Validity of Ewald’s Law in the Horizontal Semicircular Canals and the Vestibular Nuclei: Electron Microscopic Observation and Quantitative $[^{14}C]$-2-Deoxy-D-Glucose Study

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HOZAWA, J., USAMI, S. and MORI, I. Validity of Ewald’s Law in the Horizontal Semicircular Canals and the Vestibular Nuclei: Electron Microscopic Observation and Quantitative $[^{14}C]$-2-Deoxy-D-Glucose Study. Tohoku J. exp. Med., 1986, 150 (4), 467-474 — Two series of experiments were performed by using a total of 36 guinea pigs. The 1st series was to compare the influence of rotatostimulation on the bilateral cristae ampullare. After repeating the rotatostimulation with the cupulometric mode (the terminal turning velocity: 450°/sec) for 48 hr, ultrastructural changes of sensory cells were found in 9 of 17 animals with transmission electron microscopy. They showed stronger influences on the ampullopetal flow side with the exception of one animal. In the 2nd series, the difference in excitability between the bilateral vestibular nuclei was investigated on 6 guinea pigs which were exposed to the clockwise constant angular acceleration of 1°/sec$^2$ for 8 min. The autoradiographic study with $[^{14}C]$-2-deoxy-D-glucose (the 2-DG) revealed that the rotation-induced glucose uptake was increased significantly in the superior and the medial vestibular nuclei of the ampullopetal flow side. The results of the two series of experiments suggest the predominance of the ampullopetal effect not only in the horizontal semicircular canals but also in the vestibular nuclei. ——— Ewald’s law; ultrastructure; crista ampullaris; vestibular nucleus; autoradiography; rotatostimulation

“For the horizontal semicircular canal, ampullopetal flow of the endolymph is much more effective than ampullofugal, and the effective endolymphatic flow produces a nystagmus to the same side”. This is well known as Ewald’s law (Ewald 1892), and its validity has been discussed by many researchers.

The purpose of this paper is to confirm Ewald’s law not only in the horizontal

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semicircular canals but also in the vestibular nuclei. We, therefore, devised experimental methods, by which the right horizontal semicircular canal of guinea pigs was always stimulated by the ampullopetal endolymphatic flow and the left was stimulated by the ampullofugal flow. The stimulation could be given continuously for a long time sufficiently to observe its influence. By using these experimental methods, we performed the following two observations: one was to compare ultrastructural changes of sensory cells of the bilateral semicircular canals, and the other was to compare the amounts of the rotation-induced glucose uptake in the bilateral vestibular nuclei.

**MATERIALS AND METHODS**

A total of 36 normal white guinea pigs (300–600 g) showing no difference in the caloric response between the two labyrinths was divided into two groups. One group was used for the study on the horizontal semicircular canals and the other group was for the vestibular nuclear study.

*Experiment on the horizontal semicircular canals*

Seventeen guinea pigs were exposed to the repetitive rotatostimulations for 48 hr, and 10 animals were used for the control. The animal’s head, bent forward by 30°, was fixed to the center of an electrically driven turn-table to avoid the influence of centrifugal force. The table was rotated counterclockwise with a constant angular acceleration of 0.2°/sec² to the ultimate speed of 450°/sec in a darkened room. Nystagmus was not detected by

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**Fig. 1.** The experimental procedures.

a: Repetitive rotatostimulations for the study of the horizontal semicircular canals.

b: Procedures of the 2-DG study on the vestibular nuclei.

A, injection of 2-DG; B, beginning of acceleration; C, switching to the constant speed rotation; D, injection of pentobarbital; E, stop of rotation.
electronystagmography (ENG) during the rotation, and this rotation was proved to be subliminal. Immediately after stopping the rotation at the terminal speed, the postrotatory nystagmus appeared toward the right side. By this mode of rotation, the pure post-rotatory effect could be observed without the per-rotatory effect (Egmond and Jongkees 1948). Therefore, the influence of the ampullopetal and ampullofugal endolymphatic flow can be observed respectively in the right and left semicircular canals. In order to magnify this influence of the endolymphatic flow more clearly, the same rotation-mode was repeated with a pause of 25 sec for 48 hr, as shown in Fig. 1(a). After the repetitive stimulation, the caloric test (20°C, 10 ml) was performed to find out the changes of the canal function. Then, the animals were sacrificed and their labyrinths were fixed in 3% glutaraldehyde buffered in 0.07 M phosphate for more than 2 hr. The oval window was opened and the bony capsule of the ampulla in three semicircular canals were perforated to allow penetration of the fixative. Following prefixation, the specimens were postfixed in 1% osmium tetroxide, dehydrated in an ethanol series, and embedded in Epok 812. Ultrathin sections were poststained with a 2% uranyl acetate aqueous solution and were examined under a transmission electron microscope.

Experiments on the vestibular nuclei

To investigate the difference of excitability between the bilateral vestibular nuclei, the [\(^{14}\)C]-2-deoxy-D-glucose study was performed with 9 guinea pigs which were placed in identical holders. The jugular vein was surgically exposed and cannulated with polyethylene catheters to inject the 2-DG in each animal. The 2-DG obtained from New England Nuclear was administered in a dose of 50 μCi in 0.2 ml sterile saline. Forty five min after injection of the radiochemical, three resting guinea pigs were sacrificed with sodium pentobarbital for the control study. The brain was rapidly removed, and frozen in isopentane cooled to − 75°C with dry ice. The frozen tissue was mounted with the OCT compound and cut into 20 μm coronal sections at −20°C. The sections were picked up on the cover slip, dried on a hotoplate at 60°C. The tissue was then autoradiographed on Kodak single emulsion medical x-ray film. Optical densities of regions of the autoradiographs corresponding to the vestibular nuclei were measured with a SAKURA microdensitometer (Tokyo). The location of the vestibular nucleus was determined by following Tindal (1965) and Rapisarda and Bacchelli (1977). Six guinea pigs were stimulated by the rotation mode as shown in Fig. 1(b), immediately after injection of the 2-DG. Namely, the animal’s head, bent forwards by 30°, was fixed to the center of the electrically driven turn-table to avoid the influence of centrifugal force. The table was rotated clockwise with the constant angular acceleration of 1°/sec² to the ultimate speed of 450°/sec in a darkened room. Nystagmus to the right side was observed by ENG during the acceleration, and it proved that the right horizontal semicircular canal was stimulated by the ampullopetal flow. The acceleration was then switched to the constant speed-rotation at the terminal speed. Twenty min after injection of the radiochemical, the rotating animals were sacrificed by the automatic injection of pentobarbital and then the rotation was stopped. The brain was rapidly removed and frozen. The specimens were investigated by the same autoradiographic technique as that of the resting animals.

RESULTS

Influence of the repetitive rotatostimulations on the horizontal semicircular canals

The caloric response of all animals exposed to the rotation was reduced, especially in the ampullopetal side labyrinth. Namely, the ampullopetal side response decreased to 17.6% of the initial one. It was a remarkable decrease as compared with 39.2% of the ampullofugal response.
The ultrastructural changes of the crista ampullaris were classified into grade I and grade II. That is to say, Grade I: Dislocation of the kinocilium caused by cytoplasmic protrusion from the cuticle free layer and changes in the supranuclear part of the sensory cells, such as enlargement of the Golgi complex and degeneration of mitochondria were seen as the early staged findings. Grade II: Vacuol formation of degenerative mitochondria in the supranuclear part and vesiculation (or disappearance) of endoplasmic reticulum in the infranuclear part were found as the advanced changes (Fig. 2). These findings were not observed in the normal control animals, but in 9 of 17 rotating animals. The changes were more strongly observed on the ampulloptal side than on the ampullofugal side, as shown in Fig. 2, with the exception of only one animal. Results of all animals are shown in Table 1.

<table>
<thead>
<tr>
<th>Side of labyrinth</th>
<th>Animal No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ampullopetal</td>
<td>I II II II II</td>
</tr>
<tr>
<td>Ampullofugal</td>
<td>I I I I I</td>
</tr>
</tbody>
</table>

I, Grade I; II, Grade II; —, no change.

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### Table 1. Ultrastructural changes of the crista ampullaris induced by the unidirectional rotatostimulation

<table>
<thead>
<tr>
<th>Side of labyrinth</th>
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<tbody>
<tr>
<td>Ampullopetal</td>
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</tr>
<tr>
<td>Ampullofugal</td>
<td>I I I I I</td>
</tr>
</tbody>
</table>

I, Grade I; II, Grade II; —, no change.

### Table 2. Autoradiographic densities showing the regional brain glucose consumption

<table>
<thead>
<tr>
<th>Structures</th>
<th>Resting guinea pigs</th>
<th>Rotating guinea pigs</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Right</td>
<td>Left</td>
</tr>
<tr>
<td>Vestibular nucleus</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Medial nucleus</td>
<td>116.3±0.2</td>
<td>115.7±0.17</td>
</tr>
<tr>
<td>Superior N.</td>
<td>113.7±1.7</td>
<td>114.7±1.5</td>
</tr>
<tr>
<td>Lateral N.</td>
<td>110.7±4.1</td>
<td>111.0±2.4</td>
</tr>
<tr>
<td>Inferior N.</td>
<td>117.7±2.6</td>
<td>117.0±0.8</td>
</tr>
<tr>
<td>Cerebellum</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nodulus</td>
<td>109.3±2.6</td>
<td>112.3±3.9</td>
</tr>
<tr>
<td>Hemisphere</td>
<td>100.0±0.0</td>
<td>101.7±2.9</td>
</tr>
<tr>
<td>Fastigial nucleus</td>
<td>90.3±4.0</td>
<td>91.3±1.7</td>
</tr>
<tr>
<td>Dentate N.</td>
<td>101.3±3.4</td>
<td>102.3±3.3</td>
</tr>
<tr>
<td>Interpositus N.</td>
<td>110.0±4.9</td>
<td>108.0±3.6</td>
</tr>
<tr>
<td>Superior olivary nucleus</td>
<td>147.0±5.6</td>
<td>149.3±5.7</td>
</tr>
<tr>
<td>Trigeminal nucleus</td>
<td>80.3±2.5</td>
<td>83.0±2.9</td>
</tr>
</tbody>
</table>

Values are means±s.d. (resting animals, n = 3; rotating animals, n = 6).

** Significant differences between the two sides were observed in the medial and superior nuclei (p <0.01).
Fig. 2. Ultrastructural changes of the crista ampullaris induced by repetitive rotatostimulations. The changes were more remarkably observed in the ampullopetal side labyrinth (AP) than the ampullofugal side (AF).
Comparison of the rotation-induced glucose uptake in the bilateral vestibular nuclei

The relative value of autoradiographic density of the vestibular nucleus (Fig. 4) was calculated on the assumption that the density of the adjacent cerebellar hemisphere was 100. Results are shown in Table 2. Three resting guinea pigs showed no difference in the density between the bilateral vestibular nuclei. In 6 rotating guinea pigs, the superior and medial vestibular nuclei showed 37% and 24% higher densities respectively, on the ampullopetal flow side than on the ampullofugal flow side. These differences were statistically significant ($p < 0.01$).
On the other hand, the lateral and inferior vestibular nuclei revealed no significant change of the densities as compared to the resting guinea pigs. In other portions, such as the cerebellar hemisphere, nodulus, fastigial nucleus, dental nucleus and interpositus nucleus, no significant differences between the ampullopetal side and the ampullofugal side were observed.

**DISCUSSION**

The mechanism of the semicircular canals which perceive rotatostimulation has been studied by many researchers. Morphological polarization of sensory cells in the crista ampullaris described by Flock and Wersall (1962), Lindemann (1969) and Lim (1971) constituted an adequate explanation for Ewald’s law. However, it seems to us that certain differences in physical strength between the ampullopetal flow and the ampullofugal flow are related to Ewald’s law besides the morphological polarization of sensory cells. Leiri (1927) thought that the endolymph flowed easily from the narrow canal to the wide utricle, but not so easily in the reverse direction. The authors (Hozawa et al. 1965) confirmed Leiri’s opinion by recording a pigeon’s intracanalicular pressure change induced by rotatostimulation in both labyrinths. Moreover, our recent scanning electron microscopic study (Hozawa et al. 1984) suggested the predominance of the ampullopetal physical effect. Namely, the dominant morphological change of sensory cells, such as complete or incomplete loss of kinocilium and stereocilia could be observed in the tip of the crista ampullaris near the canalicular side. This area was confirmed to be profoundly influenced by an ampullopetal cupular deviation (or sliding down) by our former investigation (Hozawa et al. 1965). In our present TEM study, the strongest ultrastructural change of sensory cells could be detected in the same area, as shown in Fig. 3.

On the other hand, the fact that primary afferent neurons from the horizontal semicircular canal terminate in the medial and superior vestibular nuclei, was confirmed anatomically by Brodal and H vic (1964), Gacek (1969), Ishizuka et al. (1982) and others. Our 2-DB experiments revealed the change of autoradiographic density of this area, and could prove the result of anatomical research. Moreover, this change showed the predominant effect on the ampullopetal side vestibular nucleus. A technique involving administration of tracer amounts of radioactive 2-DG can be used to quantitate regional brain glucose consumption. Sharp (1976) and Patrickson et al. (1985) used this technique for the vestibular nuclear study. The exact measurement of autoradiographic density should be performed 45 min after the injection of 2-DG (Sokoloff et al. 1977). Although the time required for our experiments was 20 min because of the limitations of the stimulating condition, it was found to be possible to find out the difference of density between the bilateral vestibular nuclei, on referring to the time course of 2-DG concentrations in the average gray calculated by Sokoloff (1979).

From our two series of experiments, it may be concluded that the validity of
Ewald's law can be proved not only in the horizontal semicircular canals but also in the vestibular nuclei.

References


