Ion Channel Compartments in Photoreceptors: Evidence From Salamander Rods With Intact and Ablated Terminals

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MacLeish PR, Nurse CA. Ion channel compartments in photoreceptors: evidence from salamander rods with intact and ablated terminals. *J Neurophysiol* 98: 86–95, 2007. First published April 25, 2007; doi:10.1152/jn.00775.2006. Vertebrate photoreceptors are highly polarized sensory cells in which several different ionic currents have been characterized. In the present study we used whole cell voltage-clamp and optical imaging techniques, the former combined with microsurgical manipulations, and simultaneous recording of membrane current and intracellular calcium signals to investigate the spatial distribution of ion channels within isolated salamander rods. In recordings from intact rods with visible terminals, evidence for five previously identified ionic currents was obtained. These include two Ca\(^{2+}\)-dependent, i.e., a Ca\(^{2+}\)-dependent chloride current \(I_{\text{Cl(Ca)}}\) and a large-conductance Ca\(^{2+}\)- and voltage-dependent K\(^+\) or BK current \(I_{\text{BK(Ca)}}\), and three voltage-dependent currents, i.e., a delayed-rectifier type current \(I_{\text{K(V)}}\), a hyperpolarization-activated current \(I_{\text{h}}\), and a dihydropyridine-sensitive L-type calcium current \(I_{\text{Ca}}\). Of these, \(I_{\text{Cl(Ca)}}\) was highly correlated with the presence of a terminal; rods with visible terminals expressed \(I_{\text{Cl(Ca)}}\) without exception \((n = 125)\), whereas approximately 71% of rods \((40/56)\) without visible terminals lacked \(I_{\text{Cl(Ca)}}\). More significantly, \(I_{\text{Cl(Ca)}}\) was absent from all rods \((n = 33)\) that had their terminals ablated, and recordings from the same cell before and after terminal ablation led, in all cases \((n = 10)\), to the loss of \(I_{\text{Cl(Ca)}}\). In contrast, \(I_{\text{BK(Ca)}}\), \(I_{\text{K(V)}}\), and \(I_{\text{h}}\) remained largely intact after terminal ablation, suggesting that they arose principally from ion channels located in the soma and/or inner segment. The outward \(I_{\text{K(Ca)}}\) in terminal-ablated rods was reversibly suppressed on “puffing” a Ca\(^{2+}\)-free extracellular solution over the soma and was appreciably enhanced by the L-type Ca\(^{2+}\) channel agonist, Bay K 8644 \((0.1–2 \mu M)\). These data indicate that rod photoreceptors possess discrete targeting mechanisms that preferentially sort ion channels mediating \(I_{\text{Cl(Ca)}}\) to the terminal.

INTRODUCTION

Neurons and other cell types express various ion channels and receptors that are distributed within distinct compartments or domains. This differential distribution appears critical for the ability of neurons and receptor cells to receive and transduce internal or external signals and to relay appropriate physiological responses (Trimmer and Rhodes 2004). There is compelling evidence for a heterogeneity in the distribution of ion channels that underlie membrane conductances (Pyott et al. 2004; Trimmer and Rhodes 2004). For example, a combination of patch-clamp recording and optical imaging in tissue slices has shown that L-type Ca\(^{2+}\) channels are concentrated in presynaptic terminals of cerebellar basket cells (Llano et al. 1997) and retinal bipolar cells (Protti and Llano 1998), giving rise to discrete hot spots of Ca\(^{2+}\) entry. This spatial heterogeneity is not restricted to Ca\(^{2+}\) channels because patch-clamp and immunofluorescence studies have also revealed a preferential sorting of voltage-gated K\(^+\), as well as voltage- and Ca\(^{2+}\)-activated BK, channels between the soma and terminals in the CNS (Misonou et al. 2006; Southan and Robertson 1998; Trimmer and Rhodes 2004). The Ca\(^{2+}\)-activated BK channels, encoded by Slo1-α-subunits, have been well studied and their expression pattern indicates a high accumulation in axons and nerve terminals of central neurons (Misonou et al. 2006; Trimmer and Rhodes 2004). On the other hand, Ca\(^{2+}\)-activated BK channels were surprisingly found clustered in the apex of mouse inner hair cells away from the basal synaptic sites (Pyott et al. 2004), thereby raising questions about their physiological function.

Vertebrate photoreceptors represent a class of highly polarized cells whose morphology is characterized by two major specializations (see Wässle and Boycott 1991). At the apical end, the outer segment is specialized for phototransduction, whereas at the basal pole, the synaptic terminal releases neurotransmitter onto second-order cells. Several studies suggest that the light-induced voltage response and modulation of transmitter release from photoreceptors are determined, not only by events in the outer segment, but also by a variety of conductance mechanisms in the soma/inner segment and terminals (Attwell et al. 1982; Bader et al. 1982; Barnes and Hille 1989; Maricq and Kornbrodt 1988; Yagi and MacLeish 1994). In isolated light-adapted salamander rods, five currents have been identified in voltage-clamp studies (Attwell et al. 1982; Bader et al. 1982; Corey et al. 1984). These consist of two Ca\(^{2+}\)-dependent currents, i.e., Ca\(^{2+}\)-activated K\(^+\) current \(I_{\text{K(Ca)}}\) and Ca\(^{2+}\)-activated Cl\(^-\) current \(I_{\text{Cl(Ca)}}\), and three voltage-dependent currents, i.e., a hyperpolarization-activated current \(I_{\text{h}}\), a TEA-sensitive delayed-rectifier type current \(I_{\text{K(V)}}\), and a dihydropyridine-sensitive L-type calcium current \(I_{\text{Ca}}\). There is strong evidence that the L-type Ca\(^{2+}\) channel density is highest in the synaptic terminal of salamander and mammalian rod photoreceptors, giving rise to nonuniform or compartmentalized increases in intracellular calcium (Krizaj and Copenhagen 2002; Morgans et al. 1998; Nachman-Clewner et al. 1999; Steele et al. 2005). However, little is known about the relative distribution of the remaining ion channels, although Ca\(^{2+}\)-activated K\(^+\) or BK channels were reported to be highly localized in salamander rod terminals (Xu and Slaughter 2005). To address this, we used patch-clamp techniques to record whole cell currents in isolated salamander rods that were either morphologically intact or lacking termi-
nals as a result of the dissociation procedure or microablation (MacLeish and Nurse 2000). We obtained evidence for the presence of functional L-type Ca\(^{2+}\) channels in rods with ablated terminals. Interestingly, the two Ca\(^{2+}\)-dependent currents, \(I_{\text{Cl(Ca)}}\) and \(I_{\text{K(Ca)}}\), showed a different spatial distribution with \(I_{\text{Cl(Ca)}}\) being highly localized to the rod terminal. In contrast, \(I_{\text{K(Ca)}}\) and the other voltage-dependent currents, \(I_{\text{K(V)}}\) and \(I_{\text{h}}\), remained relatively unchanged in terminal-ablated rods, suggesting they arose principally from the soma and inner segment. We also used simultaneous recording of membrane current and of changes in intracellular calcium, using fura-2, to confirm which cellular compartment contributes to \(I_{\text{Cl(Ca)}}\).

METHODS

Cell dissociation

Dissociation of salamander retina was carried out using enzymatic digestion with papain, followed by trituration, as previously described (Lam 1972; MacLeish et al. 1983). The cell suspension was plated into central wells of modified 35-mm culture dishes that were previously coated with Sal-1 antibody to aid cell attachment (MacLeish et al. 1983). The well was formed by sealing a glass coverslip to the underside of the culture dish in which a hole was drilled. The surface was prepared by first adding 100 \(\mu\)l of affinity-purified goat antimouse antibody (0.2 mg/ml) for >1 h. The well was rinsed once with salt solution and then 100 \(\mu\)l of Sal-1 hybridoma supernatant were added for >1 h. Unbound antibody was removed by rinsing three times with salt solution before cells were plated. The dispersed cells were grown in a medium containing 108 mM NaCl, 3 mM KCl, 2 mM HEPES, 1 mM NaHCO\(_3\), 0.5 mM NaHPO\(_4\), 1 mM sodium pyruvate, 0.5 mM MgCl\(_2\), 0.5 mM MgSO\(_4\), 16 mM glucose, 1.8 mM CaCl\(_2\), and 100 \(\mu\)g/ml bovine serum albumin (BSA). Cells were maintained in a humidified chamber at 10°C in air for ≤3 days.

Whole cell recording

The methods followed those of Hamill et al. (1981). Briefly, dishes were mounted on a cooled stage of a Zeiss Axiovert 35 or 200M microscope equipped with phase-contrast optics. Patch-clamp pipettes were pulled on a BB-CH-PC (Mecanex S.A., Geneva, Switzerland) or Flaming/Brown P-97 (Sutter Instrument, Novato, CA) electrode puller and had a tip diameter of approximately 1 \(\mu\)m and a resistance of approximately 10 M\(\Omega\). Membrane rupture was achieved by gentle suction applied to the inside of the pipette after seal formation. For most experiments, the composition of the pipette solution was as follows (in mM): KCl 108, NaCl 10, HEPES 10, EGTA 0.05, MgCl\(_2\) 5, and ATP 1. The pH was adjusted to 7.0–7.2. For a few experiments the pipette EGTA concentration was increased to 5 mM as described in a recent study (Xu and Slaughter 2005). Also, in some experiments, 75 mM gluconate replaced chloride in the pipette. Because these pipette modifications did not affect the main conclusions of the present study, data were pooled where appropriate. In experiments designed to block K\(^-\) currents, cesium was substituted for K\(^-\) in the pipette solution and tetraethylammonium (TEA) chloride was added to the bath. Data acquisition and analysis were carried out using pClamp software version 6.0.3 or 9.2 (Axon Instruments, Union City, CA).

Intracellular Ca\(^{2+}\) measurements

Intracellular calcium levels were monitored using fura-2 and dual-wavelength stimulation (340/380 nm) while recording at 510 nm. Cells were incubated in 10 \(\mu\)g/ml fura-2 AM (Molecular Probes) for 30 min, rinsed three times with amphibian salt solution, and kept at 10°C until needed. Light stimulation and data acquisition were performed by a computer-controlled system (Stallion 4.1. Intelligent Imaging Innovations). To reduce the bleaching effects over the long recording times, cells were stimulated for 10 ms at 340 and 380 nm followed by 500 ms of no stimulation. The camera was a Zeiss AxioCam HSpm mounted on a Zeiss Axiovert 200M. The objective was a Zeiss Fluar \(\times 40\) oil-immersion NA 1.3. Images were typically binned \(4 \times 4\). Square regions of interest (ROIs) were used as permitted by the software package. For the terminal, the ROI was the smallest square that included the terminal. Background subtraction of the glass coverslip was applied to all cells. The optical recording was externally synchronized to pClamp to compare changes in calcium with electrophysiological responses. The optical system was calibrated using standards from Molecular Probes and concentration estimates were obtained using a \(K_d\) of 225 mM.

RESULTS

The polarized structural features of rod photoreceptors, including outer and inner segment, axonlike projection, and terminal enlargement, are readily retained after isolation and attachment to the substrate in vitro (MacLeish and Townes-Anderson 1988; MacLeish et al. 1983; Mandell et al. 1993). In a given culture, however, the dissociation procedure yielded a variable proportion of intact rods, rods with no discernable outer segment, and rods with terminals that were either missing or in various stages of retraction. In the present study, we also took advantage of our ability to ablate the terminals of selected rods with the tip of a patch pipette (MacLeish and Nurse 2000), without overt damage to the rest of the cell (for example, Fig. 2, A1 and A2). Thus patch-clamp recordings of membrane currents could be obtained from rods that were: 1) morphologically intact (with prominent terminals); 2) lacking visible terminals; and 3) lacking terminals, as a result of mechanical microablation under visual guidance.

General properties of \(I_{\text{Cl(Ca)}}\) in intact photoreceptors

A Ca\(^{2+}\)-dependent chloride current, \(I_{\text{Cl(Ca)}}\), has been described in vertebrate photoreceptors (Bader et al. 1982; Barnes and Hille 1989; Marcq and Korenbrot 1988; Morgans et al. 1998; Yagi and MacLeish 1994). However, irrespective of whether it is located predominantly in the soma or terminals (or both) is presently unclear. This current is usually observed as an inward tail current, after repolarizations to voltages below \(E_{\text{Cl}}\), from prolonged depolarizing steps that facilitate calcium entry. In the present study, we found that \(I_{\text{Cl(Ca)}}\) could readily be recorded from intact rods with patch pipettes containing either high (Fig. 1A) or low (not shown) chloride concentrations. These currents resembled those recorded from isolated salamander photoreceptors in previous studies (Bader et al. 1982; Barnes and Hille 1989). With high [KCl] pipettes, the peak amplitude of the inward \(I_{\text{Cl(Ca)}}\) tail current in intact rods varied typically between 100 and 500 pA (mean ± SE = 268 ± 33 pA; \(n = 15\)); the duration of \(I_{\text{Cl(Ca)}}\) measured from the beginning to the end of the tail current, showed broad variability (see Fig. 1), lasting typically between 1 and 12 s (mean = 5.5 ± 0.9 s; \(n = 12\)).

In many cells recorded with high [KCl] pipettes, \(I_{\text{Cl(Ca)}}\) was robust and its presence was revealed during moderate depolarizing steps to about −30 mV by a prominent inward current superimposed on the usual outward K\(^+\) current at these potentials (e.g., Fig. 1A, arrow). Consistent with chloride being the
main charge carrier, this inward current reversed near 0 mV (i.e., the chloride equilibrium potential), in three experiments where outward K\textsuperscript{+}/H\textsuperscript{+} currents were abolished using CsCl pipettes and bath-applied TEA (15 mM; e.g., Fig. 1B). The dependency of $I_{\text{Cl(Ca)}}$ on entry of extracellular Ca\textsuperscript{2+} was demonstrated in experiments in which Ca\textsuperscript{2+} entry was substantially reduced or abolished by “puffing” Ca\textsuperscript{2+}-free solutions over the cell ($n = 8$), or the L-type Ca\textsuperscript{2+} channel blocker, 10 \mu M nitrendipine ($n = 12$). An example of the reversible blockade of $I_{\text{Cl(Ca)}}$ (and of outward current) during perfusion with 150 \mu M cadmium is shown in Fig. 1, C1–C3.

Evidence that $I_{\text{Cl(Ca)}}$ requires the presence of a rod terminal

The frequency of successful recordings of $I_{\text{Cl(Ca)}}$ from morphologically intact rods in normal extracellular medium, with KCl- or K-gluconate–filled pipettes, was 100\% ($n = 125$). In contrast, we observed that in the majority (~71\%) of cases ($n = 40/56$), $I_{\text{Cl(Ca)}}$ was conspicuously absent in rods without visible terminals, when viewed under phase-contrast microscopy. These observations led us to hypothesize that the terminal was required for functional expression of $I_{\text{Cl(Ca)}}$ in rod photoreceptors. To test this hypothesis, we carried out repeated recordings from the same rod, one before and another after the terminal was ablated with the tip of a patch pipette. In all such cases ($n = 10$) $I_{\text{Cl(Ca)}}$ present before terminal ablation, was no longer present after ablation. Further, in 23 additional cells, the terminal was ablated before the initial recording and in each case $I_{\text{Cl(Ca)}}$ was not detectable. An example of repeated recordings from the same freshly isolated rod, where $I_{\text{Cl(Ca)}}$ was abolished after terminal ablation, is shown in Fig. 2, B1 and B2.

We used several criteria to validate that overt cell damage after terminal ablation did not account for the loss of $I_{\text{Cl(Ca)}}$ reported earlier. First, all cells were discarded when terminal ablation led to morphological changes in the cytoplasm of the soma that signaled loss of integrity, injury, or cell death; this was usually apparent within 45 s of terminal ablation and occurred in approximately 3\% of the experiments. Second, even when cells appeared “healthy” after terminal ablation, whole cell recording was discontinued if the holding current at –70 mV was >50 pA; in the majority of recordings from morphologically intact rods the holding current varied typically between 10 and 30 pA. Third, we considered acceptable, repeated recordings from the same cell where there was minimal change in magnitude of the voltage-activated outward K\textsuperscript{+} current during voltage steps to 0 mV (see example, Fig. 2D). This voltage represents the chloride equilibrium potential ($E_{\text{Cl}}$), where K\textsuperscript{+} outward current is dominant. Data from a group of five cells examined in this way are summarized in Fig. 2E, where the mean (±SE) outward current before and after

FIG. 1. Expression of Ca\textsuperscript{2+}-dependent chloride current [$I_{\text{Cl(Ca)}}$] in salamander rods. Voltage-clamp currents in response to step depolarizations from –70 mV to select voltages. A: total membrane current in control medium. $I_{\text{Cl(Ca)}}$ is seen as a prominent tail current at the end of the depolarizing steps and as an inward current at ~20 mV (smallest step, arrow). B: $I_{\text{Cl(Ca)}}$ reverses close to 0 mV. Outward currents were blocked by cesium in the pipette and TEA in the bath. Current responses changed polarity close to 0 mV, the reversal potential for chloride in this experiment. Note prominent tail current for all voltage steps. C: $I_{\text{Cl(Ca)}}$ requires external calcium. C1–C3 were obtained from the same cell before, during, and after puffing calcium-free solution. $I_{\text{Cl(Ca)}}$ disappeared in calcium-free solution along with the Cs\textsuperscript{+}-dependent outward currents during the voltage step.

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terminal ablation was not statistically different \( (P > 0.05) \) during voltage steps from \(-70\) to \(0\) mV.

During synapse maturation in vivo and in vitro, nerve cells utilize special targeting mechanisms that result in the accumulation of specific ion channels in presynaptic terminals (Misonou et al. 2006; Trimmer and Rhodes 2004). The terminal localization of \( I_{\text{Cl(Ca)}} \) in acutely isolated rods, and the fact that the majority of these rods had lost contact with their synaptic partners and other adjacent cells, led us to ask whether \( I_{\text{Cl(Ca)}} \) retained its polarized distribution after short-term culture. As exemplified in Fig. 2, \( C1 \) and \( C2 \), \( I_{\text{Cl(Ca)}} \) was still preferentially localized to the terminal even after 56 h in culture \((n = 2)\) because it was lost after terminal ablation. Thus the functional polarity of \( I_{\text{Cl(Ca)}} \) in isolated rods can be maintained in culture for \( >2 \) days without cell–cell or synaptic contact.

In addition to eliminating \( I_{\text{Cl(Ca)}} \), terminal ablation also removes an important source of Ca\(^{2+}\) entry into the photoreceptor by L-type Ca\(^{2+}\) channels located in the terminal (Krizaj and Copenhagen 1998; Steele et al. 2005; Thoreson et al. 2003). This led us to investigate further whether any residual \( I_{\text{Cl(Ca)}} \) could be unmasked in terminal-ablated rods by increasing the calcium load. To address this, we recorded from...
terminal-ablated rods in the presence of the L-type Ca$^{2+}$ channel agonist Bay K 8644 (3-pyridinecarboxylic acid, 1,4-dihydro-2,6-dimethyl-5-nitro-4-[2-(trifluoromethyl)phenyl]-, methyl ester) (Nowycky et al. 1985). As exemplified in Fig. 3 using ramp depolarizations, $I_{Cl(Ca)}$, visible in intact rods as a robust inward tail current and producing a small inward current inflection during the ramp (arrow in Fig. 3A), was negligible in rods with ablated terminals even when the Ca$^{2+}$ load was enhanced with 0.5 μM Bay K 8644 (Fig. 3, B1–B3; $n = 7$). Thus our inability to unmask $I_{Cl(Ca)}$ in terminal-ablated rods with a high calcium load points to specific targeting of $I_{Cl(Ca)}$ to the terminal.

Intracellular calcium imaging

We performed calcium imaging experiments on cells filled with fura-2 and compared the time course of the rise and fall of intracellular calcium in different cellular compartments with those of the simultaneously recorded tail current. An applied voltage step to 0 mV loaded the cell with calcium and, after repolarization to −70 mV, both the intracellular calcium transient and tail current were measured. The maximal amplitude of the tail current differed among the cells as did the maximal activity of intracellular calcium. We did, however, observe a consistent relationship between the duration of the tail current and that of the calcium changes in the terminal. Figure 4 shows the responses in three cells where it is easy to discern the greater similarity and tight correlation between the time course of the calcium changes in the terminal and time course of the tail current, as compared with calcium changes in the soma. The graph in Fig. 4D summarizes the relationship between the duration at half-maximal amplitude (t$_{1/2}$) for terminal and soma calcium concentration changes with that for the tail current. The t$_{1/2}$ for terminal calcium and for the tail current fell along a 45° line, as expected for the tight correlation between the two variables, whereas results for the soma showed much scatter and were poorly correlated. These results strengthen the claim that $I_{Cl(Ca)}$ is restricted to the terminal.

Calcium-dependent K$^+$ current $I_{K(Ca)}$ is robustly expressed in terminal-ablated rods

In contrast to $I_{Cl(Ca)}$, the other major Ca$^{2+}$-dependent current in rods, $I_{K(Ca)}$, was robustly expressed in rods with ablated terminals. At positive step potentials, the outward current in intact rods consists mainly of $I_{Cl(Ca)}$, $I_{K(Ca)}$, and voltage-activated, delayed-rectifier–type $I_{K(V)}$ currents. In the absence of selective blockers of $I_{Cl(Ca)}$, we studied K$^+$ currents in relative isolation using terminal-ablated rods because these lack $I_{Cl(Ca)}$ and the calcium current was small. As illustrated in Fig. 3, B1–B3, outward current in terminal-ablated rods was greatly enhanced (more than twofold) in the presence of Bay K 8644 ($n = 7$), indicating the presence of both dihydropyridine-sensitive L-type Ca$^{2+}$ and $I_{K(Ca)}$ currents in soma and/or inner segment. Additional experiments supported a robust expression of $I_{K(Ca)}$ in terminal-ablated rods. First, as exemplified in Fig. 5, A1–A3, when nominally Ca$^{2+}$-free solutions were applied from a “puffer” pipette positioned close to the soma, outward K$^+$ current was strongly and reversibly suppressed (see also Table 1); current suppression by low Ca$^{2+}$ occurred at all voltages positive to −50 mV. Second, a characteristic “hump” or N-shape in the current–voltage relation is
typical of Ca$^{2+}$-activated K$^+$ currents could readily be demonstrated during whole cell recordings from terminal-ablated rods ($n = 11$, see Fig. 5, A3 and A4). In a population of five cells with ablated terminals, the steady-state current at $+30$ mV was $1,448 \pm 141$ pA (mean $\pm$ SE). For three of these cells, the mean steady-state current at $+30$ mV in calcium-free solution was $1,297 \pm 81$ pA. In intact rods, the outward current was also markedly suppressed by 20–60 nM charybdotoxin (ChTx; see also, Xu and Slaughter 2005), a selective blocker of Ca$^{2+}$-activated large-conductance, maxi-K or BK channels (Galvez et al. 1990; Miller et al. 1985). The magnitude of this suppression by ChTx was approximately 73% (step to $+20$ mV), a value similar to that seen in Ca$^{2+}$-free solutions and in intact rods exposed to $150 \mu$M Cd$^{2+}$ (Table 1). Taken together, these data indicate that Ca$^{2+}$-dependent BK channels are robustly expressed in terminal-ablated rods and contrast with those reported in a recent study (Xu and Slaughter 2005).

$I_{K(V)}$ and $I_h$ are functionally expressed in terminal-ablated rods

Two other voltage-dependent currents have been described in salamander rods, i.e., a Ca$^{2+}$-insensitive, delayed-rectifier-type current [$I_{K(V)}$] and a nonselective cation current [$I_h$] that is activated by hyperpolarization (Bader et al. 1982). As exemplified in Fig. 5, A2 and A5, a detectable voltage-dependent outward current, $I_{K(V)}$, persisted when Ca$^{2+}$-dependent K$^+$ currents were blocked in terminal-ablated rods. In general, this current was considered to represent the major portion of the residual voltage-dependent current evoked during depolarizing steps in the presence of agents designed to block the Ca$^{2+}$-dependent K$^+$ current (e.g., $150 \mu$M Cd$^{2+}$), nominally Ca$^{2+}$-free solution, 10 $\mu$M nitrendipine, or 20–60 nM ChTx. Because all these agents, except ChTx, block Ca$^{2+}$ entry and therefore indirectly block $I_{Cl(Ca)}$, they provide an estimate of $I_{K(V)}$ in both terminal-ablated and intact rods. As indicated in Table 1, these treatments reduced the outward current at $+20$ mV to $\approx 200$ pA, a value less than approximately 30% of the total control outward current. The fact that the magnitude of this residual current was similar in rods with and without ablated terminals (Table 1; see also Fig. 2, D and E for step to 0 mV) suggests its predominant location was in the soma and/or inner segment. This residual $I_{K(V)}$ current appeared sensitive to 2–3 mM 4-AP (Table 1) and 15 mM TEA ($n = 2$; not shown), known blockers of delayed-rectifier K$^+$ currents in rod photoreceptors (see Bader et al. 1982; Barnes and Hille 1989).

Similarly, comparison of whole cell currents, before and after terminal ablation, revealed that $I_h$ was located in the soma.
and/or inner segment. Figure 6, A1 and A2 shows an example of recordings obtained during hyperpolarizing voltage steps in the same cell before and after terminal ablation. The amplitude and time course of the hyperpolarizing currents were virtually identical to those of \( I_h \) reported by Bader et al. (1982). The bar chart (Fig. 6B) summarizes the results for a population of three cells and shows that the magnitude of the steady-state \( I_h \) current at \(-120 \text{ mV}\) is virtually unchanged after terminal ablation.

**DISCUSSION**

In the present study we identified a compartmentalization of ion channel function in salamander rod photoreceptors, which represent a class of highly polarized sensory cells. The outer segment is specialized for phototransduction, containing photopigment and the machinery for the visual cascade that lead to regulation of cGMP-gated cationic channels during the light response. However, although up to five additional ionic conductances have been described, their relative distribution was unclear, with the exception of the voltage-gated L-type Ca\(^{2+}\) channels that appear highly concentrated in the terminal (Krizaj and Copenhagen 2002; Morgans et al. 1998; Steele et al. 2005). Using a combination of whole cell recording and microablation procedures, we show that in contrast to the remaining currents, ion channels mediating the Ca\(^{2+}\)-dependent chloride current \( I_{\text{Cl(Ca)}} \) are compartmentalized almost exclusively in the rod terminal. Furthermore, simultaneous measurements of \( I_{\text{Cl(Ca)}} \) and intracellular Ca\(^{2+}\) transients, using voltage-clamp and ratiometric fura-2 spectrofluorimetry, respectively, confirmed that intracellular [Ca\(^{2+}\)] rose more steeply in the rod terminal relative to the soma during membrane depolarization. Moreover, they revealed that the time course of decay of \( I_{\text{Cl(Ca)}} \) followed very closely that of Ca\(^{2+}\) concentration in the terminal, which had a different kinetic profile from that of the soma. Thus even though the duration of \( I_{\text{Cl(Ca)}} \) was highly variable from cell to cell (typically 1–12 s), the time to half-decay for both \( I_{\text{Cl(Ca)}} \) and terminal [Ca\(^{2+}\)] was similar within a given cell. Several studies have pointed to the complex control of intracellular Ca\(^{2+}\) dynamics and indirectly \( I_{\text{Cl(Ca)}} \) in the rod synaptic terminal that is endowed with local, specialized mechanisms that regulate intraterminal calcium (Krizaj and Copenhagen 2002; Steele et al. 2005). Because this region is specialized for synaptic transmission to second-order retinal cells, our findings highlight an important conductance that needs consideration in models of the physiological func-

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<th>Control ((n=10))</th>
<th>ChTx, 20–60 nM ((n=6))</th>
<th>Calcium Free ((n=8))</th>
<th>Cadmium, 150 (\mu)M ((n=4))</th>
<th>Nitrendipine, 10 (\mu)M ((n=6))</th>
<th>Nitrendipine, 10 (\mu)M + 4-AP, 2.3 mM ((n=3))</th>
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<td>694 ± 92</td>
<td>190 ± 31.4</td>
<td>159.3 ± 17.8</td>
<td>179 ± 31.6</td>
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Values are means ± SE. ChTx, chrybdotoxin; 4-AP, 4-aminopyridine.
tion of the synaptic terminal during the light response (Thoreson and Bryson 2004; Thoreson et al. 2003).

Evidence for selective targeting of \( I_{\text{Cl(Ca)}} \) to salamander rod terminal

The calcium-dependent chloride current \( I_{\text{Cl(Ca)}} \) is a well-described property of vertebrate photoreceptors and is activated by entry of extracellular \( \text{Ca}^{2+} \) by dihydropyridine-sensitive L-type calcium channels (Atwell et al. 1982; Bader et al. 1982; Barnes and Hille 1989; Maricq and Korenbrot 1988; Morgans et al. 1998; Thoreson et al. 2003; Yagi and MacLeish 1994), or \( \text{Ca}^{2+} \) release from intracellular stores (see Krizaj and Copenhagen 2002). In the present study, the most direct evidence for the compartmentalization of \( I_{\text{Cl(Ca)}} \) was obtained during repeated whole cell recordings from the same rod, one before and another after terminal ablation. In all such cases \( I_{\text{Cl(Ca)}} \), which was present initially, was no longer detectable after terminal ablation, although several other voltage- and \( \text{Ca}^{2+} \)-dependent currents remained largely intact. Further, attempts to reveal \( I_{\text{Cl(Ca)}} \) in terminal-ablated rods, by augmenting \( \text{Ca}^{2+} \) entry with the L-type \( \text{Ca}^{2+} \) channel agonist Bay K 8644, were unsuccessful, although this calcium load was sufficient to enhance the \( \text{Ca}^{2+} \)-dependent K⁺ or BK current. The simplest explanation for these findings is that the chloride channels underlying \( I_{\text{Cl(Ca)}} \) are specifically targeted to the photoreceptor terminal. Additional supporting evidence for compartmentalization of \( I_{\text{Cl(Ca)}} \) was obtained in comparative studies on its frequency of occurrence in rods with, versus rods without, obvious terminals when viewed under phase-contrast microscopy. \( I_{\text{Cl(Ca)}} \) was always present in rods with visible terminals and was usually (~71% of cases), but not always, absent in rods lacking visible terminals. We infer from these studies that failure to observe a visible terminal under phase-contrast microscopy is not conclusive evidence for the lack of terminal contribution to the whole cell response. It is possible for the terminal to be obscured by the soma, for example during substrate attachment, and this has actually been observed in scanning electron micrographs of isolated rods (PR MacLeish and W. Anderson, unpublished observations). Further, after tissue dissociation and plating, rod terminals often display different lengths and shapes, suggestive of varying stages of regression into the cell body. These considerations caution against inferences about channel compartmentalization based on results from cells without obvious terminals.

Physiological significance of terminal localization of \( I_{\text{Cl(Ca)}} \)

The observed, highly specific targeting of \( I_{\text{Cl(Ca)}} \) to the salamander rod terminal, a region specialized for synaptic transmission, raises questions about its physiological significance. Clearly, the physiological impact of the \( \text{Cl}^- \) channels mediating \( I_{\text{Cl(Ca)}} \) depends on the \( \text{Cl}^- \) equilibrium potential \( (E_{\text{Cl}}) \), which has been estimated to be approximately ~20 mV in salamander rods, i.e., positive to the dark resting membrane potential (~ ~46 mV; Thoreson et al. 2002). In salamander retinal slices, a feedback interaction between intracellular \( \text{Ca}^{2+} \) and these \( \text{Cl}^- \) channels has been proposed to stimulate \( \text{Cl}^- \) efflux, which in turn inhibits presynaptic \( \text{Ca}^{2+} \) channels involved in regulating neurotransmitter release (Thoreson et al. 2002).
regard, our demonstration that Ca\textsuperscript{2+} influx, causing closure of these Cl\textsuperscript{-} channels and a positive feedback amplification of the light-evoked response. This mechanism would lead to an enhanced reduction of transmitter release when the light is turned on, as well as a facilitation of release when the light is turned off, thereby increasing the signal-to-noise ratio for the receptor. Interestingly, in olfactory neurons that have an unusually high intracellular [Cl\textsuperscript{-}], and thus a relatively depolarized $E_{\text{Cl}}$, activation of $I_{\text{Cl(Ca)}}$ causes Cl\textsuperscript{-} efflux, which is thought to contribute to high-gain, low-noise amplification of the olfactory receptor potential (see Menini 1999). As proposed for cones, the channels underlying $I_{\text{Cl(Ca)}}$ may also contribute to the stabilization of the membrane potential in salamander rods. Because the estimated $E_{\text{Cl}}$ in these rods is approximately $-20$ mV (Thoreson et al. 2002), i.e., near membrane voltages that maximize Ca\textsuperscript{2+} entry, stabilization of the membrane potential in the terminal would be facilitated by a positive feedback loop. These considerations, together with our demonstration of the highly localized distribution of $I_{\text{Cl(Ca)}}$ in the rod terminal, raise the possibility that the soma and terminal regions may not be isopotential, and that this chloride conductance may allow local and autonomous regulation of membrane potential and transmitter release by the photoreceptor terminal.

**Distribution of remaining voltage-gated channels in salamander rods**

Whereas the underlying ion channels that mediated $I_{\text{Cl(Ca)}}$ in rods were preferentially sorted to the rod terminal, those that contributed to most of the remaining ionic currents did so primarily because of their location in the soma and/or inner segment. Although we could not exclude the presence of any of these conductances in the outer segment, this region is generally thought to be relatively devoid of voltage-activated currents (Barnes and Hille 1989; Fain and Lisman 1981). In particular, the major K\textsuperscript{+} currents—the Ca\textsuperscript{2+}-sensitive BK current $I_{K(\text{Ca})}$, the Ca\textsuperscript{2+}-insensitive, delayed-rectifier–like current $I_{K(V)}$, and the hyperpolarization-activated cation current $I_{h}$—were hardly affected after terminal ablation, suggesting that any terminal contribution to the whole cell current was negligible. Given the small size and surface area of the rod terminal relative to the soma and inner segment, these data do not allow firm conclusions to be drawn regarding the distribution or density of these channels within the various rod compartments. For example, we estimated that even with a uniform distribution of any particular group of ion channels over the photoreceptor surface, the contribution of the terminal region to the resulting whole cell current would be <1%. In this regard, our demonstration that $I_{K(\text{Ca})}$ remained largely intact in terminal-ablated rods conflicts with a recent study suggesting that this current is polarized preferentially to the salamander rod terminal (Xu and Slaughter 2005). In the latter study, $I_{K(\text{Ca})}$ was detectable in rods with, but not those without, visible terminals as viewed under light microscopy (see preceding **discussion**). Although our data do not exclude the possibility of BK channel expression in the terminal, such a contribution would be relatively small in our studies. At present we cannot account for this discrepancy in localization of the major component of $I_{K(\text{Ca})}$ between the two studies, other than methodological differences. Interestingly, although Ca\textsuperscript{2+}-activated BK channels are often found concentrated in synaptic terminals of central neurons (Trimmer and Rhodes 2004), they are known to be clustered in the apex of other sensory cells, e.g., mouse inner hair cells, away from the basal synaptic sites (Pyott et al. 2004; see also Kwon and Guggino 2004).

Because in our study, $I_{K(\text{Ca})}$ was robustly expressed in rods with ablated terminals and was dependent on entry of extracellular calcium through voltage-dependent L-type Ca\textsuperscript{2+} channels, the latter must also be present in the soma and/or inner segment (see also Szikra and Krizaj 2006). Indeed, in rods with ablated terminals, $I_{K(\text{Ca})}$ was substantially enhanced by Bay K 8644, an L-type Ca\textsuperscript{2+} channel agonist (Nowycky et al. 1985). Similar L-type Ca\textsuperscript{2+} channels also appear to be concentrated in the salamander rod terminal where they contribute to local “hot spots” for Ca\textsuperscript{2+} entry after membrane depolarization (Krizaj and Copenhagen 2002; Morgans et al. 1998; Nachman-Clewner et al. 1999; Steele et al. 2005).

In conclusion, our data indicate that salamander rod photoreceptors are endowed with discrete targeting mechanisms that preferentially sort ion channels mediating the anionic Ca\textsuperscript{2+}-dependent Cl\textsuperscript{-} current to the terminal region, which also acts as a local hot spot for calcium entry. The mechanisms that underlie sorting of these channels to this compartment are currently unknown, but result in a functional polarity that may contribute to stabilization of membrane potential and local regulation of neurotransmitter release at the terminal, as well as enhancement of signal-to-noise ratio for the receptor. Interestingly, this polarity persisted in rods even after isolation for 2 days in culture, suggesting that cell–cell contact was not essential, at least for its short-term maintenance. It remains to be determined whether the sorting signals are similar to those that direct membrane proteins in other polarized epithelial and neuronal cells (Trimmer 1999; Trimmer and Rhodes 2004).

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