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Minocycline cannot protect neurons against bilirubin-induced hyperexcitation in the ventral cochlear nucleus

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ABSTRACT

Excitotoxicity has been suggested to play an important role in many central nervous system diseases, particularly in bilirubin encephalopathy. Minocycline treatment has been proposed to be one of the most promising potential therapies for excitotoxicity-induced neurological disorders. However, some key questions, such as the electrophysiological effect of minocycline on neuronal excitability and hyperexcitation in pathological conditions, require clarification. In this study, using patch-clamp techniques, we showed that bilirubin increased the frequency of both spontaneous excitatory postsynaptic currents (sEPSCs) and neuronal firing in isolated ventral cochlear nucleus (VCN) neurons at postnatal days 11–14 (P11–14) in rats but it did not affect the amplitude of sEPSCs or glutamate-activated (I_{Glu}) currents. However, minocycline had no effect on sEPSC frequency or I_{Glu} amplitude. Furthermore, minocycline pretreatment did not abolish bilirubin-induced sEPSC potentiation or neuron firing. These data suggest that minocycline does not affect excitatory synaptic transmission or hyperexcitation induced by bilirubin in VCN neurons. From these results, we propose that the neuroprotective efficacy of minocycline, if it can protect neurons against neurotoxicity induced by substances like bilirubin, is mediated by either an alternative mechanism or downstream events post neuronal hyperexcitation. Certainly, additional investigation of the neuroprotective effects of minocycline is required before embarking on further clinical trials.

Introduction

Excitotoxicity is defined as the abnormal release of excitatory amino acids. Glutamate is the most prevalent excitatory neurotransmitter in the central nervous system (CNS) and significantly contributes to neurological outcomes (Sattler and Tymianski, 2001). Excessive glutamate receptor activation leads to abnormal Ca^{2+} influx and subsequent neuronal apoptosis and/or necrosis (Sattler and Tymianski, 2000; Sattler and Tymianski, 2001). Glutamate-induced excitotoxicity has been proposed as an important contributing factor in many neurological diseases, such as stroke, epilepsy, multiple sclerosis, Parkinson's disease, and kernicterus (Grojean et al., 2000; Johnston, 2005; Lau and Tymianski, 2010; McDonald et al., 1998; Silva et al., 1999). Kernicterus is a devastating neurological disorder that results from a preference of bilirubin toxicity for neurons and the regional topography of bilirubin-induced neuronal injury in the CNS (Ostrow et al., 2003). Bilirubin-induced damage to the auditory nuclei leads to auditory neuropathy, deterioration of auditory processing, and even deafness (Shapiro and Nakamura, 2001). Although the cellular mechanism of bilirubin excitotoxicity is undetermined, our recent study for the first time provided electrophysiological evidence that excessive synaptic release of glutamate and the consequent overstimulation of glutamate receptors directly contribute to bilirubin-induced hyperexcitation (Li et al., 2011). Thus, drugs that inhibit the over-release of glutamate may become potential neuroprotectants against glutamate toxicity.

Minocycline is a highly lipophilic semisynthetic derivative of tetracycline and is capable of crossing the blood–brain barrier (Zhu et al., 2002). It has been reported that minocycline can greatly ameliorate the neurological outcome of CNS injury and some neurodegenerative diseases associated with glutamate excitotoxicity through inhibition of microglial activation, proapoptotic mediators, cytochrome c release, and caspase-dependent or -independent neuronal death pathways (Pi et al., 2004; Song et al., 2006; Tikka and Koistinaho, 2001; Wang et al., 2003; Zhu et al., 2002). The neuroprotective effect of minocycline on bilirubin-induced neurotoxicity has also been reported (Geiger et al., 2007; Lin et al., 2005; Rice et al., 2011). Minocycline potently protects the central auditory system and cerebellar granule neurons against bilirubin toxicity, which may raise hope of using minocycline to attenuate and prevent bilirubin toxicity before permanent brain damage occurs (Geiger et al., 2007; Lin et al., 2005; Rice et al., 2011). However, little is known about the effect of minocycline on presynaptic glutamate release and neuronal excitability, which can be potentiated by

Abbreviations: LSO, lateral superior olive; VCN, ventral cochlear nucleus; sEPSCs, spontaneous excitatory postsynaptic currents; I_{Glu}, glutamate-activated currents; CNS, central nervous system; APV, D,L-2-amino-5-phosphonovaleric acid; NBQX, 2,3-dihydroxy-6-nitro-7-sulfamoyl-benzo[f]quinoxaline-2,3-dione.

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bilirubin. In addition, minocycline may have secondary effects in infants as it can cause stunting of bone growth and may prevent the binding of bilirubin to albumin (Buller et al., 2009), and some studies have provided conflicting evidence suggesting that minocycline is not neuroprotective but exacerbates excitotoxicity (Diguet et al., 2004a, 2004b; Goni-Allo et al., 2005; Tsuji et al., 2004). Additional work, especially electrophysiological studies, are needed to clarify the benefits of minocycline and the cellular targets involved in the protective action.

In the present study, we used patch-clamp recording techniques to investigate whether minocycline could inhibit excessive glutamate release and neuronal hyperexcitation induced by bilirubin in the ventral cochlear nucleus (VCN), one of the most sensitive auditory nuclei to bilirubin toxicity. Our results show that minocycline did not protect VCN neurons against bilirubin induced hyperexcitation, suggesting that the mechanism of minocycline protection is not through suppression of glutamatergic transmission.

Materials and methods

Experiments were performed in accordance with the guiding principles for the care and use of animals, and the study was approved by the Ethics Review Committee for Animal Experimentation at Shanghai Jiaotong University.

Preparation of VCN neurons

Isolated VCN neurons were prepared as described previously (Rhee et al., 1994). Briefly, Sprague Dawley rats (11–14 days old) were anesthetized with sodium pentobarbital (55 mg/kg, i.p.) and then decapitated. The brains were quickly removed from the skull and placed into oxygenated and ice-cold incubation solution composed of (in mM) 124 NaCl, 5 KCl, 1.2 KH_2PO_4, 1.3 $MgSO_4$, 2.4 $CaCl_2$, 24 $NaHCO_3$, and 10 glucose saturated with 95% O_2 and 5% CO_2. Brain slices containing the VCN (300 μm) were cut using a microslicer (VT-1000S, Leica Microsystems, Nussloch, Germany). The slices were allowed to recover at room temperature (21–26 °C) for at least 20–30 min and then transferred to Petri dishes (Primaria 3801; Becton Dickinson, Rutherford, NJ) filled with a standard solution containing (in mM) 150 NaCl, 5 KCl, 2 $CaCl_2$, 1 $MgCl_2$, 10 glucose, and 10 4-(2-hydroxyethyl) piperazine-1-ethanesulfonic acid (HEPES). The pH of the solution was adjusted to 7.4 with NaOH. The VCN region was identified visually using a dissecting microscope (XTL-2400; SOIEC, Shanghai, China). Neurons with attached, functional presynaptic terminals were obtained by mechanical dissociation. Mechanical dissociation of VCN neurons was accomplished using a custom-built vibration device with a fire-polished glass pipette oscillating at 50 Hz over a distance of about 0.1–0.2 mm on the surface of the VCN region for about 4 min. The isolated neurons were then adhered to the bottom of the dish for 15–20 min after the vibration and easily identified visually under the inverted microscope (TE-2000U; Nikon, Japan).

Electrophysiological measurements

All electrophysiological recordings were carried out with the whole-cell patch-clamp technique using a patch-clamp amplifier (EPC-10; HEKA, Germany). The patch electrodes were pulled from borosilicate capillary glass pipettes by a vertical pipette puller (P-9; Narishige, Tokyo, Japan) and had a resistance of 6–8 MΩ when pipettes were filled with the pipette solution. The internal pipette solution contained (in mM) 130 K gluconate, 2 $MgCl_2$, 5 KCl, 0.3 GTP, 2 ATP, 0.6 EGTA, 10 HEPES. The internal solution was adjusted to a pH of 7.2 using Tris–OH. To record spontaneous excitatory postsynaptic currents (sEPSCs), neurons were voltage-clamped at a holding potential (V_H) of −60 mV. The sEPSCs were pharmacologically isolated using bicuculine (10 μM) and strychnine (0.3 μM) to inhibit currents that might be contaminated by activation of $GABA_A$ and glycine receptors, respectively, during the recordings. To record action potentials, neurons were set at their resting membrane potential, and recordings were made in current clamp mode. Electrode capacitance and liquid junction potential were compensated. All data were sampled at 3–10 kHz and filtered at 1–3 kHz using a Dell computer equipped with Pulse 6.0 (HEKA, Lambrecht, Germany). The input resistance and series resistance were monitored continuously throughout the recording. Recordings were conducted from only one neuron per cell-culture dish. All experiments were performed at room temperature (21–26 °C).

Reagents

The reagents used in the experiments included free bilirubin, minocycline, bicuculline, strychnine, and glutamate (all from Sigma, St. Louis, MO). Bilirubin was dissolved in 0.1 M NaOH at 1 mM as a stock solution, stored in single-use aliquots in the dark at −20 °C (for less than 48 h), and diluted to the final solution prior to application. Because bilirubin is light sensitive, the bilirubin solution was protected from light at all times. The evaluation of bilirubin solution in NaOH by high performance liquid chromatography (HPLC) has revealed that the bilirubin solution has insignificant changes at 5 min, 24 h and even 48 h after preparation when stored at −20 °C. Minocycline was dissolved in ethanol and then diluted with de-ionized water and stored at 4 °C. The minocycline solution was used for no more than 3 days after preparation. The reagents were applied to isolated neurons using a Y-tube system (Nabekura et al., 1996) which could completely exchange the external solution with the reagent solution within 20 ms.

Data analyses

The number of sEPSCs and action potentials were counted and analyzed with the MiniAnalysis Program (Synaptosoft, NJ, USA). The sEPSCs (≥6 pA) and action potentials (≥40 mV) were automatically screened. The rise and decay times of the sEPSCs were checked visually and were also used as criteria for accepting the recordings as synaptic events. The average values of the sEPSC frequencies and amplitudes during the control period were scaled to 1.0. The frequency and amplitude of all synaptic events during and after the application of bilirubin or minocycline were normalized to control values. Differences in the firing rate and the frequency and amplitude of the sEPSCs were examined using Wilcoxon signed-rank tests for comparison between groups. Differences in the amplitude of the glutamate-evoked postsynaptic currents were examined using Student's paired *t*-tests. Statistical analyses were performed using SPSS 15.0 software. Values of $P<0.05$ were considered to be significant. The data are presented as means ± standard deviation.

Results

Bilirubin increased sEPSC frequency

The effect of bilirubin on glutamatergic synaptic transmission in VCN neurons was investigated first. Considering that sulfadimethoxine-injected homozygous jaundiced Gunn rats which show acute neurobehavioral abnormalities as neonates and many succumb to bilirubin encephalopathy have free bilirubin at levels of 2744–8387 nM in CNS (Daood and Watchko, 2006), we used bilirubin at a concentration of 3 μM at which bilirubin encephalopathy would occur in rats. Bilirubin (3 μM) was applied to VCN neurons while the spontaneous excitatory synaptic activity was recorded (Fig. 1A). The sEPSCs were blocked by 50 μM D,L-2-amino-5-phosphonovaleric acid (APV), a NMDA receptor antagonist, and completely abolished by a combination of APV and 10 μM 2,3-dihydroxy-6-nitro-7-sulfamoyl-benzo[f]quinoxaline-2,3-dione (NBQX), an AMPA receptor antagonist (data not shown), indicating

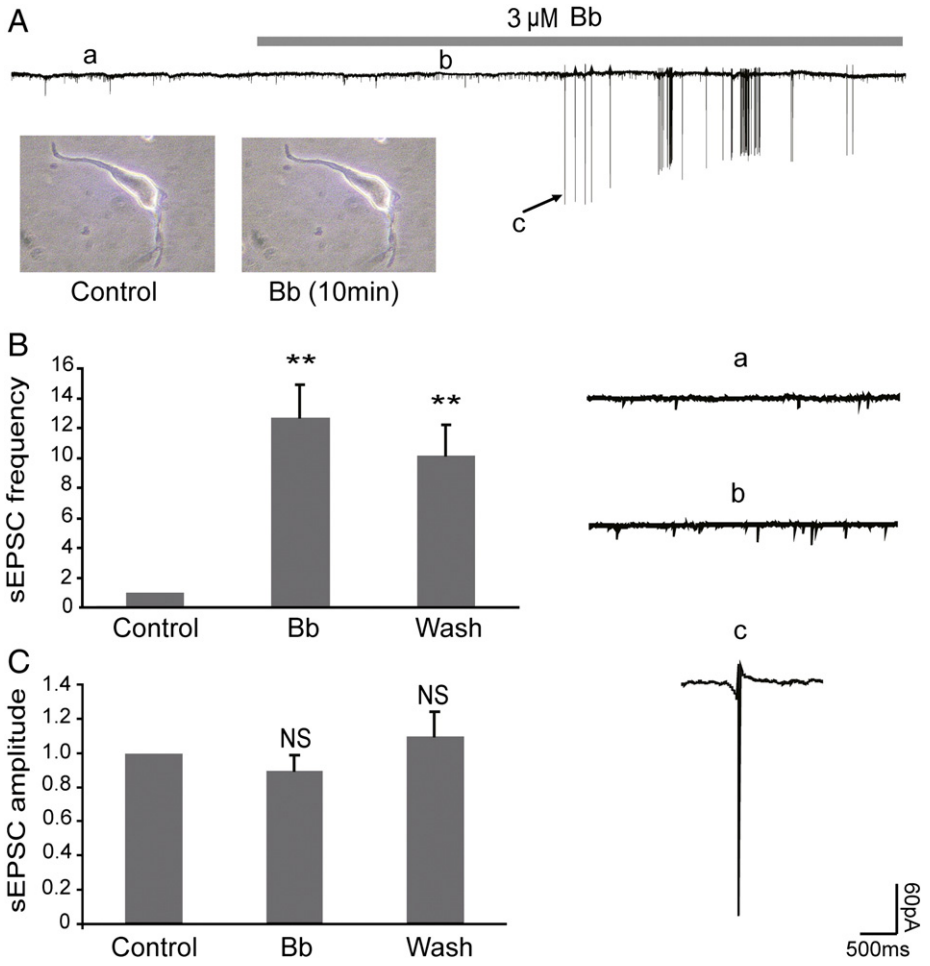

Fig. 1. Effects of bilirubin (3 μM) on the frequency and amplitude of spontaneous excitatory postsynaptic currents (sEPSCs) in VCN neurons. A: the increased incidence of sEPSCs and bidirectional currents (arrows) in a neuron during bilirubin application. Portions of the trace are also shown in higher resolution (a, b, and c). Bottom: images of the same VCN neuron before and after bilirubin treatment. B: histograms depicting average sEPSC frequency for five neurons before, during (10 min), and after (4 min) bilirubin application. C: histograms depicting average sEPSC amplitude for five neurons before, during (10 min), and after (4 min) bilirubin application. Vertical error bars represent SD; **, $P<0.01$; NS: not significant.

that the sEPSCs reflected glutamatergic activity mediated by both AMPA and NMDA receptors. After bilirubin application, the sEPSC frequency increased within 1–2 min and reached a maximum level around 5 min. The increase in sEPSC frequency lasted for at least 4 min after the removal of bilirubin (Figs. 1A, B). Like LSO neurons, on which we investigated the effect of bilirubin before (Li et al., 2011), along with the increase in sEPSC frequency, action potential currents composed of inward followed by outward currents appeared about 4–7 min after the start of the bilirubin application in all five neurons tested (Figs. 1A, C). The average sEPSC frequency during the entire 10-min bilirubin application was 1270 ± 222% of the control ($n=5$, $P<0.01$; Fig. 1B). After bilirubin was removed, sEPSC frequency was still much higher than in the control (1015 ± 322% of the control after a 4-min wash, $n=5$, $P<0.01$; Fig. 1B). However, sEPSC amplitude was not significantly affected by bilirubin (90 ± 8.8% of the control in the presence of bilirubin, 109 ± 14.1% of the control after a 4-min wash, $n=5$, $P>0.05$). These results suggest that bilirubin can increase presynaptic glutamate release in VCN neurons. During the bilirubin application period, we did not find any evidence of cell damage i.e. apoptosis and/or necrosis (e.g. shape, transparency, electrophysiological characteristics of the recorded neurons).

Minocycline did not affect presynaptic glutamate release

To determine whether minocycline affected presynaptic glutamate release, the effect of minocycline (100 μM) (Pi et al., 2004) on sEPSCs was examined. The results showed that minocycline did not significantly alter either sEPSC frequency (Mino: 149 ± 33% of the control; Wash: 122 ± 20.3% of the control, $n=5$, $P>0.05$) or amplitude (Mino: 114 ± 21% of the control; Wash: 108 ± 14% of the control, $n=5$, $P>0.05$) during 20 min of the drug application (Figs. 2A, C, D). The distribution of both the sEPSCs amplitude and the inter-mEPSC intervals was not significantly shifted (Fig. 2B).

Neither bilirubin nor minocycline affected postsynaptic glutamate receptor sensitivity

To investigate the effects of bilirubin and minocycline on the sensitivity of glutamate receptors, exogenous glutamate (20 μM) was employed. The amplitudes of the evoked glutamate current (I_{Glu}) during (95 ± 5%, $n=4$, $P>0.05$) and after (99 ± 8%, $n=4$, $P>0.05$) 10-min bilirubin application (3 μM) were not significantly different from that in the control solution (Fig. 3A). The I_{Glu} amplitude during (96 ± 9%, $n=4$, $P>0.05$) and after (95 ± 7%, $n=4$, $P>0.05$) 20-min minocycline application (100 μM) also showed no significant change from that in the control solution (Fig. 3B). These results suggest that neither bilirubin nor minocycline affected glutamate receptor sensitivity in VCN neurons.

Minocycline could not suppress sEPSC potentiation induced by bilirubin

To study whether minocycline could influence bilirubin-induced potentiation of glutamate release, we observed the effect of minocycline

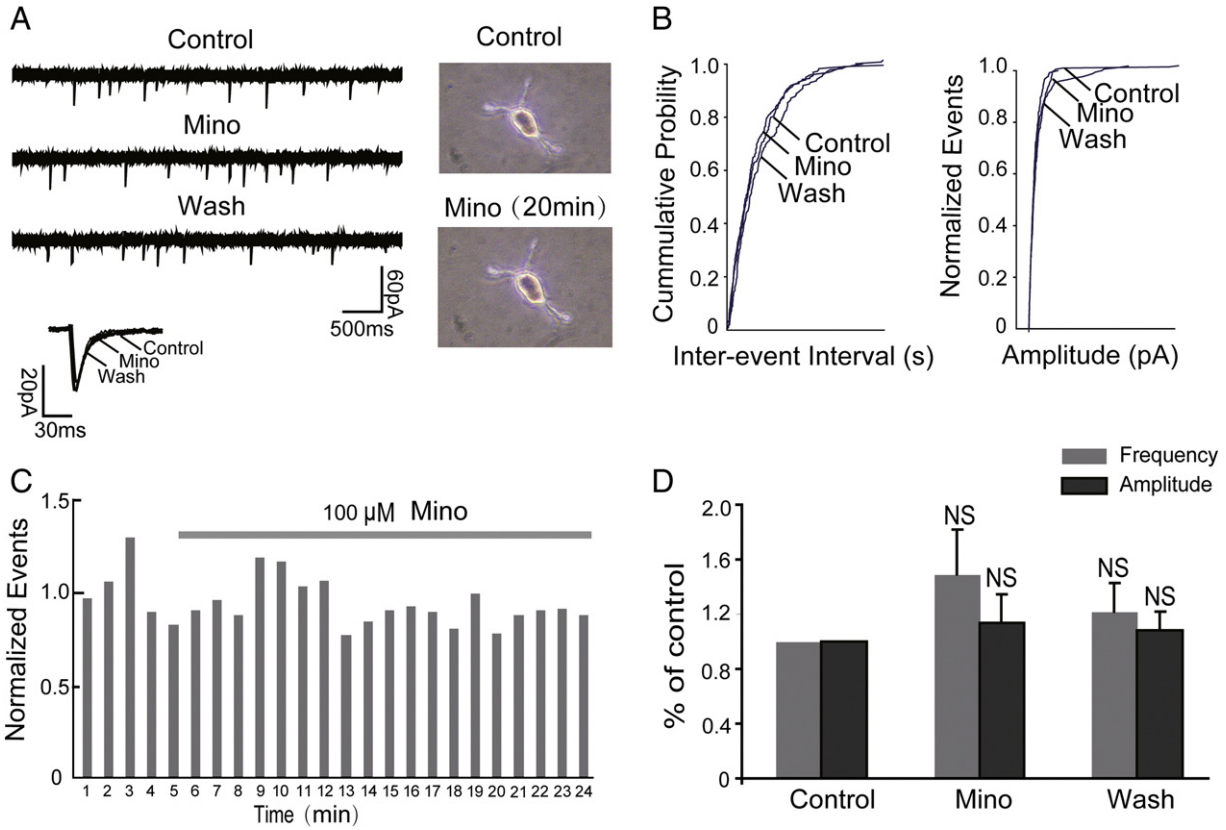

Fig. 2. Effects of minocycline (100 μM) on sEPSC frequency and amplitude in VCN neurons. A: left: a typical sEPSC trace observed before, during, and after minocycline application in a neuron. Inset: average sEPSC waveforms. Right: images of the same VCN neuron before and after minocycline treatment. B: cumulative probability distribution for inter-event intervals and current amplitudes of sEPSCs from the same neuron as in A. C: the time course of minocycline-induced changes in neuron sEPSC frequency, as in A. D: comparison of average sEPSC frequencies and amplitudes recorded from five neurons before, during (15 min), and after (4 min) minocycline application. Vertical error bars represent SD; **, $P<0.01$, NS: not significant.

on bilirubin-induced increase in sEPSC frequency. Dissociated VCN neurons were pretreated with different concentrations of minocycline (20–100 μM) (Baptiste et al., 2004; Pi et al., 2004) for different time periods before bilirubin application. Minocycline had no significant effect on sEPSC frequency, as bilirubin still considerably increased sEPSC frequency (20 μM, 1 h: 1422 ± 235%, $n=4$, $P<0.01$; 50 μM, 1 h: 1268 ± 211%, $n=4$, $P<0.01$; 100 μM, 1 h: 1499 ± 199%, $n=4$, $P<0.01$; 100 μM, 2 h: 1327 ± 322%, $n=5$, $P<0.01$; 100 μM, 3 h: 1568 ± 300%,

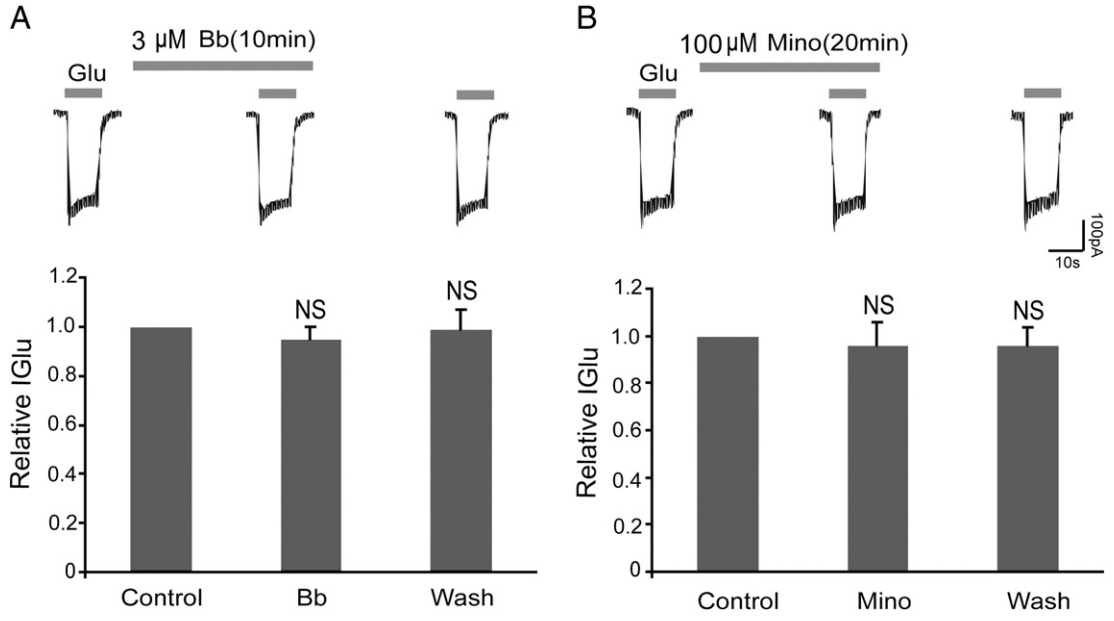

Fig. 3. The effects of bilirubin (3 μM) and minocycline (100 μM) on glutamate-evoked postsynaptic currents (I_{Glu}). A: top: typical I_{Glu} traces recorded from a VCN neuron before, during, and after bilirubin application. Bottom: normalized I_{Glu} amplitude from four neurons before, during, and after bilirubin application. B: top: typical I_{Glu} traces recorded from a VCN neuron before, during, and after minocycline application. Bottom: normalized I_{Glu} amplitude from four neurons before, during, and after minocycline application. Vertical error bars represent SD; NS: not significant.

$n=5$, $P<0.01$; Fig. 4A), just as bilirubin alone (1270 ± 222%, Fig. 1B). In the presence of minocycline, bilirubin still evoked action potential currents in all the recorded neurons. The sEPSC amplitude during bilirubin treatment alone or bilirubin treatment with minocycline pretreatment was not significantly different from that under the control condition (20 μM, 1 h: 124 ± 25%, $n=4$, $P>0.05$; 50 μM, 1 h: 112 ± 11%, $n=4$, $P>0.05$; 100 μM, 1 h: 99 ± 10%, $n=4$, $P>0.05$; 100 μM, 2 h: 98 ± 13%, $n=4$, $P>0.05$; 100 μM, 3 h: 127 ± 9%, $n=4$, $P>0.05$; Fig. 4B). These data suggest that bilirubin-induced potentiation of glutamate release was not significantly affected by minocycline.

Minocycline could not suppress neuronal hyperexcitation induced by bilirubin

Under current-clamp mode, we examined the action of bilirubin on spontaneous firing of VCN neurons. Most neurons recorded displayed spontaneous excitatory postsynaptic potentials and action potentials. Perfusing the neurons with 3 μM bilirubin dramatically increased the firing rates. The rate reached 646 ± 194% of the control level after 10 minute application ($n=4$, $P<0.01$; Fig. 5A, C). To further investigate whether bilirubin-induced hyperexcitation could be suppressed by minocycline, we examined the effect of bilirubin on action potentials in VCN neurons pretreated with minocycline (100 μM) for 3 h. The results showed that bilirubin increased action potential frequency in those neurons. The frequency was not significantly different from that under the bilirubin-alone condition (714 ± 262%, $n=4$, $P<0.01$; Fig. 5B, C). We did not observe an obvious change in the resting potential of these neurons during drug applications (data not shown).

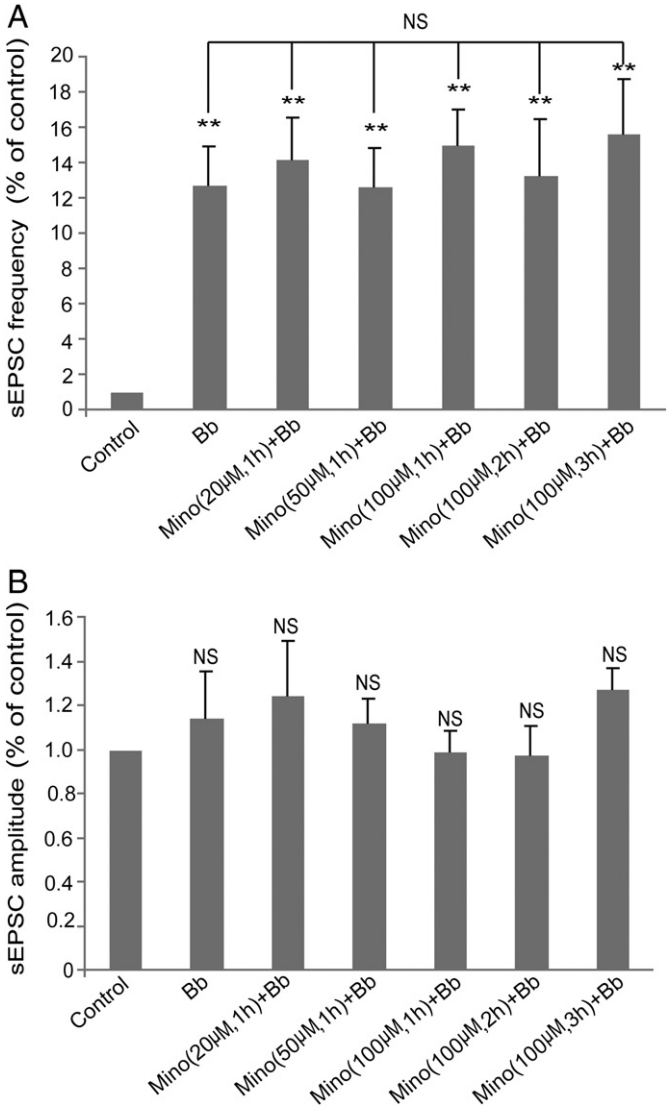

Fig. 4. The effects of minocycline pretreatment (100 μM) on bilirubin-evoked sEPSC potentiation in VCN neurons. A: summary data of sEPSC frequency before and during bilirubin application (10 min) and during bilirubin with minocycline pretreatment for different concentrations and time periods. B: summary data of sEPSCs amplitude before and during bilirubin application (10 min) and during bilirubin with minocycline pretreatment for different concentrations and time periods. Vertical error bars represent SD; **, $P<0.01$, NS: not significant.

Discussion

Glutamate excitotoxicity is a hypothesis that involves excessive synaptic release of glutamate and consequent overstimulation of glutamate receptors (Sattler and Tymianski, 2001). Excitotoxicity is proposed to be one of the main underlying mechanisms of bilirubin-induced neurological disorders (Grojean et al., 2000; Johnston, 2005; McDonald et al., 1998). Our previous study demonstrated that bilirubin increased presynaptic glutamate release and therefore evoked firing in neurons of the lateral superior olive (LSO; Li et al., 2011). The effect of bilirubin on neuronal electrophysiological activity has typically been characterized as "excitotoxic." In the present study, we found that bilirubin increased sEPSC frequency mediated by glutamate receptors and elicited VCN neuron action potentials, which is very similar to LSO neurons (Li et al., 2011). Minocycline broadly prevents neurons from excitotoxic cell death, including acute bilirubin-induced neurological dysfunction (Lin et al., 2005; Rice et al., 2011; Tikka et al., 2001). Contrary to our hypothesis that minocycline inhibited bilirubin-induced glutamatergic activity, the results of the present study showed that minocycline has no significant effects on sEPSCs, glutamate activated currents, neuronal firing, or the action of bilirubin on neuronal activity. The results suggest that minocycline was not able to inhibit either potentiation of glutamatergic synaptic transmission or neuronal hyperexcitation induced by bilirubin.

Considering the positive findings of minocycline on protection against excitotoxicity and the ability of minocycline to penetrate the blood–brain barrier, minocycline thus appears to be one of the most attractive and potentially novel therapies for many glutamate-induced neurological disorders such as bilirubin encephalopathy (Chen et al., 2000; Lin et al., 2005; Nabekura et al., 1996; Rice et al., 2011; Sanchez Mejia et al., 2001; Yrjanheikki et al., 1999). However, it is worth noting that some studies have shown inconsistent and even contradictory effects of minocycline. In these studies, minocycline had variable and even deleterious effects on many neurological disorders associated with glutamate excitotoxicity such as hypoxia–ischemia, Parkinson's disease, and Huntington's disease (Diguet et al., 2004a, 2004b; Goni-Allo et al., 2005; Tsuji et al., 2004; Yang et al., 2003). The contrasting effects of minocycline in these studies may be due in part to differences in the dose and mode of minocycline administration. However, our negative results cannot be attributed to these factors because in the present study, we consistently observed the negative effect of minocycline on glutamatergic transmission (i.e., no effect on sEPSC frequency and the sensitivity of glutamate receptors), neuronal excitability (i.e., no effect on resting membrane potential and neuron firing), and the potentiation of the neuron's excitatory activity induced by bilirubin (i.e., no effect on increased firing due to bilirubin treatment) with different administration modes (i.e., applied alone, applied with bilirubin, or pre-incubated), and exposure concentrations (i.e., 20 μM, 50 μM, or 100 μM) and period of time (i.e., 10 min, 20 min, 1 h, 2 h, or 3 h). With these parameters of minocycline application, neuroprotective effects have been observed in other studies (Baptiste et al., 2004; Pi et al., 2004; Song et al., 2006; Tikka and Koistinaho, 2001). Given that our results showed that minocycline does not influence neuronal excitability or bilirubin-induced hyperexcitation, which may directly lead to neuronal insults, additional work is required to further clarify the

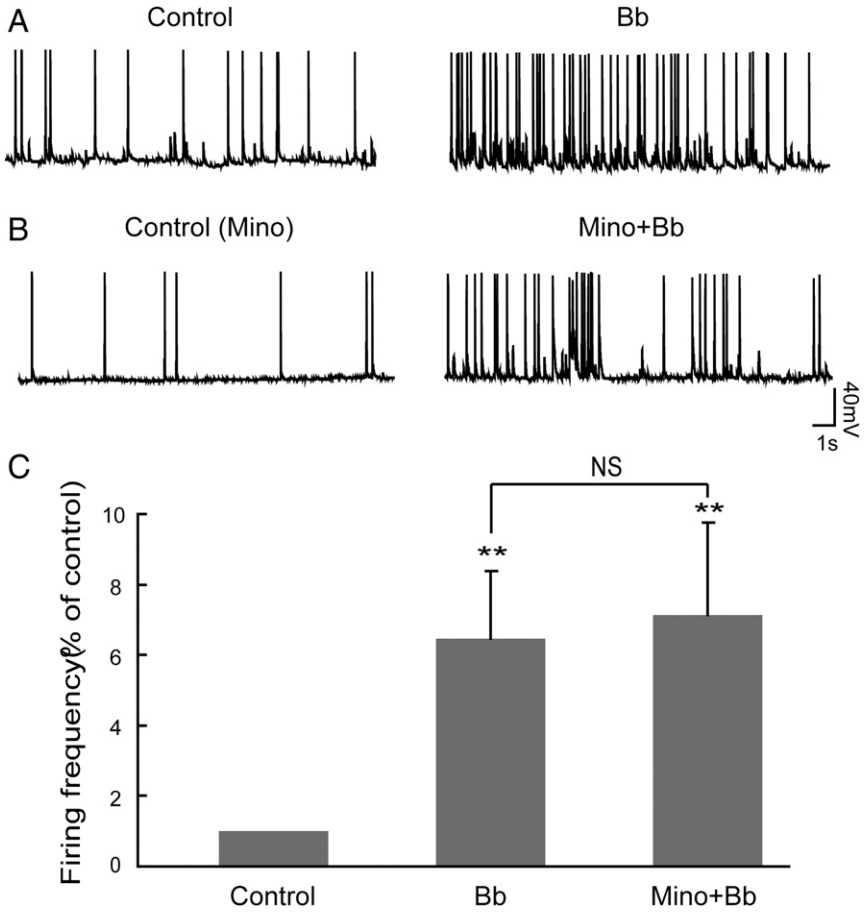

Fig. 5. The effect of minocycline pretreatment (100 μM) on bilirubin-induced potentiation of VCN neuron action potentials. A: spontaneous action potentials were recorded from a VCN neuron before and during bilirubin application. B: spontaneous action potentials were recorded from a VCN neuron before and during bilirubin application after 3 h of minocycline pretreatment. C: histograms depicting average firing frequency of four neurons before and during the bilirubin application (10 min; pretreated or not pretreated with minocycline). Vertical error bars represent SD; **, $P<0.01$, NS: not significant.

neuroprotective effects of minocycline in neurodegenerative disorders including bilirubin encephalopathy before embarking on further clinical trials.

In studies that showed neuroprotective effects of minocycline, the exact locus of minocycline protection was unclear. Our results may provide a better understanding of the mechanism of minocycline's protective effect. Previous studies confirmed that minocycline exerts neuroprotective effects independent of its anti-inflammatory properties (Maier et al., 2007). It has been reported that minocycline protects neurons against glutamate excitotoxicity through inhibition of microglial activation and proapoptotic mediators (i.e., cytochrome c release from mitochondria) as well as suppression of caspase-dependent and caspase-independent neuronal death pathways (Baptiste et al., 2004; Maier et al., 2007; Pi et al., 2004). Moreover, minocycline potently blocks bilirubin toxicity of cerebellar granule neurons through inhibition of p38 mitogen-activated protein kinase phosphorylation (Geiger et al., 2007). Based on our results in the present study showing that minocycline failed to suppress hyperexcitation induced by bilirubin, which may lead to apoptosis or necrosis, we suggest that the neuroprotective efficacy of minocycline is mediated by either an alternative mechanism or downstream events post neuronal hyperexcitation.

It has been shown that after hyperexcitation, some of the pathways by which calcium ions enter into the cell are dysfunctional (Arundine and Tymianski, 2003; Sattler and Tymianski, 2001; Szydlowska and Tymianski, 2010). Over-activation of glutamate ionotropic receptors leads to increased permeability to sodium, potassium, and most notably, calcium, which would result in membrane depolarization and further elevation of intracellular calcium. High intracellular calcium concentrations would activate numerous enzymes and lead to pathological neuronal injury (Arundine and Tymianski, 2003; Szydlowska and Tymianski, 2010). As our results showed that minocycline could not block bilirubin-induced potentiation of glutamatergic synaptic transmission and neuronal hyperexcitation, minocycline therefore should not inhibit the persistent elevation of calcium after bilirubin exposure. Consistent with this hypothesis, other studies have demonstrated that the neuroprotective actions of minocycline do not result from inhibition of glutamate receptors or rises in intracellular calcium through glutamate receptors (Baptiste et al., 2004; Pi et al., 2004; Song et al., 2006).

In conclusion, our results showed that minocycline failed to inhibit the bilirubin-induced potentiation of glutamatergic transmission and neuronal hyperexcitation in VCN neurons. These results may be helpful in determining the locus of minocycline's neuroprotective effect and also promote caution for carefully evaluating this drug before clinical use in humans, especially infants with kernicterus.

Acknowledgments

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