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Taurine protects against bilirubin-induced hyperexcitation in rat anteroventral cochlear nucleus neurons

Ning-ying Song [a,b], Chun-yan Li [a,*], Xin-lu Yin [a], Min Liang [a], Hai-bo Shi [a], Guo-ying Han [a], Shan-kai Yin [a,*]

[a] Department of Otorhinolaryngology, Affiliated Sixth People's Hospital of Shanghai Jiaotong University, 600 Yishan Road, Shanghai 200233, China
[b] Department of Otorhinolaryngology, West China Hospital, Sichuan University, Chengdu, China

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ABSTRACT

No effective medication for hyperbilirubinemia yet exists. Taurine is believed to exert a neuroprotective action. The aim of the present study was to determine whether taurine protected neurons of the rat anteroventral cochlear nucleus (AVCN) against bilirubin-induced neuronal hyperexcitation. AVCN neurons were isolated from 13 to 15-day-old Sprague–Dawley rats. The effects of bilirubin on the spontaneous excitatory postsynaptic currents (sEPSCs) and action potential currents were compared with those exerted by bilirubin and taurine together. Bilirubin dramatically increased the frequencies of sEPSCs and action potential currents, but not sEPSC amplitude. Taurine suppressed the enhanced frequency of action potentials induced by bilirubin, in a dose-dependent manner. In addition, taurine decreased the amplitude of voltage-dependent calcium channel currents that were enhanced upon addition of bilirubin. We explored the mechanism of the protective effects exerted by taurine using $GABA_A$ and glycine receptor antagonists, bicuculline and strychnine, respectively. Addition of bicuculline and strychnine eliminated the protective effects of taurine. Neither bilirubin nor taurine affected the sensitivity of the glutamate receptor. Our findings thus indicate that taurine protected AVCN neurons against bilirubin-induced neuronal hyperexcitation by activating the $GABA_A$ and glycine receptors and inhibiting calcium flow through voltage-gated channels. Thus, taurine may be effective in treatment of neonatal hyperbilirubinemia.

Introduction

Hyperbilirubinemia is triggered by accumulation of excess bilirubin in the blood. Most infants experience physiological jaundice during the first 2 weeks after birth, and some develop chronic bilirubin encephalopathy (kernicterus) (Shapiro, 2010). Deposition of bilirubin in certain brain regions causes temporary or permanent damage to neurons (Gourley, 1997; Ostrow et al., 2004; Watchko, 2006). Some structures of the central nervous system (CNS), particularly the hippocampus, the auditory nuclei, the anterior ventral cochlear nucleus (AVCN), the lateral superior olive, and the inferior colliculus, are highly sensitive to bilirubin and thus vulnerable to bilirubin-induced neurotoxicity (Li et al., 2011; McDonald et al., 1998; Shi et al., 2006). For example, the cochlear nuclear volume of homozygous (jj) Gunn rats, which exhibit congenital hyperbilirubinemia, is significantly less than that of heterozygous (Nj) rats (Conlee and Shapiro, 1991). Bilirubin directly affects the mitochondrial and plasma membranes, causing oxidative damage, and interrupts DNA and protein synthesis (Kashiwamata et al., 1980; Ostrow et al., 2004; Watchko, 2006; Yamada et al., 1977). Critically, bilirubin may cause excitotoxicity, triggering excessive influx of Na^+, Ca^{2+}, and water into the cell, in turn inducing cell swelling and death (Ostrow et al., 2004; Watchko, 2006). Glutamate, a major excitatory neurotransmitter of the CNS, contributes to bilirubin-induced excitotoxicity (Johnston, 2005). Although hyperbilirubinemia has been investigated intensively, no effective treatment for the condition has yet been identified.

Taurine is one of the most abundant free amino acids in the brain and exerts many physiological functions, including promotion of neuronal proliferation and differentiation, scavenging of free radicals, and regulation of membrane excitability (Chen et al., 1998; Godfrey et al., 2000; Hanna et al., 2004). Neuroprotection is one of the most important functions of taurine; the amino acid protects neurons from glutamate-induced excitotoxicity (Tang et al., 1996) and inhibits accumulation of excess intracellular Ca^{2+}, thus preventing neuronal damage (Kontro and Oja, 1988; Leon et al., 2009; Oja and Saransaari, 2007). Zhang et al. investigated cultured fetal neurons, and found that taurine reduced bilirubin-induced apoptotic cell death, maintained intracellular Ca^{2+} homeostasis, and aided cells to recover from bilirubin-induced damage (Zhang et al., 2010). Although the effects of taurine on neurons

Abbreviations: AVCN, anteroventral cochlear nucleus; sEPSC, spontaneous excitatory postsynaptic current; CNS, central nervous system; I_{Glu}, glutamate-evoked postsynaptic current; I_{Tau}, taurine-activated current; VGCC, voltage-dependent calcium channel.

* Corresponding authors. Fax: +86 21 64834143.
E-mail addresses: licycrystal@sina.com (C. Li), yinshankai@china.com (S. Yin).

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have been investigated extensively, the mechanism underlying the neuroprotective function remains poorly understood.

The AVCN, the first synaptic station along the central auditory pathway, plays important roles in the processing of peripheral auditory signals. In the present study, we recorded spontaneous excitatory postsynaptic currents (sEPSCs) and action potentials from AVCN neurons using the gramicidin-perforated patch-clamp technique. We found that bilirubin markedly increased the frequencies of sEPSCs, and firing, creating an excitotoxic effect. Further, we showed that taurine protected the neurons against bilirubin-induced hyperexcitation, and that addition of antagonists of the $GABA_A$ and glycine receptors eliminated this protective action. Taurine also inhibited ion flow through bilirubin-activated voltage-gated calcium channels. Together, the findings suggest that taurine may possibly be used to treat hyperbilirubinemia-induced neuronal excitotoxicity.

Materials and methods

All experimental protocols complied with institutional principles for the care and use of animals, and were approved by the Ethics Review Committee for Animal Experimentation at Shanghai Jiaotong University. All efforts were made to minimize animal suffering and the number of animals used.

Preparation of AVCN neurons

AVCN neurons were prepared as described previously (Rhee et al., 1994). Briefly, Sprague–Dawley rats (of either gender), 13–15 days old, were anesthetized with sodium pentobarbital (55 mg/kg, i.p.) and decapitated. Each brain was removed quickly and placed in ice-cold artificial cerebrospinal fluid (ACSF) containing the following (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. The brain region containing the AVCN was sectioned into coronal slices (300 μm thick) using a vibratome (VT-1000s, Leica). Brain slices were pre-incubated in ACSF saturated with 95% O_2/5% CO_2 (both v/v) at room temperature (24–26 °C) for 20–30 min. Neurons were next mechanically isolated using fire-polished glass pipettes and placed on 35-mm-diameter dishes (Corning) containing a solution comprising (in mM) 150 NaCl, 5 KCl, 2 $CaCl_2$, 1 $MgCl_2$, 10 glucose, and 10 HEPES. The pH of the solution was adjusted to pH 7.4 with Tris-base.

Reagents

Reagents used included free bilirubin, taurine, bicuculline, strychnine, and glutamate. All were purchased from Sigma (St. Louis, MO). Bilirubin was first dissolved in 0.1 M NaOH to a concentration of 1 mM, stored in single-use aliquots in the dark at −20 °C (for <24 h), and diluted to a final concentration of 3 μM immediately before use (Li et al., 2012). Bilirubin is photosensitive and all bilirubin solutions were protected from light at all times. Isolated neurons were exposed to various materials using a Y-tube system which completely replaced the existing solution with the material-containing solution within 10–20 ms (Nabekura et al., 1996).

Electrophysiological recordings

Dissociated neurons were bathed in a standard external solution and visualized using the phase-contrast mode of an inverted microscope (TE-2000U; Nikon, Tokyo, Japan). Gramicidin-perforated patch-clamp recordings were made with the aid of an amplifier (EPC 10; HEKA, Lambrecht/Pfalz, Germany). All sEPSCs were recorded in voltage clamp mode at a holding potential of −60 mV. Patch electrodes were pulled from borosilicate capillary glass using a vertical pipette puller (P-9; Narishige, Tokyo, Japan) and had a resistance of 4–7 MΩ. The patch pipette solution used to record sEPSCs and action potentials contained (in mM): 150 KCl and 10 HEPES (adjusted to pH 7.2 with Tris-base). Gramicidin was dissolved in methanol to 10 mg/ml, diluted to a final concentration of 50–80 μg/ml prior to application, and used within 2 h. Action potentials were recorded in the current clamp mode, and the injected current was zero. Voltage-dependent calcium channel (VGCC) currents were recorded in whole cells. The external solution contained 10 mM $CaCl_2$, 130 mM tetraethylammonium chloride, 5 mM 4-AP, 5 mM HEPES, 25 mM D-glucose, and 1 μM tetrodotoxin (pH 7.4); and the internal solution 105 mM CsCl, 40 mM HEPES, 5 mM D-glucose, 2.5 mM $MgCl_2$, 10 mM EGTA, 2 mM Mg-ATP, and 0.5 mM GTP (pH 7.2). Each VGCC current was activated by ramp depolarization from −80 to 60 mV in 10 mV increments, using test pulses 150 ms in duration. Capacitance and series resistance were corrected; the series resistance compensation was 80–94%. The peak current developing during step depolarization was considered to be the maximum current. Signals were filtered at 1–3 kHz, sampled at 3–10 kHz using a Dell computer equipped with Pulse 6.0 software (HEKA), and collected with the aid of the patch-clamp amplifier.

Data analysis

The numbers of sEPSCs (≥6 pA) and action potentials (≥40 mV) were automatically counted and analyzed using MiniAnalysis software (Synaptosoft, Leonia, NJ). The frequencies and amplitudes of all synaptic events (including action potentials) that developed during and after application of test materials were normalized and compared to control values using the Wilcoxon signed-rank test. The amplitudes of glutamate-evoked postsynaptic currents and peak VGCC currents were normalized to those of the control, averaged, and subjected to analysis using Student's paired *t*-test. All statistical analyses were performed with the aid of SPSS version 17.0 software (SPSS Inc., Chicago, IL). All values are expressed as means ± standard errors. A value of $P < 0.05$ was taken to indicate statistical significance.

Results

Bilirubin induced AVCN hyperexcitation

A blood bilirubin level of 3 μM causes encephalopathy in rats (Daood and Watchko, 2006). We tested the effects of bilirubin at this concentration on spontaneous firing of isolated AVCN neurons at the resting potential. Fig. 1 shows that the firing rate increased dramatically during addition of bilirubin. The firing rate was 694.0 ± 256.2% that of the control 10 min after bilirubin addition ($F = 34.543$, $P < 0.01$, $n = 6$) and remained higher than the control value (527.7 ± 311.3% of control) even 3 min after bilirubin washout. These values were not significantly different ($F = 7.569$, $P > 0.05$, $n = 6$) (Fig. 1C). Thus, bilirubin triggered hyperexcitation of AVCN neurons.

Spontaneous postsynaptic currents in dissociated AVCN neurons

Spontaneous postsynaptic currents (sPSCs) featuring two components were recorded in AVCN neurons clamped at −60 mV (Fig. 2A, top trace). The outward current (Fig. 2B, top trace) had a much longer time course and a slower decay time than did the inward sPSC (Fig. 2B, bottom trace). The former current was partially blocked by 10 μM bicuculline (a $GABA_A$ receptor antagonist) and completely blocked upon co-addition of 0.3 μM strychnine (a glycine receptor antagonist) (Fig. 2A, bottom two traces), suggesting that the outward PSCs were inhibitory in nature and were mediated by both the $GABA_A$ and glycine receptors. The inward PSCs were partially suppressed by 50 μM APV (an NMDA receptor antagonist) and completely blocked upon co-addition of 10 μM NBQX (an AMPA receptor antagonist) (Fig. 2A, top; second and third traces), indicating that the inward PSCs were excitatory and mediated by both the NMDA and AMPA receptors.