The ageing photoreceptor

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Abstract

With age many retinal neurons are lost. In humans the rod photoreceptor population in the perimacular region is subject to approximately 30% loss over life. Those that remain have been reported to suffer from extensive convolutions and localized swellings of their outer segments abnormally increasing their disc content and outer segment length. Here we examine quantitatively age-related changes in rat rod photoreceptors. The rat retina is ~97% rod dominated. Here, aged rods showed significant reductions in outer segment length. The discs in their outer segments had a similar density, irrespective of whether they were young or old, however, in aged animals a higher proportion were misregistered. Surprisingly, in all of the tissue examined, we found no evidence for any convolution of outer segments or localized swelling as reported in humans, rather all remained straight. There are methodological differences between the research reported here and that undertaken on human retinae. There are also major differences in overall retinal architecture between humans and rodents that could contribute to differences in the aging process of individual cells. If it is the case that individual photoreceptors age differently in rodents compared to humans, it may pose significant problems for the use of this animal model in studies of ageing and age related outer retinal disease.

Keywords: Retina, Ageing, Rod photoreceptor, Outer segment

Introduction

The ageing nervous system is subject to many changes, most of which are irreversible, and are reflected in shifts in function. With age there is a loss of retinal neurons including photoreceptors (Katz & Robison, 1986; Curcio et al., 1993; Weisse, 1995) and ganglion cells (Gao & Hollyfield, 1992; Curcio & Drucker, 1993). Inter-neurons between these two cell-types are also subject to age related cell loss, although quantification of this cell loss is harder to achieve (Alamouti & Funk, 2003). Retinal cell loss is reflected in a marked change in the aged electroretinogram (ERG). The magnitude of the photoreceptor response, the a-wave, is reduced and there is both a reduction in the post-receptoral response and a delay in its onset, as represented by the b-wave (Wright et al., 1985; Gresh et al., 2003; Jackson et al., 2004, 2006). Such changes are also mirrored by age related changes in acuity and visual function (Spear, 1993).

In humans 30% of rod photoreceptors are lost in the central retina between the ages of 34 and 90 years (Curcio et al., 1993). It has been reported that there are significant changes in the morphology of the photoreceptors that remain, which may further compromise retinal function. Marshall et al. (1979) undertook an extensive analysis of aged human rods in retinae from 73 human eyes spanning nine decades of life. One of the key observations of this study was that many aged rod outer segments showed extensive sinusoidal convolutions along their major axis that increased their length and their disc content by 20% to 40%. The outer segments of these cells also displayed local nodular excrescences or swellings. The study by Marshall et al. (1979) was primarily a qualitative investigation. The aim of this study is to examine and quantify age related changes in rat rod photoreceptor morphology.

Materials and methods

Female Dark Agouti (DA; inbred strain) rats of two age groups, 1 month old and 24 months old were used. All animals were bred, weaned and aged in house specifically for this project at the same time. The only difference between the groups was that the first was killed at one month whereas the second was allowed to age to 24 months. They were all group-caged and maintained in a light/dark 12:12 cycle environment. They were housed in cages on lower racks where there was no direct illumination. Young and aged animals experienced identical lighting conditions. The intensity of the in-cage illumination was 11–28 lux, depending on orientation of the light meter. Light onset was at 7 AM. Animals were killed by exposure to CO2 at approximately 11 AM after the circadian controlled peak of rod outer segment shedding (LaVail, 1980; Goldman, 1982). The animals were then perfused with a mixture of 3% glutaraldehyde and 1% paraformaldehyde in 0.08 M sodium cacodylate-HCL buffer (pH 7.4). The eyes were then removed and

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stored in the fixative. The anterior chamber and lens were removed and the eye cups rinsed in 0.1 M sodium cacodylate buffer before the eye cups were placed in 1% osmium tetroxide for 2 h. Eye cups were then dehydrated and embedded in Araldite resin. For light microscopy (LM) sections were cut at 0.75 μm, mounted on glass slides and stained with toluidine blue. For electron microscopy (EM) thin sections were cut and washed in 1% uranyl acetate and Reynolds lead citrate. EM photographic negatives taken at a magnification of ×8000 and were scanned at high resolution 1200 dpi (Agfa Duoscan T2500).

Analysis was confined to sections adjacent to the optic nerve head and within the central third of the eye cup as when viewed as a C shaped preparation. For the analysis of outer segment length LM sections were viewed at a magnification of ×1000. Digital images were captured from which measurements of individual outer segment lengths were undertaken. One eye was examined from each animal in each group (n = 5 per group). In the young group a total of 296 outer segments were measured and in the aged group a total of 200.

Measurements were also made of the number of discs per unit length in the outer segments and their relative registration. Sampling of disc numbers was undertaken in the region of the central third of the length of the outer segment. This analysis was undertaken in 4 old and 5 young animals. The total distance analyzed for the number of discs per unit length measurement was 150 μm, which represents approximately 3900 discs. The measurements were undertaken in at least 21 different locations per animal.

It was apparent on examining outer segments that some discs were misregistered. To determine the relative number of these they were only counted when the tips of the discs were separated by a minimum of 47 nanometres horizontally from an adjacent disc, which was 3.4 times the standard deviation of the normal disc alignment distances. This analysis was undertaken in the same number of animals as for the number of discs per unit length above. The total length of outer segment discs analyzed was 1259 μm in old and 1283 μm in young animals, which represents approximately 65,000 discs.

To determine the extent of age related cell loss, estimates of outer segment numbers and the thickness of the outer nuclear layer (ONL) were undertaken on sections prepared for LM. In each retina examined, the thickness of the outer nuclear layer was measured at central locations at a magnification of ×1000 on digitally stored images, which came from regions adjacent to, but not including the optic nerve head. These measurements were made in regions where the thickness of this layer appeared uniform. To determine whether changes in ONL thickness resulted from differences in cell packing density, nuclear densities were determined at the same locations. In the same regions and at similar magnifications, the number of outer segments was counted along strips approximately 60 μm wide. A total distance of 1638 μm in old and 1360 μm in young animals was analyzed. Approximately 35 OS were counted per strip, hence a total of 1380 OS in the aged and 1358 OS in the young group. Additionally the length of inner segments was determined in 4 young and 5 old animals. Student one tailed t-test was used for data analysis.

Results

There were clear differences in the outer retina between the young and aged rats. In the older animals there were approximately 30% fewer outer segments than in younger animals (Fig. 1A). This difference was statistically significant (p = 0.016). The outer nuclear layer was also approximately 20% thinner in the older than the younger animals. Again this difference was statistically significant (p = 0.027). However there was no significant difference in the packing density of ONL cells (p = 0.147). These results are shown in Fig. 1B and Fig. 2 and are consistent with age related photoreceptor loss. However, it is also possible that elements of this difference resulted from differences in eye size between the two groups as the one-month-old eye has yet to attain the full size found in older animals.

Examination of the length of the outer segments at the light microscope level revealed significant differences between the two age groups, which can be seen in Figs. 2A, 2B, and 3A, with outer segments approximately 15% shorter in the older than the younger animals, declining from a mean of approximately 31 μm to 26 μm. These differences were statistically significant (p = 0.036). Surprisingly, examination at both the light and electron microscope level failed to produce any indication of contortions in outer segments similar to that reported in humans. Every outer segment viewed at a range of eccentricities was straight. Additionally, no nodular excrescences were seen in any of the outer segments. Measurements of IS length revealed that these are 13% shorter in the aged group than in the young animals (Fig. 3B). Again this difference was statistically significant (p = 0.031).

Two additional features were examined at the electron microscope level. First, the number of discs in the outer segments per unit length was counted (Figs. 4A, 4B, 4C, and Fig. 5A). Here there was a very small difference between the groups with the older animals having slightly fewer discs, but this was not statistically significant.

Also, the relative registration of the individual discs with one another was measured to determine whether the ageing process was associated with disc alignment irregularities (Figs. 4 and 5B). Here, the aged group had a marked increase in the number of discs that were out of alignment with those adjacent to them (Figs. 4C and 5B). These registration irregularities occurred in individual discs and were rarely if ever seen in small groups of discs. There was no obvious location for these misregistered discs because they were found along the length of the outer segments. However, the differences between the two age groups in this respect were not statistically significant.

Discussion

The results of this study demonstrate that aged rods in rodents are significantly shorter than that found in their younger counterparts.
These aged photoreceptors have similar disc numbers per unit length in their outer segments, but there is an increase in the number of individual discs that are misregistered, although this was not statistically significant. There was no evidence of distorted outer segments or nodular swellings on the outer segments as reported in humans (Marshall et al., 1979).

Our data are consistent with those from other studies that have examined the extent of age related photoreceptor loss and correlated changes in function (Katz & Robison, 1986; Fox & Rubinstein, 1989; Weisse, 1995). However, an important qualification needs to be made to our data regarding age related cell loss, in that we have not controlled for changes in eye size between the groups. The one-month-old eye has probably not attained its full size, and growth related expansion would lead to a reduction in the number of cells counted per unit area.

We have also made no attempt to correlate any of these changes with the age related changes known to occur in the adjacent retinal pigment epithelium. These include changes to microvilli and rates of phagocytosis (Katz & Robison, 1986; Weisse, 1995). These may be relevant to our data but are beyond the scope of this study.

This is the first study to specifically address the question of how the rodent rod photoreceptor changes morphologically with age. However, a study by Fox and Rubinstein (1989) did include a comparison of outer segment length in rats that were 1 and 12 months of age. Here they found a small difference with aged rods being approximately 3% to 5% shorter than those found in young animals. Similarly, Li et al. (2003) measured the thickness of the ONL in rats at 1, 12 and 28 and, 31 months of age. Only minor changes were found between 1 and 12 months, but significant

Fig. 2. The outer retina in young (A & C 1-month-old) and aged (B & D 24-month-old) rats. In A and B the outer segments (OS) can be seen coursing toward the pigment epithelium away from the inner segments (IS). The outer segments were approximately 15% shorter in the aged group. This difference was statistically significant (P = 0.036). ONL = outer nuclear layer. C & D show the outer nuclear layer (ONL) in the same groups of animals. The outer nuclear layer showed similar changes between the two age groups. Here the reduction in thickness was approximately 20% in the aged group. This difference was statistically significant (P = 0.027). Scale bars = 10 μm.

Fig. 3. Bar charts of the rod outer segment and inner segment length in young (1 month) and aged rats (24 months). In both cases these structures shorten with age and in a roughly proportional manner. Differences (±SEM) found in both A and B were statistically significant (A, P = 0.036; B, P = 0.031).
reductions were found after this time. Hence, the relatively small difference in outer segment length identified by Fox and Rubinstein (1989) between the 1 and 12 months of age is most likely to be caused by the smaller age difference between the groups they used, compared with the wider time window employed here. Along with the results of Li et al. (2003) this implies that there may be a greater proportional loss of outer segment length after 12 months of age compared with earlier stages. Interestingly, Gresh et al. (2003) analyzed the number and function of aged mouse photoreceptors. One of their main conclusions was that age related cell loss alone could not account for the decline in aged photoreceptor function as determined by ERG recording. However, they did not measure outer segment length. Our finding that those cells that remain are significantly shorter may explain this discrepancy.

The results presented here are in stark contrast to those of the only other detailed morphological study of aged photoreceptors.

Fig. 4. Electron micrographs of outer segments. (A) shows the full length of a small group of outer segments in an aged animal. Irrespective of whether the animal was young or old, all of the segments were straight, with no indication of irregularities, contortions or local swellings. Scale bar = 1000 nm. Retinal pigment epithelium to the bottom. (B) shows the discs and their alignment in an individual outer segment at higher magnification. It is clear that all of the discs end at the same location at the side edge of the segment, regularly aligned. Scale bar = 100 nm. (C) However, in aged animals a greater proportion of discs ended short of the cell’s limiting membrane. An example of this is shown in the outer segment disc circled. Scale bar in C = 50 nm.

Fig. 5. (A) The number of discs counted in young (1 month-old) and aged (24 month-old) animals per micron (±SEM). The small difference between the groups with slightly less discs in the older animals was not statistically significant ($P = 0.19$). (B) In the same groups the number of discs that were misregistered in relation to those above and below them was counted (±SEM). More misregistered discs were found in the aged animals, however, again this was not statistically significant ($P = 0.15$).
Marshall et al. (1979) demonstrated in human retinae that aged rods were actually longer than those found in younger eyes and contained a greater number of discs. This was primarily caused by their having extensive sinusoidal convolutions along their major axis. No evidence of such convolutions was found in this study. Marshall et al. (1979) also reported that aged outer segments had excrescences, which appeared to be very common. Again, no such structures were found in this study.

Two main possible explanations may exist for the discrepancy between the results of this study and that of Marshall et al. (1979). First, rodents and primates have radically different retinae. The former does not use vision as its primary sensory modality, whereas the latter has the most highly developed retina among mammals (Hughes, 1977; Walls, 1942). Data provided by Marshall et al. (1979) appears to have been derived largely from the perimacular region, although precise locations were not given. The rodent not only lacks a macula, but also has only the weakest gradient in cell density between the central and peripheral retinal regions (Hughes, 1977). Hence, the architectural platform on which these age related changes are being compared is very different. Given this, it is possible their respective photoreceptors display different age related changes. If this were correct it would pose serious problems for those using the rodent as a model for retinal ageing and/or pathology.

Second, it is possible that the tissue was handled differently in the two studies. Marshall et al. (1979) examined 73 human retinae. Twenty of these came from eyes enucleated for malignant melanoma. Hence, pathological process may have taken place that impacted upon outer segments, although the authors offer cogent arguments against this. Their other eyes came from cadavers retrieved primarily for corneal grafting. No details are provided regarding the period between death and fixation. Delays here will result in tissue degradation.

Another issue that could give rise to some discrepancy is the time of death within the circadian cycle. Photoreceptors lengthen their outer segments in darkness and their apical tips are removed by the retinal pigment epithelium cells around eye opening in the morning. All rats used in this study were killed in the late morning. There is no record of when the human subjects died, or more significantly, how long their eyes had been closed prior to death. Had they been closed for significant periods, the process of stasis would have resulted in longer outer segments (Schremser & Williams, 1995), but there is no reason to believe that this would induce distortions in the outer segments or local swellings in them.

Whereas mammalian rod photoreceptors are heterogeneous, the retinal configuration in which they are housed differs markedly between human and rodent. Hence rod photoreceptor response to aging may differ because of differences in the architectural configurations of their respective retinae. It is possible that the differences found between this study and that of Marshall et al. (1979) are a combination of factors. However, until such issues are resolved such differences leave open the question of the relevance of rodent models of ageing to the human condition.

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