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Round window closure affects cochlear responses to supra-threshold stimuli

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Abstract

Objectives—The round window acts as a vent for releasing inner ear pressure and facilitating basilar membrane vibration. Loss of this venting function affects cochlear function leading to hearing impairment. The current investigation was designed to examine how the cochlea responds to supra-threshold stimuli following round window closure in an effort to identify functional changes that might be used in clinical diagnosis of round window atresia.

Study Design—Prospective, controlled, animal study.

Methods—A rat model of round window occlusion was established. With this model, the thresholds of auditory brainstem responses and the input/output functions of distortion product otoacoustic emissions and acoustic startle responses were examined.

Results—Round window closure caused a mild shift in the thresholds of the auditory brainstem response (13.5 ± 9.1 dB). It also reduced the amplitudes of the distortion product otoacoustic emissions and the slope of the input/output functions. This peripheral change was accompanied by a significant reduction in the amplitude, but not the threshold, of the acoustic startle reflex, a motor response to supra-threshold sounds.

Conclusions—In addition to causing mild increase in the threshold of the auditory brainstem response, round window occlusion reduced the slopes of both distortion product otoacoustic emissions and startle reflex input/output functions. These changes differ from those observed for typical conductive or sensory hearing loss, and could be present in patients with round window atresia. However, future clinical observations in patients are needed to confirm these findings.

Keywords

Round window; Atresia; Distortion production otoacoustic emission; Rats; Acoustic startle reflex

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Introduction

The round window acts as a vent for releasing the inner ear pressure and facilitating basilar membrane vibration. Loss of this venting function affects cochlear physiology and causes hearing dysfunction. Animal models with surgical occlusion of the round window show mild threshold shifts^{1,2}. Clinical observations in patients with round window atresia have revealed a mixed hearing loss of up to 30 to 40 dB with normal speech discrimination and a type A curve in tympanometry, but with the absence of acoustic reflexes^{3,4}. Thus far, the clinical diagnosis of round window atresia relies primarily on medical evaluation, including high-resolution CT scan and surgical exploration^{5,6}. The audiological diagnosis of this disorder is a clinical challenge because commonly used audiometric tests evaluate only the threshold, which is non-specific. The lack of specificity hinders the effort to identify round window atresia.

In the current investigation, we established an animal model with surgically induced round window closure, which we termed “round window occlusion” (RWO) to distinguish this condition from the clinical presentation of patients with round window atresia who usually have other ear abnormalities. We examined the threshold of the auditory brainstem responses (ABR) and the input/output (I/O) functions of two auditory responses: distortion product otoacoustic emissions (DPOAEs) and acoustic startle responses. For the first time, we reveal that RWO reduces the slopes of the I/O functions of DPOAE, a unique pattern that differs from the slope changes resulting from typical conductive or sensorineural hearing loss. Moreover, we reveal a reduction in the slope of the I/O function of the acoustic startle reflex, consistent with the slope changes in the DPOAE I/O functions. Since DPOAEs are routinely used clinically, we recommend this test for patients suspected of having round window atresia in order to determine whether the reduced slope is a useful metric for diagnosis of round window atresia in patients.

Materials and Methods

Animals

Sixteen adult Sprague-Dawley rats (8 female and 8 male, 150–250 g) were used. The procedures involving the use of animals were approved by the Institutional Animal Care and Use Committee of the University at Buffalo.

Procedure for RWO

The animals were anesthetized with intraperitoneal injection of ketamine (87 mg/kg) and xylazine (5 mg/kg). A skin incision was made in the post-auricular region to expose the auditory bulla of either the right or the left ear. A hole was made in the posterior wall of the bulla to expose the round window. Using a micromanipulator, a glass micropipette containing a tissue adhesive (Vetbond Tissue Adhesive, 3M) was inserted into the round window niche and then, a small drop (< 1 µl) of the tissue adhesive was injected to the round window niche to occlude the opening. A sham surgery was performed in the opposite ear by applying the same volume of saline solution onto the round window niche. The animals

were allowed to recover from the surgery. All functional tests were repeated two months after the surgery.

DPOAE test

Our procedures for collecting DPOAE have been described previously⁷. DPOAE responses were measured using a SmartEP Intelligent Hearing System (Intelligent Hearing System, Miami, FL, USA). DPOAEs were elicited by two primary tones, F_1 and F_2 , with an F_2/F_1 ratio of 1.2 and F_2 frequencies at 4, 8, 12, 16, 20, and 32 kHz. L_1 was set 10 dB higher than L_2 . DPOAE I/O functions were obtained by decreasing L_1 intensity in 5-dB steps from 70 to 25 dB SPL. The signals were delivered to the ear canal via a sealed probe connected to IHS-3738 high-frequency transducers. Sound pressure levels were measured by using an ER10B+ recording microphone (Etymotics Research, Inc., Elk Grove Village, IL). Thirty-two sweeps were presented at each test level.

Acoustic startle reflex test

The acoustic startle reflex is a short-latency response of the skeletal musculature elicited by sudden, unexpected and intense auditory stimulation. Because the reflex reflects the subject's ability to respond to a high intensity of sounds, we used the test to assess the effect of RWO on the auditory responses provoked by acoustic stimuli with levels higher than those used for the DPOAE assessment.

The test protocol has been described in detail in our previous publication⁸. Each animal was placed in a wire mesh cage with a Plexiglas bottom, and the cage was placed on a piezoelectric sensor platform in a sound attenuating chamber. The animal was exposed to a sequence of white-noise bursts ranging from 50 to 100 dB SPL. To test individual ears (either the atresia ear or the control ear), we used Pink Silicast® silicone ear mold impression material (Westone Laboratories, Inc.) to occlude the ear canal of the non-test ear, which resulted in 40–50 dB attenuation of input sounds. For startle reflex testing, ten trials were randomly presented at each stimulus intensity, and the inter-trial interval randomly varied between 18 and 22 ms. For quantification of the acoustic startle reflex movement of the rat, the voltage output (the root mean square) from the piezoelectric transducer mounted on the floor of the cage was recorded for 100 ms following the onset of the noise burst. The startle threshold was defined as the minimal level of sound that elicited a clearly detectable startle response over the noise background. The test was repeated at least twice for each condition.

ABR test

For ABR testing, the stimuli consisted of tone bursts presented at 5, 10, 20, 30, and 40 kHz (0.5 msec rise/fall Blackman ramp, 1 msec duration, alternating phase) (SigGen, Tucker-Davis Technologies, TDT, FL) presented at a rate of 21/sec. The ABR responses were recorded using stainless-steel needle electrodes that were placed subdermally over the vertex (noninverting input), post-auricular to the stimulated ear (inverting input) and post-auricular to the non-stimulated ears (ground) of the animal. The responses were filtered (100–3000 Hz), amplified, and averaged (500 sweeps) using TDT hardware (RP2.1, 100 kHz sampling rate) and software. These responses were then stored and displayed on a computer. The

ABR threshold was defined as the lowest intensity that reliably elicited a visually detectable response. All ABR thresholds were measured at least twice for each time point.

Cochlear sensory cell evaluation

To determine whether the tissue adhesive applied to block the round window was toxic to sensory cells, we examined the organ of Corti for signs of sensory cell death in five subjects using a whole mount preparation stained with propidium iodide⁹. Briefly, upon completion of the physiological evaluation, the animals were sacrificed. The cochleae were collected, fixed with 10% buffered formalin, and dissected to collect the sensory epithelium. The tissues were stained with a propidium iodide solution (5 µg/ml in phosphate buffered saline) for 10 min and were mounted on slides with an antifade medium (Prolong® Gold antifade reagent, Invitrogen Inc.). The tissues were examined with a microscope equipped with epifluorescence illumination.

Results

Preliminary analysis

We examined the middle ear cavity for signs of infection after cochlear collection and found no middle ear infection in any of the ears examined. We also inspected the round window area to assess the completeness of RWO. The tissue adhesive mass, which was solid, completely covered the round window. However, the fullness of the adhesive mass within the round window niche differed among individual ears. Based on the fullness level (1/3, 2/3 or complete; Fig. 1), we defined three levels of occlusion: partial, nearly complete and complete, respectively. Two of the 16 ears showed partial occlusion, two showed nearly complete occlusion, and the remaining 12 cochleae showed complete occlusion of the round window. We further examined the nuclear morphology of cochlear sensory cells in 10 cochleae (5 control and 5 occluded cochleae) to determine whether the application of tissue adhesive to the round window caused sensory cell death. We found no increase in the number of missing cells in the cochleae with RWO. These results indicate that the surgery and occlusion of the round window with tissue adhesive did not cause middle ear infection, nor did it affect sensory cell survival.

RWO causes a mild threshold shift of ABRs

ABR thresholds were measured before and 8 weeks after the RWO surgery. The two ears that had partial RWO showed no threshold shift relative to the thresholds measured before the surgery. The two ears that displayed nearly complete RWO exhibited mild shifts, with an average loss of 6 ± 5.48 dB for one subject and 16 ± 5.48 dB for the other, which are similar to the levels of threshold shifts observed in subjects with complete RWO. The remaining 12 cochleae with complete RWO showed 5–25 dB threshold shifts. The average shift for the ears with RWO-induced hearing loss ($n = 14$ cochleae) was 13.5 ± 9.1 dB (mean $\pm$ SD, Fig. 2A), suggesting that RWO causes a mild threshold shift of ABRs

The control ears that received only a sham surgery showed an average threshold variation of < 2.6 dB (Fig. 2B), suggesting that without RWO, the surgery itself had only a minimal influence on the ABR thresholds.

RWO suppresses the amplitude of DPOAE

To further determine how RWO affects cochlear function, we measured the DPOAE amplitudes elicited by two tones with the L_1/L_2 levels of 65/55 dB SPL and an F_2 frequency ranging from 4 to 32 kHz. RWO suppressed DPOAE amplitudes by as much as 30 dB at 16 kHz and to a lesser extent at other frequencies (Fig. 3A). In contrast, the control ears displayed only a slight reduction in the DPOAE amplitudes (Fig. 3B). This observation suggests that RWO suppresses the amplitude of DPOAE.

RWO reduces the slope of the I/O function of DPOAE

To determine whether the observed changes in the amplitude of DPOAE were dependent on the level of the stimuli, we examined the I/O functions of DPOAE. In the control ears, the I/O function exhibited a slight amplitude decrease at 8, 12, and 16 kHz (Fig. 4, upper panels), whereas the function remained unchanged at the other three frequencies. The I/O shifts after RWO appeared to be parallel to the curves observed prior the RWO surgery, a feature characteristic of conductive hearing loss¹⁰. By contrast, the RWO ears exhibited a significant reduction in the slope of the I/O function of DPOAE (Fig. 4, low panels) and the slope difference were statistically significant for 8, 12, 16 and 20 kHz (Two-way repeated measures ANOVA, $F = 7.012$, $df = 3,39$, $p < 0.001$, Tukey's test, $p < 0.05$; Fig. 5). DPOAE responses at 32 kHz were not detectable after RWO. These results suggest that RWO reduces the slope of the I/O function of DPOAE, a change that is distinct from the parallel shift observed at some frequencies in the control ears.

Assessment of the I/O function of the acoustic startle reflex

Since DPOAE saturate at moderate intensities (~70 dB SPL), we used the amplitude of the acoustic startle reflex, a short latency motor response involving auditory circuits from the cochlea to the lateral lemniscus, to assess the response of the auditory periphery to high-intensity stimuli¹¹. Figure 6A compares the mean I/O functions of the acoustic startle reflex between the RWO ears ($n = 6$) and the control ears ($n = 6$). The control ears exhibited an average startle threshold of 80 dB SPL and a gradual increase in the reflex amplitude as a function of stimulus level. The RWO ears exhibited a similar threshold level. However, the slopes of the startle I/O function from the RWO ears were less (Median = 2.30, 25–75% = 1.47–3.73) than the control ears (Median = 6.78, 25–75% = 4.10–12.60); this difference in slope was statistically significant (Fig. 6B; Wilcoxon signed rank test, $Z = 2.201$, $p = 0.031$). This suggests that RWO suppresses the growth of the startle reflex at high intensities, consistent with the reduction in the slope of the DPOAE I/O function.

Discussion

The current study examined the impact of RWO on the auditory system's responses to supra-threshold stimulation. We demonstrate that RWO for two months elevated ABR thresholds approximately 15 dB, but does not cause detectable sensory cell damage. Importantly, we demonstrate that RWO reduces the slopes of DPOAE and startle reflex I/O functions, suggesting that RWO enhances cochlear compression possibly by suppressing basilar membrane vibration.

Reported threshold shifts in animal models of RWO vary across studies. Here, we document that RWO in rats caused a mild elevation of threshold, consistent with a previously reported threshold shifts following surgical occlusion of the round window in cats¹. The average threshold shifts reported in clinical cases of round window atresia (30 to 50 dB) are greater^{3–5} than those reported in animal models of RWO. The greater threshold elevation in clinical cases of round window atresia is likely due to the combination of abnormalities in the inner ear and middle ear. Consistent with this view, several clinical observations have shown an air-bone gap of 15–25 dB in patients with overall threshold shifts of 30–40 dB^{3,12}, indicating the presence of sensorineural hearing loss. These findings suggest that clinical patients with round window atresia have more complex pathologies involving both the middle and inner ear.

The decrease in the DPOAE slope following round window closure is an important finding with clinical implications. The changes in the DPOAE I/O behavior have been observed in a variety of inner and middle ear disorders. For inner ear disorders that compromise outer hair cell function, steep I/O functions have been documented in subjects with mild to moderate levels of hearing loss^{10,13–15}. This change is attributed to the disruption of the compressive nonlinear behavior of the basilar membrane I/O function due to outer hair cell dysfunction. For middle ear disorders, changes in DPOAE are related to the severity of the pathology. Mild middle ear abnormalities cause a parallel shift of I/O functions¹⁰, whereas severe middle ear diseases abolish the response¹⁶. Here, we demonstrate that RWO decreases the slope of the I/O function, a change that is distinct from both typical middle and inner ear disorders. This finding has clinical potential for the differential diagnosis of round window atresia.

As discussed above, round window atresia in patients is often accompanied by other middle and/or inner ear abnormalities. Our results suggest that patients with purely round window atresia will have reduced DPOAE slopes whereas those with round window atresia plus middle ear pathology will have more severely depressed DPOAE I/O functions and show evidence of a threshold shift (i.e. a rightward shift of the I/O function). Therefore, it is important for future clinical studies to determine whether clinical patients who have round window atresia exhibit a similar reduction in the slope of the DPOAE I/O function. Moreover, individual variation in DPOAE behavior can hinder the detection of a slope change. In this regard, application of the current findings to the clinical setting is feasible for patients with unilateral round window atresia because a comparison of the I/O function slopes between the two ears of patients is more likely to reveal the difference.

Here, we demonstrate that DPOAEs and acoustic startle responses exhibit a similar pattern of reduction in the I/O slope, although the operating ranges for these two measures are different. DPOAE reflects the cochlear response provoked by the low to moderate levels of the stimuli (20 to 70 dB SPL), whereas the startle reflex change was observed with the higher stimulus levels (70 to 100 dB SPL). The consistency of the results suggests that the compression of cochlear responses occurs over a wide range of input sound levels. Because the reduction in acoustic startle reflex amplitude was at high sound levels, we suspect that subjects with RWO could exhibit an enhanced sound tolerance. It would be of interest to determine if behavioral recruitment tests, such as the alternate binaural loudness balance test

and the loudness discomfort level test could provide additional clues for assessing the sound tolerance level in patients with round window atresia. Moreover, future assessment of ABR I/O function may provide additional evidence for the amplitude changes induced by the high level of sound exposure.

Conclusion

The clinical diagnosis of round window closure is challenging. Here, we found a reduction in the slopes of DPOAE and startle reflex I/O functions in a rat model of RWO. This pattern of slope changes differs from the changes observed in patients with conductive or sensory hearing loss, and therefore has the potential to be used for the suggestion of round window atresia in clinical patients. Future clinical verification of these findings is needed.

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Figure 1.

Images showing round window occlusion. *A*: Normal round window without application of the tissue adhesive. *B*: A round window blocked with the tissue adhesive. *C*: Schematic drawings showing the application of the tissue adhesive as well as the partial occlusion and nearly-complete occlusion of the round window.

Figure 2.

The comparison of the average auditory brainstem response (ABR) thresholds (mean $\pm$ SD) measured pre-surgery and at 8 weeks post-surgery in the round window occlusion (RWO) ears (A) and the control ears (B). N: the number of ears used for each condition. Asterisks indicate $p < 0.001$ (two-way ANOVA, Tukey's test).

Figure 3.

Comparison of mean distortion product otoacoustic emissions (DPOAE) amplitudes ($\pm$ SD) measured pre-surgery and at 8 weeks post-surgery, using two tones at the level of 65/55 dB SPL (L_1/L_2). *A*: The ears with round window occlusion (RWO) exhibit a marked reduction in DPOAE amplitude. The dotted lines represent the noise floor. *B*: The control ears display only a slight reduction in DPOAE amplitude. The differences in the amplitude reduction between the RWO ears and the control ears were statistically significant for all the tested frequencies except for 32 kHz (two-way ANOVA, the interaction effect ($F = 7.925$, $df = 4$, 130 , $p < 0.01$)). NF: noise floor. N: the number of ears for each condition.

Figure 4.

The comparison of the input/output (I/O) functions of the distortion product otoacoustic emissions (DPOAE) before and after the sham surgery in the control ears (upper 6 panels) and in the ears with round window occlusion (RWO) (lower 6 panels). N: the number of ears for each condition.

Figure 5.

The comparison of the slopes of the input/output (I/O) functions of distortion product otoacoustic emissions (DPOAE) before and after the surgery in the round window occlusion (RWO) ears. Dots represent the slopes of individual ears. Asterisks indicate significant reductions in the slopes after the occlusion (two-way ANOVA, Tukey's test, $p < 0.05$).

Figure 6.

The input/output (I/O) functions of acoustic startle reflex amplitudes. *A*: The comparison of the I/O functions between the round window occlusion (RWO) ears and the control ears. The control ears exhibit a gradual increase in the reflex amplitudes with the increase in the stimulus level. The RWO ears, by contrast, exhibit no significant increase in the startle reflex amplitudes. *B*: The comparison of the slopes of the I/O functions between the two groups. Dots and squares represent the slopes of individual ears. The lines between the dots and squares indicate the two ears from the same animal. N: the number of ears for each condition.