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Lack of p75 receptor does not protect photoreceptors from light-induced cell death

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Abstract

Rod photoreceptors are susceptible to light-induced cell death. Previous results have suggested that the neurotrophin receptor p75 in Müller cells controls photoreceptor cell death during light-exposure by suppressing trophic factor release; and consequently, if p75 is blocked or eliminated during light-exposure, apoptosis is delayed. We explored this question by examining photoreceptor cell survival in albino p75^{-/-} mice as well as their heterozygous and homozygous littermates. Photoreceptor cell death was examined in semi-thin sections by counting the remaining rows of photoreceptors. No difference in the amount of cell death was found between p75^{+/+} and p75^{-/-} animals, whereas the single copy of p75 in the heterozygous p75^{+/-} mice provided significant neuroprotection. Cell death in the wild-type animals may indeed be mediated by p75, whereas other known apoptosis pathways may be activated in the p75^{-/-} mice. The pro-apoptotic activity of the p75 receptor may have been partially suppressed in the heterozygous p75^{+/-} mice by the silencing effect of the Trk receptor. Thus, our results suggest that p75 signaling does not mediate the main apoptosis pathway activated during light-damage.

Keywords

p75; rods; light-damage; apoptosis; neuroprotection

Photoreceptors are very stable but at the same time extremely fragile cells (Stone et al., 1999). What makes them extremely fragile is their specialization, the absorption of light and the energy requirements in the form of ATP and oxygen (Ames et al., 1992; Demontis et al., 1997). Oxygen is supplied to the photoreceptors by the vasculature of the choroid, which cannot autoregulate and thus cannot adjust to the oxygen requirement of the photoreceptors. Therefore, the large decrease in oxygen consumption that occurs when the photoreceptors go from a dark- to a light-adapted state causes a large increase in oxygen tension.

Light as an environmental factor has been shown to be toxic to rod photoreceptors if the retina is exposed to either high light intensities or to continuous light over a long period of time (reviewed in Penn and Anderson (1991)). The underlying reason for photoreceptor apoptosis is thought to be the oxidative stress induced by excessive light.

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The retina appears to be equipped with two mechanisms to protect its photoreceptors against light-induced damage, a short-term mechanism that protects against brief exposures to very bright light, and a long-term mechanism that protects against circadian light exposure. Short-term exposure to bright light increases retinal levels of antioxidants (Penn et al., 1987) and neuroprotective factors (Liu et al., 1998), whereas during a normal circadian rhythm basic fibroblast growth factor (bFGF) is upregulated during the day, the time period of higher oxygen exposure (Stone et al., 1999).

Likewise, photoreceptors can be prevented from undergoing apoptosis in response to light-damage by applying exogenous neuroprotective agents, such as the neurotrophins brain-derived neurotrophic factor (BDNF), neurotrophin 4 (NT-4), and nerve growth factor (NGF) (LaVail et al., 1998; Harada et al., 2000) and growth factors such as ciliary neurotrophic factor (CNTF), basic fibroblast growth factor (FGF-2) and insulin-like growth factor-II (IGF-II) (LaVail et al., 1992; LaVail et al., 1998). Recent experiments suggest that the majority of these rescue factors do not prevent photoreceptor cell death by activating receptors on the photoreceptors themselves, but rather by stimulating Müller glial cells (Rohrer et al., 1999; Wahlin et al., 2000; Wahlin et al., 2001). Harada et al. (2000) suggested that the Müller cells release FGF-2, which acts directly as a survival factor for photoreceptors. FGF-2 release from Müller cells was suggested to be controlled in a push-pull fashion by neurotrophin 3 (NT-3) acting via the Trk tyrosine kinase receptor TrkC, and NGF acting via the neurotrophin receptor p75, which causes increased or decreased release, and thus survival or death, respectively. In support of this hypothesis, they showed that in $p75^{-/-}$ mice on a pigmented C57BL/6J background, light-damage appeared to be slightly but significantly reduced, and that in albino Wistar rats, blocking p75 with a neutralizing antibody during the constant light-exposure delayed photo-receptor apoptosis.

We wished to follow-up the question: whether the lack of $p75^{-/-}$ is neuroprotective during light-damage. $p75^{-/-}$ mice were bred onto an albino background, the classic model for light-induced photoreceptor cell death. Photoreceptor cell death was indistinguishable between $p75^{+/+}$ and $p75^{-/-}$ animals, whereas a single copy of p75 in the $p75^{+/-}$ mice provided some neuroprotection. Our results suggest that eliminating p75 signaling does not protect against light-damage in albino mice.

Mice, homozygous for a targeted mutation in the $p75NTR$ gene ($p75^{-/-}$) (Lee et al., 1992), were obtained from Jackson Laboratories (Bar Harbor, ME) and out-crossed ($>8x$) into an albino background (ICR: Institute for Cancer Research). Experiments were performed on 3-month-old littermates of heterozygous crosses, raised under cyclic light conditions (12 hr light, 12 hr dark) at the University of California at San Francisco animal care facility. Light-exposure consisted of constant fluorescent illumination at approximately 115–175 ft-c as described previously (Faktorovich et al., 1992). All animal cages were equidistant to the light source during both the cyclic-light and the continuous-light exposure periods. Genotyping utilized mouse-tail DNA samples and PCR analyses. All experiments were in accordance with the Association for Research in Vision and Ophthalmology Guidelines for Animal Research, with approval from the University Animal Care and Use Committee.

Semi-thin sections (1 μ m) were prepared using a previously published protocol (Rohrer et al., 2001). In short, deeply anaesthetized animals (CO_2) were perfused with fixative (4% glutaraldehyde, 2% paraformaldehyde in phosphate buffered saline, pH 7.4) and eyes were enucleated. Eyes were hemisected in dorso-ventral orientation through the optic nerve and embedded in Epon/Araldite. Sections were cut with a glass knife, stained with toluidine blue solution and coverslipped using DPX medium. Number of rows of photoreceptor nuclei were counted in the central (superior and inferior, within 350 μ m of the optic nerve head) and peripheral (superior and inferior, within 350 μ m of the ora serrata) retina. Three measurements

were made per field, which were averaged to provide a single value for each area, and the four area values were averaged to give a value for the entire retina. To assure that the sections were not oblique, tissue sections were chosen where the photoreceptor outer segments and Müller cell processes crossing the inner plexiform layer were continuous in the section plane. Each group consisted of 4–7 animals. For statistical analysis, data were expressed as mean $\pm$ SEM, and a two-tailed *t*-test was used to determine significance levels.

The lack of p75 did not affect general retinal morphology, as retinas from heterozygous (p75^{+/-}) and homozygous (p75^{-/-}) animals were indistinguishable from those of their wild-type (p75^{+/+}) litter-mates (Fig. 1(A), (D), and (G)) at the light microscopy level. Potential changes in immunohistological profiles were not investigated.

To compare the rescue potential of the different levels of p75 expression, animals were placed under constant illumination (Faktorovich et al., 1992). The number of photoreceptor rows was assessed in four different areas of the retina (i.e. superior peripheral, superior central, inferior central and inferior peripheral). No regional differences were observed between the three different phenotypes (data not shown), and thus the numbers are reported as an average across the four regions (i.e. total retina). After 2 weeks of light exposure, the number of rows of photoreceptors per total retina in p75^{+/-} was down to 2.3 ± 0.25 and in p75^{-/-} to 2.2 ± 0.25 , whereas the heterozygous p75^{+/-} animals still had 4.6 ± 0.31 rows remaining ($p < 0.0005$) (Figs. 1(B), (E), and (H) and 2). After 3 weeks of constant light, p75^{+/-} animals still had more rows of photoreceptors remaining (3.0 ± 0.41) in comparison to their p75^{+/+} (1.5 ± 0.24 , $p < 0.02$) and p75^{-/-} litter-mates (0.9 ± 0.28 ; $p < 0.01$) (Figs. 1(C), (F), and (I) and 2). The effect on cones was not addressed in these experiments. Thus, the lack of two copies of p75 is not neuroprotective, whereas lacking one copy of p75 is neuroprotective with respect to photoreceptor survival.

Constant light-damage in albino mice and rats has been an accepted model for inherited photoreceptor degeneration of the retinitis pigmentosa class, a major cause of blindness worldwide. A number of survival factors have been tested to date using this animal model in an attempt to prevent photoreceptor apoptosis (LaVail et al., 1992, 1998). Harada et al. (2000) have recently suggested that the neurotrophin receptor p75 expressed in Müller cells might mediate late-stage phototoxicity by decreasing the production of FGF-2 required for photoreceptor survival, and have therefore offered that blocking p75 might increase survival of photoreceptors under those conditions. We pursued their hypothesis by outcrossing the p75^{-/-} mice into an albino strain to allow for better control of the light-damage experiments. The lack of p75-signaling was not neuroprotective during light-induced apoptosis, and light-damage was as severe as in their wild-type litter-mates. Surprisingly, the heterozygous mice were partially protected at the 2- and 3-week time points.

How could we explain these findings with our current knowledge of p75 signaling in general, and p75 signaling during light-induced cell death in particular? It has been shown that activation of p75 can result in survival or cell death, depending on the physiological state of the cell. In general, it is thought that p75 activating NF- κ B leads to the activation of the survival pathway, whereas p75 signaling via the JNK-p53-BAX pathway leads to cell death (review: (Kaplan and Miller, 2000; Mamidipudi and Wooten, 2002). However, importantly, p75 can only mediate apoptosis when the Trk tyrosine kinase receptor (Trk) is inactive (Bamji et al., 1998), arguing that Trk activation silences the p75-mediated cell death pathway. This ‘silencer of survival signals’ hypothesis has been suggested as a new concept in neurodegeneration (Venters et al., 1999; Mattson et al., 2000; Venters et al., 2000).

p75-dependent cell death has also been studied in the p75^{-/-} mouse. In sympathetic neurons, normal developmental cell death does not occur, but instead it is replaced by a later, p75-

independent period of neuronal loss (Bamji et al., 1998). Similarly, retinal ganglion cell death was reduced in the developing $p75^{-/-}$ mouse retina (Frade and Barde, 1999). Although Frade and Barde (1999) did not evaluate the number of retinal ganglion cells in the adult retina, it appears from our own sections that the RGC layer does not contain more cells (Fig. 1(G)). In contrast, generating transient transgenic retinal cells in *Xenopus laevis*, Hutson and Bothwell (2001) demonstrated that p75 increased survival and, conversely, truncated mutant p75 receptors induced cell death.

Several recent reports suggest that p75 is involved in mediating cell death in light-damage. Harada et al. (2000) demonstrated that light-damage leads to the upregulation of p75 and TrkC in Müller cells, and two recent reports indicate that p75 is upregulated in photoreceptors after light-exposure (Roque et al., ARVO abstract #3725, 2002; Srinivasan et al., ARVO abstract #3726, 2002). In vitro experiments using up- or down-stream inhibitors of the p75-signaling cascade suggest that p75 signaling may contribute to photoreceptor cell death in light-induced photoreceptor cell death. These observations are in support of previously reported experiments, which demonstrated that p75 is upregulated under pathological or inflammatory conditions in other systems (e.g. Roux et al., 1999; and reviewed by Coulson et al. (1999)).

However, in this study, we found that with respect to neuroprotection, two copies of p75 are as bad as having none, whereas one copy seems to be the least damaging. In wild-type animals ($p75^{+/+}$), p75 could mediate photo-receptor apoptosis either directly by activating the cell death pathway (Roque et al., ARVO abstract #3725, 2002; Srinivasan et al., ARVO abstract #3726, 2002), or indirectly via the proposed Müller cell pathway, depriving photo-receptor cells of their neurotrophic support (Harada et al., 2000). In the absence of p75 ($p75^{-/-}$), other pathways such as the c-fos-triggered pathway (Wenzel et al., 2000) or the proposed pathway mediated by activated transducin (Eversole-Cire et al., ARVO abstract # 3719, 2002) may dominate, and produce the apoptotic signal activated by the continued absorption of photons. In heterozygous animals ($p75^{+/-}$), the concomitant upregulation of TrkC (Harada et al., 2000) may suppress the apoptotic action of p75 and may allow for some photoreceptor survival to occur (Bamji et al., 1998). Our results also appear to be in partial agreement with the experiments reported by Harada et al. (2000). First, they reported that a p75 neutralizing antibody, which presumably only provides a partial block of the p75-mediated pathways, delayed light-induced photoreceptor cell death. These results correlate with our experiments in the heterozygous animals, which demonstrate that reducing the p75 expression level is neuroprotective. Second, a small neuroprotective effect was observed in the pigmented $p75^{-/-}$ mice, resulting in reduced retinal apoptosis following intense light exposure (their figure 7, (Harada et al., 2000)). However, due to differences in protocols (light exposure and evaluation of cell death), as well as experimental (the authors did not use littermates, guaranteeing the same genetic background in the control and experimental animals) and statistical inconsistencies (the statistical difference was minimal and the variance in the data appear to be great), these results are difficult to interpret and to compare with our results.

Taken together, these results support the idea that light-induced cell death is mediated by a number of different pathways, some of which are mediated directly by the photoreceptors and start with the absorption of photons; whereas others may involve retinal support cells like the Müller cells (Grosche et al., 1997; Liu et al., 1998; Harada et al., 2000; Walsh et al., 2001), or retinal pigment epithelium (Walsh et al., 2001; Roberts et al., 2002). However, an important goal should be to identify the gene products onto which all the pathways converge.

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Fig. 1.

Light-micrographs of plastic-embedded sections of p75^{+/+}, p75^{+/-} and p75^{-/-} mouse retinas. At 3 months of age, the outer nuclear layer of the albino mouse consists of 6–7 rows of photoreceptor nuclei, with no difference between the three genotypes (A, D, G). After 2 weeks of constant light exposure (2 weeks CL), the outer nuclear layer consists of only 2 rows of nuclei in the p75^{+/+} (B) and p75^{-/-} (H) mouse retinas, whereas the retina of the p75^{+/-} mouse still contained 4–5 row (E). By 3 weeks of constant light exposure (3 weeks CL), only one row remains in the p75^{+/+} (C) and p75^{-/-} (I) mouse retinas, whereas 2–3 rows remain in the p75^{+/-} mouse retina (F). Scale bar, 40 μ m.

Fig. 2.

Analysis of photoreceptor cell death from light-damage in p75^{+/+} (black bars), p75^{+/-} (dark gray bars) and p75^{-/-} (light gray bars) mouse retina. Values are means $\pm$ SEM. At both time points, the heterozygous p75^{+/-} mouse retina showed a significantly greater rate of survival. * $p < 0.05$; # $p < 0.005$.