Neuroscience Letters 559 (2014) 152–157

Contents lists available at ScienceDirect

Neuroscience Letters

journal homepage: www.elsevier.com/locate/neulet

Postnatal developmental changes in the medial nucleus of the trapezoid body in a mouse model of auditory pathology

Calum A. Grimsley, Shobhana Sivaramakrishnan*

Department of Anatomy and Neurobiology, Northeast Ohio Medical University, Rootstown, OH 44272, United States

HIGHLIGHTS

- Synaptic transmission was examined in the auditory brainstem of DBA/2 mice with early onset hearing loss.
- Mice exhibited very early loss of high-frequency hearing and raised hearing thresholds.
- With the onset of hearing loss, postsynaptic currents and spikes were abnormally prolonged.

ARTICLE INFO

Article history:
Received 5 August 2013
Received in revised form
13 November 2013
Accepted 26 November 2013

Keywords:
MNTB
Auditory system
AHL
DBA/2
Auditory brainstem responses

ABSTRACT

Age-related hearing loss (AHL) is a multifactorial disorder characterized by a decline in peripheral and central auditory function. Here, we examined synaptic transmission in DBA/2 mice, which carry the AHL8 gene, at the identifiable glutamatergic synapse in the medial nucleus of the trapezoid body (MNTB), a nucleus in the superior olivary complex critical for acoustic timing. Mice exhibited raised auditory brainstem thresholds by P14, soon after hearing onset. Excitatory postsynaptic currents were prolonged; however, postsynaptic excitability was normal. By P18, high-frequency hearing loss was evident. Coincident with the onset of hearing loss, MNTB principal neurons displayed changes in intrinsic firing properties. These results suggest that changes in transmission in the superior olivary complex are associated with early onset hearing loss.

1. Introduction

Genetic animal models of hearing loss arising from congenital or early onset deafness exhibit pathophysiology in the auditory brainstem. In the deaf mouse strain (dn/dn), synaptic currents at end-bulb synapses in the anterior ventral cochlear nucleus are enhanced, and high frequency stimulation evokes strong synaptic depression [15]. In congenitally deaf cats, nerve terminals at end-bulb synapses are more compact with fewer synaptic vesicles [16].

The DBA/2 mouse which expresses the AHL8 (age-related hearing loss 8) mutation [6,14] is a frequently studied model of hearing loss. Hearing loss, measured with tones up to 32 kHz, begins around P21, and progresses rapidly to an almost complete loss by P45 [23]. In the ventral cochlear nucleus, glutamatergic transmission is compromised following complete deafness at >P60; however, transmission is normal during the second postnatal week, close to hearing onset [21].

We examined synaptic transmission in the medial nucleus of the trapezoid body (MNTB), a component of the superior olivary complex, in the DBA/2 mouse. The glutamatergic MNTB synapse is made between afferents from the cochlear nucleus and MNTB principal neurons [12]. Auditory brainstem thresholds increased soon after hearing onset, by P13, with early high-frequency hearing loss at >48 kHz, by P16. Prolonged synaptic responses and loss of onset spiking in MNTB principal neurons indicated a predisposition to abnormal transmission.

2. Methods

Animal husbandry and experimental procedures were approved by the Institutional Animal Care and Use Committees at Northeast Ohio Medical University and were performed in accordance with guidelines published by the U.S. National Institutes of Health.

* Corresponding author at: Department of Anatomy and Neurobiology, Northeast Ohio Medical University, 4209 State Route 44, Rootstown, OH 44272, United States. Tel.: +1 330 325 6639; fax: +1 330 325 5916.
E-mail address: ssivaram@neomed.edu (S. Sivaramakrishnan).

0304-3940/$ – see front matter © 2013 Elsevier Ireland Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.neulet.2013.11.051

2.1. Slice preparation

We compared activity in the MNTB of DBA/2 mice with control CBA or CBA/Ca mice. 9–25 day old pups were anesthetized with isoflurane, the brain removed, and 200 μm thick transverse slices made through the MNTB. Slices were cut, stored and recorded from at 35 °C [17] in artificial cerebrospinal fluid (ACSF) containing (in mM): 120 NaCl, 3 KCl, 2 $CaCl_2$, 1.3 $MgSO_4$, 1 NaH_2PO_4, 10 $NaHCO_3$, 25 glucose, pH 7.35. Whole-cell patch clamp recordings were made under visual control using Nomarski optics and a 40× objective fitted to an upright Zeiss Axioskop. Trapezoid fibers were stimulated extracellularly with a bipolar tungsten electrode and recordings made under current- and voltage-clamp from the soma of contralateral MNTB principal neurons. Principal neurons were identified visually and by the presence of a pre-spike that preceded the EPSC.

Patch pipettes (4–5 MΩcontained (in mM): 120 K gluconate, 10 KCl, 5 NaCl, 0.2 EGTA, 0.1 $CaCl_2$, 0.3 Na-GTP, 2 Mg-ATP, 10 HEPES, pH 7.35; ~110 nM free Ca^{2+}. A junction potential correction of −11 mV was applied to all recordings. Series resistances were ≤9 MΩ and compensated by >80% (10 μs lag). EPSC decay time constants in control CBA MNTB neurons were similar in recordings with 6 and 9 MΩ series resistances ($t_{15} = 0.34$; $p = 0.74$). Average series resistances in the population were similar for CBA and DBA/2 MNTB neuron recordings (6.7 ± 2.2 MΩ and 6.5 ± 2.5 MΩ, respectively). Data from CBA and CBA/Ca mice were not statistically different and were pooled into one group. Data are reported from 138 neurons; 24 from 18 CBA mice, 36 from 12 CBA/Ca mice, and 62 from 28 DBA/2 mice.

Data collection and analyses were carried out with an EPC-10 amplifier (HEKA Elektroniks/Instrutech Corporation), Patchmaster/Fitmaster (HEKA Electroniks) and Minianalysis (Synaptosoft, Decatur, GA) software. Data were filtered at 5 kHz during acquisition. Chemicals were obtained from Sigma–Aldrich (St. Louis, MO, USA).

2.2. Analyses of synaptic responses and firing patterns

Synaptic currents were averaged in each slice over 6–10 trials at 1/5 s, then averaged across slices. PSC decay time constants (τ) were weighted from double exponential fits: $I(t) = I_f e^{-t/\tau(f)} + I_s e^{-t/\tau(s)}$, where I_f and I_s are the peak currents of the fast and slow components of the PSC. The weighted mean of the fast and slow decay time constants, $\tau(f)$ and $\tau(s)$, were calculated from the equation, $\tau = \tau_f \{I_f/(I_f + I_s)\} + \tau_s \{I_s/(I_f + I_s)\}$. Most DBA/2 PSCs were best fit by a single exponential function $I(t) = I_{max} e^{-t}/^{\tau}$, where I_{max} is the peak PSC current. In DBA/2 mice at ages <P13, decay times were fit by two exponents; thus weighted means are reported. For each EPSC, goodness of fit, calculated using Origin software, was determined from the r^2 value for the first and second time constant. Decay time constants with $r^2 > 0.98$ are reported.

Miniature synaptic currents were identified using threshold detection and analyzed with Minianalysis software (Synaptosoft). Origin software was used to construct amplitude distributions, perform Gaussian fitting and determine means and standard deviations. To determine the threshold for intrinsic MNTB firing, injected currents were altered in 5 pA increments. Threshold voltage was defined as the voltage just prior to the voltage that generated the first full spike and was identified by a small spikelet at the onset. The rate of rise of the membrane potential to threshold was measured at the voltage that evoked the first full spike. Spike widths of intrinsically evoked spikes were measured at half maximum spike height, from baseline to peak.

Results are expressed as mean ± standard error of the mean. Standard deviation, when used, is indicated in the text. Significance was determined using paired *t*-test or ANOVA; $p < 0.05$ was used as a criterion for significance and the Bonferroni correction factor applied. Normality was confirmed before statistical testing.

2.3. Measurement of auditory brainstem responses (ABRs)

Mice were anesthetized IM with ketamine (20–40 mg/kg) and xylazine (2.5 mg/kg) and monitored for anesthetic level. ABRs were recorded with two sterile tungsten wires (0.005 in. in diameter, etched to ~0.001 in. in diameter) inserted under the skin, behind an ear, and on top of the head.

Custom software (Batlab; Dr. D. Gans, NEOMED) was used to generate tone bursts and acquire ABRs. Sound was delivered through a loudspeaker placed 10 cm in front of the animal at 15° to the midline. Acoustic stimuli were digitally synthesized and downloaded onto a digital signal processing card, converted to analog signals, filtered, attenuated, summed, amplified, and sent to a loudspeaker (Tucker-Davis Technologies). Acoustic output was calibrated over 10–120 kHz with a condenser microphone placed in a position normally occupied by the animal's head. 0 dB attenuation at 4 kHz corresponded to 109 dB SPL, at 40 kHz to 101 dB SPL, at 50 kHz to 93 dB SPL and at 80 kHz to 69 dB SPL. We used a maximum tone frequency of 64 kHz. ABRs were recorded in a sound-proof chamber (Gretchken Industries) lined with foam. Sound pressure levels were corrected for speaker drop-off. Harmonic distortion was not detectable 60 dB below the signal intensity using a fast Fourier analysis of the digitized microphone signal.

ABRs were measured using 10–96 dB SPL tones in 5 or 10 dB steps. Tones were 5 ms long with a 0.5 ms cosine rise and fall. Inter-tone intervals were 15 ms. Tone frequencies were sequentially delivered from 4 to 64 kHz and repeated 300 times at 4 s^{-1} at each sound level. If the same pup was used more than once, we allowed 4–5 days between recordings. During each recording session, ABR protocols were repeated at least twice. Responses were identified as ABRs if they were ≥4 times the standard deviation of baseline noise.

3. Results

3.1. Developmental changes in DBA/2 auditory brainstem responses

The DBA/2 mouse exhibits pronounced hearing loss at >P60 [8,22]. Hearing thresholds increase at ~P25 with a loss of response at 32 kHz [23]. To determine if hearing loss began earlier, we recorded ABRs over a wider frequency range (4–64 kHz) each day between P9 and P30 (3 mice at each postnatal age).

In both CBA/Ca and DBA/2 mice, hearing onset occurred at P11, with an ABR at 16 kHz at 96 dB SPL (Fig. 1). The frequency range of CBA/Ca ABRs increased with age. Responses to 4 kHz first occurred at P16 and were maintained until P30. Responses to 64 kHz were observed by P19, and the 4–64 kHz range was maintained until P30 (Fig. 1B, top and C, top, middle). ABR thresholds decreased with age, reaching the lowest values (e.g. 36 dB SPL to the 16 kHz tone) at P18, and remained low until P30 (Fig. 1C, bottom panel).

High-frequency hearing loss occurred early in the DBA/2 mouse. Between P11 and P13, ABRs were evident between 4 and 40 or 48 kHz. The frequency range dropped with age, and the 16 kHz tone was the highest frequency that evoked an ABR at P30 (Fig. 1C, top panel). Responses at 4 kHz were normal until P30 (Fig. 1C, middle panel). By P16, the frequency range dropped, and responses to 56 kHz and higher frequencies were absent (Fig. 1C, middle panel).

ABR thresholds increased soon after hearing onset (P11). At P11, the threshold for the 16 kHz response, 96 dB SPL, was similar to control animals. Beginning at P13, ABR thresholds increased. The