TRPM1 is required for the depolarizing light response in retinal ON-bipolar cells

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The ON pathway of the visual system, which detects increases in light intensity, is established at the first retinal synapse between photoreceptors and ON-bipolar cells. Photoreceptors hyperpolarize in response to light and reduce the rate of glutamate release, which in turn causes the depolarization of ON-bipolar cells. This ON-bipolar cell response is mediated by the metabotropic glutamate receptor, mGluR6, which controls the activity of a depolarizing current. Despite intensive research over the past two decades, the molecular identity of the channel that generates this depolarizing current has remained elusive. Here, we present evidence indicating that TRPM1 is necessary for the depolarizing light response of ON-bipolar cells, and further that TRPM1 is a component of the channel that generates this light response. Gene expression profiling revealed that TRPM1 is highly enriched in ON-bipolar cells. In situ hybridization experiments confirmed that TRPM1 mRNA is found in cells of the retinal inner nuclear layer, and immunofluorescent confocal microscopy showed that TRPM1 is localized in the dendrites of ON-bipolar cells in both mouse and macaque retina. The electroretinogram (ERG) of TRPM1-deficient (TRPM1−/−) mice had a normal a-wave, but no b-wave, indicating a loss of bipolar cell response. Finally, whole-cell patch-clamp recording from ON-bipolar cells in mouse retinal slices demonstrated that genetic deletion of TRPM1 abolished chemically simulated light responses from rod bipolar cells and dramatically altered the responses of cone ON-bipolar cells. Identification of TRPM1 as a mGluR6-coupled cation channel reveals a key step in vision, expands the role of the TRP family, and provides insight into the evolution of vertebrate vision.

A recent study by Shen et al. (15) showed that the ERG b-wave is lacking in the TRPM1−/− mouse implicating this channel in photoreceptor to ON-bipolar cell synaptic transmission. Here, we present molecular, immunohistochemical, and electrophysiological evidence that TRPM1 is the mGluR6-coupled cation channel in rod-bipolar cells, and provide evidence that TRPM1 is essential for the normal response of cone ON-bipolar cells.

Results

TRPM1 Is Expressed in Retinal Bipolar Cells. Gene expression profiling studies [SI Text and Fig. S1 (16, 32)], demonstrated that TRPM1 is enriched in ON-bipolar cells, suggesting TRPM1 as a potential candidate for the long sought after channel generating the light response in these cells. We verified that the TRPM1 mRNA was expressed in ON-bipolar cells by in situ hybridization (Fig. 1A Left). By using an anti-sense probe directed against the mouse TRPM1 mRNA (bp 2,383–3,514, GenBank accession NM_001039104), a strong signal was obtained in many cells of the distal inner nuclear layer (INL), where the majority of ON-bipolar cells are located (Fig. 1A, arrows). Unlabeled cells in this region of the INL likely correspond to horizontal cells (Fig. 1A, white arrowhead is a putative horizontal cell). A control hybridization with a sense probe directed against the same region of the channel cDNA showed no signal above background in any part of the retina (Fig. 1A Right).

Immunofluorescence confocal microscopy demonstrated that the TRPM1 channel is localized to ON-bipolar cells. As shown in Fig. 1B, immunolabeling of vertical sections of mouse retina with an anti-TRPM1 antibody revealed punctate staining in the retinal neurobiology | transient receptor potential channel | visual ON-pathway

nociception, to gustation and mechanosensation (9). The founding member of the TRPM family, TRPM1, was discovered by differential display as a potential suppressor of tumor metastasis [and originally named melastatin because it is down-regulated in metastatic melanoma (10)]. TRPM1 is the product of a complex gene, spanning 58 kb and 27 exons, and exists as several isoforms, produced by mRNA splice variants (11–13). Little is known about the physiological role of TRPM1, but recently it has been reported to form constitutively active cation channels in melanocytes, where it has been proposed to function in melanin trafficking (13). The basic properties of the channel, including ion selectivity and current-voltage relationship, are similar to those reported for the mGluR6-coupled ion channel in retinal ON-bipolar cells. In the Appaloosa horse, a reduction in TRPM1 has been correlated with the loss of the ERG b-wave (14), and a recent study by Shen et al. (15) showed that the ERG b-wave is lacking in the TRPM1−/− mouse implicating this channel in photoreceptor to ON-bipolar cell synaptic transmission. Here, we present molecular, immunohistochemical, and electrophysiological evidence that TRPM1 is the mGluR6-coupled cation channel in rod-bipolar cells, and provide evidence that TRPM1 is essential for the normal response of cone ON-bipolar cells.


The authors declare no conflict of interest.

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TRPM1 is expressed by ON-bipolar cells in the mouse retina. (A) In situ hybridization of vertical sections of mouse retina with antisense (Left) and sense control (Right) TRPM1 probes. A hybridization signal is detected in many cell somata in the INL, where bipolar cell nuclei and somata are located (black arrows). Occasional unlabeled cell somata are likely horizontal cells (white arrowhead). (B) Vertical sections from a wild-type (Top) and TRPM1−/− (Bottom) retina were immunofluorescently labeled by antibodies directed against TRPM1 (green) and PKCα (red). Areas of colocalization appear yellow in the merged images (Right). onl, Outer nuclear layer; opl, outer plexiform layer; inl, inner nuclear layer; ipl, inner plexiform layer.

Fig. 1. TRPM1 is expressed by ON-bipolar cells in the mouse retina. (A) In situ hybridization of vertical sections of mouse retina with antisense (Left) and sense control (Right) TRPM1 probes. A hybridization signal is detected in many cell somata in the INL, where bipolar cell nuclei and somata are located (black arrows). Occasional unlabeled cell somata are likely horizontal cells (white arrowhead). (B) Vertical sections from a wild-type (Top) and TRPM1−/− (Bottom) retina were immunofluorescently labeled by antibodies directed against TRPM1 (green) and PKCα (red). Areas of colocalization appear yellow in the merged images (Right). onl, Outer nuclear layer; opl, outer plexiform layer; inl, inner nuclear layer; ipl, inner plexiform layer.

outer plexiform layer (OPL), as well as membrane-associated and possible intracellular staining of bipolar cell bodies. The specificity of the TRPM1 antibody was demonstrated by the absence of staining in the TRPM1−/− mouse (Fig. 1B, lower panels), as well as by labeling of HEK cells transiently transfected with TRPM1 cDNA (Fig. S2). Most of the TRPM1 labeling in the mouse retina was coincident with that for PKCα, indicating that TRPM1 is expressed in rod bipolar cells (Fig. 1B). The TRPM1-positive cells extended dendrites into the OPL, the tips of which could be labeled for mGlurR6 (Fig. S3A–C) and are apposed to labeled synaptic ribbons (Fig. S3D–F). Immunofluorescent labeling indicated that mGlurR6 is still present in the ON-bipolar cell dendrites in the TRPM1−/− retina (Fig. S4A), and that tips of TRPM1−/− rod bipolar cell dendrites are apposed to photoreceptor synaptic ribbons (Fig. S4B), suggesting that the morphology of the OPL is not grossly disrupted in the TRPM1−/− retina.

Because the TRPM1 antibody was raised against a human polypeptide, TRPM1 immunostaining was even more robust in the macaque retina (Fig. 2). In vertical sections of macaque retina, TRPM1 staining appeared as puncta in the OPL and was associated with cell plasma membranes in the distal INL. In many instances, this labeling was clearly associated with PKCα, indicating that these were rod bipolar cells (Fig. 2B). Similar to mouse, however, not all TRPM1-positive cells were immunoreactive for PKCα (arrowheads, Fig. 2B). This finding is more apparent in a horizontal optical section through the INL of a macaque retinal whole mount shown in Fig. 2 C and D (cells marked with asterisks). These cells are most likely cone ON-bipolar cells since all TRPM1-positive cells can be colabeled with Gao (Fig. S5). If this is the case, their dendritic processes should extend to cone photoreceptor pedicles in the OPL. Indeed, immunolabeling for TRPM1 is clearly associated with cone pedicles, as well as with rod terminals, shown by double labeling with the anti-TRPM1 antiserum and the cone marker, Alexafluor peanut agglutinin (PNA) (Fig. 2 E–G). Thus, TRPM1 is localized to the dendrites of both rod and cone ON-bipolar cells, where it is optimally positioned to respond rapidly to light-induced changes in the synaptic glutamate levels.

**Vision Is Impaired in the TRPM1−/− Mouse.** The expression of TRPM1 in retinal bipolar cells implies that the TRPM1 channel is important for vision. To test this hypothesis, we measured spatial frequency and contrast sensitivity thresholds of the optokinetic response (OKR) in TRPM1−/− mice (17). The spatial frequency threshold of TRPM1−/− mice was reduced 10% compared to wild-type (0.359 ± 0.004 cycles/degree for TRPM1−/−, and 0.400 ± 0.010 cycles/degree for wild-type, P < 0.05), and contrast sensitivity was reduced 3-fold (4.61 ± 0.23 for TRPM1−/−, and 14.99 ± 3.85 for wild-type, P < 0.001, measured at a spatial frequency of 0.150 cycles/degree). The OKR measurements indicate that the TRPM1−/− mice are visually im-
paired, although not profoundly so, similar to the complete congenital stationary night blindness phenotype.

Electroretinogram b-Wave Is Absent in Mice Lacking TRPM1. To investigate the physiological role of TRPM1 in the retina, we recorded the electroretinogram (ERG) from control and TRPM1$^{-/-}$ mice. The ERG responses from TRPM1$^{-/-}$ and wild-type mice were indistinguishable and have been combined to form the control group for analysis. Figure 3 shows representative ERGs from control (gray traces) and TRPM1$^{-/-}$ mice (black traces). The b-wave and oscillatory potentials, both of which are generated downstream from the photoreceptors, were absent from both rod and cone-mediated (both scotopic and photopic) ERGs of the TRPM1$^{-/-}$ mice (Fig. 3A and B). Figure 3C shows an expanded view of the beginning of the rod-isolated ERGs recorded for several flash intensities. The superimposed traces show that the negative-going rod-isolated ERG a-waves were essentially identical between TRPM1-deficient and control mice. A phototransduction model was ensemble fit to the rising phases of the rod-isolated a-waves. The derived rod phototransduction parameters (mean ± SE.) were not significantly different between control (Rmax 0.3 = −806 ± 35 μV; S = 1,158 ± 166 [cd·s/m²]−1 s−2; td = 3.9 ± 0.05 ms) and TRPM1$^{-/-}$ mice (Rmax 0.3 = −877 ± 30 μV; S = 1,221 ± 120 [cd·s/m²]−1 s−2; td = 4.1 ± 0.1 ms). Figure 3D shows an expanded view of the cone-isolated photopic ERGs. Rods have little or no cone mediated photopic ERG a-wave. In our study, the initial negative going photopic response from control mice only tracked the ERG from the TRPM1$^{-/-}$ mice for very bright flash intensities (>3 log ph cd·s/m²) (Fig. 3D). The absence of the ERG b-wave in the TRPM1$^{-/-}$ mice indicates a block in signal transmission between photoreceptors and ON-bipolar cells in these animals. The ERG, however, does not indicate whether this block resides postsynaptically, in the bipolar cell dendrites, or presynaptically, in the photoreceptor terminals. The in situ hybridization and immunofluorescence results (Figs. 1 and 2) support a postsynaptic locus for the block, but to rule out any contribution from defective glutamate release by photoreceptors in the TRPM1$^{-/-}$ retina, we recorded directly from ON-bipolar cells using a technique that bypasses the photoreceptors entirely.

Rod Bipolar Cell Response Is Absent in TRPM1$^{-/-}$ Mice. To examine the role of TRPM1 in generation of the depolarizing light response in ON-bipolar cells, we used the whole-cell patch clamp technique to record chemically simulated light responses from ON-bipolar cells in mouse retinal slices. For these experiments, the slice was bathed at all times in the mGluR6 agonist, L-2-aminophosphonobutyrate (L-AP4) to simulate darkness; pressure application of the mGluR6 antagonist, α-cyclopropyl-4-phosphonophenylglycine (CPPG), to the OPL was used to simulate a step of light. During recording, cells were filled with the dye, Alexa-488 hydrazide, which permitted morphological identification of rod and cone bipolar cells at the termination of the experiment (Fig. 4A and C). As shown in Fig. 4B, application of CPPG to the wild-type mouse retina activated a robust current with a waveform typical of rod bipolar cells (18). These currents had a peak amplitude at approximately 120 ms of −129 ± 15 pA (SEM; n = 21), which decayed rapidly to a plateau. These currents were abolished in the TRPM1$^{-/-}$ retina. In the majority of morphologically identified rod bipolar cells, CPPG application activated no measurable currents (−2.7 ± 0.55 pA; SEM; n = 25); a few cells exhibited a slowly activating current of <5 pA.

Cone ON-Bipolar Cell Response Is Dramatically Altered in TRPM1$^{-/-}$ Mice. In wild-type retina, cone ON-bipolar cells responded to CPPG with a sustained current of −75 ± 8.6 pA (n = 13) (Fig. 4D).
Approximately half of cone ON-bipolar cells in the TRPM1−/− retina showed no response to pressure application of CPPG (3/8 cells), like rod bipolar cells. The remaining cone ON-bipolar cells, however, responded to CPPG application by activating a small transient current (38 pA ± 7.6; n = 5 cells), suggesting that a subset of cone ON-bipolar cells contain additional mGluR6-coupled channels (Fig. 4D). Pressure application of a 200 μM AMPA/200 μM kainite mixture confirmed that OFF-cone bipolar cells remained responsive in TRPM1−/− mice.

Capsaicin-Sensitive Current Persists in TRPM1−/− ON-Bipolar Cells. The pharmacology of both TRPM1 and the endogenous mGluR6-coupled ion channel remain largely unknown. Recently a capsaicin-activated current has been discovered in mouse ON-bipolar cells with properties similar to the light-activated current (15), leading to speculation that the capsaicin-sensitive current may be mediated by TRPM1. As shown in Fig. 5, we found that the capsaicin-activated current was still present in at least some ON-bipolar cells in the TRPM1−/− retina. Overall, however, the magnitude of the capsaicin-activated current in ON-bipolar cells was somewhat reduced (t test P < 0.001) in TRPM1−/− mice (−14 ± 2 pA; n = 17) compared to wild-type (−27 ± 3 pA SEM; n = 15).

Discussion

The most parsimonious explanation for these data are that TRPM1 is a necessary component of the mGluR6-coupled ion channel responsible for generating the depolarizing light response in rod and cone ON-bipolar cells. Some classes of cone ON-bipolar cells, however, may express additional mGluR6-coupled ion channels, as evidenced by the residual transient current seen in some ON-cone cells from the TRPM1−/− retina. This situation is reminiscent of the light response in Drosophila photoreceptors, which consists of a sustained component mediated by trp channels and a transient component mediated by trpl channels (19). Congenital stationary night blindness (CSNB) is a group of nonprogressive retinal diseases characterized by impaired scotopic vision and a characteristic “negative” ERG, in which the b-wave is reduced or absent. In complete CSNB (CSNB1), there is a complete loss of both rod and cone mediated ERG b-waves, but the OFF pathway is preserved. CSNB1 is caused by mutations in genes expressed in ON-bipolar cells, such as nystatin (20, 21) and mGluR6 (22, 23). Based on the negative ERG measured in the TRPM1−/− retina, TRPM1 appears to be a candidate gene for CSNB1. In addition, the expression of TRPM1 by both bipolar cells and melanocytes, and its down regulation in metastatic melanoma raise the possibility that TRPM1 may be a target of the autoimmune response that causes visual deficits in melanoma-associated retinopathy, a paraneoplastic encephalopathy with reduced ON-bipolar cell function (24).

In a recent article, Sh-summary (15) described a capsaicin-activated current in ON-bipolar cells, and suggested that this current may arise from the same ion channels that generate the mGluR6-coupled transduction current. The authors demonstrate that the current is present in the TRPV1−/− retina, and suggest that it may be mediated by TRPM1, which they showed is necessary for the ERG b-wave. We show here, however, that a capsaicin-activated current is still present in many ON-bipolar cells in the TRPM1−/− mouse retina. Therefore, the precise relationship between the capsaicin-activated channel and the mGluR6-coupled channel remains to be established. In light of these findings, it is interesting to speculate that the endogenous mGluR6-coupled transduction channel may be comprised of TRPM1, which is essential for activation by mGluR6, as well as an additional type of channel subunit, which confers capsaicin sensitivity.

ON-bipolar cells share a number of conserved features with photoreceptors, including expression of recoverin, potassium channels, and ribbon synapse components; furthermore, both photoreceptors and ON-bipolar cells hyperpolarize in response to activation of their respective receptors (opsins and mGluR6). These observations have prompted the hypothesis that bipolar cells evolved from primordial photoreceptors (25, 26). Use of TRPM1, rather than a cyclic nucleotide-gated channel, as the mGluR6-coupled channel, however, indicates that the ON-bipolar cell signaling pathway is more analogous to phototransduction in Drosophila photoreceptors and melanopsin-containing intrinsically photosensitive retinal ganglion cells (ipRGCs), both of which are thought to employ trp channels (19, 27).

Materials and Methods

For complete details, refer to SI Text. Mice expressing cre recombinase under the control of the mGluR6 promoter were generated and crossed with the Z/Eg marker strain. GFP expressing ON-bipolar cells were purified by fluorescence activated cell sorting. Total RNA was isolated from the purified cells as well as from total retina, and gene expression was compared between the two samples. In situ hybridization methods for cytoskeletons have been described (28). Digoxigenin-labeled probes were detected with an anti-digoxigenin antibody conjugated to alkaline phosphatase, followed by use of the Fast Red substrate. HEK tsA-201 cells were transfected and immunostained according to standard methods. Immunofluorescent labeling of retina sections were performed as described in ref. 29. The TRPM1−/− mice were obtained from Texas A&M Institute for Genomic Medicine (Houston, TX), and the TRPM1 antibody was from Sigma-Aldrich. Whole-mount retina staining was performed by successive incubations of lightly fixed macaque retina pieces in blocking/permeabilization solution (4 h), primary antibodies (72 h), then secondary antibodies (overnight). All incubations were performed at 4 °C with washing steps in between. Immunofluorescence images were obtained with an Olympus Fluoview1000. Full-field scotopic and photopic ERGs (background = 60 candela/meter2 (cd/m2)) were recorded to flashes of increasing intensity = 3.9 to 2.0 and = 1.0 to 3.7 log ph cd/m2, respectively. Rod-isolated ERGs were obtained by subtracting scotopic cone-ERGs, obtained with a paired flash protocol from the mixed rod/cone responses (30, 31). Phototransduction parameters were derived from the ensemble fit of a P3 model to the leading edges of rod-isolated ERG a-waves (30). Optokinetic responses were measured as described in ref. 18. Patch-clamp recordings from mouse bipolar cells were performed similarly to those described (17). The external Ames solution contained 4 μM L-AP4 to activate mGluR6, and a 5-puff of 600 μM CPPG was applied to simulate a light flash.

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Fig. 5. Comparison of capsaicin-activated currents in ON-bipolar cells of wild-type and TRPM1−/− mice. Patch-clamp recordings were made in the whole-cell mode from ON-bipolar cells in the retinal slice preparation at a holding potential of −60 mV. Capsaicin (100 μM) was applied to the OPL at the time indicated via a “puffer” pipet. These traces are representative of >10 recordings from each genetic background.
promoter sequence, iCre, and the human TRPM1 cDNA, respectively; and Peter Gillespie and Jeff Karpen (Oregon Health & Science University) for critical reading of the manuscript. This work was funded by the National Institutes of Health Grants EY09534 (to R.M.D.), EY014700 (to C.W.M.), MH067094 (to R.L.B.), and RR000163.

Supporting Information

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SI Text

Generation of mGluR6-cre-GFP Mice. To identify genes expressed in ON-bipolar cells that participate in the mGluR6-activated signaling cascade, we generated a transgenic line of mice expressing Cre recombinase under the control of the mGluR6 promoter. First, the codon-improved Cre recombinase (iCre) (1) was inserted upstream of the 3’ untranslated and polyadenylation site of pUHG10.3 using common KpnI and SacI restriction sites. Second, the 637-bp Pmel-Nael mGluR6 promoter fragment was amplified from the mGluR6 5’ upstream region (2) and cloned in front of iCre-poly(A) in pBLSK(-) (Stratagene). Finally, the 9.5-kb SalI-Pmel fragment of the mGluR6 5’upstream region was added to reconstitute the full-length mGluR6 promoter. The nucleotide sequence of the amplified region was verified. The promoter construct DNA, digested with AgeI and NotI and size-purified to remove the plasmid vector sequence, was injected into fertilized mouse eggs. Two mouse lines were identified that transmitted the transgene to their progeny. These mice were crossed with two EGF reporter mouse strains, a ROSA26 mouse (3) and the Z/EG marker strain (4) (Jackson Laboratory, stock no. 004077 and no. 004178, respectively). Only one transgenic mouse line expressed Cre in the retina, as determined by immunostaining for Cre and EGFP expression in double transgenic mice. Whereas EGFP fluorescence was weak in the retina of these mice when crossed with the ROSA26 marker strain, presumably because the ROSA26 promoter is not very active in retinal bipolar cells, EGFP fluorescence was clearly visible in a majority of ON-bipolar cells from double transgenic mice with the chicken actin promoter driving EGFP (Fig. S1A).

Genetic Profiling of Retinal ON-Bipolar Cells. Retina were dissociated by mechanical trituration after limited treatment with papain (Fig. S1B). Dissociated ON-bipolar cells expressing GFP were purified by fluorescence activated cell sorting (FACS: Fig. S1C). Total RNA was prepared from the FACS-purified ON-bipolar cells, as well as from total retina. After cDNA synthesis and linear amplification, the samples were applied to an Illumina BeadChip Expression Array, and data were analyzed using the GeneSifter program (ViZxlabs). Of the 46,642 markers analyzed, the expression of 4,097 markers was reliably detected in the ON-bipolar cell preparation (P < 0.05). Importantly, this analysis also demonstrated the relative purity of the ON-bipolar cell preparation. The message for mGluR6 was enriched in the ON-bipolar cell preparation by 7.5-fold over total retina, presumably because the ROSA26 promoter is not very active in retinal bipolar cells, EGFP fluorescence was clearly visible in a majority of ON-bipolar cells from double transgenic mice with the chicken actin promoter driving EGFP. As expected, the message for PKCa was not detected in the retina of these mice, as determined by immunostaining for Cre and EGFP expression in double transgenic mice. Whereas EGFP fluorescence was weak in the retina of these mice when crossed with the ROSA26 marker strain, presumably because the ROSA26 promoter is not very active in retinal bipolar cells, EGFP fluorescence was clearly visible in a majority of ON-bipolar cells from double transgenic mice with the chicken actin promoter driving EGFP.

SI Materials and Methods

Animals. Mice were maintained on a 12-h light-dark cycle. TRPM1+/− mice were generated by Lexicon Genetics Inc. and obtained from Texas A&M Institute for Genomic Medicine (Houston, TX). Exons 2–4 were replaced by a selection cassette resulting in a nonfunctional TRPM1 allele. All experiments were conducted in accordance with National Institutes of Health guidelines and approved by the Institutional Animal Care and Use Committees at Oregon Health & Science University and Washington State University.

In Situ Hybridization. Digoxigenin-labeled probes were synthesized by runoff transcription using T3 and T7 RNA polymerase and Genius Kit components (Roche). In situ hybridization methods for cryosections have been described (5). The digoxigenin probe was detected with anti-digoxigenin antibody conjugated to alkaline phosphatase, followed by Fast Red color substrate (Roche).

Immunohistochemistry. Vertical retina sections were prepared and labeled as described in ref. 29. Sections were incubated with rabbit anti-TRPM1 antibody (1:100; Sigma-Aldrich) either alone or in combination with either mouse anti-PKCα (1:5000; Novus Biologicals), sheep anti-mGluR6 (1:100; ref. 6), mouse anti-ctip2 (anti-ribeye; 1:5000, BD Biosciences) or mouse anti-basoon (1:5000; Synaptic Systems) for either 2 h at room temperature or overnight at 4 °C. After three washes in PBS, the tissue was incubated for 1 h at room temperature with anti-rabbit IgG conjugated to either Alexa Fluor 488 or 594 (1:1,000; Invitrogen) alone or in combination with anti-mouse-Cy3 (1:500; Jackson Immunonochemicals) or PNA-Alexa Fluor 488 (1:1,000; Invitrogen), then washed again in PBS. For whole-mount macaque retina pieces, the tissue was blocked for 4 h at 4 °C, incubated with rabbit anti-TRPM1 (1:500) in combination with either mouse anti-PKCα (1:5000) or mouse anti-Gαo (1:1000; Biomol) for 72 h at 4 °C. Washed three times for 1 h each in PBS, then incubated overnight at 4 °C in secondary antibodies, before a final three washes in PBS of 1 h each. Sections and whole-retina pieces were coverslipped and imaged with an Olympus FluoView FV1000 confocal microscope using a 60×/1.42 oil-immersion objective.

Electroretinogram Recording. Mice were dark-adapted (>12 h) and prepared for recording under dim red light. Anesthesia was achieved via i.p. injection of ketamine/xylazine (100:10 mg/kg), and maintained with supplemental 30:3 mg/kg anesthesia injections every 30 min. Pupils were dilated with phenylephrine (2.5%) and tropicamide (1%), and the mouse was placed on a heating pad that maintained body temperature at 37–38 °C. The head was placed in a custom-made holder, which stabilized the head and delivered O2 (~0.25 L/min). ERGs were recorded from a custom-made contact lens electrode with its central platinum wire placed against the cornea (with a drop of methylcellulose) referenced to a platinum loop placed behind the equator of the eye. A needle platinum placed in the tail served as ground. Full-field scotopic ERGs were recorded to flash intensities of increasing intensity [−3.5 to 2.4 log scotopic candelas/second/meter2 (sc cd-s/m2)]. For flash intensities above −1.0 log sc cd-s/m2, rod-isolated ERGs were obtained by subtracting cone-ERGs, obtained with a paired flash protocol from the mixed rod/cone responses (7, 8). For flash intensities <−1.5 log sc cd-s/m2, the second flash was presented 0.7 s after a 1.6 log sc cd-s/m2 conditioning flash. At higher intensities, identical paired flashes were separated by 0.7–1.5 s. Cone-driven ERGs were also obtained photopically from flashes of increasing intensity (−1 to 3.7 log ph cd-s/m2) presented 20 min after the onset of a rod-saturating background light (60 cd/m2). ERGs were low pass filtered (−3 dB) at 300 Hz for intensities <−1.0 log sc cd-s/m2, and at 1 kHz for all other recordings. ERGs were amplified (1–10 k), high pass filtered (−3 dB at 0.1 Hz), and sampled at 2.5 kHz. A P3 model was ensemble fit to the leading edges of rod-isolated ERG a-waves (7). Derived parameters were: S [(sc cd s)−1 s−2]
a sensitivity parameter; td (ms), the delay due filtering and flash duration; and $R_{\text{maxP3}}$ (V) the maximal response.

**Optokinetic Response.** Visual acuity and contrast sensitivity were measured behaviorally from three TRPM1$^{-/-}$ mice, and four wild-type mice (one TRPM1$^{+/+}$ littermate of the TRPM1$^{-/-}$ mice, and three C57BL/6) using a virtual optokinetic system (9) (OptoMotry, CerebralMechanics). The responses of the TRPM1$^{+/+}$ mouse were indistinguishable from those of wild-type C57BL/6 mice.

**Patch-Clamp Recordings.** Detailed procedures for recording from mouse bipolar cells have been published (10). Briefly, mice were killed by cervical dislocation after isofluorane anesthesia. Eyes were removed under room light and immediately transferred to Ames solution, equilibrated with carbogen (95% O$_2$–5% CO$_2$). Retinas were dissected and adhered to nitrocellulose membrane, and approximately 200-μm slices were prepared by using a tissue chopper. Retina slices were transferred to the recording chamber on an Olympus BX51W microscope and continuously perfused with oxygenated Ames. Patch electrodes were filled with (in mM) 135 KMeSO$_4$, 6 KCl, 5 NaCl, 1 EGTA, 2 MgCl$_2$, 2 Na-ATP, and 1 Na-GTP, and 5 Na-Hepes, pH 7.4. Alexa-488 hydrazide was included to examine cell morphology at the conclusion of recording. Voltage was controlled and currents recorded with a HEKA EPC-10 amplifier and PatchMaster software. Current signals were sampled at 5 kHz and filtered at 2.5 kHz. The external Ames solution was supplemented with 4 μM L-AP4, an mGluR6 agonist to simulate the “dark” condition. A pipette attached to a Picospritzer containing Ames with 600 μM CPPG was placed on the OPL adjacent to the dendritic arbour of a target cell. After obtaining a whole-cell recording configuration, a 5-s puff application of CPPG was used to chemically simulate light responses in bipolar cells.

Fig. S1. GFP-tagged ON-bipolar cells in the mGluR6-Cre/ZEG mouse retina. (A) Vertical retina section immunolabeled for PKCα (red) and GFP (green). (B) Dissociated retinal neurons immunolabeled for PKCα (red) and GFP (green), superimposed on a Nomarski image. (C) Flow cytometry separation of GFP-labeled cells (cluster of green dots to the right) from a dissociated retina preparation. In these experiments more than half of the PKCα-labeled rod bipolar cells are GFP positive (combination of green and red fluorescence in A and B); in addition, many cone ON-bipolar cells are also GFP positive (green fluorescence only in A and B).
Fig. S2. Immunostaining of TRPM1-transfected cells. HEK tsA-201 cells were cotransfected with cDNAs encoding green fluorescent protein (GFP) and human TRPM1. (A) GFP fluorescence superimposed on a Nomarski image. (B) Immunolabeling with TRPM1 antibody labels transfected cells (arrow), but not untransfected cells (A arrowhead).
Fig. S3. TRPM1-labeled ON-bipolar cell dendrites in the OPL. Mouse retina sections were double labeled for TRPM1 (green, A and D) and either mGluR6 (red, B) or bassoon (red, E), a component of the synaptic ribbon. Merged images are shown in C and F with areas of overlap appearing yellow.
Fig. S4. ON-bipolar cell dendrites in the TRPM1$^{+/−}$ retina. (A) The distribution of mGluR6 in the TRPM1$^{+/−}$ outer plexiform layer ($−/−$, Right) is similar to wild-type ($+/+$, Left). (B) Double labeling of the TRPM1$^{+/−}$ outer plexiform layer for the rod bipolar cell marker, PKCα (green) and the synaptic ribbon protein, ribeye (red). The tips of the PKC-labeled rod bipolar cells are nested within the arcs formed by the photoreceptor synaptic ribbons (arrows).
Fig. S5. All ON-bipolar cells express TRPM1. Horizontal optical section through the inner nuclear layer of a macaque retina double labeled for TRPM1 (green) and the ON-bipolar cell marker, G\(\alpha\)o (red). The merged image is shown on the right, with areas of colocalization appearing yellow.