Age-Related Changes in the Cochlea and Cochlear Nuclei of Dogs

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ABSTRACT. To study the underlying morphological changes of presbycusis, cochlea and cochlear nuclei from twenty three dogs, ranging in age from 3 days to 17 years, were examined histologically. Dogs used in this study were house dogs kept in an environment similar to that of humans. Four types of histological changes reported in human presbycusis, that is, loss of spiral ganglion cells, atrophy of the organ of Corti, atrophy of the stria vascularis, and thickening of the basilar membrane were observed in dogs. The changes were prominent at the base of the cochlea. Less intense changes were also observed in the apex of the cochlea. The degree of these changes appeared to progress as a function of age. All four types of changes with varied intensity were found in all dogs over 12 years old. In addition to the changes in the cochlea, cochlear nuclei changes including nerve cell loss, astrogliosis and ubiquitin deposition were found in dogs over 10 years old. Hearing dysfunction was accompanied by the morphological changes, though the degree of the hearing dysfunction did not always parallel to that of morphological changes. The morphological changes seen in the cochlea and cochlear nuclei of dogs were qualitatively and quantitatively similar to those reported in aged humans, indicating that otopathologic changes in the inner ear may be due to aging plus exposure to certain environmental ototoxic factors. — KEY WORDS: aging, canine, cochlea, cochlear nucleus, presbycusis.

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There are four distinct types of human presbycusis:(1) sensory, characterized by loss of hair cells and degeneration of the organ of Corti; (2) neural, characterized by primary degeneration of neural elements, including the cells of the spiral ganglion and the fibers of the cochlear nerve; (3) strial, or metabolic, characterized by atrophy of the stria vascularis and a flat audiometric loss; and (4) inner ear conductive, or mechanical, characterized by changes in the structure and mechanical properties of the basilar membrane [18, 19]. The mechanism underlying these changes as well as the relationship between the changes are yet to be elucidated. Many individual cases do not separate into a specific type but have mixtures of these pathologic types [20]. In addition to the peripheral form of presbycusis, there is also the concept of central presbycusis, attributed to the degeneration of synapses along the central auditory pathways [1, 19, 26, 27].

Age-related morphological changes are minimum in the inner ear of the laboratory animals, including monkey [6, 7], mouse [8], rat [12], chinchilla [2, 4], rabbit [3] and guinea pig [5]. This is because these animals were kept in controlled environments of the laboratory [7]. Considering that human presbycusis consists of a variety of cochlear pathology of advanced stage, presbycusis must reflect the cumulative effects of heredity, disease, noise, ototoxic agents, and perhaps other environmental and dietary factors, superimposed upon those of the aging process itself [4, 7].

There are only a few reports on the age-related morphological changes in the inner ear of the domestic animals; Schuknecht *et al*. [21] reported pathological changes of the cochlea of a 19-year-old cat and a 20-year-old dog. Following assessment of the hearing ability of 16 dogs by recording brainstem auditory-evoked responses (BAER) to click stimuli [14], Knowles *et al*. [13] examined morphological changes of the inner ear; loss of spiral ganglion neurons was responsible for hearing loss. These studies, however, were not designed to cover full anatomical regions in the cochlear pathology. The purpose of the present paper was to examine a wide variety of the age-related morphological changes in the cochlea and cochlear nuclei of dogs reared in a similar environment to that of humans.

MATERIALS AND METHODS

Animals: Twenty three dogs, 10 males and 13 females, were used in this study (Table 1). Their ages ranged from 3 days to 17 years. All animals were house dogs with history of neither otitis or administration of ototoxic drugs. There were no sign of neurological disorders except for some degree of auditory dysfunction (Table 1). Most animals used in this study were to be euthanatized because of prolonged illness, including heart failure, renal failure and tumor in the visceral organs. Otoscopic examination revealed no abnormalities in the external ear canals or in the tympanic membranes.

Auditory testing: Subjective tests of hearing were done on each dog (Table 1). Hearing ability was judged by observations of dog's reactions to hand claps of a range of loudness. The degree of the auditory dysfunction was recorded as - (no dysfunction: immediate and reproducible response to sounds at all intensities), + (mild dysfunction: slow reaction to conversational level of sounds), ++ (moderate dysfunction: slow reaction to intense level of sounds), and +++ (severe dysfunction = complete deaf: no reaction to any levels of sounds). The result of the subjective test was verified by recording BAER to click stimuli of selected animals (Fig. 1). Briefly, clip electrodes were placed on Cz and just rostral to the base of each ear. Each of these ear electrodes were used as a reference of

Table 1. Clinical findings and pathological findings at the base of cochlea of dogs

No.	Breed	Sex	Age	Audit. dys.	SPG loss	Atrophy O. Corti	Atrophy St. vasc.	Thickening Basil. M.
1	Mongrel	M	3d	–	–	–	–	–
2	Mongrel	M	3d	–	–	–	–	–
3	Mongrel	F	3d	–	–	–	–	–
4	Pug	F	3m	–	–	–	–	+
5	Mongrel	F	4m	–	–	–	–	+
6	Shelty	M	1y	–	–	–	–	+
7	Mongrel	M	1y	–	–	–	–	+
8	Mongrel	M	5y	–	+	–	–	++
9	Beagle	F	6y	–	+	–	–	+
10	Mongrel	F	10y	–	+	–	–	+
11	Mongrel	M	11y	–	+	–	–	+
12	Mongrel	F	12y	–	+++	++	++	+++
13	Pointer	M	13y	+	+++	+++	++	+++
14	Mongrel	F	13y	+	++	++	+++	+++
15	Mongrel	F	14y	+	++	–	–	+++
16	Mongrel	F	14y	+++	++	++	+	+++
17	Mongrel	M	15y	++	+++	+++	+++	+++
18	Mongrel	F	16y	+	–	+	+	++
19	Mongrel	M	16y	+	++	+	+	+
20	Chihuahua	M	16y	++	++	+	+	+++
21	Akita	F	16y	+	+++	+++	+	+++
22	Shiba	F	17y	++	++	+	+++	+++
23	Shiba	F	17y	+	+++	+	+	+++

Audit. dys.; auditory dysfunction, SPG; spiral ganglion cell, O. Corti; organ of Corti, St. vasc.; stria vascularis, Basil. M.; basilar membrane, M; male, F; female, d; day, m; month, y; year -; none, +; mild, ++; modarate, +++; severe

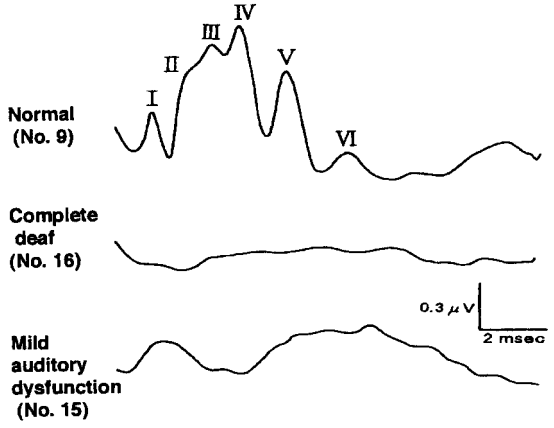

Fig. 1. BAER from dogs with normal hearing (No. 9), complete deaf (No. 16) and mild hearing dysfunction (No. 15). Note the flattened waves in dogs with hearing dysfunction compared to the wave from the dog with normal hearing ability. Horizontal bar=2.0 msec. Vertical bar=0.3 μV.

ipsilateral recording while the other was used as a ground. Click sounds were presented by earphone placed in the external auditory canal of the side to be measured with intensity of 90 dB. The acoustic stimuli were delivered at 125 msec interval in all cases. Evoked activity was sampled during the first 10 msec after each click and recorded on a magnetic tape recorder (MR-10, TEAC) at amplifier band pass between 32 Hz and 3 kHz. The responses to 1,000 click presentation were averaged and displayed on an oscilloscope and recorded on a X-Y recorder (8U11, NEC San-ei). Dogs with auditory dysfunction, judged by the subjective test, showed lowered amplitude of the waves of the BAER compared with the typical wave pattern of the BAER in dogs with normal hearing ability (Fig. 1). BAER waveforms of the normal hearing dog (No. 9) consisted of five major peaks (I, II-III, IV, V, VI). In the dog (No. 15) which was suspected to have mild auditory dysfunction, each peaks after the peak III were poorly defined and the latencies of these peaks were markedly delayed. No recognizable waves were obtained from the dog (No. 16) which was suspected to be complete deaf.

Tissue preparations: All animals were euthanatized by injection of sodium pentobarbital. For histological examination, both sides of the temporal bones were obtained immediately after death. Following removal of the brain, the temporal bones were dissected from the skull. The middle ear was opened by removal of the tympanic bulla. The stapes was removed in order to expose the round and oval windows of the labyrinth. The round window membrane was perforated with a small hook. The inner ear was fixed by gentle perilymphatic perfusion of the labyrinth with 10% neutral buffered formalin injected through the oval window membrane by a syringe. The time interval between death and fixation was less than 30 min. The whole specimen was kept in the fresh fixative in the

refrigerator at 4°C for 24 hr. The specimen was decalcified with 15% of ethylene diamine tetraacetic acid (EDTA) solution (pH 7.4) for 1–3 weeks, embedded in paraffin, and serially sectioned at the mid-modiolar plane at a thickness of 6 μm. Selected sections were stained with haematoxylin and eosin (HE), Nissl, Periodic acid schiff (PAS), and Masson trichrome, respectively.

The semiquantative data on the changes (spiral ganglion cell loss, atrophy of the organ of Corti, atrophy of the stria vascularis and thickening of the basilar membrane) in the cochlea were transferred to histograms in which black filling indicated the intensity (mild, moderate, severe) of the changes as a function of distance along the cochlear duct as measured from the basal end.

Brain was also taken immediately after death. The samples were then fixed in 10% neutral buffered formalin for 3 days. Transverse section of the brain stem including both sides of the dorsal and ventral cochlear nuclei was trimmed, routinely processed and embedded in paraffin wax. Sections cut at 6 μm were examined histologically (after HE staining) and immunohistochemically.

Immunohistochemistry: Serial sections from the cochlear nuclei were immunolabelled using the primary antibodies: anti-synaptophysin monoclonal antibody (Boehringer Mannheim, Mannheim, Germany), anti-glial fibrillary acidic protein (GFAP) monoclonal antibody (Dako, Glostrup, Denmark) and anti-ubiquitin polyclonal antibody (Dako). After blocking endogeneous peroxidase activity with 3% H_2O_2 in phosphate-buffered saline (PBS), sections were treated with 5% normal goat serum, incubated with primary antibodies overnight at 4°C and then sequentially incubated with biotinylated goat anti-mouse IgG (Dako) (1 in 500 PBS) for the monoclonal antibodies and with biotinylated goat anti-rabbit IgG (Dako) (1 in 500 PBS) for the polyclonal antibodies for 2 hr at room temperature, and in peroxidase-conjugated streptavidin (1 in 500 PBS) for 1 hr. Primary antibodies were diluted with PBS containing Triton-X 100 0.3% (1 in 10 for synaptophysin; 1 in 100 for GFAP; 1 in 200 for ubiquitin). After the first and second incubations, the sections were given three 10-min washes in PBS and then developed with 0.02% 3,3'-diaminobenzidine tetrahydrochloride and H_2O_2 (DAB-H_2O_2). The sections were counterstained with methyl green or haematoxylin. As negative control, sections were also incubated with PBS or with either non-immune mouse serum for the monoclonal antibody or rabbit serum for the polyclonal antibody instead of the primary antibody.

RESULTS

In this study, histological changes similar to those reported in human presbycusis were found in the organ of Corti, spiral ganglion, stria vascularis and basilar membrane; human presbycusis has been classified into the four distinct types by the pattern of changes in the four anatomical regions of the cochlea [18, 19]. Lipofuscin accumulation was also observed in the cytoplasm of a variety of cells in both cochlea and cochlear nuclei. These histological changes were associated with hearing impairment; intensity of histological changes, however, did not necessarily parallel to that of hearing dysfunction (Table 1). Signs of auditory dysfunction first appeared at around 12 years of age. Pathological changes, some of which had already began beforehand, became also prominent at around 12 years of age (Table 1). Histological findings of each side of samples were essentially the same in both quality and quantity.

Loss of spiral ganglion cells: A loss of spiral ganglion cells, which first appeared at 5 years of age, was prominent at the base of the cochlea (Fig. 2). In many cases, the degree of spiral ganglion cell loss paralleled to that of the

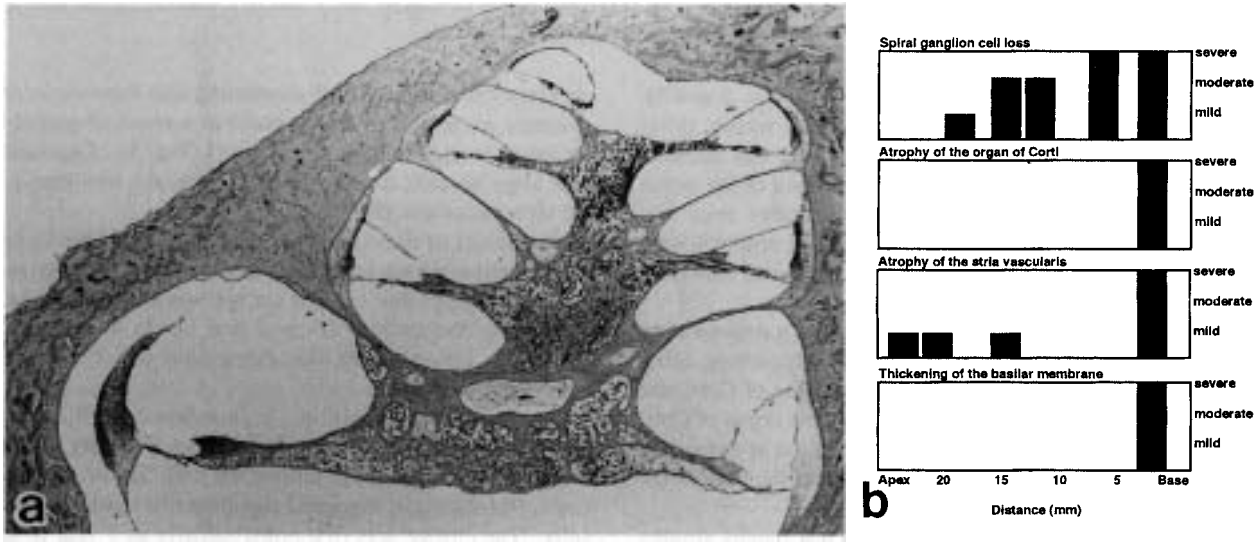

Fig. 2. No. 13, 13-year-old dog. (a) Midmodiolar view of the cochlea shows the loss of spiral ganglion cells, advanced atrophic changes in the organ of Corti and in the stria vascularis and thickened basilar membrane. Note these changes are predominant at the base. HE. × 30. (b) Cytocochleogram shows prominent localization of the changes at the base.

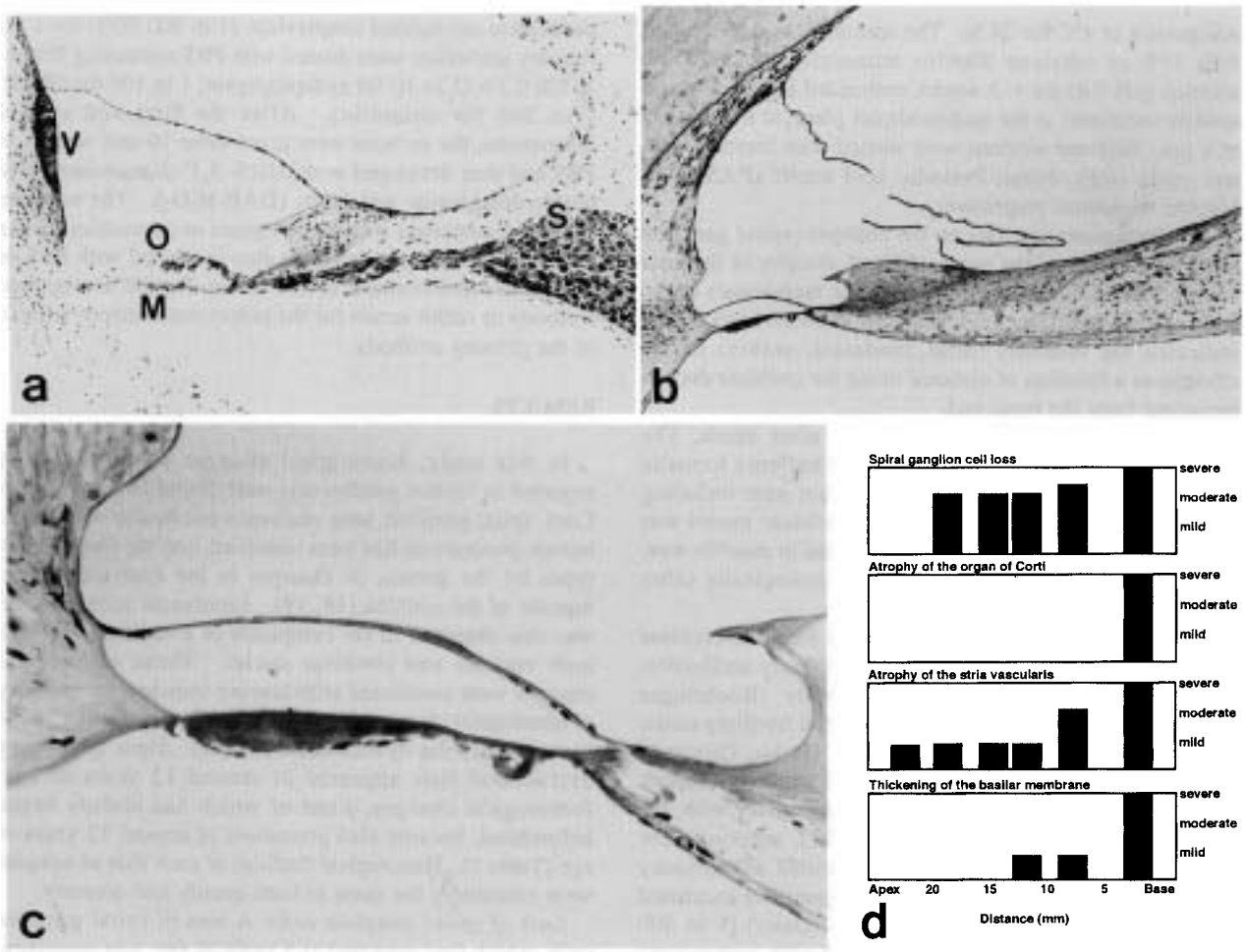

Fig. 3. (a) Midmodiolar section at the base of cochlea from a 1-year-old dog (No. 7), showing normal structure of the organ of Corti (O), spiral ganglion cells (S), stria vascularis (V) and basilar membrane (M). HE. × 70. (b) Midmodiolar section at the base of cochlea from a 15-year-old dog (No. 15), showing complete loss of both spiral ganglion cells and organ of Corti, atrophy of the striva vascularis and thickened basilar membrane. HE. × 70. (c) Higher power view of b). HE. × 340. d) Cytocochleogram from a 15-year-old dog (No. 15). Note the prominent localization of the changes at the base.

pathology in the organ of Corti (Table 1) (Figs. 2 and 3). There were, however, occasional cases in which spiral ganglion cell loss occurred at certain part of the cochlear duct, where no signs of degenerative changes of the organ of Corti were observed. Myelinated fiber loss and infiltration of macrophages containing lipid droplets were found in the severe lesions of spiral ganglion cell loss (Fig. 4).

Atrophy of the organ of Corti: There was a degeneration of both inner and outer hair cells and supporting cells, showing distortion and flattening of the organ of Corti; the change was followed by complete loss of the organ of Corti (Fig. 3). These changes first became evident at around 12 years of age (Table 1) and was prominent at the base of the cochlea (Figs. 2 and 3).

Atrophy of the stria vascularis: There was patchy atrophy of the stria vascularis (Figs. 2, 3 and 5). The change, which was severe in the apical and extreme basal regions of the cochlear duct, consisted of shortening and thinning of the structure at the mid-modiolar plane as a result of partial or complete loss of cellular components (Fig. 5). Deposition of a large amount of melanin-like pigments was found in the stria vascularis (Fig. 5).

Thickening of the basilar membrane: Signs of thickening of the basilar membrane first became evident as early as 3 months of age (Table 1). The change was also prominent at the base of the cochlea (Figs. 2 and 3). In the advanced stage, the thickened basilar membrane was hyalinized, showing PAS positive.

Lipofuscin accumulation: Accumulation of lipofuscin pigments was noted in the cytoplasm of a variety of cells including hair cells and supporting cells in the organ of Corti, fibroblasts in the spiral ligament and spiral ganglion cells. The change was first noted as early as 1 year of age and the quantity of the accumulated pigments increased with advancing age. Slightly basophilic cytoplasmic deposits,

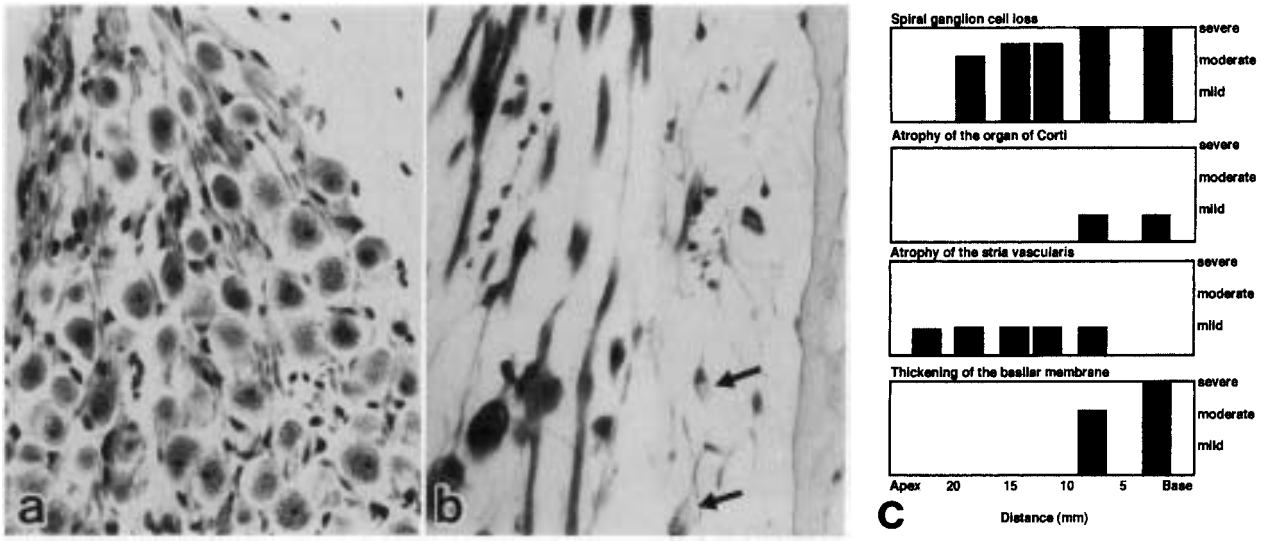

Fig. 4. Midmodiolar section at the base of cochlea. Spiral ganglion cells of a 1-year-old dog (No. 7)(a) and a 17-year-old dog (b). Note the marked decrease of spiral ganglion cells and the presence of lipid-laden macrophages (arrows) in (b). HE. (a) × 450. (b) × 530. (c) Cytocochleogram from a 17-year-old dog (No. 23). Note the difference in the intensity of the changes between the four anatomical regions.

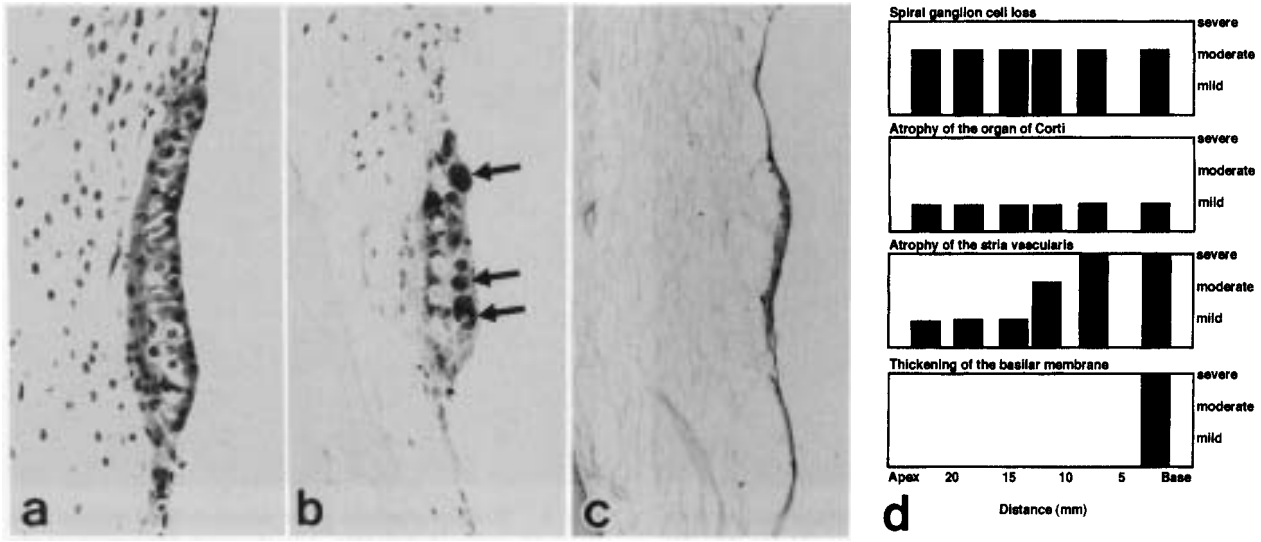

Fig. 5. Midmodiolar section at the base of cochlea. Stria vascularis of a 1-year-old dog (No. 6)(a) , a 16-year-old dog (No. 21) (b) and a 17-year-old dog (No. 22)(c). Note a large amount of melanin-like pigments (arrows) in (b) and loss of stria vascularis in (c). HE. × 270. (d) Cytocochleogram from a 17-year-old dog (No. 22) showing moderate intensity of spiral ganglion cells throughout the cochlear canal.

which were PAS-positive, were occasionally found in the outer supporting cells in the organ of Corti and in the fibroblasts in the ligamentum spirale (Fig. 6)

Aged changes in the cochlear nuclei: Nerve cell loss and increased density of glial cells were noted in both dorsal and ventral cochlear nuclei of dogs older than 10 years (Figs. 7 and 8). Lipofuscin accumulation, deposition of ubiquitin-positive granules (Fig. 9) in the neuropil, GFAP-positive astrocytic gliosis (Fig. 8) and decreased number of synaptophysin-positive granules at the periphery of the dendrites (Fig. 7) were also demonstrated in the nuclei. These changes appeared to become prominent with advancing age. The intensity of the cochlear pathology including spiral ganglion cell loss did not always parallel to that of the changes in the cochlear nuclei.

DISCUSSION

Based on the histological and physiological findings, human presbycusis has been classified to four predominant

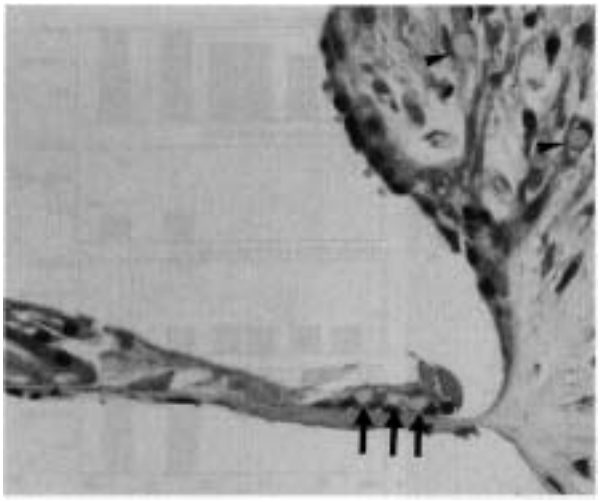

Fig. 6. Midmodiolar section at the base of cochlea of a 15-year-old dog (No. 17). Note the presence of slightly basophilic substance in the cytoplasm of outer supporting cells (arrows) and fibroblasts (arrow heads) in the spiral ligament. HE. × 530.

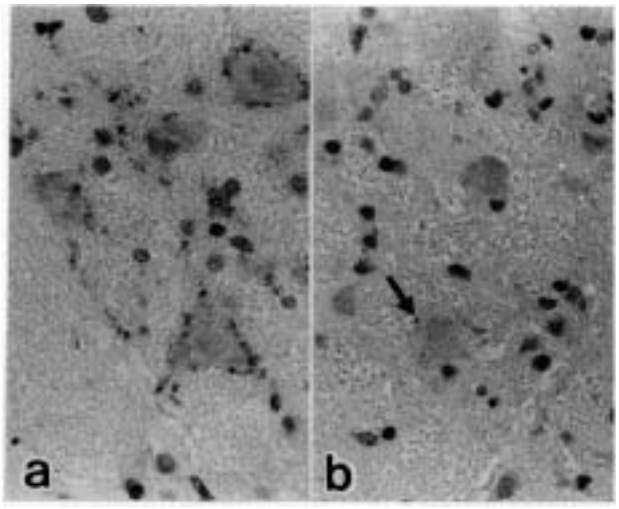

Fig. 7. Transverse section of the dorsal cochlear nucleus from a 1-year-old dog (No. 7) (a) and a 16-year-old dog (No. 21) (b). Note decreased number of both neurons and synaptophysin positive granules (an arrow) at the periphery of the neuronal cell somata in (b). Synaptophysin immunostaining. × 430.

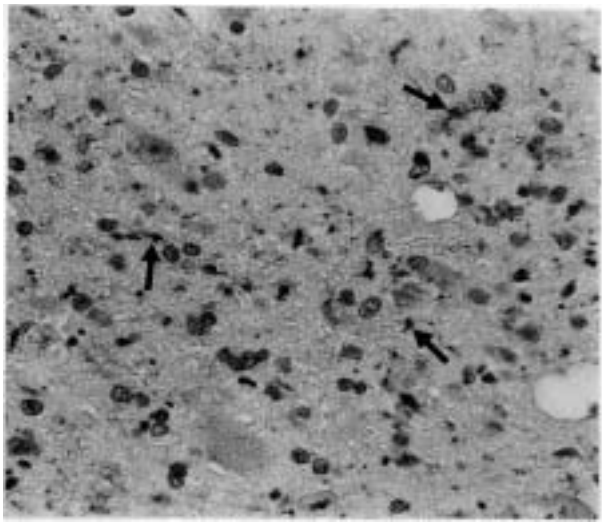

Fig. 8. Transverse section of the dorsal cochlear nucleus from a 15-year-old dog (No. 17) showing gliosis. Note the increase in the GFAP positive filamentous structure (arrows). GFAP immunostaining. × 430.

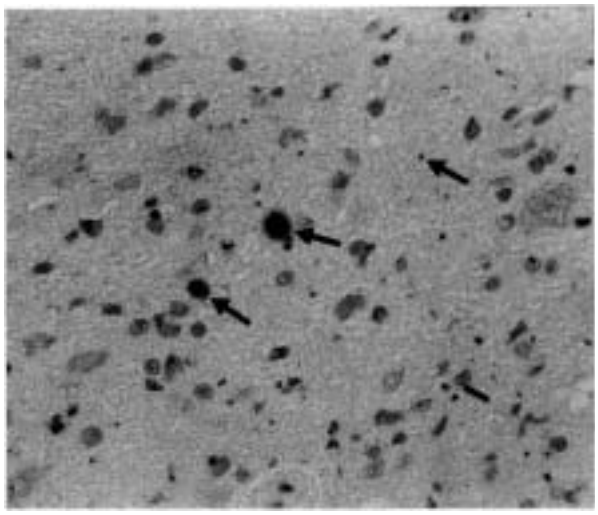

Fig. 9. Transverse section of the dorsal cochlear nucleus from a 15-year-old dog (No. 17) showing deposition of the ubiquitin positive granules (arrows) in the neuropil. Ubiquitin immunostaining. × 530.

pathologic types, these being sensory, neural, strial and cochlear conductive; an abrupt high-tone loss signals sensory presbycusis, a flat threshold pattern is indicative of strial presbycusis, loss of word discrimination is characteristic of neural presbycusis and the hearing loss characterized by a gradually decreasing linear distribution pattern of threshold loss on the audiometric scale is identified as cochlear conductive presbycusis [18, 19]. Many individual cases do not separate into a specific type but have mixtures of these pathologic types and are termed mixed presbycusis [20]. There is also a concept of central presbycusis, in which changes in the cochlear nuclei, such as loss of neurons and neurotransmitter are responsible for the auditory impairment [1, 19, 26, 27]. In the present study, morphological changes in the dogs with hearing dysfunction consisted of almost all types of lesions, indicating mixed presbycusis as classified in humans.

As to the pathology responsible for the age-related hearing impairment, there were no consistent pattern in the combination of each type of lesions. No consistent correlation was noted between the type of the audiometric

curve and the localization of lesions in the cochlea of humans [25]. In the present study, no fixed type of change correlated to auditory dysfunction, indicating that type of presbycusis as well as the underlying mechanism of presbycusis may differ between the dogs.

In the aging ear of Rhesus monkeys, ganglion cell loss was not always associated with hair cell degeneration, suggesting that ganglion cell loss is not necessarily secondary to hair cell loss, but may occur independently [7]. Following the study of inner ear from 80 chinchillas, Bohne *et al.* [4] also reported that ganglion cell loss occurred in areas where the changes in the sensory cells were minimum. Such findings were also recorded in human cochlea [10]. This is in accord with the present study, in which there were some cases showing ganglion cell loss without a sign of hair cell degeneration. In addition, there was a discrepancy in the intensity of the lesions between the cochlea and cochlear nuclei in dogs. Taken together, changes in each anatomical region in the auditory pathway including cochlear nuclei in the brain stem may occur dependently or independently.

In the entire length of the cochlea, from the top to the basal end, certain area may be susceptible to some type of pathological changes, though the underlying mechanism is not known. Sensory and neural degeneration limited to the basal end was observed in four pairs out of nine pairs of human temporal bones examined [10]. Soucek *et al*. [24] also reported predominant atrophy of the organ of Corti at the end of basal coil. In contrast, hair cell degeneration was consistent at the apex in humans [11]. In accelerated senescence mice, hair cell loss was localized exclusively in the apex and base [17]. A similar pattern of damage, greater at the apex and base with middle relatively spared, was also reported in rats [12]. In the present study, spiral ganglion cell loss and hair cell loss dominated in the base and changes of stria vascularis in both apex and base. Difference in species, type of changes and age may be responsible for the different results. Lack of changes in the basal or apical ends reported may be, in part, a reflection of the ages of the animals studied as opposed to the damage that may occur with advanced years.

Age-related changes including neuronal loss [23], gliosis [22] and deposition of ubiquitin-positive granules [9, 16] have been demonstrated in the brain of dog. Liu *et al*. [15] reported the age-related decrease of synaptic density in the cerebral cortex of humans. In the present study, similar age-related changes were demonstrated in the cochlear nuclei of dogs. Marked loss of spiral ganglion cells, which project neurons to the cochlear nuclei, may be responsible for the age-related changes including neuronal loss and lowered synaptic density in the aged dogs.

In this study, histological changes both qualitatively and quantitatively similar to those reported in human presbycusis were found in the organ of Corti, spiral ganglion, stria vascularis and basilar membrane of dogs. In addition, atrophic changes were also noted in the cochlear nuclei. The morphological changes seen in the inner ear of aged experimental animals including chinchillas were qualitatively similar to those seen in the temporal bones of aging humans, although the magnitude of the changes was considerably less [4]. Hawkins *et al*. [7] also found a simple, localized, age-related degeneration of the inner ear in 15 monkeys ranging in age from 4 to 31 year and reared in controlled laboratory conditions. House dogs used in the present study were reared in a similar environment to that of humans. In this context, the damage found in aging cochlea of human and dog may be due to aging plus exposure to one or more ototraumatic agents [4, 7].

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