MINI-REVIEW

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Phototransduction in Retinal Ganglion Cells

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The mammalian retina contains a small number of retinal ganglion cells that express melanopsin, a retinal based visual pigment, and generate a depolarizing response to light in the absence of rod and cone driven synaptic input; hence they are referred to as intrinsically photosensitive retinal ganglion cells (ipRGCs†). They have been shown to be comprised of a number of sub-types and to provide luminance information that participates primarily in a variety of non-imaging forming visual functions. Here I review what is currently known about the cascade of events that couple the photoisomerization of melanopsin to the opening of a non-selective cation channel. While these events conform in a general sense to the prevailing model for invertebrate phototransduction, in which visual pigment signals through a G protein of the G_q class and a phospholipase C cascade to open a TRPC type ion channel, none of the molecular elements in the melanopsin transduction process have been unequivocally identified. This has given rise to the possibility that the underlying mechanism responsible for intrinsic photosensitivity is not same in all ipRGC sub-types and to the recognition that signal transduction in ipRGCs is more complex than originally thought.

INTRODUCTION

In 2002, little more than a hundred years after Cajal's first description of rod and cone visual receptors in the eye [1] a third class of photoreceptor cells were discovered hiding in plain sight as ganglion cells on the opposite side of the retina from the rods and cones [2,3]. They comprise a small subset of the ganglion cell population (< 1 percent in primates) that express melanopsin, a retinal based photopigment, and generate a depolarizing light response with an increase in spike activity that persists in the absence of rod and cone driven synaptic input as well as after being removed from the retina by microdissection [2]. It is clear that these cells contain all the necessary molecular machinery to convert light into an electrical signal and are thus referred to as intrinsically photosensitive retinal ganglion cells (ipRGCs). For the three most recent reviews see [4-6].

BIOLOGICAL FUNCTIONS OF iPRGCs

ipRGCs provide luminance information that plays a critical role in the pupillary light reflex, the synchronization of behavioral rhythms with the circadian light-dark cycle, sleep regulation and mood [7-12]. While these non-image-forming functions were initially thought to be mediated by a single (M1) cell type, ipRGCs are now know to be a diverse group that can be subdivided according to differences in morphology, physiology, and central projections into five subtypes (M1 through M5) in nocturnal rodents [13], three subtypes (M1, M2, and a novel 3rd type) in the tree shrew [14], an evolutionary intermediate between rodents and primates, and two subtypes (M1 and M2) in primates and humans [15,16].

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The increase in the assortment of ipRGC cell types has expanded their list of possible functions to include participation in color vision [15,17] and spatial perception [18-20].

MECHANISMS OF LIGHT TRANSDUCTION IN iPRGCs

The central question of our discussion is: How do ipRGCs convert (transduce) light into an electrical signal? This process begins with a light-sensitive protein, melanopsin, a G protein coupled receptor first identified in frog dermal melanocytes [21], hence the name, and subsequently shown to be selectively expressed in a small number of mammalian retinal ganglion cells [22] with intrinsic photosensitivity [3]. Further studies showed that ipRGCs in melanopsin knock out mice are light insensitive and cells that are normally insensitive to light are made light-sensitive by the heterologous expression of melanopsin [23-28].

The steps in the transduction cascade that follow the light activation of melanopsin are less well understood. They consist of a G protein stimulated effector enzyme that, via the generation an intracellular second messenger signal, opens a non-selective cation channel to give rise to a depolarizing light response.

The consensus—based in large part on the fact that melanopsin is more similar to invertebrate than vertebrate visual pigment [22] and by analogy to the phototransduction cascade in most invertebrates [29,30]—is that melanopsin signals through a G protein of the G_q class and phospholipase C [25,26,28,31-33], as opposed to rods and cones, which use G_t (transducin) and phosphodiesterase.

This does not, however, seem to be the whole story. Melanopsin is able to activate transducin in a heterologous expression system [34] and has been shown to be capable of signaling through a cascade that includes cyclic nucleotides but not PLC [24]. There are also conflicting reports about which members of the $G_{q/11}$ gene family (G_q, G_{11}, G_{14}, and G_{15}) are expressed in ipRGCs [32,35-37] as well as disagreement about the effects of genetic inactivation of $G_{q/11}$ genes on their intrinsic photosensitivity [35,36] raising speculation about the possibility that ipRGCs are able to utilize a $G_{q/11}$-independent phototransduction cascade [35].

The evidence that phospholipase C (PLC) is the effector enzyme in the transduction cascade is also rather slim. It is based on two observations made in a single study [32]; ipRGCs express PLCβ4 (the PLC isozyme that is a key participant in invertebrate phototransduction) and the light response of cultured ipRGCs is blocked by U73122, a PLC antagonist that is not, however, particularly selective, in that it has effects on numerous other cellular proteins including phospholipase D and Ca-ATPase, as well as Ca^{2+} and K^+ channels [38].

The second messenger that is generated by the effector enzyme to mediated downstream excitation in the melanopsin transduction cascade is not known. Light responses have been reported to be present in excised patches of ipRGC membrane. While this was considered evidence that rules out a soluble cytoplasmic messenger [32], that is not necessarily the case. Electrical responses resulting from light-evoked changes in cGMP, a diffusible cytoplasmic messenger, have been shown to persist in excised patches from rod outer segment membrane [39]. With respect to this issue it also noteworthy that photocurrents in ipRGC decline during whole cell recording but not during perforated patch recording, suggesting the loss of cytoplasmic components by whole cell dialysis [40]. Attempts to identify the second messenger, whether it be diffusible or membrane delimited have failed. The exogenous application of likely candidates produced by PLC activity, IP3, and diacylglycerol (DAG), had no effect on phototransduction in ipRGCs [32], nor did depletion of intracellular Ca stores [41,42].

The last step in the melanopsin transduction cascade is a non-selective ion channel that opens and produces a depolarizing potential change. The leading candidate for this job is a member of transient receptor potential (TRP) channel family, especially the TRPC subfamily, which are thought to be the phototransduction channels in *Drosophila* that are activated via a G protein-coupled phospholipase C (PLCβ4) cascade [29]. The evidence supporting their participation in the melanopsin transduction process include the elimination of the photoresponse by TRP-channel blockers and the presence of TRPC channel protein and/or mRNA in ipRGCs [32,41-43]. While light evoked responses persist in the absence of functional expression of either homomeric TRPC3, TRPC6, or TRPC7 [44], there are, however, subtle changes in the response consistent with the suggestion the transduction channels in ipRGCs are heteromultimeric assemblies formed by different combinations of subunits drawn from the TRPC subfamily.

OUTLOOK

It is clear that our understanding of the melanopsin driven transduction process is incomplete. While the prevailing view is that it somehow fits the common template for invertebrate phototransduction in which light-activated visual pigment excites a G_q type G protein causing in turn the opening of TRP ion channels via a signaling pathway that involves PLC, none of the steps in this sequence of events are understood in detail nor unequivocally supported by experiment. Whatever the molecular elements in the transduction cascade maybe they appear

to pre-exist in non-ipRGC retinal ganglion cells as shown by finding that viral vector mediated expression of melanopsin rendered conventional ganglion cells intrinsically photosensitive [45].

The diversity of ipRGC cell types as well as a high degree of cell-to-cell heterogeneity in the biophysical properties of a single (M1) ipRGC cell class [46] raises the possibility that the transduction process underlying intrinsic photosensitivity is not the same in all ipRGCs. In support of this point of view a recent study (Jiang, Z, et al. Invest Ophthalmol Vis Sci 2017; 58(8): ARVO Abstract 4127) reports that light evoked responses in the presence of synaptic blockers persist in M2 and M4 type ipRGCs in mice lacking genes for the expression of PLCβ4, as well as TRPC1,3,4,5,6, and 7. Photoresponses in wild-type M2 and M4 ipRGCs were eliminated by ZD7288, a HCN-channel blocker, but not by Ruthenium Red, a wide-spectrum TRPC-channel antagonist. Both M2 and M4 cell types express HCN channels and the voltage dependence of the photocurrents they generate are consistent with hyperpolarization-activated current (I_h). Finally, photo-release of cage cyclic nucleotide produced an inward current that had properties similar to those of the intrinsic photocurrent suggesting that cyclic nucleotides as well as HCN channels participate in the intrinsic photosensitivity of M2 and M4 ipRGCs.

While these results are described in a meeting abstract and consequently await confirmation and further exposition they nevertheless breathe new life into the melanopsin saga by introducing a serious alternative to the invertebrate transduction model that has, with limited success, dominated the search for the source of ganglion cell intrinsic photosensitivity since its discovery in 2002. In any case, with or without this new chapter in the story, it is clear that the answer to "How do They Work" is more complex than originally imagined.

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