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Quantitative Study of Lipofuscin Accumulation in Cochleae of Patients With Presbycusis.

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Abstract

Presbycusis is a major disability for the elderly. Its mechanisms are little known. Presbycusis is accompanied by accumulation of lipofuscin which is considered a reliable, well visualized marker. In this study we quantified the areas occupied by lipofuscin in the three segments of spiral ganglion of the cochlea from subjects with presbycusis. We found the greater accumulation in the anterior basal segment compared to the posterior basal (junction of basal and middle turns) and upper segments. This may be due to greater functional and metabolic activity in the anterior basal segment. Indeed, the basal segment of the cochlea is exposed to all vibrations, whereas the apical segment is exposed to low frequencies only. The increase of lipofuscin in the basal segment indicates that the process of aging might be accelerated in this segment in comparison to other segments.

Introduction

PRESBYCUSIS is characterized by gradual, progressive, bilateral, symmetrical, sensorineural hearing loss related to advancing age, with the patient's having no history of ear or systemic disease that may affect hearing. Zwaardemaker in 1891 was the first to report in the literature this disorder [1]. He termed his general observation "presbycusis" ("Presbys" means old and "Akouses" means hearing). The disorder initially affects the perception of sounds of high frequency sounds and later affects lower frequencies. The specific causes of presbycusis are not known. However, it has been suggested that such factors as hypertension [2] and diet [3] could be associated with the condition.

Atrophy of the spiral ganglion cells is the most common histopathological finding in the aging cochlea. Schuknecht compared it with changes in the general number of neurons in the aging central nervous system [4,5]. The changes are most prominent in the basal segment of the cochlea, where high-frequency sound is perceived [6]. Crow and Guild showed a positive correlation between the degree of high tone hearing loss and the degree of neuronal loss [7].

In this study, we measured the areas occupied by lipofuscin in ganglion cells in the three segments of the cochlea i.e.anterior basal, posterior basal (junction of basal and middle turns) and upper segments. Lipofuscin is considered a reliable, well visualized marker that reflects the aging process in neurons.

Material and Methods

We investigated histological sections prepared from celloidin-embedded temporal bones from 15 patients with presbycusis. Age of the subjects ranged from 58 to 95 years. We used mid modiolar sections because they contain anterior basal, posterior basal and upper spiral ganglion segments in the same preparations.

We modified the technique for observation of lipofuscin in celloidin-embedded sections under the fluorescence microscope because output of fluorescence of lipofuscin is low in the sections while fluorescence of background is high. For that celloidin was removed from the sections by immersion in ether for 24 hours, then in a mixture of alcohol and ether (1 part : 3 parts) for 25 minutes, in absolute alcohol for 3 minutes, and in 50% alcohol for 3 minutes. After the sections were washed in distilled water and dried, they were mounted into glycerine for 30 minutes. Excess of glycerine was then allowed to flow off the slide. Glycerine-gelatine was used to embed the sections. Fluorescence was observed with a Nikon microscope equipped with a high-pressure HBO 100 mercury lamp and epi-illumination system.

With a computer system, which included a digitizing tablet and a video camera, we measured the cross sectional area of the ganglion cells and the areas occupied by lipofuscin in the three different segments of the spiral ganglion. We calculated the differences between the segments. For that we used two parameters, namely the percentage of cells containing lipofuscin and also the percentage of areas occupied by lipofuscin in relation to the cross sectional area of the cells containing Lipofuscin. We used statistical methods for comparison of the data obtained, namely one-way analysis of variance.

Results

Fig. (1) shows the fluorescing lipofuscin granules in ganglion cells of the anterior basal segment. They emit fluorescence of yellow colo against greenish background. Lipofuscin is accumulated in the

cytoplasm usually near one of the poles of the cells. The number of granules varies but rarely exceeds ten and the granules may fuse together forming larger bodies.

We used the relative amount of lipofuscin in the three segments of the cochlea (percentage of total area occupied by lipofuscin) to consider the degenerating effect associated with lipofuscin accumulation. The one-way analysis of variance on this variable revealed a statistically significant difference in the three segments (F= 5.03 $p < .01$).

The difference between the percentage of cells containing Lipofuscin in the three segments of the spiral ganglion (anterior basal, posterior basal and upper segment) was statistically significant according to oneway analysis of variance (F = 50.48, $p < 0.001$); i.e. the anterior basal segment had the greatest percentage of cells containing lipofuscin (mean = 43.0%); the posterior basal segment had the second greatest percentage (mean = 20.97%); and the upper area had the least (mean = 10.34%).

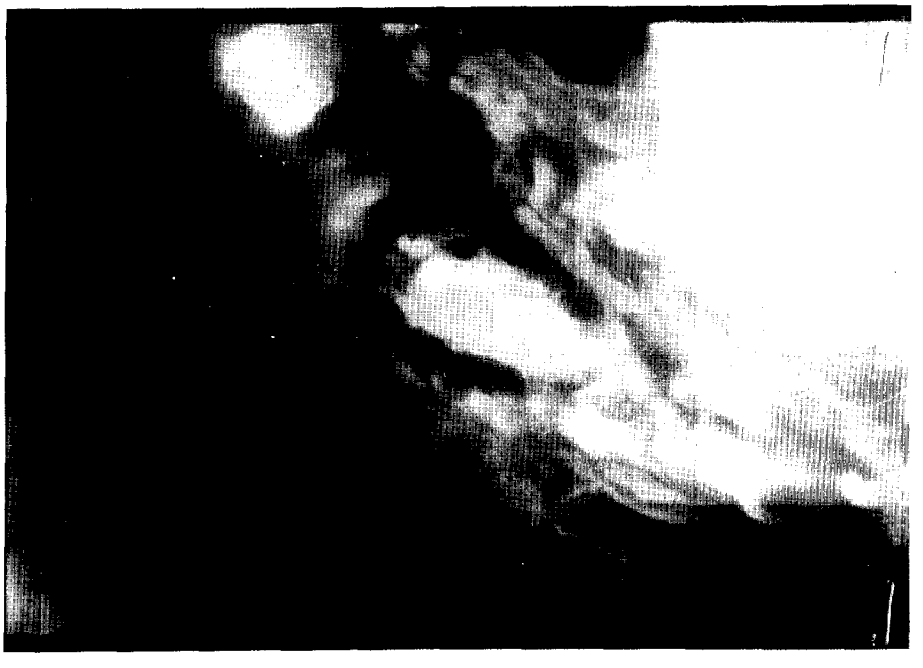

Fig. 1. Unstained section showing the anterior basal segment of the spiral ganglion showing intracytoplasmic fluorescing lipofuscin granules [x400]. They tend to accumulate near one pole of the cells.

Discussion

Lipofuscin, which is one of the most characteristic phenomena in aging cells, is considered an indicator of aging. Its origin is still unknown but may be from mitochondria [8, 9, 10] or degenerated Golgi complexes [11]. Now it is generally believed that lipofuscin is of lysosomal origin due to its high content of acid phosphatase [12, 13], and that lipofuscin represents waste products of metabolism. It seems that presbycusis is a part of the whole body aging process, a process that is not yet well defined. The primary impact may be metabolic, and lipofuscin accumulation may be an indication of this metabolic disturbance. Cockayne syndrome, the triad of dwarfism, retinal atrophy, and deafness, has the same histopathological and audiological findings of presbycusis [14], thus supporting this metabolic etiology. The rate of lipofuscin accumulation depends on the functional activity of the cell. It increases with increased functional activity and consequently the metabolic rate of the cell [15]. This also may explain the increase in lipofuscin concentration in neurons and glial tissues in people exposed to noise [16].

The findings are in principle in good agreement with the travelling wave theory of hearing, which states that the basal segment of the cochlea is maximally exposed to all frequencies, whereas the apical segment is exposed to low frequencies only. The increase of lipofuscin in the basal segment may indicate that the process of aging might be accelerated in this segment in comparison to other segments. However, the concentration of lipofuscin pigment in cells does not seem to depend on age alone. Factors such as protein deficiency [17], vitamin E deficiency [18], and administration of prostaglandins and papaverine [19] as well as hypoxia and ACTH [20] were reported to increase lipofuscin levels in cells. Sohal & Donata [21, 22] demonstrated a faster rate of accumulation of lipofuscin in flies subjected to a high level of physical activity (flying) compared to flies maintained under conditions of relatively low physical activity. A decrease in tissue lipofuscin content was reported after administration of the drug centrophenoxin [19]. Although lipofuscin accumulation was thought to impede functional capacity or to lead to degeneration of ganglion cells with consequent hearing loss of corresponding frequencies, it is now believed that it is an adaptive mechanism to relative hypoxia using carotenoids as a final oxidant instead of oxygen [23, 24].

Thus, lipofuscin can serve as an indicator of metabolic derangement and is not the direct cause of degeneration. Consequently, trials to decrease lipofuscin concentration as a treatment of presbycusis would not result in any functional recovery.

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