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Modulation of major voltage- and ligand-gated ion channels in cultured neurons of the rat inferior colliculus by lidocaine[1]

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Key words

lidocaine; tinnitus; inferior colliculus; sodium channel; potassium channel; glycine receptor; $GABA_A$ receptor; *N*-methyl-*D*-aspartate receptor; whole-cell patch-clamp

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Abstract

Aim: The purpose of the present study was to explore how lidocaine as a therapeutic drug for tinnitus targets voltage- and ligand-gated ion channels and changes the excitability of central auditory neurons. **Methods:** Membrane currents mediated by major voltage- and ligand-gated channels were recorded from primary cultured neurons of the inferior colliculus (IC) in rats with whole-cell patch-clamp techniques in the presence and absence of lidocaine. The effects of lidocaine on the current-evoked firing of action potentials were also examined. **Results:** Lidocaine at 100 μmol/L significantly suppressed voltage-gated sodium currents, transient outward potassium currents, and the glycine-induced chloride currents to 87.66%±2.12%, 96.33%±0.35%, and 91.46%±2.69% of that of the control level, respectively. At 1 mmol/L, lidocaine further suppressed the 3 currents to 70.26%±4.69%, 62.80%±2.61%, and 89.11%±3.17% of that of the control level, respectively. However, lidocaine at concentrations lower than 1 mmol/L did not significantly affect GABA- or aspartate-induced currents. At a higher concentration (3 mmol/L), lidocaine slightly depressed the GABA-induced current to 87.70%±1.87% of that of the control level. Finally, lidocaine at 100 μmol/L was shown to significantly suppress the current-evoked firing of IC neurons to 58.62%±11.22% of that of the control level, indicating that lidocaine decreases neuronal excitability. **Conclusion:** Although the action of lidocaine on the ion channels and receptors is complex and non-specific, it has an overall inhibitory effect on IC neurons at a clinically-relevant concentration, suggesting a central mechanism for lidocaine to suppress tinnitus.

Introduction

Tinnitus is an auditory phantom sensation without external auditory stimulation, and is a prevalent otological problem that affects a large population of the world[1,2]. Currently, there is no effective therapeutic strategy for the treatment of tinnitus, but sometimes local anesthetics are clinically used to relieve its symptoms[3]. Local anesthetics are a broad family of drugs that are composed of 3 components, including a lipophilic aromatic ring, an intermediate ester or amide chain, and terminal amine. They are classified into 2 groups: the amine group and the ester group. Procaine, an ester-type local anesthetic, was the first local anesthetic used for tinnitus-suppressing effects[4]. Lidocaine, an amine-type local anesthetic currently used for anti-arrhythmic therapy, was then introduced into tinnitus treatment and has been reported to ameliorate tinnitus of sufferers when administered intravenously[5,6]. However, the underlying mechanism for the tinnitus-suppressing effects of lidocaine remains obscure.

The understanding of tinnitus generation will help us to understand how lidocaine relieves tinnitus symptoms. Unfortunately, the neural mechanisms of tinnitus are also poorly understood. Several hypotheses have been proposed for explaining the generation of tinnitus. One of these hypotheses claims that the central auditory system is

involved in the perception of tinnitus[7,8]; however, whether the tinnitus generator is located peripherally or centrally is still unknown. More and more researchers tend to believe that, at least in some cases, tinnitus has a central rather than peripheral origin[9–12]. For example, tinnitus still persists in some patients following acoustic removal with sectioning of the auditory nerve[13]. Because the common dose of lidocaine, which can pass the blood–brain barrier immediately after intravenous injection[8,14], is approximately 1.5 mg/kg body weight for tinnitus treatment[3,14-16], a possibility exists that the pharmacological target of lidocaine therapy for tinnitus with a central origin is located in the central auditory system. Some studies suggest direct pharmacological actions of lidocaine on the central auditory system[17]. For example, the V wave of the auditory brainstem response[18], which is associated with the activity of the inferior colliculus (IC), and salicylate-induced discharge of IC neurons[19] can be inhibited by lidocaine. There is also a suppressive effect of lidocaine on tinnitus in patients following the translabyrinthine removal of vestibular schwannoma[3], suggesting a central target of lidocaine. The purpose of the present study was to explore how lidocaine, as a therapeutic drug for tinnitus, targets ion channels and changes the excitability of central auditory neurons. Because evidence from a number of studies suggests that the IC is a potential candidate for the locus of tinnitus generation[19–21], we performed experiments in primary cultured neurons of rat IC with whole-cell patch-clamp techniques to examine how lidocaine affects the functions of major voltage- and ligand-gated channels, as well as current-evoked neuronal firing in this auditory region.

Materials and methods

Cell culture The use and care of the animals in this study followed the guidelines and protocols of the Institutional Animal Care and Use Committee of University of Science and Technology of China.

Primary cultures of dissociated neonatal rat IC neurons were prepared as described previously[22]. In brief, Wistar rats, aged P0 (either sex), were decapitated; the whole brain was transferred to iced Hanks' solution. The IC (Figure 1A) was then collected and placed under a microscope. The tissue was incubated with 0.25% trypsin (Sigma, St Louis, MO, USA) for 15 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 (1.5×10^6 cell/mL) on poly-*L*-lysine (Sigma, USA)-coated cover glasses and grown in DMEM with *L*-glutamine plus 10% fetal bovine serum, 10% F-12 nutrient mixture, 100 U/mL penicillin, and 100 μg/mL streptomycin (Gibco, USA) for 24 h. Neuron-basal medium (1.5 mL) with 2% B27 (Gibco, USA) was replaced every 3–4 d. Treatment with 5-fluoro-5′-deoxyuridine (20 μg/mL; Sigma, USA) on d 4 after plating was used to block the cell division of non-neuronal cells, which helped to stabilize the cell population. The cultures were maintained at 37 °C in a 5% CO_2 humidified atmosphere. Cells were used for electrophysiological recordings 7–20 d after plating (Figure 1B).

Solutions and drugs The standard external solution contained (in mmol/L): 150 NaCl, 5 KCl, 1 $MgCl_2$, 2 $CaCl_2$, 10 glucose, and 10 HEPES, and was titrated to a pH of 7.4 with Tris base. The standard external solution was used for recording glycine-induced currents (I_{Gly}), gamma aminobutyric acid (GABA)-induced currents (I_{GABA}), and the current-evoked firing of action potentials. The external solution for recording aspartate (Asp)-induced currents (I_{Asp}) contained (in mmol/L): 150 NaCl, 5 KCl, 2 $CaCl_2$, 10 glucose, 10 HEPES, and 1 μmol/L glycine, and was titrated to a pH of 7.4 with Tris base. The external solution for recording Na^+ currents (I_{Na}) contained (in mmol/L): 150 NaCl, 5 KCl, 1 $MgCl_2$, 2 $CaCl_2$, 10 glucose, 10 HEPES, 5 4-aminopyridine (4-AP), 0.2 $CdCl_2$, and 0.1 $NiCl_2$, and was titrated to a pH of 7.4 with Tris base. The external solution for recording transient outward K^+ currents (I_A) contained (in mmol/L): 150 NaCl, 5 KCl, 1 $MgCl_2$, 2 $CaCl_2$, 10 glucose, 10 HEPES, 0.0003 tetrodotoxin (TTX), 0.2 $CdCl_2$, and 20 tetraethylammonium chloride (TEACl), and was titrated to a pH of 7.4 with Tris base. The osmolarity of all the bath solutions was adjusted to 310–320 mOsm/L with sucrose and a microosmometer (model 3300; Advanced Instruments, Pomona, CA, USA).

The patch pipette solution for whole-cell patch recording contained (in mmol/L): 120 KCl, 30 NaCl, 1 $MgCl_2$, 0.5 $CaCl_2$, 5 ethylene glycol-bis-(2-aminoethylether)-tetraacetic acid (EGTA), 2 Mg-ATP, and 10 HEPES. The pH of the internal solution was adjusted to 7.2 with KOH. The pipette solution for recording Na^+ currents contained (in mmol/L): 120 CsCl, 20 TEACl, 2 $MgCl_2$, 10 EGTA, 2 Mg-ATP, and 10 HEPES. The pH of the internal solution was adjusted to 7.2 with CsOH. The pipette solution for recording transient outward K^+ currents contained (in mmol/L): 70 KCl, 1 $CaCl_2$, 2 $MgCl_2$, 70 KF, 10 EGTA, 2 Mg-ATP, and 10 HEPES. The pH of the internal solution was adjusted to 7.2 with KOH. The pipette solution for recording the current-evoked firing of action potentials con-

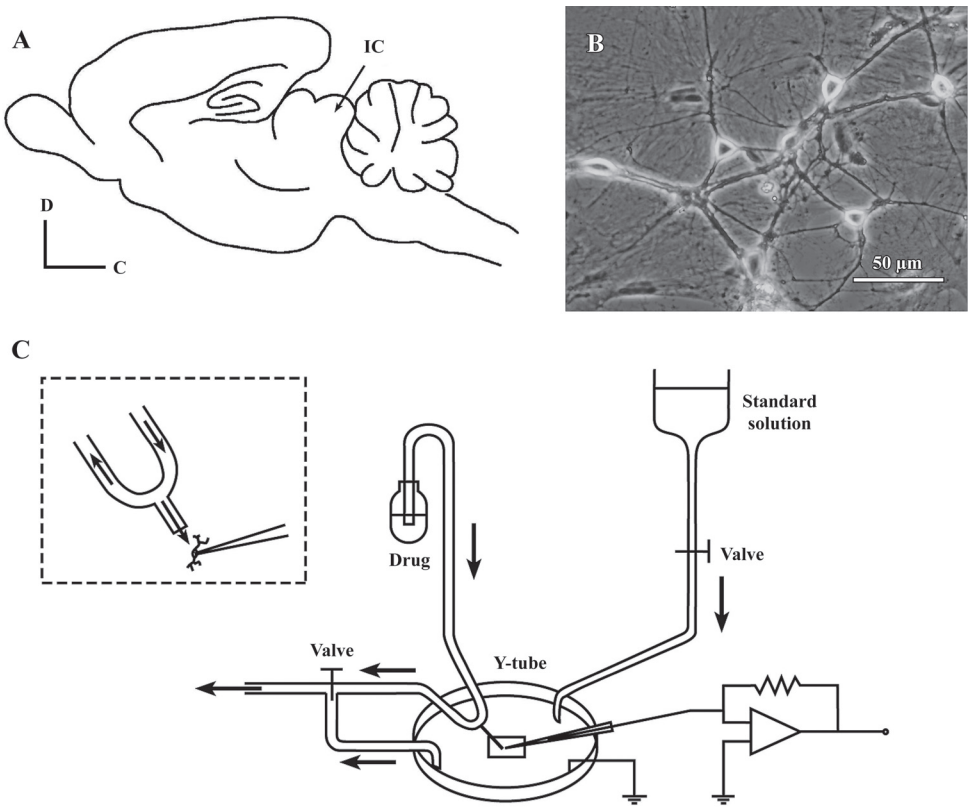

Figure 1. Preparation of cell culture for IC neurons and the Y-tube drug delivery system. (A) schematic drawing showing a lateral view of the rat brain. Arrow indicates the location of IC. (B) representative morphologies of cultured IC neurons. (C) drug delivery to the neurons being recorded with the Y-tube method. Diagram was redrawn from Murase *et al*[23] with modification. C, caudal; D, dorsal.

tained (in mmol/L): 135 K-gluconate, 15 KCl, 5 NaCl, 0.5 EGTA, 10 HEPES, and 2 Mg-ATP. The pH of the internal pipette solution was adjusted to 7.2 with KOH.

The drugs used in this study were all purchased from Sigma, unless otherwise specified. The drugs were first dissolved in ion-free water and then diluted to the final concentrations in the standard external solution just before use. The drugs were applied throughout the experiments with a rapid application technique termed the "Y-tube" method[23] (Figure 1C). The Y-tube was made from polyethylene, and its outlet tip (0.1 mm diameter) was approximately 0.2 mm away from the cell being recorded. The drugs were drawn through the arms of the Y-tube with gentle suction. Some bath fluid was drawn in through the stem so that no agent was applied unintentionally. The system allows for a complete exchange of external solution surrounding a neuron within 20 ms.

Electrophysiological recordings A patch-clamp amplifier (Axopatch 200B; Axon Instruments, Union City, CA, USA) was used for the whole-cell voltage-clamp recordings. Data were sampled and analyzed using a Digidata 1320A interface and a computer installed with Clampex and Clampfit software (version 9.0.1; Axon Instruments, USA). Patch pipettes were pulled from glass capillaries with an outer diameter of 1.5 mm on a 2-stage puller (PP-830; Narishige, Tokyo, Japan). The resistance between the recording electrode filled with pipette solution and the reference electrode was 2–6 MΩ. In most experiments, 70%–90% series resistance compensation was applied, and the membrane potential was held at –60 mV. When recording Na^+ currents, data were filtered at 2 kHz and digitized at 10 kHz with membrane potential holding at –80 mV. Fast and slow capacitances were also neutralized. Data were sampled at 5 kHz and filtered by a low-pass Bessel filter set at 1 kHz when recording K^+ currents. In addition, currents were corrected for leak and residual capacitance transients by a P/4 protocol. All of the experiments were carried out at room temperature (22–25 °C).

Data analysis All data are shown as the mean±SEM, with statistical significance assessed by Student's *t*-test. Statistically significant differences were assumed to be $P<0.05$ and $P<0.01$ for all data. *P* and *n* represent the value of significance and the number of neurons tested, respectively.

Results

Lidocaine reversibly inhibited the I_{Na} Under voltage-

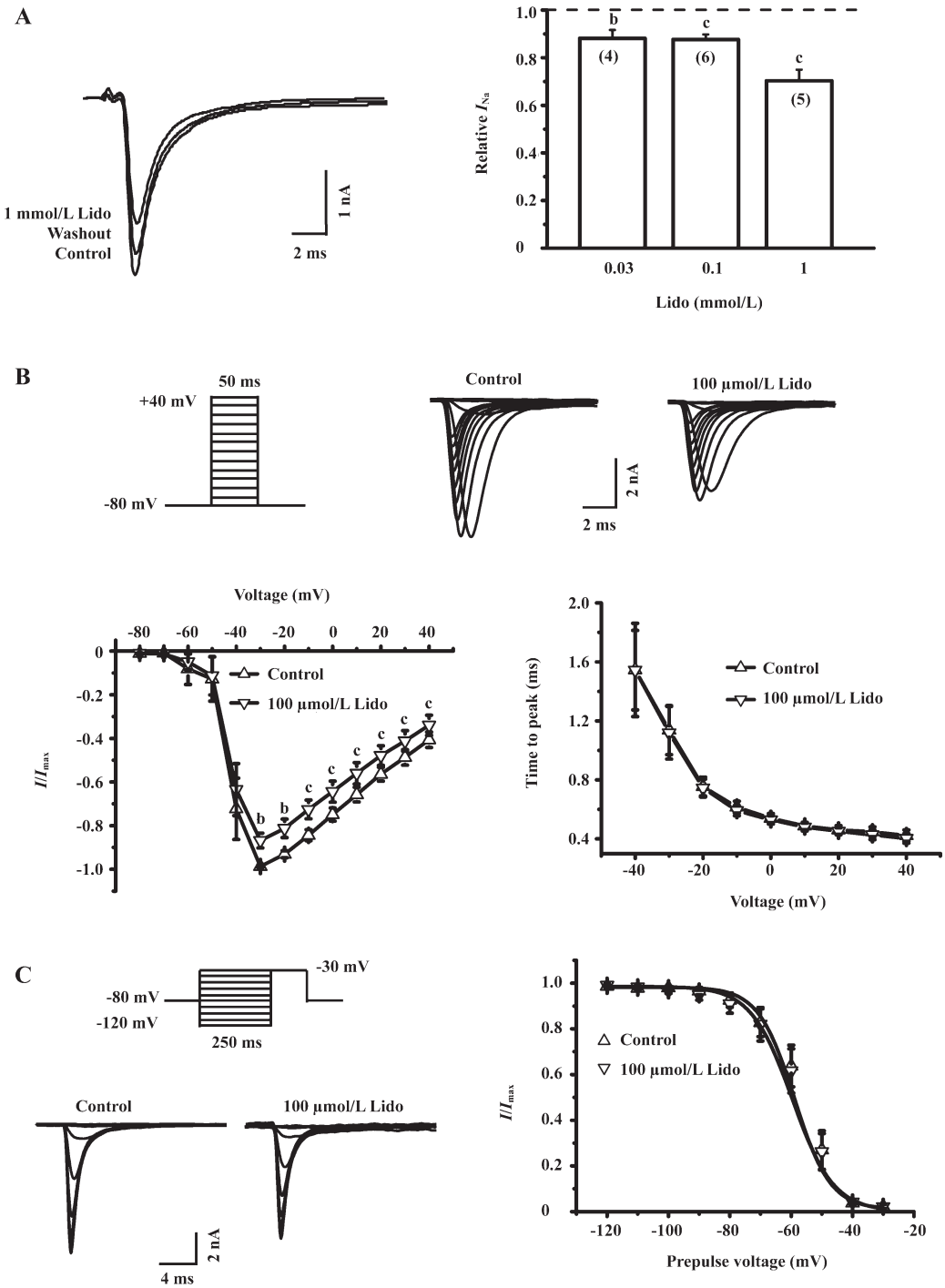

Figure 2. Inhibitory effects of lidocaine (Lido) on the I_{Na}. (A) I_{Na} was inhibited by the application of lidocaine at various concentrations (0.03, 0.1, and 1 mmol/L). Duration of drug treatment for each concentration was 30 s. Depressing effect was reversible following 1 min washout. Sample sizes are indicated in parentheses. Vertical bars represent 1 standard error. (B) lidocaine reduced the peak amplitude of the I_{Na} without significant left or right shift in the current-voltage curve (lower left panel, n=5) and the activation time of the I_{Na} (lower right panel, n=7). Upper left panel shows step pulses used for recording the I_{Na}. Upper right panel shows sample curves of the I_{Na}. Vertical bars represent±SEM. (C) lidocaine did not significantly alter the inactivation curves of the I_{Na} (right panel, n=8, P>0.05). I_{Na} amplitudes were normalized to those in the control conditions and fitted with a Boltzmann function. Left upper panel shows step pulses used for recording the inactivation curves of the I_{Na}. Left lower panel shows sample traces of the inactivation curves. Vertical bars represent±SEM. ^{b}P<0.05, ^{c}P<0.01.

clamp conditions, the I_{Na} was recorded in cultured rat IC neurons, which were held at –80 mV and depolarized to –30 mV for 20 ms (Figure 2A, left panel). This current was completely and reversibly blocked by 0.3 μmol/L TTX, suggesting that it was mediated by TTX-sensitive, voltage-gated sodium channels. To investigate the effects of lidocaine on the I_{Na}, all tested neurons were treated with lidocaine for approximately 30 s before assessing. Lidocaine at 100 μmol/L reduced the I_{Na} amplitude to 87.66%±2.12% of that of the control level (n=6, P<0.01; Figure 2A, right panel). After washout, the I_{Na} could be recovered to 98.91%±2.19% of that of the control level, indicating a reversible effect of lidocaine on the I_{Na}. Moreover, the I_{Na} was reduced by lidocaine to a larger extent as the drug concentration was increased from 0.03 to 1 mmol/L (Figure 2A, right panel).

The effect of lidocaine on the current-voltage curve of voltage-gated sodium channels was evaluated. The I_{Na} was evoked by step pulses (50 ms wide) from –80 to +40 mV in 10 mV increments (Figure 2B, upper panel). The sodium current was normalized to the maximal current and plotted against evoking potentials. Each of the curves was fitted with a Boltzmann function (Figure 2B, lower left panel). The application of 100 μmol/L lidocaine reduced the peak amplitude of the I_{Na} without any significant left or right shift in the current-voltage curve of the I_{Na} (n=5, P>0.05). In addition, the application of 100 μmol/L lidocaine did not affect the activation time of the I_{Na} (n=7, P>0.05), which is defined as the time between the onset of evoking potentials and the peak amplitude of the I_{Na} (Figure 2B, lower right panel).

To further investigate whether lidocaine could also affect the inactivation of voltage-gated sodium channels, we applied step pulses from –120 to –30 mV in 10 mV increments to IC neurons (Figure 2C, left panel). The I_{Na} was normalized to the amplitude of the maximal current, and points were fitted with a Boltzmann function. The value of $V_{1/2}$, at which the conductance of the I_{Na} reaches half its maximum, was not significantly shifted by the presence of 100 μmol/L lidocaine (–56.03±0.74 *vs* –58.97±1.44 mV, n=8, P>0.05; Figure 2C, right panel), indicating that lidocaine does not significantly change the inactivation properties of voltage-gated sodium channels.

Lidocaine reversibly inhibited the I_A At a holding potential of –70 mV, the I_A of IC neurons was elicited by stepping the membrane potential to +50 mV for 200 ms after a –110 mV prepulse (Figure 3A, left panel). The extracellular application of 0.1, 0.3, and 1 mmol/L lidocaine significantly reduced the amplitude of I_A to 96.33%±0.35%, 91.36%±1.64%, and 62.80%±2.61% of that of the control level (n=5, P<0.05), respectively (Figure 3A, right panel). The effects could be removed by the washout of lidocaine for 1 min, indicating that the lidocaine-induced reduction in I_A is reversibly inhibited.

To obtain the conductance-voltage curves, we recorded the I_A by using 250 ms depolarizing voltage pulses with 10 mV increments from –60 to +50 mV following a 150 ms prepulse at –110 mV (Figure 3B, upper panel). The I_A was converted into conductance by using the equation G=I/(V–V_K), where V refers to the evoking potential, and V_K refers to the potassium equilibrium potential. The conductance was then normalized and plotted against the membrane potential. Each of the curves was fitted with a Boltzmann function: $G/G_{max}=(1+\exp[V-V_{1/2}]/k)^{-1}$, where G is the conductance, G_{max} is the maximum conductance, V is the command potential, $V_{1/2}$ is the potential for half-maximal activation, and k is the slope factor. In the absence of lidocaine, the I_A was shown to start activation from approximately –60 mV, and its peak amplitude increased with depolarizing pulse steps. This activation pattern was not significantly influenced by 100 μmol/L lidocaine (Figure 3B, middle panel). In addition, lidocaine at 100 μmol/L also did not significantly change the $V_{1/2}$ (n=7, P>0.05; Figure 3B, lower panel). These results indicate that lidocaine does not change the steady-state activation of the I_A.

To investigate the effects of lidocaine on the steady-state inactivation properties of the I_A, we applied a 200 ms test command pulse of +30 mV following 150 ms hyperpolarizing prepulses from –110 to –10 mV in 10 mV increments to IC neurons (Figure 3C, upper panel). The I_A recorded with this protocol was normalized and fitted with a Boltzmann function. In the absence of lidocaine, the I_A was shown to start inactivation from approximately –100 mV and decrease with hyperpolarizing pulse steps. This inactivation pattern was not significantly influenced by 100 μmol/L lidocaine (Figure 3C, middle panel). In addition, lidocaine at 100 μmol/L also did not significantly shift the $V_{1/2}$ of the I_A inactivation curve (–69.36±2.14 *vs* –74.08±1.79 mV, n=7, P>0.05; Figure 3C, lower panel). These results indicate that lidocaine does not change the steady-state inactivation of the I_A.

Lidocaine reversibly inhibited the I_{Gly} Under the condition of voltage-clamp at a holding potential of –60 mV, the extracellular application of 100 μmol/L glycine induced an inward current in all tested neurons, and this current could be almost completely blocked by strychnine, a specific antagonist of the glycine receptor (data

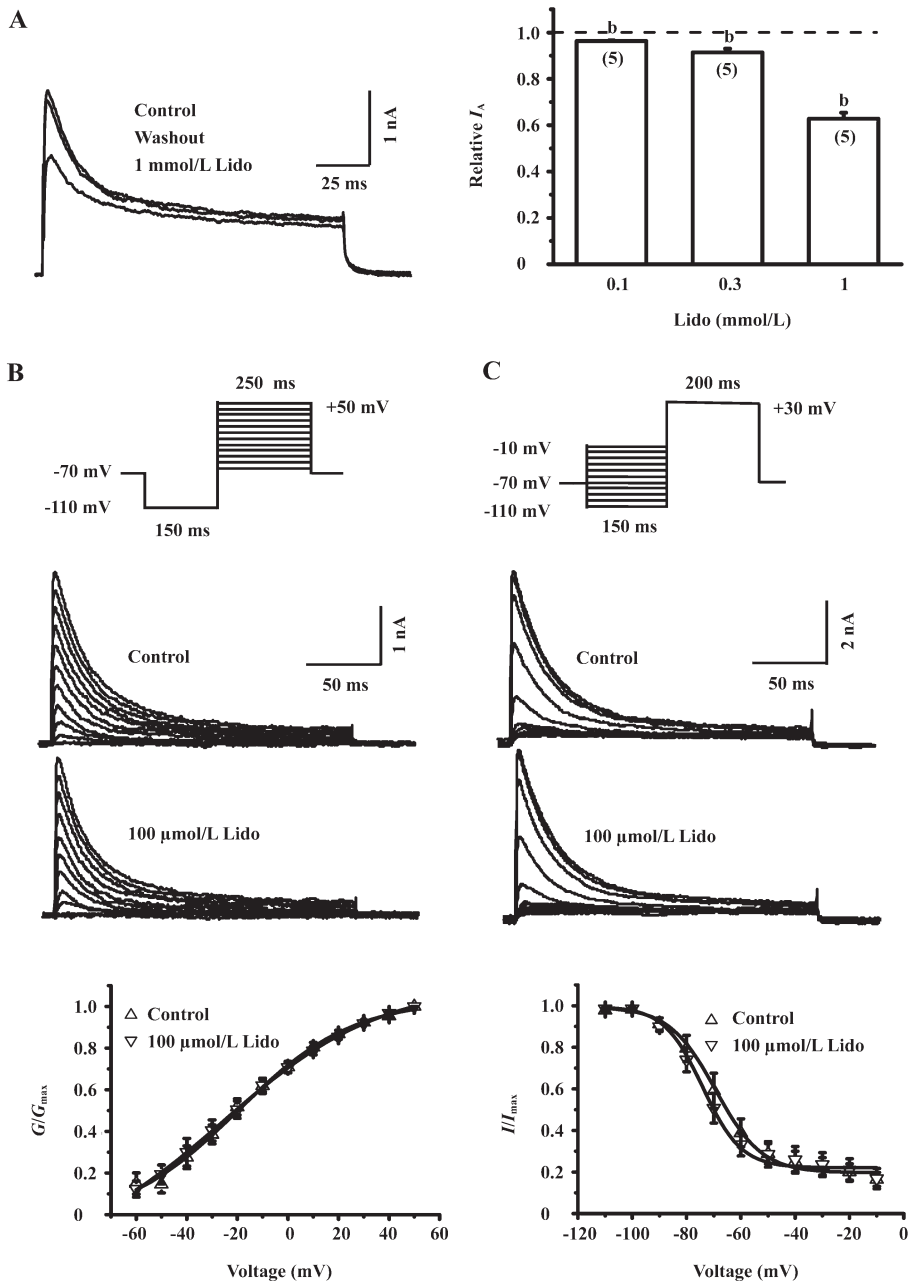

Figure 3. Effect of lidocaine on the I_A. (A) I_A was inhibited by the application of lidocaine at various concentrations (0.1, 0.3, and 1 mmol/L, right panel). Duration of the drug treatment for each concentration was 30 s. Left panel shows sample traces of the I_A before and after the application of 1 mmol/L lidocaine. Depressing effect was reversible following 1 min washout. Sample sizes are indicated in parentheses. Vertical bars represent 1 standard error. $^bP<0.05$. (B) activation curve of the I_A was not changed by 100 μmol/L lidocaine (lower panel, n=7, $P>0.05$). I_A amplitudes were normalized to those under control conditions and fitted with a Boltzmann function: $G/G_{max}=(1+\exp[V-V_{1/2}]/k)^{-1}$. Upper panel shows step pulses used for recording the activation curves of the I_A. Middle panel shows sample traces of the activation curves before and after the application of lidocaine. Vertical bars represent±SEM. (C) inactivation curve of the I_A was also not changed by lidocaine (lower panel, n=7, $P>0.05$). I_A amplitudes were normalized to those under control conditions and fitted with a Boltzmann function: $I/I_{max}=(1+\exp[V-V_{1/2}]/k)^{-1}$. Upper panel shows step pulses used for recording the inactivation curves of the I_A. Middle panel shows sample traces of the inactivation curves before and after the application of lidocaine. Vertical bars represent±SEM. G, conductance for activation; I, current for inactivation.

not shown). Figure 4A (left panel) shows sample traces of the I_{Gly} in the absence and presence of lidocaine at various concentrations. Lidocaine itself at different concentrations did not evoke any detectable current in these neurons; however, lidocaine at 0.1 and 1 mmol/L slightly but significantly reduced the I_{Gly} to 91.46%±2.69% and 89.11%±3.17% of that of the control level, respectively (n=5, $P<0.05$). Lidocaine appeared to suppress the I_{Gly} to a larger extent at a higher concentration (3 mmol/L; Figure 4A, right panel). The inhibitory effect of lidocaine on the I_{Gly} could recover after washout (Figure 4A, left panel).

Lidocaine did not affect the I_{GABA} at lower concentrations To study the effect of lidocaine on the I_{GABA}, we recorded the currents induced by 30 μmol/L GABA in the absence and presence of lidocaine at 3 different concentrations (0.1, 1, and 3 mmol/L; Figure 4B, left panel). At a holding potential of –60 mV, the extracellular application of GABA induced an inward current in all tested neurons, and this current could be completely blocked by 10 μmol/L bicuculline, a specific

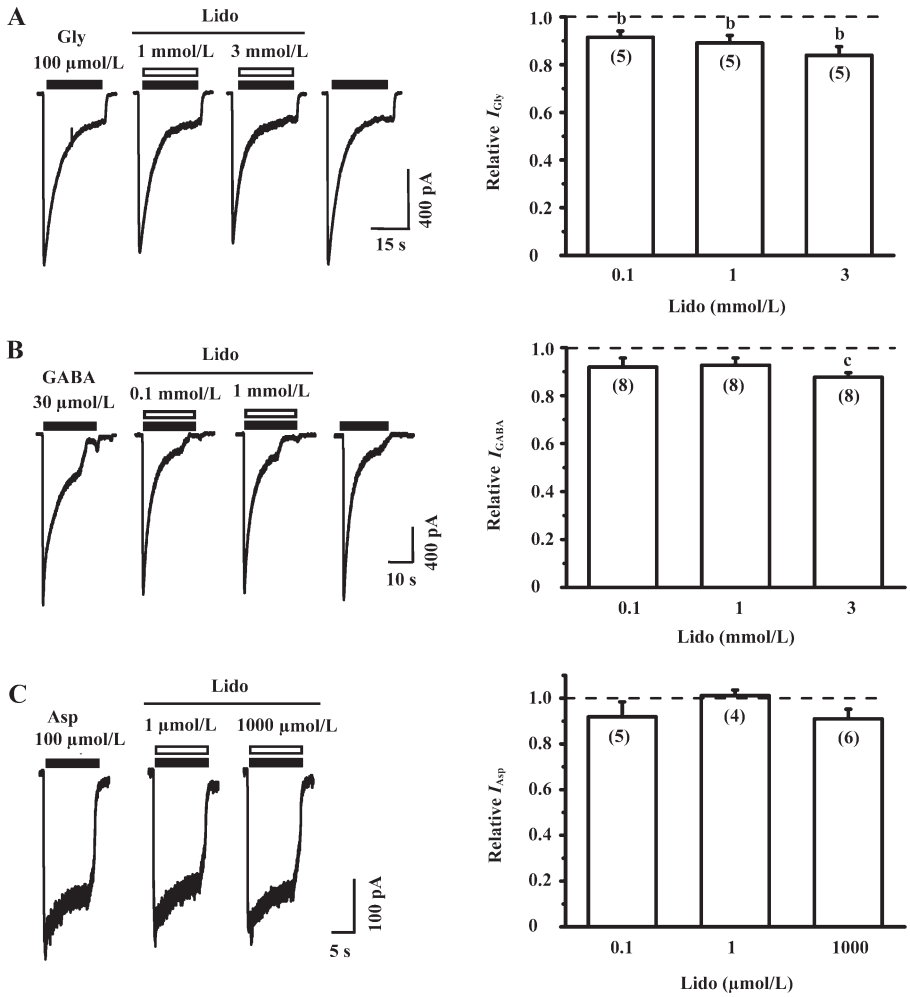

Figure 4. Effects of lidocaine on the I_{Gly}, I_{GABA}, and $I_{Asp.}$ (A) lidocaine (0.1, 1, and 3 mmol/L) slightly but significantly depressed the I_{Gly} (right panel). Left panel shows sample traces of the I_{Gly} before and after the application of lidocaine. Glycine (Gly) concentration was 100 μmol/L. (B) lidocaine did not significantly change the I_{GABA} at a lower concentration (0.1 and 1 mmol/L), but reduced the I_{GABA} at a higher concentration (3 mmol/L, right panel). Left panel shows sample traces of the I_{GABA} before and after the application of lidocaine. (C) lidocaine did not significantly change the I_{Asp} (right panel). Left panel shows sample traces of the I_{Asp} before and after the application of lidocaine. I_{Asp} could be abolished by APV, an NMDA receptor antagonist. All the currents were normalized to those under control conditions. Sample sizes are indicated in parentheses. Vertical bars represent 1 standard error. $^{b}P<0.05$, $^{c}P<0.01$.

antagonist of $GABA_A$ receptors (data not shown), indicating that the I_{GABA} recorded in the present study was mainly mediated by $GABA_A$ receptors. Lidocaine at lower concentrations (0.1 and 1 mmol/L) did not have any significant effect on the I_{GABA}, but slightly and significantly reduced the I_{GABA} to 87.70%±1.87% of that of the control level (n=8, P<0.01) at a higher concentration (3 mmol/L; Figure 4B, right panel).

Lidocaine did not affect the I_{Asp} At the holding potential of –40 mV, 100 μmol/L Asp could activate *N*-methyl-*D*-aspartic acid (NMDA) receptors in the Mg^{2+}-free extracellular solution containing 1 μmol/L glycine to induce an inward current (Figure 4C, left panel). The I_{Asp} could be completely blocked by 50 μmol/L 2-amino-5-phosphonovaleric acid (APV; a specific NMDA receptor antagonist), indicating that the I_{Asp} recorded in the present study was mainly mediated by NMDA receptors. Lidocaine at 0.1, 1, and 1000 μmol/L did not significantly change the I_{Asp} (n=4−6, P>0.05; Figure 4C, right panel), indicating that lidocaine does not affect NMDA receptors in IC neurons.

Lidocaine depressed the current-evoked firing of action potentials Because lidocaine had complex and non-specific actions on ion channels and receptors, as shown by the present experiments (Figures 2−4), we sought to determine whether these effects of lidocaine would increase or decrease the excitability of cultured IC neurons. To do so, we recorded the firing rate of action potentials evoked by injecting a positive electrical current in the absence and presence of 100 μmol/L lidocaine under a current-clamp mode. Only neurons that had a resting membrane potential lower than –50 mV (–57.30±1.30 mV, n=5) and did not fire spontaneously were selected for this experiment. Usually action potentials can be evoked by a 20 pA depolarizing current (Figure 5A), and the mean firing rate of recorded neurons is 10.20±3.07 Hz (n=5). Lidocaine at 100 μmol/L did not change the current–voltage relationship (Figure 5B), but significantly depressed the firing rate of action

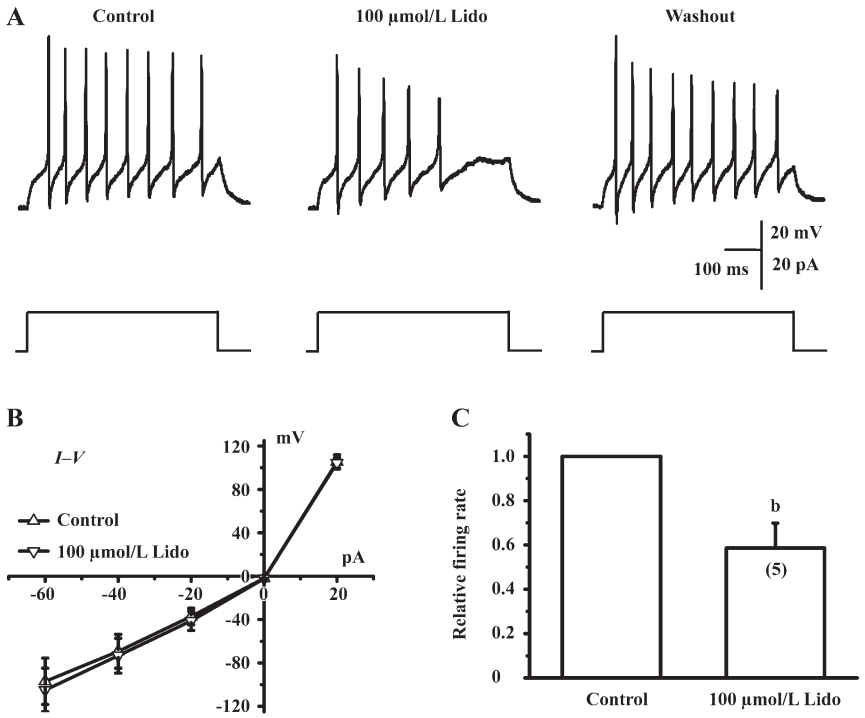

Figure 5. Lidocaine depressed the current-evoked firing of action potentials. (A) sample traces of action potentials recorded before and after the application of 100 μmol/L lidocaine (upper panel). Firing of action potentials was evoked by a 20 pA depolarizing current (lower panel), and the mean firing rate of recorded neurons is 10.20±3.07 Hz (n=5). (B) lidocaine did not change the current-voltage relationship (n=5, P>0.05). (C) statistical results showing that the relative number of action potentials evoked by the current injection was significantly reduced by application of lidocaine. Sample size is indicated in parentheses. Vertical bar represents 1 standard error. ^{b}P<0.05.

potentials in IC neurons to 58.62%±11.22 % of that of the control level (n=5, P<0.05; Figure 5A and C).

Discussion

In the present study, we investigated the effects of lidocaine on major types of ion channels and receptors in IC neurons of the auditory system, including voltage-gated sodium channels, transient outward potassium channels, glycine receptors, $GABA_A$ receptors, and NMDA receptors. In addition, we examined whether lidocaine influenced the neuronal excitability by current-evoked firing of action potentials in IC neurons. Lidocaine at concentrations lower than 1 mmol/L significantly suppressed the I_{Na}, I_A, and I_{Gly} to 87.66%±2.12%, 96.33%±0.35%, and 91.46%±2.69% of that of the control level, respectively (Figures 2A, 3A, 4A). At a higher concentration (1 mmol/L), lidocaine further suppressed the 3 currents to 70.26%±4.69%, 62.8%±2.61%, and 89.11%±3.17% of that of the control level, respectively (Figure 2A, 3A, 4A). However, lidocaine at concentrations lower than 1 mmol/L did not significantly affect the I_{GABA} (Figure 4B) and the I_{Asp} (Figure 4C), but slightly depressed the I_{GABA} to 87.70%±1.87% of that of the control level at a higher concentration (3 mmol/L) (Figure 4B). These results demonstrate that the effects of lidocaine on ion channels and receptors in the central auditory system are complex and non-specific. However, lidocaine at 100 μmol/L significantly suppressed the current-evoked firing of action potentials to 58.62%±11.22% of that of the control level (Figure 5), indicating that lidocaine seems to reduce the overall excitability of IC neurons. We suggest that lidocaine at low concentrations (30–100 μmol/L, clinically relevant concentration) reduces the excitability of IC neurons to suppress tinnitus, probably through the inhibition of voltage-gated sodium channels. Alternatively, lidocaine may act on other types of ion channels, receptors[24–26], or synaptic transmissions[27], which were not examined in the present study, to suppress tinnitus.

Effects of lidocaine on voltage-gated channels Voltage-gated sodium channels are essential for action potential generation and its propagation, since they contribute to the rapid rising phase and initial falling phase of action potentials. Therefore, voltage-gated sodium channels play an important role in regulating the excitability of neurons. Voltage-gated sodium channels have 3 distinct states: closed or resting, open, and inactivated. The conformational state in which these channels reside depends on the transmembrane potential[28,29]. It is generally believed that the blockade effect of lidocaine on sodium channels may result from the drug's interaction with the open state or the inactivated state of the channel[30,31]. Consistent with previous findings, our results show that lidocaine can block the voltage-gated sodium channel, although the inhibition induced by lidocaine

is less than that reported by others[30,32]. However, our study demonstrates that lidocaine has no effects on the voltage dependence of the activation state and the time to peak, suggesting that the drug may not interact with the S4 segments, which act as a voltage sensor to initiate activation in response to depolarization signals[33].

Potassium channels are essential for controlling electrical properties and the excitability of neurons, including setting the resting membrane potential, action potential duration, and firing patterns[34]. They also play a vital role in processing auditory information in auditory brainstem nuclei[35]. The transient outward potassium channel has been identified in IC neurons[36], and changes in the amplitude or kinetics of the I_A will alter neuronal electrical properties, such as spike repolarization and action potential firing, and influence neuronal excitability[37]. In the present study, lidocaine did not change the amplitude of the I_A at a low concentration (100 μmol/L, clinically relevant concentration; Figure 3A), although it significantly reduced the amplitude of the I_A at a high concentrations (1 mmol/L, too high to be considered a clinically relevant concentration; Figure 3A, right panel). In addition, lidocaine at a lower concentration did not change its steady-state activation and inactivation (Figure 3B, 3C). Thus, at a clinical dose used for tinnitus treatment, transient outward potassium channels may not be involved in producing suppressive effects on tinnitus. The depression of the I_A at a higher concentration (1 mmol/L) may contribute to increase neuronal excitability and can cause neurotoxicity.

Effects of lidocaine on ligand-gated ion channels As major inhibitory neurotransmitters in the mammalian central nervous system, glycine and GABA play an important role in processing auditory information. Glutamate is a major excitatory neurotransmitter and plays an important role in processing auditory information as well. In the present study, lidocaine concentrations lower than 100 μmol/L were found to inhibit the I_{Gly} (Figure 4A), but not influence the I_{GABA} (Figure 4B) and the I_{Asp} (Figure 4C). Because the I_{Asp} could be completely blocked by APV, a specific NMDA receptor antagonist (data not shown), the I_{Asp} recorded in the present study was mainly mediated by NMDA receptors. Taken together, these results indicate that lidocaine acts on glycine receptors, but not on GABA receptors or NMDA receptors. The degree to which lidocaine suppresses the I_{Gly} was small (up to 91.5% of that of the control level at 100 μmol/L; Figure 4A), which suggests that lidocaine at a clinical concentration does not significantly raise the excitability of the auditory system.

Possible neural basis for the suppressive effects of lidocaine on tinnitus One hypothesis suggests that tinnitus is related to an imbalance between inhibitory and excitatory neurotransmissions in the central nervous system, that is, tinnitus is a consequence of overexcitability in the central auditory system[38]. If this theory holds, an effective therapy for tinnitus should be reducing the excitability of the overexcited auditory system. Our data suggest that lidocaine at a low dose in tinnitus treatment may reduce the excitability in the central auditory system by inhibiting voltage-gated sodium channels. Our data also suggest that transient outward potassium channels, $GABA_A$ receptors, and NMDA receptors may not be activated by lidocaine at a low dose. The reduced excitability of IC neurons by lidocaine at lower concentrations has been demonstrated by the drug's suppressive effects on the current-evoked firing of action potentials (Figure 5). However, our data also suggest that lidocaine may also increase the excitability of the auditory system by inhibiting potassium channels and glycine receptors, particularly when lidocaine is at a high dose. This notion is consistent with the observation that lidocaine at a high dose can cause convulsion[39]. Although our data indicate that the effects of lidocaine on the ion channels and receptors are complex and non-specific in the central auditory system, this drug may nevertheless reduce the overall excitability to suppress tinnitus. Alternatively, lidocaine may act on other types of ion channels, receptors[24–26], or synaptic transmissions[27], which were not examined in the present study, to suppress tinnitus. Further experiments, such as differential responses of excitatory and inhibitory neurons to lidocaine, as well as the effects of lidocaine on alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors, are required to provide further insight into its therapeutic mechanism for suppressing tinnitus in the auditory system.

Author contributions

Lin CHEN designed the research, Mu YU performed the experiment and analyzed the data, and Mu YU and Lin CHEN wrote the paper.

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