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).
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 cochlea. 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 reverse 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 \((\text{Actb})\) 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 \((\text{CT}_{\text{target}} - \text{CT}_{\beta-\text{actin}} = \Delta\text{CT})\). Relative amounts of the target gene in the bilateral cochlear ablation group were normalized to the sham control group \((\Delta\text{CT}_{\text{sham control}} = \Delta\Delta\text{CT})\). The change of target gene expression was calculated as \(2^{-\Delta\Delta\text{CT}}\).
The following PCR primers are used: BDNF, forward, 5'-GGTCACACGCGAGATAAA-3', reverse, 5'-CCGAACATACGATTGGGT-3'; TrkB, forward, 5'-GACGCTATAAGCAGCC-3', reverse, 5'-GCGTACCCTGAAGCTCAT-3'; β-actin (Actb), forward, 5'-GGAGATTACGTGCCCTGGCTCCTA-3', reverse, 5'-GACTCATGCTACTCCTGCTGCTG-3'.

2.5. Immunohistochemistry

It is well accepted that the gene's mRNA expression level certainly does not always correlate with its protein level (Vogel and Marcotte, 2012), so immunohistochemistry was used to detect the protein expression. Animals from the bilateral cochlear ablation group (n = 4) and age-matched sham control group (n = 2) after postoperative survival periods of 2, 4, 6, 8 weeks were deeply anesthetized with 5% chloral hydrate and perfused transcardially with 0.9% sodium chloride, followed by 4% paraformaldehyde in phosphate-buffered saline (PBS). Brains were removed and further postfixed in the same fixative overnight at 4 °C. Brains were washed with PBS and dehydrated in gradient alcohol, immersed in dimethylbenzene, and embedded in paraffin, sectioned in the anterior to posterior direction until the gyrus of the hippocampus could be observed. Sections were initially cut at 10 μm and every fifth section was stained with hematoxylin and eosin (H&E) to identify the starting point where the CA3 region of the hippocampus could be observed lying directly opposite to the perirhinal sulcus. The auditory cortex extends 1.02 mm anterior and 2.14 mm posterior from this point (Paxinos and Watson, 1998). Using the microtome, serial paraffin sections of 4 μm thickness were obtained.

A streptavidin-peroxidase (SP) method was used for immunohistochemical detection. Brain sections were immersed in 3% hydrogen peroxide at room temperature for 30 min to quench endogenous peroxidase activity, rinsed in 0.01 M PBS, blocked with normal goat serum at 37 °C for 30 min, and incubated with primary antibodies (rabbit anti-rat BDNF polyclonal antibody, Santa Cruz, SC-20981; rabbit anti-rat TrkB polyclonal antibody, Bioworld, BS1431; dilution ratio 1: 100) at 4 °C overnight. On the following day, sections were washed with 0.01 M PBS, incubated with goat anti-rabbit antibody for 30 min at 37 °C, dropped with Horse Radish Peroxidase (HRP) conjugated pronase avidin working solution for 30 min at 37 °C and then 3,3'-diaminobenzidine (DAB) substrate was applied. The staining reactions were stopped by distilled water. All sections were counterstained with hematoxylin, dehydrated with gradient alcohol and sealed with neutral resin. For negative control, the primary antibody was replaced with 0.01 M PBS. Stained sections were observed and images were taken under an optical microscope. Image-pro-plus 5.1 software was used to measure the optical density values.

2.6. Statistical analysis

The data were shown as mean ± standard deviation. A one-way ANOVA test was used to determine the statistical differences among all groups at different postoperative survival periods, and a student t-test was used to compare between cochlear ablation and sham control groups at each postoperative survival period. Statistical analysis was performed with SPSS 13.0 software and P < 0.05 was considered as a statistically significant difference.

3. Results

3.1. Auditory brainstem response (ABR) after bilateral cochlear ablation

Two weeks after the surgery, ABRs were measured to confirm successful elimination of peripheral hearing by the bilateral cochlear ablation procedure. In the control animals after the sham surgery, the ABR recordings showed typical five-peak (I–V) waves evoked by click stimuli (Fig. 1A) and the average threshold was 20 ± 3.78 dB SPL (Fig. 1C). In contrast, the bilateral cochlear removal completely eliminated all ABR waves (Fig. 1B) with the average threshold 90.68 ± 5.78 dB SPL, which was significantly higher than that in the sham control animals (Fig. 1C). These results indicate that there was no activity generated by auditory nerve and auditory nuclei in the brainstem and middle brain after the bilateral cochlear ablation.

3.2. Time-dependent change of BDNF mRNA and protein expression in the auditory cortex after bilateral cochlear ablation

To determine the regulation of the BDNF mRNA expression levels in the auditory cortex after the bilateral cochlear ablation, the BDNF mRNA levels were measured by RT-qPCR in animals at 2, 4, 6 and 8 weeks after the bilateral cochlear removal and normalized to time-matched sham surgery controls. The relative BDNF mRNA expression levels were initially reduced to approximately half of time-matched sham controls at 2 weeks but later increased to 1.87-fold of time-matched sham controls at 4 weeks and returned to similar levels as in sham controls 6 weeks after surgery (Fig. 2A). At 8 weeks, the BDNF mRNA levels in study animals were further decreased to approximately half of time-matched sham controls (Fig. 2A). The BDNF protein expression levels in the auditory cortex were also investigated by immunohistochemistry with an antibody specifically against BDNF. The auditory cortex in rats comprises 6 layers from exterior to interior: molecular layer, outer granulose cells layer, outer vertebral somatic cells layer, inner granulose cells layer, inner vertebral somatic cells layer and polymorphic cells layer. The immunoreactivity of BDNF was observed in all cell layers except for the molecular layer and localized to the somata (Fig. 2B). The optical density of BDNF immunostaining in neurons of the auditory cortex was quantified and compared between groups. The average expression of BDNF protein in study animals was significantly reduced at 2 weeks (0.311 ± 0.051 vs. sham controls 0.543 ± 0.05, p < 0.05) but dramatically increased at 4 weeks (0.623 ± 0.032 vs. sham controls...
0.334 ± 0.026, p < 0.05) as compared to time-matched sham controls (Fig. 2C). At 6 weeks, the BDNF protein level in study animals returned to the same level as in sham controls (0.342 ± 0.046 vs. sham controls 0.332 ± 0.027). However, the expression of BDNF was further reduced and significantly lower than sham controls (0.226 ± 0.021 vs. sham controls 0.328 ± 0.031, p < 0.05) at 8 weeks (Fig. 2C).

### 3.3. The temporal alteration of TrkB mRNA and protein expression in the auditory cortex after bilateral cochlear removal

The change of TrkB mRNA and protein expression in the auditory cortex post bilateral cochlear removal was also examined with RT-qPCR and immunohistochemistry. The relative TrkB mRNA expression levels were initially reduced to almost half of time-matched sham controls at 2 weeks but increased to 1.54-fold of time-matched sham controls at 4 weeks (Fig. 3A). At 6 weeks, the TrkB mRNA levels in study animals were still increased to 1.2-fold of sham controls but decreased to half of time-matched sham controls at 8 weeks (Fig. 3A). Regulation of TrkB protein levels after cochlear removal was analyzed by immunohistochemistry and the optical density of TrkB-positive neurons was quantified in the auditory cortex (Fig. 3B and C). The average expression of TrkB protein in auditory cortex of study animals dropped significantly at 2 weeks (0.343 ± 0.039 vs. sham controls 0.546 ± 0.029, p < 0.05) but dramatically increased at 4 weeks (0.619 ± 0.026 vs. sham controls 0.323 ± 0.022, p < 0.05) as compared to time-matched sham control group (Fig. 3C). At 6 weeks, the TrkB protein level in study animals returned to the similar level as in sham controls (0.311 ± 0.039 vs. sham controls 0.325 ± 0.025). However, at 8 weeks, the expression of TrkB in study animals was further reduced and significantly lower than sham controls (0.236 ± 0.056 vs. sham controls 0.323 ± 0.026, p < 0.05) (Fig. 3C).

The temporal alteration of TrkB mRNA and protein expression coincided with the change of BDNF in the auditory cortex post bilateral cochlear removal.

### 4. Discussion

In the present study, we have shown that the bilateral cochlear ablation in rats leads to the initial decrease of BDNF-TrkB mRNA and protein expression in the auditory cortex at 2 weeks and subsequent increase at 4 weeks followed by the reduction at 6 and 8 weeks, as compared to the age-matched sham control groups.
Auditory experience plays an important role in the development of the central auditory system. It has been shown in rats that pure tone exposure from postnatal day 11–14 altered sound intensity representations in the primary auditory cortex (A1) (de Villers-Sidani et al., 2007). The attenuation or elimination of peripheral auditory signals especially during the critical period leads to the morphological and functional changes in the central auditory pathway. In order to study the plasticity of the auditory center, cochlear ablation is a common method to establish the in vivo aural deprivation model. In a mouse model of bilateral cochlear ablation, the volume of cochlear nucleus was dramatically reduced due to the significant loss of neurons 4 months after auditory deprivation (Zhang et al., 2009). Another study has shown that cochlear ablation in gerbil pups at P10 results in impaired GABAergic transmission in pyramidal neurons in the thalamorecipient auditory cortex at later stages, which contributes to auditory plasticity when deafness (Kotak et al., 2008).

Furthermore, auditory deprivation also changes the expression of an array of genes and proteins in the auditory cortex. A very recent study has shown specific neurofilament proteins in the rat auditory cortex have significantly increased 6 and 12 weeks after bilateral auditory deprivation but have not changed after inducing unilateral auditory deprivation (Park et al., 2016). A microarray study has revealed many gene transcripts have altered in the primary auditory cortex after the bilateral cochlear ablation during the early critical period, including neural plasticity genes such as BDNF whose gene expression level is initially decreased at 2 weeks but increased at 4 weeks and further reduced at 12 weeks (Oh et al., 2007). This study coincides with our results on the change of BDNF mRNA transcripts in the auditory cortex after cochlear removal. It is well accepted that the gene's mRNA expression level certainly does not always correlate with its protein level. In addition, we further demonstrate the change of BDNF protein followed the same trend as for its gene transcript.

The spatial and temporal expression of BDNF mRNA and BDNF protein is regulated by development and neuronal activity (Lu, 2003). The level of BDNF in the rat auditory cortex varies at different developmental stages as measured by an enzyme-linked immunosorbent assay (ELISA), which is low before and during the critical period (postnatal day 7 and 12 respectively) and increases in adults (postnatal week 14) (Zhou et al., 2011). The immunohistochemical staining of BDNF in the control rats from the present study has shown a higher expression at 2 weeks as compared to 4, 6, and 8 weeks after the sham operation, which further confirms age-dependent expression of BDNF in the normal auditory cortex. The activity-dependent regulation of BDNF expression is supported by previous studies (Lu, 2003). The secretion of BDNF is also controlled by secretion pathways in neurons in a neuronal activity and Ca\(^{2+}\)-dependent manner (Lessmann and Brigadski, 2009). The increase of intracellular Ca\(^{2+}\) concentration activates calcium-dependent kinases such as calcium/calmodulin-dependent protein kinase IV (CaMKIV) phosphorylating cyclic AMP/Ca\(^{2+}\)-responsive element-binding protein (CREB), which further triggers the transcription of...
The BDNF mRNA expression in the hippocampus is markedly increased in rats with experimental seizure (Ernfors et al., 1991). In addition, chronic electrical stimulations with cochlear implants have markedly induced BDNF protein levels in the contralateral auditory cortex of deafened rats (Tan et al., 2008). In contrast, BDNF levels in the auditory cortex are reduced after noise exposure, which suggests cortical BDNF can be modulated by the neuronal activity (Zhou et al., 2011). In the present study, high levels of BDNF mRNA and protein expression in the auditory cortex of rats with bilateral cochlear ablation occurred 2 weeks later than those of sham control animals. It suggests that deprivation of peripheral auditory signals during the critical period shifts or delays the BDNF expression profile in the auditory cortex.

BDNF is not only important for neuronal survival and differentiation, but also involved in the activity-dependent synaptic plasticity (Mizui et al., 2016). Blocking of endogenous BDNF signaling in the rat auditory cortex during the critical period decreased the selectivity of neurons to tone frequencies and impair topographic order in the auditory cortex (Anomal et al., 2013). As BDNF itself is a diffusible molecule, the activity-dependent and synapse-specific modulation of plasticity by BDNF is fine-tuned via locally regulated TrkB receptor signaling. The change of neuronal activity modulates synthesis and transport of TrkB mRNA, insertion and translocation of TrkB receptor on the cell membrane as well as trafficking to synapses (Nagappan and Lu, 2005). Our findings in the present study have demonstrated that the change of TrkB gene and TrkB protein expression in the auditory cortex, closely correlates with that of BDNF in rats after sham operation or bilateral cochlear removal, although the TrkB receptor subtypes have not been examined. The completion of spatiotemporal cortical resolution in the central auditory system is initiated by peripheral auditory input that triggers BDNF-TrkB dependent cortical inhibition. The bilateral cochlear ablation during the critical period delays the normal expression of BDNF-TrkB in the auditory cortex, which results in a failure of increased cortical inhibition. Therefore, children with profound hearing loss who received cochlear implants at younger age may have more appropriate outcome in speech and communication than children who received their implants later (Hayes et al., 2009; Svirsky et al., 2004).

5. Conclusions

In this study, we have shown that auditory deprivation during the critical period leads to the delayed expression of BDNF and TrkB in the rat auditory cortex, and this delay could be due to cross-modal plasticity post bilateral cochlear ablation. This result implies that the BDNF-TrkB signaling may participate in the peripheral activity-dependent plasticity in the auditory cortex. Further studies are required to investigate the role of the BDNF-TrkB signaling pathway in the structural and functional maturation of the auditory cortex.
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