Age-related quantitative changes in mitochondria of satellite cell sheaths enveloping spinal ganglion neurons in the rabbit

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Abstract

We studied mitochondria in the satellite cell sheaths which envelope the spinal ganglion neurons of rabbits aged 12, 42, and 79 months. While the mean cytoplasmic volume of satellite cell sheaths did not change significantly with age, the mean percentage of cytoplasmic volume occupied by mitochondria decreased with age. This decrease is mainly due to a reduction in the total mitochondrial mass and only in minor part is a consequence of lipofuscin accumulation. Mitochondrial structure did not change, while mitochondrial size increased with age. Comparison between mitochondria in nerve cell bodies and those in satellite cell sheaths showed that: (1) the mean percentage of cytoplasmic volume occupied by mitochondria was greater in nerve cell bodies than satellite cell sheaths and the ratio between these two percentages remained constant with advancing age; (2) the total mitochondrial mass was much greater in nerve cell bodies than satellite cell sheaths and the ratio between these two values increased with age; (3) the extent of increase of mitochondrial size with age was similar in nerve cell bodies and satellite cell sheaths. The results of the present study suggest that: (1) the ability of satellite cell sheaths to produce energy decreases with age; (2) the decreased ability of sensory neurons in old animals to meet high energy demands may be partly due to the diminished contribution of their associated satellite cell sheaths.
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1. Introduction

In the sensory ganglia of adult animals, each nerve cell body is usually enveloped by a satellite cell sheath, which is sharply separated from the sheaths encircling the adjacent neurons by intervening connective tissue (Fig. 1). Thus, each nerve cell body with its satellite cell sheath constitutes a discrete unit (for reviews see [19,20]). Some data suggest that satellite cells support the metabolism of the neuron with which they are associated (for more details see [19]). Therefore, in sensory ganglia a nerve cell body and its associated satellite cell sheath constitute not only a structural unit, but probably also a metabolic one. In view of the close interactions between each sensory ganglion neuron and its satellite cell sheath, studies on age-related changes in the former must be coupled with similar studies on the latter. In view of the central role that mitochondria are cur-

rently thought to play in the ageing process (for reviews see [4,9,15,17,25,26]), we have previously quantitatively investigated age-related changes in mitochondria of rabbit spinal ganglion neurons [12]. We now continue this research by studying the age-related changes in the mitochondria of the satellite cell sheaths that envelope these neurons. As far as we are aware, no studies have been published on this topic.

2. Materials and methods

Rabbits (*Oryctolagus cuniculus*) aged 12 months (two animals, 3.4–3.5 kg body weight), 42 months (two animals, 3.6–3.8 kg body weight) and 79 months (two animals, 4.0–4.2 kg body weight) were used. The rabbits were treated according to the European Community Council Directive (86/609/EEC) for the care and use of laboratory animals. The dates of birth of these animals were documented; all had been raised by a specialist rabbit breeder with particular attention to hygiene and regular veterinary inspections and had been fed an unrestricted diet. Because the life span of the normal healthy *Oryctolagus* is 60–72 months [6] or 84–96

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Fig. 1. Electron micrograph showing the nerve cell body (N_1) of a spinal ganglion neuron enveloped by its satellite cell sheath (sc). This sheath is sharply separated from the sheaths encircling the adjacent nerve cell bodies (N) by intervening connective tissue (c). The neuron-satellite cell boundary is outlined in ink. v, blood vessel. Spinal ganglion from a young adult rabbit. Scale bar: 2 μm.

months [27], the 12-month-old rabbits we studied were young adults, the 42-month-old rabbits were middle-aged animals and the 79-month-old rabbits were aged animals. Furthermore, the end of fertility is usually considered to mark the onset of senescence and female rabbits are not normally fertile after 60 months, so that the 79-month-old animals are to be considered aged also from this point of view.

The animals were perfused transcardially with a solution containing 2% formaldehyde and 2% glutaraldehyde in 0.1 M sodium cacodylate buffer (pH 7.3) under deep anaesthesia with Nembutal (80 mg/kg). After fixation for about 3 h, the thoracic spinal ganglia were removed, washed in cacodylate buffer (0.2 M, pH 7.3) for 2 h and then postfixed on ice for 1.5 h in 2% OsO_4, buffered with 0.1 M sodium cacodylate. The specimens were washed in distilled water, stained with 2% aqueous uranyl acetate, dehydrated in alcohol and embedded in Epon–Araldite resin. Several semithin sections were prepared from each ganglion and stained

with 0.5% toluidine blue in 1% sodium borate. They were then examined in the light microscope to check the quality of fixation. Only the best preserved ganglia were used and neither the nerve cell bodies nor the perineuronal satellite cell sheaths in these ganglia showed signs of swelling or shrinkage. Overall, 60 ganglia (10 for each animal) were used for this study.

Isotropic uniform random (IUR) sections were obtained following the orientator procedure [14]. For each ganglion a single IUR thin section (about $0.25\,mm \times 0.20\,mm$) was photographed under the electron microscope. Each section was photographed in its entirety at a magnification of 2500× and the negatives printed to a final magnification of 10,000×. A montage of 60–70 prints was necessary to reconstruct each section. A transparent sheet containing a series of points spaced at 1 mm intervals was randomly positioned over the photomontage. Next a sheet of cardboard containing uniform-sized ($10\,cm \times 5\,cm$), systematically arranged

windows was placed over the transparent sheet and photomontage. For each section, all the points falling on the cytoplasm of the perineuronal satellite cell sheaths (P_s) within each window were counted, as were all the points lying on the mitochondria (P_{sm}). The percentage of cytoplasmic volume occupied by mitochondria (V_{vsm}) was calculated from the following formula: $V_{vsm} = P_{sm} \times 100/P_s$. The mean value of V_{vsm} was calculated for each rabbit. To obtain information on the size of the mitochondria, the number of test points lying on each mitochondrion within each window was determined. The mean number of points lying on a mitochondrion was then calculated for each animal. This number gives an indication of the size of the mitochondria under study. The method we used ensures that all satellite cell sheaths have the same chance of being sampled, irrespective of their size and the size of the nerve cell bodies with which they are associated.

The part of each ganglion left after the preparation of the IUR thin section was used to determine the volume of perineuronal satellite cell sheaths. To this end the circle-fitting method [21] was employed. This method assumes that the nerve cell body is spherical and that its enveloping satellite cell sheath is a closely fitting spherical shell. Full details of this method are given in the original paper [21]. From the mean volume of the satellite cell sheaths (nuclei excluded) and the percentage of that volume occupied by mitochondria, the total mitochondrial mass within the cytoplasm of the perineuronal satellite cell sheaths was calculated.

The values obtained for the rabbits in each age group were compared to establish whether they differed significantly. The mean values of the age groups were then compared. The statistical comparisons employed the two-tailed Student's t-test (differences with $P < 0.05$ were considered significant) and for each value the 95% confidence limits were calculated. All data analyses were carried out using a statistical graphics program (Statgraphics software STSC).

3. Results

We found that in perineuronal satellite cell sheaths mitochondrial structure conformed to the literature descriptions of these organelles (e.g. see [20]) and did not differ in the three age groups. In particular, swollen or degenerating mitochondria were absent in all preparations. We found that the mean percentage of cytoplasmic volume occupied by mitochondria decreased significantly with increasing age (Fig. 2), with a difference of about 27% between young adult and old rabbits. The mean cytoplasmic volume of perineuronal satellite cell sheaths did not change significantly with age, while the total mitochondrial mass within these sheaths decreased by about 34% in old rabbits compared to young adults (Fig. 3). The mean number of points falling on a mitochondrion significantly increased with age (Fig. 4).

Comparison of the present data on mitochondria in satellite cell sheaths with those from the nerve cell bodies of the
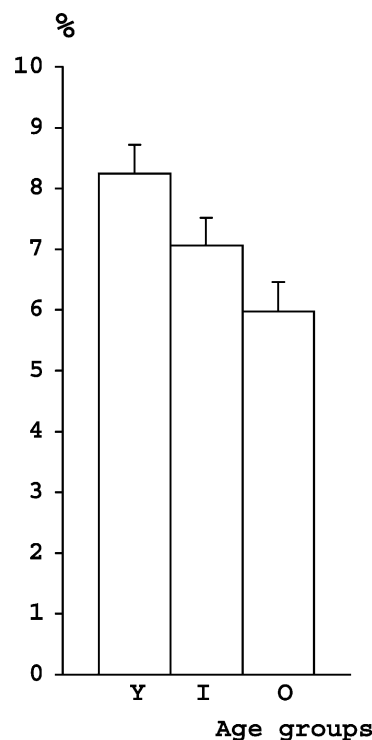

Fig. 2. Percentage of cytoplasmic volume occupied by mitochondria in the perineuronal satellite cell sheaths of young adult (Y), intermediate (I), and old (O) rabbits. Values are mean ± S.E.M. The differences between Y and I, between I and O, and between Y and O are significant ($P < 0.05$).
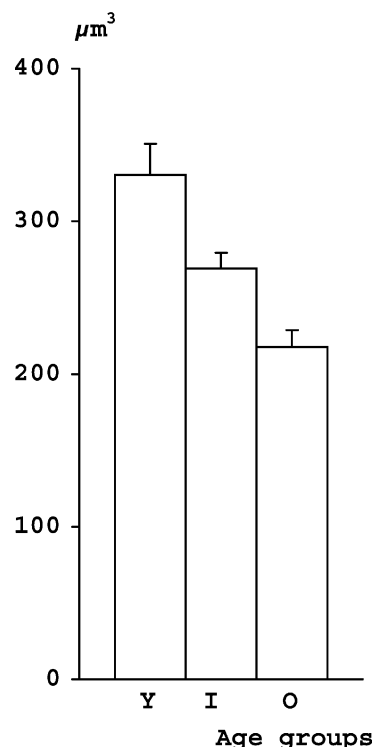

Fig. 3. Total mitochondrial mass (μm^3) within the perineuronal satellite cell sheaths of young adult (Y), intermediate (I), and old (O) rabbits. Values are mean ± S.E.M. The differences between Y and I, between I and O, and between Y and O are significant ($P < 0.05$).

No

4 ⊢

3 ⊢

2 ⊢

1 ⊢
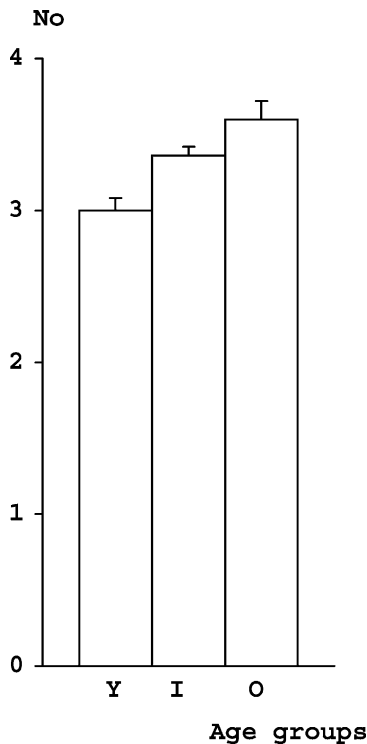
0 ⊢

Y   I   O

Age groups

Fig. 4. Number of points falling on a mitochondrion in the perineuronal satellite cell sheaths of young adult (Y), intermediate (I), and old (O) rabbits. Values are mean ± S.E.M. The differences between Y and I, between I and O, and between Y and O are significant ($P < 0.05$).

same ganglia [12] showed the following. The mean percentage of cytoplasmic volume occupied by mitochondria was greater in nerve cell bodies than satellite cell sheaths. The ratio between these two percentages (1.2:1) was the same in all three age groups. The total mitochondrial mass was much greater within nerve cell bodies than satellite cell sheaths. The ratio between these two values increased with age: 4.5:1 in young adults, 6.2:1 in middle-aged animals, and 8.1:1 in old rabbits. Mitochondria increased in size with age both in the nerve cell bodies and the satellite cell sheaths. In each age group, the mean size of these organelles was the same in neurons and satellite cells.

4. Discussion

In the perineuronal satellite cell sheaths of young adult rabbits the mitochondria occupied 8.24% of the cytoplasmic volume. This percentage is similar to that (7.41%) reported for the Schwann cells of myelinated fibres [22]. As far as we are aware, quantitative studies on mitochondria of the perineuronal satellite cells of sensory ganglia have been concerned only with the rat vestibular ganglion [3,13]. The mitochondria of the satellite cells of this ganglion were found to occupy 19.4% of the cytoplasmic volume [13], a value much higher than those reported above. The reasons for this difference are unclear.

In the present study, we found that in the satellite cell sheaths of rabbit spinal ganglia the percentage of cytoplasmic volume occupied by mitochondria decreased significantly with increasing age. This result differs from those of a study carried out on the satellite cells of the rat vestibular ganglion [3], in which it was reported that the mean percentage of cytoplasmic volume occupied by mitochondria did not change with increasing age.

Previous work in our laboratory [11] showed that in the perineuronal satellite cell sheaths of rabbit spinal ganglia the percentage of cytoplasmic volume occupied by lipofuscin is very low, although it does increase with age (0.29% at 12 months and 2% at 79 months). Hence, the decrease in the percentage volume of mitochondria we observed in old animals is only in minor part due to lipofuscin accumulation. It is, therefore, clear that, in the satellite cells we studied, the ratio between the total mitochondrial mass and the functionally active volume of cytoplasm (i.e. the total cytoplasmic volume minus lipofuscin volume) decreases with age. This decrease suggests that in the perineuronal satellite cell sheaths mitochondrial degradation with age is greater than the production of new mitochondria. However, we never observed degenerating mitochondria.

Our data showed that the mean number of points falling on a mitochondrion significantly increased with age. Since mitochondria did not appear swollen in any age group, this finding indicates that mitochondria increase in size with age. Age-related increases in mitochondrial size have been reported in the rat cerebellum [2] and in human [24] and mouse [28] liver (for review see [18]); however, Herbener [7] found no such changes in mouse heart and liver.

We did not observe degenerated neurons or neurons undergoing degeneration, not even in old rabbits. This suggests that the mitochondrial changes observed in neurons [12] and their associated satellite cells (present paper) of spinal ganglia do not lead to significant neuronal loss. This suggestion is consistent with observation of no reduction [5,10,16] or only a small reduction [1] in the number of spinal ganglion neurons with advancing age.

A linear relationship between the total mitochondrial mass and the maximum rate of oxygen consumption has been demonstrated in muscle tissue [8]. If this were also the case in sensory ganglia, the ability of satellite cell sheaths to produce energy would decrease with age. Hence, their ability to support the metabolism of the neuron would also decrease. One may, therefore, hypothesize that the decreased ability of the neuron in old animals to meet high energy demands (e.g. see [23]) may be partly due to the diminished contribution of the satellite cell sheath.

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