Relief of Mg^{2+} -Dependent Inhibition of TRPM1 by $PKC\alpha$ at the Rod Bipolar Cell Synapse

Melissa Ann F. Rampino¹ and Scott A. Nawy^{1,2}

¹Dominick P. Purpura Department of Neuroscience and ²Department of Ophthalmology and Visual Sciences, Albert Einstein College of Medicine, Bronx, New York 10461

In the retina, light onset hyperpolarizes photoreceptors and depolarizes ON bipolar cells at the sign inverting photoreceptor–ON bipolar cell synapse. Transmission at this synapse is mediated by a signaling cascade comprised of mGluR6, a G-protein containing $G_{\alpha o}$, and the cation channel TRP melastatin 1 (TRPM1). This system is thought to be common to both the rod- and ON-cone-driven pathways, which control vision under scotopic and photopic conditions, respectively. In this study, we present evidence that the rod pathway is uniquely susceptible to modulation by $PKC\alpha$ at the rod–rod bipolar cell synapse. Decreased production of DAG (an activator of PKC) by inhibition of PIP_2 (phosphatidylinositol-4,5-bisphosphate) hydrolysis caused depression of the TRPM1 current. Conversely, addition of a DAG analog, 2-acetyl-1-oleoyl-sn-glycerol (OAG), potentiated the current in rod bipolar cells but not in ON-cone bipolar cells. The potentiating effects of OAG were absent both in mutant mice that lack $PKC\alpha$ expression and in wild-type mice in which enzymatic activity of $PKC\alpha$ was pharmacologically inhibited. In addition, we found that, like other members of the TRPM subfamily, TRPM1 current is susceptible to voltage-independent inhibition by intracellular magnesium, and that modulation by $PKC\alpha$ relieves this inhibition, as the potentiating effects of OAG are absent in low intracellular magnesium. We conclude that activation of $PKC\alpha$ initiates a modulatory mechanism at the rod–rod bipolar cell synapse whose function is to reduce inhibition of the TRPM1 current by magnesium, thereby increasing the gain of transmission at this synapse.

Introduction

In the retina, parallel ON pathways driven by rod and cone photoreceptors convey light information under scotopic and photopic conditions, respectively. Both the rod–rod bipolar cell (RBC) and the cone–ON cone bipolar cell (CBC) synapses are thought to use the same postsynaptic mechanism involving mGluR6 (Nakajima et al., 1993, Nomura et al., 1994), the G-protein G_o (Vardi, 1998, Nawy, 1999), and the cation channel TRP melastatin 1 (TRPM1) (Morgans et al., 2009, Nakajima et al., 2009, Shen et al., 2009, Koike et al., 2010). Photoreceptors tonically release glutamate in the dark, and the rate of release lessens upon absorption of light, causing mGluR6 to shut off and allowing TRPM1 to open.

Despite similarities in the RBC and ON CBC transduction machinery, it is not known whether all aspects of this process are the same at both synapses. Immunohistochemical data show that RBCs express the protein $PKC\alpha$, but this protein is absent in ON CBCs (Kosaka et al., 1998). This distinctive property of RBCs has generated interest in understanding the function of $PKC\alpha$, par-

ticularly as a modulator of TRPM1 (Ruether et al., 2010). In fact, recent electroretinogram (ERG) data from mice that lack $PKC\alpha$ show delays in the rise and decay phases of the b-wave, suggesting a role for $PKC\alpha$ in activation and termination of the TRPM1 response (Ruether et al., 2010). However, because ERGs relay information about total retinal output, the effects of loss of $PKC\alpha$ at the rod–bipolar cell synapse are likely obscured by the effects of loss of this protein on the entire circuit, and therefore they do not address the specific function of $PKC\alpha$ on the mGluR6/TRPM1 cascade. In addition to modulation by PKC, TRP channels can be regulated by other components of the phosphatidylinositol-4,5-bisphosphate (PIP_2)–PLC–PKC signaling pathway (Runnels et al., 2002, Leung et al., 2008, Daniels et al., 2009, Peng et al., 2010).

TRP channels are also commonly inhibited by Mg^{2+}_i (Nadler et al., 2001, Kerschbaum et al., 2003, Fleig and Penner, 2004, Kraft and Harteneck, 2005, Macianskiene et al., 2008). Studies of TRPM7 modulation have shown that high $[Mg^{2+}]_i$ suppresses TRPM7 currents (Nadler et al., 2001, Kerschbaum et al., 2003), and both TRPM7 and TRPM6 have been implicated in cellular Mg^{2+} homeostasis (Schmitz et al., 2003, Chubakov et al., 2005). Interestingly, in both TRP and NMDA channels there is evidence that both voltage-dependent and -independent forms of Mg^{2+} inhibition can be alleviated through modulatory mechanisms involving $PKC\alpha$ and other components of the PIP_2 –PLC–PKC signaling pathway (Chen and Huang, 1992, Macianskiene et al., 2008, Parnas et al., 2009).

Here, using simulated light responses, we examined the role of $PKC\alpha$, as well as the effects of Mg^{2+}_i in TRPM1 regulation. We found that activation of $PKC\alpha$ potentiates the TRPM1 current in

Received May 27, 2011; revised July 25, 2011; accepted July 31, 2011.

Author contributions: M.A.F.R. and S.A.N. designed research; M.A.F.R. performed research; M.A.F.R. analyzed data; M.A.F.R. and S.A.N. wrote the paper.

This research was supported by National Eye Institute Grant EY010254 and a nonrestricted grant from Research to Prevent Blindness. We thank Dr. Ron Gregg for his kind donation of the $PKC\alpha^{-/-}$ mice; and the members of the Nawy laboratory, as well as Dr. Reed Carroll, for helpful discussions.

Correspondence should be addressed to Melissa Ann F. Rampino, 1410 Pelham Parkway South, Room 525, Bronx, NY 10461. E-mail: melissaann.rampino@phd.einstein.yu.edu.

DOI:10.1523/JNEUROSCI.2655-11.2011

Copyright © 2011 the authors 0270-6474/11/3113596-08\$15.00/0

rod-driven but not cone-driven ON bipolar cells. We provide evidence that the current is inhibited by Mg_i^{2+} and that PKC α may relieve this inhibition. Based on our findings, we propose that regulation of TRPM1 function is accomplished in part by a balance of Mg_i^{2+} and PKC α activation.

Materials and Methods

Retinal slice preparation. Retinal slices were prepared from 3- to 5-week-old female wild-type (WT) C57BL/6 (Charles River Laboratories) or PKC $\alpha^{-/-}$ 129/SV (courtesy of Dr. Ron Gregg, University of Louisville, Louisville, KY) mice. Animals were anesthetized with isoflurane and killed by cervical dislocation in accordance with the policies set forth on animal use and euthanasia by the Albert Einstein College of Medicine Animal Institute. Eyes were removed and enucleated, and whole retinas were isolated. Retinal dissections were performed in chilled Ames medium supplemented with sodium bicarbonate, and pH was buffered by bubbling with 95% CO₂/5% O₂. Whole retinas were placed on nitrocellulose paper (Millipore) in a slicing chamber with ganglion cells oriented downward and cut to 150- μ m-thick slices using a tissue slicer. Slices were then transferred to an adjacent recording chamber, secured with vacuum grease for recording, and perfused with Ames medium bubbled with 95% CO₂/5% O₂ at room temperature at a rate of 3–5 ml/min.

Solutions and recording. Patch pipettes were pulled from fire-polished thin-walled capillary tubing (World Precision Instruments) to resistances of 5–8 M Ω using a two-stage vertical puller (Model PP-830, Narishige). Recordings had input resistances of $\sim$ 1 G Ω and series resistances of 5–20 M Ω .

To block inhibitory currents, Ames medium was supplemented with the following (in μ M): 1 strychnine, 100 picrotoxin, 50 6-tetrahydropyridin-4-yl methylphosphonic acid (TPMPA). Four micromolar L-AP4 was added to activate mGluR6 receptors. The internal solution for most experiments contained the following (in mM): 100 gluconic acid, 40 HEPES, 2.5 EGTA, 4 Mg-ATP, and 1 Li-GTP. $MgCl_2$ in the internal solution ranged from 3 to 4 mM for normal $[Mg^{2+}]_i$ (resulting in free $[Mg^{2+}]_i$ of 1.7–2 mM) and 0–1 mM for low $[Mg^{2+}]_i$ (resulting in free $[Mg^{2+}]_i$ of 0.5–1 mM); pH was adjusted to 7.3 using cesium hydroxide. The free $[Mg^{2+}]_i$ was calculated using WebMaxCStandard chelation calculator (<http://www.stanford.edu/~cpatton/webmaxc.htm>). In experiments in which we differentiated between rod and ON cone bipolar cells, Alexa Fluor 488 was added to the internal solution at a concentration of 50 μ M.

Experimental drugs were added to the internal solution and were dissolved either in DMSO [U73122, U73343, Wortmannin, 2-acetyl-1-oleoyl-sn-glycerol (OAG), and RHC 80267], to a final DMSO concentration of 0.1%, or in double-distilled H₂O (diC8-PIP₂, PKC inhibitor peptide 19–36). Experimental drug concentrations are specified in Results. LY341495 or RS- α -cyclopropyl-4-phosphonophenyl glycine (CPPG), mGluR antagonists, was prepared fresh daily in Ames medium to final concentrations of 500 and 10 μ M, respectively and were applied to the bipolar cell dendrites by pressure application into the outer plexiform layer. Application of either mGluR antagonist allowed for displacement of L-AP4 (an mGluR agonist), which produced a simulated synaptic response due to shutting off of mGluR6. Unless otherwise noted, all voltage-clamp recordings were performed at a holding potential of +38 mV (corrected for the solution junction potential, which was measured to be +12 mV). Inhibitory feedback responses in RBCs were evoked by puffing glutamate at a concentration of 50 μ M into the inner plexiform layer (IPL) near the RBC terminals to activate the A17 amacrine cells. During experiments in which we compared rise and decay kinetics in WT and PKC $\alpha^{-/-}$ mice, a local perfusion system containing our standard external solution was used to facilitate drug diffusion in an attempt to control for variability in diffusion rates between different slice preparations. Drugs were purchased from Sigma-Aldrich, except for L-AP4, LY341495, CPPG, U73122, U73343, and PKC inhibitor peptide 19–36, which were purchased from Tocris Bioscience.

Data analysis and acquisition. Recordings were obtained using Axograph X (John Clements), and data were analyzed using Axograph X and Kaleidograph (Synergy Software). Percentages of increase/decrease were calculated as the ratio of the mean normalized response amplitude at 5 min to the response at break-in.

Results

Inhibition of PLC depresses the TRPM1 current

We simulated light responses from mouse ON bipolar cells by bathing retinal slices in the mGluR6 agonist L-AP4, which activates the mGluR6 cascade and closes TRPM1, as is observed in darkness. Light onset was simulated by pressure application of an mGluR antagonist (LY341495 or CPPG), which displaces L-AP4 from the receptor and shuts off mGluR6 signaling. To establish a role for PLC in modulating the TRPM1 current, we added the PLC inhibitor U73122 (10 μ M) to the intracellular solution. We observed a rapid depression of the current to $50.61 \pm 4.78\%$ of the response at break-in following 5 min of dialysis with U73122 (Fig. 1*A,C,F*) ($n = 9$; $p < 0.0001$, compared with vehicle alone at 5 min). This effect was not seen when using U73343, an inactive analog of this drug (Fig. 1*B,C*) ($n = 9$; mean current amplitude after 5 min was $105.55 \pm 6.35\%$ of the original amplitude).

The depression of the TRPM1 current could be caused by increased levels of PIP₂. To test this possibility, we dialyzed cells with wortmannin, which, at the concentration used in this study (5 μ M), prevents PIP₂ synthesis by blocking PI4 kinase activity (Sorensen et al., 1998; Xie et al., 1998). If excess PIP₂ were inhibiting the channel, we would expect to observe a potentiation of the current upon addition of wortmannin to the intracellular solution. However, wortmannin reduced the current to $54.17 \pm 8.55\%$ of the amplitude at break-in (Fig. 1*D,F*) ($n = 5$). To confirm that excess PIP₂ had no direct effect on the amplitude of the current, we added a nonhydrolyzable analog of PIP₂, diC8-PIP₂, to the intracellular solution. The addition of diC8-PIP₂ had no significant effect on TRPM1 current amplitude after 5 min of recording (Fig. 1*E,F*) ($94.02 \pm 9.58\%$ of the current at break-in; $n = 6$).

Exogenous DAG potentiates the TRPM1 current in ON bipolar cells

The observations that both depletion of PIP₂ and inhibition of PLC depresses TRPM1 current suggest that a metabolite downstream of PIP₂ modulates the ON bipolar cell synaptic channel. Both DAG and IP₃ have been implicated in modulation of several members of the TRP family of channels. In particular, evidence suggests that phospholipids like DAG may play a role in modulating current amplitude through multiple mechanisms (Leung et al., 2008; Tu et al., 2009). To explore this possibility, we augmented endogenous levels of DAG by adding OAG, a cell-permeable DAG analog, to the intracellular solution. Consistent with the idea that DAG enhances the TRPM1 current, dialysis with OAG potentiated the current to $192.58 \pm 1.15\%$ of the response at break-in (Fig. 2*A,B*) ($n = 11$; $p < 0.0001$), with no effect on the baseline current. We next tested the possibility that addition of exogenous DAG could rescue the effects of PLC inhibition on TRPM1 current. In the presence of 100 μ M OAG, U73122 (10 μ M) failed to inhibit the TRPM1 current (Fig. 2*C,D*) (response at 5 min = $98.53 \pm 9.40\%$ of response at break-in; $n = 5$). These data support the idea that inhibition of PLC depresses the TRPM1 current due to depletion of DAG, and that this effect can be reversed with addition of exogenous DAG.

There are several mechanisms that could account for modifications of the ON bipolar cell synaptic current by DAG. One such mechanism involves the use of polyunsaturated fatty acids (PUFAs). PUFAs are produced by the cleavage of DAG by the enzyme DAG lipase and have been shown to play a role in the regulation of TRP channels (Leung et al., 2008). We therefore tested the possibility that PUFAs modulate TRPM1 by inhibiting DAG lipase. Figure 2, *E* and *F*, shows representative traces and a

summary of the effects of dialyzing the cell with the DAG lipase inhibitor RHC 80267 ($n = 5$). At concentrations of 10 and 15 μM , RHC 80267 did not significantly increase the current amplitude over time ($136.48 \pm 17.24\%$ of the response at break-in), implying that PUFAs do not modulate TRPM1 current. Reducing hydrolysis of DAG by inhibiting DAG lipase increases free intracellular DAG, and these higher levels of DAG may account for the slight potentiation that we observe.

Potentiation by DAG is PKC dependent

DAG is an activator of PKC (Nishizuka, 1984; Nishizuka et al., 1984), which is expressed at high levels in certain classes of ON bipolar cells (Greferath et al., 1990; Fyk-Kolodziej et al., 2002). We therefore tested the possibility that DAG potentiates TRPM1 current by activating PKC α . Many ion channels contain PKC phosphorylation sites that render them susceptible to phosphorylation-induced changes in function. We used a PKC inhibitory peptide (10 μM) to determine whether preventing PKC phosphorylation would affect the ability of exogenous DAG to potentiate the current. Dialysis of the PKC inhibitory peptide in combination with OAG prevented the OAG-mediated increase in the current amplitude (Fig. 3*A,C*) ($n = 6$; $127.4 \pm 16.99\%$ of the response at break-in), which suggests that the activation of PKC is required for modulation.

As an independent approach, we measured the effects of OAG in a mouse model in which PKC α is knocked out. In the PKC $\alpha^{-/-}$ model, OAG had no effect on the current amplitude (Fig. 3*B,C*) ($n = 5$, peak response at 5 min = $109.70 \pm 8.80\%$ of the response at break-in), while control mice with the same genetic background as the PKC α -null mice still exhibited potentiation in the presence of OAG (response at 5 min increased to $168.98 \pm 3.894\%$ of the response at break-in; $n = 8$, $p = 0.001$ compared with control response at 5 min; data not shown).

We next determined whether PKC α potentiated TRPM1 currents independently of the magnitude of the current, or whether it shifted the dose–response function for TRPM1 activation. We performed dose–response experiments by varying the length of application of the mGluR6 antagonist. Figure 3*D* compares the responses from WT and PKC $\alpha^{-/-}$ mice to antagonist puff durations of 5, 10, 100, 200, 300, and 1000 ms. The responses in WT mice were larger than those in PKC $\alpha^{-/-}$ mice, but when we normalized the responses to the maximum response for each puff duration, there was clearly no difference in the dose–response function between WT and PKC $\alpha^{-/-}$ mice. This suggests that PKC α performs a linear scaling of the TRPM1 current without altering the sensitivity of the channel to weak stimuli.

Modulation by PKC α is specific to the rod pathway

Because RBCs express PKC α , while ON CBCs do not, we wanted to determine whether DAG-mediated potentiation of TRPM1 was rod pathway specific. (Greferath et al., 1990). We identified rod- and cone-driven bipolar cells based on the shape of their

Figure 1. Inhibition of PLC depresses the TRPM1 current. *A*, LY341495-induced current responses from an ON bipolar cell at break-in (black trace) and after 5 min of dialysis (gray trace) with the PLC inhibitor U73122 (10 μM , $n = 9$). *B*, ON bipolar cell current responses at break-in (black trace) and after 5 min of dialysis (gray trace) with the inactive PLC inhibitor analog U73343 (10 μM , $n = 9$). *C*, Time course summarizing the effects of U73122 (circles) on the TRPM1 current amplitude, compared with the response in the presence of U73343 (triangles) and in control conditions in which the cell was dialyzed with DMSO (squares; $n = 8$). Error bars indicate SD of the peak amplitude from the mean value at each time point; mean value at 5 min in the presence of U73122 compared with control was significantly different (Student's *t* test, $*p < 0.0001$). *D*, ON bipolar cell current responses at break-in (black trace) and after 5 min of dialysis (gray trace) with the PI4 kinase inhibitor wortmannin (5 μM , $n = 5$). *E*, ON bipolar cell current responses at break-in (black trace) and after 5 min of dialysis (gray trace) with the nonhydrolyzable PIP₂ analog diC8-PIP₂ (100 μM , $n = 6$). *F*, Histogram representing the mean TRPM1 response amplitudes at 5 min normalized to the mean response at break-in under control conditions and in the presence of U73122, wortmannin, and diC8-PIP₂. Compared with control conditions, wortmannin caused significant current depression after 5 min (Student's *t* test, $*p = 0.02$); no change was observed in the presence of diC8-PIP₂.

axon terminals and the depth of termination into sublamina b of the inner plexiform layer (Fig. 4*A*) (Kolb and Famiglietti, 1974; Saito and Kujiraoka, 1982; Hartveit, 1997; Ghosh et al., 2004). In cells confirmed to be RBCs, 100 μM OAG produced a greater than twofold potentiation of TRPM1 current (Fig. 4*B,D*) (amplitude at 5 min = $207.14 \pm 13.17\%$ of the amplitude at break-in; $n = 16$). It has been reported that there are two morphologically distinct subtypes of RBCs that differ in their axon stratification pattern (Wu et al., 2004). We observed similar amounts of potentiation among all cells that we confirmed to be RBCs. Conversely, we saw no effect of OAG in ON CBCs over the course of 5 min (Fig. 4*C,D*) (amplitude at 5 min = $102.62 \pm 17.03\%$ of response at break-in; $n = 6$). These findings are consistent with the known expression pattern of PKC α in mouse ON bipolar cells.

Activation of PKC α relieves Mg²⁺-dependent inhibition of TRPM1

Several members of the TRPM subfamily of channels are susceptible to blocking or inhibition by intracellular Mg²⁺; this property is also shared by TRP channels outside of the TRPM subfamily (Chen and Huang, 1992; Nadler et al., 2001; Voets et al., 2004; Li et al., 2007; Macianskiene et al., 2008; Parnas et al., 2009), and there is evidence that membrane phospholipids or constituents of phospholipid signaling pathways may regulate the effects of Mg²⁺ on these channels (Chen and Huang, 1992; Macianskiene et al., 2008; Parnas et al., 2009). To test the possibility that TRPM1 might be similarly regulated by Mg²⁺, we recorded

Figure 2. Exogenous DAG potentiates the TRPM1 current in ON bipolar cells. **A**, ON bipolar cell responses at break-in (black trace) and after 5 min of dialysis (gray trace) with the DAG analog OAG (100 μ M, $n = 11$). **B**, Comparison of ON bipolar cell responses at break-in and after 5 min of dialysis with OAG (triangles represent individual cells; squares represent the mean at each time point). Error bars indicate the SD of the peak amplitude from the mean (Student's t test; $*p < 0.0001$). **C**, ON bipolar cell responses at break-in (black trace) and after 5 min of dialysis (gray trace) with both U73122 and OAG (10 and 100 μ M, respectively; $n = 5$). **D**, Time course depicting the effects of dialysis with U73122 + OAG compared with U73122 alone. Responses for each time point were normalized to the mean peak response at break-in. Error bars indicate SD from the mean peak response. Statistical significance was determined by comparing the difference in the normalized response at 5 min in the presence of U73122 + OAG to U73122 alone (Student's t test, $*p = 0.002$). **E**, ON bipolar cell responses at break-in (black trace) and after 5 min of dialysis (gray trace) with the DAG lipase inhibitor RHC 80267 (10–15 μ M; $n = 5$). **F**, Individual (triangles) and mean (squares) responses from ON bipolar cell at break-in and after 5 min of dialysis with RHC 80267. Error bars indicate the SD of the peak amplitude from the mean.

from RBCs using two ranges of $[Mg^{2+}]_i$: the range of 1.7–2 mM free Mg^{2+} (which is the concentration range used for all previous experiments); and a lower range of 0.5–1 mM Mg^{2+} (Fig. 5A). At a holding potential of +50 mV, we observed a highly significant correlation between response amplitude and Mg^{2+} concentration in both rod- and cone-driven ON bipolar cells. The mean peak amplitude in 0.5–1 mM Mg^{2+} was 18.50 ± 2.09 pA for RBCs ($n = 7$) and 19.79 ± 2.64 pA for ON CBCs. In the presence of 1.7–2.0 mM Mg^{2+} , the mean peak response amplitude dropped to 8.80 ± 1.05 and 10.47 ± 1.59 pA, respectively ($n = 16$ and 8, respectively), indicating that TRPM1 that is expressed at both the rod–RBC synapse and the cone–ON CBC synapse is inhibited by increasing concentrations of Mg^{2+} ($p = 0.001$ and 0.004 for RBCs and ON CBCs in 0.5–1 mM free Mg^{2+} compared with 1.7–2 mM Mg^{2+}).

To determine whether PKC α might act to relieve Mg^{2+} inhibition of TRPM1 current in the rod pathway, we next compared the effects of OAG in RBCs in the presence of different $[Mg^{2+}]_i$ values. In 0.5–1.0 mM Mg^{2+} , OAG had no significant effect on the amplitude of the TRPM1 current (Fig. 5B) ($n = 9$; mean response amplitudes before and after dialysis were 19.06 ± 3.06 and 18.445 ± 3.96 pA respectively; $p = 0.8203$). The different effects of

Figure 3. Potentiation by OAG is PKC α dependent. **A**, ON bipolar cell responses from wild-type mice at break-in (black trace) and after 5 min of dialysis (gray trace) with a PKC inhibitor peptide and OAG (10 and 100 μ M, respectively; $n = 10$). **B**, ON bipolar cell responses from PKC $\alpha^{-/-}$ mice at break-in (black trace) and after 5 min of dialysis (gray trace) with OAG (100 μ M; $n = 10$). **C**, Histogram comparing the ON bipolar cell responses to LY341495 under control conditions and after dialysis with OAG in WT mice in which PKC function is uninterrupted, in PKC $\alpha^{-/-}$ mice, and in WT mice under conditions of pharmacological inhibition of PKC. **D**, Dose–response curve depicting the mean ON bipolar cell responses to the mGluR antagonist CPPG of varying puff durations in wild-type and PKC $\alpha^{-/-}$ mice ($n = 7$ for both WT and PKC $\alpha^{-/-}$ mice). Puff lengths ranged from 5 to 1000 ms. Responses were normalized to the maximum response for each puff length. Both plots were fitted with the Hill equation; Hill coefficients for WT and PKC $\alpha^{-/-}$ were 0.998 and 0.995, respectively. Inset, Raw responses to CPPG at 5, 10, 100, 200, 300, and 1000 ms for ON bipolar cells in WT (left) and PKC $\alpha^{-/-}$ (right) mice. Calibration: 5 pA, 1 s.

OAG on RBCs dialyzed with 0.5–1 mM free Mg^{2+} compared with cells dialyzed with 1.7–2.0 mM free Mg^{2+} was highly significant (Fig. 5C) ($n = 9$ and 16 respectively; $p = 0.001$). In addition, TRPM1 current amplitudes of WT RBCs in 1.7–2 mM Mg^{2+} and OAG were nearly identical to the current amplitudes observed in 0.5–1 mM Mg^{2+} either in the presence or absence of OAG. This is consistent with the idea that the effects of PKC α activation on the TRPM1 current are occluded in low Mg^{2+} .

Inhibition of RBCs by Mg^{2+} is not voltage dependent

Based on classic examples of Mg^{2+} channel inhibition, intracellular magnesium could act via a voltage-dependent block of the TRPM1 channel pore or through an inhibition of the channel that is independent of voltage (Mayer et al., 1984; Gwanyanya et al., 2004). To determine whether Mg^{2+} inhibition of RBC current is dependent on voltage, we performed whole-cell recordings in 1.7–2 and 0.5–1 mM Mg^{2+} and ramped the membrane potential from -60 to $+70$ mV while alternating between puffs of LY341495 to open TRPM1 and no puffs (Fig. 6). TRPM1 current was isolated by subtraction of the control ramp from the ramp obtained in the presence of LY341495. Voltage-dependent inhibition would be indicated by inward rectification in the presence of 1.7–2 mM Mg^{2+} , with smaller current at positive voltage due to block of the TRPM1 pore by Mg^{2+} , as it exits the channel. Conversely, the current–voltage (I – V) relationship in 0.5–1 mM Mg^{2+} would display little or no inward rectification. However, although comparison of the I – V relationship of the raw responses shows that the magnitude of TRPM1 current is significantly larger in 0.5–1.0 mM Mg^{2+} (mean amplitude at -60 and $-60 = 19.97 \pm 2.74$ and 10.63 ± 1.63 pA in 1.7–2 mM Mg^{2+} , and -42.14 ± 3.98 and 24.79 ± 3.89 pA in 0.5–1 mM Mg^{2+} , respec-

tively), there was no difference in the rectification properties in the two ranges of Mg_i^{2+} (Fig. 6A). This is shown more clearly in Figure 6B, where the I - V relations have been normalized to allow for comparison of overall shape. These data indicate that the effects of Mg_i^{2+} on TRPM1 are independent of voltage.

The ramps used here are of relatively short duration, spanning the complete range of voltage in 100 ms. When measured in this way, the I - V relationship describing TRPM1 is relatively linear. On the other hand, previous measurements of TRPM1 made use of voltage steps, each lasting for many seconds, and revealed a strong outward rectification of the I - V (Shen et al., 2009). Thus, the I - V characteristics of a channel can change dramatically depending upon the protocol used to measure the relationship.

PKC α activation at the rod-RBC synapse does not affect the kinetics of the simulated light response

A recent study of the ERG b-wave, an indicator of ON bipolar function (Stockton and Slaughter, 1989) found that both the time to peak and decay time were slowed in PKC $\alpha^{-/-}$ mice compared with wild-type mice (Ruether et al., 2010). To test the possibility that the effect of PKC α on the b-wave is due to modulation of TRPM1 current, we compared the kinetics of the current in wild-type and PKC $\alpha^{-/-}$ animals. Both the rise and decay of individual responses could be reasonably well fitted with a single exponential. We saw no difference in the mean rise time (time constant = 0.2103 ± 0.05 s and 0.2098 ± 0.034 s for WT and PKC $\alpha^{-/-}$ mice, respectively), and a small but statistically insignificant change in the mean decay time (time constant = 1.029 ± 0.51 s and 1.268 ± 0.10 s for WT and PKC $\alpha^{-/-}$ mice, respectively). We also recorded RBC responses in current-clamp and saw no difference in either the rise or the decay kinetics between the two (Fig. 7B) (WT mean rise time = 0.116 ± 0.042 s, decay time = 0.776 ± 0.308 s, $n = 8$; PKC $\alpha^{-/-}$ mean rise time = 0.089 ± 0.059 s, decay time = 1.241 ± 1.379 s, $n = 4$). It should be noted that the time of decay of the response elicited by local application of mGluR6 antagonist was much slower than the decay times reported in the ERG measurements. Thus, recovery from local mGluR6 antagonist application is likely to be limited by events upstream from G-protein activation and TRPM1 channel closure, such as removal of the antagonist from the tissue, or antagonist unbinding from the mGluR6 receptor.

The b-wave might reflect the influence of other synaptic pathways on the shape of the light response in bipolar cells in addition to the intrinsic TRPM1 current, as PKC α is widely expressed throughout RBCs, including the axon terminal. We therefore considered the possibility that PKC α plays a role in regulating inhibitory feedback from amacrine cells onto RBCs, and that disruption of this process could account for some of the reported changes in the ERG b-wave. We evoked feedback inhibitory currents in RBCs by puffing glutamate into the IPL near the RBC terminal to activate neighboring amacrine cells (Fig. 7C,D)

Figure 4. Modulation by PKC α is rod pathway specific. **A**, Example of an RBC (left) and an ON CBC (right). Bipolar cells were filled with Alexa Fluor 488 ($50 \mu M$), and were classified based on the shape of their axon terminals (RBC terminals were more bulbous, while ON CBC terminals were more diffuse) and the depth of extension into the IPL (RBCs extended into sublamina b, while ON CBCs terminated higher in the IPL, in sublamina a). **B**, RBC response at break-in (black trace) and after 5 min of dialysis (gray trace) with OAG ($100 \mu M$; $n = 16$). **C**, CBC response at break-in (black trace) and after 5 min of dialysis (gray trace) with OAG ($100 \mu M$; $n = 6$). **D**, Time course depicting the effects of dialyzing RBCs and ON CBCs with OAG. Error bars indicate SD from the mean peak response. Statistical significance was determined by comparing the difference in the normalized mean response RBC response at 5 min in the presence of OAG with that of the normalized mean ON CBC response (Student's t test, $*p = 0.003$).

(Chávez and Diamond, 2008, Chávez et al., 2010). Currents were identified as GABAergic IPSCs by their reversal potential and susceptibility to block by picrotoxin and TPMPA (data not shown) (Hartveit, 1999, Molnar and Werblin, 2007, Chávez and Diamond, 2008, Chávez et al., 2010). Interestingly, IPSCs were significantly lower in amplitude on average in the absence of PKC α . In WT animals, the mean IPSC amplitude was 22.52 ± 3.44 pA, while that of the PKC $\alpha^{-/-}$ animals was 8.00 ± 0.90 pA ($n = 17$ for WT and $n = 13$ for PKC $\alpha^{-/-}$; Student's t test, $p = 0.0007$). Activators of PKC have been shown to modulate GABAA receptors on bipolar cells (Gillette and Dacheux, 1996), albeit in the opposite direction, but effects on GABAC receptors have not been reported. Our finding leaves open the possibility that PKC α can regulate inhibitory feedback at RBC terminals, potentially providing an explanation for the discrepancy between effects of PKC α on the b-wave and those reported here, but a more definitive conclusion will require further study.

Discussion

Our results show that the DAG analog OAG robustly potentiates TRPM1 current evoked by pharmacologically inhibiting mGluR6. Potentiation is prevented by intracellular dialysis with a PKC inhibitory peptide, and in mice lacking expression of PKC α , indicating a role for this kinase. Analysis of the effects of PKC α activation as a function of Mg_i^{2+} suggests that PKC α may potentiate TRPM1 current by providing relief from Mg^{2+} inhibition. OAG had no effect when dialyzed into ON bipolar cells in internal solutions with free Mg_i^{2+} calculated to be 0.5 – 1.0 mM. Higher free Mg_i^{2+} caused greater inhibition of TRPM1, and the current could be rescued by activation of PKC α . Measurements of free Mg_i^{2+} in mammalian neurons using Mg^{2+} -sensitive dyes have ranged from 0.3 to 1.5 mM (Brocard et al., 1993, Li-Smerin et al.,

Figure 5. Activation of PKC α relieves Mg^{2+} -dependent inhibition of TRPM1. **A**, Comparison of peak RBC and ON CBC responses from WT mice in low (0.5–1 mM) Mg^{2+}_i and normal (1.7–2 mM) Mg^{2+}_i . Both RBC and ON CBC response amplitude decreased with increasing $[Mg^{2+}]_i$; for both cell types, there was a significant difference between the mean amplitude of the responses in 0.5–1 mM $[Mg^{2+}]_i$ conditions compared with the 1.7–2 mM range (Student's t test; RBCs: 1.7–2 mM Mg^{2+}_i , $n = 16$; 0.5–1 mM, $n = 7$, $p = 0.001$; ON CBCs: 1.7–2 mM Mg^{2+}_i , $n = 9$, 0.5–1 mM, $n = 11$. There was no significant difference in the mean peak response amplitude between RBCs and ON CBCs at either $[Mg^{2+}]_i$ (1.7–2 mM Mg^{2+}_i : $p = 0.21$; 0.5–1 mM Mg^{2+}_i : $p = 0.75$). **B**, Summary of the effect of OAG in WT RBCs in 0.5–1 mM $[Mg^{2+}]_i$. No significant difference was observed between the response amplitude at break-in and after 5 min of dialysis with OAG (100 μM ; $n = 9$). **C**, Histogram summarizing the different effects of OAG in WT RBCs in low and high $[Mg^{2+}]_i$. Both the mean peak response at 5 min normalized to the response amplitude at break-in (histogram) and individual normalized peak responses (filled circles) are plotted for each $[Mg^{2+}]_i$ condition. Statistical significance was determined by comparing normalized peak response at 5 min in low and high $[Mg^{2+}]_i$ ($n = 9$ and 11 for low and high $[Mg^{2+}]_i$, respectively; Student's t test, $*p = 0.001$). **D**, Comparison of effect of OAG on the peak RBC responses from WT mice in normal (1.7–2 mM) and low (0.5–1 mM) $[Mg^{2+}]_i$. In WT RBCs, addition of OAG potentiated the current to $200.06 \pm 1.05\%$ of the current at break-in (Student's t test, high, $n = 16$, low, $n = 9$; $*p = 0.001$). No significant difference was observed in the mean amplitudes in PKC $\alpha^{-/-}$ RBCs in the two $[Mg^{2+}]_i$ conditions ($p = 0.6954$).

2001, Henrich and Buckler, 2008). To our knowledge, there are no published measurements of free Mg^{2+} in ON bipolar cells, but extrapolation from other neurons suggests that the $[Mg^{2+}]_i$ is sufficient to constitutively inhibit TRPM1.

Differences between the rod and ON-cone pathways

Although there is a consensus that TRPM1 is required for generation of the light response in ON bipolar cells (Morgans et al., 2009, Nakajima et al., 2009, Shen et al., 2009, Koike et al., 2010), it is less clear whether both RBCs and CBCs express the same channel, or if they express separate isoforms. In fact, two distinct isoforms of TRPM1 have been identified in the retina (Oancea et al., 2009), although their expression pattern is not known. Ca^{2+} -dependent desensitization of TRPM1 in RBCs, but not CBCs, has been reported in mouse retina (Berntson et al., 2004), consistent with the possibility of multiple TRPM1 isoforms that are differentially activated by Ca^{2+} -calmodulin, but a direct interaction TRPM1 with the Ca^{2+} -calmodulin complex has yet to be demonstrated.

In the present study, we have found that potentiation by OAG of TRPM1 occurs in RBCs exclusively, consistent with the known expression of PKC α in ON bipolar cells. Analysis of the response amplitude of ON CBCs in 1.7–2 and 0.5–1 mM Mg^{2+}_i indicates that the TRPM1 channel that is expressed in both rod- and cone-

Figure 6. Inhibition of RBCs by Mg^{2+} is not voltage dependent. **A**, **B**, Top, Voltage ramp protocol. Membrane potential was stepped to -60 for 800 ms before a 100 ms ramp from -60 to $+70$ mV (shaded box). LY341495 was applied so that the current peak reached a steady state before starting the ramp. **A**, Bottom, I - V relationship of the raw peak responses in the presence of 1.7–2 mM Mg^{2+}_i (solid line; $n = 9$) and 0.5–1 mM Mg^{2+}_i (dashed line; $n = 90$). Error bars indicate SEM. Mean current amplitude at -60 mV was -19.97 ± 2.74 pA for 1.7–2 mM Mg^{2+}_i and -42.14 ± 3.98 pA for 0.5–1 mM Mg^{2+}_i , $p = 0.0004$; mean current amplitude at $+60$ mV was 10.63 ± 1.63 pA for 1.7 mM Mg^{2+}_i and 24.79 ± 3.89 pA for 0.5–1 mM Mg^{2+}_i , $p = 0.007$, Student's t test. **B**, Bottom, I - V relationship of the responses in the presence of 1.7–2 mM Mg^{2+}_i (solid line) and 0.5–1 mM Mg^{2+}_i (dashed line) normalized to the peak response at -60 mV.

Figure 7. PKC α activation at the rod-RBC synapse does not affect the kinetics of the simulated light response. **A**, Mean normalized, scaled TRPM1 voltage-clamp responses in WT ($n = 17$) and PKC $\alpha^{-/-}$ mice ($n = 13$). **B**, Mean normalized, scaled TRPM1 current-clamp responses in WT ($n = 8$) and PKC $\alpha^{-/-}$ mice ($n = 4$). **C**, Representative RBC IPSCs in WT ($n = 17$) and PKC $\alpha^{-/-}$ mice ($n = 13$). **D**, Histogram summarizing the mean peak amplitude of IPSCs in RBCs from WT and PKC $\alpha^{-/-}$ mice; filled circles represent peak responses plotted for each. Statistical significance was determined by comparing the mean peak amplitude (22.52 ± 3.44 pA and 8.00 ± 0.90 pA for WT and PKC $\alpha^{-/-}$, respectively; Student's t test, $*p = 0.0007$).

driven ON bipolar cells is similarly inhibited by intracellular Mg^{2+} , implying that the major difference between these two pathways is the ability of PKC α to relieve this inhibition in RBCs. It is therefore unclear whether CBCs do not exhibit this regulation simply because they do not express PKC α , or because they express a variant of TRPM1 that is not susceptible to PKC α modulation. One approach to address this issue would be to expose the CBC TRPM1 channel to activated PKC α catalytic subunit.

However, the present study does not address the question of whether the target of PKC α is TRPM1 or an intermediate protein. Therefore, failure to observe potentiation of TRPM1 current in CBCs could result from lack of expression of intermediate proteins rather than differences in the TRPM1 channel itself.

PKC α activation and expression

PKC α is expressed at both the dendrites and the axon terminals of the RBC, and there is evidence for adaptation state-dependent expression at one or the other of these cellular compartments. In goldfish, it has been shown that PKC α is localized to the soma and dendrites in the light-adapted state, but that it translocates to the axon terminals in the dark (Vaquero et al., 1996, Gabriel et al., 2001, Behrens et al., 2007). Regulation of PKC α distribution by adaptation state provides a potential mechanism by which PKC α could be selectively turned on in RBC dendrites. In the present study, all experiments were performed in light-adapted retinas, and so further experiments performed on dark-adapted retinas will be required to determine whether the effect of OAG-activated PKC α is heightened or dampened under dark-adapted conditions. Our results suggest that PKC α may also play a role in regulation of GABAergic feedback onto RBC terminals. During recruitment to the dendrites, a corresponding decrease in PKC α levels at the axon terminal might decrease the amplitude of the IPSC at the RBC–amacrine cell reciprocal synapse, enhancing transmission from RBCs to downstream ganglion cells (Völgyi et al., 2002, Freed et al., 2003, Eggers and Lukasiewicz, 2006, Chávez et al., 2010).

Comparison of the effects of PKC α on TRPM1 current and the b-wave

Ruether et al. (2010) recently reported that both the rise and decay time of the ERG b-wave are delayed in PKC α ^{−/−} mice, without any obvious change in amplitude. While the results presented here and those reported by Ruether et al. (2010) do not overlap, they are also not mutually exclusive. The pharmacological method that we employ to elicit TRPM1 currents has the advantage of allowing us to focus on our synapse of interest and isolate the postsynaptic mechanism at this synapse. In this study, we were therefore able to focus on the role of PKC α in signal transmission and channel function solely at the RBC dendrites. While the authors of the aforementioned study observed no significant changes in the b-wave amplitude, we provide strong evidence that there are significant changes in the amplitude of the TRPM1 current across a range of levels of PKC α activation.

When we compared relative latencies of the response in the WT and the PKC α ^{−/−} mice, we did not detect changes in the kinetics of this response. However, we acknowledge that our method of eliciting currents may present technical barriers to determining absolute response kinetics. For instance, it is possible that the rate of the simulated light response is limited by the rate at which the mGluR agonists and antagonists diffuse through the tissue. If this is the case, we cannot accurately determine whether or not we see changes in the response kinetics that parallel those shown by Ruether et al. (2010).

We conclude that at this synapse, PKC α amplifies the rod-driven signal, but that perhaps the effect that we present here is masked in the ERG by other changes attributable to the loss of PKC α that are upstream or downstream of the rod–RBC synapse. Studies by Dong and Hare (2000) suggest that both the amplitude and kinetics of ERG b-wave can be affected by the input from third-order neurons. It is therefore possible that, in addition to technical and methodological differences, some of the discrepan-

cies between our findings and those reported by Ruether et al. (2010) may be due to the fact that there are greater network effects caused by loss of PKC α that manifest in changes in the ERG b-wave kinetics.

References

- Behrens UD, Borde J, Mack AF, Wagner HJ (2007) Distribution of phosphorylated protein kinase C alpha in goldfish retinal bipolar synaptic terminals: control by state of adaptation and pharmacological treatment. *Cell Tissue Res* 327:209–220.
- Berntson A, Smith RG, Taylor WR (2004) Postsynaptic calcium feedback between rods and rod bipolar cells in the mouse retina. *Vis Neurosci* 21:913–924.
- Brocard JB, Rajdev S, Reynolds IJ (1993) Glutamate-induced increases in intracellular free Mg²⁺ in cultured cortical neurons. *Neuron* 11:751–757.
- Chávez AE, Diamond JS (2008) Diverse mechanisms underlie glycinergic feedback transmission onto rod bipolar cells in rat retina. *J Neurosci* 28:7919–7928.
- Chávez AE, Grimes WN, Diamond JS (2010) Mechanisms underlying lateral GABAergic feedback onto rod bipolar cells in rat retina. *J Neurosci* 30:2330–2339.
- Chen L, Huang LY (1992) Protein-Kinase-C Reduces Mg²⁺ Block of Nmda-Receptor Channels as a Mechanism of Modulation. *Nature* 356:521–523.
- Chubanov V, Gudermann T, Schlingmann KP (2005) Essential role for TRPM6 in epithelial magnesium transport and body magnesium homeostasis. *Pflügers Arch* 451:228–234.
- Daniels RL, Takashima Y, McKemy DD (2009) Activity of the neuronal cold sensor TRPM8 is regulated by phospholipase C via the phospholipid phosphoinositol 4,5-bisphosphate. *J Biol Chem* 284:1570–1582.
- Dong CJ, Hare WA (2000) Contribution to the kinetics and amplitude of the electroretinogram b-wave by third-order retinal neurons in the rabbit retina. *Vision Res* 40:579–590.
- Eggers ED, Lukasiewicz PD (2006) Receptor and transmitter release properties set the time course of retinal inhibition. *J Neurosci* 26:9413–9425.
- Fleig A, Penner R (2004) The TRPM ion channel subfamily: molecular, biophysical and functional features. *Trends Pharmacol Sci* 25:633–639.
- Freed MA, Smith RG, Sterling P (2003) Timing of quantal release from the retinal bipolar terminal is regulated by a feedback circuit. *Neuron* 38:89–101.
- Fyk-Kolodziej B, Cai W, Pourcho RG (2002) Distribution of protein kinase C isoforms in the cat retina. *Vis Neurosci* 19:549–562.
- Gabriel R, Lesauter J, Silver R, Garcia-España A, Witkovsky P (2001) Diurnal and circadian variation of protein kinase C immunoreactivity in the rat retina. *J Comp Neurol* 439:140–150.
- Ghosh KK, Bujan S, Haverkamp S, Feigenspan A, Wässle H (2004) Types of bipolar cells in the mouse retina. *J Comp Neurol* 469:70–82.
- Gillette MA, Dacheux RF (1996) Protein kinase modulation of GABA currents in rabbit retinal rod bipolar cells. *J Neurophysiol* 76:3070–3086.
- Greferath U, Grünert U, Wässle H (1990) Rod bipolar cells in the mammalian retina show protein kinase C-like immunoreactivity. *J Comp Neurol* 301:433–442.
- Gwanyanya A, Amuzescu B, Zakharov SI, Macianskiene R, Sipido KR, Bolutina VM, Verecke J, Mubagwa K (2004) Magnesium-inhibited, TRPM6/7-like channel in cardiac myocytes: permeation of divalent cations and pH-mediated regulation. *J Physiol* 559:761–776.
- Hartveit E (1997) Functional organization of cone bipolar cells in the rat retina. *J Neurophysiol* 77:1716–1730.
- Hartveit E (1999) Reciprocal synaptic interactions between rod bipolar cells and amacrine cells in the rat retina. *J Neurophysiol* 81:2923–2936.
- Henrich M, Buckler KJ (2008) Effects of anoxia, aglycemia, and acidosis on cytosolic Mg²⁺, ATP, and pH in rat sensory neurons. *Am J Physiol Cell Physiol* 294:C280–C294.
- Kerschbaum HH, Kozak JA, Cahalan MD (2003) Polyvalent cations as permeant probes of MIC and TRPM7 pores. *Biophys J* 84:2293–2305.
- Koike C, Obara T, Uriu Y, Numata T, Sanuki R, Miyata K, Koyasu T, Ueno S, Funabiki K, Tani A, Ueda H, Kondo M, Mori Y, Tachibana M, Furukawa T (2010) TRPM1 is a component of the retinal ON bipolar cell transduction channel in the mGluR6 cascade. *Proc Natl Acad Sci U S A* 107:332–337.
- Kolb H, Famiglietti EV (1974) Rod and cone pathways in the inner plexiform layer of cat retina. *Science* 186:47–49.

- Kosaka J, Suzuki A, Morii E, Nomura S (1998) Differential localization and expression of alpha and beta isoenzymes of protein kinase C in the rat retina. *J Neurosci Res* 54:655–663.
- Kraft R, Harteneck C (2005) The mammalian melastatin-related transient receptor potential cation channels: an overview. *Pflugers Arch* 451:204–211.
- Leung HT, Tseng-Crank J, Kim E, Mahapatra C, Shino S, Zhou Y, An L, Doerge RW, Pak WL (2008) DAG lipase activity is necessary for TRP channel regulation in *Drosophila* photoreceptors. *Neuron* 58:884–896.
- Li M, Du J, Jiang J, Ratzan W, Su LT, Runnels LW, Yue L (2007) Molecular determinants of Mg²⁺ and Ca²⁺ permeability and pH sensitivity in TRPM6 and TRPM7. *J Biol Chem* 282:25817–25830.
- Li-Smerin Y, Levitan ES, Johnson JW (2001) Free intracellular Mg²⁺ concentration and inhibition of NMDA responses in cultured rat neurons. *J Physiol* 533:729–743.
- Macianskiene R, Gwanyanya A, Verecke J, Mubagwa K (2008) Inhibition of the magnesium-sensitive TRPM7-like channel in cardiac myocytes by nonhydrolysable GTP analogs: involvement of phosphoinositide metabolism. *Cell Physiol Biochem* 22:109–118.
- Mayer ML, Westbrook GL, Guthrie PB (1984) Voltage-dependent block by Mg²⁺ of NMDA responses in spinal cord neurones. *Nature* 309:261–263.
- Molnar A, Werblin F (2007) Inhibitory feedback shapes bipolar cell responses in the rabbit retina. *J Neurophysiol* 98:3423–3435.
- Morgans CW, Zhang J, Jeffrey BG, Nelson SM, Burke NS, Duvoisin RM, Brown RL (2009) TRPM1 is required for the depolarizing light response in retinal ON-bipolar cells. *Proc Natl Acad Sci U S A* 106:19174–19178.
- Nadler MJ, Hermosura MC, Inabe K, Perraud AL, Zhu Q, Stokes AJ, Kurokaki T, Kinet JP, Penner R, Scharenberg AM, Fleig A (2001) LTRPC7 is a MgATP-regulated divalent cation channel required for cell viability. *Nature* 411:590–595.
- Nakajima Y, Iwakabe H, Akazawa C, Nawa H, Shigemoto R, Mizuno N, Nakanishi S (1993) Molecular characterization of a novel retinal metabotropic glutamate receptor mGluR6 with a high agonist selectivity for L-2-amino-4-phosphonobutyrate. *J Biol Chem* 268:11868–11873.
- Nakajima Y, Moriyama M, Hattori M, Minato N, Nakanishi S (2009) Isolation of ON bipolar cell genes via hrGFP-coupled cell enrichment using the mGluR6 promoter. *J Biochem* 145:811–818.
- Nawy S (1999) The metabotropic receptor mGluR6 may signal through G(o), but not phosphodiesterase, in retinal bipolar cells. *J Neurosci* 19:2938–2944.
- Nishizuka Y (1984) The role of protein kinase C in cell surface signal transduction and tumour promotion. *Nature* 308:693–698.
- Nishizuka Y, Takai Y, Kishimoto A, Kikkawa U, Kaibuchi K (1984) Phospholipid turnover in hormone action. *Recent Prog Horm Res* 40:301–345.
- Nomura A, Shigemoto R, Nakamura Y, Okamoto N, Mizuno N, Nakanishi S (1994) Developmentally regulated postsynaptic localization of a metabotropic glutamate receptor in rat rod bipolar cells. *Cell* 77:361–369.
- Oancea E, Vriens J, Brauchi S, Jun J, Splawski I, Clapham DE (2009) TRPM1 forms ion channels associated with melanin content in melanocytes. *Sci Signal* 2:ra21.
- Parnas M, Katz B, Lev S, Tzarfaty V, Dadon D, Gordon-Shaag A, Metzner H, Yaka R, Minke B (2009) Membrane lipid modulations remove divalent open channel block from TRP-like and NMDA channels. *J Neurosci* 29:2371–2383.
- Peng H, Lewandrowski U, Müller B, Sickmann A, Walz G, Wegierski T (2010) Identification of a protein kinase C-dependent phosphorylation site involved in sensitization of TRPV4 channel. *Biochem Biophys Res Commun* 391:1721–1725.
- Ruether K, Feigenspan A, Pirngruber J, Leitges M, Baehr W, Strauss O (2010) PKC α is essential for the proper activation and termination of rod bipolar cell response. *Invest Ophthalmol Vis Sci* 51:6051–6058.
- Runnels LW, Yue L, Clapham DE (2002) The TRPM7 channel is inactivated by PIP(2) hydrolysis. *Nat Cell Biol* 4:329–336.
- Saito T, Kujirakawa T (1982) Physiological and morphological identification of two types of on-center bipolar cells in the carp retina. *J Comp Neurol* 205:161–170.
- Schmitz C, Perraud AL, Johnson CO, Inabe K, Smith MK, Penner R, Kurokaki T, Fleig A, Scharenberg AM (2003) Regulation of vertebrate cellular Mg²⁺ homeostasis by TRPM7. *Cell* 114:191–200.
- Shen Y, Heimel JA, Kamermans M, Peachey NS, Gregg RG, Nawy S (2009) A transient receptor potential-like channel mediates synaptic transmission in rod bipolar cells. *J Neurosci* 29:6088–6093.
- Sorensen SD, Linseman DA, McEwen EL, Heacock AM, Fisher SK (1998) A role for a wortmannin-sensitive phosphatidylinositol-4-kinase in the endocytosis of muscarinic cholinergic receptors. *Mol Pharmacol* 53:827–836.
- Stockton RA, Slaughter MM (1989) B-wave of the electroretinogram. A reflection of ON bipolar cell activity. *J Gen Physiol* 93:101–122.
- Tu P, Kunert-Keil C, Lucke S, Brinkmeier H, Bouron A (2009) Diacylglycerol analogues activate second messenger-operated calcium channels exhibiting TRPC-like properties in cortical neurons. *J Neurochem* 108:126–138.
- Vaquero CF, Velasco A, de la Villa P (1996) Protein kinase C localization in the synaptic terminal of rod bipolar cells. *Neuroreport* 7:2176–2180.
- Vardi N (1998) Alpha subunit of Go localizes in the dendritic tips of ON bipolar cells. *J Comp Neurol* 395:43–52.
- Voets T, Nilius B, Hoefs S, van der Kemp AW, Droogmans G, Bindels RJ, Hoenderop JG (2004) TRPM6 forms the Mg²⁺ influx channel involved in intestinal and renal Mg²⁺ absorption. *J Biol Chem* 279:19–25.
- Völgyi B, Xin D, Bloomfield SA (2002) Feedback inhibition in the inner plexiform layer underlies the surround-mediated responses of AII amacrine cells in the mammalian retina. *J Physiol* 539:603–614.
- Wu SM, Gao F, Pang JJ (2004) Synaptic circuitry mediating light-evoked signals in dark-adapted mouse retina. *Vision Res* 44:3277–3288.
- Xie LH, Takano M, Kakei M, Okamura M, Noma A (1999) Wortmannin, an inhibitor of phosphatidylinositol kinases, blocks the MgATP-dependent recovery of Kir6.2/SUR2A channels. *J Physiol* 514:655–665.