The above suggested that SS inhibits Fyn-mediated Y1472 phosphorylation. Consistent with the results, studies on cardiomyocytes and monocytes cultured in vitro also found that SS inhibits angiotensin- or platelet-derived growth factor activation of Src (which belongs to the same Src kinase family as Fyn) in a concentration-dependent manner, and the most obvious effect is 5–20 mM (Wang and Brecher 2001; Perez et al. 2002). Other studies have found that the internalization of NMDAR generally occurs in a manner similar to the production of clathin, which is mainly mediated through the interaction between the NR2 subunit C-terminal internalized motif YEKL and AP2. In rat cerebellum and hippocampus (Yaka et al. 2002; Prybylowski et al. 2005), neuronal activity induces the phosphorylation of the YEKL motif Y1472 site of NR2B by Fyn, thus inhibiting AP2-mediated NMDAR internalization. Moreover, the phosphorylation of NR2B-S1480 by CK2 destroys the binding of the receptor and membrane-associated guanylate kinase (MAGUK), causing NMDAR not to bind to Fyn, reducing the phosphorylation of NR2B-Y1472 and promoting internalization (Sanz-Clemente et al. 2010). However, only the changes in Fyn and Y1472 could be detected in this

experiment (Figs. 6, 7). Therefore, we speculated that SS mediates NMDAR internalization relying on GABA$_A$R, and the Fyn-dependent phosphorylation of Y1472 participates in the regulation of this process.

In conclusion, we assessed firstly that Fyn-dependent phosphorylation of Y1472 is a pathway of SS action on SGNs. The inter-receptor crosstalk acts as a compensatory, counterbalancing mechanism to dampen NMDAR-mediated excitotoxicity, which not only breaks the traditional idea that the receptor is a single and independent functional unit but also provides a new idea for studying the intrinsic mechanisms of ototoxicity. However, there are still many components in the molecular mechanism of SS toxicity, and further studies on cell electrophysiology and molecular pharmacology are needed.

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