Auditory Disorder in the Cerebellar Vermis Defect (cvd) Rats

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ABSTRACT

The cerebellar vermis defect (cvd) rat is a mutant characterized by a cerebellar vermis defect and fused cerebellar hemisphere. This mutant rat also exhibits heterotopic dysplastic cerebellum, especially in the cerebello-pontine junctional zone. We focused on the auditory system of the cvd rat. The homozygous cvd rats exhibited bilaterally indistinct ABR (auditory brainstem response) waves or prolongation of latency in each ABR component. The number of the spiral ganglion neurons in the Rosenthal's canal was apparently decreased in the homozygous rats. Present study indicates the homozygous cvd rats had auditory disorders concerned with cerebellar malformation.

The cerebellar vermis defect (cvd) rat is a mutant characterized by a cerebellar vermis defect and fused cerebellar hemisphere (8). This mutant rat also exhibits heterotopic dysplastic cerebellum, especially in the cerebello-pontine junctional zone (8). These characters are inherited by a simple autosomal recessive mode (9). Therefore, the cvd mutant may provide a useful animal model for studying the pathogenesis of the cerebellar vermis defect and cerebellar cortical dysplasia.

The histogenesis of cerebellum in the cvd rat has been investigated; many cerebellum-constituting cells penetrated into the pons, the external granule cells (EGCs) aggregated perivascularly, and the aggregated EGCs migrated radially around the vessels, resulting in the heterotopic and dysplastic cerebellum (10). However, the details of the morphological and functional abnormalities have not been reported in the cvd rat except for the cerebellum. Auditory disorders were reported in some mutant rats, such as white spotting (Ws) rat (5) and spontaneously epileptic (SER) rat (7). In the present study, we focused on the auditory system of the cvd rat.

MATERIALS AND METHODS

The cvd rats originated from spontaneously ataxic rats found in the LEW strain. The mutant colony has been kept as a hybrid strain with both LEW and Donryu genetic backgrounds (8). We mated heterozygous (+/cvd) females and heterozygous (+/cvd) or homozygous (cvd/cvd) males, and obtained the homozygous (cvd/cvd), heterozygous (+/cvd) and wild type (+/+) rats used in this study. The used rats were applied to mate with proven-heterozygous rats to check the genotype. Four homozygous, 3 heterozygous, and 3 wild type rats aged at 16 weeks were examined.

Auditory brainstem response (ABR) was recorded on the animals treated with a sedative xylazine hydrochloride (0.1 mg/kg, I.M.), which was known to have no effect on ABR pattern (2). Click stimuli of alternating polarity generated by 4 kHz single sine waves were used at a rate of 20 Hz by an acoustic stimulator (Nihon Kohden, SMP-3100). Clicks were delivered through an inner type earphone (SONY, MDR-E464), which was attached directly to the
external auditory meatus of rats. Small stainless steel needle electrodes were inserted subcutaneously on the parietal region and unilateral retroauricular areas, and a ground electrode was put on the other retroauricular areas. ABRs were measured separately for each ear using the Neuropack MEB-5504 (Nihon Kohden, Japan), and took an average from 1000 responses. Right ear was stimulated at intensities of 135, 120, 110, 90, 70, 60, 50 and 40 dB SPL (sound pressure level). Left ear was stimulated with 135, 120, 110 dB SPL. Hearing thresholds were determined from the lowest intensity yielding reliable components.

After the ABR examination, rats were sacrificed under ether anesthesia. The cochlear organs and brain were removed and processed for histopathological examination. The detail methods for the cochlear organs had been reported by Wada et al. (14). The tissue samples were fixed in 10% neutral buffered formalin for 24 h, and the cochlear organs were decalcified in 10% formic acid solution. Then the specimen were dehydrated in graded ethanol and embedded in paraffin. The sections were routinely stained with hematoxylin and cosin (HE). The cochlear organs were examined with axial sections. The cross-section area of Rosenthal’s canal and the number of spiral ganglion cells were measured.

A specific Tanabe Seiyaku’s Animal Care and Use Committee have approved this experimentation.

RESULTS

All of the heterozygous and wild type cvd rats had robust ABRs with the expected thresholds. The ABR thresholds of the heterozygous and wild type rats were 50 dB SPL. On the other hand, 3 of the 4 homozygous rats exhibited bilaterally indistinct ABR waves. Because of all the ABR waves were disappeared in the 3 homozygous cvd rats, typical waves shown in Fig. 1. The other homozygous rat showed prolongation of latency in each ABR components and higher auditory threshold (60 dB SPL) comparing with that of heterozygous or wild type rats.

The cerebellar vermis of the homozygous cvd rats was absent and cerebellar hemispheres were fused in the midline. Histologically dysplastic cerebellum was distributed in the lateral of the midbrain and beneath the fused cerebellar hemispheres. Any other CNS (central nervous system) in the homozygous rats was almost normal. In the cochlea, though Corti’s organ was almost normal, the area of Rosenthal’s canal (spiral canal of cochea) and the number of spiral ganglion cells were decreased in all the homozygous cvd rats (Table 1). Neither inflammatory cells nor degenerated cells were present throughout the scala tympani (Fig. 2). The spiral ganglion neurons of heterozygous and wild type rats were well preserved. No abnormality was found in the auditory system of heterozygous and wild type rats.

DISCUSSION

It is generally considered that the waves I, II, III and IV of the ABR are evoked from the cochlear nerve, cochlear nuclei, superior olives and inferior colliculi, respectively (1, 4, 13). Thus, the ABR recording is a useful method for detection of dysfunction site in the auditory pathway. All the ABR waves disappeared in the 3 homozygous cvd rats and only one homozygous rat showed prolongation of latency in...
Table 1  The cross-section area of Rosenthal’s canal and the number of neuronal ganglion cells

<table>
<thead>
<tr>
<th></th>
<th>Area of Rosenthal’s canal (mm²)</th>
<th>Number of neuronal ganglion cells / Rosenthal’s canal</th>
<th>n</th>
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<tbody>
<tr>
<td>Wild type</td>
<td>0.0435 ± 0.0112</td>
<td>143 ± 15</td>
<td>n=32</td>
</tr>
<tr>
<td>Heterozygous</td>
<td>0.0385 ± 0.0253</td>
<td>127 ± 38</td>
<td>n=34</td>
</tr>
<tr>
<td>Homozygous</td>
<td>0.0135 ± 0.0314</td>
<td>16 ± 15</td>
<td>n=20</td>
</tr>
</tbody>
</table>

Fig. 2  Axial view of the cochlea from heterozygous (A) and homozygous (B) rats. Note the prominent loss of spiral ganglion cells in the homozygous rats. Bar = 200 µm. C: Higher power view of A. D: Higher power view of B. Bar = 40 µm. H-E stain.

Each ABR component. On the other hand, all the homozygous cvd rats showed loss of spiral ganglion cells and cerebellar malformation. Therefore, the ABR dysfunctions were suggested relevance to the histological disorder. The cvd rats have dysplastic cerebellum in the cerebello-pontine junctional areas contiguous to auditory pathway in the CNS (8). It appeared that heterotopic cerebellum affected to the acoustic function in the CNS of cvd rats.

The histopathological examination revealed loss of spiral ganglion cells in the Rosenthal’s canal of the homozygous rats. Although, there were some reports on aging showing ganglion cell loss without a sign of hair cell degeneration (3, 6, 11). It is reported that age-related hearing loss starts from 14 months of age in the rats (12). The present study was performed in young cvd rats. Thus, aging effect may be ruled out in the present study. As no degenerative and reactive change was found in the cochlear organ and the CNS of the auditory pathway, the loss of spiral ganglion cells is considered to be a secondary atrophy followed with the auditory disorder.

In conclusion, the present study indicates that the homozygous cvd rats had auditory disorders concerned with cerebellar malformation.

REFERENCES
