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Auditory deprivation modifies the expression of brain-derived neurotrophic factor and tropomyosin receptor kinase B in the rat auditory cortex

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Abstract

The development and plasticity of central auditory system can be influenced by the change of peripheral neuronal activity. However, the molecular mechanism participating in the process remains elusive. Brain-derived neurotrophic factor (BDNF) binding with its functional receptor tropomyosin receptor kinase B (TrkB) has multiple effects on neurons. Here we used a rat model of auditory deprivation by bilateral cochlear ablation, to investigate the changes in expression of BDNF and TrkB in the auditory cortex after auditory deprivation that occurred during the critical period for the development of central auditory system. Reverse transcription-quantitative polymerase chain reaction (RT-qPCR) and immunohistochemistry methods were adopted to detect the mRNA and protein expression levels of BDNF and TrkB in the auditory cortex at 2, 4, 6 and 8 weeks after surgery, respectively. The change in the expression of BDNF and TrkB mRNAs and proteins followed similar trend. In the bilateral cochlear ablation groups, the BDNF-TrkB expression level initially decreased at 2 weeks but increased at 4 weeks followed by the reduction at 6 and 8 weeks after cochlear removal, as compared to the age-matched sham control groups. In conclusion, the BDNF-TrkB signaling is involved in the plasticity of auditory cortex in an activity-dependent manner.

Keywords: Central plasticity; Brain-derived neurotrophic factor; Tropomyosin receptor kinase B; Auditory deprivation; Auditory cortex

1. Introduction

Hearing loss during development leads to profound changes in sound discrimination speech perception and language acquisition (Sanes and Bao, 2009; Sanes and Woolley, 2011). The normal hearing function relies on the precise transmission of external auditory signals from peripheral auditory organs to the central auditory system. Accumulating evidence in recent years has shown that manipulation of peripheral auditory input subsequently produces plastic changes in the central auditory pathway of humans and animals (Butler and Lomber, 2013; Chen and Yuan, 2015). However, the plasticity of central auditory system is not maintained the same degree throughout life. There are specific time windows referred to as critical periods when certain plastic changes in the auditory center must occur and cannot be completely compensated later in life (Kral, 2013). In rats, the onset of auditory function as measured by auditory brainstem response (ABR) starts on postnatal day (P)12–14 and reaches to the adult level at round P22 days (Geal-Dor et al., 1993). During this critical period (P12–22) for auditory development, the plasticity of the rat auditory cortex is considerably higher than during other periods. It has been shown that at least five mechanisms may contribute to the central auditory plasticity in the sensitive/critical periods based on previous work on animals and humans (Kral, 2013).

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To study the plasticity of auditory center after aural deprivation, one of the most common *in vivo* animal models is complete or partial ablation of cochleae. The auditory deprivation leads to changes of gene and protein expression, synaptic transmission, morphology and functions in the central auditory system including the auditory cortex (Hildebrandt et al., 2011; Janz and Illing, 2014; Lee and Godfrey, 2014; Oh et al., 2007; Park et al., 2016).

Brain-derived neurotrophic factor (BDNF), the best characterized neurotrophin, is expressed and released both in the peripheral and central auditory system, which is dependent on neural activity (Singer et al., 2014). The onset of BDNF expression in the rodent auditory cortex is between P8 and P15 (Baquet et al., 2004). BDNF binds to its high-affinity receptor, tropomyosin receptor kinase B (TrkB) forming homodimers, and activates several complex intracellular signal transduction cascades. The early auditory experience that initiates the long-lasting inhibitory potentiation in the auditory cortex is critical for achieving spatiotemporal resolution in the central auditory system. The increased intra-cortical inhibition is largely dependent on the cortical BDNF levels and can be blocked by TrkB receptor inhibitor (Xu et al., 2010). Since the expression of BDNF and TrkB have been shown to be strongly affected by neuronal activity (Nagappan and Lu, 2005), our study aimed to investigate the alteration in mRNA and protein expression of BDNF and TrkB in the rat auditory cortex at 2, 4, 6 and 8 weeks after bilateral cochlear ablation.

2. Materials and methods

2.1. Experimental animals

Forty-eight male Special-Pathogen-Free (SPF) rats, 2 weeks old, were used in this study, provided by the department of experimental animal, Hebei Medical University. All animal experiments were performed following approved protocol for care and use of animals by the Laboratory Animal Care of Hebei Medical University (Hebei Province, China). Otoscopic examinations were performed to exclude any visible middle ear infection.

2.2. Cochlear ablation

Experimental animals were deeply anaesthetized with 5% chloral hydrate (8 mg/kg body weight). Bilateral cochlear removal was performed in rats (n = 32) placed on a heating pad under the dissecting microscope. A retro-auricular skin incision was made and the musculi colli and facial nerve were separated. A small hole was drilled and expanded on the bulla until the cochlea was visible. The bony cochlear wall was penetrated and the modiolus and osseous spiral lamina were destroyed. After surgery, the skin was sutured and animals were allowed to recover and housed under the same conditions as before surgery. In age-matched sham-operated control animals (n = 16), the skin was incised but the bulla was not opened. The use of sham-operated animals as controls is to examine the incidental effects due to for example anesthesia and surgical trauma. After postoperative survival periods of 2, 4, 6, 8 weeks, the cochlea-ablated and sham control rats were used for immunohistochemistry and revere transcription-quantitative polymerase chain reaction (RT-qPCR) analysis as below.

2.3. Auditory brainstem response (ABR)

Before surgery and 2 weeks after surgery, click-evoked ABR recordings were performed in all animals to test hearing thresholds (ICS, CHARTR, USA). All recordings were completed in a sound-attenuating, electrically shielded room. Rats were anaesthetized with intraperitoneal injections of 5% chloral hydrate and three recording electrodes were placed on different locations of experimental animals (Vertex-positive; Pinna-negative; Apex nasi-ground). Click stimuli with a duration of 100 μs were presented to a microphone which was placed at the opening of external acoustic meatus. The system was calibrated so that the maximum sound level maintained at 97 dB sound pressure level (SPL). Click stimuli were presented at a rate of 21.1 clicks per second, and the recorded responses were amplified 50 K times. The auditory threshold level was determined by recording the evoked responses at 15−97 dB SPL (15, 20, 40, 60, 80, 97 dB SPL) from the maximum intensity of 97 dB SPL. The auditory threshold was defined as the minimal click stimulus intensity that evoked response amplitude with more than 0.25 μV for wave V of the ABR trace. If the ABR waveform is not visible at the maximum stimulus level 97 dB SPL, we define the ABR threshold as 97 dB SPL. The ABR threshold is below 21 dB SPL in both bilateral cochlear ablation and sham control groups before surgery.

2.4. Reverse transcription-quantitative polymerase chain reaction (RT-qPCR)

After postoperative survival periods of 2, 4, 6, 8 weeks, animals from the bilateral cochlear ablation group (n = 4) and age-matched sham control group (n = 2), were deeply anaesthetized with 5% chloral hydrate and decapitated. Brains were extracted, the auditory cortex was dissected out bilaterally and immediately frozen in liquid nitrogen and stored at −80 °C. Total RNA was isolated with Trizol reagent. The quality of total RNA was checked with electrophoresis. cDNAs were generated by reverse transcription of total RNAs with oligo(dT) primers. Quantitative PCR was carried out on an ABI Prism 7500 PCR instrument. The PCR cycle parameters included 95 °C for 10 min, followed by 40 cycles consisting of 95 °C for 15 s, 55 °C for 30 s and 72 °C for 15 s for 1 min. The relative quantification of transcript expression was carried out using the comparative CT (cycle threshold) method. β-actin (*Actb*) was used as the reference gene. The CT of the target gene was calibrated against that of β-actin amplified in parallel from the same sample ($CT^{target} - CT^{\beta\text{-actin}} = \Delta CT$). Relative amounts of the target gene in the bilateral cochlear ablation group were normalized to the sham control group ($\Delta CT^{ablation} - \Delta CT^{sham\ control} = \Delta\Delta CT$). The change of target gene expression was calculated as $2^{-\Delta\Delta CT}$.

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