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Research paper

Auditory function and cochlear morphology in the German waltzing guinea pig

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Abstract

The German waltzing guinea pig is a new strain of animals with a recessively inherited inner ear defect resulting in deafness and severe vestibular dysfunction. Measurements of auditory brainstem responses (ABRs) demonstrated that the homozygotes (*gw*/*gw*) are deaf while the heterozygotes (*gw*/+) have normal hearing. In the *gw*/*gw* cochlea, a collapse of Reissner's membrane leads to the absence of scala media. Melanin pigment accumulation was clearly observed in the *gw*/*gw* stria vascularis, and both the height and width of stria vascularis were significantly reduced. Ultrastructural observations further detailed the disorganization of stria vascularis in the *gw*/*gw* animals: marginal cells lacked basolateral infoldings; intermediate cells (melanocytes) were scarce and degenerated; and basal cells were difficult to identify. The level of degeneration of the organ of Corti varied between individual *gw*/*gw* animals. The density of spiral ganglion neurons was significantly decreased in old (1–2 years of age) *gw*/*gw* animals. In contrast, no pathological changes were observed in the cochleae of *gw*/+ animals. Our data suggest that the degeneration originates in the stria vascularis (most likely in the melanocytes), and that this is the primary cause for inner ear defects in the German waltzing guinea pig. Here, we describe the auditory function and cochlear morphology in this spontaneously mutated guinea pig strain.

Keywords: Deafness; Inner ear; Melanocytes; Phenotype; Recessive genes; Stria vascularis

1. Introduction

The mammalian inner ear is a complex sensory system comprising the auditory and vestibular organs. Various specialized cells within the hearing organ, in particular sensory hair cells, are required to orchestrate the acquisition of sound. The delicate inner ear structure is vulnerable to both exogenous insults (e.g. noise trauma, ototoxic agents) and intrinsic genetic alterations, which consequently cause hearing impairment. Among these, genetic factors account for at least 50% of hearing disorders (Reardon, 1992). Understanding human pathology of inherited types of deafness and their underlying molecular mechanisms is largely impeded by the limited access to the human inner ear and availability of human tissues. In addition, detailed electrophysiological and developmental studies are not

Abbreviations: ABRs, auditory brainstem responses; TEM, transmission electron microscopy; +/+, wild-type animals; *gw*/+, heterozygous animals; *gw*/*gw*, homozygous animals; EP, endocochlear potential; RW, round window; OW, oval window; SM, scala media; SV, scala vestibuli; ST, scala tympani; RM, Reissner's membrane; StV, stria vascularis; TM, tectorial membrane; IHC, inner hair cell; cp, cuticular plate; aff, afferent nerve endings; eff, efferent nerve endings; tj, tight junction; SpP, spiral prominence; SpL, spiral ligament; MC, marginal cell; mit, mitochondria-rich basolateral infoldings; IC, intermediate cell; BC, basal cell; bv, blood vessels; R, Rosenthal's canal

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readily carried out in human inner ears. Thus, animal models closely mimicking human hearing disorders are particularly useful. The correlation between waltzing (circling) behavior and hereditary hearing loss in various animal mutants was noticed by auditory researchers already in the early 20th century. Identification of defective genes in deaf animal mutants has revealed direct clues to the etiology of human genetic deafness. A typical example is that positional cloning of the *Myo7a* gene in *shaker-1* mutant mice (Gibson et al., 1995) facilitated the identification of mutations in the homologous human gene *MYO7A*, which is responsible for non-syndromic deafness DFNB2 (Liu et al., 1997a) and DFNA11 (Liu et al., 1997b), as well as for Usher syndrome type 1B (Weil et al., 1997). Thus, it is extremely valuable to identify and characterize new animal mutants with inherited hearing and balance problems.

The guinea pig is established as an important animal model for research on cochlear physiology. The time course of guinea pig inner ear development is similar to that of the human, with an auditory function that develops prenatally (Horner et al., 1987). Moreover, both human and guinea pig populations are characterized by genomic heterogeneity, which is lacking in the spontaneously deaf mice occurring in large inbred populations. Two previously known guinea pig strains displaying hereditary hearing loss and typical waltzing/circling behavior have been quite extensively described. The first strain of waltzing guinea pigs, the Kansas strain, was described already in the 1920s (Ibsen and Risty, 1929). The Kansas strain followed a pattern of autosomal recessive inheritance. The homozygous waltzing guinea pigs appeared deaf from birth. Morphological studies of the cochlea in the homozygotes showed variable degeneration of the organ of Corti (Lurie, 1939; Lurie, 1941). Unfortunately, neither the inner ear degeneration nor its molecular basis has been fully investigated since the Kansas strain of waltzing guinea pig is not available anymore. Another strain of waltzing guinea pigs, the NIH strain, was extensively studied by Ernstson (1970, 1971a,b, 1972)). The NIH strain displays a dominant mode of inheritance with a recessive lethal effect (Ernstson, 1970). The homozygotes exhibit progressive hearing loss, which becomes severe two weeks after birth (Ernstson, 1972; Canlon et al., 1993). Most hair cells in the cochlea of this waltzing guinea pig appeared normal at birth, but were missing at 2–3 months of age, and the hair cell loss was followed by degeneration of spiral ganglion neurons (Ernstson, 1971a). There was no apparent atrophy of the stria vascularis. Early pathological features consisted of hair bundle fusion and protrusion of the cuticular plate in both cochlear and vestibular hair cells (Ernstson et al., 1969, 1971b). Transmission electron microscopy (TEM) studies revealed unique actin filament rods within type I vestibular hair cells of the NIH strain waltzing guinea pig. Despite extensive studies, the causative genetic defect has not been identified for either of the two strains.

The third and most recent strain of waltzing guinea pigs arose spontaneously and was originally discovered in 1996 in Germany, thus named the German waltzing guinea pig. The autosomal recessive pattern of inheritance was determined by systematic breeding experiments (Skjönsberg et al., 2005). However, the underlying genetic substrate is still unknown. Homozygotes (*gw*/*gw*) are deaf at birth and display circling behavior. Heterozygotes (*gw*/+) are symptom-free and have normal hearing, and they interestingly and paradoxically exhibit resistance to noise exposure, while more susceptible to gentamicin (Skjönsberg et al., 2005; Halsey et al., 2005). Cochlear structures in heterozygotes did not differ from those in wild-type guinea pigs (+/+). We describe in this study general phenotype, auditory function, cochlear morphology and morphometric analysis in the German waltzing guinea pig.

2. Materials and methods

2.1. Experimental animals

Pigmented homozygous (*gw*/*gw*) and heterozygous (*gw*/+) animals from the German waltzing guinea pig strain were used in the present study. Heterozygous animals (*gw*/+) were identified by controlled breeding (Skjönsberg et al., 2005). Wild-type (+/+) pigmented guinea pigs from the commercially available strain (“Sahlin strain”; Bio Jet Service, Uppsala, Sweden) were used as controls. The age of the animals varied from newborn up to 2 years of age. All procedures followed approved protocol for care and use of animals (approval N465/03).

2.2. Auditory function assessment

Methods used in the present study have been described in detail previously (Skjönsberg et al., 2005). In short, auditory brainstem responses (ABRs) were recorded from the right ear at discrete frequencies ranging from 1 kHz to 12.5 kHz (depending on genotype and age). Animals were anesthetized (ketamine 50 mg/kg and xylazine 10 mg/kg, i.p.) and needle electrodes inserted subcutaneously on the vertex (positive), behind the recorded ear (negative) and in the hind leg (ground). Sinusoidal tone bursts (20 stimuli/s) were produced by a Tucker Davis Technologies system (TDT system II hardware and BioSig 32 version 3.12 software) and delivered to a TDH-39 earphone connected to the ear canal via a short plastic tube. Up to 2000 responses (gain 100000) at each level and frequency were averaged and filtered through a band-pass filter (30–3000 Hz) and a 50 Hz notch filter. In heterozygous animals (*gw*/+) the stimuli were initially presented at 90 dB SPL (frequencies 2–12.5 kHz), and then decreased in 10 dB-steps. Measurements started at postnatal day 7 and continued weekly until 12 weeks of age. In the threshold region, the stimuli were varied in 5-dB steps and the hearing threshold in each animal was defined as the lowest level where a reproducible wave was observed. In homozygous