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Short Communication

Hereditary deafness occurring in cd/1 mice

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Different strains of mice provide a valuable research tool for studying both hereditary and acquired forms of deafness. The cd/1 strain has been found to demonstrate hereditary cochlear pathology. The characteristics of hearing loss in cd/1 mice have not previously been reported. In this investigation auditory thresholds were obtained by measuring evoked brain stem responses in subjects of three different ages: 3 weeks, 10 weeks and 6 months. The results were compared with thresholds obtained from CBA/Ca mice (which have normal hearing) and C57BL/6 mice (which are known to have a genetically determined pre-senile progressive cochlear hearing loss). A significant hearing loss was observed which progressed from high to low frequencies, and with age. Extensive degeneration was observed throughout the organ of Corti. cd/1 mice may provide a useful model for studying genetically determined deafness.

Mice; Genetic; Deafness; Cochlea; ABR.

Introduction

Of the hundreds of mice strains relatively few have been studied for hearing (Erway, 1990). While some strains such as CBA/J and CBA/Ca hear normally into advanced age (Henry and Chole, 1980), others such as C57BL/6 develop an early sensorineural and/or conductive hearing loss that is genetically determined (Henry and Lepkowski, 1978).

The advantages of studying hereditary deafness in strains of mice are, firstly, that the histopathology found in deaf mice demonstrates features similar to many found in humans (Mikaelian, 1979; Chole and Henry, 1983; Henry, 1983); secondly, there is considerable conservation of the genomes between mice and humans, so that genetic analysis of loci involving hearing impairment in mice may provide valuable clues to the location of equivalent loci in humans (Steel, 1990), and thirdly, mice breed relatively rapidly and therefore, once a phenotype of interest is discovered, it is possible to determine the genetic traits of that phenotype and to create an inbred colony for genetically-determined studies.

One strain which often demonstrates cochlear pathology is the cd/1 mouse (Hill, 1981). This strain has been widely used in non-auditory research and is readily available. The purpose of this study was to assess the functional and structural characteristics of the peripheral auditory system and their changes with time from pubescence to maturity.

Method

A total of 20 cd/1 mice of different ages, ten CBA/Ca mice (aged 6 months) known to have normal hearing (Henry and Chole, 1980), and ten C57BL/6 mice (age 6 months) known to have cochlear hearing loss (Henry and Chole, 1980; Willott, 1986) were obtained from Charles River Laboratories for use in this study. Experimental methods were reviewed and approved by the University of Michigan Committee on Use and Care of Animals, and the subjects were maintained throughout the study in IACUC approved facilities.

Hearing thresholds were assessed by evoked auditory brain stem responses (ABR). Mice were anesthetized with ketamine 80 mg/kg and xylazine 14 mg/kg, placed in an acoustically and electrically shielded booth, and protected from heat loss using a heating pad. Acoustic stimuli were delivered via a modified aural speculum placed just inside the ear canal without distorting the meatus. The stimulus consisted of tone bursts of alternating polarity with a trapezoidal envelope of 15 ms overall duration, linear rise and fall times of 1 ms, and presented at a rate of 10/s. The frequencies tested were 8, 12, 16, 20, 24 and 32 kHz. Stimuli were measured calibrated by a 1/2

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inch Bruel & Kjaer microphone with calibrated probe tube, which was mounted in the speculum. Electrophysiological responses were recorded from subcutaneous needle electrodes placed at the vertex and at the ipsilateral retro-auricular region, with the ground behind the opposite ear. For each measurement, 1024 sweeps were averaged after 1000 times amplification and 300–3000 Hz bandwidth filtering. Responses to stimuli at intensity intervals of 10 dB were evaluated. The threshold was defined as being 5 dB better than the intensity of the last reproducible response.

Thresholds were obtained from cd/1 mice aged 3–4 weeks ($N = 8$), ten weeks ($N = 4$), and six months ($N = 8$). For each age group the mean threshold was calculated for each frequency tested. The results were compared with the mean thresholds obtained from 6 month old CBA/Ca and C57BL/6 subjects.

Four 6 month old cd/1 mice were used for anatomical studies. They were anesthetized as described for ABR testing and systemically perfused with a fixative containing 4% paraformaldehyde and 1% glutaraldehyde in 0.15 M cacodylate buffer, pH 7.4. The cochleas were post-fixed in 1% osmium tetroxide and dissected as surface preparation for light microscope (LM) examination, or, prepared for scanning electron microscopy (SEM) as previously described (Raphael and Altschuler, 1991).

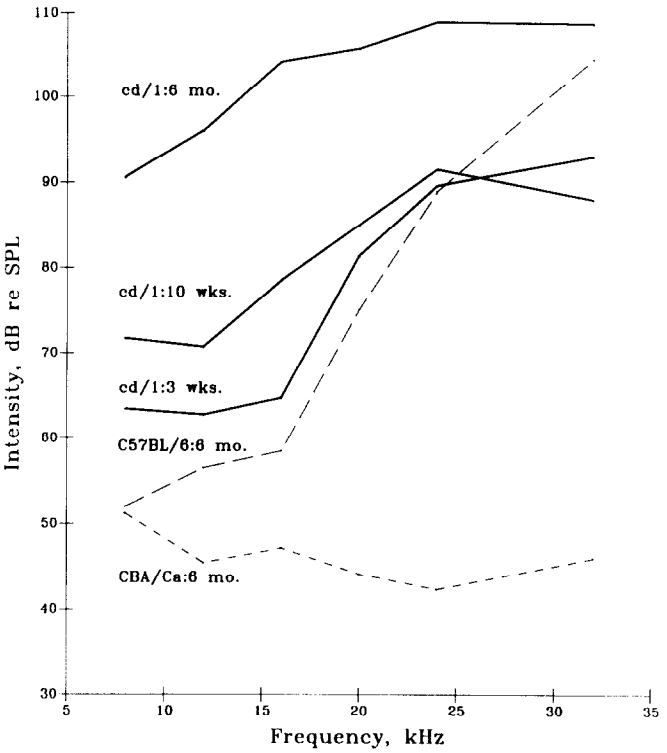

Fig. 1. Mean ABR threshold functioning for 6 month CBA/Ca mice ($N = 10$), 6 month C57BL/6 mice ($N = 10$), 3–4 week cd/1 mice ($N = 8$), 10 week cd/1 mice ($N = 4$) and 6 month cd/1 mice ($N = 8$).

Results

Mean thresholds and the standard deviations observed at each frequency tested are presented in Fig. 1 and Table I. The results from the CBA/Ca subjects represent normal hearing. The 6 month old C57BL/6 subjects demonstrated a significant high-tone hearing loss, as described by previous investigators (Henry and Chole, 1980; Henry, 1982; Shnerson and Pujol, 1981; Willott, 1986). The cd/1 subjects have a significant hearing loss that is age-dependent. At 3 weeks, their threshold sensitivity is elevated throughout the frequency range of hearing compared to CBA/Ca subjects. For low frequencies, this loss is 10–15 dB, while at highest frequencies it may be more than 40 dB. The hearing loss increases with age and by six months cd/1 subjects show a 40 dB loss for low frequencies and more than 60 dB at high frequencies. Significant between-animal variation in the hearing loss was observed in the cd/1 strain, as seen in the high standard deviations (Table I). This was also seen in the higher test frequencies for the C57BL/6 subjects.

In all four cd/1 mice which were used for anatomical studies, the middle ears were found to be structurally normal. During dissection of the tissue for LM or SEM, it became evident that the tectorial membrane was present throughout the cochlear duct. In the basal

TABLE I

MEAN THRESHOLDS: dB SPL (1 s.d.)

	Frequency kHz					
	8	12	16	20	24	32
STRAIN/AGE						
cd/1: 3 wks ($N = 8$)	63.4 (20.2)	62.7 (25.9)	64.7 (28.2)	81.4 (21.5)	89.6 (12.7)	93.0 (9.3)
cd/1: 10 wks ($N = 4$)	71.7 (20.1)	70.7 (22.1)	78.5 (18.9)	85.0 (11.7)	91.5 (4.3)	88.0 (0)
cd/1: 6 mo. ($N = 8$)	90.5 (23.7)	96.0 (19.2)	104.1 (10.6)	105.7 (9.4)	108.9 (5.6)	108.7 (6.7)
C57BL/6: 6 mo. ($N = 10$)	52.0 (6.2)	56.5 (6.5)	58.5 (8.3)	75.2 (9.5)	89.0 (8.5)	104.5 (7.1)
CBA/Ca: 6 mo. ($N = 10$)	51.2 (4.2)	45.5 (3.5)	47.2 (5.2)	44.2 (3.2)	42.4 (2.8)	46.0 (3.5)

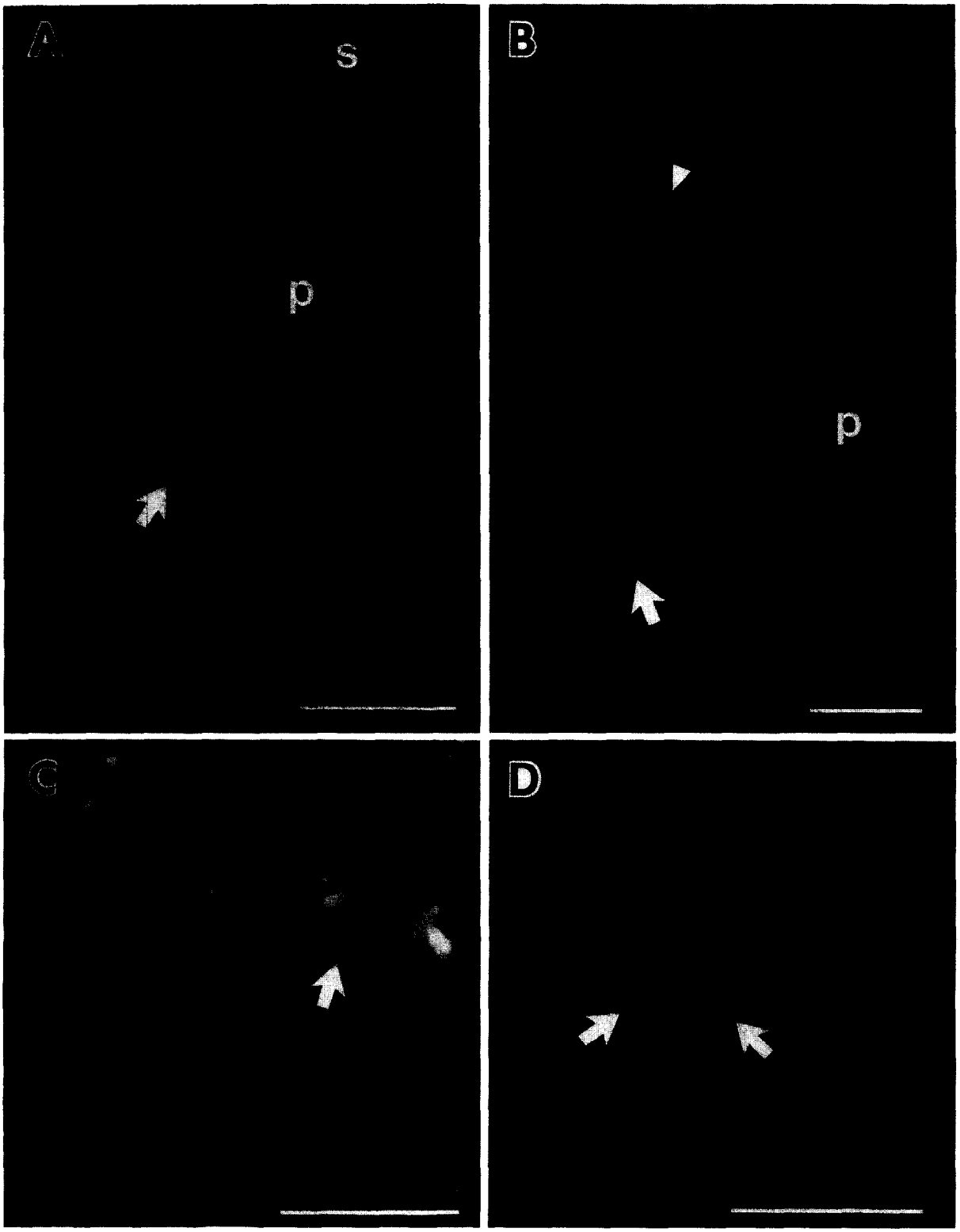

Fig. 2. SEM micrographs showing reticular lamina in a six months old cd/1 mouse cochlea. Upper part of micrographs corresponds with lateral aspect of reticular lamina. (A) An area in apical turn where all hair cells are missing. Scars replace inner hair cells (arrow) and outer hair cells (s). The area of inner pillar cells (p) remains organized. (B) Near the helicotrema, inner (arrow), outer (arrow-head) and pillar (p) cells are present. Note that cells do not appear normal. (C) Distorted inner hair cells (arrow) are seen in area where no outer hair cells are present. (D) Two degenerating inner hair cells. Supporting cells invade sub-apical space of one inner hair cell (arrows). Scale bars = 5 mm

turn the tectorial membrane was completely detached from the epithelial surface and, in the apical turn, it was very lightly attached.

Whole-mount preparations of the organ of Corti were examined at the LM level. In the basal turn of the cd/1 mouse cochlea, no hair cells or supporting cells were present. The basilar membrane was covered with a simple epithelium and the vas spirale was present. Approximately 50% of the inner, and 80% of the outer hair cells were missing in the apical turn.

SEM analysis revealed that pillar cells were present throughout the apical turn, even where no hair cells had remained (Fig. 2A). This is in contrast to findings after drug-induced cochlear degeneration in man and guinea pig (Johnsson et al., 1981; Raphael and Altschuler, 1991a), where complete absence of hair cells was concomitant with complete degeneration of the organ of Corti. Another unexpected difference, in comparison to scars after drug- or noise-induced injury, is that the scar cells which replaced outer hair cells in the cd/1 mouse cochlea were not heavily covered by microvilli (Fig. 2A, compare to Raphael and Altschuler, 1991b).

The rate of surviving inner and outer hair cells was highest near the helicotrema (Fig. 2B). Quantitation of hair cell loss performed with SEM was in agreement with LM results. Qualitatively, however, SEM revealed that hair cells which remained in the apical turn, did not appear normal (Fig. 2B–D). It is likely, therefore, that the degenerative process in the organ of Corti was still advancing at six months.

Conclusion

Cd/1 mice were found to have a significant degree of cochlear hearing loss, that was progressive with age. At six months, the organ of Corti was completely degenerated in the basal turn and severely damaged in the apical turn. This extensive degeneration observed at six months was consistent with the severe threshold changes found at this time. Cd/1 mice may prove to be a useful model for studying genetically determined hearing loss. For this purpose, the genetic trait of the deafness phenotype should be investigated and an inbred line carrying this phenotype in a defined genetic background should be established.

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