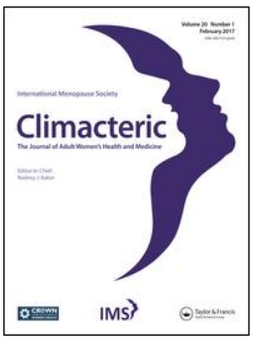

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ORIGINAL ARTICLE

Lower expression of prestin and MYO7A correlates with menopause-associated hearing loss

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ABSTRACT

Objective: This study aimed to test the hearing function and to investigate the effect of estrogen on prestin and MYO7A expression in hair cells of the cochlea in ovariectomized rats.

Study design: We compared the hearing function systematically in normal female Sprague–Dawley rats and in ovariectomized rats with or without exogenous β-estradiol administration by auditory brainstem response measurements and distortion product otoacoustic emissions. In addition, a correlation analysis between the functional parameters and cochlear histology was carried out.

Results: There was a significant auditory threshold shift in the high-frequency range in the ovariectomized rats. Prestin and MYO7A expression was lowered in the cochlea of ovariectomized rats. These effects could be recovered by subcutaneous administration of β-estradiol.

Conclusion: Low estrogen levels may lead to reduced expression of prestin and MYO7A in cochlea, leading to a menopause-related hearing loss.

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Hearing loss; menopause; estrogen; prestin; MYO7A

Introduction

Estrogen levels affect the auditory function in healthy women[1–3]. The decline in hormone levels after menopause is known to trigger hearing loss[4]. It has been reported that the administration of exogenous estrogen and hormone replacement therapy can impart hearing protection in menopausal women[5,6]. In a previous study, we demonstrated a rapid high-frequency hearing loss in women after menopause, which was associated with decreased serum anti-Mullerian hormone. This might be possibly due to the reduced endogenous estrogen levels[7]. However, the mechanism of low estrogen-induced hearing loss is poorly understood.

The estrogen receptor (ER) is distributed in the hair cells of the cochlea. ERα is involved in transmission of the cochlear and vestibular signals, and ERβ may have neuroprotective effects on the inner ear[8]. The high-frequency hearing loss is due to the loss of high-frequency signals during the transmission of the cochlea. So, the impact of ERα on menopausal hearing loss is important. Damage and dysfunction of hair cells have a direct correlation with hearing loss. Human myosin VIIA (MYO7A) is a member of the unconventional myosin superfamily of proteins. It is an actin-binding molecular motor that uses the enzymatic conversion of adenosine triphosphate to adenosine diphosphate and inorganic phosphate to provide energy for movement[9,10]. Mutations in MYO7A are responsible for Usher syndrome – a condition characterized by congenital hearing loss in humans. There is a known association between sarcopenia and hearing thresholds in postmenopausal women[11]. Muscle strength in menopausal women declines with age due to the reduction in ovarian hormone secretion[12]. Prestin is a motor protein, expressed in the basolateral plasma membrane of cochlear outer hair cells (OHCs). It underlies the generation of somatic, voltage-driven mechanical force which is the basis for the exquisite sensitivity, frequency selectivity, and dynamic range of mammalian hearing[13,14]. Clinical research shows that low estrogen levels and abnormal hair cell function can lead to a high-frequency hearing loss. Prestin is also known to be affected by hormones (thyroid hormone, melatonin, etc.)[15,16]. Therefore, it may be hypothesized that low estrogen levels may affect MYO7A and prestin in the cochlea, leading to hearing impairment.

In this study, we systematically compared the hearing function between normal female Sprague–Dawley (SD) rats and ovariectomized rats treated with or without β-estradiol. Hearing function was monitored by auditory brainstem response measurements (ABRs) and distortion product otoacoustic emissions (DPOAEs). In addition to this, a correlation analysis between the functional parameters and cochlear histology was carried out.

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Supplemental data for this article can be accessed here.

Materials and methods

Animals and husbandry

Forty-five female SD rats from the laboratory animal science department of Peking University Health Science Center aged 10 weeks and weighing 200 ± 5 g were used for the study. The rats had no prior noise exposure or history of ototoxic drug treatments. The protocol was approved by the laboratory animal welfare committee. The animal experiments were performed in accordance with the National Institutes of Health guidelines and the guidelines from Directive 2010/63/EU of the European Parliament on the protection of animals used for scientific purposes.

All of the animals were acclimated to the animal housing for 1 week before the experiment. They had free access to water and food, and were housed in collective cages (five rats per cage) at a controlled temperature of 22 ± 2 °C under a 12-h light/dark cycle (light from 8:00 am to 8:00 pm). All of the animals were fed rodent chow without soybean, and their food intake was monitored daily during the experimental period. The animals were randomly divided into three groups ($N = 15$ per group): sham-operated (SHAM), ovariectomized (OVX) and ovariectomized with β-estradiol oil hypodermic injection (E). The hearing function in all animals was monitored by ABR and DPOAE. Ten rats were randomly selected from each group and their body weight (BW) measured weekly. Rats in the OVX and E groups underwent bilateral oophorectomy. The surgery was performed under aseptic conditions and general anesthesia (1% sodium pentobarbital, 40 mg/kg intraperitoneal injection). Rats in the SHAM group underwent similar surgery – the abdomen was opened to expose the ovaries and then closed without resecting the ovaries. After ovariectomy, vaginal smears were examined for 5 days and estrous cycles were observed to determine that there was no estrous cycle in ovariectomized rats. All three groups received hypodermic injections every day between 9:00 am and 10:00 am for 4 weeks. Group E received 0.025 μg/g BW β-estradiol (Sigma, E8875) per day. The β-estradiol was dissolved in sterile sesame oil (Acros, 241002500). The SHAM and OVX groups received an equivalent volume of sterile sesame oil. The operation flow is shown in Supplementary Figure S1.

Serum estradiol concentration and uterine wet weight measurement

Serum estradiol concentrations were determined using a radioimmunoassay. A kit for estradiol detection was purchased from the Beijing North Institute of Biological Technology (China). The tests were carried out following the manufacturer's instructions.

After sacrifice, the abdominal fat pad and total uterus (including the vagina) were collected after removing the surrounding connective tissue, muscles, and other debris. The organs were weighed and the absolute wet-weight in grams was recorded. To account for differences in BW between animals, the relative weight (% BW) was calculated from BW at sacrifice and the uterine wet-weight using the following equation[17]:

$$\text{Relative organ weight } (\%\ \text{BW}) = \text{weight of uterus at sacrifice} \times 100.$$

Table 1. Estrogen (serum).

	Sum of squares	*df*	*Mean square*	F	p-*Value*
Between groups	5947.177	2	2973.588	17.674	0.000
Within groups	7066.547	42	168.251		
Total	13,013.724	44			

ABR and DPOAE measurements

Auditory brainstem response measurements

ABRs were recorded in animals placed in a soundproof and anechoic room using acoustic free-field stimulation. The walls and ceiling inside the room were covered with phono-absorbent cones (soundproof room, ambient noise in line with GB/T16403). The SD rats were anesthetized with sodium pentobarbital (1% sodium pentobarbital, 40 mg/kg, intraperitoneal injection) and placed on a heating pad. The ABR threshold value was examined in each group before ovariectomy, 14 days after surgery, and 28 days after surgery before execution. The hearing was evaluated using Tucker-Davis Technologies hardware and software (TDT System III, Alachua, FL, USA). ABRs were subcutaneously recorded with sterile electrode needles. The reference was inserted beneath the pinna of the ear, the ground beneath the opposite ear, and the active electrode beneath the skin at the vertex. The scan intensity was 20–110 dB sound pressure level and the rise/fall time was 1 ms. Stimulus levels varied in descending steps of 5 dB. At each level, 1024 responses were averaged. The scan time was 10 ms. The hearing threshold was confirmed until wave III disappeared. Three repeat measurements near the threshold were performed to ensure data accuracy.

Distortion product otoacoustic emissions

At 16 weeks, 10 SD rats were randomly selected from each group for DPOAE measuring. DPOAE recordings were performed in an anechoic and soundproof room. During DPOAE recordings, the rats were anesthetized and placed on a heating pad to maintain body temperature at 38 °C. DPOAEs were measured in the right ear of the animals. To test the functional status of the OHCs, the DPOAEs were recorded with a low-noise microphone system (Etymotic probe ER-10B+; Etymotic Research, Elk Grove Village, IL, USA). DP-grams (the function of the DPOAE level on increasing stimulus frequency) were recorded with a resolution of four points per octave over the frequency range from 1 to 32 kHz (1 kHz, 2 kHz, 4 kHz, 8 kHz, 12 kHz, 16 kHz, 20 kHz, 28 kHz, 32 kHz). During DPOAE recording, in addition to the DPOAE amplitude, F1 and F2 intensities in the outer ear canal were also monitored to confirm the correct placement of the recording probe. Acoustic stimuli (two primary tones with frequency ratio F2/F1 = 1.21 and level

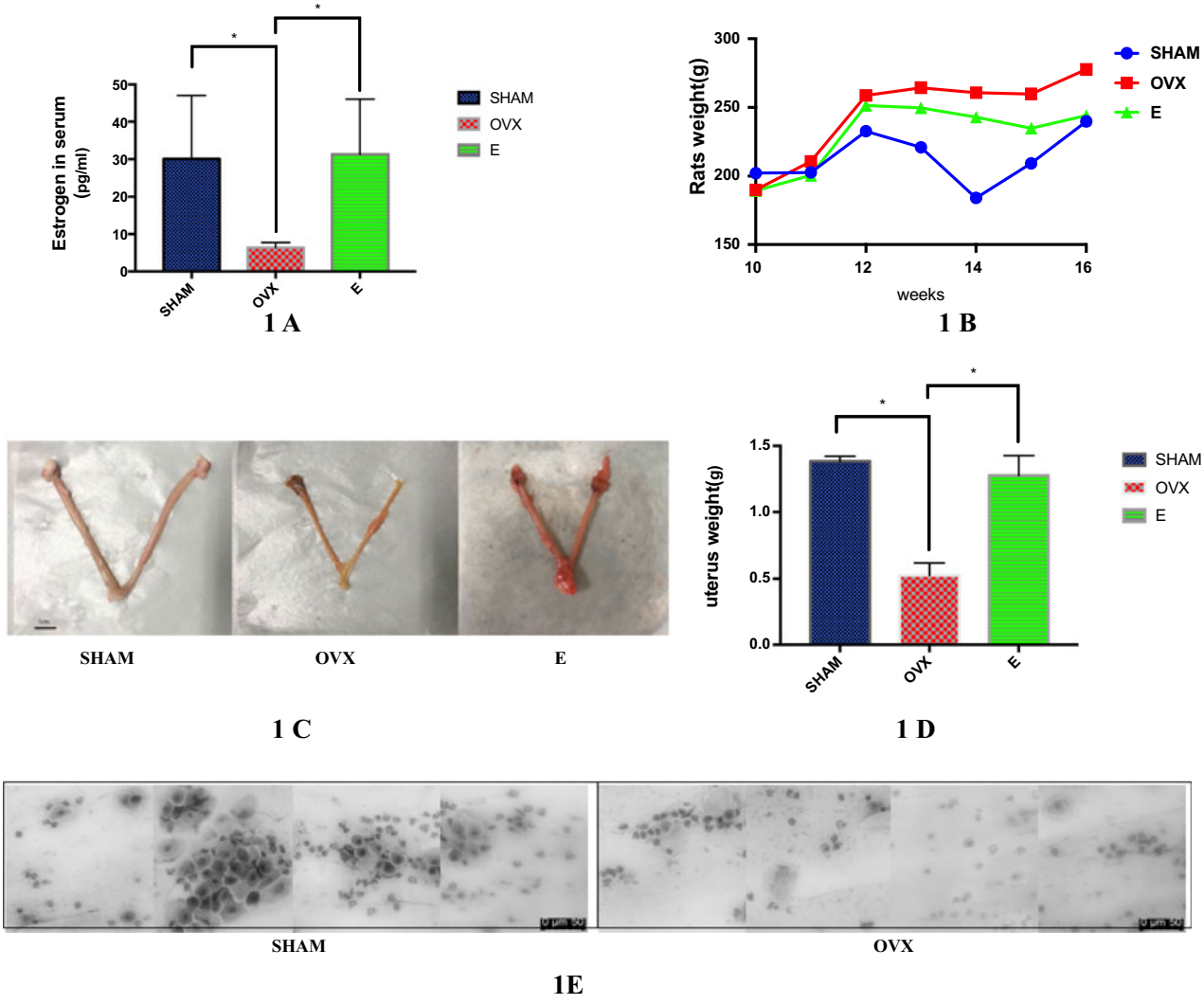

Figure 1. Generation of the ovariectomized rat model. (**A**) Serum estrogen levels in SHAM, OVX, and E groups evaluated using radioimmunoassay. Data represented as mean ± standard deviation (SD). A significant reduction in estrogen levels was observed in the OVX group, confirming successful ovariectomy. $^{*}p < 0.00$. (**B**) Changes in body weight (BW) in the three experimental groups over the experimental period. OVX and E groups showed a decrease in BW after ovariectomy (week 10). BW was restored in the E group with exogenous β-estradiol injections. (**C**) Representative images of uteruses resected from the experimental animals. Ovariectomy altered the morphology of the uterus in the rats. (**D**) Uterine weight in the experimental animals. Data represented as mean ± SD. OVX group showed a reduction in uterine weight, which was recovered with exogenous β-estradiol injections in E group. $^{*}p < 0.00$. (**E**) After ovariectomy, vaginal smears were performed on SHAM and OVX groups. Vaginal smear of SHAM group had an estrous cycle (proestrous, estrous, postestrous, diestrous), while OVX group only had diestrous. E, ovariectomized with β-estradiol oil hypodermic injection; OVX, ovariectomized; SHAM, sham-operated.

Table 2. Rat weight.

		Sum of squares	*df*	*Mean square*	F	p-*Value*
10 weeks	Between groups	110.208	1	110.208	3.624	0.067
	Within groups	851.585	28	30.414		
	Total	961.794	29			
11 weeks	Between groups	18.881	1	18.881	0.155	0.697
	Within groups	3416.853	28	122.030		
	Total	3435.735	29			
12 weeks	Between groups	120.000	1	120.000	0.322	0.575
	Within groups	10,441.200	28	372.900		
	Total	10,561.200	29			
13 weeks	Between groups	690.240	1	690.240	0.864	0.360
	within groups	22,362.121	28	798.647		
	Total	23,052.362	29			
14 weeks	Between groups	5964.300	1	5964.300	2.860	0.102
	Within groups	58,387.067	28	2085.252		
	Total	64,351.367	29			
15 weeks	Between groups	496.133	1	496.133	0.379	0.543
	Within groups	36,674.533	28	1309.805		
	Total	37,170.667	29			
16 weeks	Between groups	210.145	1	210.145	0.209	0.651
	Within groups	28,143.963	28	1005.142		
	Total	28,354.108	29			

Table 3. Uterine wet weight.

	Sum of squares	*df*	*Mean square*	F	p-*Value*
Between groups	6.580	2	3.290	302.909	0.000
Within groups	0.456	42	0.011		
Total	7.036	44			

L1 = L2 = 65 dB) were generated by a TDT System III (RP2 processor; sampling rate 100 kHz) and presented to the ear canal with a custom-made piezoelectric stimulator connected to the probe using a 10-cm-long silastic tube. The magnitude of F1, F2, the DP (DP = 2F1 − F2) cubic distortion product, and the noise floor of the frequency bins surrounding the DP component were measured from fast Fourier transformation. The difference between the amplitude of DP and noise floor surrounding the DP (the maximum one of the surrounding three amplitudes) was the result of the DPOAE from the three groups.

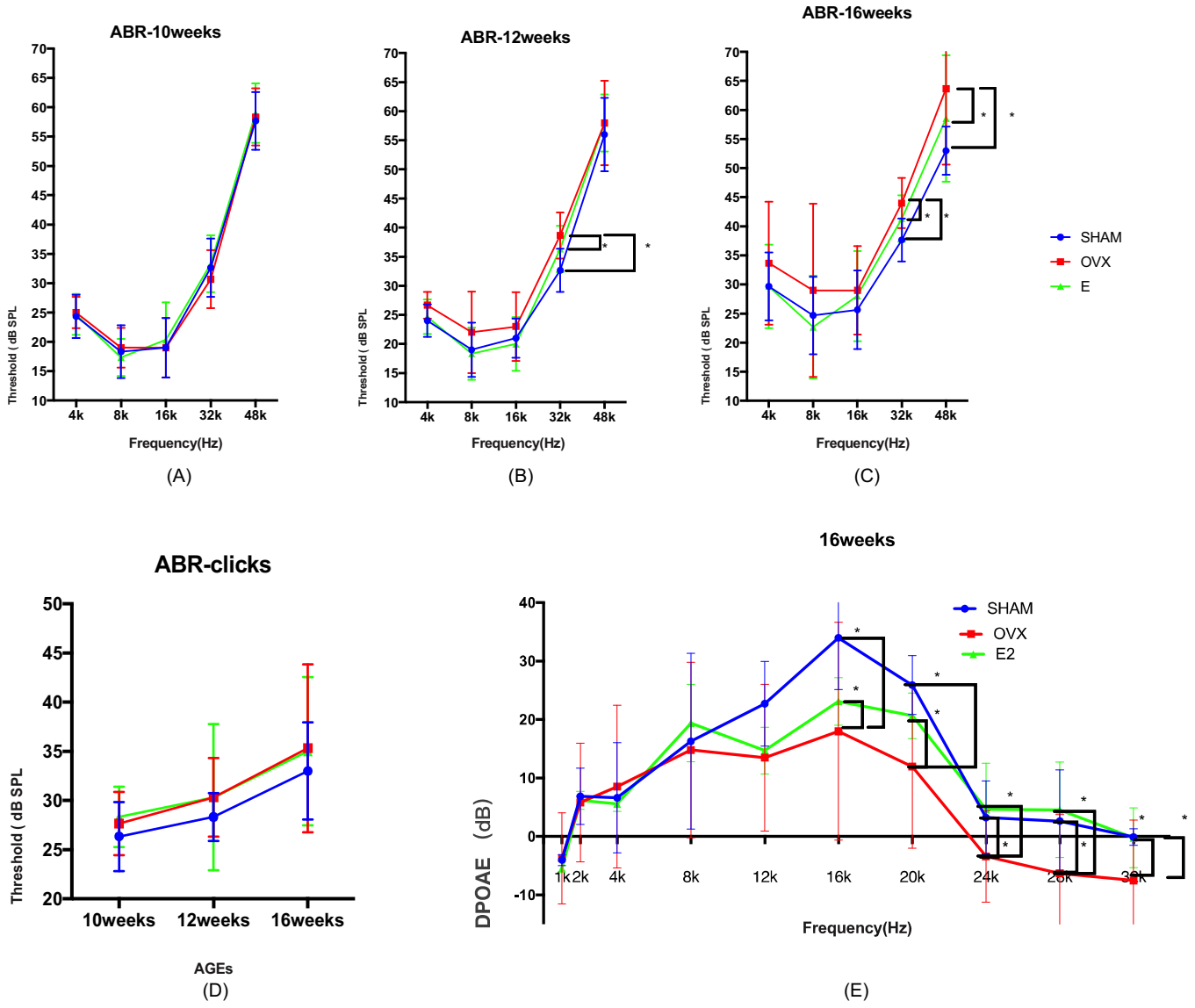

Figure 2. Hearing function assessment in ovariectomized rats. ABR thresholds (measured in decibels) as a function of frequency in the three experimental groups at (**A**) 10 weeks (baseline), (**B**) 12 weeks, and (**C**) 16 weeks. (**D**) ABR-CLICK measurements from the three experimental groups at 10, 12, and 16 weeks. (**E**) DPOAE measurements in the three experimental groups at 16 weeks. OVX group had a significant change in both ABR and DPOAE, which was recovered with exogenous β-estradiol injections in group E. $^{*}p < 0.05$. ABR, auditory brainstem response measurement; DPOAE, distortion product otoacoustic emission; E, ovariectomized with β-estradiol oil hypodermic injection; OVX, ovariectomized; SHAM, sham-operated.

Table 4. ABR-CLICK at 10, 12, and 16 weeks between the three groups.

		Sum of squares	*df*	*Mean square*	F	p-*Value*
10 weeks	Between groups	31.111	2	15.556	1.452	0.246
	Within groups	450.000	42	10.714		
	Total	481.111	44			
12 weeks	Between groups	40.000	2	20.000	0.778	0.466
	Within groups	1080.000	42	25.714		
	Total	1120.000	44			
16 weeks	Between groups	47.778	2	23.889	0.464	0.632
	Within groups	2163.333	42	51.508		
	Total	2211.111	44			

Table 5. Auditory brainstem response tone burst at 10 weeks between the three groups.

Frequency		*Sum of squares*	*df*	*Mean square*	F	p-*Value*
4 kHz	Between groups	3.333	2	1.667	0.150	0.861
	Within groups	466.667	42	11.111		
	Total	470.000	44			
8 kHz	Between groups	21.111	2	10.556	0.756	0.476
	Within groups	586.667	42	13.968		
	Total	607.778	44			
16 kHz	Between groups	17.778	2	8.889	0.289	0.751
	Within groups	1293.333	42	30.794		
	Total	1311.111	44			
32 kHz	Between groups	57.778	2	28.889	1.190	0.314
	Within groups	1020.000	42	24.286		
	Total	1077.778	44			
48 kHz	Between groups	13.333	2	6.667	0.270	0.765
	Within groups	1036.667	42	24.683		
	Total	1050.000	44			

Fluorescence immunohistochemistry and confocal imaging

All animals were sacrificed 28 days after ovariectomy. Immediately after the animals were sacrificed, the temporal

Table 6. Auditory brainstem response tone burst at 12 weeks between the three groups.

Frequency		*Sum of squares*	*df*	*Mean square*	F	p-*Value*
4 kHz	Between groups	43.333	2	21.667	2.967	0.062
	Within groups	306.667	42	7.302		
	Total	350.000	44			
8 kHz	Between groups	114.444	2	57.222	1.873	0.166
	Within groups	1283.333	42	30.556		
	Total	1397.778	44			
16 kHz	Between groups	70.000	2	35.000	1.547	0.225
	Within groups	950.000	42	22.619		
	Total	1020.000	44			
32 kHz	Between groups	274.444	2	137.222	9.005	0.001
	Within groups	640.000	42	15.238		
	Total	914.444	44			
48 kHz	Between groups	40.000	2	20.000	0.512	0.603
	Within groups	1640.000	42	39.048		
	Total	1680.000	44			

Table 7. Auditory brainstem response tone burst at 16 weeks between the three groups.

Frequency		*Sum of squares*	*df*	*Mean square*	F	p-*Value*
4 kHz	Between groups	160.000	2	80.000	1.213	0.308
	Within groups	2770.000	42	65.952		
	Total	2930.000	44			
8 kHz	Between groups	314.444	2	157.222	1.368	0.266
	Within groups	4826.667	42	114.921		
	Total	5141.111	44			
16 kHz	Between groups	87.778	2	43.889	.804	0.454
	Within groups	2293.333	42	54.603		
	Total	2381.111	44			
32 kHz	Between groups	303.333	2	151.667	9.414	0.000
	Within groups	676.667	42	16.111		
	Total	980.000	44			
48 kHz	Between groups	857.778	2	428.889	4.167	0.022
	Within groups	4323.333	42	102.937		
	Total	5181.111	44			

Table 8. Distortion product otoacoustic emissions at 16 weeks between the three groups.

Frequency		*Sum of squares*	*df*	*Mean square*	F	p-*Value*
1 kHz	Between groups	17.678	2	8.839	0.429	0.655
	Within groups	556.154	27	20.598		
	Total	573.832	29			
2 kHz	Between groups	5.822	2	2.911	0.068	0.935
	Within groups	1158.211	27	42.897		
	Total	1164.032	29			
4 kHz	Between groups	44.892	2	22.446	0.237	0.791
	Within groups	2556.303	27	94.678		
	Total	2601.195	29			
8 kHz	Between groups	109.258	2	54.629	0.331	0.721
	Within groups	4451.770	27	164.880		
	Total	4561.027	29			
12 kHz	Between groups	503.135	2	251.568	3.347	0.050
	Within groups	2029.447	27	75.165		
	Total	2532.582	29			
16 kHz	Between groups	1330.871	2	665.435	4.516	0.020
	Within groups	3978.402	27	147.348		
	Total	5309.273	29			
20 kHz	Between groups	993.294	2	496.647	6.321	0.006
	Within groups	2121.349	27	78.568		
	Total	3114.643	29			
24 kHz	Between groups	370.595	2	185.297	3.415	0.048
	Within groups	1464.976	27	54.258		
	Total	1835.571	29			
28 kHz	Between groups	669.653	2	334.827	4.096	0.028
	Within groups	2207.263	27	81.750		
	Total	2876.916	29			
32 kHz	Between groups	363.284	2	181.642	3.989	0.030
	Within groups	1229.364	27	45.532		
	Total	1592.648	29			

bones were removed. The stapes was then luxated and the oval and round windows were opened. A small fenestra was made in the bony shell of the cochlear apex, and the cochlea was gently perfused with 4% (wt/vol) paraformaldehyde and then immersed in the 4% paraformaldehyde overnight. Specimens were decalcified for approximately 3 days in 0.1 M ethylenediamine tetraacetic acid at 37 °C. Once the tissue was soft enough for sectioning, the inner ears were embedded in OCT compound (Tissue-Tek, SAKURA-4583) and frozen at −80 °C. The tissue blocks were sectioned into 40-μm cryosections and stored at −80 °C.

Each specimen was sectioned at a thickness of 10 μm with a freezing microtome (Leica, CM1950). The sections were then rinsed with 0.1% (wt/vol) PBS solution (pH 7.43) three times, and immersed in 0.25% (vol/vol) Triton X for 30 min. Subsequently, they were incubated at 4 °C overnight with the primary antibody (ERα, Thermo-MA513304; prestin, Santa-sc-293212; MYO7A, Abcam-ab3401), diluted as per the manufacturer's instructions. Some sections were not incubated with the primary antibody and were used to check the specificity of the secondary antibodies. After rinsing with 0.1% PBS solution and treatment with 3% (vol/vol) normal goat serum for 1 h, the sections were incubated with the secondary antibody for 1 h. They were then rinsed with 0.1% PBS (three times, 5 min each). The samples were mounted with 4′,6-diamidino-2-phenylindole.

The slides were observed under a confocal laser scanning microscope (Leica, TCS SP8) and photographs were obtained. The slides were photographed with the Corti's organ in the same orientation at a magnification of 63× under the same brightness settings to obtain comparable micrographs. The staining intensity was calculated from image analysis using ImageJ. Twenty sections were chosen randomly in every cochlea and the staining intensities at the basal turn, second turn, and apical turn were measured in each cochlea. All analyses of immunostaining were carried out in a randomized double-blind manner.

Western blotting

Crude cochlear tissue lysates were prepared using RIPA lysis buffer (Merck Millipore, Billerica, MA, USA) following the manufacturer's instructions. Proteins were separated by 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis and transferred to a polyvinylidene difluoride membrane. Non-specific protein binding was prevented with the addition of blocking buffer (5% milk, 20 mM Tris–HCl [pH 7.4], 150 mM NaCl, and 0.1% Tween-20) and blotted with specific primary antibodies in the blocking buffer at 25 °C for 90 min, followed by incubation with the secondary antibody for 60 min. Proteins were visualized using horseradish peroxidase-conjugated secondary antibodies (Jackson Immuno Research, West Grove, PA, USA).

Statistical analyses

Correlation analyses were performed using a commercial statistical software package (SPSS 24).

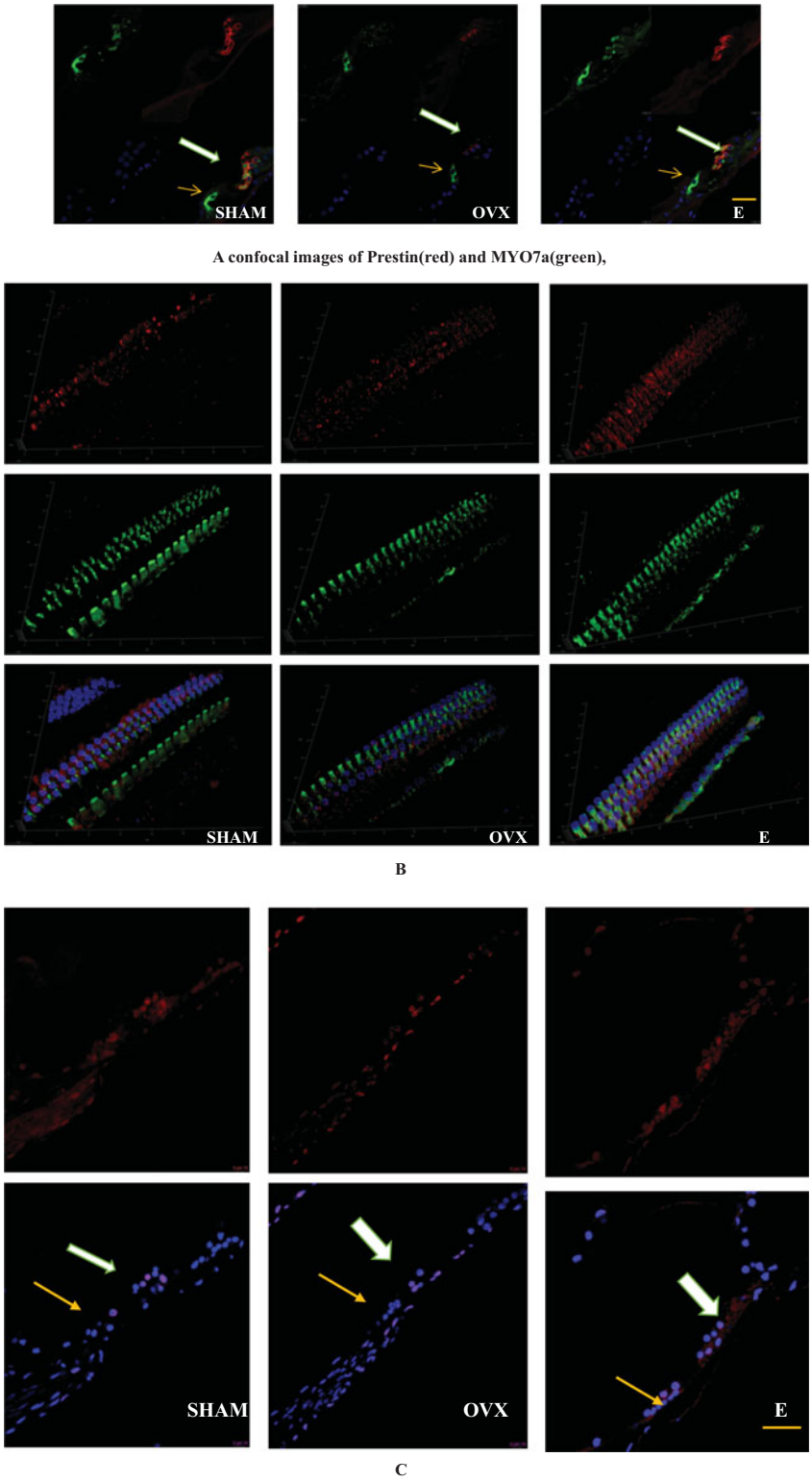

Figure 3. Immunohistochemistry studies. (**A**) Expression of MYO7A (green) and prestin (red) in frozen sections of cochlea. Nuclei shown in blue. (**B**) Expression of MYO7A (green) and prestin (red) in basilar membrane of cochlea in 3D and 2D. Nuclei shown in blue. (**C**) Expression of ERα (red) in frozen sections of cochlea. Yellow arrow points to inner hair cells and white arrow points to outer hair cells. Straight yellow line in lower right corner indicates 20 μm. E, ovariectomized with β-estradiol oil hypodermic injection; MY07A, human myosin VIIA; OVX, ovariectomized; SHAM, sham-operated.

Results

Effect of estradiol on the uterus, body weight, and estrogen in serum and vaginal smears

As shown in Table 1, estrogen levels were significantly lower in the OVX group compared to the SHAM group. Subcutaneous estrogen injections recovered the serum estrogen levels in the E group. The reduction in serum estrogen levels in the OVX group confirmed the success of ovariectomy (Figure 1(A)).

After the ovariectomy, as presented in Table 2, the weight of ovariectomized rats was increased, and the weight of

Table 9. The gray scale of western-blotting in three groups.

		Sum of squares	*df*	*Mean square*	F	p-*Value*
ERα	Between groups	0.116	2	0.058	249.545	0.000
	Within groups	0.001	6	0.000		
	Total	0.117	8			
MYO7a	Between groups	0.270	2	0.135	106.156	0.000
	Within groups	0.008	6	0.001		
	Total	0.278	8			
Prestin	Between groups	0.168	2	0.084	332.813	0.000
	Within groups	0.002	6	0.000		
	Total	0.170	8			

ER, estrogen receptor; MY07A, human myosin VIIA.

ovariectomized rats gradually decreased to the same level as the SHAM group. According to the published data concerned, there was no significant difference in weight between the three groups but there was a trend of weight change (Figure 1(B)).

The uterine morphology was altered in the OVX group (Figure 1(C)), and according to the data presented in Table 3 there was a significant difference between the OVX and SHAM groups, while there was no significant difference between the E and SHAM groups (Figure 1(D)). In addition, vaginal smears were examined for 5 days after the vatiectomy and estrous cycles were observed to determine that there was no estrous cycle in ovariectomized rats (Figure 1(E)). Long-term studies are necessary to evaluate the effect of estrogen on the uterus in ovariectomized rats[18,19].

Audiological evaluations

ABR tone burst

ABR-CLICK is the best stimulus signal for synchronous excitation of the auditory nerve, which can test the auditory threshold, but cannot detect the threshold at a specific frequency. ABR tone burst (ABR-TB) and DPOAE can detect the auditory threshold of a specific frequency in rats, and the change of DPOAE amplitude is correlated with the function of OHCs. The mean hearing thresholds for the right ear at baseline and at follow-up are presented in Tables 4–7, which Figure 2(A–D) relate to. At baseline, the mean thresholds at all frequencies were better than 20 dB hearing level. At follow-up, in the OVX and E groups, the mean thresholds at 32kHz and 48 kHz were about 40 dB hearing level and were not significantly different from the SHAM group. After hypodermic estrogen treatment for 4 weeks, there were significant differences in high frequencies (32 kHz and 48 kHz) between the OVX and SHAM groups, but not between the SHAM and E groups. At the low frequencies (4 kHz, 8 kHz, and 16 kHz), the thresholds were generally better than at the high frequencies and no significant differences between the three groups were observed.

Distortion product otoacoustic emissions

Figure 2(E), which relates to Table 8, represents DPOAEs for each of the three groups at the end of the study period (16 weeks). DPOAE levels between the groups were similar in the low-frequency range at week 16. In the high-frequency range, the OVX group showed significantly lower DPOAE compared to the SHAM and E groups. DPOAEs in the high-frequency group were partially recovered with estrogen administration in the E group.

Results of ABR-CLICK and ABR-TB indicated that the hearing threshold of the OVX group did not change under multiple frequency stimuli, but increased under high frequencies. On the other hand, after estrogen supplementation (group E), the high-frequency hearing threshold was protected (no significant difference from the SHAM group). In addition, the results of DPOAE suggest that the hearing threshold level increase at high frequency was due to the function of OHCs. Two weeks after ovariectomy, the rats' hearing did not change significantly. Six weeks after ovariectomy, there was a significant difference between the SHAM and OVX groups, suggesting that hearing loss would not happen with the short-term change in estrogen.

Fluorescence immunohistochemistry and confocal imaging

ERα, prestin, and MYO7A expression in the cochlea was examined by immunocytochemistry. The OVX group showed lower expression of ERα, prestin, and myosin compared to the SHAM group. Estrogen administration restored the expression of these proteins in the E group (Figure 3).

Western blotting

ERα, prestin, and MYO7A expression in the cochlea was assayed by western blot analysis of three biological replicates. As shown in Table 9 (Figure 4), the OVX group showed a significant decrease in ERα, prestin, and MYO7A expression compared to the SHAM group. Estrogen administration recovered the expression of these proteins.

Discussion

This study demonstrates an induction of high-frequency hearing loss in ovariectomized rats. This is consistent with our previous results which showed a rapid high-frequency hearing loss in women after menopause[7,20]. Recent studies have revealed a network of proteins interacting with prestin in the OHCs and responsible for sensory amplification. These proteins participate in biotransformation, transmembrane folding and localization, and signal transduction[21]. A decrease in prestin expression is associated with hearing loss[22]. Prestin is also a biomarker of cisplatin ototoxicity in the OHCs[23]. Our study revealed a decrease in prestin expression in ovariectomized rats that was restored with subcutaneous administration of estrogen. A similar decrease in the expression of MYO7A was also observed in the ovariectomized rats. MYO7A mutation or loss of expression has been reported to cause otology diseases[24,25]. Estrogen is also known to affect the expression of myosin in other tissues and organs[26,27]. Our results confirm that hearing loss after menopause may be due to the low estrogen levels affecting the expression of prestin and MYO7A.

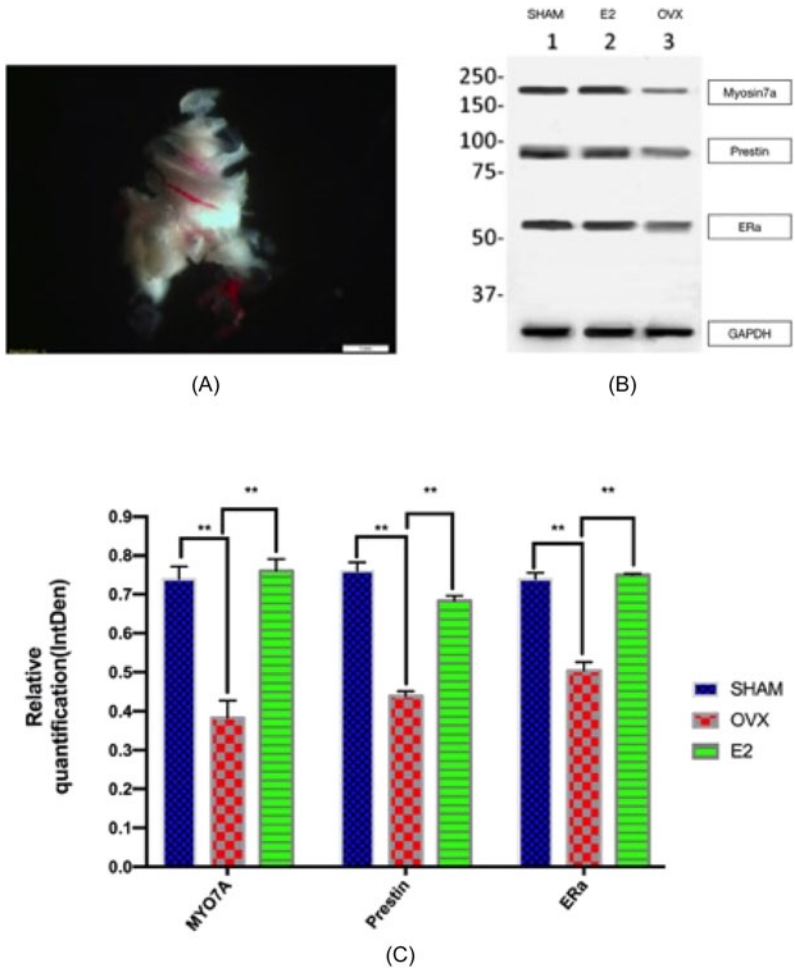

Figure 4. MYO7A and prestin expression in the whole cochlea. (**A**) Representative picture of a rat cochlea. (**B**) Representative western blot gel showing the expression of various proteins in the whole cochlear extract from the three experimental groups. (**C**) Densitometric quantification of western blot data. Data represented as mean ± standard deviation. OVX showed a decrease in the expression of ERα, MYO7A, and prestin which was recovered by exogenous β-estradiol injections in the E group. $^{**}p < 0.001$. E, ovariectomized with β-estradiol oil hypodermic injection; ER, estrogen receptor; GAPDH, glyceraldehyde 3-phosphate dehydrogenase; MY07A, human myosin VIIA; OVX, ovariectomized; SHAM, sham-operated.

Previous studies on the mechanisms of hearing loss after menopause focused on population studies and the effect of hormone replacement therapies. This is the first study to detect the downregulation of prestin and MYO7A in the cochlea using an ovariectomized rat model.

Conclusion

Our results indicate that low levels of estrogen may decrease the expression of prestin and MYO7A, leading to hearing loss. Advancing age and the reduction in estrogen level may hasten hearing loss, and exogenous administration of estrogen might have a protective effect against hearing loss after menopause.

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