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NEUROTROPHINS INDUCE NEUROPROTECTIVE SIGNALING IN THE RETINAL PIGMENT EPITHELIAL CELL BY ACTIVATING THE SYNTHESIS OF THE ANTI-INFLAMMATORY AND ANTI-APOPTOTIC NEUROPROTECTIN D1

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1. SUMMARY

The integrity of retinal pigment epithelial cells is critical for photoreceptor cell survival and vision. The essential omega-3 fatty acid, docosahexaenoic acid, attains its highest concentration in the human body in photoreceptors. Docosahexaenoic acid is the essential precursor of neuroprotectin D1 (NPD1). NPD1 acts against apoptosis mediated by A2E, a byproduct of phototransduction that becomes toxic when it accumulates in aging retinal pigment epithelial (RPE) cells and in some inherited retinal degenerations. Here we also describe that neurotrophins, mainly pigment epithelium-derived factor, induce NPD1 synthesis and its polarized apical secretion, suggesting paracrine and autocrine bioactivity of this lipid mediator. In addition, DHA elicits a concentration-dependent and selective potentiation of pigment epithelial-derived factor-stimulated NPD1 synthesis and release through the apical RPE cell surface. The bioactivity of signaling activated by PEDF and DHA demonstrates synergistic cytoprotection when cells were challenged with oxidative stress, resulting in concomitant NPD1 synthesis. Also, DHA and PEDF synergistically activate anti-apoptotic protein expression and decreased pro-apoptotic Bcl-2 protein expression and caspase 3 activation during oxidative stress. Thus, DHA-derived NPD1 protects against RPE cell damage mediated by aging/disease-induced A2E accumulation. Also, neurotrophins are regulators of NPD1 synthesis and of its polarized apical efflux from RPE cells. Therefore, NPD1 may elicit autocrine and paracrine bioactivity in cells located in the proximity of the interphotoreceptor matrix.

2. INTRODUCTION

Phospholipids, enriched with docosahexaenoyl chains (22:6, n-3), and the phototransduction molecular components are assembled at the base of the photoreceptor cell outer segment (Rodriguez de Turco et al., 1997). The oldest photoreceptor disks, at the tip of the photoreceptor, are then shed and phagocytized by the retinal pigment epithelial (RPE) cells. The RPE cells supply the photoreceptors with nutrients, including all-trans retinol, the precursor to the visual pigment chromophore for vision and docosahexaenoic acid (DHA), from the essential fatty acid family of the omega-3 series. Both are continuously recycled between the RPE and the cone and photoreceptor cells (Bazan, 2006). The RPE cell also secretes neurotrophins that are necessary for photoreceptor and RPE cell survival (Hu and Bok, 2001; LaVail et al., 1992; LaVail et al., 1998; Politi et al., 2001; Valter et al., 2005).

3. A2E-MEDIATED RPE CELL APOPTOSIS IS ATTENUATED BY NPD1

We have further investigated the A2E-mediated RPE cell damage. This bispyridinium bisretinoid is a byproduct of phototransduction that becomes toxic when it accumulates in RPE

cells as a lipofuscin component. A2E accumulation in RPE cells occurs during aging (Bui et al., 2006; Cideciyan et al., 2004; Radu et al., 2004; Sparrow et al., 2003) in animal models of age-related macular degeneration as well as in the disease itself (Bui et al., 2006; Cideciyan et al., 2004; Radu et al., 2004).

As a result of A2E accumulation, RPE cells undergo apoptosis preceding photoreceptor cell dysfunction and death (Cideciyan et al., 2004). A2E (20 μ M), added to ARPE-19 cells in the presence of light and O₂, was converted into oxiranes (epoxides) (Radu et al., 2004; Sparrow et al., 2003) and promoted apoptosis (Mukherjee et al., in press). NPD1 was cytoprotective against A2E-induced ARPE-19 cell apoptosis, displaying a wide window of cytoprotection after A2E addition. NPD1 (50 nM), even 6 hrs after A2E, exerted cytoprotection. Moreover, the involvement of Bcl-2 proteins suggests that NPD1 action target the premitochondrial stage of the apoptotic cascade. The extended NPD1 window of protection indicates that the attenuation of the apoptotic cascade targets committed cell death events only relatively late upon exposure of the cells to A2E/oxiranes.

4. NEUROTROPHINS ARE ACTIVATORS OF THE SYNTHESIS AND APICAL NPD1 RELEASE FROM RPE CELLS

Neurotrophins participate in photoreceptor survival (LaVail et al., 1992; LaVail et al., 1998; Politi et al., 2001; Valter et al., 2005). Using human RPE cells grown to confluence and a high degree of differentiation displaying apical-basolateral polarization (Hu and Bok, 2001), we have found that neurotrophins are NPD1 synthesis agonists (Mukherjee et al., in press). Figure 1 illustrates neurotrophin (PEDF)-mediated NPD1 synthesis and bioactivity. These studies also allowed to show that human RPE cells in primary culture also synthesize NPD1, initially described in the ARPE-19 cell line (Mukherjee et al., 2004). In addition, the use of human RPE cells in barrier-forming monolayers allows us to address the issue of “sidedness” of NPD1 release. Several neurotrophins (PEDF, BDNF, CNTF, FGF, GDNF, LIF, NT3, and Persephin) with bioactivities that promote neuronal and/or photoreceptor cell survival are agonists of NPD1 synthesis. These neurotrophins trigger synthesis and release of NPD1 through the apical surface of the cell. Pigment epithelium-derived factor (PEDF) was by far the most potent stimulator of NPD1 synthesis. PEDF, a member of the serine protease inhibitor (serpin) family, was initially identified in the same human retinal pigment epithelial cells (Tombran-Tink and Barnstable, 2003) used in these studies (Mukherjee et al., in press). PEDF or ciliary neurotrophic factor (CNTF), when added to the basal medium in increasing concentrations, evoke much less NPD1 release on the apical side. Conversely, if these neurotrophins are added to the apical medium, they exert concentration-dependent increases in NPD1 release only on the apical side. These findings have relevance to retinal physiology and pathology because, when RPE cell polarization in the plane of the epithelium is disrupted, certain growth factors are believed to participate in an injury/inflammatory response, including the mediation of angiogenesis as in age-related degenerations (Bhutto et al., 2006; Kannan et al., 2006; Rattner and Nathans, 2006).

5. DHA POTENTIATES PEDF-INDUCED NPD1 SYNTHESIS AND RELEASE

DHA exerts a remarkable potentiation by PEDF of NPD1 release to the apical media (Figure 1). In contrast, much less NPD1 was found in the media bathing the basolateral side of the cells. Much less apical NPD1 release was observed when PEDF was applied to the media bathing the basolateral RPE surface. Regardless of the side of the cell where PEDF is added, the amount of NPD1 release through the basolateral side is similar (Mukherjee et al., in press). Moreover, the addition of DHA to either side of the cell monolayer selectively synergized PEDF-induced NPD1 release only through the apical side. Arachidonic acid, another polyunsaturated fatty acid, did not stimulate NPD1 synthesis. The polarity of actions

for the neurotrophin-mediated response suggests that NPD1 may function, at least in part, as an autocrine and paracrine signal on cells that surround the interphotoreceptor matrix, namely the photoreceptor cells and Müller cells. Moreover, the apical side of the RPE participates in the recognition and shedding of photoreceptors during outer segment phagocytosis (Bazan, 2006; Rattner and Nathans, 2006). Furthermore, interphotoreceptor matrix proteins may be acceptors of NPD1, to facilitate its diffusion and to target it to cellular site(s) of action.

6. DHA AND PEDF PROTECTS RPE CELLS FROM OXIDATIVE STRESS WITH CONCURRENT NPD1 SYNTHESIS

To study the consequences of the DHA/PEDF synergy in terms of bioactivity as well as to define the downstream signaling induced, we exposed ARPE-19 cells to oxidative stress. ARPE-19 cells, as human RPE cell primary cultures, up-regulated NPD1 synthesis in the presence of PEDF. Also, significant cytoprotection and increased NPD1 formation occurred synergistically when PEDF was added along with DHA under conditions of oxidative stress-induced apoptotic cell death triggered by serum starvation/H₂O₂/TNF α (Mukherjee et al., in press).

7. CONCLUSIONS

Because neurotrophins elicit pleiotropic bioactivity, inducing multiple pathways, it is likely that there are major signaling routes activated to promote RPE cellular integrity and preserve function. DHA-NPD1 represents a major signaling mechanism for neurotrophins in the RPE cell.

Neurotrophins, mainly PEDF, are NPD1 synthesis agonists and selective activators of the apical efflux of the lipid mediator in human RPE cells in monolayer cultures. In addition, DHA greatly potentiates PEDF-induced RPE cytoprotection against oxidative stress, with concomitant NPD1 synthesis. The synergy with PEDF and DHA indicates that the availability of the NPD1 initial precursor is critical for its synthesis.

The regulation of apoptosis is complex and comprises multiple checkpoints. The ability of DHA to potentiate PEDF bioactivity on the Bcl-2 family of proteins (Figure 1) expression indicates that the pre-mitochondrial stage of the apoptotic cascade checkpoint is involved in cytoprotection, with concomitant NPD1 formation (Mukherjee et al., in press). These findings are in agreement with studies in human neural progenitor cells (Lukiw et al., 2005). The regulation of pro- and anti-apoptotic proteins during a relatively prolonged window of protection (about 6 hrs in cells in culture) will help to further define NPD1 survival bioactivity in the RPE cell. These events are clinically significant because it may allow the exploration of therapeutic interventions for retinal degenerative diseases.

In retinitis pigmentosa, the expression of a mutation and the initiation of the disease is in most instances a slow process. We have an incomplete understanding of the events triggered by the expression of mutations causing retinitis pigmentosa, although impaired RPE cell function does take place. Thus, RPE cell protection may contribute to preserve photoreceptor cell integrity. Moreover, the identification of these events will allow the design of therapeutic alternatives to delay the initiation and progression of retinitis pigmentosa. The relatively wide window of NPD1-mediated cytoprotection against A2E-induced RPE cell apoptosis (Mukherjee et al., in press) may allow the identification of potentially useful therapeutic alternatives.

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Figure 1.

Illustration depicting NPD1 (neuroprotectin D1, 10S,17S-DHA) synthesis and bioactivity. Neurotrophins, mainly PEDF (pigment epithelium-derived factor) are NPD1 synthesis agonists in primary RPE cells in culture (Mukherjee et al., in press). Arrows indicate that PEDF may be secreted from the RPE cell or be derived from another cell. A putative PEDF receptor is indicated. A PLA₂ (phospholipase A₂) that mediates the cleavage of docosahexaenoyl chains from phospholipids located in RPE membranes and /or phagocytized photoreceptor membranes (POS, photoreceptor outer segment) is illustrated. Likely, a very specific pool of DHA-containing phospholipids is the precursor of docosanoids. A 15-lipoxygenase-1-like enzyme mediates NPD1 synthesis. Because PEDF has antiangiogenic properties, it is likely

that NPD1-evoked synthesis by this neurotrophin may participate in this process. NPD1 potently downregulates IL-1 β -induced COX-2 expression (Mukherjee et al., 2004) as well as other proinflammatory genes (Lukiw et al., 2005). NPD1 action on Bcl-2 proteins may be mediated by an autocrine route or by an intracellular action. NPD1 apically released may exert bioactivity on cells in the proximity of the interphotoreceptor matrix.