Calcium-Activated Potassium Conductances in Retinal Ganglion Cells of the Ferret

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Wang, Guo-Yong, David W. Robinson, and Leo M. Chalupa. Calcium-activated potassium conductances in retinal ganglion cells of the ferret. J. Neurophysiol. 79: 151–158, 1998. Patch-clamp recordings were made from isolated and intact retinal ganglion cells (RGCs) of the ferret to examine the calcium-activated potassium channels expressed by these neurons and to determine their functional role in the generation of spikes and spiking patterns. Single-channel recordings from isolated neurons revealed the presence of two calcium-sensitive potassium channels that had conductances of 118 and 22 pS. The properties of these two channels were shown to be similar to those ascribed to the large-conductance calcium-activated potassium channel (BK_{Ca}) and small-conductance calcium-activated potassium channel (SK_{Ca}) channels in other neurons. Whole cell recordings from isolated RGCs showed that apamin and charybdotoxin (CTX), specific blockers of the SK_{Ca} and BK_{Ca} channels, respectively, resulted in a shortening of the time to threshold and a reduction in the hyperpolarization after the spike. Addition of these blockers also resulted in a significant increase in spike frequency over a wide range of maintained depolarizations. Similar effects of apamin and CTX were observed during current-clamp recordings from intact alpha and beta ganglion cells, morphologically identified after Lucifer yellow filling. About 20% of these neurons did not exhibit a sensitivity to either blocker, suggesting the presence of functionally distinct subgroups of alpha and beta RGCs on the basis of their intrinsic membrane properties. The expression of these calcium-activated potassium channels in the majority of alpha and beta cells provides a means by which the activity of these output neurons could be modulated by retinal neurochemicals.

INTRODUCTION

Calcium-activated potassium currents have been reported to play important roles in the regulation of neuronal activity. In particular, these currents were shown to 1) contribute to the repolarizing phase of the action potential (Adams et al. 1982); 2) control the repetitive discharge of spikes (Constanti and Sim 1987; Lancaster and Pennefather 1987; Pennefather et al. 1985; Schwindt et al. 1988); and 3) participate in various forms of oscillatory membrane behavior (Bourque 1988). Single-channel studies have revealed several types of calcium-activated potassium channels, which can be divided into two distinct groups on the basis of their pharmacological and biophysical properties: large-conductance calcium-activated potassium channel (BK_{Ca}) and small-conductance calcium-activated potassium channel (SK_{Ca}) (Barrett et al. 1982; Blatz and Magleby 1987; Lipton and Tauck 1987; Marty 1981; Maruyama et al. 1983; Pallotta et al. 1981). The BK_{Ca} channels can be blocked by charybdotoxin, have a high unitary conductance, and display sensitivity to both voltage and submicromolar concentrations of charybdotoxin (CTX) (Barrett et al. 1982; Blatz and Magleby 1987; Marty 1981; Maruyama et al. 1983; Pallotta et al. 1981). The current passing through these channels has been implicated in action potential repolarization and the fast hyperpolarization after the spike (Adams et al. 1982). In contrast, SK_{Ca} channels have a low unitary conductance, are voltage- and CTX-insensitive, and are activated by nanomolar concentrations of calcium (Blatz and Magleby 1987). The current flowing through these channels is sensitive to apamin and was shown to underlie the slow afterhyperpolarization (AHP) that in many cells is responsible for action potential frequency adaptation (Lancaster et al. 1991; Madison and Nicoll 1984).

Recent studies have documented a number of different conductances in retinal ganglion cells (reviewed in Ishida 1995), but as yet little is known about the role of calcium-activated potassium currents in these neurons. In this study we have identified two channels expressed by postnatal ferret RGCs that have characteristics similar to BK_{Ca} and SK_{Ca} described in other mammalian neurons (e.g., Blatz and Magleby 1987). To our knowledge, this is the first demonstration that both types of calcium-activated potassium channels are expressed in mammalian retinal ganglion cells (RGCs). Although Lipton and Tauck (1987) found three potassium channels in single-channel recordings from rat RGCs, only the BK_{Ca} conductance was identified. Furthermore, by making whole cell recordings from both isolated and intact RGCs, in the present study we show that both calcium-activated potassium currents contribute to spike repolarization and regulate the frequency of spiking activity.

METHODS

Isolation and plating of RGCs retrogradely labeled with rhodamine latex beads

All surgical procedures were carried out in compliance with National Institute of Health guidelines and in accordance with protocols approved by the campus animal use committee. The methods for the retrograde labeling and isolation of ferret RGCs were the same as those previously described in detail for the cat and will not be repeated here (Skaliora et al. 1993, 1995). Postnatal ferrets, aged between postnatal day (P)30 and P46, were used as experimental animals because in preliminary experiments we found that at this stage the cells were optimal for patch-clamp recordings. RGCs were dissociated and stored in solution containing a 1:1 ratio of L-15 medium (Sigma) and EMEM (Eagle’s minimum essential medium; Sigma), supplemented with 10^{-7} M insulin (Sigma) and an antibiotic-antimycotic agent (Gibco 600-5240AG). After dissociation, the cell suspension was immediately sieved through a nylon mesh (Tetko Nitex 3-250/50) to remove cell clots and tissue debris. The retinal cells were then plated onto coated coverslips and...
stored in a humidified incubator (5% CO₂; 36.5°C) until ready for use. To ensure that the retinal cells had become adhered to the coverslips, recordings were not attempted until 6 h after plating.

The coverslips (12-mm circle, Fisher) were cleaned in 95% alcohol, autoclaved, and subsequently coated with a 10% poly-D-lysine solution. The coated coverslips were then rinsed in sterile, double distilled water and placed in sterile 24-well tissue culture plates (Falcon). Just before plating, the coverslips were washed several times in culture medium and conditioned in an incubator for 20 min. Recordings were made from these neurons 6–72 h after culturing and no changes were evident in functional properties during this period.

RGCs, backfilled with rhodamine latex beads, were visualized in the recording chamber by using an IM35 Zeiss epifluorescence microscope (×400; filters: BP 510–560, FT 580; LP 590) equipped with a mercury vapor lamp. Differential interference contrast (DIC) optics were used to view the cell during the recording period. All neurons from which recording were obtained had translucent cell bodies with nongranular appearance, clear surfaces, and often retained stumps of broken processes.

Preparation of retinal whole-mounts

After deep anesthesia of postnatal ferrets with barbiturate, the retinas were removed and placed in oxygenated EMEM (Sigma, M-7278) at 25°C, continuously bubbled with oxygen. A small piece of retina was placed ganglion cell layer up in the recording chamber and stabilized with an overlying piece of filter paper. A 2-mm hole in the filter paper provided access for the recording electrode. Cells were visualized through this opening with a ×40 objective mounted on a fixed-stage upright epifluorescence microscope (Nikon) equipped with a mercury vapor lamp.

The retina was bathed in the chamber with EMEM, heated with a Peltier device, and continuously bubbled with 95% O₂–5% CO₂. A calibrated thermocouple monitored the temperature in the recording chamber, which was maintained at 35°C. Patch electrodes were filled with a solution containing 140 mM KCl, 10 mM N-2-hydroxyethylpiperazine-N’-2-ethanesulfonic acid (HEPES), 50 mg/ml Nystatin, 200 mg/ml Pluronic, and 2% Lucifer yellow, pH 7.4. By the end of the recording, the soma and the dendritic arborizations were usually completely labeled. In some cases, complete filling required additional application of a hyperpolarizing potential (200 mV) for ~5 min. Once adequate filling was achieved, the retina was removed and fixed in 4% paraformaldehyde for 6–8 h at 4°C. The retina, with filter paper still attached, was then mounted on a slide and labeled cells were subsequently viewed with a BioRad MRC-600 confocal microscope equipped with an argon laser mounted on an Olympus microscope. Optical sections were recorded in sequential steps of 3–5 μm and the resulting images were then compiled to provide a Z-series montage depicting the entire perikaryon.

Solutions

For the cell-attached patch and isolated whole cell recordings, the bath solution contained (in mM) 130 NaCl, 5.9 KCl, 2.5 CaCl₂, 1 MgCl₂, 10 HEPES-NaOH, and 22 glucose; pH 7.35. During inside-out recordings the bath solution was changed and contained (in mM) 140 KCl, 1 MgCl₂, variable CaCl₂, 10 HEPES-NaOH, and 22 glucose; pH 7.35. For the single-channel recording, the electrode solution comprised (in mM) 140 KCl, 2.5 CaCl₂, 1 MgCl₂, 10 HEPES-NaOH, and 22 glucose; pH 7.35. For whole cell recordings from isolated retinal ganglion cells, the electrodes were filled with a solution containing (in mM) 140 KCl, 10 HEPES, 50 mg/ml Nystatin, 200 mg/ml Pluronic, and 2% Lucifer yellow, pH 7.4. Apamin (1 μM) and charybdotoxin (CTX, 0.02 μM) were administered to the bath through a gravity fed line. At these concentrations both apamin and charybdotoxin were reported to specifically block calcium-activated potassium channels without affecting other types of K⁺ conductances (Meves 1992; Pineda et al. 1992). The high affinity of apamin and CTX for their respective channels made it difficult to wash out the drug so only partial recovery toward control could be obtained.

Electrophysiology

The cell-attached, inside-out, and whole cell patch variations of the patch-clamp technique were utilized to examine calcium-activated potassium currents in isolated ferret RGCs. Patch electrodes with resistances between 5 and 10 MΩ were pulled from thick-walled 1.5-mm OD borosilicate glass on a Sutter Instruments P-87 puller. High-resistance seals were obtained by moving the patch electrode onto the cell membrane and applying gentle suction. After the formation of a high-resistance seal between the electrode and the cell membrane, transient currents caused by pipette capacitance were electronically compensated by the circuit of the Axopatch 1C amplifier.

Both single-channel currents and membrane potentials were recorded with an Axopatch 1C patch-clamp amplifier and data were low-pass filtered and digitized at rates between 1 and 4 kHz before being stored on an IBM computer for subsequent off-line analysis. These recordings were carried out at room temperature (24°C).

Patch pipettes with a tip resistance between 7 and 12 MΩ were pulled from thick-walled 1.5-mm OD borosilicate glass on a Sutter Instruments P-87 puller. Current-clamp recordings were made with an Axopatch 1-D patch-clamp amplifier. The data were low-pass filtered and digitized at rates between 1 and 4 kHz before storage on an IBM computer for subsequent off-line analysis. Recordings were obtained by patching onto cells with clear, nongranular cytoplasm. Immediately after the whole cell configuration was attained, the resting membrane potential was read off the amplifier. The value of the resting potential was monitored regularly throughout the experiment and if significant changes were observed, recordings were terminated.

RESULTS

The cell-attached, inside-out patch and whole cell variations of the patch-clamp technique were utilized to characterize the properties and functional roles of calcium-activated potassium currents in ferret retinal ganglion cells. Successful recordings were made from 112 cells obtained from 33 postnatal ferrets aged between postnatal day (P)30 to P46. During this period, there were no age-related changes in the recordings. Two different calcium-activated potassium channels were identified in these neurons with properties similar to those ascribed to the BKCa and SKCa channels. The characteristics and functional roles of these conductances are described in the following sections.

Identification of calcium-activated potassium channels

The cell-attached and inside-out patch techniques were used to examine the expression and single-channel properties of calcium-activated potassium conductances in isolated RGCs unequivocally identified by retrograde labeling with rhodamine latex beads. Figure 1A shows a cell-attached patch recording obtained with 140 mM K⁺ in the electrode and 5.9 mM K⁺ in the bathing solution at a number of command potentials. When 60 mV was applied to the outer surface of this patch, a large inward current was seen to pass through the channel. The amplitude of this single-channel current became progressively smaller as the command potential became more negative and reversed around ~40 mV. The addition of 5 μM BAY-K8644, a calcium current agonist, to the bathing solution resulted in an increase in the P_{open} and duration of channel openings, suggesting that this channel...
is sensitive to increases in intracellular Ca\textsuperscript{2+}. The $P_{\text{open}}$ and mean open time, calculated when 60 mV was applied to the patch, were 0.015 ± 0.002 (SD) and 3.51 ± 0.03 ms in the absence of BAY-K8644 and 0.038 ± 0.004 and 4.22 ± 0.05 ms ($n = 4$) in the presence of the agonist.

Under identical recording conditions a second channel (Fig. 1B), also sensitive to bath application of 5 $\mu$M BAY-K8644, was observed. Application of the calcium channel agonist also caused an increase in the $P_{\text{open}}$ from 0.087 ± 0.008 to 0.249 ± 0.08 and mean open time from 1.02 ± 0.02 to 2.54 ± 0.06 ms ($n = 3$) in these channels. The amplitude of the single channel current passing through this channel, however, was much smaller than that passing through the channel illustrated in Fig. 1A. In spite of their reduced size, single-channel currents were readily distinguished above the baseline noise (inset to Fig. 1B). The few records in which this was not the case were excluded from analysis.

To determine the conductance of these two channels, the amplitude of the single-channel current ($I$) was measured at a number of different command potentials ($V_{\text{com}}$) and the conductance of each channel was determined by fitting a regression line through the data. Figure 1C shows the summary of such an analysis for seven cell-attached patches, four containing the large opening channels, and three containing the smaller opening channels. Plotted for each group is the mean single-channel current (SD), which was fitted with a regression line. The large opening channel (●) had a slope conductance of 118 ± 1.4 pS ($n = 4$) and the smaller opening channels (○) had a conductance of 22 ± 1.2 pS ($n = 3$). The probability of opening $P_{\text{open}}$ was also determined over a range of command potentials between −40 and 40 mV. The $P_{\text{open}}$ for the large conductance channel showed a voltage dependence with values ranging from 0 at −40 mV to 0.018 at 40 mV ($n = 4$). In contrast, the small conductance channel expressed little voltage dependence with $P_{\text{open}}$ values ranging from 0.103 at −40 mV to 0.104 at 40 mV ($n = 3$). The properties of these conductances are similar to those reported for the BK\textsubscript{ca} and SK\textsubscript{ca} channels in other neurons (Barrett et al. 1982; Blatz and Magleby 1987; Lipton and Tauck 1987; Marty 1981; Maruyama et al. 1983; Pallotta et al. 1981), so this terminology will be used throughout the remainder of this paper.

To further demonstrate the sensitivity of the large and small conductance channels, inside-out patch recordings were obtained with different Ca\textsuperscript{2+} concentrations at the cytosolic face of the membrane. Figure 2, A and B, shows inside-out recordings of a BK\textsubscript{ca} channel obtained from the same patch at a holding potential of −40 mV, with 140 mM K\textsuperscript{+} in both the bathing and the electrode solution. In Fig. 2A the intracellular face of the patch was bathed in 1 nM Ca\textsuperscript{2+} and the $P_{\text{open}}$ is plotted as a function of time in Fig. 2C. Increasing the Ca\textsuperscript{2+} concentration at the intracellular face 10-fold (Fig. 2B) resulted in an increase in the number and duration of single channel openings and the subsequent $P_{\text{open}}$ is plotted as a function of time in Fig. 2D. Similar results were obtained in five patches. In low calcium concentration the mean $P_{\text{open}}$ was 0.05 ± 0.002 ms and the mean dwell time in the open state was 3.13 ± 0.07 ms. Raising the calcium concentration 10-fold at the cytosolic face of the patch increased the mean $P_{\text{open}}$ to 0.11 ± 0.012 ms and lengthened the mean open dwell time to 4.47 ± 0.05 ms ($n = 5$).

A qualitatively similar result was observed in three patches containing the small conductance channel (SK\textsubscript{ca}). As may be seen in Fig. 3, increasing the Ca\textsuperscript{2+} concentration on the intracellular face of an inside-out patch from 1 nM (Fig. 3A) to 0.01 $\mu$M (Fig. 3B) caused a marked increase in the number and duration of SK\textsubscript{ca} channel openings. Figure 3, C and D, shows the $P_{\text{open}}$ as a function of time for the SK\textsubscript{ca} channel in 1 nM and 0.01 $\mu$M Ca\textsuperscript{2+}, respectively. The mean open time in the presence of low Ca\textsuperscript{2+} was 0.005 ± 0.001 ms and increased to 0.05 ± 0.002 ms when the calcium concentration at the cytosolic face of the patch was increased 10-fold. The open state dwell times were 0.98 ± 0.02 ms to 2.98 ± 0.04 ms in 1 nM and 0.01 $\mu$M Ca\textsuperscript{2+}, respectively ($n = 3$).

**Roles of BK\textsubscript{ca} and SK\textsubscript{ca} channels in the generation of spikes and spiking patterns**

Whole cell patch-clamp recordings were made to examine the roles of the BK\textsubscript{ca} and SK\textsubscript{ca} channels in spike generation.
For this purpose, current-clamp recordings were obtained from 50 RGCs with a mean resting membrane potential of $-53 \pm 11$ mV. These cells were depolarized to elicit only a single spike and the spikes generated in this manner had a slow rise to threshold and were followed by a brief hyperpolarizing phase (Fig. 4, ---). The addition of 1 μM apamin, a well-characterized SKCa channel blocker, to the bathing media resulted in a shortening of the time to threshold and a reduction in the hyperpolarization after the spike (Fig. 4A, •••). Similar results were seen in five cells with

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**FIG. 2.** A and B: inside-out patch recordings of large conductance channel (BK) at $-40$ mV with 140 mM K$^+$ in both bath and electrode solution. A−B, top: each panel shows a small part of records below on an expanded time scale. Calcium concentration in bathing solution was 1 nM in A and 0.01 μM in B. Single-channel open probability in each calcium concentration is shown in C and D, respectively.

**FIG. 3.** A and B: inside-out patch recordings of small conductance channel (SK) at $-40$ mV with 140 mM K$^+$ in both bath and electrode solution. A−B, top: a small part of records below on an expanded time scale. Calcium concentration in bathing solution was 1 nM in A and 0.01 μM in B. Single-channel open probability in each calcium concentration is shown in C and D, respectively.
the time to threshold and the hyperpolarization after a spike decreasing significantly ($P < 0.01$, two tailed $t$-test) by $1.7 \pm 0.53$ ms and $3.5 \pm 0.7$ mV, respectively. The spike width was unaffected by the application of $1 \text{ M}$ apamin with values of $3.8 \pm 0.31$ ms and $3.6 \pm 0.29$ ms in the absence and the presence of the blocker, respectively. In two of seven neurons, the addition of $1 \text{ M}$ apamin had no effect on either the time to threshold or the hyperpolarization after the spike.

The BK$_{Ca}$ channel blocker charybdotoxin (CTX), added at a concentration of $0.02 \text{ M}$, resulted in a more pronounced decrease in the time to threshold and reduced the size of the hyperpolarization by a greater amount than did apamin (Fig. 4B). The time to threshold and the hyperpolarization after a spike both decreased significantly ($P < 0.01$, two-tailed $t$-test) by $2.1 \pm 0.48$ ms ($n = 7$) and $5.8 \pm 1.0$ mV ($n = 7$), respectively, on application of the BK$_{Ca}$ channel blocker. The spike width ($3.7 \pm 0.43$ ms; $n = 7$) was once again unaffected by the application of CTX ($3.9 \pm 0.33$ ms). Application of CTX had no effect on the time to threshold or the hyperpolarization after the spike in two of nine cells. As was the case with apamin, these cells could not be distinguished on the basis of soma size from the RGCs that were sensitive to this blocker.

The roles of the large and small conductance calcium-activated potassium channels in the generation of spiking patterns were examined by injecting maintained currents in the presence and absence of apamin and CTX (Fig. 5). The Fig. 5A, left, illustrates the response of a RGC to a 400 ms current injection of $170 \text{ pA}$ from a holding potential of $-67$ mV. The neuron responded with a sustained burst of action potentials, the frequency of which increased with the addition of $0.02 \text{ M}$ CTX to the bathing solution (Fig. 5A, right). A qualitatively similar result was obtained in a different neuron when $1 \text{ M}$ apamin was added to the bathing solution (Fig. 5B). The effect of both drugs was consistent over a wide range of stimulus amplitudes as shown in the two panels of Fig. 5, C and D, where spike frequency is plotted as a function of injected current magnitude. Bath application of apamin and CTX (○) increased the spike frequency at all maintained depolarizations that generated sustained spiking patterns. Such an increase in spike frequency on application of the SK$_{Ca}$ and BK$_{Ca}$ channel blockers was observed in $81\%$ of the cells tested with CTX ($21/26$) and $75\%$ of those tested with apamin ($18/24$). To quantify this change in spike frequency, we calculated the increase in spike rate in the presence of the blocker normalized to the spike rate when no blocker was added. At stimulus amplitudes in the midrange of those utilized to activate the cell, the mean increase with CTX application was $33 \pm 15\%$ ($n = 21$) and with apamin it was $30 \pm 9\%$ ($n = 18$).

Furthermore, by examining the mean firing frequency during the first and last 200 ms of the test depolarization we sought to determine whether or not this increase in overall firing rate (described above) was consistent throughout the duration of the stimulus. The application of CTX and apamin resulted in $41$ and $51\%$ increases in firing rate during the initial 200 ms, although during the last 200 ms these rates increased by $42$ and $40\%$, respectively.

### Functional roles of calcium-activated potassium currents in intact RGCs

The observation that a number of isolated cells did not express a CTX- or an apamin-sensitive conductance raised...
the possibility that there may be differences among morphologically defined cell classes in terms of expression of functional calcium-activated potassium conductances. Because the isolation procedure removes most of the dendritic processes, a distinction among different cell classes could not be made. To determine whether or not there is a relationship between the expression of calcium-activated potassium conductances and ganglion cell class, whole cell recordings were made from the intact retina. Specifically, the effects of apamin and CTX on the spontaneous activity and responses to injected currents were examined. Successful recordings were obtained from 31 intact RGCs (8 alpha and 23 beta cells), which had a mean resting membrane potential of $-57 \pm 7$ mV. Figure 6 shows the confocal reconstructions of the two morphological cell types, alpha and beta, from which recordings were made.

In general, recordings from the intact retina yielded spike discharges of a higher frequency than those obtained from the isolated RGCs (compare discharges shown in Fig. 5 with those in Fig. 7). Most likely, this reflects the fact that recordings from the isolated cells were done at room temperature while those from the intact retina were carried out close to body temperature. It is also possible that the loss of dendrites in the isolated cells contributed to this difference. Nevertheless, as will be described below, the effects of Ca-activated potassium channel blockers were essentially the same on isolated and intact RGCs.

Figure 7 shows that the effects of apamin and CTX on the responses of intact retinal ganglion cells to maintained depolarizations. The responses of a beta cell to a 1 s depolarizing current (130 pA), in the absence and presence of 1 $\mu$M apamin, are shown in Fig. 7, A and B, respectively. Addition of the blocker to the bathing solution clearly increased the frequency of discharge (Fig. 7C). This was observed in 71% (10/14) of the ganglion cells examined and the magnitude of this effect at the midpoint of the applied stimulus amplitudes was $27 \pm 9\%$ ($n = 10$). A qualitatively similar result ($26 \pm 9\%, n = 13$) was obtained in 76% (13/17) of the RGCs when 0.02 $\mu$M CTX was added to the bathing solution (Fig. 7D). This increase in overall firing rate observed in response to the specific channel blockers was consistent throughout the duration of the maintained depolarization. The application of CTX and apamin resulted in 22 and 18% increases in firing rate during the initial 200 ms and during the last 200 ms the rates increased by 42 and 41%, respectively. Such effects were obtained in both alpha and beta cells. Specifically, 3 of 5 alpha cells and 10 of 12 beta cells were sensitive to CTX, whereas 2 of 3 alpha and 8 of 11 beta cells were apamin sensitive.

**DISCUSSION**

In the present study we have identified two types of calcium-activated potassium channels expressed by ferret retinal ganglion cells. Whole cell recordings from isolated and intact ganglion cells showed that both conductances regulate the frequency of spike discharges in response to maintained depolarizations. Activation of these channels leads to an increase in both the time to spike threshold and in the hyperpolarization after the spike, decreasing the rate of sustained discharges. Recordings from the intact retina revealed that the majority of alpha and beta cells expressed these two types of calcium-activated potassium conductance.

**Identification of calcium-activated potassium channels**

By using the cell-attached and inside-out variations of the patch-clamp technique, two types of calcium-activated...
potassium channels were identified in isolated postnatal ferret RGCs. In the cell-attached mode, with 140 mM potassium in the electrode, these channels had conductances of 118 and 22 pS, and were sensitive to changes in intracellular calcium induced by application of the calcium-current agonist Bay-K8644. The larger conductance channel also expressed a voltage dependence between −40 mV and 40 mV, whereas the small conductance channel did not show such voltage-dependence. Inside-out patch recordings revealed that increasing the calcium concentration at the intracellular face of the patch resulted in an increase in the $P_{\text{open}}$ and in the time spent in the open state for both channels.

Postnatal ferret RGCs therefore express two channels with properties similar to those described for the BK$_{\text{Ca}}$ and SK$_{\text{Ca}}$ calcium-activated potassium channels in other cells (Barrett et al. 1982; Blatz and Magleby 1987; Marty 1981; Maruyama et al. 1983; Pallotta et al. 1981). Interestingly, only a BK$_{\text{Ca}}$ channel with a conductance of 115 pS was reported in rat RGCs (Lipton and Tauck 1987), which is virtually identical to the 118 pS reported here. This suggests the possibility that the expression of these channels may differ between carnivore and rodent ganglion cells.

Roles of BK$_{\text{Ca}}$ and SK$_{\text{Ca}}$ channels in the generation of spikes and spiking frequency

The contribution of currents passing through the BK$_{\text{Ca}}$ and SK$_{\text{Ca}}$ channel to the generation of spikes and spiking patterns was determined by making whole cell current-clamp recordings in isolated neurons unequivocally identified by retrograde labeling with rhodamine latex beads. The addition of the specific channel blockers CTX (BK$_{\text{Ca}}$) and apamin (SK$_{\text{Ca}}$) resulted in a shortening of the time to threshold and a reduction in the hyperpolarizations after the spike. The addition of CTX or apamin, however, had no effect on spike width. The decrease in time to threshold could indicate that sufficient calcium enters the cell during spike initiation to activate both BK$_{\text{Ca}}$ and SK$_{\text{Ca}}$ channels. It is also possible that these channels are active at rest because we found that very low intracellular Ca$^{2+}$ concentration (1 nM) were capable of activating both conductances.

The overall effect of decreasing the time to threshold and the degree of hyperpolarization after the spike was a decrease in the interspike interval and a concomitant increase in the frequency of spike discharge. BK$_{\text{Ca}}$ and SK$_{\text{Ca}}$ mediated currents therefore appear to play a role in setting the frequency of spike discharge attainable by RGCs. Furthermore it is conceivable that the overall activity of RGCs could be raised or lowered by retinal neurochemicals that directly modulate these conductances or regulate the amount of calcium entering the cell during a spike. Somatostatin, for example, a neuropeptide localized in the carnivore retina (White et al. 1990; White and Chalupa 1991), was shown to increase the light-evoked activity of rabbit RGCs (Zalutsky and Miller 1990) and inhibit voltage-gated calcium currents in other systems (Dryer et al. 1991; Inoue and Yoshii 1992; Meriney et al. 1994; Narahashi et al. 1987).

Expression of BK$_{\text{Ca}}$ and SK$_{\text{Ca}}$ by alpha and beta cells

About 19% of the neurons tested were not sensitive to CTX and 25% were not sensitive to apamin. In these ganglion cells the calcium-activated potassium channels were either absent or were present in numbers too few to have a functional effect. Within the age range studied, there was no indication that the presence or absence of these conductances was related to maturational state. Furthermore, sensitivity to CTX and apamin was not related to morphological cell class, suggesting that there may be functional subgroups of alpha and beta cells distinguished on the basis of their expression of calcium-activated potassium channels. Neurons that do not express BK$_{\text{Ca}}$ or SK$_{\text{Ca}}$ channels would tend to be more excitable than those that do, for the reasons discussed above. The functional significance of such heterogeneity in channel expression to the processing of visual information in the intact retina remains to be established. Interestingly, we have recently found heterogeneity in the intrinsic temporal properties of alpha and beta cells, suggesting functional subclasses not reflected in the traditional classification system based on alpha/Y and beta/X criteria (Robinson and Chalupa 1997). Conceivably, the differential expression of calcium-activated potassium channels demonstrated here could relate to such temporal differences. In future experiments it should be feasible to assess the merits of these ideas by examining light-evoked responses in combination with current-clamp recordings in retinal whole-mounts.

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