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Gated currents generate single spike activity in amacrine cells of the tiger salamander retina

(lateral interactions)

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ABSTRACT **Amacrine cells form the neural networks mediating the second level of lateral interactions in the vertebrate retina. Members of a prominent class of amacrine cells, found in most vertebrates, respond at both the onset and termination of steps of illumination with a single, large transient depolarization. We show here how specific relationships between membrane currents control this single spike activity. Using whole-cell patch clamp on living retinal slices, we studied the membrane currents in amacrine cells. The currents elicited by depolarizing voltage steps could be separated into three main ionic components: a transient inward voltage-gated sodium current, a relatively small sustained inward voltage-gated calcium current, and a calcium-dependent outward current. A specific relationship between the sodium and potassium current alone appears to preclude repetitive spike activity. Potassium current is activated at potentials positive to −20 mV, but the sodium inactivation, between −60 and −20 mV, does not intersect potassium activation. Therefore, a steady depolarizing current step elicits an initial spike but then the membrane cannot be sufficiently hyperpolarized by potassium current to remove sodium inactivation and the cell remains refractory.**

It is now well-established that neurons in the distal part of the vertebrate retina generate only graded potentials in response to illumination (1–3). Photoreceptors, horizontal cells, and bipolar cells all behave in this way. The first cell to be encountered that appears to generate spike-like activity is the amacrine cell. Its response consists typically of a brief transient, including a single spike. The mechanisms mediating the transition from sustained activity in the distal to transient activity in the proximal region are unknown. We show here that the amacrine cell membrane itself acts to amplify and accentuate the transient component of its synaptic inputs through voltage-gated currents.

Using whole-cell patch clamp (4) in retinal slices (5), we have examined the membrane currents in amacrine cells. Our results suggest that gated currents in the membrane generate a large, transient depolarization and allow only a single spike to be generated in response to the onset of depolarizing current steps. We show here how gated currents in the amacrine cell membrane mediate the change-detection function.

MATERIALS AND METHODS

Preparation. All recordings were made in the retinal slice (5). Briefly, a small section of retina, still in the eyecup, was placed against a Millipore filter, to which it held tenaciously. The retina–filter combination was sliced by a razor blade at 150-μm intervals. Each slice was held with Vaseline on a slide. The preparation and electrode were viewed with a Zeiss 40× LWD water-immersion objective. Cells were patched without the use of enzymes.

Recording System. Patch electrodes of 5-MΩ resistance were made on a modified David Kopf electrode puller (David Kopf, New Haven, CT) using borosilicate glass of 1.5 mm outer diameter (WPI Instruments, Waltham, MA; TW150). The resistance increased 2- to 3-fold during recording, which was performed by using conventional whole-cell patch electronics (4) with a 1-GΩ feedback resistor in the current-to-voltage converter. Data were digitized, stored, and processed using an IBM PC/XT with Data Translation (Marlboro, MA) analog interface board.

Solutions. Patch electrodes normally contained (in mM): 116 potassium gluconate, 4 Hepes, 0.1 $CaCl_2$, and 1.0 EGTA to buffer Ca to 0.01 μM at pH 7.4. The extracellular medium contained (in mM): 108 NaCl, 2 KCl, 3 $CaCl_2$, 1 $MgCl_2$, and 4 Hepes at pH 7.5. For staining, the electrode contained 3% Lucifer yellow CH in a buffered solution of 116 mM LiCl. Solutions were changed with a peristaltic pump in a 1.0-ml bathing chamber. Solution change time was about 1 min.

Cell Identification. All amacrine cells were studied in the retinal slice and identified by their position within the depth of the slice. Of nearly 100 cells studied, 10% were stained with Lucifer yellow to confirm identification. These cells had a characteristic morphology: a 14- to 18-μm cell body with a single stout dendrite branching into stratified processes extending laterally for up to several hundred microns. They resemble the type 1 amacrine cells described by Wong-Riley (6). We never observed dye coupling between amacrine cells.

RESULTS

Whole-Cell Currents. Fig. 1*A* shows examples of the currents elicited by depolarizing voltage steps, from a holding potential of −65 mV. Twenty-millivolt polarizations from the resting level, like the depolarizing step to −45 mV, and a hyperpolarizing step to −85 mV (not shown here) elicited currents of <10 pA, indicating that the input resistance in these cells was >2 GΩ, orders of magnitude higher than found with conventional intracellular electrodes (7, 8). When the membrane was depolarized to −25 mV, a transient inward current was elicited. Further depolarization to −15 mV elicited an even larger, faster transient inward current exceeding 1 nA in magnitude in some cells. Finally, a step to −5 mV elicited a slowly developing, sustained outward current. The current–voltage curves derived at the peak of the inward transient and during the plateau are shown in Fig. 1*B*.

Separating Membrane Currents. The membrane currents could be pharmacologically decomposed into three major components: a transient inward, a sustained inward, and a

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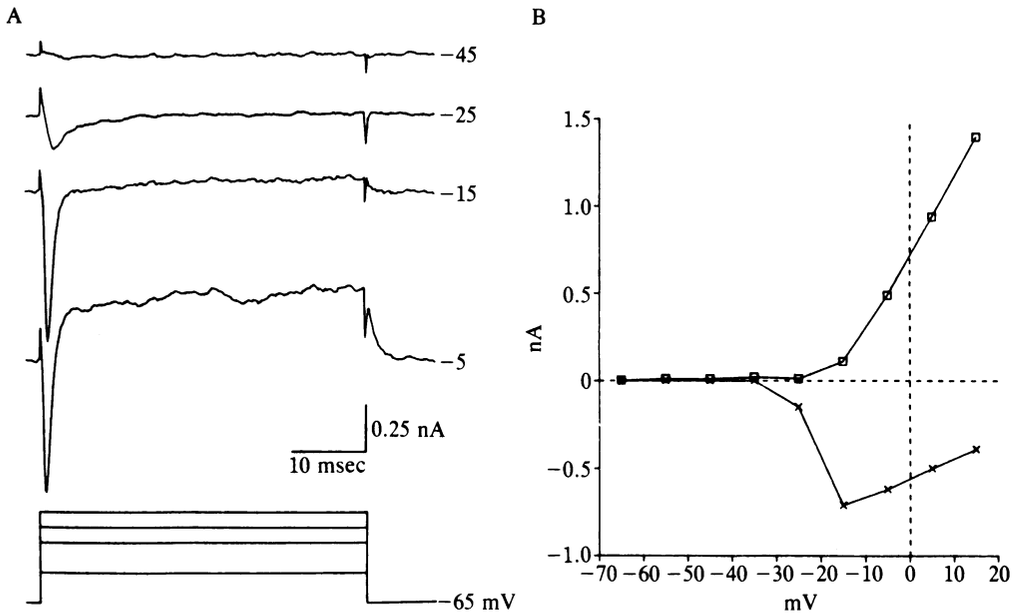

FIG. 1. (*A*) Currents under whole-cell patch clamp. Responses have been displaced vertically for clarity. The voltage step levels are indicated to the right of the traces. Holding potential was −65 mV. The normally large capacitive current was reduced electronically during the recording by feeding back a capacitatively coupled in-phase signal. (*B*) Current–voltage relations at the peak of the inward transient (×) and 40 msec after the onset of the voltage step (□). Note the sharp knee in the steady-state curve near −10 mV where the slope conductance increases 50-fold.

sustained outward current. When all three currents were blocked, the membrane showed no measurable gated currents but had a linear current–voltage relation with slope resistance near 2 GΩ between −80 and +10 mV. An outward current remained at potentials more positive than +10 mV. The compositions of the bathing and electrode solutions used to isolate the currents are given in the figure captions.

Transient Inward Current. The transient inward current shown in Fig. 2*A* was measured in solutions that blocked the other two currents. The sustained outward (potassium)

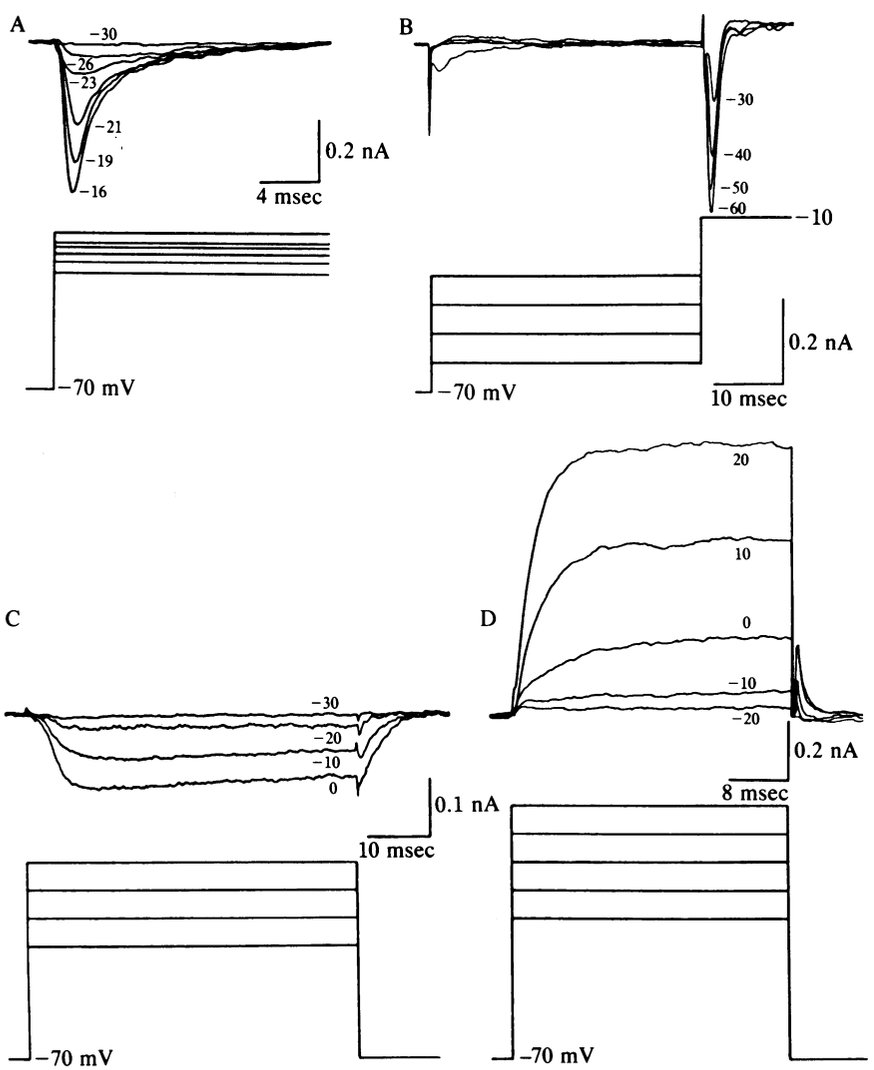

FIG. 2. Decomposition of membrane currents into three ionic components. Currents are shown in the upper traces; voltage steps are shown in the lower traces. Numbers to the left in the lower traces are the holding potentials. Numbers near the curves in the upper traces are the step potentials at which each current was measured. (*A*) The transient inward current. Here the sustained inward current was reduced with extracellular 3 mM $MgCl_2$ and 1 mM $CaCl_2$. The outward current was blocked by replacing 116 mM potassium with cesium in the electrode. Depolarizations beyond −16 mV elicited smaller currents. (*B*) Inactivation of transient inward current. Conditioning levels preceded test steps. Currents began to inactivate near −60 mV and were almost completely inactivated near −30 mV. (*C*) Sustained inward current. Here transient inward current was blocked by replacing all extracellular sodium with choline. Outward current was blocked by replacing all potassium with cesium in the electrode. (*D*) Sustained outward current. Currents were derived from subtractions in the presence and absence of 20 mM tetraethylammonium, which blocked about 75% of the outward current. Choline replaced sodium to eliminate transient inward current.

current was blocked by substituting cesium for potassium in the patch electrode and allowing diffusion from the electrode to the cytoplasm. The sustained inward (calcium) current was blocked with high extracellular magnesium and low calcium. The remaining transient inward current began to activate at about −30 mV and was fully activated at −15 mV. In some cells this current component alone was >1 nA, similar in magnitude and kinetics to the transient inward current measured without blockers in Fig. 1.

Inactivation of the transient inward current was measured by preceding a depolarizing test step that spanned the activation range with a series of conditioning potential steps. The conditioning steps were taken in the range from −70 to −30 mV, more negative than the −30 to −15 mV potential range over which this current activated. Fig. 2*B* shows the currents elicited from each conditioning level and indicates that inactivation begins near −60 mV and is nearly complete at potentials more positive than −30 mV. The conditioning step at −30 mV elicited some inward current, thereby diminishing the response to the subsequent test step, so the positive end of the inactivation curve was only approximated here.

This transient inward current is probably carried by sodium because it was unaffected by the presence of cobalt or the removal of calcium from the bathing medium but was entirely abolished when sodium was completely replaced by choline or when tetrodotoxin was added to the extracellular medium. By using data in the range from −20 to +10 mV, it had an extrapolated reversal potential near +50 mV, a reasonable value for the sodium equilibrium potential.

Sustained Inward Current. The sustained inward current is shown in Fig. 2*C*. Here the transient inward current was blocked by replacing extracellular sodium with choline. The outward current was eliminated by substituting cesium for potassium in the patch electrodes. Analysis of the tail currents showed this current to activate between −30 and 0 mV with maximum amplitude of about 0.1 nA at 0 mV.

The sustained inward current was eliminated with 1 mM extracellular cobalt, or by removing calcium, and it was enhanced in the presence of 4 mM barium. These findings, when compared with previously established criteria (9, 10), suggest that it is carried by calcium.

Sustained Outward Current. The sustained outward current shown in Fig. 2*D* could be observed in the absence of extracellular sodium (sodium replaced by choline), but it was eliminated by replacing potassium with cesium in the electrode or by substituting 20 mM tetraethylammonium for equimolar sodium in the bath. The outward current was also eliminated by agents that blocked the calcium current, so it appears to be a calcium-dependent potassium current (11). Tetraethylammonium blocked about 75% of the total outward current, so the currents were measured by subtracting those measured in choline alone from those measured in choline plus tetraethylammonium. The currents obtained through this subtraction were consistently smaller than the total outward current measured without blockers, as in Fig. 1.

The sustained outward current appeared to activate over the potential range from −20 to +20 mV. A measure of its activation curve, derived from the tails of the responses to voltage steps, is shown in Fig. 3 by dividing the measured current by the driving force for potassium.

Current-Clamped Voltage Response. The light response of these amacrine cells is characterized by a single spike (1, 2). A sustained current step also elicited a single spike, as shown in Fig. 3, indicating that single spiking is a fundamental characteristic of the amacrine cell active membrane and not simply due to a brief synaptic input during the light response.

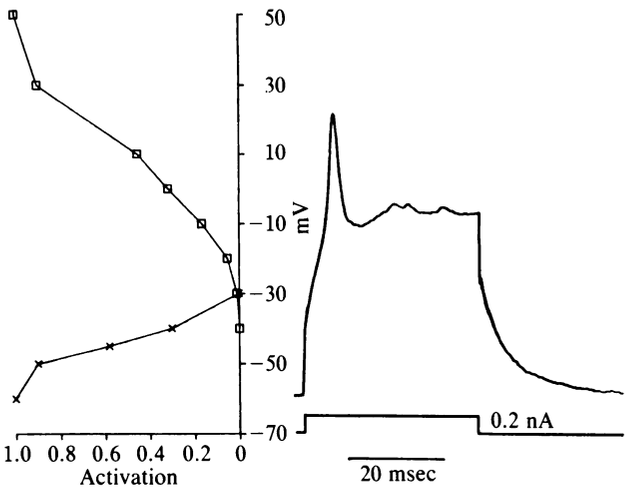

FIG. 3. Currents underlying the spike and plateau of the amacrine cell response. (*Right*) Voltage response to a sustained current step shows a single spike and a steady plateau level. Resting level was −58 mV. The spike reached a peak potential of +20 mV with a plateau level near −10 mV. (*Left*) A measure of potassium activation (□) (measured current divided by driving force) and sodium inactivation (×) plotted on the same voltage axis as the response. The plateau level (−10 mV) falls just below the potentials where potassium current begins to activate suggesting that it places a "ceiling" on the plateau level. But this relatively positive level for the initiation of potassium current precludes a repolarization to a potential that would allow removal of sodium inactivation. Therefore, the cell remains refractory following an initial spike and is capable of generating only one spike.

DISCUSSION

Three important features of the amacrine response—namely, the existence of a spike, its nonrepetitive nature, and its sustained plateau—can be adequately accounted for by the gated currents above.

The Spike. The 35 mV spike with <3 msec rise time can be accounted for by the transient inward current shown in Fig. 2*A*. This current, normally close to 1 nA with a duration of 3 msec, could depolarize the membrane capacitance of typically 15 pF by 100 mV.

The Plateau. The plateau in the voltage response near −10 mV in Fig. 3 is coincident with the level of activation of outward potassium current shown in Fig. 2*D*. This activation leads to an abrupt decrease in slope resistance of nearly 50 times, from >2000 MΩ to <40 MΩ. A well-defined maximum potential near −10 mV is thereby generated by the gating of potassium current.

Nonrepetitive Spike. The single spike can be accounted for by the special relationship between potassium activation and sodium inactivation. These two domains do not intersect, as shown on the left in Fig. 3. Therefore, in the presence of a sustained input, the membrane can never be sufficiently repolarized by the potassium current to eliminate sodium inactivation. Using the steady-state values of the inward and outward currents measured here, even a synaptic input as small as 10 pA will be sufficient to keep the membrane from hyperpolarizing beyond −30 mV. At that potential, sodium current would be mostly inactivated, so a depolarization from this level will not generate enough inward current to initiate a second spike.

Conclusions. The amacrine cell network is activated by temporal changes in the visual scene (1, 2) and acts to inhibit every type of ganglion cell in the tiger salamander retina (3). The change in sensitivity is mediated by transient synaptic current, accentuated by a single spike. This study shows that the single spike in amacrine cells is mediated by gated

currents in the amacrine cell membrane. The specific relationship between the potential ranges for sodium inactivation and the generation of potassium currents in the amacrine cell membrane leads to interactions between these currents that preclude multiple spiking.

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