Ionic Mechanisms Mediating Oscillatory Membrane Potentials in Wide-Field Retinal Amacrine Cells

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INTRODUCTION

Amacrine cells constitute the most diverse cell class in the vertebrate retina. For example 43 morphological types of amacrine cells (ACs) have been identified in the fish (roach) (Wagner and Wagner 1988) and 29 types in the mouse retina (Wagner and Wagner 1988) and 29 types in the mouse retina. For example 43 morphological types of amacrine cells (ACs) have been identified in the fish (roach) (Wagner and Wagner 1988) and 29 types in the mouse retina (Wagner and Wagner 1988). Other populations of ACs are capable of producing membrane potential oscillations in response to light stimuli and/or in the dark (Djamgoz et al. 1990; Sakai and Naka 1988; Teranishi et al. 1987). The oscillations were thought to arise as a result of network activity. The role of these oscillations is not understood in terms of retinal information processing but could be involved, for example, in information transfer across large dendritic fields and/or in transmitter release. In other systems, oscillations spread information over long distances signaling information of “importance” to other neurons (Llinas 1988). In coupled systems, oscillations synchronize the network, and if the cells are “tuned,” broadcast the relevant signal most efficiently (Gray et al. 1989). Recently, single GABAergic wide-field ACs (WFAC) isolated from the white bass retina were shown to generate membrane potential oscillations in response to extrinsic depolarization (Solessio et al. 2002). These oscillations also occurred in response to glutamate analogues; glutamate is the endogenous neurotransmitter impinging on WFACs. Thus the ability of these WFACs to generate oscillations is an intrinsic feature of the cell and not due to network properties.

The goal of the present study was to determine the underlying ionic mechanisms responsible for generating the oscillations.
tions. Because the oscillations may be involved in signal processing (Gray et al. 1989; Llinas 1988; Sakai and Naka 1988), it is important to understand how they come about to understand the functional properties of the cell. Much is known about the oscillations in other neuronal systems (Amir et al. 2002; Hudspeth 1986; Hutcheon and Yarom 2000), but the basis for this type of intrinsic oscillatory behavior in amacrine cells is unknown. We found that the oscillations in WFACs arose as a result of the complex interplay between voltage-dependent calcium currents and voltage- and calcium-dependent potassium currents. This is similar to what has been observed in hair cells in the sacculus (Hudspeth and Lewis 1988b) and in CNS neurons (Hutcheon and Yarom 2000) but with some interesting differences.

Methods

Isolation procedure

Isolated WFACs maintained in culture were used for all experiments. The cell-isolation procedure used was similar to that previously described in detail (Pfeiffer-Linn and Lasater 1998). All procedures were performed in accordance with the United States Animal Welfare Act and the National Institutes of Health Guide for the Care and Use of Laboratory Animals. Briefly, white bass (Morone chryspops) were dark-adapted for 2 h and then rapidly killed. The eyes were enucleated and hemisected, then the retinas were removed from the eyecups under dim red illumination. This was followed by 20-min incubation in papain (2.5 U/ml, Worthington, Freehold, NJ) at room temperature followed by washing several times in fresh modified L-15 (Sigma, St. Louis, MO). The retinas were then triturated and the cells plated onto cell culture dishes. Cells were maintained in an incubator (10°C) for 1–5 days in modified L-15 cell culture medium until used.

Experimental solutions

Before each experiment, the L-15 culture medium in the culture dish was replaced with bass Ringer consisting of (in mM) 130 NaCl, 2.9 KCl, 1.23 MgCl2, 0.44 K2HPO4, 10 MOPS, and 8.4 Tris-sulfate; the pH adjusted to 7.4 with NaOH. The standard patch pipette solution consisted of (in mM) 130potassium gluconate, 4 NaCl, 4 KCl, 0.2 EGTA, 2 MgCl2, and 10 HEPES, and 7.8 μM CaCl2.

In experiments studying Ca influx through voltage-gated channels, the normal Ringer solution was modified by increasing CaCl2 concentration to 10 mM and adding 1 μM TTX to block sodium, 5 mM 4-aminopyridine (4-AP) and 10 mM TEA to block potassium currents. Osmolarity was kept constant by removal of equimolar NaCl. Also, in such experiments we used a cesium-based pipette solution to maximally reduce the potassium currents. This solution consisted of (in mM) 106.72 Cs-gluconate, 4 NaCl, 1 CaCl2, 1 MgCl2, 8.4 HEPES, and 0.2 EGTA, supplemented with 4 ATP and 20 phosphocreatine and 50 μM creatine phosphate. The pH was adjusted with gluconic acid to 7.4. All chemicals were purchased from Sigma. The liquid-junction potentials introduced by the cesium gluconate solution were compensated for offline.

Solutions containing ω-Aga-TX (50 nM), ω-conotoxin MVIC (1 μM), ω-conotoxin GVIA (1 μM) all from Alomone Labs, Jerusalem, Israel), nifedipine (100 μM, Sigma-RBI, Natick, MA), CoCl2 (4 mM), diltiazem (100 μM), and ζ-Bay-K (1 μM; all from Sigma) were focally delivered to cells via a 12-reservoir pressure ejection system controlled by a personal computer (DAD-12, Adams). Because of its light sensitivity, the container holding nifedipine was shielded from light with aluminum foil.

Recording procedures and data analysis

Whole cell recordings (Hamill et al. 1981) were made with micropipettes pulled on a two-stage puller (model PP-83, Narishige Instruments, Tokyo) from borosilicate tubing (Druumond Scientific, Broomall, PA) and were used unpolished. The electrode tip resistance was typically 7–9 MΩ when measured in the bath solution. Series resistance and capacitance were compensated for electronically. Voltage and current signals were recorded and low-pass filtered at 2 kHz with an Axopatch 200A amplifier (Axon Instruments, Foster City, CA) then sampled at 10 kHz. Data acquisition was controlled by a personal computer interfaced to a Digidata 1200 or Digidata 1322A (Axon Instruments) data-acquisition system driven by the pClamp suite of programs (Axon Instruments). After establishing a whole cell configuration, the cells were held at a potential (HP) of −70 mV unless indicated otherwise. Under voltage-clamp conditions, the stimuli consisted of 90-ms-long incremental and decremental voltage pulses between −120 and +70 mV. Under current-clamp conditions, the stimuli consisted of a series of incremental depolarizing pulses ranging from 0 to 1.5 nA. The pulses were 150 ms in duration and applied from the holding potential of the cell unless indicated otherwise. For clarity, all current-clamp figures except Fig. 1 show only responses to 0-, 0.5-, 1-, and 1.5-nA depolarizing current pulses.

Analysis and plotting of data were performed with Clampfit (Axon Instruments), SigmaPlot (SPSS, Chicago, IL) and Excel. Plots show average responses ± SDs and number of observations is indicated in the figure and/or in the legends. Statistical analysis of the data were performed by using Student’s t-test (paired comparison), and the P value of <0.05 was considered to be significant.

Quantification of the oscillations

The oscillations were quantified by using the distance of the “line” tracing the membrane voltage change during the depolarizing current steps. The distance was calculated as follows

\[ I = \frac{1}{n} \sum_{i=1}^{n} |x_i - x_{i-1}| \]

Intensity (I) represents the duration and the amplitude of the oscillations, T: the observation period, and x: membrane potential at time t. Using this approach, a cell that produced fewer or attenuated oscillations will have a shorter “line” length than one that generated sustained oscillations.

Immunofluorescence techniques

All antibodies against the calcium channel subunits were rabbit polyclonal obtained commercially from Alomone Labs, except for the α1F antibody, which was raised in sheep (Morgans 2001). The monoclonal anti-synaptin was purchased from Sigma. Cells were isolated and maintained on con-A-coated coverslips for 2–4 days before staining. Isolated cells were fixed in 4% paraformaldehyde solution for 15 min, incubated in 10% normal donkey serum in 0.1 M PB 0.5% Triton X-100 for 1 h at 4°C, and then transferred to either a solution of a single antibody or a mixture of two primary antibodies. The antibodies were used at a 1:500 to 1:1,000 dilution. After overnight incubation in the antibody mix, isolated cells were washed in 0.1 M PB and transferred to a cocktail of the secondary antibodies [donkey anti-rabbit IgG coupled to fluorescein (FITC), donkey anti-mouse IgG rhodamine (TRITC) or IgG fluorescein] at 1:100 dilution in 0.1 M PB 0.5% Triton X-100 for 1 h. Immunostained sections were examined by confocal microscopy. A Zeiss LSM510 was used to image the cells and to optically section the cells at 1-μm intervals. Slices were combined to make a composite image or single slices were analyzed. Control sections were obtained by omitting the primary antibody.
RESULTS

Identification of WFAC in isolation

Cells were identified as WFACs in culture by their unique, distinctive morphology (Solessio et al. 2002). WFACs reported on in this study typically had a small, often triangular shaped cell body (~15 μm or less), with characteristic long, thick processes extending for ~250 μm and were typically branched. Although processes were sometimes truncated during the isolation procedure, it was not unusual to find cells in culture whose dendritic spread extended between 300 and 400 μm (see Fig. 5).

Basic characteristics of the oscillations

Small, depolarizing current steps evoked passive membrane depolarizations (Fig. 1, +0.1 nA). With further increase of the depolarizing current steps, activation of voltage-dependent mechanisms was observed. Characteristically, oscillation of the membrane potential was always present if the membrane depolarization reached ~43 ± 4 mV (n = 10, see Fig. 1, +0.2 nA and up). This is in close agreement with the activation potentials of the voltage-gated sodium ($I_{Na}$) and Ca$^{2+}$ currents ($I_{Ca}$) in WFACs (Solessio et al. 2002). The magnitude, duration and frequency of the oscillations increased with membrane depolarization (see Fig. 1). Blocking the voltage-dependent Na$^+$ channels with TTX did not eliminate the response, whereas the Ca$^{2+}$ channel blocker Cd$^{2+}$ or a mixture of K$^+$ channel blockers (TEA and 4-AP) effectively blocked the oscillations (Solessio et al. 2002). These results suggested that oscillatory membrane potentials (OMPs) result from the interplay of Ca$^{2+}$ and K$^+$ currents. This likely includes a Ca$^{2+}$-dependent potassium current. In support of this notion, blockade of the voltage-gated Ca$^{2+}$ currents with Cd$^{2+}$ dramatically reduced the outward K$^+$ currents (Solessio et al. 2002), strongly indicating the presence of $K_{Ca}$ in WFACs. We went on to study in detail the ionic mechanisms underlying the OMPs with a focus on the calcium and potassium currents.

Contribution of Ca$^{2+}$ to the oscillations

Like Cd$^{2+}$, 4 mM Co$^{2+}$ blocked the oscillations (n = 15) (Fig. 2A), except for the first peak. The first peak was attenuated or absent in the presence of TTX suggesting it was Na$^+$ based (Solessio et al. 2002). In addition, the application of Co$^{2+}$ also resulted in a 10- to 15-mV hyperpolarization of the cell. Note also the large increase in the membrane potential in

![FIG. 1. Oscillatory potentials of wide field amacrine cells (WFACs) elicited by depolarizing current steps of increasing amplitude. The duration and frequency of the oscillatory membrane potentials (OMPs) increased with depolarization. Voltage traces are shifted vertically for clarity. Bottom: application of depolarizing pulse. Magnitude of depolarizing current indicated with each trace. Holding potential was ~70 mV.](http://jn.physiology.org/)

![FIG. 2. Role of Ca$^{2+}$ in the generation of the oscillatory potentials in WFACs. A: $4 \text{ mM Co}^{2+}$ in the extracellular solution blocked the oscillations and increased the cell input resistance. B and C: elevation of extracellular Ca$^{2+}$ (10 mM) enhances the oscillations. Note that with weak intracellular Ca$^{2+}$-buffering (0.2 mM EGTA), the oscillations are strongly attenuated or dampened (B). With stronger buffering (10 mM EGTA) no attenuation was observed (C).](http://jn.physiology.org/)
response to the depolarizing step in the presence of Co^{2+} in comparison to the corresponding control traces. These are due to a block of calcium leak into the cell and an increase in input resistance, respectively.

Next we determined the effect of elevated extracellular Ca^{2+} concentration on the oscillations. Under control conditions, cells were bathed in normal Ringer containing 2.1 mM Ca^{2+}. High-Ca^{2+} (10 mM) Ringer solution was flushed over the cells during the recording and then washed away with normal Ringer. Elevation of the extracellular [Ca^{2+}] markedly increased both the duration and the amplitude of the oscillations (n = 17, Fig. 2B). However, the frequency remained unaltered. Interestingly, the threshold potential for the generation of the OMPs did not change significantly (not illustrated). Recovery was achieved within a few minutes of washout. Note the pronounced dampening of the oscillations in 10 mM Ca^{2+}-containing Ringer solution (Fig. 2B). Elevation of the Ca^{2+}-buffer EGTA in the pipette from 0.2 to 10 mM eliminated this dampening (Fig. 2C), suggesting a Ca^{2+}-dependent downregulation of the oscillatory response components.

Based on these results, we concluded that Ca^{2+} influx through voltage-gated channels was essential to the generation of OMPs in WFACs. On the one hand, elevated extracellular Ca^{2+} facilitated oscillatory responses in WFACs. On the other hand, Ca^{2+} entering the cells also triggered a Ca^{2+}-dependent process that dampened the oscillations.

**Inactivation of Ca^{2+} currents**

We investigated whether or not the voltage-gated Ca^{2+} current was subject to Ca^{2+}-dependent inactivation. With a Cs^{+}-based pipette solution and TTX, TEA, 4-AP, and 10 mM Ca^{2+} containing extracellular solution, 50-ms-long depolarizing voltage steps evoked a very large, sustained inward current (not illustrated). The current amplitude ranged from 1 to 3 nA. For cells with an input capacitance of 56.0 ± 7.6 pF (n = 13), the peak current density was 53.7 ± 12.4 pA/pF at −10 mV. To study the inactivation properties of the Ca^{2+} currents, membrane potentials were stepped from −70 to 0 mV for 400 ms. Using long voltage steps, a marked relaxation of I_{Ca} became apparent over time (Fig. 3A, control). We were able to dissect two forms of inactivation: a time-dependent component, which was always present, and a Ca^{2+}-dependent component, which required Ca^{2+} as the charge carrier. These two mechanisms were distinguished in two ways: first by adding 10 mM bis-(o-aminophenoxy)-N,N,N′,N′-tetraacetic acid (BAPTA; Fig. 3A, BAPTA) to the intracellular solution and second by using Ba^{2+} as a charge carrier (Fig. 3A, barium; Eckert and Tillotson 1981; Gleason et al. 1994). For such an experiment, the tip of the pipettes were filled with 0.2 mM EGTA containing Cs^{+}-based intracellular solution, then backfilled with this solution supplemented with 10 mM BAPTA to avoid immediate BAPTA diffusion into the cells. Control recordings (Fig. 3A, control) were made immediately after breaking into the cells and then repeated after 5–10 min to allow BAPTA to diffuse into the cell. Therefore the cell was washed with Ringer in which Ca^{2+} was substituted with equimolar Ba^{2+}.

When only 0.2 mM EGTA was present, the Ca^{2+} current declined by 98% at the end of the 400-ms voltage step (Fig. 3A, control), but after exposure to BAPTA the decline was only 31% for the same cell (Fig. 3A). This finding is similar to that observed in the case of cloned human L-type channels having only the α1D pore-forming subunit (Bell et al. 2001). Replacement of Ca^{2+} by equimolar Ba^{2+} in the extracellular solution resulted in a significant increase in the peak inward current (Fig. 3A). That is a well-documented characteristic of calcium channels when Ba^{2+} enters into the cell through high-voltage-activated (HVA)-type channels (Hagiwara and Ohmori 1982; Soldatov et al. 1997). Using Ba^{2+} left only the time-dependent portion of inactivation, which was responsible for a 15% decline in current during the 400-ms voltage step in the cell shown in Fig. 3A.

Thus in WFACs, for a population of cells the time-dependent decline of the sustained Ca^{2+} current during a 400-ms voltage step recorded in the presence of Ba^{2+} averaged around 15% (14.5 ± 5.0%, n = 13, Fig. 3B). On top of this, Ca^{2+} entering through voltage-dependent channels triggered a more pronounced decline of the Ca^{2+} current, 62.4 ± 13.1% (n = 13, Fig. 3B, control), which was markedly reduced by 10 mM BAPTA (22.2 ± 20.0%, n = 8, Fig. 3B).

In our current-clamp experiments, 150-ms-long depolarizing pulses were employed. The corresponding decline of I_{Ca} at 150
ms for the above population of cells was 5.0 ± 3.2 and 7.0 ± 3.3% for BAPTA and Ba, respectively, whereas it was 19.7 ± 5.9% for control. Hence, in WFACs, significant Ca^{2+}-dependent inactivation of the HVA Ca^{2+} current occurs and is likely to play an important role in the attenuation of the OMPs.

**Pharmacological identification of the voltage-gated Ca^{2+} currents**

Known Ca^{2+} channel antagonists and agonists were used to characterize the type of calcium channel(s) involved in the generation of the OMPs. After control measurements, drugs were applied for 1 min before their effect was tested on $I_{Ca}$. More than one drug was tested on the same cell only if the currents recovered to within ±10% of the original current amplitude. The data are summarized in Fig. 4, A and B.

In WFACs, the relatively minor inactivation of $I_{Ba}$ taken together with an increase in current amplitude when Ba$^{2+}$ replaced Ca$^{2+}$ in the extracellular solution suggested that a significant portion of $I_{Ca}$ flows through L-type channels. L-type Ca$^{2+}$ currents have also been defined pharmacologically by their sensitivity to low concentrations of 1,4-dihydropyridine (DHP) antagonists (e.g., nifedipine, nitrendipine) and agonists such as Bay K 8644 (Nowycky et al. 1985b; Sanguinetti and Kass 1984). In WFACs, Bay K 8644 (1 μM), which prolongs the opening time of L-type channels (Nowycky et al. 1985a), enhanced the peak $I_{Ca}$ by 130 ± 25% ($n = 8$, Fig. 4B) and shifted the activation of Ca$^{2+}$ current by 10 mV toward more negative potentials (Fig. 4C). This is consistent with the Bay K effect seen in other preparations (Avila and Dirksen 2000). The effectiveness of different L-type specific antagonists varied. A saturating dose of the DHP nifedipine (100 μM) (Varming et al. 1997) reduced the current amplitude only by 21.8 ± 7.3% ($n = 10$). But another L-type channel blocker, the benzothiazepine (+) diltiazem (100 μM) (Kraus et al. 1998) reduced the current by 51.3 ± 9.1% ($n = 21$). This pharmacological profile most closely resembles that of α1D and α1F subunit-containing Ca$^{2+}$ channels present in rod and cone photoreceptors (Kourennyi and Barnes 2000; Wilkinson and Barnes 1996).

The N-type Ca-channel blocker ω-conotoxin GVIA (1 μM) attenuated $I_{Ca}$ by 29.6 ± 5.5% ($n = 14$). The less specific, P/Q/N-type Ca$^{2+}$-channel blocker ω-conotoxin MVIIC (1 μM) (McDonough et al. 1996) blocked 22.7 ± 9.9% ($n = 7$) of the total Ca$^{2+}$ current. Because the potent, reversible P/Q-type blocker ω-Aga-TK (50 nM) (Teramoto et al. 1993) exerted no effect on $I_{Ca}$ amplitude in six cells tested (8.7 ± 4.5%, $P > 0.05$), we concluded that both the ω-conotoxins (GVIA and MVIIC) acted on N-type channels. In support of this notion, GVIA treatment evoked an average block of the $I_{Ca}$ that was not significantly different from the MVIIC-evoked inhibition ($P > 0.05$).

When the N-type current component was blocked with 1 μM GVIA, the remaining portion of $I_{Ca}$ was enhanced by 339 ± 2% ($n = 8$) by Bay K (Fig. 4B). A mixture of MVIIC (1 μM) and diltiazem (100 μM) reduced the total Ca$^{2+}$ current by 83.8 ± 11.3% ($n = 7$). By adding GVIA (1 μM) to the MVIIC/diltiazem cocktail, we could not increase the inhibition of the total $I_{Ca}$ (79 ± 10.5%, $n = 19$). This further supports our notion that ω-conotoxins GVIA and MVIIC exert their effect on the same, presumably N-type, Ca$^{2+}$ channel in WFACs. The nonspecific Ca$^{2+}$ channel blocker Co$^{2+}$ (4 mM) eliminated $I_{Ca}$ almost completely (97.1 ± 1.9%, $n = 11$, Fig. 4A). The diltiazem and the ω-conotoxins were used at saturating doses based on past studies (Hillyard et al. 1992; Kraus et al. 1998; Olivera et al. 1985). Therefore we wondered whether any portion of the $I_{Ca}$ that remained after using the various cocktails of antagonists reflected T-type channel activity. Nickel (Ni$^{2+}$) at 100 μM has been shown in some systems to selectively block T-type Ca$^{2+}$ channels (Carbone and Swandulla 1989). In WFACs, it seems to be a nonspecific blocker. Ni$^{2+}$ eliminated ~50% of the total $I_{Ca}$ in the absence of other blockers ($n = 4$, not illustrated). When tested after using the diltiazem/conotoxin mixture, it eliminated 46.4 ± 9.6% ($n = 6$) of the residual $I_{Ca}$. This is not what one would expect if it were acting on a single type of channel. Moreover, we were unable to dissect out a transient component by using a stimul

**FIG. 4.** Pharmacology of the Ca$^{2+}$ current. A: effect of Ca$^{2+}$ channel antagonists. Aga, ω-agatoxin IVA (1 μM); Nif, nifedipine (100 μM); MVIIC (1 μM); GVIA (1 μM); Dilt, diltiazem (100 μM); M + Dilt, MVIIC (1 μM) + diltiazem (100 μM); Mix, MVIIC (1 μM) + diltiazem (100 μM) + GVIA (1 μM). Peak amplitude of $I_{Ca}$ recorded after 1-min drug exposure was normalized to that obtained immediately after break in. B: Bay K 8644 (1 μM) effect on $I_{Ca}$. Bay K evoked a ∼2.3-fold increase of the $I_{Ca}$. In the presence of GVIA, the normalized enhancement was more pronounced (∼3.4-fold). This is consistent with the finding that the L-type channel contribution to the total Ca$^{2+}$ current in WFACs is ~50–60%. C: current-voltage relationship showing that Bay K 8644 (1 μM) elevated the Ca$^{2+}$ current amplitude at all holding potentials above the threshold for activation and shifted the activation by ~10 mV ($n = 5$). ○, control; ●, Bay K 8644.

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protocol that would maximally activate or inactivate that current relative to the others \((n = 11)\). Thus we concluded that T-type channel activity in WFACs is unlikely.

To summarize, pharmacological investigation of the \(I_{Ca}\) in WFACs revealed at least two separate components. The larger portion—\(\sim 50-60\%\)—was mediated by L-type channels and a smaller, but still substantial, portion—\(\sim 30-40\%\)—of the voltage-gated \(Ca^{2+}\) enters the WFACs through N-type channels. Based on the present pharmacological data, it is possible that R-type currents were responsible for the residual \(I_{Ca}\) contributing \(\leq 10-15\%\) of the total \(Ca^{2+}\) current in WFACs.

**Ca\(^{2+}\) channel immunoreactivity in WFACs**

Next, we immunohistochemically localized the voltage-gated \(Ca^{2+}\) channel subunits present in the WFACs. We used antibodies directed against the \(\alpha1A\) (P/Q-type), \(\alpha1B\) (N-type), \(\alpha1E\) (R-type), pore-forming subunits of the intermediate conductance channels. Antibodies against the \(\alpha1C\) (cardiovascular L-type) were used to confirm the identity of the cells as WFACs (Solessio et al. 2002).

In concert with our pharmacological data, WFACs stained positively with anti-\(\alpha1B\) and anti-\(\alpha1F\) (Fig. 5), indicating N-type and retinal L-type \(Ca^{2+}\) channels. We found that N-type staining localized in close proximity to the cell body (Fig. 5, C and D), whereas the anti-\(\alpha1F\) labeled in a diffuse pattern (Fig. 5B) near the cell body and over the processes. No staining was observed with the anti-\(\alpha1E\) nor the rest of the antibodies tested. Thus the immunohistochemical data do not support the presence of the P/Q or R-type channels. Because the specificity of the antibodies used has not been established previously in fish, these data are still open to consideration.

**Contribution of different types of \(Ca^{2+}\) channels to the OMPs**

The purpose of these experiments was to determine if any of the effective \(Ca^{2+}\) current blockers could inhibit a particular aspect of the oscillations or selectively change the amplitude and/or frequency. Neither \(\omega\)-conotoxin GVIA (1 \(\mu\)M, \(n = 15\), Fig. 6A) nor \(\omega\)-conotoxin MVIC influenced the OMPs (\(n = 5\), not illustrated), suggesting that N-type channels do not contribute to the mechanism underlying the oscillatory behavior under our experimental conditions.

We found that the L-type \(Ca^{2+}\) channel antagonist diltiazem (100 \(\mu\)M) was almost as potent an inhibitor of the OMPs as \(Co^{2+}\) (Fig. 6B, compare with Fig. 2A). As shown in the preceding text, Bay-K was found to enhance the \(I_{Ca}\) in WFACs. Nevertheless, it failed to enhance the oscillations and, in some cases, slightly reduced both the duration and the frequency of the OMPs (\(n = 10\), Fig. 5C, see DISCUSSION for details).

Next we asked the question, do pharmacologically distinct \(Ca^{2+}\) channels contributed to the OMPs to different degrees under different conditions? To determine this, we bathed the cells in normal 2.1 mM \(Ca^{2+}\)-containing Ringer, then enhanced the oscillations by puffing high (10 mM)-\(Ca^{2+}\)-containing Ringer over the WFACs (Fig. 7, control trace). Co-application of 100 \(\mu\)M diltiazem with the high-\(Ca^{2+}\) Ringer eliminated the enhancement. Moreover, puffing 4 mM \(Co^{2+}\) during the long depolarizing step in the presence of diltiazem did not alter the membrane potential (Fig. 7). Thus we concluded that N-type currents did not contribute either to the OMPs enhanced by high extracellular \(Ca^{2+}\) or to the late (flat) phase of the membrane voltage response (\(n = 5\)).

Large, unattenuated OMPs were observed whenever the cells were superfused with high extracellular \(Ca^{2+}\) and the solution in the recording pipette was supplemented with 10 mM BAPTA (Fig. 8B). As we demonstrated earlier, this occurred because BAPTA markedly reduced the inactivation of the \(Ca^{2+}\) current in WFACs (Fig. 3, A and B). But the question arises as to which \(Ca^{2+}\) current component’s inactivation is
inhibited by BAPTA? In other words, does the L-type or a non L-type (N-type) voltage-gated Ca\(^{2+}\) channel provide the Ca\(^{2+}\) that underlies the nonattenuated OMPs? To determine this, we applied 100 \(\mu M\) diltiazem with the high Ca\(^{2+}\) Ringer again to WFACs preloaded with 10 mM BAPTA (Fig. 8C). Diltiazem eliminated the enhanced oscillations (Fig. 8, C and E, \(n = 6\)). This indicates that the oscillations in WFACs, whether dampened or not, are evoked by Ca\(^{2+}\) entering into the cells exclusively via \(\alpha 1F\) subunit containing L-type channels and that the L-type channels are subject to substantial Ca\(^{2+}\)-dependent inactivation.

**Role of \(I_K\) currents**

Next, studies were undertaken to determine the role of specific potassium channel types in the generation of the OMPs. Pharmacological investigation of the voltage-gated K\(^+\) currents in WFACs revealed at least three pharmacologically distinct components. Extracellular application of the nonspecific K\(^+\) channel blocker TEA (10 mM) reduced the outward current by 56.2 \(\pm\) 19.9\% (Fig. 9, \(n = 5\)), whereas 4-AP (5 mM) did so by 22.6 \(\pm\) 20\% (Fig. 9, \(n = 5\)). Known blockers of calcium-activated potassium channels were then tested. It is not clear if WFACs express small-conductance (SK) \(K_{(Ca)}\) channels, but apamin (5 \(\mu M\)), a selective (SK) \(K_{(Ca)}\) channel antagonist, produced a 14.8 \(\pm\) 9.9\% reduction of the outward current. ACs are known to posses large-conductance (maxi or BK) \(K_{(Ca)}\) channels (Mitra and Slaughter 2002), and the tremorgenic fungal toxin, penitrem A, selectively blocks (BK) type \(K_{(Ca)}\) channels (Knaus et al. 1994). When applied to the WFACs, penitrem A (1 \(\mu M\)) reduced K\(^+\) currents by 17 \(\pm\) 8.3\% (Fig. 9, \(n = 7\)). Other than TEA, the effect of the preceding potassium channel blockers on the total potassium current was not impressive.

Nonetheless, these agents were tested on the OMPs in an effort to determine whether or not particular potassium channels played a role in OMP generation. When applied prior to evoking OMPs, 4-AP (5 mM) had no effect (Fig. 10B), but a similar application of 10 mM TEA completely eliminated the oscillations in the same cell (Fig. 10C). Extracellular application of 1–5 \(\mu M\) of apamin, to block the (SK) \(K_{(Ca)}\) channels, under control conditions only slightly reduced the duration of OMPs in 12 cells tested (Fig. 11A). However, if OMPs were enhanced by washing the cells with 10 mM Ca\(^{2+}\)-containing Ringer, the same dose of apamin eliminated the enhancement independent of intracellular Ca\(^{2+}\) buffering (\(n = 7\), see Fig. 11, B and C, respectively). Taking these findings together with the preceding results, it is likely that apamin-sensitive (SK) \(K_{(Ca)}\) channels are functionally coupled to L-type Ca\(^{2+}\) channels, and this interaction is responsible for increasing the duration or generating more sustained OMPs in WFACs.

Application of either 10 mM TEA (Fig. 10C) or 4 mM Co\(^{2+}\) (Fig. 2A) eliminated the OMPs completely, indicating that apamin-insensitive members of the \(K_{(Ca)}\) current family play a role in generating the apamin-resistant initial phase of the oscillations. When tested on WFACs under control conditions, penitrem A (500 nM to 1 \(\mu M\)) completely and reversibly eliminated the OMPs (Fig. 11D, \(n = 10\)). In addition, the mixture of penitrem A (500 nM) and apamin (5 \(\mu M\)) totally eliminated the OMPs in
DISCUSSION

WFACs isolated from the teleost retina generate dampened MPOs in response to depolarizing current injections as well as to application of glutamate or its analogues (Solessio et al. 2002). The present set of experiments were undertaken to provide a detailed insight into the ionic mechanisms underlying the generation and modulation of these oscillations in an effort to better understand the functional properties of ACs.
WFACs also reduced the total K\(^+\)/H\(_{11001}\) eliminates the OMPs completely (Fig. 2A). Also, they open rapidly after the activation of Ca\(^{2+}\) channels but quickly inactivate as membrane potential and/or intracellular Ca\(^{2+}\) ([Ca\(^{2+}\)]\_i) availability drops (Sah and Davies 2000; Vergara et al. 1998; Weiger et al. 2002). In contrast, the (SK) \(K_{(Ca)}\) channels are influenced little by membrane potential and therefore conduct current dependent only on the intracellular Ca\(^{2+}\) levels (Xia et al. 1998).

Most often, \(K_{(Ca)}\) channels are coupled to specific voltage-gated Ca\(^{2+}\) channels (see Sah and Davies 2000 for review). We found that OMPs were eliminated by the L-type \(Ca^{2+}\) channel blocker diltiazem and that was independent of oscillation duration or dampening (Figs. 6B, 7, and 8C). So in WFACs both (SK) and (BK) \(K_{(Ca)}\) channels must be functionally coupled to L-type voltage-gated \(Ca^{2+}\) channels to mediate oscillations (Davies et al. 1996; Marrion and Tavalin 1998; Wisignda and Dryer 1994). This situation is very similar to that described in bull-frog saccular hair cells (Hudspeth and Lewis 1988b). There a calcium current interacts with an inactivating, transient potassium current and a calcium-activated potassium current, probably the BK type, to produce dampened oscillations.

Dampening of the oscillations

Including high concentrations of Ca\(^{2+}\) buffers (either EGTA or BAPTA) in the pipette solution diminished the dampening of the oscillations (Figs. 2C and 8B, respectively). Because the gating of the (SK) \(K_{(Ca)}\) channels is independent of protein phosphatases and kinases including Ca\(^{2+}\)-dependent ones (Xia et al. 1998), Ca\(^{2+}\)-dependent inactivation of (SK) \(K_{(Ca)}\) channels in WFACs is not a source for attenuation of the OMPs. Although the (BK) \(K_{(Ca)}\) channels are known to be subject to multiple modulatory factors, their gating is voltage and Ca\(^{2+}\) dependent, and Ca\(^{2+}\)-dependent downregulation of the channel has not been reported (Weiger et al. 2002). Note though that even in the presence of intracellular BAPTA and 10 mM extracellular Ca\(^{2+}\), (BK) \(K_{(Ca)}\) currents seems to inactivate based on the oscillatory pattern (it becomes dampened) when apamin blocked the (SK) \(K_{(Ca)}\) channels (Fig. 11C). In addition, apamin only slightly reduced the duration of the OMPs under normal conditions (Fig. 11A). Based on this we concluded that in WFACs, (BK) \(K_{(Ca)}\) channels do inactivate and it is somewhat faster than the Ca\(^{2+}\)-dependent inactivation of the L-type \(Ca^{2+}\) currents. By the time the (BK) \(K_{(Ca)}\) currents inactivate, the (SK) \(K_{(Ca)}\) currents turn on. However, the inactivation of \(I_{Ca}\) is suppressing the OMPs under normal conditions. Taken together this suggests that the Ca\(^{2+}\)-dependent inactivation of \(I_{Ca}\) in WFACs is the likely cause of dampening in the oscillations.

In this study, we found that WFACs membrane potential oscillations were mediated by a feed-back loop between voltage-gated Ca\(^{2+}\) and \(K_{(Ca)}\) currents (see following text). This is similar to what has been demonstrated in other systems, for example in saccular hair cells (Hudspeth 1986; Roberts et al. 1990), but different currents seem to be involved. We found that blocking the voltage-gated Ca\(^{2+}\) channels in WFACs eliminates the OMPs completely (Fig. 2A). In an earlier study, we showed that blocking voltage-gated \(Ca^{2+}\) channels in WFACs also reduced the total K\(^+\) current by \(-50\%\) (Solessio et al. 2002), suggesting current flow through calcium-activated K\(^+\) channels. Here we found that both (SK) and (BK) \(K_{(Ca)}\) channels participate in the generation of the oscillations.

Significantly, we found there is interplay between the two channels to generate the OMPs: BK channels seem to be necessary to initiate the oscillations, whereas SK channels control the duration. This pattern of contribution fits with their biophysical characteristics. The (BK) \(K_{(Ca)}\) channels are gated by Ca\(^{2+}\) with a \(K_{(Ca)}\) that is steeply voltage-sensitive being in the nanomolar range at +20 to +40 mV and several micromolar near the resting potential (Latorre et al. 1989; Weiger et al. 2002). We also, they open rapidly after the activation of Ca\(^{2+}\) channels but quickly inactivate as membrane potential and/or intracellular Ca\(^{2+}\) ([Ca\(^{2+}\)]\_i) availability drops (Sah and Davies 2000; Vergara et al. 1998; Weiger et al. 2002). In contrast, the (SK) \(K_{(Ca)}\) channels are influenced little by membrane potential and therefore conduct current dependent only on the intracellular Ca\(^{2+}\) levels (Xia et al. 1998).

Ionic currents mediating the oscillations in WFACs

FIG. 11. Typical effect of the small-conductance \(K_{(Ca)}\) blocker apamin and the (BK) \(K_{(Ca)}\) blocker penitrem A on the oscillations. A: under control conditions (2.1 mM Ca\(^{2+}\) Ringer, 0.2 mM [EGTA]), 1–5 μM apamin slightly reduced the duration of oscillations. However, it reduced the sustained oscillations evoked by high extracellular Ca\(^{2+}\) independent of intracellular Ca\(^{2+}\) buffering (B and C). B: 0.2 mM [EGTA], C: 10 mM [BAPTA], D: extracellular application of 500 nM penitrem A on WFACs eliminated the OMPs evoked under control conditions (0.2 mM [EGTA], 2.1 mM [Ca\(^{2+}\)]\_i).

In WFACs, the inactivation of (SK) \(K_{(Ca)}\) channels in close resemblance to that described in cardiac cells (Imredy and Yue 1994), smooth muscle cells (Giannatassio et al. 1991) and neurohypophysial nerve endings (Lemos and Nowycky 1989). That is, it begins without delay after
depolarization and leads to an almost complete inactivation in 500 ms. This is ~100-fold faster than the Ca\(^{2+}\)-dependent inactivation of \(I_{\text{Ca}}\) reported in retinal BC synaptic terminals (Qian et al. 1999) and ~40-fold faster than in rods (Rabl and Thoreson 2002). This suggests that Ca\(^{2+}\)-dependent inactivation of the L-type current is a prominent and general feature of different types of retinal neurons, nevertheless, the kinetics of the process vary, which may reflect different functional needs. In the case of the WFAC, the frequency of the oscillations is tied to the time constant of inactivation. It must be rapid for \(I_{\text{Ca}}\) to participate effectively in the generation of the oscillations.

It is of interest that in WFACs with high internal BAPTA not only the dampening of the positive oscillatory peaks disappeared, but the dampening of the negative peaks as well. This provides a second line of evidence supporting the notion that the (SK) \(K_{\text{Ca}}\) current only follows the \(I_{\text{Ca}}\) in WFACs because the steady amplitude of the repolarizing peaks must be tied to the (SK) \(K_{\text{Ca}}\) currents. If \(I_{\text{Ca}}\) is more sustained, then the hyperpolarization provided by (BK) and more importantly (SK) \(K_{\text{Ca}}\) currents are steady in amplitude as well and does not decline (e.g., Figs. 8, B and D, and 11C). This predicts that any modulatory effect reducing the inactivation of the \(I_{\text{Ca}}\) in WFACs would enhance the duration of the OMPs.

### Relationship between L-type Ca\(^{2+}\) and \(K_{\text{Ca}}\) channels in WFACs

In the present experiments, we found that apamin-sensitive (SK) \(K_{\text{Ca}}\) contributed to the oscillations only if they were enhanced by high extracellular Ca\(^{2+}\) (Fig. 11, B and C) or by increasing intracellular buffering with BAPTA (Fig. 3B). Interestingly, this suggests that under “normal” conditions (2.1 mM extracellular Ca\(^{2+}\)), when WFACs are depolarized, the \([\text{Ca}^{2+}]_\text{i}\) does not reach a level in the cell sufficient to fully activate the (SK) \(K_{\text{Cas}}\) current. This could result from a high Ca\(^{2+}\)-buffering capacity due to intrinsic Ca\(^{2+}\)-binding proteins, which are abundant in the WFACs (Solessio et al. 2002) and/or from the inactivation of the Ca\(^{2+}\) current (Fig. 3A).

The preceding findings are somewhat puzzling. The calculated free \([\text{Ca}^{2+}]_\text{i}\) with 10 mM EGTA in the pipette is 6.5 \(	imes\) 10\(^{-9}\) M and about the same when using BAPTA, which has a similar \(K_D\) but binds Ca\(^{2+}\) 100 times faster (Wang et al. 1997; Zhang et al. 1995). This \([\text{Ca}^{2+}]_\text{i}\), is far below the half-activation concentration (0.31–0.33 \(\mu\)M) for all known members of the (SK) \(K_{\text{Cas}}\) channel family (Xia et al. 1998). Consequently one might expect that at least the fast-Ca\(^{2+}\) chelator BAPTA could adequately mop up Ca\(^{2+}\) to prevent \(K_{\text{Cas}}\) current activation. This in turn would eliminate the oscillations. In fact, intracellular BAPTA resulted in a diametrically opposite effect; the OMPs were greatly enhanced. This apparent conflict allows some predictions to be made about the physical relationship between voltage-gated Ca\(^{2+}\) and \(K_{\text{Cas}}\) channels in WFACs.

Several studies have proposed various Ca\(^{2+}\) microdomain hypotheses suggesting that regions of elevated free Ca\(^{2+}\) are highly localized to the immediate vicinity of individual Ca\(^{2+}\) channels. But their activation often times does not lead to a significant increase in the bulk \([\text{Ca}^{2+}]_\text{i}\) of the cytosol (Imredy and Yue 1992; Llinas et al. 1995). In addition, convincing evidence from other systems supports the notion that functional coupling between voltage-gated Ca\(^{2+}\) channels and \(K_{\text{Cas}}\) conductances requires physical co-localization (Gola and Crest 1993; Roberts et al. 1990; Robitaille et al. 1993). This is in agreement with our finding that intracellular perfusion with BAPTA could not prevent Ca\(^{2+}\) binding to the \(K_{\text{Cas}}\) channel’s Ca\(^{2+}\) sensor—which is likely to be calmodulin (Sah and Davies 2000; Saimi and Kung 2002)—so the \(K_{\text{Cas}}\) channels opened. Calcium binding by buffers, either endogenous or introduced, are simply not fast enough to act within a distance <50 nm of the Ca\(^{2+}\) channels to strongly influence \([\text{Ca}^{2+}]_\text{i}\), which can rise to 100 \(\mu\)M in <100 \(\mu\)s (Neher 1998); although in some cases, it has been suggested that BAPTA exerts some effects as close as 30 nm to the pore (Budde et al. 2002). In any case, this suggests that in WFACs, L-type Ca\(^{2+}\) channels are clustered together with \(K_{\text{Cas}}\) channels, packed next to each other similar to the arrangement in hair cells (Roberts et al. 1990) and the distance between them is <50 nm.

In WFACs both high EGTA and BAPTA eliminated the attenuation of the oscillations as well as the Ca\(^{2+}\)-dependent inactivation of the L-type Ca\(^{2+}\) currents. In some systems, Ca\(^{2+}\)-dependent inactivation results from calmodulin activation (Ivanina et al. 2000; Sun et al. 2000). One model of this proposes that calmodulin is tethered to the Ca\(^{2+}\) channel complex and attaches to the channel only after Ca\(^{2+}\) binding (Peterson et al. 1999). Because high BAPTA and EGTA were found equally potent in eliminating the attenuation of the OMPs, we propose that the Ca\(^{2+}\) sensor for the Ca\(^{2+}\)-dependent inactivation of L-type Ca\(^{2+}\) channels in WFACs is localized \(\approx100\) nm away from the channel. According to Neher (1998), \(\approx100\) nm is the distance from the channel where the “slow” EGTA can exert its effect and be as potent as BAPTA. This model explains with physical distances how BAPTA could prevent the (tethered) calmodulin-dependent inactivation of the L-type channels while it didn’t eliminate Ca\(^{2+}\) activation of the calmodulin serving as the putative internal Ca\(^{2+}\) sensor for the \(K_{\text{Cas}}\) channels (Saimi and Kung 2002) in WFACs.

### Membrane potential oscillations, cell resonance, and the different roles of \(I_{\text{Ca}}\) in WFACs

As a general rule, membrane potential oscillations can be produced in a neuron whose membrane exhibits resonant properties (Hudspeth and Lewis 1988a). Resonance arises from the interactions between active resonant currents influenced by the “passive” filtering characteristics of the membrane (Hutcheon and Yarom 2000). Currents that actively oppose changes in membrane voltage and that activate slowly relative to the membrane time constant can produce resonance in a cell. Calcium-dependent \(K^+\) currents meet these criteria (Hudspeth and Lewis 1988a). The Ca\(^{2+}\) dependence of these channels ensures that they will open with a delay, and because their reversal potential falls near the foot of their activation curve, they actively oppose change in membrane potential. To produce oscillations, a resonant cell must also possess an amplifying current that can interact with the resonant current. The amplifying current is the opposite of the resonant current: it activates rapidly and accelerates membrane potential change because its reversal potential is close to the peak of its voltage-activation relationship (Hutcheon and Yarom 2000). Here we have shown that in WFACs \(I_{\text{Ca}}\) fulfills these criteria.

The Ca\(^{2+}\) current plays a dual role in the generation of WFAC membrane oscillations. First, it provides Ca\(^{2+}\) for the activation of the resonant \(K_{\text{Ca}}\) currents. Second, as described...
in the preceding text, \( I_{\text{Ca}} \) fits the role of the amplifying current. So blocking the voltage-gated \( \text{Ca}^{2+} \) inflow eliminates the OMPs by not only reducing the \( K_{(\text{Ca})} \) currents but by eliminating the amplifying conductance as well.

The “passive” filtering characteristics of the membrane are set by the parallel leak conductance and the capacitance of the membrane that attenuates responses to inputs at high frequencies. For the WFAC, the membrane time constant (RC), calculated under conditions where the membrane exhibit passive responses (e.g., depolarizations below \(-40 \text{ mV}\)), is on the order of 25 ms. This value was obtained from measurements of the cell resistance as inferred from the \( I-V \) relationship (\( R = -500 \text{ M}\Omega) and using an average capacitive value of 50 pF. This yields a low-pass cutoff frequency \( f_l = 1/(2\pi RC) \approx 6 \text{ Hz} \). A similar value can be obtained by fitting a rising exponential to the voltage response in current-clamp mode (RC = \(-20 \text{ ms}\)) while the cell is responding passively (membrane potentials below \(-40 \text{ mV}\)) before oscillating.

The activation time constant for the potassium channels (pooled) depends on the \( \text{Ca}^{2+} \) concentration. We can estimate an “upper limit” for this value by fitting an exponential function to the potassium currents obtained with the \( \text{Ca}^{2+} \) and \( \text{Na}^+ \) channels blocked. This will give a time constant reflecting no calcium-dependent potassium channels, which is likely to be a little faster than the fastest time \( K_{(\text{Ca})} \) currents can activate (Sah and Davies 2000). For the WFAC, this value is \(-5 \text{ ms}\), which translates into a cutoff for the high-pass filter of \( f_H = 30 \text{ Hz} \) (Hutcheon and Yarom 2000). Therefore the resonance characteristics of these cells computed as the product of the first-order transfer functions for the low-pass (6 Hz) and high-pass (30 Hz) transfer functions results in a band-pass or notch filter (Fig. 12A). A problem with this is immediately evident. The cutoff frequency of the low-pass filter is lower than that of the high-pass filter. As a result, the system presents a poorly tuned transfer function that peaks at 10 Hz, nowhere near the 80- to 100-Hz range of the observed oscillations.

Alternatively, we can consider that by the time the potassium channels activate, the \( \text{Ca}^{2+} \) currents are already activated because their activation takes \(<0.5 \text{ ms} \) (Solessio et al. 2002). So rather than considering only the passive cell resistance, we must also consider the \( \text{Ca}^{2+} \) conductance in determining the membrane resistance. Doing so gives a cell conductance of \( 38 \times 10^{-9} \text{ S} \) or equivalently a membrane resistance of \( 25 \text{ M}\Omega \). With a 50-pF membrane capacitance, that makes the RC = 1.3 ms to give an estimated \( f_H \) of 120 Hz. The shift of the cutoff to a higher frequency yields a highly tuned transfer function (Fig. 12B). The frequency of resonance under these conditions is \(-100 \text{ Hz} \), in line with our observations. At 300–400 Hz, the frequency of the attenuating responses, the system is working mostly as a low-pass filter and therefore introducing marked attenuation in the oscillations. So the \( \text{Ca}^{2+} \) currents are not only amplifying the oscillations but also contribute to setting the range of oscillations by its impact on the cell conductance. Therefore the current can be considered as a band-pass amplifier, much like what has been seen in hair cells (Hudspeth and Lewis 1988a).

The changing \( I-K_{(\text{Ca})} \) and the \( \text{Ca}^{2+} \)-dependent modulation of the \( \text{Ca}^{2+} \) currents will change the position of the cutoff frequencies and as a result change the oscillatory properties of the cells. For example, the Bay-K doubles the \( K_{(\text{Ca})} \) conductance. This should bring a shift in the cutoff frequency of the low-pass component and an increment in the oscillatory response; however, the opposite was observed. This was most likely due to a concomitant decrease in rise time of the \( I-K_{(\text{Ca})} \) that raised the value of the cutoff of the high-pass filter, resulting in attenuation of the oscillations. The reduced duration is likely to be the consequence of the increased \( \text{Ca}^{2+} \)-dependent inactivation of the \( I_{\text{Ca}} \).

**WFACs and oscillations: functional implications**

WFACs isolated from the teleost retina possess at least two pharmacologically distinct \( \text{Ca}^{2+} \) channels: N-type and L-type, with differential distributions. N-type channels were found on, and in close proximity to, the cell body, whereas the L-type channels seemed to be evenly distributed over the cell surface particularly on the dendrites. Because the N-type current did not seem to contribute to the oscillations, its physiological role was not investigated here. Nevertheless, their localization allows us to make some predictions about their function. In lower vertebrates, transient WFACs are functionally polarized, so that they are sensitive to excitatory (glutamatergic) inputs near and at the soma, while they respond to inhibitory inputs more distally along their dendrites (Maguire 1999). Thus it is possible that N-type channels are functionally coupled to glutamate receptors to boost depolarizations, whereas the L-type
currents are functionally coupled to (BK) and apamin-sensitive (SK) $K_{Ca}$ channels to mediate oscillations as we demonstrated in the preceding text. While (BK) $K_{Ca}$ channels seem to trigger the OMPs, it is the apamin-sensitive (SK) $K_{Ca}$ channel activation that keeps it going. The characteristic features of the OMPs, frequency, duration, and amplitude, are greatly modulated by Ca$^{2+}$. The frequency of the OMPs is set by the RC properties of the membrane, where $R$ is influenced by the activation potential and the magnitude of $I_{Ca}$. The duration is regulated by the Ca$^{2+}$-dependent inactivation of the L-type Ca$^{2+}$ channels. The amplitude is connected to the L-type Ca$^{2+}$ channels as well because first they provide the Ca$^{2+}$ to activate the resonant $K_{Ca}$ currents and second, $I_{Ca}$ serves as an amplifier of the resonance leading to self-sustained oscillations.

Future work will explore the role of the oscillations in AC function. The OMPs themselves are likely to be the means by which retinal WFACs process and distribute visual information through the dendritic tree of the cell. It is likely these cells are electrically coupled (Sakai and Naka, 1988), and the OMPs may play a role in synchronizing their activity. If this is the case, because of the similarity between the frequencies of OMPs in WFACs and the oscillations seen in the electoretinogram (ERG) (Solessio et al. 2002), it is tempting to speculate whether or not these ERG oscillations originate in WFACs. Supporting this view, spontaneous subthreshold membrane potential oscillations were observed in goldfish BCs in a slice preparation (Protti et al. 2000) under particular ambient lighting conditions. So it is quite possible that OMPs in synaptically connected amacrine and BCs interact to drive each other at certain resonant frequencies. These combined oscillations are then expressed in the ERG.

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