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Nitric Oxide Modulation of GABAergic Synaptic Transmission in Mechanically Isolated Rat Auditory Cortical Neurons

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The auditory cortex (A1) encodes the acquired significance of sound for the perception and interpretation of sound. Nitric oxide (NO) is a gas molecule with free radical properties that functions as a transmitter molecule and can alter neural activity without direct synaptic connections. We used whole-cell recordings under voltage clamp to investigate the effect of NO on spontaneous GABAergic synaptic transmission in mechanically isolated rat auditory cortical neurons preserving functional presynaptic nerve terminals. GABAergic spontaneous inhibitory postsynaptic currents (sIPSCs) in the A1 were completely blocked by bicuculline. The NO donor, S-nitroso-*N*-acetylpenicillamine (SNAP), reduced the GABAergic sIPSC frequency without affecting the mean current amplitude. The SNAP-induced inhibition of sIPSC frequency was mimicked by 8-bromoguanosine cyclic 3',5'-monophosphate, a membrane permeable cyclic-GMP analogue, and blocked by 2-(4-carboxyphenyl)-4,4,5,5-tetramethylimidazoline-1-oxyl-3-oxide, a specific NO scavenger. Blockade of presynaptic K^+ channels by 4-aminopyridine, a K^+ channel blocker, increased the frequencies of GABAergic sIPSCs, but did not affect the inhibitory effects of SNAP. However, blocking of presynaptic Ca^{2+} channels by Cd^{2+}, a general voltage-dependent Ca^{2+} channel blocker, decreased the frequencies of GABAergic sIPSCs, and blocked SNAP-induced reduction of sIPSC frequency. These findings suggest that NO inhibits spontaneous GABA release by activation of cGMP-dependent signaling and inhibition of presynaptic Ca^{2+} channels in the presynaptic nerve terminals of A1 neurons.

Key Words: Auditory cortex, Nitric oxide, Synaptic transmission, GABA

INTRODUCTION

The central auditory nervous system delivers acoustic information from the cochlear nucleus to the superior olivary complex, lateral lemniscus, inferior colliculus, medial geniculate body, and auditory cortex. The primary auditory cortex (A1) processes acoustic information, such as the selective coding of frequency and amplitude modulation (Schreiner et al., 2000), as the ability to process the temporal sequences of sound, similar to those found in communication signals, disappears following the ablation of auditory cortex in cats and primates (Hupfer et al., 1977; Neff, 1977). In general, while glutamatergic excitatory synaptic transmission mediates acoustic information from the cochlear nucleus to the auditory cortex, GABAergic inhibitory synaptic transmission finely tunes the neuronal excitability within the auditory cortex, as approximately 25% of auditory cortical neurons is thought to be GABAergic ones (Hendry et al., 1987; Prieto et al., 1994). Similarly, increased neural noise in the aged auditory cortex due to loss of GABAergic inhibition is likely to impair normal acoustic coding functions (Ling et al., 2005), suggesting that the GABAergic inhibitory system plays an important role in the processing of acoustic information within the auditory cortex.

Nitric oxide (NO) is a gas molecule with free radical properties that functions as a transmitter molecule in both the peripheral and central nervous systems. In the central nervous system, NO is associated with a variety of different behavioral functions, including learning and memory formation, feeding, sleep, reproduction, as well as in sensory and motor function. NO might affect these behavioral functions by modulating neuronal electrical activities, such as neurotransmitter release, synaptic plasticity, and receptor functions (Bredt and Snyder, 1989; Stamler et al., 1997; Ko and Kelly, 1999). For example, NO hinders the activity of NMDA receptors, thus altering neurotransmission by glutamate (Manzoni et al., 1992). More importantly, NO plays important roles in synaptic plasticity as a retrograde messenger at central synapses, and can modulate neurotransmitter release from presynaptic terminals in the brain (Boulton et al., 1994; Ozaki et al., 2000; Klyachko et al., 2001; Kraus and Prast, 2002). However, it is still unknown whether NO can regulate synaptic transmission onto audi-

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ABBREVIATIONS: A1, primary auditory cortex; SNAP, S-nitroso-*N*-acetylpenicillamine; 8-Br-cGMP, 8-bromoguanosine cyclic 3',5'-monophosphate; Carboxy-PTIO, 2-(4-carboxyphenyl)-4,4,5,5-tetramethylimidazoline-1-oxyl-3-oxide; 4-AP, 4-aminopyridine.

tory cortical neurons. In the present study, therefore, we have investigated the effect of NO on spontaneous GABAergic transmission in mechanically isolated rat auditory cortical neurons with preserved functional presynaptic nerve terminals.

METHODS

Preparation

All experimental procedures using animals were approved by the Institutional Care and Use Committee of School of Dentistry, Kyungpook National University, and were performed in accordance with the guiding principles for the care and use of animals approved by the Council of the National Institutes of Health Guide for the Care and Use of Laboratory Animals. All efforts were made to minimize the number of animals as well as suffering. Sprague-Dawley rats (12- to 16-day-old) of both sexes were decapitated under ketamine anesthesia (100 mg kg^{-1}, i.p.). Brains were quickly removed and transversely sliced at a thickness of 400 μm using a microslicer (Vibratome® 1000, Warner Instruments, Hamden, CT, USA). Before mechanical dissection, slices were preincubated for at least 1 h at room temperature (22~25°C) in a solution saturated with 95% O_2 and 5% CO_2. For dissociation, slices were transferred to a 35-mm plastic culture dish (Primaria 3801, Becton Dickinson, Rutherford, NJ, USA), and the A1 was identified under a binocular microscope (SMZ-1, Nikon, Tokyo, Japan). Mechanical dissociation was accomplished using a custom-built vibration device and a fire-polished glass pipette oscillating at approximately 50~60 Hz. The tip of the pipette was lightly placed on the surface of the A1 and vibrated horizontally (0.3~0.5 mm displacement) for approximately 2 minutes. Slices were removed, and the mechanically dissociated neurons were allowed to settle and adhere to the bottom of the dish for at least 15 minutes before commencement of recording.

Electrical measurements

All electrical measurements were performed using conventional whole-cell patch recordings and a patch-clamp amplifier (Axopatch 200B, Molecular Devices, Union City, CA, USA). Neurons were voltage clamped at a holding potential (V_H) of −60 mV. Patch pipettes were made from borosilicate capillary glass (1.5 mm outer diameter, 0.9 mm inner diameter, G-1.5, Narishige, Tokyo, Japan) by use of a pipette puller (P-97, Shutter Instrument Co., Novato, CA, USA). The patch pipette was positioned on the neuron using a water-driven micromanipulator (WR-60, Narishige, Tokyo, Japan). The resistance between the recording electrode filled with the internal pipette solution and the reference electrode was 4~6 MΩ. The neurons were visualized with phase-contrast equipment on an inverted microscope (Ti-S, Nikon, Tokyo, Japan). Membrane currents were filtered at 1 kHz, digitized at 4 kHz, and stored on a computer equipped with pCLAMP 10 (Molecular Devices). All experiments were performed at room temperature (22~25°C).

Data analysis

Spontaneous inhibitory postsynaptic currents (sIPSCs) were counted and analyzed using the MiniAnalysis program (Synaptosoft Inc., Decatur, GA, USA), as described previously (Jang et al., 2001). Briefly, sIPSCs were screened automatically using an amplitude threshold of 10 pA, and then visually accepted or rejected based upon their rise and decay times. Basal noise levels during voltage-clamp recordings were typically <8 pA. The amplitudes and interevent intervals of large numbers of sIPSCs obtained from a single neuron were examined by constructing all-point cumulative probability distributions and compared using the Kolmogorov-Smirnov (K-S) test with Stat View software (SAS Institute Inc., Cary, NC, USA). Mean amplitudes and frequencies of sIPSCs were normalized to the control conditions and are reported as means±standard error of the mean (SEM). Significant differences in the mean frequency and amplitude were tested using Student's two-tailed paired *t*-test, using absolute values rather than normalized ones. Values of $p<0.05$ were considered significant.

Solutions

The ionic composition of the incubation solution was (in mM): NaCl 124, KCl 2, KH_2PO_4 1, $MgCl_2$ 1, $CaCl_2$ 2, glucose 10, and $NaHCO_3$ 26. The pH was adjusted to 7.4 by continuous bubbling with 95% O_2 and 5% CO_2. The standard external solution was (in mM): NaCl 150, KCl 3, $MgCl_2$ 1, $CaCl_2$ 2, glucose 10, and *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulphonic acid (HEPES) 10. The pH was adjusted to 7.4 with tris-hydroxymethyl aminomethane (Tris-base). The composition of the internal pipette solution for sIPSC recording was (in mM): CsCl 110, TEA-Cl 30, EGTA 5, adenosine 5'-triphosphate magnesium salt (Mg-ATP) 5, guanosine 5'-triphosphate trisodium salt (Na3-GTP) 0.4, and HEPES 10. The pH was adjusted to 7.2 with Tris base. For sIPSC recording, external solutions contained 300 nM TTX, 3 μM 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX), and 10 μM DL-2-amino-5-phosphonovaleric acid (AP5) to block voltage-dependent Na^+ channels and glutamatergic excitatory synaptic currents.

Drugs and chemicals

The drugs used in the present study were AP5, Mg-ATP, Na3-GTP, HEPES, Tris-base, dimethyl sulfoxide, ethylene glycol-bis (β-aminoethyl ether)-*N,N,N'N'*-tetraacetic acid (EGTA), $CdCl_2$ (from Sigma, St. Louis, MO, USA), and (S)-Nitroso-*N*-acetylpenicillamine (SNAP), 8-bromoguanosine cyclic 3',5'-monophosphate sodium salt (8-bromo-cGMP), 2-(4-carboxyphenyl)-4,4,5,5-tetramethylimidazoline-1-oxyl-3-oxide potassium salt (carboxy-PTIO), 4-aminopyridine (4-AP), tetrodotoxin (TTX), bicuculline, CNQX (from Tocris, Bristol, UK). Drugs were added to external solutions at the final concentrations shown in the text and the vehicle concentrations never exceeded 0.01%. Drugs were applied using a rapid application system termed the "Y-tube method," described elsewhere (Akaike and Harata, 1994).

RESULTS

GABAergic spontaneous inhibitory postsynaptic currents in isolated A1 neurons

Mechanically isolated A1 neurons were bipolar or pyramidal in shape and retained short portions of their proximal dendrites. When the neurons were voltage-clamped at V_H

−60 mV in the presence of 300 nM TTX, 3 μM CNQX, and 10 μM AP5, prominent sIPSCs were observed in dissociated A1 neurons. These sIPSCs were completely abolished by bath application of 50 μM bicuculline, an antagonist of $GABA_A$ receptors (Fig. 1A). Fig. 1B shows typical GABAergic sIPSCs at various V_H values. The reversal potential (−5 mV) of these sIPSCs, estimated from the current-voltage (I-V) relationship, was very similar to the theoretical Cl^- Nernst equilibrium potential (E_{Cl}) of −4 mV calculated using the extracellular and intracellular Cl^- concentrations of 161 mM and 140 mM, respectively. Thus the spontaneous events were identified as GABAergic sIPSCs mediated by $GABA_A$ receptors.

NO-induced inhibition of GABAergic sIPSCs is mediated by a cGMP-dependent mechanism

To test the effect of NO on synaptic GABA release onto A1 neurons, an NO donor, S-nitroso-*N*-acetylpenicillamine (SNAP), was used. Superfusion of SNAP (100 μM) decreased the frequency of GABAergic synaptic events in most A1 neurons. However, SNAP did not change the rate of rise and decay time constant (Fig. 2A). Fig. 2B shows cumulative probability plots for inter-event intervals and current amplitudes of sIPSCs. SNAP shifted the distribution curve of sIPSC frequency to the right, indicating a reduction of sIPSC frequency. SNAP decreased the mean sIPSC frequency to 80.3±7.1% of the mean control frequency ($p < 0.05$, n=20; Control, 0.36±0.11 Hz; SNAP, 0.33±0.12 Hz), but the mean amplitude was not affected (93.3±3.8% of the control amplitude, p=0.091, n=20; Control, 47.32±5.53 pA; SNAP, 44.34±5.98 pA; Fig. 2C). These results indicate that SNAP acts presynaptically to inhibit the release probability of GABA at the synapse.

To test whether a general increase in cGMP level mimics the effects of SNAP, we bath-applied the membrane per-

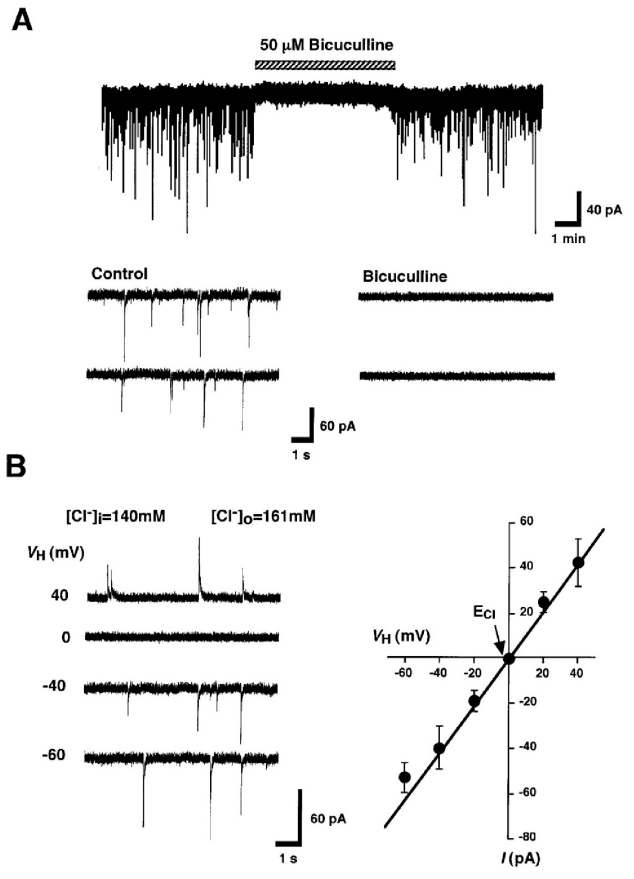

Fig. 1. GABAergic sIPSCs recorded from mechanically dissociated A1 neurons. (A) A representative trace of sIPSCs recorded before, during, and after the application of 50 μM bicuculline at a V_H of −60 mV. The external solution contained 300 nM TTX, 3 μM CNQX and 10 μM AP5. Insets represent typical traces with an expanded time scale. (B) Traces of sIPSCs recorded at V_H of −60, −40, 0, and +40 mV. Intracellular and extracellular Cl^- concentrations were 140 mM and 161 mM, respectively. I-V curve for the mean amplitude of sIPSCs recorded at various V_Hs. Each point is the mean of four neurons.

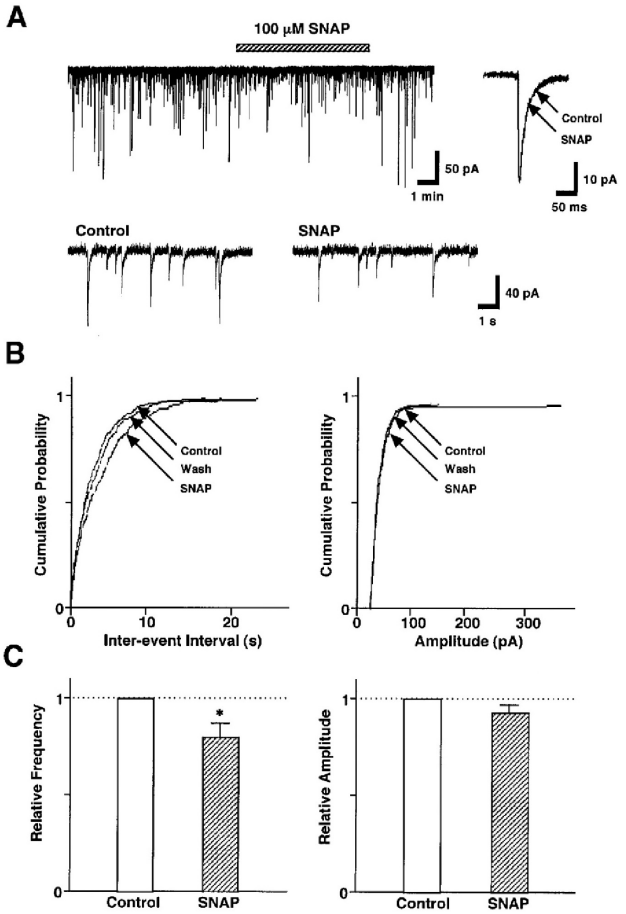

Fig. 2. SNAP decreases GABAergic sIPSCs by presynaptic mechanisms. (A) A representative trace of sIPSCs recorded before, during, and after the application of 100 μM SNAP. Insets, represent typical traces with an expanded time scale (lower) and SNAP-induced changes in the rate of rise and decay time constant (upper). (B) Cumulative probability distributions for inter-event interval (left) and amplitude (right) of GABAergic sIPSCs recorded from the same neuron. p values indicate the results of K-S tests for frequency and amplitude. (C) Each column is the mean of 20 neurons. All frequencies and amplitudes are normalized to those of control sIPSCs. Asterisks represent a statistically significant difference (*$p < 0.05$, paired two-tailed *t*-test).

meable cyclic-GMP analogue, 8-bromoguanosine cyclic 3',5'-monophosphate (8-Br-cGMP). 8-Br-cGMP (100 μM) decreased the sIPSC frequency in a manner similar to SNAP itself, but did not change the rate of rise and decay time constant (Fig. 3A). In addition, 8-Br-cGMP shifted the distribution curve of sIPSC frequency to the right, indicating a reduction of mIPSC frequency (Fig. 3B). 8-Br-cGMP reduced the mean sIPSC frequency to 80.8±6.1% of the control frequency ($p<0.05$, n=6; control, 0.79±0.26 Hz; 8-Br-cGMP, 0.65±0.21 Hz) without affecting the mean amplitude (95.3±3.5% of the control amplitude, p=0.239, n=6; Control, 60.66±12.71 pA; 8-Br-cGMP, 59.31±14.30 pA; Fig. 3C).

To determine whether the effect of SNAP on sIPSCs was mediated through NO release, a specific NO scavenger, 2-(4-carboxyphenyl)-4,4,5,5-tetramethylimidazoline-1-oxyl-3-oxide (carboxy-PTIO), was employed. The neurons were superfused with an external solution containing carboxy- PTIO for 5 minutes before simultaneous superfusion of SNAP (Fig. 4A). Carboxy-PTIO (1 μM) blocked the inhibitory effect of SNAP (100 μM) on sIPSC frequency (96.5±12.4% of the carboxy-PTIO condition, p=0.646, n=5; carboxy-PTIO, 0.99±0.39 Hz; carboxy-PTIO+SNAP, 0.95±0.37 Hz) without affecting sIPSC amplitude (97.0±7.9% of the carboxy-PTIO condition, p=0.722, n=5; carboxy-PTIO, 63.33±17.88 pA; carboxy-PTIO+SNAP, 60.14±17.10 pA; Fig. 4C). Carboxy-PTIO itself did not affect sIPSC frequency (108.9±16.0% of the control frequency, p=0.516, n=5; Control, 0.93±0.38 Hz; carboxy-PTIO, 0.99±0.39 Hz) or amplitude (95.5±6.9% of the control amplitude, p=0.554, n=5; Control, 66.66±18.78 pA; carboxy-PTIO, 63.33±17.88 pA; Fig. 4C). These results suggest that SNAP modulation of GABAergic synaptic transmission in the A1 is mediated by a cGMP-dependent mechanism.

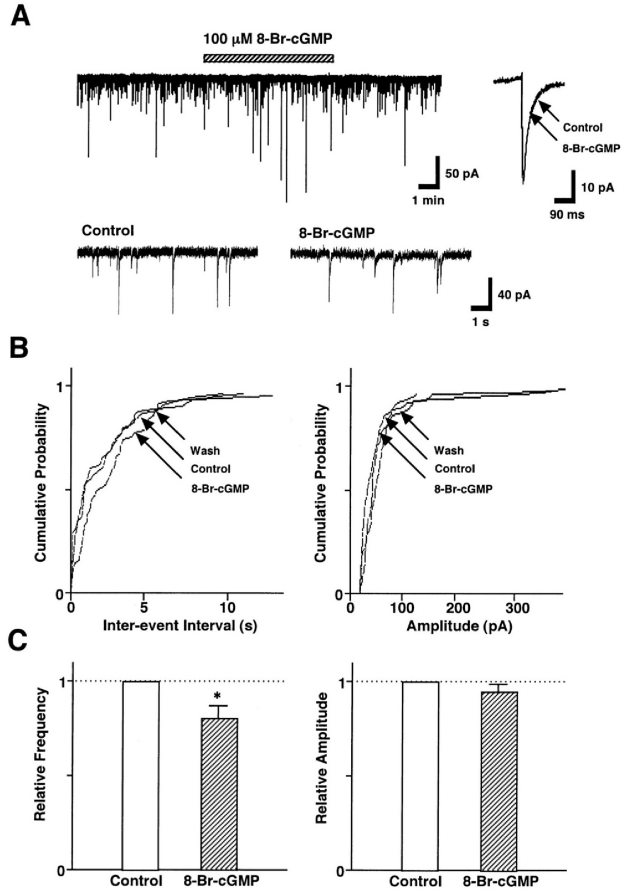

Fig. 3. SNAP-induced presynaptic inhibition of GABAergic sIPSCs is coupled to the cGMP-dependent signal transduction pathway. (A) A representative trace of sIPSCs recorded before, during, and after the application of 100 μM 8-Br-cGMP. Insets, represent typical traces with an expanded time scale (lower) and 8-Br-cGMP-induced changes in the rate of rise and decay time constant (upper). (B) Cumulative probability distributions for inter-event interval (left) and amplitude (right) of GABAergic sIPSCs recorded from the same neuron. p values indicate the results of K-S tests for frequency and amplitude. (C) Each column is the mean of 6 neurons. All frequencies and amplitudes are normalized to those of control sIPSCs. Asterisks represent a statistically significant difference (*$p<0.05$, paired two-tailed t-test).

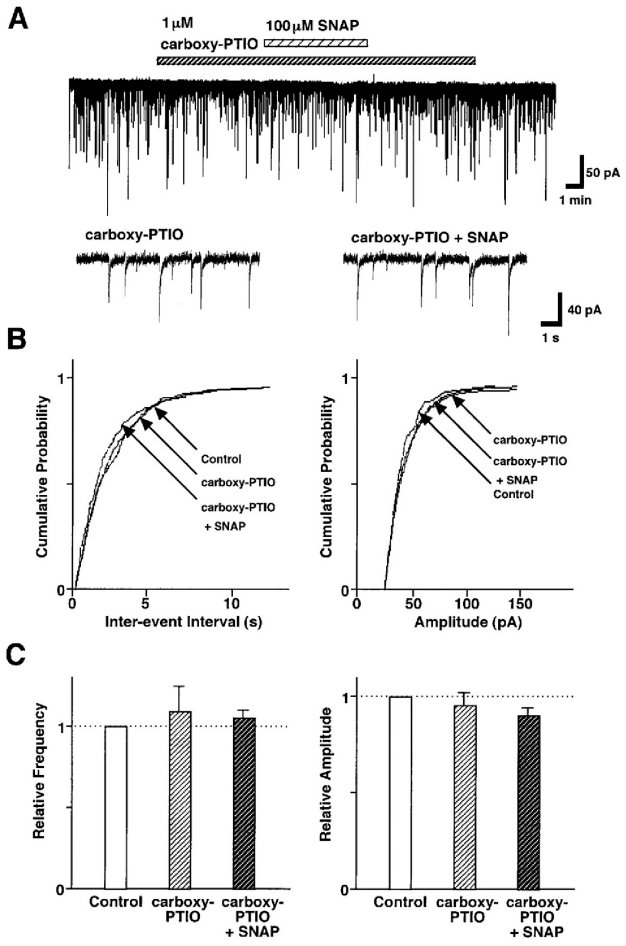

Fig. 4. Effect of carboxy-PTIO on the SNAP-induced inhibition of GABAergic sIPSCs. (A) Representative recording traces of sIPSCs observed before and during the application of 100 μM SNAP in the absence or presence of 1 μM carboxy-PTIO. Insets represent typical traces with an expanded time scale. (B) Cumulative distributions for inter-event interval (left) and amplitude (right) of sIPSCs recorded from the same neuron. p values indicate the results of K-S tests for frequency and amplitude. (C) Each column is the mean of 5 neurons. All frequencies and amplitudes are normalized to those of control sIPSCs.

NO inhibits GABAergic sIPSCs by regulation of presynaptic Ca^{2+} channels

K^+ channels are present at presynaptic terminals in the brain and may be involved in the effect of SNAP on synaptic GABA release. To determine whether presynaptic activation of K^+ channels is responsible for the inhibitory effect of SNAP on GABAergic sIPSC frequency, we tested the effects of 4-AP, which is known to block K^+ channels. 4-AP (100 μM) significantly increased sIPSC frequency to 1,026.7±202.0% of the control frequency ($p<0.05$, n=9; Control, 0.37±0.13 Hz; 4-AP, 2.53±0.50 Hz) without affecting the mean amplitude (121.6±11.3% of the control amplitude, p=0.094, n=9; Control, 59.92±9.93 pA; 4-AP, 67.70±8.58 pA; Fig. 5C). However, in the presence of 4-AP, SNAP still reversibly decreased sIPSC frequency to 66.4±7.7% of the frequency in the 4-AP condition ($p<0.05$, n=9; 4-AP, 2.53±0.50 Hz; 4-AP +SNAP, 1.50±0.28 Hz), but did not affect the mean sIPSC amplitude (91.7±5.7% of the 4-AP condition, p=0.181, n=9; 4-AP, 67.70±8.58 pA; 4-AP+SNAP, 61.83±8.66 pA; Fig. 5C). These results indicate that activation of presynaptic K^+ channels is unlikely to contribute to SNAP-induced inhibition of GABAergic sIPSCs.

Ca^{2+} influx through voltage-dependent Ca^{2+} channels (VDCCs) plays an important role in neurotransmitter release from presynaptic nerve terminals (Wu and Saggau, 1994). To test the involvement of presynaptic VDCCs in the SNAP-induced inhibition of spontaneous GABA release, the effect of Cd^{2+}, a general VDCC blocker, on the SNAP-induced decease in sIPSC frequency was observed. Superfusion with 100 μM Cd^{2+} decreased not only sIPSC frequency (43.7±6.0% of the control frequency, $p<0.05$, n=6; Control, 0.43±0.12 Hz; Cd^{2+}, 0.18±0.06 Hz), but also sIPSC amplitude

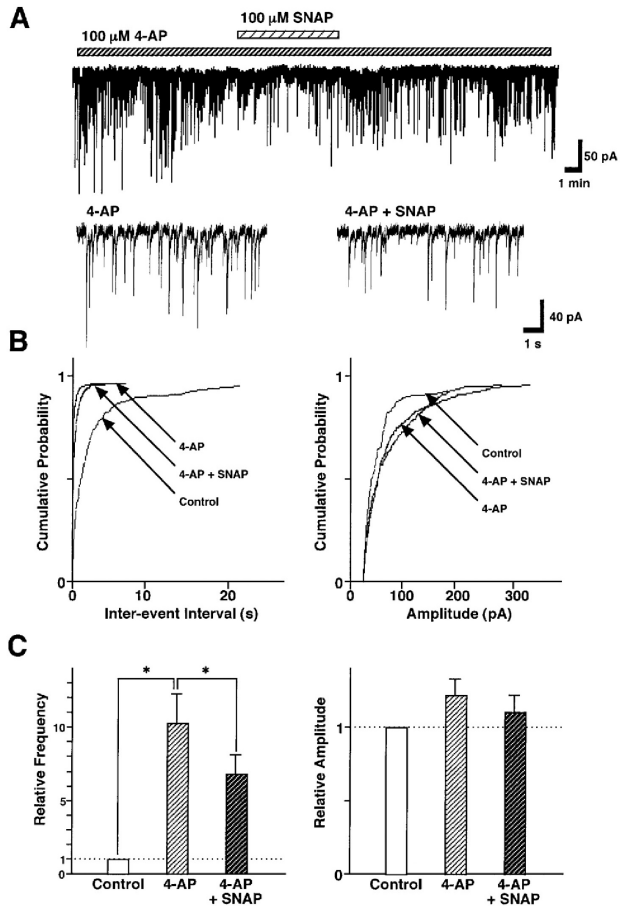

Fig. 5. Effect of the 4-aminopyridine on the SNAP-induced inhibition of GABAergic sIPSCs. (A) Representative recording traces of sIPSCs show the change in the SNAP effect in the presence of 100 μM 4-AP, a K^+ channel blocker. Insets represent typical traces with an expanded time scale. (B) Cumulative distributions for inter-event interval (left) and amplitude (right) of sIPSCs in the same neuron. p values indicate the results of K-S tests for frequency and amplitude. (C) All amplitudes and frequencies are normalized to the control sIPSCs. Each column is the mean of 9 neurons. Asterisks represent a statistically significant difference (*$p<0.05$, paired two-tailed *t*-test).

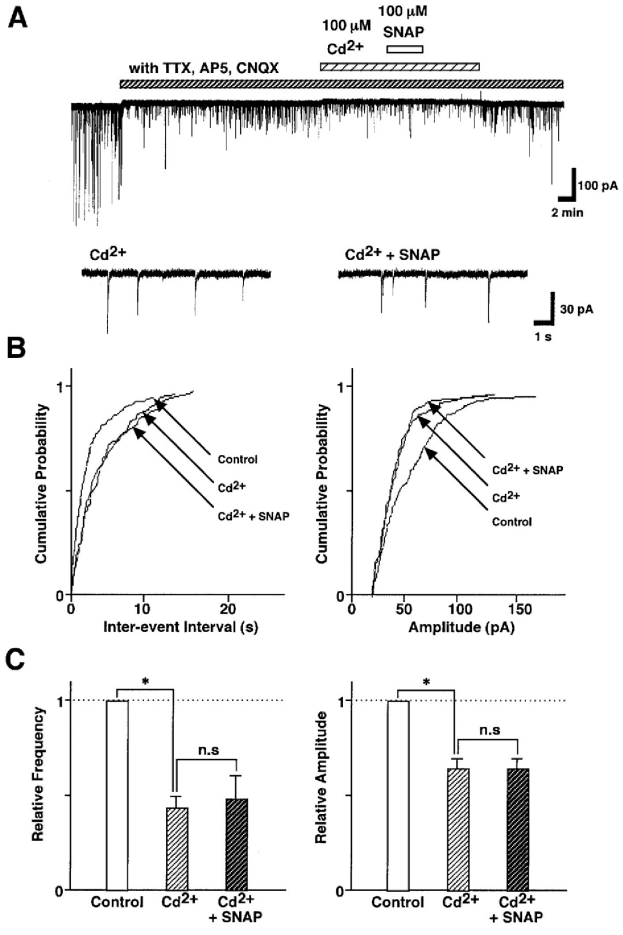

Fig. 6. SNAP-induced inhibition of GABAergic sIPSCs is related to the presynaptic VDCCs. (A) Representative recording traces of sIPSCs shows the change in the SNAP effect in the external solution containing 100 μM Cd^{2+}, a non-selective Ca^{2+} channel blocker. Insets represent typical traces with an expanded time scale. (B) Cumulative distributions for inter-event interval (left) and amplitude (right) of sIPSCs in the same neuron. p values indicate the results of K-S tests for frequency and amplitude. (C) All amplitudes and frequencies are normalized to the control sIPSCs. Each column is the mean of 6 neurons. Asterisks represent a statistically significant difference (*$p<0.05$, paired two-tailed *t*-test). n.s indicates $p>0.05$.

(64.4±5.2% of the control amplitude, $p<0.05$, n=6; Control, 46.03±6.05 pA; Cd^{2+}, 29.96±4.68 pA; Fig. 6C). In addition, Cd^{2+} completely blocked SNAP-induced inhibition of GABAergic sIPSC frequency (110.0±21.4% of the Cd^{2+} condition, p=0.320, n=6; Cd^{2+}, 0.18±0.05 Hz; Cd^{2+}+SNAP, 0.15±0.03 Hz) without altering the current amplitude (101.2±6.9% of the Cd^{2+} condition, p=0.865, n=6; Cd^{2+}, 29.96±4.68 pA; Cd^{2+}+SNAP, 29.03±3.41 pA; Fig. 6C). These results suggest that the SNAP-induced inhibition of spontaneous GABA release is related to the Ca^{2+} influx through presynaptic VDCCs.

DISCUSSION

The present study was designed to investigate the mechanisms underlying the inhibitory effect of NO on presynaptic GABA release in rat auditory cortical neurons. Our principal finding is that NO suppressed inhibitory transmission in auditory cortical neurons. It appears that NO inhibits GABAergic sIPSCs by activation of cGMP-dependent signaling and inhibition of presynaptic Ca^{2+} channels in the presynaptic nerve terminals of auditory cortical neurons.

NO-cGMP mechanisms are involved in the presynaptic modulation in the hippocampus, supraoptic nucleus, and nucleus accumbens (Boulton et al., 1994; Ozaki et al., 2000; Kraus and Prast, 2002), but the role of cGMP in the effect of NO on synaptic neurotransmitter release remains controversial. NO and cGMP inhibit synaptic transmission through actions exerted at the presynaptic level. In this regard, cGMP reduces fast glutamatergic synaptic transmission in the rat hippocampus (Boulton et al., 1994). The cGMP analogue, 8-Br-cGMP, also decreases GABA and glutamate release to magnocellular neurons in the supraoptic nucleus (Ozaki et al., 2000). On the other hand, cGMP can produce an excitatory effect on the neurotransmitters release. For example, NO and cGMP can facilitate glutamate and GABA release in the nucleus accumbens (Kraus and Prast, 2002). The apparent contradiction between these findings may simply be a reflection of the multiple NO-sensitive mechanisms that control Ca^{2+} signals in neurons. In the present study, an NO donor, SNAP, decreased GABAergic sIPSC frequency without affecting the amplitude distribution (Fig. 2), indicating that NO acts presynaptically to inhibit spontaneous GABA release from the presynaptic nerve terminals of A1 neurons. In addition, this effect was completely blocked by carboxy-PTIO, a specific NO scavenger, and mimicked by 8-Br-cGMP, a membrane permeable cyclic-GMP analogue (Fig. 3 and 4). Thus, the effect of NO on GABAergic presynaptic nerve terminals in the A1 seems to be mediated primarily by a cGMP-dependent signaling.

NO produces its biological effect through distinct signal transduction pathways (Stamler et al., 1997; Ahern et al., 2002). NO activates soluble guanylyl cyclase to produce cyclic guanosine monophosphate (Arnold et al., 1977), and three principal targets of cGMP are protein kinase G (Jaffrey and Snyder, 1995), cyclic-nucleotide-gated channels (Zagotta and Siegelbaum, 1996), and cyclic-nucleotide phosphodiesterase (Pineda et al., 1996; Kraus and Prast, 2002). Although several different signaling pathways may be involved in NO-mediated prestsynaptic modulation, the NO/cGMP/PKG pathway and K^+ channels seems critical to NO-induced presynaptic GABA release in the paraventricular nucleus of the hypothalamus (Li et al., 2004; Yang et al., 2007). In addition, postsynaptic voltage-gated K^+ channels of rat auditory cortical neurons were activated by increased levels of NO (Lee et al., 2008). However, the present findings do not suggest a role for K^+ channels in the presynaptic NO modulation of GABAergic sIPSCs (Fig. 5).

Ca^{2+} influx through the voltage-dependent Ca^{2+} channels participates in neurotransmitter release and is activated when Na^+-dependent action potentials invade the axon terminal. However, neurotransmitters can undergo spontaneous vesicle fusion with the presynaptic membrane, independent of conducted electrical signals. In fact, miniature inhibitory postsynaptic currents (mIPSCs) are recorded in the presence of TTX, which blocks Na^+-dependent action potentials. Since the frequency of mIPSCs in the presence of TTX is unaffected by further removal of extracellular Ca^{2+} or by the application of Cd^{2+}, these events do not depend on extracellular Ca^{2+} entry and/or VDCCs. In contrast, in the present study, the application of Cd^{2+} significantly decreased both sIPSC frequency and amplitude (Fig. 6). This result indicates that Ca^{2+} influx passing through presynaptic VDCCs partially contributes to the generation of sIPSCs.

Spontaneous release of GABA from the GABAergic presynaptic terminals is generally mediated by L- and P/Q-type Ca^{2+} channels (Jang et al., 2001). NO interferes with Ca^{2+} channel activity through the activation of a cytosolic guanylyl cyclase and subsequent elevation of cGMP/PKG levels. A PKG-mediated inhibition of L- and P/Q-type Ca^{2+} channel activity occurs in chick cardiac cells (Tohse and Sperelakis, 1991), guinea-pig smooth muscle cells (Tewari and Simard, 1997), rat pinealocytes (Chik et al., 1995), and rat insulinoma RINm5F cells (Grassi et al., 1999). In cultured visual cortex, 8-Br-cGMP and the specific PKG activator, SP-8-Br-PET-cGMPS, cause a decrease in the spontaneous frequency of excitatory postsynaptic currents, which is associated with diminished voltage-dependent Ca^{2+} channel currents (Wei et al., 2002). In the present study, Cd^{2+}, a general VDCC blocker, completely blocked NO-induced inhibition of GABAergic sIPSCs (Fig. 6). These results indicate that NO suppresses GABAergic sIPSCs perhaps, by inhibiting presynaptic Ca^{2+} channels. However, further studies are needed to definitively identify the signal transduction mechanism for NO-induced inhibition of GABAergic transmission.

Age-related changes have been observed throughout the external ear, middle ear, inner ear, and the central auditory nervous system, with changes in the inner ear and auditory nervous system contributing to age-related hearing loss. Accurate temporal processing depends on the ability of inhibitory circuits to sharpen responses to rapid time-varying signals (Liang et al., 2002). Thus, age-related central sensory processing deficits could be attributable, at least in part, to decrements in GABA inhibitory neurotransmission (Caspary et al., 1990; Caspary et al., 2008). Indeed, the auditory midbrain (inferior colliculus) shows significant age-related changes related to GABA neurotransmission in rats (Raza et al., 1994). Age-associated changes arise from the production of free-radicals, including the superoxide, hydroxyl anions, nitric oxide, and peroxynitrite (Knight, 1995; Peinado, 1998). As the amount of NO increases with age, NO can damage the central auditory nervous system, causing age-related hearing loss (Huh et al., 2008). Aging and partial damage to the peripheral sensory systems of mammals appear to result in plastic pre- and postsynaptic changes in the inhibitory neurotransmitter systems of the primary sensory pathways. The exact nature of these

changes is dependent upon the anatomic location and function of the inhibitory circuits within the particular primary sensory system. Previously, we showed that aging increased NO production in rat A1 neurons (Lee et al., 2008). In addition, the present study indicate that NO suppressed GABA neurotransmission in A1 neurons. Taken together, these findings from A1 neurons suggest that age-related disruption in GABA neurotransmission is associated with increased NO production. Therefore, increased NO production may be related to age-related hearing loss in A1 neurons. However, in this study, we have used single auditory cortical neurons isolated from 2-week-old rats, primarily because the mechanical isolation of single neurons from a brain area including auditory cortex is difficult in older rats.

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