Journal Club

Editor's Note: These short, critical reviews of recent papers in the *Journal*, written exclusively by graduate students or postdoctoral fellows, are intended to summarize the important findings of the paper and provide additional insight and commentary. For more information on the format and purpose of the Journal Club, please see http://www.jneurosci.org/misc/ifa_features.shtml.

Role of Melastatin-Related Transient Receptor Potential Channel TRPM1 in the Retina: Clues from Horses and Mice

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Review of Shen et al.

Parallel processing is one of the main features of the mammalian visual system, with distinct neuronal circuits carrying information related to either increases in light illumination (On pathway) or decreases in light illumination (Off pathway). This segregation of light information occurs very early, at the synapse between photoreceptors and bipolar cells within the retina. Photoreceptors respond to light with a hyperpolarization and subsequent decrease in glutamate release. This decrease in glutamate release from photoreceptors results in a depolarization of On bipolar cells, but causes a hyperpolarization of Off bipolar cells.

Slaughter and Miller (1981) were the first to demonstrate a pharmacological mechanism separating components of the On and Off signaling pathways within the retina. They demonstrated that 2-amino-4-phosphonobutyric acid (2-APB) blocked the light responses of On but not Off bipolar cells. Furthermore, they demonstrated that 2-APB produced this effect by acting as an agonist to hyperpolarize On bipolar cells via closure of an ion channel, similar to the endogenous effects of glutamate released by photoreceptors in the dark. Subsequent work characterized several features of the signal transduction pathway, namely that glutamate released by photoreceptors activates the metabotropic glutamate receptor mGluR6 in On bipolar cells (Masu et al., 1995), that the signaling requires Gαo (Dhingra et al., 2000), and that this signaling eventually leads to closure of the transduction channel and hyperpolarization of the On bipolar cell in the dark (Yamashita and Wässle, 1991). The identity of this cation selective, On bipolar cell transduction channel has, until recently, remained a mystery.

Two candidates for the On bipolar cell transduction channel have been postulated: the cyclic-nucleotide-gated (CNG) channels and the transient receptor potential (TRP) channels. CNGs were proposed as the transduction channels largely because cGMP was found to potentiate the On bipolar cell transduction current. However, findings that mGluR6 coupled better to G_o than transducin, coupled with lack of immunocytochemical evidence for CNGs indicated that perhaps cGMP is playing only a modulatory role in signal transduction (Snellman et al., 2008). TRP channels have been proposed to act as the transduction channel in On bipolar cells primarily because they are desensitized by Ca^{2+}, can be modulated by components of G-protein signaling cascades, and may act as the transduction channel in the intrinsically photosensitive retinal ganglion cells (Snellman et al., 2008). In a recent issue of *The Journal of Neuroscience*, Shen et al. (2009) investigated the identity of the cation channel responsible for On bipolar cell signal transduction, and more specifically tested the hypothesis that the transduction channel in On bipolar cells is a member of the transient receptor potential channel vanilloid type (TRPV) family. They based this hypothesis on previous findings that both the On bipolar transduction channel and TRPV1 are moderately Ca^{2+} permeable and show Ca^{2+}-mediated desensitization.

The authors first tested the effects of TRPV channel antagonists on the transduction current of one subtype of On bipolar cell, the rod bipolar cell. The authors found that the noncompetitive, nonspecific TRPC and TRPV family antagonist ruthenium red and the TRPV1-specific antagonists capsazepine and SB366791 reduced the rod bipolar cell transduction current to a fraction of its original value. However, 2-aminoethoxydiphenylborate, a TRPC family antagonist but TRPV family agonist, potentiated the transduction current. Collectively, these results supported the idea that a member of the TRP, and more specifically the TRPV family, mediates the transduction current in rod bipolar cells.

The authors next examined whether TRPV1 agonists capsaicin and anandamide activate a transduction-like current in rod bipolar cells. Indeed, application of capsaicin elicited a current in every rod bipolar cell tested, and this current was blocked by coapplication of the antagonist capsazepine. Additionally, anandam-

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ide elicited a response in rod bipolar cells that was blocked by coapplication of capsazepine. Importantly, conductances activated by capsaicin and the mGluR antagonist LY341495 showed similar outward rectification and reversal potentials. The pharmacological and current–voltage data obtained from these experiments indicated that the transduction channel in rod bipolar cells possesses properties consistent with those previously reported for TRPV1 channels.

The authors further went on to test the idea that mGluR6 and capsaicin-generated currents are generated by the same population of channels. To test this, they examined whether the responses generated by capsaicin and mGluR antagonist showed the occlusion that would be predicted if these compounds were activating the same population of channels. Indeed, at high concentrations, coapplication of these two compounds showed the predicted occlusion, suggesting the same transduction channel is activated by both compounds.

Shen et al. (2009) had thus far demonstrated that (1) antagonists of TRP channels and, more specifically, antagonists thought to be specific for TRPV1 channels blocked the transduction current in rod bipolar cells and (2) that agonists of TRP channels, including those thought to be specific for TRPV1 channels, activated the transduction current in rod bipolar cells. Based on these findings, the authors set out to test the specific hypothesis that the transduction current in rod bipolar cells was mediated by TRPV1.

Surprisingly, upon recording from rod bipolar cells in a TRPV1−/− mouse model, the authors found the transduction pathway of rod bipolar cells to be unperturbed, with responses to mGluR antagonist, capsaicin, and anandamide being comparable to wild type (WT). The authors also performed electroretinograms in both TRPV1−/− and WT mice to compare the b-wave, which represents the On bipolar component of the retinal response to an increase in illumination. They found that the b-wave was similar in TRPV1−/− and WT mice.

The normal b-wave of TRPV1−/− mice seems particularly surprising in light of the pharmacological data pointing to TRPV1 as a likely candidate for the rod bipolar cell transduction channel. However, recent studies have uncovered the melastatin-related TRP channel family member TRPM1 as a possible candidate for rod bipolar cell transduction. Appaloosa horses with congenital stationary night blindness show markedly reduced expression of mRNA encoding TRPM1, as well as a reduced b-wave in ERGs (Bellone et al., 2008). Additionally, recent work has shown TRPM1 mRNA expression in mouse On bipolar cells (Kim et al., 2008). To test whether TRPM1 could be mediating the transduction current in rod bipolar cells, Shen et al. (2009) measured the ERG in a TRPM1−/− mouse model and found a complete lack of b-wave (i.e., On bipolar cell response) but normal a-wave (photoreceptor response), indicating a severe deficit in On bipolar cell function.

Collectively the study by Shen et al. (2009) provides strong support for the idea that a TRP channel is mediating the transduction in rod bipolar cells. Furthermore, they provide evidence that this transduction is not mediated by TRPV1 but is possibly mediated by TRPM1. However, one notable set of experiments lacking in this paper are recordings of the light-evoked current of the rod bipolar cells in the TRPM1−/− retina, as well as pharmacological manipulations using mGluR antagonists and TRPV1 agonists to activate the transduction current in rod bipolar cells of the TRPM1−/− mouse. These manipulations would further confirm that functional rod bipolar cell signaling is absent in the TRPM1−/− mouse, and provide direct functional evidence that lack of the TRPM1 channel in rod bipolar cells eliminates the transduction current. Shen et al. (2009) do not provide reasoning for the somewhat confusing result that the TRPV1 agonists continue to activate the transduction current in the rod bipolar cells of the TRPV1−/− mouse, making these manipulations in the TRPM1−/− mouse even more important to perform. Should these TRPV1 agonists fail to exert effects on rod bipolar cells in the TRPM1−/− mouse model, the data would imply that perhaps these pharmacological agents can also act at TRPM1 subunits and that "TRPV1-specific" drugs such as capsaicin may not be as specific as previously believed.

The specific identification of a possible transduction channel for rod bipolar cells raises several important future questions. First, the physiological and pharmacological properties, as well as the signaling pathways that gate and modulate TRPM1 have yet to be described. Human melanocyte cell lines have been previously used to study TRPM1 (Oancea et al., 2009), and it would be of interest to transfect mGluR6 into some of these cell lines to determine whether mGluR6 can directly modulate this channel. mGluR6 localizes to the tips of the dendrites in On bipolar cells (Vardi et al., 2000), and if TRPM1 is definitively described as the transduction channel in rod bipolar cells, then the localization of TRPM1 on the bipolar cell membrane becomes an important question. Finally, TRPM1 is not only expressed in retina but in brain as well (Fonfria et al., 2006). It will be interesting to determine whether a similar signal transduction mechanism operates in brain neurons where TRPM1 is expressed.

In terms of relevance to human disease, congenital stationary night blindness is not only found in the Appaloosa horse, but in humans as well. Though several mutations have been identified in humans as potential causes for congenital stationary night blindness, none have been linked to TRPM1 (Bellone et al., 2008). As research in this area progresses, it is possible that TRPM1 (or a dysfunction in its signaling) will be found to play a role in the human form of this disease. Thus, a deeper understanding of the transduction pathway in rod bipolar cells could aid in the development of treatment strategies for this disease.

Though important questions remain, the study by Shen et al. (2009) takes a critical step forward in identification of the thus far elusive transduction channel of the depolarizing bipolar cells of the On vertical pathway within the vertebrate retina. These findings implicate TRPM1 in the transduction of light signals via On bipolar cells to downstream targets in the retina and eventually the brain. Further, the signal transduction mechanism uncovered in the retina may extend to other neuronal systems and may be an important target for study of congenital stationary night blindness in humans.

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