Voltage-Dependent Na\(^+\) Currents in Mammalian Retinal Cone Bipolar Cells

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Pan, Zhumo-Hua and Hui-Juan Hu. Voltage-dependent Na\(^+\) currents in mammalian retinal cone bipolar cells. J Neurophysiol 84: 2564–2571, 2000. Voltage-dependent Na\(^+\) channels are usually expressed in neurons that use spikes as a means of signal coding. Retinal bipolar cells are commonly thought to be nonspiking neurons, a category of neurons in the CNS that uses graded potential for signal transmission. Here we report for the first time voltage-dependent Na\(^+\) currents in acutely isolated mammalian retinal bipolar cells with whole cell patch-clamp recordings. Na\(^+\) currents were observed in ~45% of recorded cone bipolar cells but not in rod bipolar cells. Both ON and OFF cone bipolar cells were found to express Na\(^+\) channels. The Na\(^+\) currents were activated at membrane potentials around −50 to −40 mV and reached their peak around −20 to 0 mV. The half-maximal activation and steady-state inactivation potentials were −24.7 and −68.0 mV, respectively. The time course of recovery from inactivation could be fitted by two time constants of 6.2 and 81 ms. The amplitude of the Na\(^+\) currents ranged from a few to >300 pA with the current density in some cells close or comparable to that of retinal third neurons. In current-clamp recordings, Na\(^+\)-dependent action potentials were evoked in Na\(^+\)-current-bearing bipolar cells by current injections. These findings raise the possibility that voltage-dependent Na\(^+\) currents may play a role in bipolar cell function.

INTRODUCTION

Retinal bipolar cells are second-order neurons that relay the signal from photoreceptors to amacrine and ganglion cells in the retina. It is commonly believed that bipolar cells are nonspiking neurons and that signals propagate in a passive manner in these cells. However, the recent findings of spontaneous and light-evoked Ca\(^{2+}\)-spikes in Mb1 retinal bipolar cells in the goldfish suggest that bipolar cells may be more excitable than what has been previously thought (Burrono and Lagnard 1997; Proti et al. 2000; Zenisek and Matthews 1998). Nevertheless, voltage-dependent Na\(^+\) currents have not been previously reported in retinal bipolar cells. However, an early study found that a portion of mammalian bipolar cells displayed immuno-reactivity to Na\(^+\) channel α subunits (Miguel-Hidalgo et al. 1994).

Bipolar cells are classified into ON and OFF types based on their response polarity to light (Kaneko 1970; Werblin and Dowling 1969). Bipolar cells are also divided into rod and cone bipolar cells based on their synaptic inputs. In mammals, only a single type of rod bipolar cells, ON type, has been reported (Boycott and Dowling 1969; Boycott and Kolb 1973; Dacheux and Raviola 1986; Greferath et al. 1990). On the other hand, multiple subtypes of cone bipolar cells have been reported (Euler and Wässle 1995; Famiglietti 1981; Kolb et al. 1981; Pourcho and Goebel 1987). Most of the previous studies of membrane currents in bipolar cells were performed in lower vertebrates (Connaughton and Maguire 1998; Karschin and Wässle 1985; Lasater 1988; Maguire et al. 1989; Tessier-Lavigne et al. 1988). Studies of mammalian bipolar cells were mainly limited to rod bipolar cells (Gillette and Dacheux 1995; Karschin and Wässle 1990). The properties of voltage-activated membrane currents in mammalian cone bipolar cells are less clear.

We previously reported the capability of distinguishing mammalian rod and cone bipolar cells after enzymatic dissociation (Pan 2000). We characterized and compared voltage-activated membrane channels between these two types of bipolar cells. Here we report that a portion of cone bipolar cells in the rat retina display voltage-dependent Na\(^+\) currents. A brief report of this work has been presented in abstract form (Pan 1999).

METHODS

Bipolar cells were isolated from ≥4-wk-old Long Evans rats by dissociation methods previously described (Pan 2000; Pan and Lipton 1995). In brief, animals were deeply anesthetized with CO\(_2\) and killed by decapitation. Retinas were removed and placed in a Hanks’ solution (normal Hanks’) containing (in mM) 138 NaCl, 1 NaHCO\(_3\), 0.3 Na\(_2\)HPO\(_4\), 5 KCl, 0.3 KH\(_2\)PO\(_4\), 1.25 CaCl\(_2\), 0.5 MgSO\(_4\), 0.5 MgCl\(_2\), 5 HEPES, and 22.2 glucose, with phenol red, 0.001% vol/vol; adjusted to pH 7.2 with 0.3 N NaOH. The retinas were incubated for ~50 min at 34–37°C in an enzyme solution that consisted of the Hanks’ solution, supplemented with 0.2 mg/ml Dl-cysteine, 0.2 mg/ml bovine serum albumin, and 1.6 U/ml papain, adjusted to pH 7.2 with 0.3 N NaOH. Following several rinses in Hanks’ solution, the retinas were mechanically dissociated by gently triturating with a glass pipette. The resulting cell suspension was plated onto culture dishes. Cells were used for recordings within 5 h after dissociation.

Bipolar cells were identified based on their characteristic morphology (Karschin and Wässle 1990; Pan and Lipton 1995; Yeh et al. 1990). Identification of rod and cone bipolar cells has been previously described (Pan 2000). In brief, rod bipolar cells had long and thick axons and usually retained axon terminals with relative large synaptic boutons. Their dendritic trees were thick and bush-like. Cone bipolar cells, on the other hand, had sparser dendritic trees and thinner axons. Synaptic boutons were small or absent.

Recordings with patch electrodes in the whole cell configuration were made by standard procedures (Hamill et al. 1981) at room temperature.
temperature (20–25°C) with an EPC-9 amplifier and PULSE software (Heka Electronik, Lambrecht/Pfalz, Germany). Electrodes were coated with silicone elastomer (Sylgard, Dow Corning, Midland, MI) and fire-polished. The resistance of the electrode was 7–14 MΩ. Series resistance ranged from 12 to 40 MΩ and was not routinely compensated. Cell capacitance was canceled and recorded by PULSE software. In some recordings, leak currents were subtracted with an on-line P/4 protocol provided by PULSE software.

Na⁺ currents in morphologically identified cone bipolar cells

When cone bipolar cells were depolarized to −50 mV or more positively from the holding potential of −70 or −80 mV, fast transient inward currents were frequently observed. An example of a morphologically identified cone bipolar cell that displayed such currents is shown in Fig. 1A.

The fast transient currents were still present in the recording solution containing 4 mM Co²⁺ and without added Ca²⁺ (Fig. 1, B and C). The currents were activated around −50 to −40 mV and reached their peak around −20 to 0 mV from the holding potential of −80 mV (Fig. 1B). The rapid activation and inactivation of the currents resemble the typical pattern of Na⁺ currents. Furthermore the currents were reversibly blocked by TTX (0.5–1 μM) as exemplified in Fig. 1C (n > 10). These results indicate that the fast transient currents are Na⁺ currents.

GABA<sub>C</sub> receptor-mediated currents in Na⁺-current-bearing bipolar cells

To further ensure that the recorded Na⁺-current-bearing cells were bipolar cells, we examined GABA<sub>C</sub> receptor-mediated currents in these cells. This is because bipolar cells but not...
third-order neurons in the rat retina were reported to express GABA<sub>C</sub> receptors, which are insensitive to bicuculline, a GABA<sub>A</sub> receptor antagonist (Euler and Wässle 1998; Feigenspan et al. 1993; Pan 2000). Over 80 Na<sup>+</sup>-current-bearing cone bipolar cells were examined. All of them displayed GABA-evoked currents in the presence of bicuculline. As a typical example shown in Fig. 2, the presence of Na<sup>+</sup> currents in this cone bipolar cell was confirmed by depolarizing voltage pulses (Fig. 2A). In the same cell, co-application of GABA (100 μM) and bicuculline (200 μM) evoked a sustained inward current. Recordings were made in normal Hanks' containing Co<sup>2+</sup> (4 mM). The cell was held at −80 mV.

![Image](http://jn.physiology.org/)

**FIG. 2.** Demonstration of the presence of GABA<sub>C</sub> receptor currents in a Na<sup>+</sup>-current-bearing cone bipolar cell. A: the presence of voltage-dependent Na<sup>+</sup> currents was confirmed by test pulses of −50 to −10 mV. B: co-application of GABA (100 μM) and bicuculline (200 μM) evoked a sustained inward current. Recordings were made in normal Hanks' containing Co<sup>2+</sup> (4 mM). The cell was held at −80 mV.

**Ca<sup>2+</sup> currents in Na<sup>+</sup>-current-bearing bipolar cells**

Mammalian cone bipolar cells have been reported to express both low-voltage-activated (LVA) Ca<sup>2+</sup> currents and L-type high-voltage-activated Ca<sup>2+</sup> channels (de la Villa et al. 1998; Hartveit 1999; Pan 2000; Satoh et al. 1998). We have previously reported that the LVA Ca<sup>2+</sup> currents of rod and cone bipolar cells display distinct activation and inactivation kinetics (Pan 2000). When recordings were made in normal Hanks’, inward currents with slower activation and inactivation than those of above-described Na<sup>+</sup> currents were also observed in cone bipolar cells. These were Ca<sup>2+</sup> currents since the currents were blocked by Co<sup>2+</sup> but not by TTX (Fig. 3A). In many of these cells, however, Na<sup>+</sup> currents were significantly larger than Ca<sup>2+</sup> currents when cells were depolarized from −80 mV and recorded in the physiological Ca<sup>2+</sup> concentration. A typical example is shown in Fig. 3B. The I-V relationships for the peak Na<sup>+</sup> (▲) and Ca<sup>2+</sup> (●) currents are shown in Fig. 3C. The amplitude of the peak Ca<sup>2+</sup> currents did not find to show too much variation among cone bipolar cells with the average value of 17.4 ± 5.6 pA (mean ± SD; n = 9). When recordings were made in high-Ca<sup>2+</sup> (10 mM) solution, as expected, Ca<sup>2+</sup> currents become much larger (also see Pan 2000). For comparison, the currents shown in Fig. 3D were recorded in high-Ca<sup>2+</sup> from the same cell shown in Fig. 3B. For this cell, the Na<sup>+</sup> currents were masked by the Ca<sup>2+</sup> currents and were barely noticed. But, for many other cells, the presence of Na<sup>+</sup> currents could still be observed in high-Ca<sup>2+</sup> solution (data not shown). Voltage-activated Ca<sup>2+</sup> currents in Na<sup>+</sup>-current-bearing bipolar cells appeared to be activated at slightly more negative potentials (around −60 to −50 mV) than that of Na<sup>+</sup> currents (around −50 to −40 mV as described in the preceding text). Furthermore the inactivation kinetics of the LVA Ca<sup>2+</sup> currents resembles that of the LVA Ca<sup>2+</sup> current of cone bipolar cells previously described (Pan 2000).
Na⁺ currents in both ON and OFF cone bipolar cells

Since Na⁺ currents were observed only in a portion of cone bipolar cells, we investigated whether Na⁺ channels were specifically expressed only in the ON or OFF type of bipolar cells. It has been known that ON and OFF bipolar cells express different glutamate receptors: metabotropic glutamate receptors in ON bipolar cells and ionotropic glutamate receptors in OFF bipolar cells. Particularly activation of the metabotropic glutamate receptors by L-2-amino-4-phosphonobutyric acid (L-AP4), a selective agonist of metabotropic glutamate receptors in OFF cone bipolar cells, closes cGMP-gated channels (Nawy and Jahr 1990; Shiells and Falk 1990; Slaughter and Miller 1981). Mammalian OFF cone bipolar cells have been reported to express kainate subtype of glutamate receptors (DeVries and Schwartz 1999). Thus we examined the response of Na⁺-current-bearing cone bipolar cells to kainate, L-AP4, or glutamate. In these recordings, cGMP (1 mM) was added in the electrode solution. Cells were usually held at −70 mV. Both types of responses were observed among Na⁺-current-bearing cone bipolar cells. Figure 4A illustrates a typical example of Na⁺-current-bearing cone bipolar cells responding to L-AP4 (2 μM) or glutamate (1 mM) with an outward current or a decrease of the holding inward current (n = 31). A typical example of Na⁺-current-bearing cone bipolar cells responding to kainate (300 μM) with an inward current is shown Fig. 4B (n = 19). These results indicate that Na⁺ channels are expressed in both ON and OFF cone bipolar cells.

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Na⁺-current distribution and amplitude

Na⁺ currents were observed in ~45% of recorded cone bipolar cells. For example, among 493 recorded cone bipolar cells, 223 of them displayed Na⁺ currents. In contrast, Na⁺ currents were never observed in rod bipolar cells (n = 700). Although it was not possible for us to tell whether a cone bipolar cell could have Na⁺ currents by its morphology, Na⁺ currents were more frequently observed in the cone bipolar cells with a smaller soma and longer axon. Consistent with this observation, the average whole cell capacitance of cone bipolar cells with Na⁺ currents was 2.46 ± 0.41 pF (mean ± SD; n =
In contrast, the average whole cell capacitance of cone bipolar cells without Na\textsuperscript{+} currents was 2.74 ± 0.44 pF \((n = 109)\). These two values are significantly different \((P < 0.001; \text{by } t\text{-test})\).

Na\textsuperscript{+} currents were observed in cone bipolar cells that did not retain axon terminals. In a few cases, we were able to identify cone bipolar cells without axon and Na\textsuperscript{+} currents were also observed in some of these cone bipolar cells. Furthermore, we did not notice there were any correlations between the amplitude of the Na\textsuperscript{+} current and the presence or absence of axon or axon terminals. Our results suggest that the Na\textsuperscript{+} channels are at least located in the soma.

When recordings were made in normal Hanks', the peak Na\textsuperscript{+} current of cone bipolar cells ranged from a few to >300 pA. Figure 5 shows the distribution of the peak Na\textsuperscript{+} currents and the current densities for 28 cone bipolar cells. The Na\textsuperscript{+} current density was obtained by dividing the peak current by the cell membrane capacitance. The average Na\textsuperscript{+} current is 93.1 ± 95.8 pA \((n = 28)\). The average Na\textsuperscript{+} current density is 39.1 ± 44.6 pA/pF \((n = 28)\). As shown in Fig. 5, in some cells, Na\textsuperscript{+} current densities are >100 pA/pF.

Voltage dependence of activation and inactivation

Voltage dependence of activation was calculated from the I-V relationships of the Na\textsuperscript{+} currents. The averaged data \((●)\) for five cells were shown in Fig. 6A \((\text{right})\). The half-maximum activation potential and the slope factor were −24.7 and 5.9 mV, respectively. Steady-state inactivation was determined by conditioning pulses ranging from −100 to −30 mV followed by a test pulse at −10 mV. The averaged data \((●)\) from six cells were also shown in Fig. 6A \((\text{left})\). The half-maximum inactivation potential and the slope factor were −68 and 11.8 mV, respectively.

The activation and inactivation kinetics of the Na\textsuperscript{+} currents were determined by measuring the time to peak and the decay time constant at different test potentials. The decay time constants were obtained by fitting the decay current with a single exponential. The average values of the time to peak \((n = 8)\) and decay time constant \((n = 8)\) were plotted versus the test potentials in Fig. 6, B and C.

Recovery from inactivation

The recovery of Na\textsuperscript{+} currents from inactivation was determined by a series of paired pulses (Fig. 7A). In each paired-pulse, cells were first depolarized from the holding potential of −80 to −10 mV for 100 ms (condition pulse) followed by a test pulse (20 ms to −10 mV) with a varied time delay. Paired pulses were applied once every 5 s. B: the time course of Na\textsuperscript{+} current recovery from inactivation. The ratio values of the peak Na\textsuperscript{+} currents evoked by test pulse and condition pulse are plotted in relation to time delay. The data points are mean values and the error bars represent SE obtained from 8 cells. —, the fitting to the sum of 2 exponential functions with time constant of 6.2 and 81 ms.

FIG. 7. Recovery of Na\textsuperscript{+} currents from inactivation. A: paired-pulse protocols used to determine the time course of recovery from inactivation. In each paired pulse, cells were first depolarized from the holding potential of −80 to −10 mV for 100 ms (condition pulse) followed by a test pulse (20 ms to −10 mV) with a varied time delay. Paired pulses were applied once every 5 s. B: the time course of Na\textsuperscript{+} current recovery from inactivation. The ratio values of the peak Na\textsuperscript{+} currents evoked by test pulse and condition pulse are plotted in relation to time delay. The data points are mean values and the error bars represent SE obtained from 8 cells. —, the fitting to the sum of 2 exponential functions with time constant of 6.2 and 81 ms.

FIG. 8. Demonstration of Na\textsuperscript{+}-dependent spikes in a cone bipolar cell. A: the presence of voltage-dependent Na\textsuperscript{+} currents was confirmed in voltage-clamp recordings. B: in current-clamp mode, spikes were evoked by current pulses (400 ms) from the holding current of −3.8 pA to 5 and 10 pA. C: after the application of TTX (500 nM), no spikes were evoked by the same current pulses. Recordings were made in normal Hanks' and electrode solution contained K-gluconate.
**Na⁺-dependent action potentials in cone bipolar cells**

We further determined whether Na⁺-dependent spike activities could be present in cone bipolar cells. The recordings were made in normal Hanks'. K-glucuronate was used in intracellular solution without blocking K⁺ currents (see METHODS for details). Under these recording conditions, Na⁺-dependent action potentials were observed. An example of such recordings is shown in Fig. 8. The cone bipolar cell was first recorded in the voltage-clamp mode to confirm the presence of voltage-dependent Na⁺ currents in this cell (Fig. 8A). Then the recordings were switched to the current-clamp mode resulted in the cell being clamped at −3.8 pA. Stepwise current pulses were applied to depolarize the cell from the holding current of −3.8 pA to 5 and 10 pA for 400 ms. A single spike was observed at the beginning of the depolarization evoked by current injections (Fig. 8B). After an application of 500 nM TTX, the same current injections did not evoke any spike (Fig. 8C). Similar results were observed in four other cone bipolar cells.

**DISCUSSION**

**Voltage-dependent Na⁺ currents in cone bipolar cells**

In this study, we report voltage-dependent Na⁺ currents in rat retinal bipolar cells with patch-clamp recordings. Bipolar cells were identified by their characteristic morphology. Two lines of evidence further ensure that the Na⁺-current bearing cells recorded in this study were bipolar cells. First, these cells showed bicuculline-insensitive GABA, or GABA emojis; receptors mediated, responses. GABA emojis; receptors have been reported to be expressed in bipolar cells but not in third-order neurons in the rat retina (Eulner and Wässle 1998; Feigenspan et al. 1993; Pan 2000). Second, we also showed that a portion of these cells responded to L-AP4 or glutamate with a decrease in conductance, a unique property of ON bipolar cells. Thus the Na⁺ currents observed in this study could not be recorded from retinal third-order neurons.

The bipolar cells displaying Na⁺ currents were morphologically identified as cone bipolar cells, which have been previously described in detail (Pan 2000). In addition, the LVA Ca²⁺ currents in these Na⁺-current bearing bipolar cells displayed the characteristic properties of the LVA Ca²⁺ currents of cone bipolar cells (Pan 2000), further supporting that Na⁺ currents are expressed in cone bipolar cells.

It should be mentioned that the present study was performed on acutely dissociated bipolar cells. Large Na⁺ currents were also observed shortly after the dissociation (<20 min). Moreover, Na⁺ currents were never observed in rod bipolar cells even though a large number of rod bipolar cells were recorded. Therefore it is very unlikely that the Na⁺ current could arise as some type of culture artifact.

Taken together, our results indicate that voltage-dependent Na⁺ currents are expressed in cone bipolar cells of the rat retina. Markedly, a significant portion (~45%) of cone bipolar cells was observed to show Na⁺ currents in this study. The magnitude of the Na⁺ currents among cone bipolar cells varied widely. The variation was not found to be correlated to the presence or absence of the axon and axon terminals. Furthermore the Na⁺ currents were found to be expressed in both on and off of cone bipolar cells. However, it remains to be determined whether Na⁺ channels are expressed in specific subtypes of ON and OFF cone bipolar cells.

The finding of Na⁺ currents is consistent with an early report of immunolocalization of Na⁺ channel α subunits in a portion of cat and monkey bipolar cells (Miguel-Hidalgo et al. 1994). Surprisingly, however, this is the first report of voltage-activated Na⁺ currents in retinal bipolar cells with electrophysiological recordings. Why have voltage-dependent Na⁺ currents of bipolar cells not been observed in previous studies? There are several possible reasons. First, because only a portion of cone bipolar cells express Na⁺ currents, these bipolar cells might have been missed in previous recordings. Second, bipolar cells displaying Na⁺ currents recorded in previous studies might have been considered to be third-order neurons. Finally, voltage-dependent Na⁺ currents may only be expressed in mammalian cone bipolar cells. In fact, most of the previous studies of membrane currents of bipolar cells were carried out in lower vertebrates (Connaughton and Maguire 1998; Kaneko and Tachibana 1985; Lasater 1988; Maguire et al. 1989; Tessier-Lavigne et al. 1988). Studies of mammalian retinal bipolar cells were previously mainly performed on rod bipolar cells (Gillette and Dacheux 1995; Karschin and Wässle 1990) which do not express Na⁺ currents as further confirmed in this study.

**Properties of Na⁺ currents**

The biophysical properties of the Na⁺ currents in cone bipolar cells are largely similar to that of Na⁺ currents reported in retinal third-order neurons (Barnes and Werblin 1986; Hidaka and Ishida 1998; Kanaeda and Kaneko 1991; Lipton and Tauck 1987). The potential range of the peak current occurring from −20 to 0 nV is also similar to that of retinal third-order neurons (Barnes and Werblin 1986; Lipton and Tauck 1987). Both the activation and inactivation are rapid and voltage dependent. In addition, there is an overlap in the activation and steady-state inactivation curves (from −40 to −30 mV), suggesting Na⁺ currents may be constantly activated within this potential range. Recovery of Na⁺ currents from inactivation is rapid. Thus a brief hyperpolarization of membrane potential could partially remove the inactivation of Na⁺ channels.

Under normal physiological conditions, the amplitude of Na⁺ currents of cone bipolar cells in at least more than one order of magnitude smaller than that of retinal ganglion cells. However, since bipolar cells are much smaller than retinal ganglion cells, the difference in the Na⁺ current density between cone bipolar cells and retinal third-order cells, such as ganglion cells, does not appear to be so large. Na⁺ current densities in retinal ganglion cells were reported to be 100–300 pA/pF (Hidaka and Ishida 1998; Lipton and Tauck 1987). In fact, Na⁺ current densities observed in some cone bipolar cells in this study are close to or comparable to these values (see Fig. 5). Bipolar cells with such a large Na⁺ current density would be expected to generate Na⁺ spikes. Indeed, we showed in this study that Na⁺-dependent action potentials could be evoked by current injections.

However, under our vitro current-clamp recording conditions, only a single spike could be observed. Interestingly, such property resembles that of amacrine cells in vivo. As having been demonstrated previously for retinal amacrine cells (Elia sof et al. 1987), the lack of the capability to fire a series of...
action potentials could be due to the intrinsic properties of the channel itself and/or other membrane currents, such as voltage-activated K\(^+\) and Ca\(^{2+}\)-activated currents.

The dark membrane potentials of bipolar cells are believed to be around −40 to −45 mV with ON bipolar cells depolarizing and OFF bipolar cells hyperpolarizing in responding to light stimulation. The steady-state inactivation curve of the Na\(^+\) current suggests that most of the Na\(^+\) channels in ON bipolar cells would be in the inactivated state(s). On the other hand, the light-evoked hyperpolarization may partially remove the inactivation of the Na\(^+\) channels in OFF bipolar cells. Therefore the voltage-activated Na\(^+\) channels would be more likely to be activated in OFF cone bipolar cells in vivo.

**Functional implications of expression Na\(^+\) channels in bipolar cells**

What might be the possible role of expression Na\(^+\) channels in bipolar cells? First, as mentioned in the preceding text, because there is an overlap in the activation and steady-state inactivation curves, Na\(^+\) currents might be constantly activated around bipolar cell resting membrane potentials, which could support cells’ electrical excitability. Second, activation of Na\(^+\) channels, or Na\(^+\) spikes, in bipolar cells may speed up the membrane depolarization and shape light-response waveform and, in turn, affect Ca\(^{2+}\) current activation and transmitter release at the axon terminals.

Furthermore Na\(^+\) channels might play a role in bipolar cell signal propagation. It is commonly thought that the space constant of bipolar cells is much longer than the axons of bipolar cells and, thus the membrane potentials can propagate from dendrites to axon terminals passively without significant loss. However, at least in rats, both the dendrites and axons of most cone bipolar cells after dissociation appeared to be markedly thinner than that of rod bipolar cells (Pan 2000). In addition, membrane resistance of bipolar cells in vivo has been reported to be lower than the value obtained in vitro probably due to cell coupling and constant activation of membrane ion channels (Tessier-Lavigne et al. 1988). Together these factors could significantly reduce the space constant for certain groups of bipolar cells. Particularly, as mentioned in the preceding text, voltage-dependent Na\(^+\) currents were more frequently observed in cone bipolar cells with smaller soma and longer axon. Therefore it is possible that the expression of Na\(^+\) channels in bipolar cells may serve as a mechanism for boosting or facilitating membrane potential propagation.

However, a previous study reported that co-application of TTX and cadmium was not found to show effect on the multi-quantal release of glutamate from mouse bipolar cells in light-adapted retinal slices (Tian et al. 1998). This appears to suggest that retinal bipolar cells don’t generate spontaneous Na\(^+\) or Ca\(^{2+}\) activities under the studied conditions. On the other hand, a recent study reported that Mblind type bipolar cells in dark-adapted goldfish retinal slices were capable of generating light-evoked Ca\(^{2+}\) spikes (Pretti et al. 2000). Further study will be needed to determine the functional role of the expression of voltage-gated Na\(^+\) currents in bipolar cells. Particularly it would be interesting to determine whether Na\(^+\) currents could shape the light response or generate light-evoked Na\(^+\) spikes in mammalian cone bipolar cells in vivo. Furthermore, experiments with paired recordings of bipolar cells and third-order neurons in retinal slices may be able to determine whether Na\(^+\) currents play a role in bipolar cell signal propagation.

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