Indian J Otolaryngol Head Neck Surg
(December 2013) 65(Suppl 3):S582–S586; DOI 10.1007/s12070-012-0561-2

ORIGINAL ARTICLE

Otoacoustic Emission Responses of the Cochlea to Acute and Total Ischemia

Yavuz Selim Yıldırım · Fadlullah Aksoy · Orhan Ozturan · Bayram Veyseller · Hasan Demirhan

Received: 13 December 2011 / Accepted: 30 March 2012 / Published online: 28 August 2012

Abstract In the present experimental study, we sought to monitor distortion product otoacoustic emissions (DPOAEs) as an indicator of cochlear function, after sudden, total, and irreversible interruption of cochlear blood flow, to provide information on the time course of cochlear response to ischemia. Twenty rats with normal hearing function were included. Complete and abrupt ischemia was provided by decapitation. DPOAEs at 3–8 kHz frequencies were recorded at baseline and exactly every consecutive minute after decapitation, until emissions in all frequencies disappeared completely. Mean DPOAE values decreased significantly and progressively after decapitation for all frequencies. The mean duration of emissions was 8.20 ± 1.96 min (minimum 3 min, maximum 11 min). The longest durations of DPOAEs were observed with 4 and 5 kHz frequencies, and 3 and 6 kHz had the shortest durations. The outer hair cells exposed to acute ischemia seem to exhibit a rapid functional loss; thus, cautious handling of the cochlear vasculature and surrounding structures is necessary in surgical interventions. Additionally, our results provide some idea of the normal tolerance range of the cochlea to ischemia, which could be useful for future studies.

Keywords Otoacoustic emissions · Outer hair cell · Cochlear blood flow · Cochlear ischemia · Rat

Introduction

Cochlear ischemia is one of the most important causes of sensorineural hearing loss. Although most causes of sudden hearing loss are still unknown, vascular causes are usually regarded to be responsible for the development of this mostly unilateral condition. When its circulatory pattern is considered, the cochlea might be expected to be an end-organ that is very sensitive to alterations in blood circulation [1]. Common vascular disorders that affect the cochlea include thromboembolic events, vasoconstriction, hypotension, and hemoconcentration. Factors such as decreased cardiac output reduce systemic oxygenation and result in local tissue ischemia. Moreover, noise-related hearing loss and presbyacusia have also been reported to be associated with cochlear hypoxia and ischemia [2]. Such clinical conditions are known to be associated with reduced cochlear blood flow [3].

The human cochlea contains about 16,000 hair cells. The number of outer hair cells is 3.5 times greater than the inner hair cells. Otoacoustic emissions (OAEs) are the side-products of active amplification of the outer hair cells, and are defined as the energy transmitted from the cochlea to the outer ear canal through the middle ear. Generally, two types of otoacoustic emission are used: transient evoked otoacoustic emissions and distortion product OAEs (DPOAEs). When the cochlea is simultaneously exposed to two stimuli with different frequencies (f1 and f2) and

This study was presented in annual meeting of the CORLAS in Brugges, Belgium, 5–7 September, 2011.

Y. S. Yıldırım (✉)
Department of Otorhinolaryngology, Medical Faculty, Bezmialem Vakif University, 46300 Karaelbistan, Kahramanmaraş, Turkey
e-mail: dryavuzselim@yahoo.com

F. Aksoy · O. Ozturan · B. Veyseller
Department of Otorhinolaryngology, Medical Faculty, Bezmialem Vakif University, Istanbul, Turkey

H. Demirhan
Department of Otorhinolaryngology, Islahiye State Hospital, Gaziantep, Turkey

intensities (L1 and L2), acoustic energy emissions at one or more frequencies are obtained. This is called DPOAE and can be measured with the aid of a microphone placed into the external auditory canal. DPOAE measurement is a more convenient method for use in clinical studies of adult patients, because it allows evaluation of higher frequencies, it has a large frequency range, and it can be used for diagnostic purposes, using low-intensity stimuli (65/55 dB SPL) [4]. Sensitivity of the recording device and the noise level are also important factors that affect the detection threshold for DPOAEs [5].

Outer hair cell electromotility is very sensitive to extreme noise, ototoxic agents, and hypoxic and anoxic events. Under such conditions, outer hair cell function rapidly changes and it either decreases or disappears, depending on the severity of injury [6]. Vascular occlusion or injury that results in cochlear function loss can develop during cerebellopontine angle tumor surgery. Auditory brainstem responses and electrocochleography are used to monitor hearing during such surgeries. However, these methods may result in delayed detection of several alterations, including ischemia. In contrast, measurement of DPOAEs is the most convenient method for the monitoring of hearing, because it immediately detects changes in cochlear blood flow [7]. Thus, DPOAEs rapidly provide information on the blood flow and function of the cochlea, and are used in intraoperative monitoring to prevent ischemic injury [8]. Although there are studies that have measured cochlear function by means of DPOAEs during transient cochlear ischemic episodes, cochlear function in response to sudden and complete ischemia induced by decapitation has rarely been studied.

The aim of the present experimental study was to monitor DPOAEs to investigate the longevity and tolerance range of the cochlea after sudden, total, and irreversible interruption of cochlear blood flow.

Materials and Methods

The present experimental study was conducted in one session following the guidelines published in the Guide for the Care and Use of Laboratory Animals (DHEW publication NIH 85–23, revised 1996, Office of Science and Health Reports, DRR/NIH, Bethesda, MD) and approved by the Ethic Committee on Animal Research. In total, 20 Sprague–Dawley female rats, weighing 250–400 g and 3–6 months old, were used. All rats had normal-appearing external auditory canals and tympanic membranes, with positive Preyer's reflexes. None of the rats had been used in another study and all had been grown under similar conditions. All experiments were done in the same room under low-noise conditions and constant room temperature and environment.

Anesthesia of the rats was provided using intraperitoneal ketamine (50 mg/kg) and xylazine (10 mg/kg). One of the ears of each rat was randomly selected to be used in the experiments. Ten minutes after anesthesia, the external auditory canal and tympanic membrane were examined under a microscope, and the cerumen was removed. An Echoport ILO292 and ILOv6 software (Otodynamics Ltd, Hatfield, Herts, UK) were used for DPOAE recordings. Prior to testing, acoustic calibration was done. Following appropriate configuration of stimuli waveform, DPOAEs were measured in diagnostic mode by giving non-linear clicks. The ratio of f2/f1 was kept at 1.22. Stimuli intensities were L1 and L2 for f1 and f2 frequencies, respectively, and L1–L2 was kept at 10 dB SPL (L1 = 65 dB SPL, L2 = 55 dB SPL). DPOAEs were created by presenting two stimuli to the external ear canal from two different speakers (for f1 and f2, respectively), and emissions were recorded by a microphone in the probe placed in the canal. Continuous measurements were done for f2 frequencies (3–8 kHz), without removing the probe from the ear. Following baseline measurements, rats were decapitated in the cervical region using a guillotine, and DPOAE measurements were continuously recorded until emissions completely disappeared for all frequencies. Beginning from the decapitation time, DPOAE measurements were saved exactly at every consecutive minute.

SPSS for Windows (version 11.5) was used for statistical analysis. Dependent and independent variables of the experiment were DPOAEs and time, respectively. DPOAEs for 3–8 kHz frequencies were recorded. The mean DPOAE value of all frequencies was used for analysis. Baseline and post-decapitation values were compared using Wilcoxon signed rank test. p Values <0.05 were deemed to indicate statistical significance.

Results

Course of DPOAEs over time: At 1 min after decapitation, mean DPOAEs decreased significantly, compared with baseline, for all frequencies ($p < 0.05$). Mean values at 2–9 min were also decreased, compared with baseline, for all frequencies (3–8 kHz). The decrease in DPOAEs was continuous over time until 11 min, so that at each of these time points the value was significantly decreased compared with that at the previous time point for all comparisons and all frequencies ($p < 0.001$; Figs. 1, 3).

Complete disappearance of DPOAEs: the mean duration of emissions was 8.20 ± 1.96 min after decapitation (minimum 3 min, maximum 11 min). The longest duration of DPOAEs was observed at 4 and 5 kHz frequencies, and 3 and 6 kHz frequencies had the shortest duration. Complete disappearance rate of emissions over time is depicted in Fig. 2.

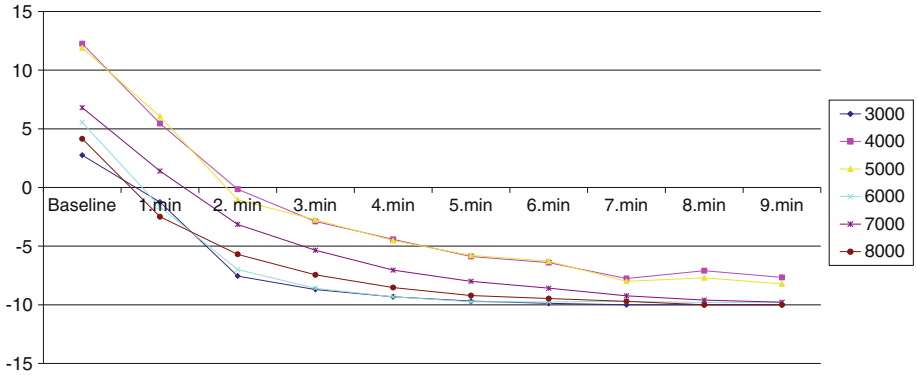

Fig. 1 Time course of mean DPOAE levels for each frequency at baseline and after decapitation

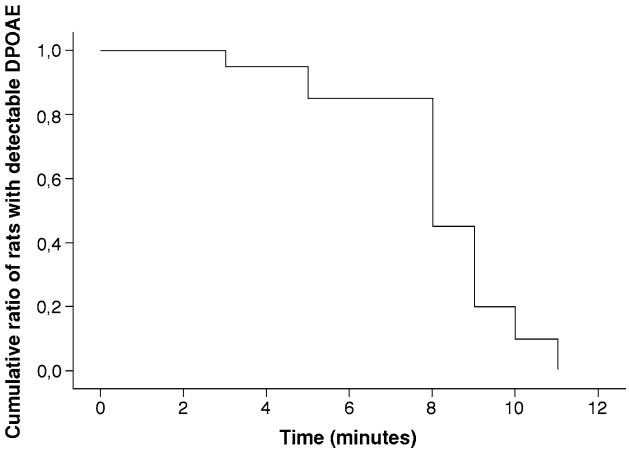

Fig. 2 Complete disappearance of emissions over time

Discussion

Small changes in blood flow can have a large impact on very tiny end-organs like the cochlea, and evaluation of blood flow to these organs represents a challenge [9]. Hypoxic ischemia is an important pathogenic factor for the development of internal ear disorders [10]. Cochlear ischemia and blood flow have been studied for many years. The first study was conducted in 1959 by Pearlman et al. [11]. They performed an extensive study of functional and histological cochlear alterations during and after reversible labyrinthine ischemia in the guinea-pig. They showed that cochlear outer hair cells were the most sensitive elements to ischemia.

Because the human cochlea is deeply located in the temporal bone, it is not readily accessible for investigation. Laser doppler flowmetry has confirmed blood flow impairment as a potential cause of hearing loss. Human studies that have measured cochlear blood flow with laser doppler flowmetry have also found reductions in patients with idiopathic sensorineural loss, and during cochlear implantation and intravenous propofol anesthesia [12]. Additionally, Meniere's disease, sudden hearing loss, and perilymphatic fistula are all associated with reduced cochlear blood flow [13].

In chronic conditions that lead to the death of outer hair cells, the process is slow and progressive over time. However, in acute cellular injury (e.g., exposure to sudden sound trauma), the process is rather rapid. Outer hair cell death mostly resembles the apoptotic process at the cellular level. DPOAE amplitudes are very sensitive to the complete interruption of cochlear blood flow through direct occlusion of the internal auditory artery. The latency between complete interruption of cochlear blood flow and detectable histological changes in outer hair cells ranges between 15 and 30 min [8].

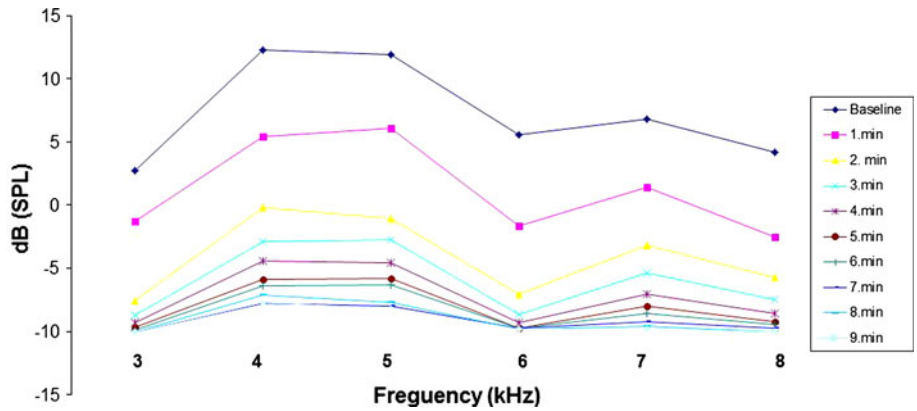

Fig. 3 The time course of mean DPOAE levels for each frequency at baseline and after decapitation

There are few previous reports on total and sudden cochlear ischemia. In the study of Lopez-Gonzalez et al. [14], rats were randomized to among three groups and exposure to one of the following: melatonin, an antioxidant mixture, and a combination of melatonin and antioxidant mixture. That study compared decapitation and chloroform inhalation anesthesia as a method of sacrifice. SPL was 70 dB and measurements were done between 1 and 6 kHz. Groups were analyzed in terms of OAE duration and sacrifice method. All three groups had dissimilar DPOAE levels. In contrast to the decapitation group, death did not occur immediately after the termination of the heart beat in the inhalational anesthesia group; thus, DPOAEs were detectable for a longer period. Therefore, the nature of death is important for postmortem detection of DPOAE activity [14]. Decapitation is an ideal method of sacrifice to provide sudden and effective ischemia. During decapitation, antegrade blood flow ceases bilaterally, suddenly and completely. This way of sudden and complete interruption of blood flow is much more efficient than other sacrifice methods [15]. Consistent with these studies, in the present study, rapid and significant reductions were detected in DPOAEs representing outer hair cell function, with acute and complete interruption of cochlear blood flow using decapitation. This method of sacrifice leading to total and complete interruption of cochlear blood flow, resulted in the rapid disappearance of DPOAEs. To our knowledge, this is the first reported study to examine the cochlear response to abrupt and irreversible ischemia induced by decapitation of rats using DPOAE measurements.

When DPOAE measurements are made following high-intensity stimuli ($\geq$70 dB SPL), robust emissions can be detected, despite cochlear functional impairment [16]. DPOAEs that respond to high-level stimuli should be evaluated cautiously. DPOAEs in response to high-level stimuli can arise from passive cochlear mechanics; thus, they might not be reliable for the monitoring of cochlear function. Hence, DPOAEs that arise in response to high-intensity stimuli are the responses of passive cochlear structures and/or stimulus artifacts [17]. In rats, DPOAEs in response to low-intensity and high-intensity stimuli (>65 dB SPL) should be evaluated separately. DPOAEs that arise from active cochlear structures as a response to low-intensity stimuli are more valuable because they physiologically and directly reflect outer hair cell activity. Experimentally induced cochlear blood flow impairment has a more rapid impact on DPOAEs in response to low-intensity compared with high-intensity stimuli. Thus, the stimulus intensity that best reflects the actual status of the cochlea is 65/55 dB SPL, and low-intensity stimulus is recommended for clinical use [18]. In the present study, low-intensity stimuli of 65/55 dB SPL were used. Total ischemia was provided by decapitation and measurements were taken at 3–8 kHz frequencies. Considering the high level of noise in low frequencies and relatively higher sensitivity of rat audition to frequencies above 3 kHz, a lower frequency limit of evaluation was set at 3 kHz in the present study. Rats have been found to be more susceptible to hearing loss at mid-frequencies (3–12 kHz) [19].

In the study of Lopez-Gonzalez et al. [14], the upper frequency limit of evaluation was set at 6 kHz, using high-intensity primary stimuli (70 dB SPL) that has potential passive cochlear effects, and the use of chloroform anesthesia instead of decapitation as the method of sacrifice, could all have had unfavorable effects on the homogeneity of the results. Additionally, as the noise level was not indicated on the charts of that study, the lower threshold for the appearance of DPOAEs is unclear.

Nishizaki et al. sampled and histologically fixed internal ear structures at 0, 10, 20 and 30 min after cervical dislocation of rats. Positive TUNEL staining of the outer hair cells was observed at 10 min, consistent with autolysis rather than apoptosis. Thus, 10 min after death, autolysis and functional loss occur in cochlear structures [20]. In our study, DPOAEs as early as 3–11 min (mean 8.20 min) completely disappeared following decapitation; that is, they became completely nonfunctional, which is in accordance with the findings of Nishizaki et al. [20] OAEs cease completely when there is not a sufficient number of functional cells to produce emissions.

In the present study, we found a statistically significant decrease in DPOAE amplitude at 1 min, compared with baseline. Abrupt interruption of cochlear blood flow as a result of decapitation caused very rapid functional loss of outer hair cells, within seconds, which was reflected in reduced DPOAE amplitude and response. It should be borne in mind that hearing loss due to cochlear ischemia episodes can be seen, even when the auditory nerve is not directly injured. Cautious handling of cochlear vascular structures during surgical treatment of cerebellopontine angle pathologies is important for preserving residual hearing capacity. Intraoperative incidence of thrombus formation, vascular injury and vasospasm might impair physiological activity of the cochlea. Time is of the utmost importance in terms of preserving cellular viability. The duration of cochlear ischemia and tolerance of the cochlea to total ischemia are important, particularly in the setting of the surgical treatment of cerebellopontine angle pathologies and development of sudden hearing loss as a result of vascular etiologies.

The results give a strong indication of the normal tolerance range of the cochlea to total abrupt ischemia, which represents, more or less, the normal duration range. On the other hand, the effect of reperfusion on the restoration of outer hair cell function and the effect of certain agents, such as melatonin, on the resistance of these cells to ischemia warrant further investigations.

Conflict of interest None.

References

1. Abrashkin KA, Izumikawa M, Miyazawa T, Wang CH, Crumling MA, Swiderski DL et al (2006) The fate of outer hair cells after acoustic or ototoxic insults. Hear Res 218:20–29
2. Amarjargal N, Mazurek B, Haupt H, Andreeva N, Fuchs J, Gross J (2008) Effects of SERCA and PMCA inhibitors on the survival of rat cochlear hair cells during ischemia in vitro. Physiol Res 57:631–638
3. Suckfüll M, Mees K (1998) Hemoconcentration as a possible pathogenic factor of sudden hearing loss. Eur Arch Otorhinolaryngol 255:281–284
4. Kemp DT (2002) Otoacoustic emissions, their origin in cochlear function, and use. Br Med Bull 63:223–241
5. Rhode WS (2007) Distortion product otoacoustic emissions and basilar membrane vibration in the 6–9 kHz region of sensitive chinchilla cochleae. J Acoust Soc Am 122:2725–2737
6. Brownell WE (1990) Outer hair cell electromotility and otoacoustic emissions. Ear Hear 11:82–92
7. Telischi FF, Stagner B, Widick MP, Balkany TJ, Lonsbury-Martin BL (1998) Distortion-product otoacoustic emission monitoring of cochlear blood flow. Laryngoscope 108:837–842
8. Morawski K, Telischi FF, Niemczyk K (2006) A model of real time monitoring of the cochlear function during an induced local ischemia. Hear Res 212:117–127
9. Austin RE Jr, Hauck WW, Aldea GS, Flynn AE, Coggins DL, Hoffman JI (1989) Quantitating error in blood flow measurements with radioactive microspheres. Am J Physiol 257:280–288
10. Riva C, Donadieu E, Magnan J, Lavieille JP (2007) Age-related hearing loss in CD/1 mice is associated to ROS formation and HIF target proteins up-regulation in the cochlea. Exp Gerontol 42:327–336
11. Perlman HB, Kimura M, Fernandez C (1959) Experiments on temporary obstructions of the internal auditory artery. Laryngoscope 69:591–613
12. Scheibe F, Haupt H, Berndt H, Magnus S, Weymar P (1990) Laser light transmission and laser Doppler flow measurements on the human, rat, and guinea pig cochlea. Eur Arch Otorhinolaryngol 247:20–23
13. Nakashima T, Hattori T, Sone M, Sato E, Tominaga M, Sugiura M (2004) Blood flow measurements in the ears of patients receiving cochlear implants. Ann Otol Rhinol Laryngol 113:426–430
14. Lopez-Gonzalez MA, Guerrero JM, Rojas F, Osuna C, Delgado F (1999) Melatonin and other antioxidants prolong the postmortem activity of the outer hair cells of the organ of Corti: its relation to the type of death. J Pineal Res 27:73–77
15. Nakashima T, Naganawa S, Sone M, Tominaga M, Hayashi H, Yamamoto H et al (2003) Disorders of cochlear blood flow. Brain Res Rev 43:17–28
16. Avan P, Bonfils P, Gilain L, Mom T (2003) Physiopathological significance of distortion-product otoacoustic emissions at 2f1–f2 produced by high-versus low-level stimuli. J Acoust Soc Am 113:430–442
17. Ozturan O, Oysu C (1999) Influence of spontaneous otoacoustic emissions on distortion product otoacoustic emission amplitudes. Hear Res 127:129–136
18. Gorga MP, Stover L, Neely ST (1996) The use of cumulative distributions to determine critical values and levels of confidence for clinical distortion product otoacoustic emission measurements. J Acoust Soc Am 100:968–978
19. Lataye R, Campo P, Loquet G (1999) Toluene ototoxicity in rats: assessment of the frequency of hearing deficit by electrocochleography. Neurotoxicol Teratol 21:267–276
20. Nishizaki K, Yoshino T, Orita Y, Nomiya S, Masuda Y (1999) TUNEL staining of inner ear structures may reflect autolysis, not apoptosis. Hear Res 130:131–136