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Outer Segments of Retinal Photoreceptors – A Review in the Light of Novel Findings

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Authors' contributions

This work was carried out in collaboration between all authors. Author RHWF and CR' designed the underlying study and designed the formation of this review. Author US performed and managed the lab work and statistical analysis of the underlying studies. Author RHWF wrote the first draft of the manuscript. Author RHWF and CR managed the literature searches. All authors read and approved the final manuscript.

Review Article

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ABSTRACT

Aims: Recent studies have demonstrated that all molecules of the respiratory chain are present in the photoreceptor outer segment (see Calzia et al., Biol Cell 2013). Furthermore, our group (Roehlecke et al., PLoS One 2013) could show that, after blue light stress of the retina, the outer segments are significant sources of reactive oxygen species (ROS) – even more so than the mitochondria in the inner segment. These two new findings have also important implications for degenerative diseases of the retina.
Methodology: In this respect we revisited the literature regarding the photoreceptor reactions after blue light and radical stress. Furthermore, we refer to the common features of mitochondria and outer segments.
Results: In the light of the recent findings many unique features of the photoreceptors get understandable: they are characterized by excessive oxygen consumption - even higher than that of other neuronal cells. Photoreceptors possess, in addition to their mitochondria – rich neuronal component an outer segment packed with stacks of membrane discs harbouring the photo pigments, respiratory enzymes and enzymes of the visual cycle.
Therapeutic use of red and near infrared light is often explained by ameliorating the

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mitochondrial function; therefore, we also discuss this topic with respect to photoreceptor outer segments.
Conclusion: Revisiting the functional anatomy with the above mentioned new findings is very important also for the interpretation of the neurodegenerative diseases like age related macular disease (AMD).

Keywords: Photoreceptor; outer segments; blue light; oxidative stress; retina; red light.

1. INTRODUCTION

Degenerative diseases of the retina emerged as a major point in health sciences and socio-economics and are now in the focus of eye research [1,2]. Blue light and oxidative stress are discussed as important pathogenetic drivers besides other factors like genetic predisposition, immune and metabolic diseases [3]. A decline of the closely linked compartments of photoreceptors, retinal pigment epithelium and choroid is now discussed as initial damage in age related macular disease - the most common retinal degenerative disease in industrial nations [4-6].

In this regard, we want to compare here the functional morphology of photoreceptor outer segments with that of mitochondria and look for common features as well as therapeutic possibilities like the application of red or infrared light [7-10].

2. RETINA AND PHOTORECEPTORS – NOVEL ASPECTS

2.1 Functional Anatomy of the Photoreceptors in the Retina

The photoreceptors in the human retina are specialized nerve cells possessing two completely different cell compartments: The inner (neural) part (ellipsoid, perikaryon and axon with the synapses) and the outer part (outer segment). Furthermore, the outer segment is embedded into a micro–milieu which is totally different from that of the neural part of the cell.

The neural part is supplied by a microcirculatory unit (the retinal capillaries) typical for the central nervous system. It is characterized by capillaries with a small lumen and a tight endothelium, while glia cells (Müller cells) reside in the immediate vicinity (for literature, see [11]).

In contrast the outer segments are embedded within the interphotoreceptor matrix. This matrix contains special proteins and hyaluronic acid [12-14]. Literally, the outer segments “bathe” within this matrix supplied by plasma from the “ocean” of blood flowing in the chorocapillaries (fenestrated capillaries) and larger choroidal vessels (for literature, see [11]). The membrana limitans externa (sealing zone made by tight junctions between the Müller cells and the outer end of the photoreceptor inner segment) serves as watershed zone between both regions.

Within the outer segments, membrane discs harbour the visual pigments. During photo-transduction radicals are formed: radicals originating in the rhodopsin cycle transform all-*trans*-retinal into di-retinoid-pyridinium-ethanolamine (A2E). This metabolite then accumulates as most dangerous component of lipofuscin in the retinal pigment epithelium

(RPE) [15-18]. Interestingly, A2E blocks cytochrome c oxidase in the mitochondria [19]. Thus, the radical product A2E itself is blocking the respiratory chain and leads (as a vicious cycle) to an increased deviation of electrons producing again new reactive oxygen species (ROS).

This and the high content of polyunsaturated fatty acids (PUFAs) render the outer segment discs susceptible for ROS [1]. Damage to the whole photoreceptors is circumvented by a regeneration of the outer segments by steady renewal and shedding of discs. This prevents the accumulation of toxic oxidation products in the outer segments. About 10 of the average 700 discs in the outer segments are shed per day. Then they are phagocytised by the RPE. This has happened about 3 billion times in the eyes of a 70 year old person over his or her lifetime [20-22].

The choroid is situated externally to the retina and its derivative the outer leaflet of the developing optic cup, the RPE. Abundant vessels (vascular sponge) characterize the choroid. The vessels are regulated only minimally via the concentration of oxygen - like it is the case for brain and inner retinal vessels. Thus, very high concentrations of oxygen can reach RPE and outer segments, independent of the oxygen consumption – a fact that makes this system prone to oxidative stress [3].

However, even more important than the absolute oxygen partial pressure (pO_2) in the choroid is the pO_2 gradient under physiological conditions and in pathologic states of relative hypoxia. Stefansson et al. [23] report that under physiologic conditions "the pO_2 decreases almost linearly with the distance from the chorocapillaries (capillaries of the choroid) to the inner portion of the photoreceptors". Interestingly, at the inner portion of the photoreceptors, the pO_2 can reach 0 mmHg in the dark and is a little higher in the light. Hindrances in the diffusion through Bruch´s membrane situated between RPE and choroid will contribute to this lowering of the pO_2.

2.2 Mitochondria in Photoreceptors

The outermost part of the inner segment (the ellipsoid), is the location of the photoreceptor mitochondria. Here mitochondria are densely packed, closely to the pO_2 source - the choroid. As in other tissues, the mitochondria are moving actively to the location of highest pO_2 [24]. Mitochondria deliver the vast amount of energy, which is needed for the steady synthesis of the outer segment discs. The photoreceptors consume via mitochondria 3-4 times more energy than all other retinal neurons or cells in the central nervous system. They are probably the cells with the highest oxygen consumption of all cells in the human body [25,26]. Moreover, the mitochondria are organelles that are especially susceptible to oxidative stress [27] as they harbor the enzymes of the respiratory chain, which handle electrons. Under normal circumstances, this works without any major leakage of free radicals. However, if the mitochondria are under stress or if they are pre-damaged by multiple small genetic failures then radicals or products of oxidative radicals (mostly ROS) can spread out into the cell [28]. Therefore, damages of the mitochondrial DNA will occur with increasing frequency as age advances.

However, due to their "ROS defense system" (several enzymes that remove superoxide, H_2O_2 and organic hydroperoxides, see Starkov [29]), mitochondria can also be a sink for ROS. On the other hand, intermittent hypoxia (see above, pO_2 gradient) and accumulation of succinate, for example, can result in an increased mitochondrial ROS generation upon re-oxygenation [29].

In addition to mitochondria, numerous other sources are present in the cell, e.g. membrane bound NADH and NADPH oxidases, so the impact of oxidative stress can elicit enhanced ROS production from different sites [30]. In our recent study of mouse retinal whole mounts, we could show that blue light induces ROS in outer segments via NADPH oxidase as well as possibly via mitochondria-like activity of the outer segments [31]. The cross talk between NADPH oxidases and mitochondria-like activity may stimulate the NADPH oxidases. Here, an example of such a cross-talk between NADPH oxidases and mitochondria has been recently shown with SOD-2 depletion causing an increase in NADPH oxidase activity, whereas SOD-2 over expression reduces activation of NADPH oxidases and NADPH-generated ROS [32]. See also Bhatt et al. [30].

2.3 Effect of Blue Light on Mitochondria of Retinal Cells

The effect of short wavelength light on the metabolism of the mitochondria has been an important topic of experimental *In vitro* and *In vivo* studies. Indeed, these studies could show that blue light impact leads to an enhanced production of radicals in mitochondria [33-36]. Enzymes of the respiratory chain like flavins and cytochrome oxidases can absorb at wavelengths of 440 – 450 nm and they can cause the production of ROS and oxidative stress [3,37-39]. Thus, after blue light exposure, more electrons deviate from the respiratory chain in the mitochondria, resulting in further damage. Chromophores in general, especially the cytochromes, can be sources of ROS [3,38,39]. On the other hand, inhibiting the mitochondrial transport chain in RPE cells or addition of mitochondria-specific antioxidants blocks ROS formation and cell death [38].

What does this mean for the retina as a whole? The photoreceptors are densely packed with mitochondria in their inner segment, especially in the ellipsoid. Presumably, the discs of the outer segments are loaded with radicals by these mitochondria. In addition, the discs are themselves possible sources of radical production and indeed vast amounts of radicals are produced if photoreceptors are exposed to blue light [40]. In a study on isolated frog rods, Demontis et al. showed that rhodopsin in the outer segment, when activated by blue light, can produce oxidative radicals which can also lead to lipid peroxidation [41]. On the other hand, a robust amount of reactive oxygen species is also produced in the ellipsoid when cultured photoreceptor cells (without a true outer segment) are exposed to blue light [40].

Consistently, radical production after blue light exposure can also be observed in other retinal cells like the ganglion cells, which contain numerous mitochondria: Studies of Osborne et al. showed that blue light was ineffective regarding radical damage in cells which are depleted of mitochondria [36].

2.4 Ectopic Enzymes of the Respiratory Chain within the Photoreceptor Outer Segments

Panfoli et al. [42-44] were the first authors who found that enzymes of the respiratory chain are located in the membranes of the photoreceptor outer segments. These authors mentioned that the activity of respiratory chain complexes in outer segment fractions was comparable to that found in retinal mitochondria-enriched fractions. In isolated outer segments, they showed that a proton potential difference exists across the disc membranes - formed as double membranes like the double membranes of the mitochondria. Regarding the highly energy consuming process of photo-transduction and the rapid increase of energy demand in light and dark cycles, Calzia et al. [45] argue that it would be doubtful that ATP

and phosphocreatine can diffuse from the inner segment mitochondria to the outer third of the outer segments with a proper timing, although only these are active in the rhodopsin cycle (see also [46]). With an overall the O_2 consumption of the outer segments three – fold greater than the inner retina, this seems unlikely, because the connecting stalk between inner and outer is very thin and contains a cilium [47]. The above mentioned paper of the Panfoli group [48] even contains evidence that parts of the respiratory complexes come from mitochondrial membranes fused with the newly formed membranes of the outer segment discs. It is clear that most of these novel findings regarding analogies between outer segments and mitochondria come from the group of Panfoli or the collaborators with them. So more results must be gathered regarding this topic.

However as another corroborating fact, we could show in our recent paper [31] that dyes that mark double membranes separating a high proton gradient, which was traditionally thought to be exclusively the case in mitochondria, also mark the outer segments of photoreceptors.

2.5 Proton Pumps, Photoreceptors and Mitochondria

At this time one can only speculate whether this unique staining behavior of the outer segment membranes is due to the proton electrochemical potential difference across the disk membrane, which had already been detected by Uhl and Desel in 1989 [49]. Since then it has become evident that rhodopsins are capable of pumping protons, albeit with low efficiency [50].

What is the connection between light perception proton pumping and possible mechanisms of ATP production?

There is a common ancestral pathway for both photosynthesis and light perception: proton pumps such as bacteriorhodopsin – driven pumps are found at the root of the development of eubacteria and halobacteria [51]. In addition, an electron transport chain is already established in early evolution [52]. In lower eukaryotic organisms including fungi similar microbial rhodopsins are widespread [53]. Thus as far as proton transport is concerned, the essential features of the bacteriorhodopsin pumping mechanism have been conserved in evolution not only for energy production but also light sensing [54].

To follow the common line of light sensing and electron transfer as source of energy one should recall the concept of endosymbiosis between the bacterial ancestors of oxygen reductors and the first cells [55]. In the primitive earth atmosphere, the first anaerobic eukaryotes became endangered by the raising oxygen levels due to the oxygen production by primitive algae. By incorporating the ancestors of mitochondria they could use the formerly toxic oxygen for ATP – production of the “pro-mitochondria”. *Vice versa* the “pro-mitochondria” received metabolic substrates and shelter from the host cell.

Thus, the gap is not large between light sensing rhodopsin and proton driving rhodopsin and also the electron transfer chain, which is designed more refined in mitochondria. The evolutionary relationship is similarly close for the main endosymbiotic organelles: mitochondrion and chloroplast. Here, a “dual-targeting” of many proteins can be found with modern methods [56].

Along the evolutionary path to the highly specialized photoreceptors of vertebrates, many modes of coupling photo-acceptor molecules like rhodopsin to the transduction chain were elaborated (e. g. Amphioxus has four kinds of eyes with different mechanisms, for review

see [57]). Also the arrangement of opsin proteins in the membranes has been refined: the density of opsin in rods as a cilium – like photoreceptor is nearly ten times higher than that of microvillar membranes (e.g. in rhabdomers of insect photoreceptors; [57]). During the evolution of rods, which came later than cones, a shut-off mechanism has evolved for rods in bright light, which reduces the ATP consumption in mammals by as much as five fold (see [58]). The energy consumption in the vertebrate retina is therefore lower than in the compound eyes of insects.

2.6 Effect of Blue Light on the Retina - Pathological Consequences

On the other hand the human retina has concentrated all high-resolution vision to the “fovea centralis” within the “macula lutea”. The fovea with its “foveola” is only 500 micrometer in diameter and is characterized by the feature that all inner retinal layers are pushed aside. This means that the light directly hits the photoreceptors at this location. However, only blue to violet light impinges on the retina because the cornea absorbs shorter wavelengths. At higher age the shortest transmitted wavelengths (about 400 nm) shift to longer ones because the lens gets more and more yellow and later brownish.

This is why ophthalmologists talk about a “blue light hazard” for the retina and not an “UV – light hazard” – although shorter wavelengths would exert even higher damages to the retina. In addition, filter experiments show the damaging effect of blue light in RPE cells (see [59]). As mentioned above, after exposure to blue light, the photoreceptors generate ROS by their outer segments as well as by their inner segments with their mass of mitochondria.

The action curve of blue light damage, the so-called blue light hazard, has a peak around 440 nm. Here, it seems very probable that the impact at this wavelength is dominated by the chromophor A2E [60]. If A2E has absorbed a photon, especially of the wavelength 430 – 440, then free radicals are generated, mostly ROS [61,62]. Consistently Wielgus et al. were able to show, that when albino rats were exposed to blue light (450 nm, 3,1mW cm $^{-2}$), the oxidized form of A2E was specifically increased. This form seems to be especially responsible for the damaging process of retinal cells [63]. Furthermore, A2E is able to generate other toxic oxidative products after adsorption of blue light (for review, see [64]). This results in a damaging cascade within the cell and causes expression of inflammatory and angiogenic substances [65-68]. Thus, A2E inhibits important functions of the cell and is able to increase the apoptosis of the RPE.

In the RPE it has been shown that a significantly higher rate of cell death occurs in lipofuscin or chromophor A2E loaded cells in vitro, when these cells were exposed to blue light (430 ± 30 nm) than when they were exposed to white light (390 till 750 nm) [69]. However, Tanito et al. [70] found that an intensified exposition to white light also induced protein and lipid modifications. This reaction is mediated by 4-HNE and 4-hydroxyhexanal. Both are reactive aldehyds, which are produced during enzymatic oxidation of n -6 und n -3 non-saturated fatty acids.

Here, the high content of PUFAs renders the outer segment discs susceptible for ROS [71]. This alters the structure of the membranes that harbor also the visual pigments leading to gross morphological changes in the outer segments [72]. Thus, the first signs of the most devastating neurodegenerative retinal disease – the age related macular disease (AMD) - can be explained.

In addition, the RPE has a high ROS production by its function of phagocytising the shedded oxidized disc membranes. By this, the RPE accumulates lipofuscin and A2E (see above). In ophthalmology, the accumulation of lipofuscin in the RPE (and later outside as bulk – "Drusen") is then a visual sign of the more advanced AMD.

2.7 Therapeutical Options by Red Light?

Quite opposite to the action of blue light, red or infrared light can have positive (protective) effects to different tissues and organs – a fact which is described in an increasing amount of recent studies [9,34,73-81].

Several studies have also shown the positive effects of red or infrared light for regeneration processes in the retina [7,8,10,82-84]. Here also, the mitochondrion and the respiratory chain within the mitochondria seems to play a major role ([85], see [86] for review). Long – wave parts of the light spectrum are present in all continuous spectra of natural light sources like sun or fire but also in incandescent or halogen lamps (however, not in "strip lamps" – they have their peaks of the spectrum more in the short – wave range).

As one causal explanation, recent studies reveal that red and NIR light is absorbed by the heme structures and copper centers of the cytochrome c oxidase (Cco – complex IV in the respiratory chain). Here absorption maxima exist in the range of 760 – 900 nm with peaks at 767, 791 and 880 nm [75]. A dimeric copper complex with four ligands is absorbing in the 810 – 820 nm range (see also [87]). Copper atoms in the redox active centers in the Cco show absorption peaks at 620, 680, 760 and 820 nm [74] and have a maximum of biological activity at an adsorption wavelength of 670 and 830 nm with a nadir in both spectra of around 728 nm (see [88]).

In addition to an acceleration of electron transfer, Ball et al. [89] found that low intensity light stimulates nitrite-dependent nitric oxide (NO) synthesis, not only oxygen consumption by Cco. Here, Cco acts as an alternative intracellular source of cellular NO, by catalyzing nitrite dependent NO synthesis (NO_2- reductase activity of Cco) [89,90]. This NO related activity of Cco is found in many phyla and species including mammals. Interestingly, besides the well known activity of NO as vasodilator, part of the Cco – produced NO can act inside the cell as signaling mediator in low concentrations and functions primarily under hypoxic conditions. In either case, red or NIR light can again stimulate this activity.

In the light of the newly found analogy between photoreceptors and mitochondria it is possible that the positive protective effects found by Tang et al. [79]; Begum et al. [82] and Albarracin et al. [7] are also due to an amelioration of the metabolic situation within the outer segment and not only by an enhancement of the respiratory chain in photoreceptor mitochondria.

3. CONCLUSION

Many aspects of the unique situation of the photoreceptors must be revisited in the light of the two novel findings regarding the outer segments – the presence of respiratory chain enzymes and their role as main source of ROS after blue light impingement.

This new interpretation has also many implications for therapy: to treat neurodegenerative diseases of the retina like AMD we have to consider now any option to ameliorate the

oxygen supply of the photoreceptors like improving choroidal microcirculation (treat overall circulation and metabolic parameters), reducing the diffusion barriers (treat the metabolic load like e.g. lipofuscin and oxidation products) and improving the function of respiratory enzymes (reduce A2E and lipofuscin) (see [19]). Furthermore, the use of anti-oxidative agents is now a very important therapy option (see [2]) as well as the treatment by red or infrared light during early stages of retinal degenerative diseases (see above).

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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