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### Cross-talk between NMDA and GABA<sub>A</sub> receptors in cultured neurons of the rat inferior colliculus

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Neuronal ion channels of different types often do not function independently but will inhibit or potentiate the activity of other types of channels, a process called cross-talk. The *N*-methyl-*D*-aspartate receptor (NMDA receptor) and the  $\gamma$ -aminobutyric acid type A receptor (GABA<sub>A</sub> receptor) are important excitatory and inhibitory receptors in the central nervous system, respectively. Currently, cross-talk between the NMDA receptor and the GABA<sub>A</sub> receptor, particularly in the central auditory system, is not well understood. In the present study, we investigated functional interactions between the NMDA receptor and the GABA<sub>A</sub> receptor using whole-cell patch-clamp techniques in cultured neurons from the inferior colliculus, which is an important nucleus in the central auditory system. We found that the currents induced by aspartate at 100  $\mu\text{mol L}^{-1}$  were suppressed by the pre-perfusion of GABA at 100  $\mu\text{mol L}^{-1}$ , indicating cross-inhibition of NMDA receptors by activation of GABA<sub>A</sub> receptors. Moreover, we found that the currents induced by GABA at 100  $\mu\text{mol L}^{-1}$  ( $I_{\text{GABA}}$ ) were not suppressed by the pre-perfusion of 100  $\mu\text{mol L}^{-1}$  aspartate, but those induced by GABA at 3  $\mu\text{mol L}^{-1}$  were suppressed, indicating concentration-dependent cross-inhibition of GABA<sub>A</sub> receptors by activation of NMDA receptors. In addition, inhibition of  $I_{\text{GABA}}$  by aspartate was not affected by blockade of voltage-dependent  $\text{Ca}^{2+}$  channels with  $\text{CdCl}_2$  in a solution that contained  $\text{Ca}^{2+}$ , however,  $\text{CdCl}_2$  effectively attenuated the inhibition of  $I_{\text{GABA}}$  by aspartate when it was perfused in a solution that contained  $\text{Ba}^{2+}$  instead of  $\text{Ca}^{2+}$  or a solution that contained  $\text{Ca}^{2+}$  and 10  $\text{mmol L}^{-1}$  BAPTA, a membrane-permeable  $\text{Ca}^{2+}$  chelator, suggesting that this inhibition is mediated by  $\text{Ca}^{2+}$  influx through NMDA receptors, rather than voltage-dependent  $\text{Ca}^{2+}$  channels. Finally, KN-62, a potent inhibitor of  $\text{Ca}^{2+}$ /calmodulin-dependent protein kinase II (CaMKII), reduced the inhibition of  $I_{\text{GABA}}$  by aspartate, indicating the involvement of CaMKII in this cross-inhibition. Our study demonstrates a functional interaction between NMDA and GABA<sub>A</sub> receptors in the inferior colliculus of rats. The presence of cross-talk between these receptors suggests that the mechanisms underlying information processing in the central auditory system may be more complex than previously believed.

**inferior colliculus, *N*-methyl-*D*-aspartate receptor,  $\gamma$ -aminobutyric acid type A receptor, whole-cell patch-clamp, cross-talk**

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Neuronal receptor ion channels of different types are activated by specific ligands called neurotransmitters, and the

activation of these channels by their corresponding neurotransmitters may appear to be an independent process. However, accumulating evidence indicates that this is not the case. The activation of specific ion channels is often

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modulated by the activation of other ion channels [1–3]. This so-called “cross-talk” between two different types of ion channels means that the activation of one channel can inhibit or potentiate the activation of another. Experimentally, cross-talk can be demonstrated by recording the channel currents from two different ion channels while specific agonists of these channels are applied simultaneously or sequentially. Previous studies have shown that a number of receptors can engage in cross-talk. For example, cross-talk occurs between adenosine triphosphate (ATP) P2X and 5-hydroxytryptamine (5-HT) receptors in rat pheochromocytoma cells [1], between glycine and  $\alpha$ -amino-3-hydroxy-5-methyl-4-isoxazolepropionate (AMPA) receptors in rat spinal neurons [2], between *N*-methyl-*D*-aspartate (NMDA) and dopamine D1 receptors [3], and between glycine and  $\gamma$ -aminobutyric acid (GABA) receptors in rat spinal dorsal horn neurons [4].

NMDA and GABA<sub>A</sub> receptors are the major excitatory and inhibitory synaptic receptors in the central nervous system (CNS), respectively. The NMDA receptor is one of three subtypes of ionotropic glutamate receptors, i.e., the AMPA receptor, the kainate receptor and the NMDA receptor [5,6]. The GABA type A receptor (GABA<sub>A</sub> receptor) is a principal inhibitory receptor and gate, which is distinct but homologous to a class of chloride-permeable ion channels. Activation of NMDA receptors mediates excitatory neurotransmission and plays a fundamental role in both physiological and pathological processes in the CNS. Activation of GABA<sub>A</sub> receptor leads to an inward flow of chloride ions and a hyperpolarizing neuronal response, which is crucial for normal brain function, such as nociceptive messages [7,8] and auditory signal processing [9,10]. Both NMDA and GABA<sub>A</sub> receptors are widely distributed throughout the CNS [11,12], including in the inferior colliculus (IC), which is a major synaptic integration center and relay station of the central auditory pathway in mammals. Moreover, auditory processing in the IC involves both NMDA and GABA<sub>A</sub> receptors [13,14].

It has been reported that the activation of NMDA receptors suppresses the function of GABA<sub>A</sub> receptors in rat cerebellum granule cells, in hippocampal neurons and in the rat sacral dorsal commissural nucleus. Suppression of GABA<sub>A</sub> receptor responses by the activation of NMDA receptors is a Ca<sup>2+</sup>-dependent process, and enzymes such as calcineurin, Ca<sup>2+</sup>/calmodulin-dependent protein kinase II (CaMKII) and NO-synthase are involved in the process [15–17]. Currently, however, the interaction between these two receptors has not been systematically studied. In particular, relatively little is known concerning the interaction between NMDA receptors and GABA<sub>A</sub> receptors in central auditory regions. In the present study, we investigated the cross-talk between NMDA and GABA<sub>A</sub> receptors and the possible mechanisms underlying this cross-talk in cultured IC neurons using whole-cell patch-clamp recording techniques. Our results show that activation of GABA<sub>A</sub> recep-

tors suppresses NMDA receptor-mediated currents and activation of NMDA receptors conversely reduces GABA<sub>A</sub> receptor-mediated currents, indicating the presence of cross-talk between NMDA and GABA<sub>A</sub> receptors in the central auditory system.

## 1 Materials and methods

### 1.1 Cell culture

All procedures conducted with animals in this study followed the guidelines and protocols approved by the Institutional Animal Care and Use Committee of the University of Science and Technology of China. All efforts were made to minimize the number of animals used and any pain or distress in the animals used.

The neurons used for cell culture were dissociated from the IC of newborn Wistar rats (postnatal day 0) as previously described [18]. Briefly, the IC was dissected from the brainstem under a dissection microscope. The tissue was incubated with 0.25% trypsin (Sigma, St Louis, MO, USA) for 10 min at 37°C and mechanically dissociated by trituration with a Pasteur pipette in Dulbecco's modified Eagle's medium (DMEM; Gibco, Carlsbad, CA, USA). The isolated neurons were plated ( $1.5 \times 10^6$  cell mL<sup>-1</sup>) on poly-L-lysine (Sigma, St Louis, MO, USA)-coated cover glasses and grown for 24 h in a DMEM mixture with L-glutamine, 10% fetal bovine serum, 10% F-12 nutrient, 100 U mL<sup>-1</sup> penicillin, and 100  $\mu$ g mL<sup>-1</sup> streptomycin (Gibco, USA). Then, we placed the cells in a neuron-basal medium (1.5 mL) containing 2% B27, which was replaced every 3 d. To block the division of non-neuronal cells and stabilize the cell population, we treated the neurons with 5-fluoro-5'-deoxyuridine (20  $\mu$ g mL<sup>-1</sup>; Sigma, USA) on the fourth day after plating. The cultures were maintained at 37°C in 5% CO<sub>2</sub> and 95% humidified air, and the cells were used for electrophysiological recordings 9–14 d after plating.

### 1.2 Electrophysiology

Whole-cell voltage-clamp recordings were carried out using a patch-clamp amplifier (Axon 200B, Axon Instruments, USA). Patch pipettes were pulled from glass capillaries with an outer diameter of 1.5 mm on a two-stage puller (PC-10, Narishige, Japan). The resistance between the recording electrode filled with pipette solution and the reference electrode was 2–5 M $\Omega$ . In the experiments, 70%–90% series resistance was compensated, and the membrane potential was held at –60 mV throughout the experiment under voltage clamp conditions. Data were sampled using a Digidata 1320A interface and analyzed using a computer installed with Clampex and Clampfit software (Version 9.0.1, Axon Instruments, USA). All experiments were performed at room temperature (22–25°C). The data were sampled at 5

kHz and low pass filtered at 2 kHz. All measurements were carried out after stabilization of the aspartate (Asp) responses or the GABA responses.

### 1.3 Solutions and drugs

The standard external solution consisted of (in mmol L<sup>-1</sup>): 150 NaCl, 5 KCl, 1 MgCl<sub>2</sub>, 2 CaCl<sub>2</sub>, 10 glucose, and 10 HEPES. The pH was adjusted to 7.4 using Tris base. In some experiments, we needed to remove the MgCl<sub>2</sub> and add 1 μmol L<sup>-1</sup> Gly to the external solution, replace the CaCl<sub>2</sub> with BaCl<sub>2</sub>, or add 10 μmol L<sup>-1</sup> of CdCl<sub>2</sub> to the external solution. The osmolarity of all bath solutions was adjusted to 310–320 mOsm L<sup>-1</sup> with sucrose (model 3300; Advanced Instruments, Pomona, CA, USA).

The patch pipette solution for whole-cell patch recording consisted of (in mmol L<sup>-1</sup>): 120 KCl, 30 NaCl, 1 MgCl<sub>2</sub>, 0.5 CaCl<sub>2</sub>, 5 ethylene glycol-bis-(2-aminoethylether)-tetra acetic acid (EGTA), 2 Mg-ATP, and 10 HEPES. The pH was adjusted to 7.2 with Tris base. In some experiments, 10 mmol L<sup>-1</sup> BAPTA, a membrane-permeable Ca<sup>2+</sup> chelator, was added to the pipette solution.

Unless otherwise specified, the drugs used in the present study were all purchased from Sigma, Inc., USA. All drugs were applied with a rapid application technique which was termed the ‘Y-tube’ method [19]. With this system, we can completely exchange the external solution surrounding a neuron within 20 ms.

### 1.4 Statistical analysis

Clampfit software (Version 9.2, Axon Instruments, USA) and Origin 8.0 software (OriginLab Corporation, USA) were used for data analysis. Statistical significance between

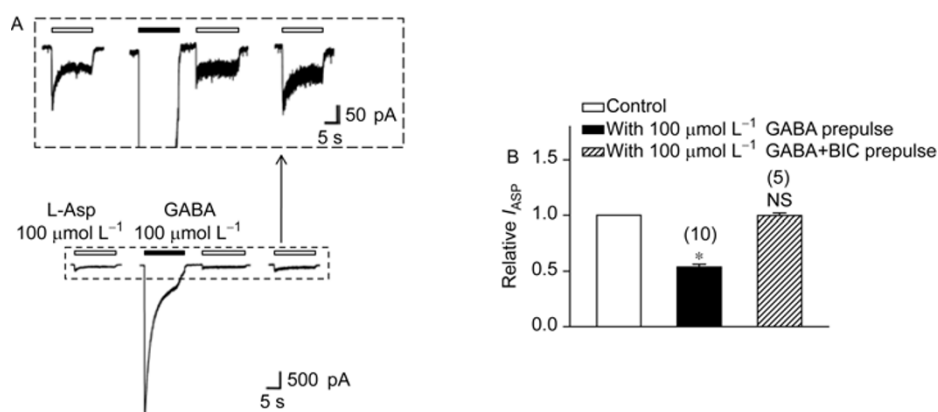
two groups was assessed with a Student’s *t*-test.  $P < 0.01$  was considered to be statistically significant. All the data are represented as the mean ± the standard error of the mean (SEM). *P* and *n* represent the value of significance and the number of neurons, respectively.

## 2 Results

### 2.1 Activation of GABA<sub>A</sub> receptors suppressed NMDA receptor-mediated responses

Both NMDA receptor-mediated current and GABA<sub>A</sub> receptor-mediated current could be recorded in the IC neurons. In the presence of Mg<sup>2+</sup>-free external solution plus 1 μmol L<sup>-1</sup> glycine, Asp at 100 μmol L<sup>-1</sup> induced an inward current (*I*<sub>Asp</sub>) at the holding potential of -60 mV. This *I*<sub>Asp</sub> could be completely blocked by 100 μmol L<sup>-1</sup> APV (D-2-amino-5-phosphonovaleate), a specific NMDA receptor antagonist, indicating that the *I*<sub>Asp</sub> recorded in our experiments is mediated by NMDA receptors. Similarly, a current evoked by application of GABA (*I*<sub>GABA</sub>) could be completely blocked by bicuculline (10 μmol L<sup>-1</sup>), a specific GABA<sub>A</sub> receptor antagonist, indicating that the *I*<sub>GABA</sub> recorded in the IC neurons is mediated by GABA<sub>A</sub> receptors. Moreover, *I*<sub>Asp</sub> and *I*<sub>GABA</sub> also could be observed from the same neurons.

We observed cross-inhibition between NMDA and GABA<sub>A</sub> receptors in IC neurons. When 100 μmol L<sup>-1</sup> GABA was applied following application of 100 μmol L<sup>-1</sup> Asp, we found that the amplitude of *I*<sub>Asp</sub> was 53% ± 3% of control 5 s after application of GABA, and the effect was reversible. Pre-perfusion of 10 μmol L<sup>-1</sup> bicuculline for 3 min blocked the inhibitory effect of GABA on the *I*<sub>Asp</sub> (Figure 1). As the time interval between sequential application

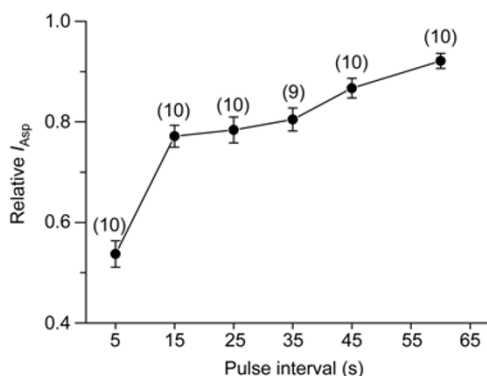


**Figure 1** GABA-induced reversible suppression of *I*<sub>Asp</sub>. **A**, *I*<sub>Asp</sub> was reversibly suppressed by a preceding 100 μmol L<sup>-1</sup> GABA administration in an IC neuron. The interval between GABA and Asp application was 5 s. A solid arrow indicates an enlargement of the current traces. **B**, Statistics showing the normalized suppression of 100 μmol L<sup>-1</sup> Asp-evoked currents by preapplication of 100 μmol L<sup>-1</sup> GABA or by pre-application of 100 μmol L<sup>-1</sup> GABA following pre-perfusion of 10 μmol L<sup>-1</sup> bicuculline for 3 min. In this and the subsequent figures, the horizontal bars indicate the time course of drug applications, vertical bars represent the mean ± SEM and the number of experiments is shown in parentheses. \*,  $P < 0.01$ , NS indicates no statistically significant difference.

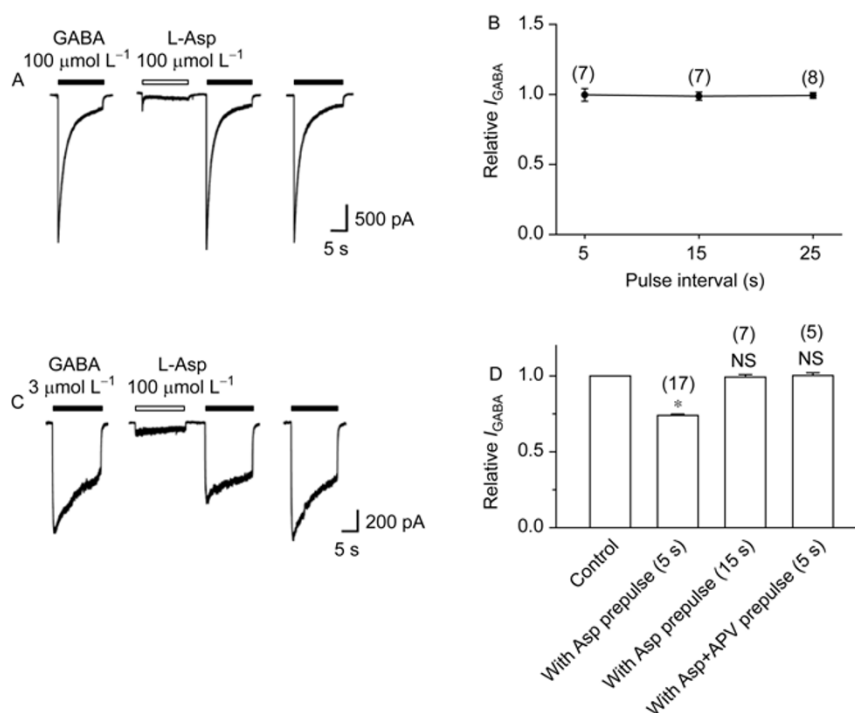
of GABA and Asp was increased, the inhibition progressively decreased (Figure 2), indicating that the recovery of  $I_{Asp}$  was dependent upon elapsed time.

## 2.2 The inhibition of $I_{GABA}$ by activation of NMDA receptors was state-dependent

To determine whether activation of NMDA receptors also affects the activity of GABA<sub>A</sub> receptors, we examined the effects of activation of NMDA receptors on  $I_{GABA}$ . We found that activation of NMDA receptors suppressed  $I_{GABA}$ ,



**Figure 2** Time-dependent recovery of  $I_{Asp}$  expressed as the normalized amplitude of  $I_{Asp}$  and measured as the time interval between sequential application of GABA and Asp increased. All points shown are the mean $\pm$ SEM.



**Figure 3** The effects of pre-application of Asp on  $I_{GABA}$  are state-dependent. A and B, pre-application of  $100 \mu\text{mol L}^{-1}$  Asp did not significantly change the amplitude of  $100 \mu\text{mol L}^{-1}$  GABA-evoked currents ( $I_{GABA}$ ) over various pre-application time intervals (5 s/15 s/25 s). C and D,  $I_{GABA}$  induced by  $3 \mu\text{mol L}^{-1}$  GABA were reversibly suppressed by a preceding  $100 \mu\text{mol L}^{-1}$  Asp administration. The interval between Asp and GABA application was 5 s. Pre-perfusion of  $100 \mu\text{mol L}^{-1}$  APV for 6 min blocked this inhibition. When the interval was increased to 15 s, the inhibition of  $I_{GABA}$  was no longer detectable. The symbols and bars in B and the vertical bars in D represent the mean $\pm$ SEM. \*,  $P < 0.01$ , NS indicates no statistically significant difference.

and the inhibition of  $I_{GABA}$  was dependent on the GABA concentration. When a higher concentration of GABA ( $100 \mu\text{mol L}^{-1}$ ) was applied, pre-application of  $100 \mu\text{mol L}^{-1}$  Asp did not significantly change the amplitude of  $I_{GABA}$  over various pre-application time intervals (5 s/15 s/25 s) (Figure 3A and B). However, when a lower concentration of GABA ( $3 \mu\text{mol L}^{-1}$ ) was applied, the amplitude of  $I_{GABA}$  was  $73\% \pm 1\%$  of control 5 s after application of  $100 \mu\text{mol L}^{-1}$  Asp (Figure 3C and D). Pre-perfusion of  $100 \mu\text{mol L}^{-1}$  APV for 6 min blocked  $I_{Asp}$  and the effect of Asp inhibition on  $I_{GABA}$  (Figure 3D). When we increased the time interval between the sequential application of Asp and GABA, the inhibition of  $I_{GABA}$  was no longer detectable when the interval reached 15 s (Figure 3D). Given that the recovery of  $I_{GABA}$  is rapid, we choose a time interval between the application of Asp and GABA of 5 s and a concentration of GABA of  $3 \mu\text{mol L}^{-1}$  for all subsequent experiments.

## 2.3 Intracellular $\text{Ca}^{2+}$ is involved in the suppression of the activation of NMDA receptors by $I_{GABA}$

Recent studies have shown that changes in the intracellular  $\text{Ca}^{2+}$  concentration influence the function of GABA receptors [20,21]. To investigate whether the Asp-induced suppression of the GABA response is  $\text{Ca}^{2+}$  dependent, we substituted the  $\text{Ba}^{2+}$  for  $\text{Ca}^{2+}$  in the external solution. In this  $\text{Ca}^{2+}$ -free extracellular solution, the Asp-induced suppression

of the GABA response was robustly reduced (Figure 4). Furthermore, the inhibitory effect of Asp on  $I_{\text{GABA}}$  disappeared when  $10 \text{ mmol L}^{-1}$  BAPTA was added to the pipette solution. These results indicate that an increase in the intracellular  $\text{Ca}^{2+}$  concentration contributes to the inhibition of  $I_{\text{GABA}}$ . To investigate whether  $\text{Ca}^{2+}$  influx through voltage-dependent  $\text{Ca}^{2+}$  channels (VDCCs) also induces an inhibition of  $I_{\text{GABA}}$ , we added  $10 \mu\text{mol L}^{-1}$   $\text{CdCl}_2$ , a VDCCs blocker, to the extracellular solution. Following this manipulation, the amplitude of  $I_{\text{GABA}}$  was inhibited by the Asp pre-application to the same extent ( $72\% \pm 3\%$ ) as that of control ( $73\% \pm 1\%$ ) (Figure 4). These results suggest that the  $\text{Ca}^{2+}$  mediated inhibition is specific for  $\text{Ca}^{2+}$  influx through NMDA receptor channels, but not VDCCs.

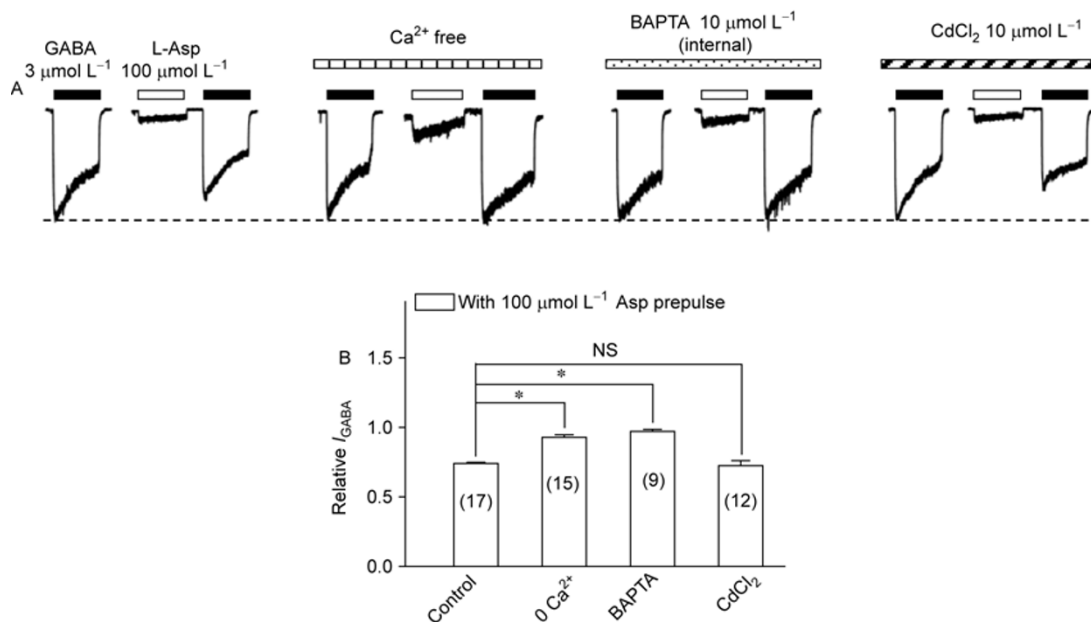
#### 2.4 CaMKII may mediate the suppression of $I_{\text{GABA}}$

Since previous studies have shown that CaMKII is involved

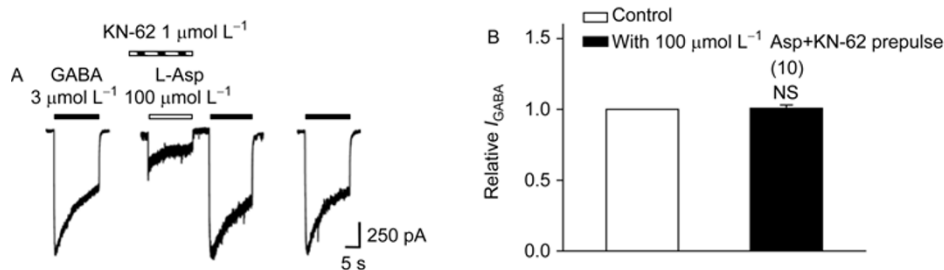
in the suppression of  $I_{\text{GABA}}$  by activation of NMDA receptors in SDCN neurons [17], we examined the possible role CaMKII plays in the inhibition of  $I_{\text{GABA}}$  by activation of NMDA receptors in IC neurons. When stable GABA responses were obtained, and  $1 \mu\text{mol L}^{-1}$  potent inhibitor of CaMKII KN-62 was perfused for 8 min, we did not observe any inhibition on  $I_{\text{GABA}}$  by application of Asp (Figure 5), indicating that CaMKII contributes to Asp-induced suppression of  $I_{\text{GABA}}$  in IC neurons.

### 3 Discussion

In the present study, we demonstrated that activation of the NMDA receptor and the  $\text{GABA}_A$  receptor can result in a mutual inhibition between the two receptors in cultured IC neurons. These results indicate the presence of cross-talk between the NMDA and the  $\text{GABA}_A$  receptors in this central



**Figure 4** Involvement of intracellular  $\text{Ca}^{2+}$  in the Asp-induced depression of  $I_{\text{GABA}}$ . A, raw current traces showing a reduction in the effects of  $100 \mu\text{mol L}^{-1}$  Asp on  $3 \mu\text{mol L}^{-1}$  GABA in a  $\text{Ca}^{2+}$ -free bath or following addition of  $10 \text{ mmol L}^{-1}$  BAPTA. The effects of Asp were absent when  $10 \mu\text{mol L}^{-1}$   $\text{CdCl}_2$  was added to the extracellular solution. The amplitudes of  $I_{\text{GABA}}$  were normalized to the amplitudes before Asp application. B, pooled percent suppression of  $3 \mu\text{mol L}^{-1}$  GABA-evoked currents by  $100 \mu\text{mol L}^{-1}$  Asp in the conditions indicated under the corresponding columns. Vertical bars indicate the mean  $\pm$  SEM. \*,  $P < 0.01$ , NS indicates no statistically significant difference.



**Figure 5** Involvement of CaMKII in Asp suppression of  $I_{\text{GABA}}$ . A, KN-62 ( $1 \mu\text{mol L}^{-1}$ ), a potent inhibitor of CaMKII, markedly decreased the inhibitory effects of  $100 \mu\text{mol L}^{-1}$  Asp on  $I_{\text{GABA}}$ . B, Summary of the normalized  $I_{\text{GABA}}$  in the presence of  $1 \mu\text{mol L}^{-1}$  KN-62. Vertical bars represent the mean  $\pm$  SEM. NS indicates no statistically significant difference.

auditory region. Our findings are consistent with those reported by previous workers in other areas of the central nervous system [15,16,22]. For example, prior activation of GABA<sub>A</sub> receptors inhibits NMDA receptor-mediated whole-cell currents in cultured rat hippocampal neurons [23]. However, our study provides more detailed information regarding how the two receptors interact with each other. In this regard, our findings detail the role of VDCCs and Ca<sup>2+</sup> ions in this cross-talk. Specifically, here we show that the inhibition of  $I_{GABA}$  by aspartate is not affected by blockade of VDCCs, but the inhibition is blocked when the Ba<sup>2+</sup> for Ca<sup>2+</sup> in the external solution or when 10 mmol L<sup>-1</sup> membrane-permeable Ca<sup>2+</sup> chelator BAPTA is added to the pipette solution (Figure 4), suggesting that this inhibition is mediated by Ca<sup>2+</sup> influx through NMDA receptor channels rather than VDCCs. In addition, the potent inhibitor of CaMKII KN-62 reduced the inhibition of  $I_{GABA}$  by aspartate, indicating the involvement of CaMKII in the cross-inhibition. Finally, our findings suggest that the typical functions of the NMDA and GABA receptors are required for cross-talk between them because the GABA-induced suppression of  $I_{Asp}$  was attenuated by bicuculline, a specific antagonist of the GABA<sub>A</sub> receptor, and the Asp-induced suppression of  $I_{GABA}$  was attenuated by APV, a specific antagonist of the NMDA receptor.

Our results confirm the important role of Ca<sup>2+</sup> ions in the modulation of NMDA receptor activity and interactions between NMDA and GABA<sub>A</sub> receptors [24,25]. It has been suggested that elevations of intracellular Ca<sup>2+</sup> contribute to the inhibitory effects of NMDA receptor activation on  $I_{GABA}$  [16,17]. In neurons, the [Ca<sup>2+</sup>]<sub>i</sub> is elevated by release of Ca<sup>2+</sup> from intracellular stores and/or influx of extracellular Ca<sup>2+</sup> through membrane ion channels. In our study, there are three observations that indicate the involvement of an influx of Ca<sup>2+</sup> through NMDA receptors in the inhibition of  $I_{GABA}$ . First, in the absence of extracellular Ca<sup>2+</sup>, the Asp-mediated inhibition of  $I_{GABA}$  diminished. Second, strong buffering of [Ca<sup>2+</sup>]<sub>i</sub> through addition of BAPTA prevented Asp-induced suppression of  $I_{GABA}$ . Third, consistent with previous findings, Asp-induced suppression of  $I_{GABA}$  did not require the activation of VDCCs because Asp still inhibited  $I_{GABA}$  even after Cd<sup>2+</sup> blocked the VDCCs [20,24,26]. It is probable that [Ca<sup>2+</sup>]<sub>i</sub> entering through VDCCs cannot rise to a sufficient level to affect  $I_{GABA}$ .

What are the downstream signaling events involved in the cross-inhibition between GABA<sub>A</sub> and NMDA receptors? In hippocampal cells, a study has shown that intracellular Ca<sup>2+</sup> suppresses the GABA-evoked response by activating a phosphatase [27]. However, other studies have shown that dephosphorylation is involved in NMDA-induced suppression of the GABA-evoked response [15,16]. In our studies, pretreatment with KN-62, a potent inhibitor of CaMKII, completely abolished the inhibition of Asp on  $I_{GABA}$  (Figure 5), indicating that the elevation of [Ca<sup>2+</sup>]<sub>i</sub> induced by NMDA receptor activation could suppress the GABA re-

sponse by activation of CaMKII, which is a multifunctional enzyme which could catalyze the phosphorylation of various proteins, such as nitric oxide synthase, GABA-modulin, GABA<sub>A</sub> receptors, phospholipase A2, acetyl-CoA carboxylase and calcineurin [28]. Based on our findings, we believe that the activation of CaMKII by the elevation of [Ca<sup>2+</sup>]<sub>i</sub> may suppress the GABA response through phosphorylation of sites on GABA<sub>A</sub> receptors, such as on the beta 1 subunit or the gamma 2L/2S subunits [29]. In our experiments, the suppression of  $I_{GABA}$  by Asp gradually diminished 15 s after the removal of Asp. This transient effect of Asp inhibition on  $I_{GABA}$  may be because of transient phosphorylation by activated CaMKII. Meanwhile, Ca<sup>2+</sup> and calmodulin may activate intracellular Ca<sup>2+</sup>-dependent protein phosphatases and allow for a rapid recovery of  $I_{GABA}$  through dephosphorylation [30].

The mechanism underlying the GABA-induced suppression of  $I_{Asp}$  has not been fully established. It has been shown the direct protein-protein coupling enables functional cross-talk between G protein-coupled dopamine D5 receptors and ligand-gated GABA<sub>A</sub> receptors [31]. It has also been shown that NMDA receptor-mediated functions are modulated by dopamine D1 receptors through direct protein-protein interactions [3]. These studies suggest the possibility that other receptors, including NMDA receptors and GABA<sub>A</sub> receptors, may engage in functional cross-talk through a similar mechanism. We suggest that the GABA<sub>A</sub> receptor may form a complex with the NMDA receptor that affects NMDA receptor-mediated functions through direct protein-protein interactions. However, fully elucidating the mechanisms underlying the GABA-induced suppression of  $I_{Asp}$  would require further study.

The interaction between NMDA and GABA<sub>A</sub> receptors may have important implications for central auditory functions. First, this cross-inhibition may exert a neuroprotective effect. When the central auditory system is hyperactive, as is the case during overexposure to sound, GABAergic interneurons may be activated and a large amount of GABA may be released from presynaptic terminals. Increased amount of GABA may then suppress NMDA receptor responses through cross-inhibition, which may prevent the hyperexcitability of postsynaptic neurons. Second, the existence of cross-inhibition between the GABA<sub>A</sub> receptor and the NMDA receptor suggests a GABAergic autoregulation mechanism may exist in the central auditory system because studies have shown that activation of GABA<sub>A</sub> receptors increases intracellular Ca<sup>2+</sup> concentrations in some brain regions [32,33]. Third, the inhibition of NMDA receptor-mediated responses by the activation of GABA<sub>A</sub> receptors may regulate the temporal properties of excitatory postsynaptic potentials in the IC. Similarly, the inhibition of GABA<sub>A</sub> receptor-mediated responses by the activation of NMDA receptors may regulate the temporal properties of inhibitory postsynaptic potentials in the IC. Thus, the interaction between these two types of receptors may play a role

in maintaining the balance between inhibition and excitation in this system. Indeed, the inhibition of synaptically activated NMDA receptors by the activation of GABA<sub>A</sub> receptors has been reported to be important for maintaining the temporal precision of responses in the central auditory system [34]. Taken together, the presence of cross-talk between these two types of channels suggests that the mechanisms mediating information processing in the central auditory system may be more complex than previously believed.

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- 1 Nakazawa K, Inoue K, Koizumi S. Facilitation by 5-hydroxytryptamine of ATP-activated current in rat pheochromocytoma cells. *Pflugers Arch*, 1994, 427: 492–499
- 2 Xu T L, Li J S, Jin Y H, et al. Modulation of the glycine response by Ca<sup>2+</sup>-permeable AMPA receptors in rat spinal neurones. *J Physiol*, 1999, 514 (Pt 3): 701–711
- 3 Lee F J, Xue S, Pei L, et al. Dual regulation of NMDA receptor functions by direct protein-protein interactions with the dopamine D1 receptor. *Cell*, 2002, 111: 219–230
- 4 Li Y, Wu L J, Legendre P, et al. Asymmetric cross-inhibition between GABA<sub>A</sub> and glycine receptors in rat spinal dorsal horn neurones. *J Biol Chem*, 2003, 278: 38637–38645
- 5 Hollmann M, Heinemann S. Cloned glutamate receptors. *Annu Rev Neurosci*, 1994, 17: 31–108
- 6 Seeburg P H. The TINS/TiPS Lecture. The molecular biology of mammalian glutamate receptor channels. *Trends Neurosci*, 1993, 16: 359–365
- 7 Harvey R J, Depner U B, Wassle H, et al. GlyR alpha3: an essential target for spinal PGE<sub>2</sub>-mediated inflammatory pain sensitization. *Science*, 2004, 304: 884–887
- 8 Ahmadi S, Lippross S, Neuhuber W L, et al. PGE(2) selectively blocks inhibitory glycinergic neurotransmission onto rat superficial dorsal horn neurones. *Nat Neurosci*, 2002, 5: 34–40
- 9 Casseday J H, Ehrlich D, Covey E. Neural tuning for sound duration: role of inhibitory mechanisms in the inferior colliculus. *Science*, 1994, 264: 847–850
- 10 Brand A, Behrend O, Marquardt T, et al. Precise inhibition is essential for microsecond interaural time difference coding. *Nature*, 2002, 417: 543–547
- 11 Stephenson F A. The GABA<sub>A</sub> receptors. *Biochem J*, 1995, 310 (Pt 1): 1–9
- 12 Betz H. Glycine receptors: heterogeneous and widespread in the mammalian brain. *Trends Neurosci*, 1991, 14: 458–461
- 13 Adams J C, Wenthold R J. Distribution of putative amino acid transmitters, choline acetyltransferase and glutamate decarboxylase in the inferior colliculus. *Neuroscience*, 1979, 4: 1947–1951
- 14 Ma C L, Kelly J B, Wu S H. AMPA and NMDA receptors mediate synaptic excitation in the rat's inferior colliculus. *Hear Res*, 2002, 168: 25–34
- 15 Chen Q X, Wong R K. Suppression of GABA<sub>A</sub> receptor responses by NMDA application in hippocampal neurones acutely isolated from the adult guinea-pig. *J Physiol*, 1995, 482(Pt 2): 353–362
- 16 Robello M, Amico C, Cupello A. A dual mechanism for impairment of GABA<sub>A</sub> receptor activity by NMDA receptor activation in rat cerebellum granule cells. *Eur Biophys J*, 1997, 25: 181–187
- 17 Wang D, Lu H, Xu T. Mediation by calcium/calmodulin-dependent protein kinase II of suppression of GABA(A) receptors by NMDA. *Sci China C-Life Sci*, 2000, 43: 655–662
- 18 Tang Z Q, Lu Y G, Zhou K Q, et al. Amiloride attenuates glycine-induced currents in cultured neurones of rat inferior colliculus. *Biochem Biophys Res Commun*, 2006, 350: 900–904
- 19 Murase K, Ryu P D, Randic M. Excitatory and inhibitory amino acids and peptide-induced responses in acutely isolated rat spinal dorsal horn neurones. *Neurosci Lett*, 1989, 103: 56–63
- 20 Mougnot D, Feltz P, Schlichter R. Modulation of GABA-gated chloride currents by intracellular Ca<sup>2+</sup> in cultured porcine melanotrophs. *J Physiol*, 1991, 437: 109–132
- 21 Martina M, Kilic G, Cherubini E. The effect of intracellular Ca<sup>2+</sup> on GABA-activated currents in cerebellar granule cells in culture. *J Membr Biol*, 1994, 142: 209–216
- 22 Stelzer A, Shi H. Impairment of GABA<sub>A</sub> receptor function by *N*-methyl-*D*-aspartate-mediated calcium influx in isolated CA1 pyramidal cells. *Neuroscience*, 1994, 62: 813–828
- 23 Xu J, Liu Y, Zhang G Y. Neuroprotection of GluR5-containing kainate receptor activation against ischemic brain injury through decreasing tyrosine phosphorylation of *N*-methyl-*D*-aspartate receptors mediated by Src kinase. *J Biol Chem*, 2008, 283: 29355–29366
- 24 Mulle C, Choquet D, Korn H, et al. Calcium influx through nicotinic receptor in rat central neurones: its relevance to cellular regulation. *Neuron*, 1992, 8: 135–143
- 25 Ghosh A, Greenberg M E. Calcium signaling in neurones: molecular mechanisms and cellular consequences. *Science*, 1995, 268: 239–247
- 26 Pitler T A, Alger B E. Postsynaptic spike firing reduces synaptic GABA<sub>A</sub> responses in hippocampal pyramidal cells. *J Neurosci*, 1992, 12: 4122–4132
- 27 Chen Q X, Stelzer A, Kay A R, et al. GABA<sub>A</sub> receptor function is regulated by phosphorylation in acutely dissociated guinea-pig hippocampal neurones. *J Physiol*, 1990, 420: 207221
- 28 Braun A P, Schulman H. The multifunctional calcium/calmodulin-dependent protein kinase: from form to function. *Annu Rev Physiol*, 1995, 57: 417–445
- 29 McDonald B J, Moss S J. Differential phosphorylation of intracellular domains of gamma-aminobutyric acid type A receptor subunits by calcium/calmodulin type 2-dependent protein kinase and cGMP-dependent protein kinase. *J Biol Chem*, 1994, 269: 18111–18117
- 30 Xu T L, Dong X P, Wang D S. *N*-methyl-*D*-aspartate enhancement of the glycine response in the rat sacral dorsal commissural neurones. *Eur J Neurosci*, 2000, 12: 1647–1653
- 31 Liu F, Wan Q, Pristupa Z B, et al. Direct protein-protein coupling enables cross-talk between dopamine D5 and gamma-aminobutyric acid A receptors. *Nature*, 2000, 403: 274–280
- 32 Obrietan K, van den Pol A N. GABA neurotransmission in the hypothalamus: developmental reversal from Ca<sup>2+</sup> elevating to depressing. *J Neurosci*, 1995, 15: 5065–5077
- 33 Takebayashi M, Kagaya A, Hayashi T, et al. gamma-Aminobutyric acid increases intracellular Ca<sup>2+</sup> concentration in cultured cortical neurones: role of Cl<sup>-</sup> transport. *Eur J Pharmacol*, 1996, 297: 137–143
- 34 Wu S H, Ma C L, Kelly J B. Contribution of AMPA, NMDA, and GABA(A) receptors to temporal pattern of postsynaptic responses in the inferior colliculus of the rat. *J Neurosci*, 2004, 24: 4625–4634

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