Microscopic details of age related changes in rat optic nerve

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Abstract

Background: Age-related changes in the number and density of optic nerve fibres were studied in 12-month-old (adult) and 24-month-old (aged) male Wistar rats.
Methods: Two-micrometer-thick resin-embedded optic nerve cross-sections obtained from two different age groups were stained with toluidine blue and examined under a light microscope at low (5x) and high (500x) magnification. The optic nerve cross-sectional area, and the number of nerve fibres with diameters less or higher than 1 µm were evaluated by means of computerized image analysis and statistical analysis of results.
Results: Retrobulbar optic nerve cross-sectional area decreased in relation to ageing. The number of optic nerve fibres with a diameter of less than 1 µm decreased by about 39% in 24-month-old rats versus 12 month-old animals ($P < 0.05$) while only a swelling was observed in nerve fibres with diameters higher than 1 µm ($P > 0.05$).
Conclusions: Data suggest that age-related impairment of nerve cell population also occurs at the optic nerve level. Our data allow us to hypothesize that all major components of the rat optic paths are sensitive to the aging process.

Keywords: *rat, optic nerve, nerve fibres, aging, animal models*

1.Introduction

Vision and quality of life related to vision acquires particular relevance in the elderly. Most common causes of visual loss in elder patients are physiological deterioration of the visual system and increased incidence of ocular pathology. Increasing evidence seems to suggest that the fall in visual acuity and in visual impairment occurring in old age elder people cannot be solely attributed to the opacity of the cornea or the lens, but it is, can be, at least in part, dependent on age-related changes at the level of the optic nerve pathways [1-4]. In fact, age-dependent loss of nerve cells has been reported in the various layers of the retina retinal layers [5-8]. and changes occurring in the visual cortex too. Age-related changes in the visual cortex have also been described [9,10]. However, the age-related changes in the optic pathway with o1d age are a matter of discussion debated [11,12].

Some authors described an age-re1ated impairment pattern of changes in human optic nerve morphology [13,14] as well as a decrease in the number of optic nerve axons in the elderly [15,16]. No data are yet available, to our knowledge, concerning age-dependent changes in rat optic nerve microscopic details or in the number of nerve axons, although the rat is widely used as a model for the study of neuronal development and ageing [7,15]. However, age-related optic nerve modifications should be expected since the number of rat retinal ganglion cells, which represent the origin of optic nerve fibres [10,11]; is reduced as these animals age in rats, as in other animals [16,18].

For these reasons, we studied the age-related changes occurring in the rat optic nerve.

2. Materials and Methods

Twelve adult (12-month-old), and 12 elderly (24-month-old) male Wistar rats were used. Experiments were performed in agreement with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research and in compliance with the Italian law on animal care no. 116/1992 and the EEC/609/86. The average life span of the rat colony used in the present study was 26 months. The animals were weighed, and anaesthetized with pentobarbital (5 mg/kg body weight perfused through the ascending aorta with a fixative containing 2% glutaraldehyde and 2% formaldehyde in 0.1 M sodium cacodylate buffer).

Table 1. Comparison between adult rats and aged rats concerning optic nerve cross-sectional area and total number of nerve fibres.

Optic nerve	Adult rats (-12) 12 months old	Aged rats (n-12) 24 months old	p
Cross section area 2 x 105	2.65 ± 0.21	1.91 ± 0.14	<0.01
Total nerve fibres	94750 ± 2580	68340 ± 2420	<0.05
Nerve fibres diameter less than 1 µm	65120 ± 2312	46820 ± 1980	<0.05
Nerve fibres diameter between 1 and 1.5 µm	13789 ± 914	12980 ± 926	>0.05
Nerve fibres diameter highter than 1.5 µm	9650 ± 864	9390 ± 817	>0.05

The right eyeball, together with the optic nerve, was removed and placed in the same fixative. The optic nerve was then cut just at the level of its attachment to the eyeball, washed in the above buffer and fixed with the perfusion solution at 4°C overnight. The samples were then post-fixed in 1% OsO4 in cacodylate buffer, rinsed, dehydrated and embedded in Historesin (LKB, Sweden).

Cross-sections (2-µm-thick) were obtained using a motorized microtome, mounted on microscope slides and stained with toluidine blue. Each section was photographed with a Zeiss II photomicroscope using a 1x or a 100x objective and 5x ocular optovar to obtain a magnification of 5x and 500x. Separate computerized photos were taken for each section (more of 500).

The total area of the optic nerve, as well the number of nerve fibres and their diameters were evaluated from 5 sections and 5 microscopic fields for each animal, using a 2000 Quantimet computerized image analyzer. These data formed the basis of our statistical analysis. For the evaluation of the nerve fibres diameter, the protocol described by Johnson et al.5 was followed.

3. Results

The optic nerve cross-sectional area is significantly decreased in 24 month old rats ($P < 0.01$). In addition, the total number of nerve fibres was gradually decreased in aged animals by about 39% compared to younger rats ($P < 0.05$) (table 1). No topographic differences in the age-dependent reduction of the number of optic nerve fibres were observed at the level of the three different sections of the nerve studied (central, nasal and temporal).

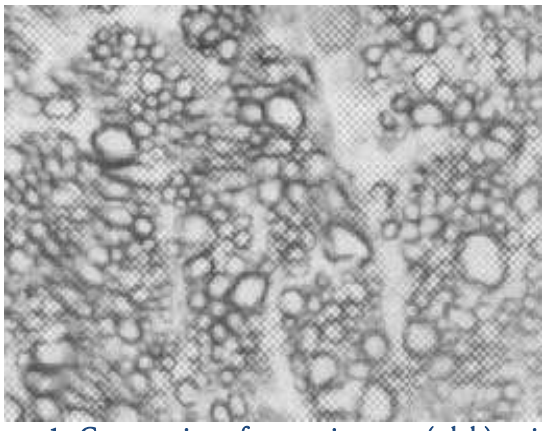

Figure 1. Cross section of rat optic nerve (adult) stained with Toluidine blue. We can observe numerous thin and some thick nerve fibres. Fig. 1 can be compared with Fig.2. (Magnification 500x).

Only nerve fibres with a diameter less than 1 µm were significantly reduced in aged rats: 39% less in elderly versus adult rats ($P < 0.05$). Nerve fibres with a diameter between 1 and 1.5 µm, or with diameters exceeding 1.5 µm, were unaffected by ageing ($P > 0.05$) and show only a swelling of diameters (table 1). Numerous cross sections of rat nerve fibres were studied by light microscopy.

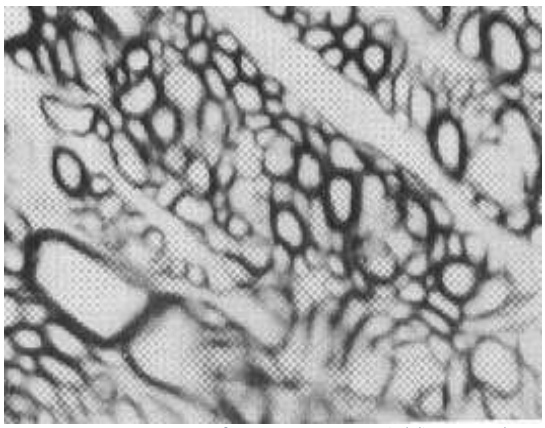

Figure 2. Cross section of rat optic nerve (old) stained with Toluidine blue. We can observe a specific loss of thin nerve fibres and swelling of the thicker ones. (Magnification 500x).

Two samples of these sections were reported in figures 1 and 2. It seems that the age-dependent loss of rat optic nerve fibres is not a generalized phenomenon, but involves only thinner nerve fibres which behave differently than thicker ones, as described for human optic nerve (Johnson et al. 1987) [6].

4.Discussion

The present results provide direct evidence that the optic nerve of elderly rats undergoes age-dependent changes consisting in a significant decrease in nerve cross-sectional area as well as a decrease in the number of nerve fibres with a diameter of less than 1 µm. These results are consistent with age-related changes described in human optic nerve [3,5,19]. The decrease in the cross-sectional area of the nerve of aged animals suggests an age-related increase in the meningeal membrane components similar to that described in man [4,17] .

The data of our study agree with those reported in the literature, which show a significant age-related reduction in the number of ganglion cells [15,18]. Furthermore, in the rat, the process of age-related reduction of nerve fibers involves only the thinner ones, unlike what occurs in the human optic nerve5. These results suggest that changes in visual acuity that have been highlighted in rats may be specific for some functions rather than for others [8,18] .

5.Conclusion

The context of the above data allows us to compare it with data published by other authors concerning the loss of nerve cells in the different layers of the retina , as well as in the visual cortex of aged rats [8,11,15,18]. Our data allow us to hypothesize that all the main components of the rat optic pathways are sensitive to the ageing process.

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