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AGE-RELATED MITOCHONDRIAL DAMAGE IN THE B-TYPE CELLS OF THE RAT TRIGEMINAL GANGLIA*

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Aging is associated with signs of sensory impairment and neurological symptoms. Advancing age is characterized by increased thresholds of thermal, tactile and vibratory sensations. One important cause of the sensory disturbances has been stated to be the loss of neurons. Decreases have been observed in the number of peripheral nerve fibers and in the number of neurons in the spinal ganglia of rats.

In the present study, the cytoplasmic organelles of the neurons of the trigeminal ganglia were examined in young and senescent rats in order to reveal the cause of cell loss during aging. Mitochondrial alterations, swelling and loss of internal cristae were observed from 23 week of age in the B-type neurons of the trigeminal ganglia. Other cytoplasmic elements were intact. Mitochondrial damage was never seen in A-type neurons and satellite glial cells.

It was concluded that the ultrastructural changes in the mitochondria of the B-type cells may contribute to the nervous disturbances that occur in senescent individuals. The diminution of mitochondrial damage and the protection of B-type neurons through the use of nerve growth factors may prevent the sensory impairment late in life.

Keywords: Aging – capsaicin – mitochondrial alterations – sensory disturbances – sensory ganglia

INTRODUCTION

Aging is associated with signs that are similar to peripheral neuropathy [9, 23]. Axonal dystrophy, degeneration and demyelination of sensory nerve cells have been reported in senescent animals [2, 16, 42]. As consequences of these changes, increased thresholds of thermal, tactile and vibration perceptions may be observed [13, 31].

One of the main reasons for the sensory deficits in senescent animals has been considered to be the loss of primary sensory neurons. Earlier results yielded contradictory cell counts. The cell numbers in the mouse L_3 spinal ganglion [18, 25] and the trigeminal ganglia of the rat [5] did not reveal changes that correlated with the age of the animals. Duchen and Scaravilli [12] found a lower cell number in the

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mouse dorsal root ganglia at birth than later. No significant change was found in the cell number in the thoracic ganglia from birth to 70 years in humans. In contrast, Emery and Singhal [14], Gardner [15] and Scharf and Blumenthal [32] described significant cell losses in the human dorsal root ganglia after 35 years of age. Moreover, a small decrease (12%) was found in the number of lumbar and thoracic dorsal root ganglion neurons, with higher thresholds for nociceptive and tactile stimuli in aged rats [3]. However, there was no correlation between the degree of cell loss and the extent of nervous disturbances among the aging animals [3].

During aging, a number of alterations have been detected in the peripheral nerve fibers in rats. For example, glycogen granules, unidentified granulo-filamentous bodies and lipofuscin accumulate in the axoplasm. The axoplasmic organelles, such as the mitochondria, also display damage [17]. Tubular or filamentous inclusions, glycogen particles and electron-dense globules have been observed among the internal cristae or in the matrix of the mitochondria of the spinal ganglia in rats [43]. It has been suggested that the changes may result from an age-related dysfunction of the specific oxidative metabolisms.

The present study was undertaken in order to compare the subcellular cytoplasmic organelles in young and senescent rats and to reveal possible changes in the A- and B-type neurons of the trigeminal ganglia. Previous studies had suggested that only B-type cells are vulnerable to different noxious agents; accordingly, we set out to study which neurons display mitochondrial alterations, and to what extent, in aging rats.

MATERIALS AND METHODS

All animal experiments were carried out in accordance with the European Communities Council Directive of 24 November 1986 (86/609/EEC) and according to the Animal (Scientific Procedures) Act 1998 (Hungary) and in adherence to the rules of the Ethics Committee on Animal Research at the University of Pécs.

Animals

Wistar and Long Evans male rats were used. Two rats in each age group were perfused at the ages of 6, 12, 20, 23, 26 and 52 weeks. There was no significant difference between the results from the two different species.

Perfusion

The rats were anesthetized with ketamine, and then perfused transcardially, first with 0.1 M phosphate buffer (PB, pH 7.4) followed by 4% paraformaldehyde containing 1% glutaraldehyde in 0.1 M PB.

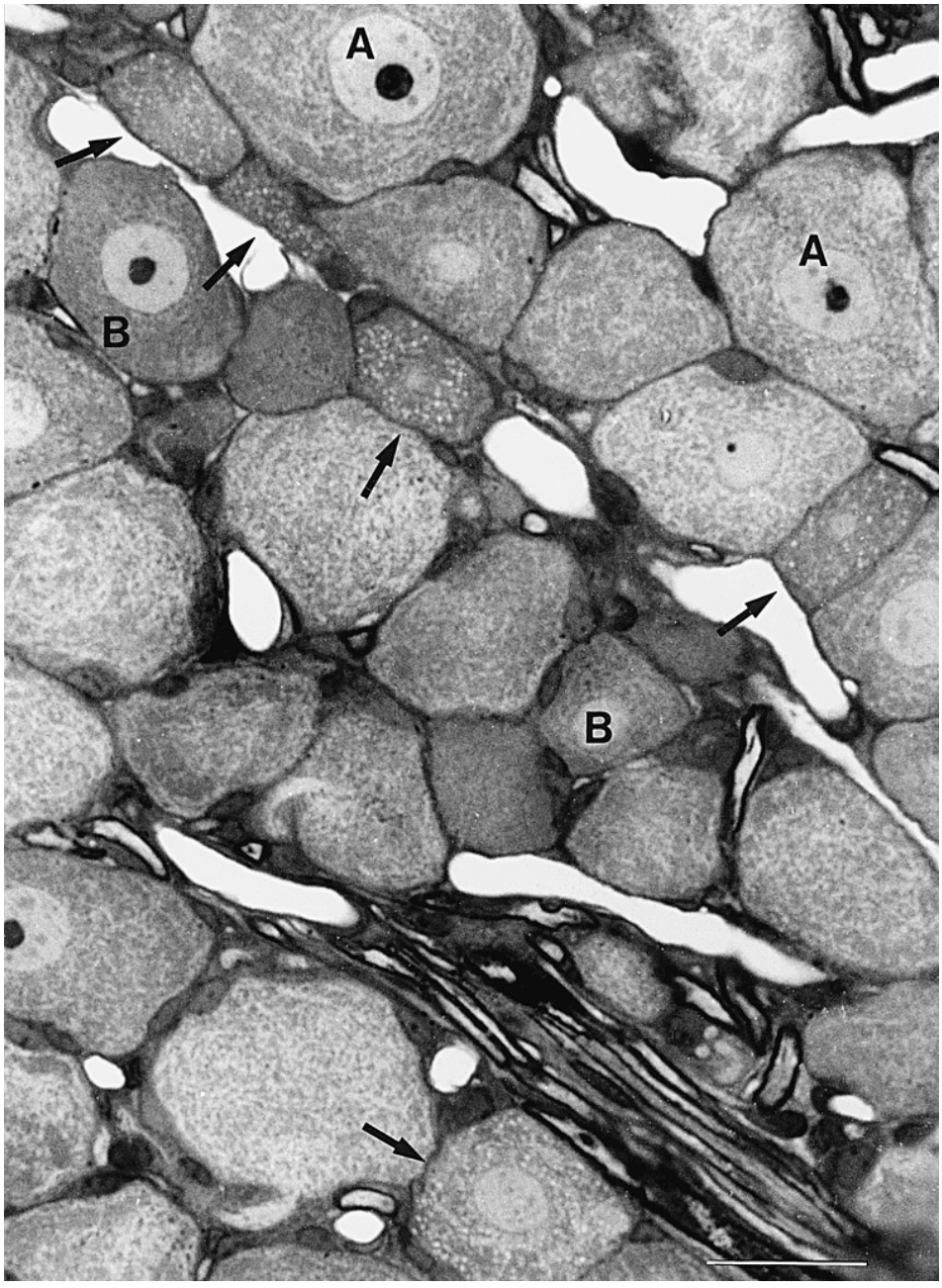


Fig. 1. Photomicrograph of the trigeminal ganglion from a 26-week-old rat. The A-type cells (A) are large and display a light cytoplasm, whereas the B-type cells (B) are smaller and display a darker cytoplasm. In the cytoplasm of the relatively small B-type cells a large number of regular light holes appear (arrows). In the electron microscope these light holes proved to be swollen mitochondria with broken inner cristae. Bar = 10 μm

Light microscopy

The ganglia were removed, dehydrated, embedded in paraffin and serially sectioned at 8 microns. The sections were mounted on gelatin-coated slides in serial order and stained with 1% cresyl violet, dehydrated, cleared and covered with DePex.

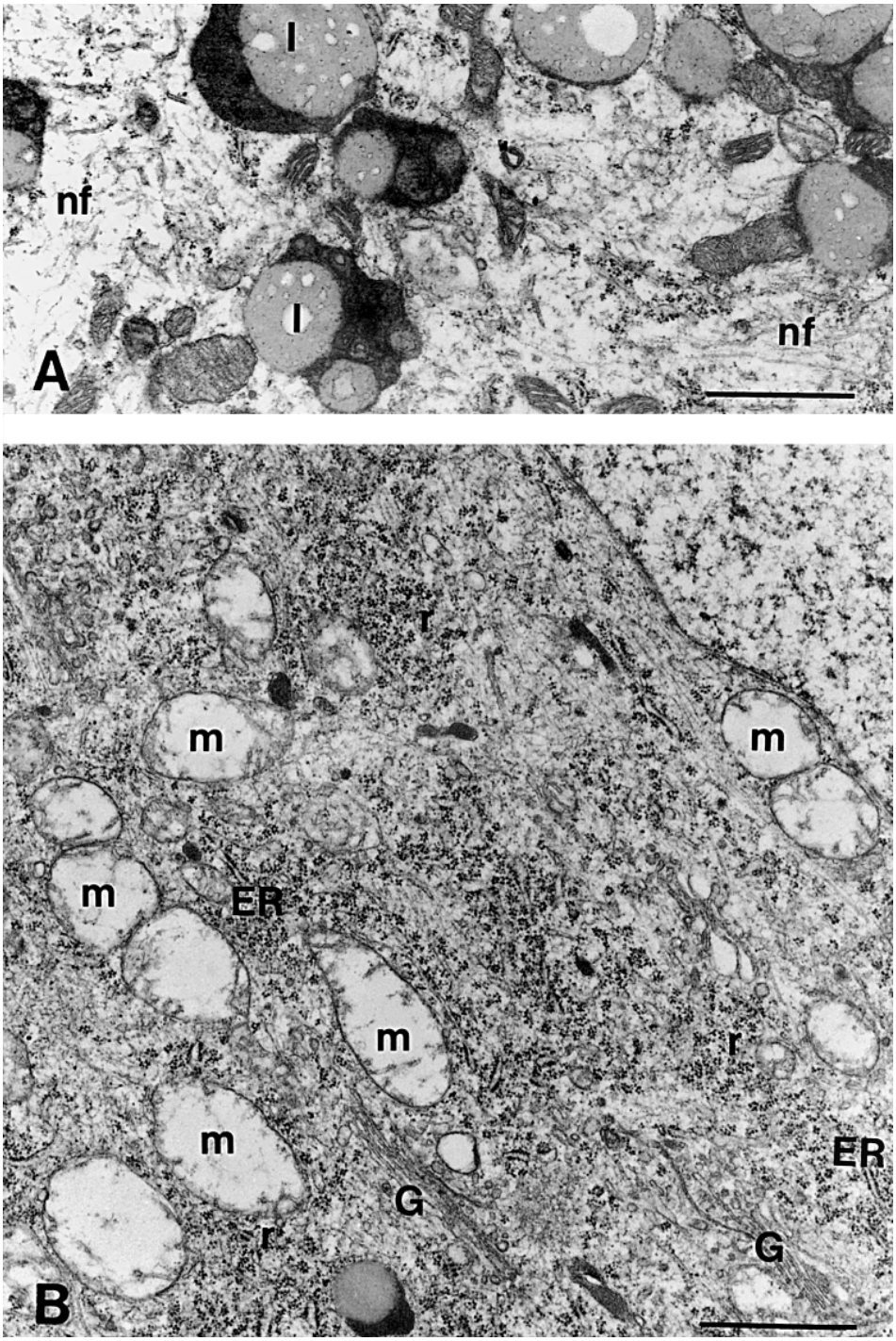
Electron microscopy

After removal of the trigeminal ganglia, small blocks were cut that were suitable for electron microscopic embedding. The blocks were postfixed for 1–3 hours in 4% paraformaldehyde. Following postfixation, the blocks were washed several times in 0.1 M PB, then treated with 1% OsO_4 dissolved in 0.1 M PB for 30 min. Blocks were stained with uranyl acetate during dehydration in 70% alcohol, and embedded in Durcupan (Sigma) according to the routine electron microscopic procedure. Semithin sections were cut with a Leica ultramicrotome and stained with toluidine blue, and a selected area was thin-sectioned. Thin sections were stained with uranyl acetate and lead citrate, and examined in a JEOL 1200 electron microscope. Numerous semithin and thin sections from the trigeminal ganglia of 3- to 20-week-old animals were available from earlier examinations to characterize the normal ultrastructure of the ganglion neurons.

RESULTS

In the trigeminal ganglia, two types of neurons can be distinguished: the large, light A-type ganglion cells and the small, dark B-type cells [1, 26]. These neurons differ in both their light and electron microscopic characteristics. The A-type neurons have a light cytoplasm (Fig. 1) and large groups of organized lamellae of rough endoplasmic reticulum (Nissl bodies) interspersed with a large number of lightly-staining neurofilament bundles. The B-type neurons display more densely-staining cytoplasm (Fig. 1) with fewer neurofilaments, but with many free ribosomes and dispersed, short lamellae of rough endoplasmic reticulum and a large Golgi apparatus. The B-type cells contain large numbers of mitochondria that surround the cell nucleus. The B-type cells differ greatly in size: many of them are similar in size to the A-type cells, although the majority of them are significantly smaller [40, 41].

Fig. 2. Electron micrographs of cells of the trigeminal ganglion of a 26-week-old rat. (A) The electron micrograph shows several large lipofuscin granules (l) in the cytoplasm of an A-type cell, where the mitochondria appear to be normal. Note the numerous neurofilament bundles (nf) characteristic of A-type cells. – (B) Electron micrograph of a B-type cell from the trigeminal ganglion of a 26-week-old rat. Several swollen mitochondria (m) appear among the normal cytoplasmic organelles, e.g. the lamellae of rough endoplasmic reticulum (ER), the Golgi apparatus (G) and groups of free ribosomes. Bar = 0.5 μm for A and B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The neurons of the ganglia of 6- to 12-week-old rats were free of pathological changes. Beginning from week 12, lipofuscin pigments gradually accumulated in both the A-type and the B-type neurons (Fig. 2A), as described earlier [6]. More lipofuscin could be observed in the older groups, and the older the animal was, the more lipofuscin was detected. Not all of the cells contained lipofuscin, but in the affected cells large portions of the cytoplasm were occupied by lipofuscin. This was especially charcteristic for A-type cells, which exhibited large lipofuscin bodies (Fig. 2A). The B-type cells contained smaller and fewer lipofuscin bodies. Other signs of ultrastructural changes in the trigeminal neurons were not detected until week 23. From that time on, a selective mitochondrial swelling with disorganized cristae was seen in a few B-type neurons (Fig. 2B). Some mitochondria of B-type cells displayed slight swelling with a partial loss of cristae, while severe mitochondrial swelling was associated with the complete loss of internal cristae. The pronounced mitochondrial lesions of the B-type neurons could also be observed in the semithin sections (Fig. 1). In the light microscope, multiple mitochondrial swellings appeared as light, round holes in the cytoplasm.

In the affected B-type cells all mitochondria appeared to be damaged (Fig. 2B). The mitochondrial cristae had often disappeared completely. Other cytoplasmic organelles, such the rough endoplasmic reticulum, the Golgi apparatus and the free ribosomes and also the nucleus of the affected cells had normal morphology (Fig. 2B). Mitochondrial damage was never seen in the A-type cells or in the satellite glial cells. Apoptotic or necrotic signs of cell degeneration were never observed in neurons with damaged mitochondria although such characteristics would have easily been detected in the semithin sections, where hundreds of neurons are present in a single section. No cells with necrotic or apoptotic signs were observed in the thin sections. (In contrast, there was a small decrease in cell number in the trigeminal ganglia of the 1-year-old animals; this decrease was around 15%, i.e. close to the range that can be considered the error in the method.) Older animals have not been examined yet, but it can be expected that in 2-year-old animals a more marked cell loss will be found.

DISCUSSION

Ultrastructural alterations in the mitochondria of the trigeminal B-type neurons of aging rats has been reported in the present study. Mitochondrial swelling and a partial loss of cristae were seen from week 23 of age (with a small loss of neurons). This damage was not observed in the A-type neurons or satellite cells, and apoptotic or necrotic signs were not observed in the affected cells with mitochondrial damage. The mitochondrial lesion did not correlate with the appearance of lipofuscin (age-pigment), because lipofuscin appeared in both the A and B cells. Further, the first lipofuscin granules appeared as early as weeks 13–15, whereas mitochondrial swelling was not detected before week 23 in the control rats.

It is interesting, that the mitochondrial damage was very similar to that described after systemic capsaicin treatment (50 mg/kg s.c.) in neonatal or adult animals [10, 24, 32, 33, 35, 37–41]. Selective mitochondrial swelling of immature sympathetic nerve cells similar to that in the B cells of capsaicin-treated and aged animals has been described in rats treated with adrenergic neuron-blocking agents such as bretylium tosylate [7] or guanethidine [27, 28].

In harmony with the mitochondrial lesion and consequent cell loss, increased thresholds for tactile and thermal stimuli have been described in senescent rats [3, 13, 31]. The impairment of neuronal functions after vanilloid treatment, e.g. antinociception to noxious chemicals or hot stimuli and the impairment of temperature regulation are in accord with the selective damage of the capsaicin-sensitive neural population [4, 19–22, 34, 36–38]. Capsaicin increases the intracellular calcium concentration by opening the VR1 receptor cation channel [4, 8, 34] and the mitochondria overloaded by calcium are damaged [11]. Under calcium-free conditions, mitochondrial swelling does not occur [29]. It is interesting that nerve growth factor prevented the capsaicin-induced neuronal loss when capsaicin was applied neonatally [40]. Other studies suggested that nerve growth factors may protect neurons from the effects of capsaicin [30]. Therefore, it can be proposed that the age-related cell loss in the trigeminal ganglia and the consequent sensory deficits may be prevented by the application of nerve growth factors. Additionally, the changes in the sensory ganglia of aging rats offer a model for study of those factors that may prevent or delay naturally-occurring age-dependent cell death.

Another intriguing question is the possible differences between the mitochondria of the A- and B-type cells, because only the B-type cells are sensitive to different noxious effects both in the neonatal period and in adulthood, as well as through aging. It may be suggested that the effects of noxious agents speed up the process that results in mitochondrial damage in the aged animals. The inhibition of such a process may lead to protection of the B-type neurons. Since the loss of B-type neurons has been described for humans, it may be speculated that sensory deficits can be prevented by preserving an intact number of cells in the sensory ganglia.

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