On bipolar cells are connected to photoreceptors via a sign-inverting synapse. At this synapse, glutamate binds to a metabotropic receptor which couples to the closure of a cation-selective transduction channel. The molecular identity of both the receptor and the G protein are known, but the identity of the transduction channel has remained elusive. Here, we show that the transduction channel in mouse rod bipolar cells, a subtype of On bipolar cell, is likely to be a member of the TRP family of channels. To evoke a transduction current, the metabotropic receptor antagonist LY341495 was applied to the dendrites of cells that were bathed in a solution containing the mGluR6 agonists 1-AP4 or glutamate. The transduction current was suppressed by ruthenium red and the TRPV1 antagonists capsazepine and SB-366791. Furthermore, focal application of the TRPV1 agonists capsaicin and anandamide evoked a transduction-like current. The capsacin-evoked and endogenous transduction current displayed prominent outward rectification, a property of the TRPV1 channel. To test the possibility that the transduction channel is TRPV1, we measured rod bipolar cell function in the TRPV1−/− mouse. The ERG b-wave, a measure of On bipolar cell function, as well as the transduction current and the response to TRPV1 agonists were normal, arguing against a role for TRPV1. However, ERG measurements from mice lacking TRPM1 receptors, another TRP channel implicated in rod bipolar transduction suggested that the cation channel may be a member of the CNG family of channels, based on the observation that cGMP strongly potentiates the current (Nawy and Jahr, 1994) and the transient receptor potential (TRP) channels have emerged as candidates for the transduction channel (Shiells et al., 1981; Slaughter and Miller, 1981).

**Introduction**

Glutamate hyperpolarizes On bipolar cells by closing a cation-selective channel (Shiells et al., 1981; Slaughter and Miller, 1981). The glutamate receptor (Nakajima et al., 1993; Nomura et al., 1994) and the G protein (Yardi et al., 1993; Nawy, 1999; Dhingra et al., 2000) that mediate this response have been identified, but the cation channel has not. Two major families of cation-selective channels are the cyclic nucleotide-gated channels (CNG) (Cra-ven and Zagotta, 2006) and the transient receptor potential (TRP) channels (Ramsey et al., 2006). Previous studies of On bipolar transduction suggested that the cation channel may be a member of the CNG family of channels, based on the observation that cGMP strongly potentiates the current (Nawy and Jahr, 1990; Shiells and Falk, 1990). However, it was later shown that the channel is unlikely to be gated directly by cGMP, but rather that cGMP has a modulatory role (Nawy, 1999; Snellman and Nawy, 2004).

In the vertebrate retina, pharmacological evidence suggests that a member of the TRP channel family is likely expressed in light-sensitive ganglion cells (Warren et al., 2006; Hartwick et al., 2007; Sekaran et al., 2007). In On bipolar cells, two types of TRP channels have emerged as candidates for the transduction channel. One candidate is TRPV1, which is expressed predominantly in the peripheral nervous system and mediates heat sensation. Both TRPV1 and the On bipolar cell transduction channel are moderately permeable to Ca2+ with a Ca2+/Na+ permeability ratio of 9.6 in TRPV1 channels expressed in oocytes (Caterina et al., 1997) and 4.9 in salamander On bipolar cells (Nawy, 2000). The entry of Ca2+ activates a negative feedback pathway leading to desensitization of both the On bipolar cell transduction current (Shiells and Falk, 1999; Nawy, 2000; Bernston et al., 2004; Naray, 2004) and the response to heat and capsaicin mediated by TRPV1 (Liu and Simon, 1996; Caterina et al., 1997; Koplas et al., 1997; Piper et al., 1999). Here, we present evidence that the transduction channel can be activated by both capsaicin and anandamide, compounds that are thought to be specific agonists for TRPV1. Another candidate channel is the founding member of the family of melastatin-related TRP channels (TRPM1). Recent
studies of Appaloosa horses have demonstrated that a dramatic reduction in the expression of mRNA encoding TRPM1 is a possible cause of night blindness and a reduced b-wave in the ERGs (Sandmeyer et al., 2007; Bellone et al., 2008). Both are indicative of a disruption of ON bipolar cell function, implying that TRPM1 may play a role in mGLR6 signal transduction. We, therefore, set out to characterize the functional properties of the transduction channel and to further evaluate the possibility that it is composed of TRPV1 or TRPM1 subunits.

Materials and Methods

Preparation of slices. Retinal slices from 4- to 6-week-old C57BL/6 mice (Charles River) and TRPV1 knock-out mice (TRPV1\textsuperscript{-/-}), The Jackson Laboratory) were prepared as described previously (Snellman and Nawy, 2004). Briefly, after killing, whole retinas were isolated and placed on a cellulose acetate/nitrate membrane filter (Millipore), secured with vacuum grease to a glass slide adjacent to the recording chamber. Slices were cut to a thickness of 100 μm using a tissue slicer (Stoelting), transferred to the recording chamber while remaining submerged, and viewed with a Nikon E600FN upright microscope equipped with a water-immersion 40× objective and differential interference contrast optics.

Solutions and drug application. Slices were continuously perfused with Ames media bubbled with 95% O\textsubscript{2}/5% CO\textsubscript{2}. Whole-cell recording solutions were composed of (in mM) 120 NaCl, 5 KCl, 2 CaCl\textsubscript{2}, 1 MgCl\textsubscript{2}, 10 glucose, and 25 HEPES, pH 7.4. Whole-cell record- ing and analysis. Whole-cell recordings were obtained with an Axopatch 1D amplifier (Molecular Devices). Currents were acquired at a sampling rate of 2 kHz with Axograph X software and an Apple G5 computer, low-pass filtered at 50 Hz (Frequency Devices) and digitized with an ITC-18 interface (Heka). Holding potentials were corrected for the liquid junction potential, which was measured to be 10 mV with the standard K\textsuperscript{+} gluconate pipette solution. Recordings were discarded if the series resistance exceeded 20 MΩ. Data were analyzed off-line with Axograph X and Kaleidagraph (Synergy Software). Plots of normalized conductance of the transduction channel versus voltage were fit with a Boltzmann relation of the form \( g = \frac{g_{\text{max}} - g_{\text{min}}}{1 + \exp(V_{m} - V_{1/2})/k} + g_{\text{min}} \) where \( g_{\text{max}} \) is the maximum conductance, \( g_{\text{min}} \) is the minimum conductance, \( V_{m} \) is the voltage at which the conductance is half of maximum, and \( k \) is the slope factor RT/2F, where \( R \) is the valance of the gating charge. Holding potential for all cells was +40 mV, unless indicated otherwise.

ERGs were recorded to flash stimuli presented to the dark-adapted eye from TRPV1+/− mice using a previously described procedure (Gregg et al., 2007) and from TRPM1+/− mice using a procedure that was generally similar but used a different anesthetic (urethane, 2 g/kg), maximum stimulus duration (5 ms), and sampling rate (10 kHz). The a-wave was measured at 8 ms from the prestimulus baseline, whereas the b-wave was measured from the a-wave trough to the positive peak. To test the hypothesis that the transduction channel is a member of the TRP family, we first examined the effects of compounds known to antagonize TRP channels on the rod bipolar cell transduction current.

Results

The rod bipolar cell transduction current is inhibited by TRP antagonists

To test the hypothesis that the transduction channel is a member of the TRP family, we first examined the effects of compounds known to antagonize TRP channels on the rod bipolar cell transduction current. To evoke a transduction current, rod bipolar cells were bathed in either 1 mM glutamate or 4 mM t-AP4 and then exposed to brief applications of the mGLR agonist LY341495 (100 μM). Blockade of mGLR6 resulted in the opening of the transduction channel which, at positive voltages, generated an outward current (Fig. 1A) (mean amplitude, 30.4 ± 3.0 pA; general Valve), and the mGLR6 agonist t-AP4 (4 μM) was added to the bath. In other experiments, drugs were applied via a fast-flow apparatus (Snellman and Nawy, 2004), and glutamate was used as an mGLR6 agonist. Drugs and chemicals were purchased from Sigma, with the exceptions of t-AP4 and LY341495 (Tocris Bioscience) and AM251 (Caymen Chemical).

Figure 1. The rod bipolar cell transduction current is blocked by antagonists of TRPV1. A, Response to 100 μM LY341495 (delivered via fast-flow apparatus) before (left) and after (center) a 3 min application of 10 μM ruthenium red. Right, Response of another cell to a 1 s puff of LY341495 delivered through a puffer pipette alone (top) or during simultaneous application of ruthenium red from a second puffer pipette (middle). Ruthenium red was applied alone for 10 s before obtaining the middle trace. The inhibition of ruthenium red was readily reversed using this approach (bottom). Calibration: 10 pA, 2 s. B, Response to LY341495 before and after a 5 min application of 100 μM 2-APB. C, D, Responses to 1 s puffs of LY341495 (100 μM) before and after 5 min bath application of 20 μM capsaicin (C) and 20 μM SB366791 (D). D. Responses to LY341495 typically showed partial recovery after removal of antagonists, as shown in the right panel of D. Traces in C and D are from different cells. E, Summary of results. The number of cells for each experiment is indicated above each bar.
TRPV1 agonists evoked a current with properties that are similar to the transduction current

Our results suggest that TRPV1 agonists are capable of blocking the gating of the transduction channel by the endogenous activator of the channel. We, therefore, tested the possibility that TRPV1 agonists can activate the rod bipolar cell transduction current. Application of 10 μM capsaicin, the prototypical TRPV1 agonist (Caterina et al., 1997), elicited a response in every rod bipolar cell that we examined (Fig. 2A) (mean amplitude, 14.8 ± 1.4 pA; n = 41). To examine the specificity of capsaicin, we applied it to Off bipolar cells, which were identified morphologically by dye filling and physiologically by their lack of response to LY341495. Application of capsaicin to Off bipolar cells produced no detectable response (n = 4; data not shown). We also recorded from rod bipolar cells in mice that were 8–9 d old. At this age, there was no detectable response to LY341495 or capsaicin (n = 4; data not shown), suggesting that the transduction cascade was not yet functionally developed. Finally, the response to capsaicin was completely blocked by capsazepine (n = 2) (Fig. 2A).

The endocannabinoid anandamide, another agonist of TRPV1 receptors (Caterina et al., 1997; Jordt and Julius, 2002; van der Stelt et al., 2005), also elicited a response in rod bipolar cells (Fig. 2B) (mean amplitude, 9.6 ± 1.7 pA; n = 8). The response to anandamide was inhibited by capsazepine (34.8% of control; n = 2) but was unaffected by the cannabinoid-1 receptor antagonist AM251 (105.4% of control; n = 3), indicating that it is not attributable to activation of cannabinoid receptors.

To more closely compare the transduction current and the current elicited by capsaicin, we measured the relationship between current and voltage by varying the holding potential from −80 to +80 mV in 20 mV increments. Figure 2C, D, E, F. Plots of normalized conductance for the cells of the transduction channel and the capsaicin-gated channel. Lines are the fits to a Boltzmann function (see Materials and Methods). Plots were obtained from the same sets of cells whose I–V relations are summarized in C and D, using the equation $g = g_{\text{max}} V_{\text{rev}}/(V_{\text{rev}} - V_m)$, where $V_{\text{rev}} = 0$ mV, to obtain the conductance for each cell.
TRPM1 and TRPV1 are a component of the transduction cascade, we recorded from et al., 2008). To address the possibility that one or both channels are a member of the family of TRP channels, perhaps TRPM1. The b-wave, which is thought to be generated by the opening of On bipolar cell synaptic channels, is normal in a TRPV1 knock-out mouse but completely eliminated in a mouse lacking functional TRPM1 channels. Our results are consistent with a recent study showing expression of TRPM1 RNA in mouse On bipolar cells (Kim et al., 2008). To date, a physiological characterization of TRPM1 has yet to be reported, and so it is unclear if this TRP channel can be gated by endovanilloids or whether TRPM1 currents rectify as do those of many other TRP channels. Although it is tempting to speculate that the current evoked by endovanilloids in rod bipolar cells is attributable to the opening of TRPM1 channels, confirmation of this hypothesis will require further investigation of the functional properties of TRPM1.
Our findings are consistent with the findings of several recent studies of bipolar cell function in Appaloosa horses. In these horses, there is a link between a specific pattern of coat coloration and congenital stationary night blindness (Sandmeyer et al., 2007). Animals with this coloration lack the ERG b-wave, indicating a loss of function of On bipolar cells, although the structure of the retina appears normal (Witzel et al., 1978). Genetic analysis of this phenotype revealed decreased expression of mRNA encoding TRP channel TRPM1 (Bellone et al., 2008). Of course, the loss of On bipolar cell function could potentially result from a number of underlying etiologies other than a mutation in the transduction channel (McCall and Gregg, 2008). Nevertheless, an intriguing possibility, based on the results presented here and previous work on the Appaloosa horse, is that the transduction channel in the dendrites of rod bipolar cells is composed of TRPM1, either as a homomer or in association with other TRP channels.

References