Original Paper

Cells Tissues Organs

Cells Tissues Organs 2007;186:141–146
DOI: 10.1159/000104408

Accepted after revision: March 5, 2007

Mitochondria in Perineuronal Satellite Cell Sheaths of Rabbit Spinal Ganglia: Quantitative Changes during Life

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Key Words

Aging · Dorsal root ganglia · Perineuronal satellite cells · Peripheral neuroglia · Sensory ganglia

Abstract

We studied quantitative changes in mitochondria of perineuronal satellite cell sheaths (SCSs) of rabbit spinal ganglia from young to extremely advanced age (1, 3.6, 6.7 and 8.8 years). The mitochondrial structure did not differ in the four age groups, while mitochondrial size increased progressively and significantly with age. The mean percentage of cytoplasmic volume occupied by mitochondria decreased progressively and significantly from young to old animals. This decrease was mainly due to a progressive and significant reduction in the total mitochondrial volume. Lipofuscin accumulation had a negligible influence on this reduction. These results suggest that the ability of SCSs to produce energy decreases with age and that the reduced ability of spinal ganglion neurons to respond to high energy demands in old age may be in part due to the diminished contribution of perineuronal satellite cells.

Introduction

In view of the central role attributed to mitochondria in the aging process [for reviews, see Miquel et al., 1980; Wallace, 1992; Kadenbach et al., 1995; Ozawa, 1995; Cortopassi and Wong, 1999; Walter et al., 1999], we have quantitatively investigated age-related changes in mitochondria of spinal ganglion neurons in the rabbit [Ledda et al., 2001; Martinelli et al., 2006]. In spinal ganglia, each nerve cell body is usually enveloped by its own satellite cell sheath (SCS), thus constituting a structural and probably also a metabolic unit [for reviews, see Pannese, 1981, 1994]. Because of the close interaction between the spinal ganglion neuron and its SCS, we have previously performed studies on age-related changes in the former and similar studies in the latter [Martinelli et al., 2003]. We have now resumed our investigations on age-related changes in mitochondria of rabbit spinal ganglion SCSs, extending our study to more specimens of the previously considered age groups and also examining animals of extremely advanced age.

Abbreviation used in this paper

SCS	satellite cell sheath

C.M. and P.S. contributed equally to this study.

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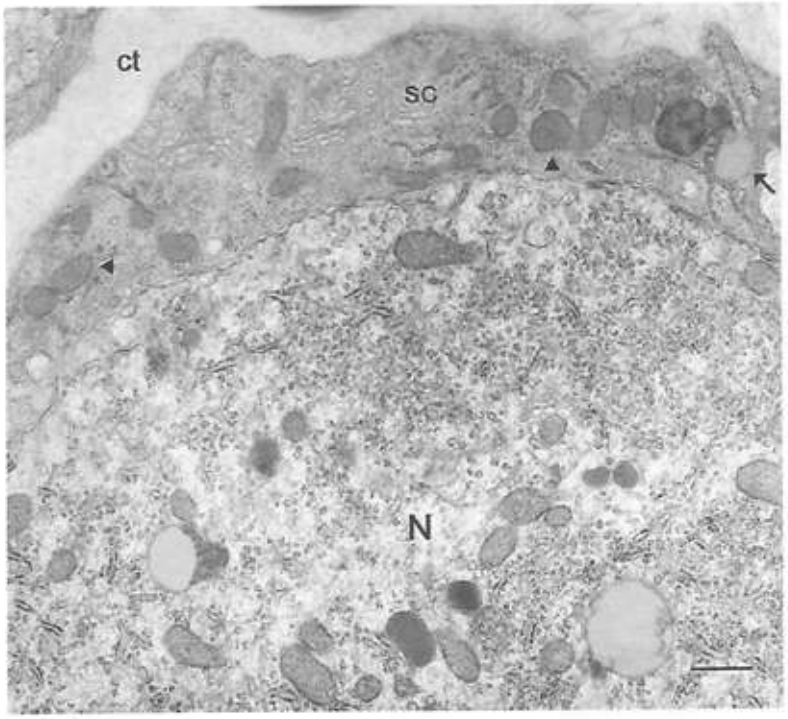

Fig. 1. Electron micrograph showing a portion of an SCS (sc) enveloping a nerve cell body (N) of a spinal ganglion of a rabbit aged 8.8 years. Two of the mitochondria and a lipofuscin body within the SCS are indicated by arrowheads and arrow, respectively. ct = Connective tissue. Scale bar = 0.5 μm.

Materials and Methods

The present study was carried out in rabbits *(Oryctolagus cuniculus)* of both sexes. Rabbits aged 1 year (3 animals, 3.4–3.5 kg body weight), 3.6 years (3 animals, 3.6–3.8 kg body weight), 6.7 years (3 animals, 4.0–4.2 kg body weight) and 8.8 years (3 animals, 4.2–4.5 kg body weight) were used. The rabbits were cared for according to the European Community Council Directive (86/609/EEC) on the use of laboratory animals. In all animals, the dates of birth 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 mean life span of the normal healthy *Oryctolagus* is approximately 5.6 years [Harkness and Wagner, 1983] and the maximal life span is approximately 8 years [Weisbroth et al., 1974], the 1-year-old rabbits we studied were young, the 3.6-year-old rabbits were adult, the 6.7-year-old rabbits were old, and the 8.8-year-old animals were very old.

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 anesthesia with Nembutal (80 mg/kg i.p.). After fixation, the thoracic spinal ganglia were removed and routinely processed for electron microscopy. The quality of fixation was checked by examining semithin and thin sections. Only the best-preserved ganglia were used, and neither the nerve cell bodies nor the SCSs in these ganglia showed signs of swelling or shrinkage. Overall, 96 ganglia (8 for each animal) were used for this study.

Isotropic uniform random sections were obtained following the orientator procedure [Mattfeldt et al., 1990; Pannese et al., 1997]. The percentage of cytoplasmic volume occupied by mitochondria and the size of mitochondria were determined using the stereological method described previously [Martinelli et al., 2003]. This method ensures that all SCSs 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 isotropic uniform random thin section was used to determine the volume of SCSs. To this end, the circle-fitting method [Pannese et al., 1972] was employed. Full details of this method are given in the original paper [Pannese et al., 1972]. 280 SCSs in young animals, 275 in adult animals, 281 in old animals and 266 in very old rabbits were studied. From the mean volume of the SCSs (nuclei excluded) and the percentage of that volume occupied by mitochondria, the total mitochondrial volume within the cytoplasm of the SCSs was calculated for each rabbit.

The values obtained for the 3 rabbits in each age group were compared by one-way ANOVA to establish whether they differed significantly. Subsequently, the mean values obtained for each age group were compared by one-way ANOVA. When ANOVA revealed significant differences, the post hoc Tukey test for multiple comparisons was applied. Values were expressed as means ± SE. Both for ANOVA and post hoc Tukey test, differences were considered significant if p was <0.05. All data analyses were carried out using SPSS 11.0 software.

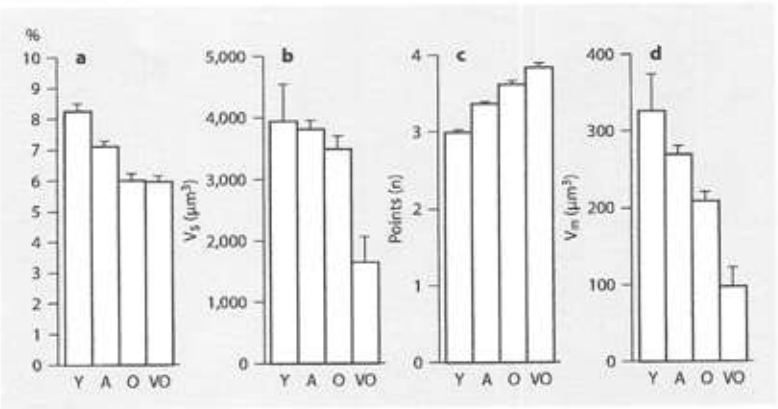

Fig. 2. a Percentage (%) of cytoplasmic volume occupied by mitochondria in SCSs. **b** Volume (V_s, μm^3) of SCSs. **c** Number of points falling on each mitochondrion in SCSs. **d** Total mitochondrial volume (V_m, μm^3) in SCSs. Y = Young rabbits (aged 1 year); A = adult rabbits (aged 3.6 years); O = old rabbits (aged 6.7 years); VO = very old rabbits (aged 8.8 years). Values are means ± SE. Significant differences are shown in tables 1 and 2.

Table 1. SCSs: mean percentage of cytoplasmic volume occupied by mitochondria and mean size of mitochondria

Rabbit	Age months	Ganglia examined, n	Points lying on the cytoplasm (P_s), n	Points lying on mitochondria (P_{sm}), n	Cytoplasmic volume occupied by mitochondria (V_{vsm}), % (means ± SE)	Points lying on each mitochondrion, n (means ± SE)
Values for each rabbit						
1	12	8	57,040	4,536	8.260 ± 0.367	3.026 ± 0.059
2	12	8	54,594	4,574	8.227 ± 0.421	3.038 ± 0.067
3	12	8	49,528	3,946	8.261 ± 0.427	2.906 ± 0.060
4	42	8	117,526	8,278	7.176 ± 0.304	3.346 ± 0.043
5	42	8	106,978	7,004	6.990 ± 0.389	3.368 ± 0.045
6	42	8	152,866	9,676	7.076 ± 0.318	3.382 ± 0.050
7	79	8	46,536	2,856	5.932 ± 0.303	3.632 ± 0.080
8	79	8	46,636	3,264	6.065 ± 0.296	3.628 ± 0.079
9	79	8	45,412	2,898	5.945 ± 0.614	3.588 ± 0.087
10	104	8	49,124	3,238	5.938 ± 0.338	3.856 ± 0.106
11	104	8	41,162	2,484	6.030 ± 0.384	3.886 ± 0.143
12	104	8	58,390	3,302	5.894 ± 0.325	3.794 ± 0.074
Values for each age group						
Young	12	24	161,162	13,056	8.247 ± 0.236[a, b]	2.992 ± 0.036[a, b]
Adult	42	24	377,370	24,958	7.083 ± 0.194[b]	3.364 ± 0.027[c]
Old	79	24	138,584	9,018	5.981 ± 0.251	3.616 ± 0.047
Very old	104	24	148,676	9,024	5.945 ± 0.200	3.832 ± 0.062

[a] $p < 0.05$ vs. adult, [b] $p < 0.05$ vs. old and very old, [c] $p < 0.05$ vs. very old.

Results

The structure of mitochondria did not differ in the four age groups. In particular, swollen or degenerating mitochondria were absent in all preparations (fig. 1). The mean percentage of cytoplasmic volume occupied by mitochondria, the mean cytoplasmic volume of SCSs, the mean size of mitochondria and the total mitochondrial volume did not significantly differ between the 3 rabbits in each age group (tables 1, 2).

The mean percentage of cytoplasmic volume occupied by mitochondria decreased significantly from young to old animals, but there was no significant change between old and very old animals (fig. 2a); the difference between

Table 2. Mean cytoplasmic volume of SCSs and total mitochondrial volume within SCSs

Rabbit	Age months	Ganglia examined, n	Cytoplasmic volume, μm^3 (means ± SE)	Total mitochondrial volume within the cytoplasm, μm^3 (means ± SE)
Values for each rabbit				
1	12	8	4,165.972 ± 1,253.695	344.109 ± 103.355
2	12	8	3,863.981 ± 1,236.281	317.890 ± 101.709
3	12	8	3,795.451 ± 378.594	313.542 ± 31.276
4	42	8	3,692.502 ± 239.947	264.974 ± 17.219
5	42	8	3,867.564 ± 286.667	270.343 ± 20.038
6	42	8	3,892.164 ± 216.195	275.410 ± 15.298
7	79	8	3,382.222 ± 331.630	200.633 ± 19.672
8	79	8	3,593.640 ± 381.815	217.954 ± 23.157
9	79	8	3,482.387 ± 406.578	207.028 ± 24.171
10	104	8	1,544.696 ± 254.032	91.724 ± 15.084
11	104	8	1,673.699 ± 764.906	100.924 ± 46.124
12	104	8	1,660.352 ± 751.242	97.861 ± 44.278
Values for each age group				
Young	12	24	3,939.901 ± 593.278[a]	324.924 ± 48.928[a, b]
Adult	42	24	3,801.334 ± 149.938[a]	269.248 ± 10.620[a]
Old	79	24	3,479.025 ± 214.649[a]	208.080 ± 12.838
Very old	104	24	1,639.604 ± 417.551	97.474 ± 24.823

[a] $p < 0.05$ vs. very old and [b] $p < 0.05$ vs. old.

young and very old rabbits was about 28% (table 1). The mean cytoplasmic volume of SCSs did not significantly change from young to old animals, but was markedly reduced in the very old animals (fig. 2b); the difference between young and very old rabbits was about 58% (table 2). The mean size of mitochondria increased progressively and significantly with age (fig. 2c), with a difference of about 28% between young and very old rabbits (table 1). The total mitochondrial volume within the cytoplasm of SCSs declined progressively and significantly with age (fig. 2d), with a difference of about 70% between young and very old rabbits (table 2).

Discussion

In the SCSs of young rabbits, the mitochondria occupied little more than 8% of the cytoplasmic volume, a similar percentage to that found in Schwann cells of myelinated fibers [Pannese et al., 1988]. A literature search revealed that quantitative studies on mitochondria in the SCSs of sensory ganglia have been performed only on the rat vestibular ganglion. In the satellite cells of this ganglion, mitochondria occupied 19.4% of the cytoplasmic volume [Lyon and Carney, 1990], being much higher than the value we found in rabbit spinal ganglia. The reasons for this marked difference remain to be determined.

Lipofuscin accumulation is not appreciably involved in the decrease in the mean percentage of cytoplasmic volume occupied by mitochondria in the SCSs, since the cytoplasmic volume occupied by lipofuscin in the SCSs of rabbit spinal ganglia varies from 0.29 to 2% [Ledda et al., 1999]. This decrease with age is rather due to the imbalance between mitochondrial degradation and the production of new mitochondria in favor of the former. A comparison of the present data on the mean percentage of cytoplasmic volume occupied by mitochondria in perineuronal sheaths with those from the nerve cell bodies of the same ganglia [Martinelli et al., 2006] showed that in all four age groups this percentage was greater in nerve cell bodies than perineuronal sheaths. The ratio between these two percentages (1.3:1) did not change throughout life.

In our previous study on mitochondria of the rabbit SCSs [Martinelli et al., 2003], three age groups were studied. The mean cytoplasmic volume of these sheaths did not significantly change from young to old rabbits. In the present study, we also examined very old animals in addition to the three age groups. The SCS volume markedly decreased from old to very old age. This new finding shows that in studies on aging it is advisable to examine

extremely old animals in order to obtain complete information on the aging process.

In a previous study, the mean mitochondrial size increased significantly with age [Martinelli et al., 2003], and our present results reveal that this increase continues into extremely advanced age. Mitochondrial size also rises with age in the nerve cell bodies of rabbit spinal ganglia [Martinelli et al., 2006]. A comparison of the present findings with data on nerve cell bodies in the same ganglia shows that in each age group, the mean size of these organelles is the same in SCSs and neurons. Age-related increases in mitochondrial size have also been reported at other sites (e.g. rat cerebellum [Bertoni-Freddari et al., 1993] and human and mouse liver [Tauchi and Sato, 1968; Wilson and Franks, 1975; for a review, see Ozawa, 1997]). The age-related increase in mitochondrial size is probably attributable to a decreased rate of mitochondrial division.

Another finding of this study was the progressive and significant decrease in the total mitochondrial volume in the cytoplasm of SCSs with increasing age. This decrease was particularly marked in the oldest animals. Since the total mitochondrial volume decreases and the mitochondrial size increases with age, it is likely that the number of mitochondria decreases with age. A linear correlation between the maximum rate of oxygen consumption and the total mitochondrial volume has been found in muscle tissue [Hoppeler et al., 1987]. If a similar correlation occurs in spinal ganglia, the implication is that the ability of SCSs to produce energy and hence support neuronal metabolism decreases with age. The reduced neuronal metabolism [Meier-Ruge et al., 1976; Mann et al., 1978; van den Bosch de Aguilar and Vanneste, 1980; Finch and Morgan, 1990] and the reduced ability of the neuron to respond to high energy demands [Sylvia and Rosenthal, 1979] in old age might be due in part to a diminished contribution from the perineuronal satellite cells. However, the mitochondrial changes found in neurons [Martinelli et al., 2006] and in perineuronal satellite cells (present study) of spinal ganglia were not associated with significant neuronal loss, as shown by the lack of degenerated or degenerating neurons even in extremely old animals. In other animal species, the number of spinal ganglion neurons does not decrease (humans [Emery and Singhal, 1973; Ohta et al., 1974] and rats [La Forte et al., 1991]) or only slightly declines (rats [Bergman and Ulfhake, 1998]) in senescence.

Acknowledgments

The authors wish to thank Mr. F. Redaelli for photographic assistance.

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