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Synaptic Transmission Mediated by Internal Calcium Stores in Rod Photoreceptors

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Retinal rod photoreceptors are depolarized in darkness to approximately −40 mV, a state in which they maintain sustained glutamate release despite low levels of calcium channel activation. Blocking voltage-gated calcium channels or ryanodine receptors (RyRs) at the rod presynaptic terminal suppressed synaptic communication to bipolar cells. Spontaneous synaptic events were also inhibited when either of these pathways was blocked. This indicates that both calcium influx and calcium release from internal stores are required for the normal release of transmitter of the rod. RyR-independent release can be evoked by depolarization of a rod to a supraphysiological potential (−20 mV) that activates a large fraction of voltage-gated channels. However, this calcium channel-mediated release depletes rapidly if RyRs are blocked, indicating that RyRs support prolonged glutamate release. Thus, the rod synapse couples a small transmembrane calcium influx with a RyR-dependent amplification mechanism to support continuous vesicle release.

Key words: **retina; ryanodine receptors; calcium channels; glutamate; synaptic transmission; rod**

Introduction

In most neurons in the CNS, arrival of an action potential opens voltage-gated calcium channels, causing a brief burst of exocytosis (Katz and Miledi, 1967). Vesicle release sites within 10 nm of calcium channels sense 100–200 μM calcium during exocytosis (Llinas et al., 1992). These synapses reliably signal digital information, arrival of a spike, and timing between spikes.

In contrast, retinal photoreceptors and bipolar cells convey analog signals encoding the visual world. Photoreceptors continuously release glutamate in darkness, and light produces an intensity-dependent reduction of release (Trifonov, 1968; Dowling and Ripps, 1973). These neurons have numerous synaptic specializations to maintain transmitter release, notably synaptic ribbons that may function as conveyor belts for vesicle delivery to release sites (von Gersdorff, 2001).

Vertebrate photoreceptors have membrane potentials near −40 mV in darkness, their state of near-maximal neurotransmitter release. At this potential, the voltage-gated calcium channels are at the base of their activation curve. This low level of calcium channel activation has led investigators to explore synaptic release mechanisms that do not depend on voltage-gated calcium channels. Proposals include calcium-independent transport-mediated release (Schwartz, 1986), increased synaptic gain attributable to gap junctions (Attwell et al., 1987), and cGMP-regulated calcium influx (Rieke and Schwartz, 1994). However, calcium entry through voltage-gated channels seems necessary for transmitter release (Dacheux and Miller, 1976). An alternative possibility is that calcium influx recruits calcium from internal stores. In many neurons, transmitter release is influenced by intracellular calcium release in addition to calcium influx (Hua et al., 1993; Llano et al., 1994; Bouchard et al., 2003).

Ryanodine-sensitive stores play an active role in controlling presynaptic calcium dynamics in sensory neurons such as inner hair cells (Kennedy and Meech, 2002). Salamander rods possess both IP_3 and ryanodine receptors (RyRs) at their synaptic terminals (Peng et al., 1991; Krizaj et al., 2003), and caffeine can transiently elevate calcium at rod inner segments and synaptic terminals (Krizaj et al., 1999, 2003).

The direct involvement of internal stores in transmitter release is unresolved (Bouchard et al., 2003). Investigations indicate that presynaptic calcium-induced calcium release (CICR) can modulate neurotransmitter release at central synapses. Notably, Ca^{2+} sparks in cerebellar Purkinje cell terminals generate large multivesicular spontaneous IPSCs (Llano et al., 2000). Spontaneous transmitter release and augmented evoked release have also been attributed to CICR in the hippocampus (Emptage et al., 2001; Galante and Marty, 2003).

Our experiments indicate that CICR is necessary for transmitter release at rod terminals. Although calcium influx alone can evoke transmitter release, this occurs at supraphysiological voltage levels (approximately −20 mV). At physiological potentials, RyRs are required for transmitter release. Even at −20 mV, calcium influx only supports phasic rod transmitter release, whereas a ryanodine-sensitive mechanism promotes sustained release. Furthermore, calcium influx-generated release was susceptible to paired-pulse depression, whereas sustained release was more robust. In addition, RyRs support spontaneous miniature EPSCs (mEPSCs) and can coordinate multivesicular release under conditions of high-release probability (P_r). Thus, RyR-mediated release provides for tonic glutamate exocytosis that is well suited for information coding at the rod synapse.

Materials and Methods

Slice preparation. Larval tiger salamanders (*Ambystoma tigrinum*) were obtained from Kons Scientific (Germantown, WI) and Charles Sullivan

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(Nashville, TN) and were kept in tanks maintained at 4°C on a 12 h light/dark cycle. The animals were decapitated, and the eyes were enucleated. All procedures were performed in accordance with the United States Animal Welfare Act and the *NIH Guide for the Care and Use of Laboratory Animals* (publication 85-23) and were approved by the Animal Care Committee of the State University of New York. The retina was removed and placed vitreal side down on 0.22 μm pore membrane filters (Millipore, Bedford, MA) and then sliced at 250 μm intervals using a tissue slicer (Stoelting, Woods Lane, IL). For dark-adapted experiments, slicing and recording were performed under infrared light. An infrared-sensitive CCD camera was used to display the image on an external monitor for viewing.

Electrophysiology. A single slice was transferred to the recording chamber attached to an upright Olympus Optical (Tokyo, Japan) BX51WI fluorescent microscope, equipped with a LUMPlanFI/IR 60× water-immersion lens. The external solution contained the following (in mM): 111 NaCl, 2.5 KCl, 1.8 $CaCl_2$, 1 $MgCl_2$, 5 HEPES, and 10 dextrose, pH 7.8. When recording calcium currents, 40 mM tetraethylammonium (TEA)-Cl was substituted for 40 mM NaCl. The preparation was superfused continuously with Ringer's solution using a gravity-fed perfusion system. Ringer's solution could be exchanged with drug-containing solutions within 1–3 s. The standard pipette solution contained the following (in mM): 105 K-gluconate, 5 KCl, 1 $MgCl_2$, 5 EGTA, 5 HEPES, 4 ATP-Na_2, and 0.5 GTP-Na_3, pH 7.4 using KOH. For recording calcium currents, pipette solution contained the following (in mM): 105 Cs-methanesulfonate, 5 TEA-Cl, 1 $MgCl_2$, 5 EGTA, 5 HEPES, 4 ATP-Na_2, and 0.5 GTP-Na_3, pH 7.4 using CsOH.

Photoreceptors were held at a membrane potential of −80 mV unless otherwise specified. Bipolar cells were voltage clamped at −70 mV, and the chloride reversal potential of these cells was −60 mV. At the specified holding potential, inputs to rod-dominated bipolar cells are excitatory and glutamatergic (Maple et al., 1999). Some experiments were done in the presence of 50 μM picrotoxin and 10 μM strychnine to eliminate GABAergic and glycinergic feedback. All chemicals were obtained from either Tocris Cookson (Ballwin, MO) or Sigma (St. Louis, MO).

Most of the single-cell patch-clamp experiments were done using an Axopatch 200B amplifier (Molecular Devices, Union City, CA). A Multiclamp 700B amplifier (Molecular Devices) and the Multiclamp 700B interface were used for simultaneous dual patch-clamp experiments. Clampex 9 (pClamp9; Molecular Devices) was used to control the voltage command outputs, acquire data, and trigger the light stimulus and perfusion system (built in-house) for all experiments. Membrane currents and voltages were filtered at 10 kHz with a Bessel filter and sampled at 1–10 kHz using the Digidata 1322 analog-to-digital board (Molecular Devices). Junctional potential differences were corrected. The light sources were Stanley red and green light-emitting diodes.

Data analysis and statistics. Igor Pro 5.0 (WaveMetrics, Lake Oswego, OR) and Clampfit 9.0 were used for data analysis. The template search program in Clampfit 9.0 was used for detection and analysis of spontaneous EPSCs. The plots of normalized conductance (g_{norm}) versus membrane voltage (V_m) were fitted using the Boltzmann equation $g_{norm} = 1/(1 + \exp[(-zF/RT) \times (V_m - V_{1/2})])$, where $V_{1/2}$ is the voltage at which channels are half-maximally activated, z is the charge, R is gas constant, and $T = 295$°K. Statistical significance of peak amplitude and interevent interval distributions were tested using the paired two-tailed t test (Prism; GraphPad Software, San Diego, CA), and significance was accepted at $p < 0.05$. Data are presented as mean ± SEM.

Results

Ryanodine receptor inhibition blocks the rod–bipolar cell synapse

To examine the importance of ryanodine receptors at the rod synapse, 100 μM ryanodine was applied to the retinal slice preparation. This concentration of ryanodine blocks ryanodine receptors (Waterhouse et al., 1987; Bouchard et al., 2003). It suppressed light-evoked synaptic communication between photoreceptors and both ON and OFF bipolar cells (Fig. 1). Bipolar cells in the salamander retina have been classified as rod or cone dominated (Pang et al., 2004). This study identified three distinctive properties of rod-dominated OFF bipolar cells: noisy dark currents, high levels of spontaneous mEPSCs, and sustained light responses with small overshoots at light offset. The light response waveform presented in Figure 1*A* is typical of a rod-dominated OFF bipolar cell. In the same slice, a cone-dominated OFF bipolar cell showed a pronounced overshoot to light offset, less synaptic noise, and very little sustained response to the same light intensity. Light suppresses photoreceptor glutamate release, and the application of ryanodine had a similar effect. Ryanodine eliminated the light-driven EPSC in the OFF bipolar and produced an outward current in the dark (Fig. 1*A*), both characteristics of suppression of the synapse. The dark current in the presence of ryanodine is close to the light-evoked current, indicating that the effect of ryanodine is similar to the suppression of rod glutamate release. The spontaneous mEPSCs were also suppressed by ryanodine. Similar results were obtained in nine rod-dominated OFF bipolar cells. The mean suppression of the light response was 89.7 ± 3.3%. Ryanodine had a similar effect on cone-dominated synaptic input to OFF bipolar cells (data not

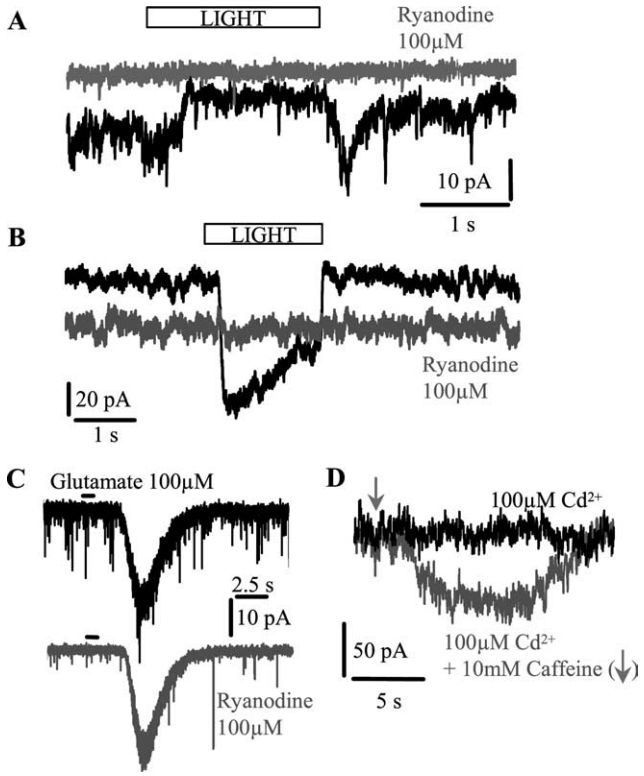

Figure 1. RyRs mediate glutamatergic synaptic input to bipolar cells. ***A***, Light response of a rod-dominated OFF bipolar cell voltage clamped at −70 mV (dark trace) to a 2 s light stimulus. Ryanodine (100 μM; gray trace) application eliminated the light response, generated an outward current that aligns with the outward current produced by light, and suppressed synaptic noise. ***B***, Light response of an ON bipolar cell held at −70 mV (dark trace) to a 2 s light stimulus. Ryanodine (100 μM; gray trace) suppressed the light response and produced an inward current in alignment with the decay phase of the light response, corresponding to constitutive opening of the metabotropic glutamate receptor cationic channels that are normally closed in the presence of glutamate in darkness. ***C***, Glutamate at 100 μM was applied for 1 s (represented by bar above current trace) while recording an OFF bipolar cell clamped at −70 mV (dark trace). During treatment with 100 μM ryanodine, the puff of glutamate was reapplied (gray trace). Note that the glutamate current was unchanged in the presence of ryanodine, although synaptic noise was reduced. ***D***, Cadmium (100 μM) was applied to the retina to block voltage-gated calcium channel-dependent synaptic transmission while recording from an OFF bipolar cell clamped at −70 mV (dark trace). Then, 10 mM caffeine was applied in the presence of cadmium, producing an EPSC in the OFF bipolar cell (gray trace).

shown), although this phenomenon was not pursued in the present study. The reduction in the dark current, suppression of mEPSCs, and loss of the light response indicate that ryanodine inhibits the rod to OFF bipolar synapse.

In similar experiments in ON bipolar cells, application of 100 μM ryanodine suppressed light responses (92.04 ± 2.1% mean suppression; $n = 12$). In voltage-clamp mode, ryanodine produced an inward current and suppression of the light response (Fig. 1*B*). The ON bipolar cell synapse is unusual in that glutamate released from photoreceptors in the dark acts to close channels in the bipolar cell (Slaughter and Miller, 1981). During light stimulation, photoreceptor glutamate release is reduced and an inward cationic current is generated. Thus, the depolarization and inward current produced by ryanodine are consistent with the suppression of photoreceptor synaptic input to ON bipolar cells. The inward current produced by ryanodine does not reach the peak of the light-evoked current but instead is close to the late, plateau phase of the ON bipolar response. This is characteristic of agents that block glutamate action at the ON bipolar synapse (Awatramani and Slaughter, 2000).

To test whether ryanodine application was affecting postsynaptic glutamate receptors, exogenous glutamate was applied while recording from an OFF bipolar cell (Fig. 1*C*). Glutamate produced an inward current, and this current was not suppressed by 100 μM ryanodine ($n = 5$), nor did ryanodine suppress glutamate-regulated currents in ON bipolar cells ($n = 2$; data not shown).

Other modulators of internal calcium release also affected ON and OFF bipolar cell light responses in a manner comparable with ryanodine. Ca^{2+}-ATPase pumps are present on the endoplasmic reticulum and are required for store refilling after release. Blockers of Ca^{2+}-ATPases, thapsigargin (50 μM) and cyclopiazonic acid (CPA) (50 μM), reduced light responses in ON bipolar cell (66.43 ± 4.9% suppression; $n = 7$) and OFF bipolar cell (66.19 ± 5.7% suppression; $n = 6$). The effects of thapsigargin and CPA on internal calcium release depend on the rate of Ca^{2+} turnover from the stores, which may explain why they were less effective than ryanodine in suppressing glutamate release during these experiments (Treiman et al., 1998).

Photoreceptor transmitter release has opposite polarity effects on ON and OFF bipolar cells, and RyR inhibition also had opposite effects on the membrane currents of ON and OFF bipolar cells. These effects of RyR inhibition are consistent with block of transmitter action, yet ryanodine did not alter the glutamate sensitivity of postsynaptic neurons. The simplest explanation of these results is that ryanodine acts presynaptically at the photoreceptor terminal to reduce transmitter release. Furthermore, the results suggest that photoreceptor transmitter release depends on release of calcium from internal stores. If internal stores are necessary, might their activation be sufficient for photoreceptor transmitter release? To test this, 10 mM caffeine, a methylxanthine that acts as an agonist at ryanodine-sensitive stores, was used (Neering and McBurney, 1984). Caffeine causes calcium release from ryanodine-sensitive stores in rod terminals (Krizaj et al., 2003). Voltage-dependent calcium channels were blocked by pretreating the retinal slice with 100 μM cadmium, thereby eliminating rod transmitter release. In the presence of cadmium, activation of RyRs with 10 mM caffeine stimulated photoreceptor release, as evidenced by EPSCs observed in OFF bipolar cells (Fig. 1*D*) ($n = 5$). The action of caffeine could be blocked by 100 μM ryanodine. Thus, internal calcium stores can initiate rod neurotransmitter release independent of voltage-gated calcium channels.

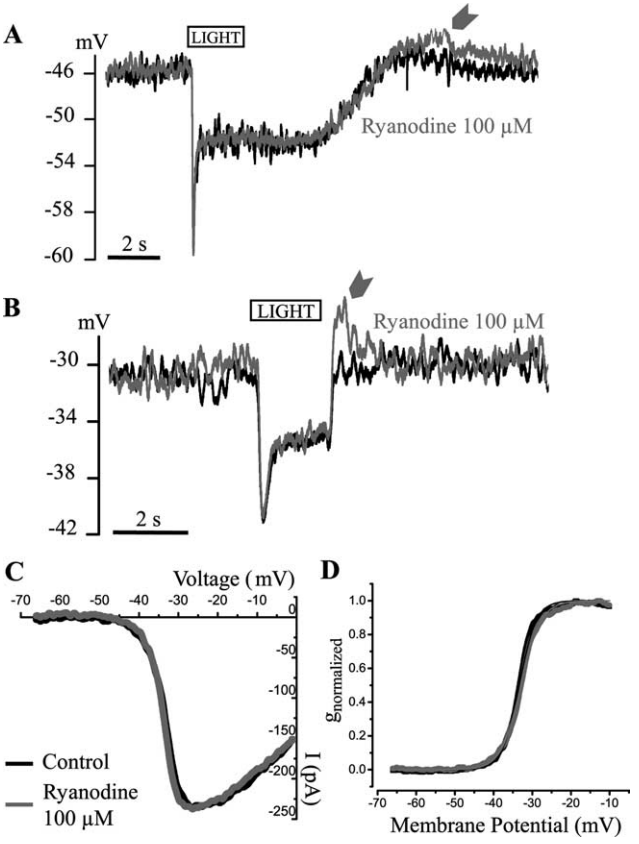

Figure 2. RyR inhibition does not suppress photoreceptor light response or calcium current. ***A***, Light response of a rod (dark trace) held in current clamp ($I = 0$) to a 2 s green light stimulus. Application of 100 μM ryanodine (gray trace) did not suppress the response to light but produced a slight increase in the overshoot at light offset (arrow). ***B***, Light response of a cone (dark trace) held in current clamp ($I = 0$) to a 2 s red light stimulus. Application of 100 μM ryanodine (gray trace) did not suppress light response but increased the overshoot at light offset (arrow). ***C***, A representative current–voltage relationship of calcium current recorded from rod photoreceptors (dark trace). The rod was held at −80 mV and ramped to 0 mV in 100 ms. Currents were measured by subtracting control traces from 100 μM Cd^{2+} substituted traces. No significant change was observed during application of 100 μM ryanodine (gray trace). ***D***, Activation curves ($g_{normalized}$ vs V) plotted by fitting current–voltage data to the Boltzmann equation (see Materials and Methods), yielding a $V_{1/2}$ of −33.4 mV (control, dark trace) compared with −32.9 mV (100 μM ryanodine, gray trace).

Ryanodine does not block photoreceptor light responses or calcium currents

Ryanodine-sensitive internal calcium release might be directly involved in control of rod glutamate release or it might interfere with release indirectly. Two potential indirect mechanisms are suppression of rod light responses or reduction in transmembrane presynaptic calcium currents. Ryanodine (100 μM) did not suppress rod light responses (Fig. 2*A*) ($n = 7$). Because rod-dominant bipolar cells are primarily but not exclusively rod driven, the effect of ryanodine on light responses of cones was ascertained. Ryanodine (100 μM) did not suppress cone light responses (Fig. 2*B*) ($n = 3$). Ryanodine did produce a small increase in the overshoot at light offset in some rods and cones, but this would not account for the suppression of postsynaptic responses.

The entry of calcium through voltage-gated calcium channels is a prerequisite for normal photoreceptor neurotransmitter release (Dacheux and Miller, 1976). Therefore, the effect of ryanodine on calcium entry through voltage-gated calcium channels at the rod terminal was considered. Calcium currents were evoked

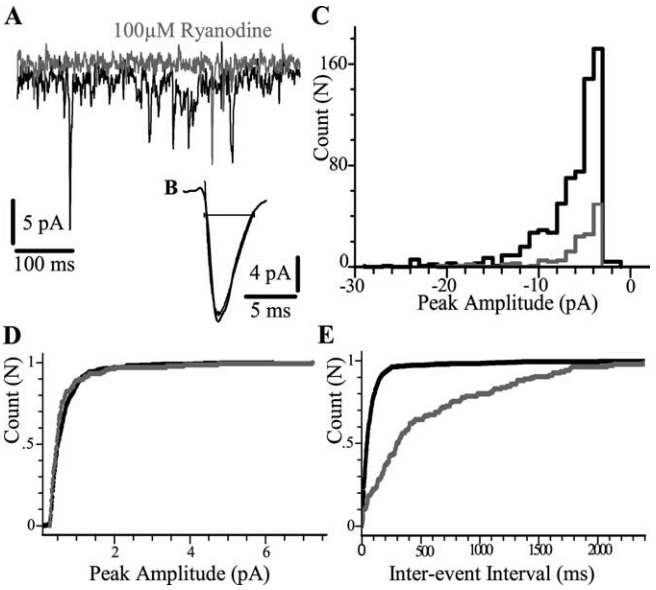

Figure 3. RyRs mediate spontaneous mEPSCs. ***A***, Spontaneous EPSCs recorded from a rod-dominated OFF bipolar cell clamped at −70 mV in control conditions (dark trace) and after application of 100 μM ryanodine (gray trace). ***B***, Example of EPSC (black trace) fit to an EPSC template. ***C***, Histogram of spontaneous EPSC amplitudes recorded during 40 s under control conditions (black) and in the presence of 100 μM ryanodine (gray). The EPSCs varied in amplitude and had a skewed distribution with mean amplitude of −6.96 ± 0.02 pA in control and −6.91 ± 0.06 pA in 100 μM ryanodine. ***D***, ***E***, The normalized cumulative amplitude and interevent interval distributions in control (dark) and after application of 100 μM ryanodine (gray).

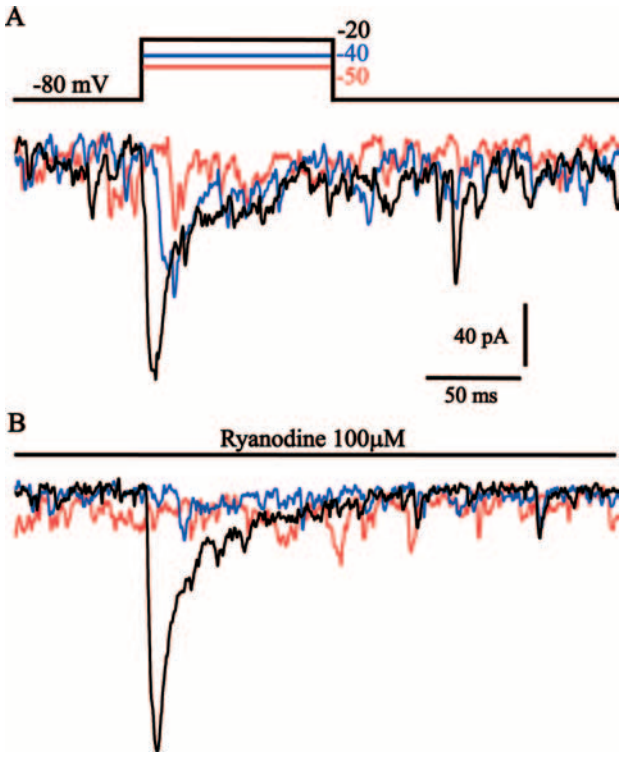

Figure 4. RyR control of rod transmitter release depends on presynaptic voltage. ***A***, In dual recordings of a rod and an OFF bipolar cell, the rod was clamped at −80 mV and then stepped to −50, −40, or −20 mV for 100 ms. This produced postsynaptic currents in an OFF bipolar cell clamped at −70 mV (red, blue, and black traces, respectively). ***B***, After treatment with 100 μM ryanodine, the same stimulation protocol was repeated.

by depolarizing rods from −80 to 0 mV during a 100 ms ramp. The *I–V* curve and activation curve ($g_{normalized}$ vs V) were plotted for control conditions and after application of 100 μM ryanodine (Fig. 2*C*,*D*). The activation curve was fit to the Boltzmann equation (see Materials and Methods), yielding a $V_{1/2}$ of −33.4 mV (control) compared with −32.9 mV (100 μM ryanodine). When comparing $V_{1/2}$ values over trials, we found no significant difference between control and ryanodine traces. In six rods tested, the mean $V_{1/2}$ values were −31.1 ± 2.2 mV in control versus −31.9 ± 2.7 mV in 100 μM ryanodine ($p = 0.33$). Also, ryanodine does not have any discernable effects on isolated rod photoreceptor calcium currents (data not shown). The experiments indicate that ryanodine is not suppressing synaptic transmission by inhibiting the light response or the voltage-gated calcium channels in rod synaptic terminals. Thus, ryanodine is probably acting directly to reduce glutamate release at the rod synapse.

Ryanodine suppresses miniature EPSC frequency

Under dark-adapted conditions, rod-dominated OFF bipolar cells have prominent discrete spontaneous mEPSCs (Maple et al., 1994; Pang et al., 2004). We recorded spontaneous mEPSCs in OFF bipolar cells held at −70 mV (Fig. 3*A*). The mEPSCs were detected by a template and fit to a exponential rise and decay with time-to-peak of 1.52 ± 0.01 ms and half-width of 2.77 ± 0.02 ms (Fig. 3*B*). Ryanodine had little effect on the peak amplitudes of the mEPSCs (Fig. 3*C*,*D*). The mean peak amplitude of the mEPSCs was −6.96 ± 0.02 pA in control and −6.91 ± 0.06 pA in the presence of ryanodine. However, 100 μM ryanodine reduced the mean frequency of events from 10.1 ± 1 to 1.8 ± 0.2 Hz. The decay of the interevent interval histogram could be fit to a single exponential, indicating a Poisson process (time constants of control vs 100 μM ryanodine, 63.7 ± 2 vs 210.6 ± 30 ms). Similar results were obtained in four other trials. Application of ryanodine decreased overall event frequency from 16.1 ± 5 to 3.5 ± 1 Hz ($n = 4$; $p = 0.01$). However, the mEPSC waveform was unchanged by ryanodine treatment, and the normalized cumulative amplitude distribution for control and ryanodine were similar (control vs ryanodine: time-to-peak, 1.76 ± 0.02 vs 1.77 ± 0.01 ms and half-width, 3.28 ± 0.21 vs 3.27 ± 0.26 ms, $n = 4$, $p = 0.68$; mean amplitudes, −6.28 ± 0.45 vs −6.15 ± 0.41 pA, $n = 4$, $p = 0.74$). Hence, application of ryanodine primarily reduces frequency of observed mEPSCs. This is additional evidence that ryanodine acts by blocking presynaptic release.

Paired recordings of rods and bipolar cells reveal ryanodine-sensitive slow release

In our recordings, the calcium current in rods became apparent approximately −45 mV and reached its peak close to −20 mV (Fig. 2*C*). The dark membrane potential of rods is approximately −40 mV, a point at which relatively few calcium channels are open. Sustaining glutamate release at this membrane potential might require ryanodine receptors to serve as transducers to augment internal calcium. This was tested by paired recordings from rods and OFF bipolar cells. Rods were depolarized to different potentials (100 ms step from −80 to −50, −40, and −20 mV) while recording EPSCs in OFF bipolar cells held at −70 mV (Fig. 4*A*). The extent of depolarization determined latency and rise time of postsynaptic currents, suggesting that rate of exocytosis is highly dependent on the degree of calcium channel activation. For a depolarization to −20 mV, EPSC onset was within 1–3 ms of the presynaptic depolarizing step, and 10–90% rise rime was 1.91 ± 0.14 ms ($n = 7$ pairs). In comparison, after depolarizing the rod to −40 mV, EPSC onset was at 6–12 ms, and 10–90% rise

time was 15.03 ± 1.8 ms ($n = 6$ pairs). In the OFF bipolar cell in Figure 4, ryanodine reduced the standing inward current by 20 pA, indicative of the loss of tonic glutamatergic input (probably from other photoreceptors). Notably, ryanodine produced an intensity-dependent suppression of evoked responses (Fig. 4*B*). Steps to −50 mV did not always produce EPSCs, perhaps attributable to the low probability of Ca^{2+} channel openings and CICR at those potentials. In the example in Figure 4, the step to −50 mV produced a delayed EPSC, and ryanodine application eliminated it. Ryanodine greatly reduced the EPSC evoked when the rod was depolarized to −40 mV. The total charge carried by EPSCs produced by steps to −40 mV was suppressed by 79 ± 2.8% ($n = 6$ pairs). However, when the rod was depolarized to −20 mV, ryanodine produced less inhibition of the OFF bipolar cell EPSC. In this case, the peak current was not suppressed, only the later phase of the EPSC was inhibited, and the total charge of the EPSC was reduced by 32 ± 3% ($n = 7$ pairs). The reduction in total charge reflects that the EPSC declined more rapidly in the presence of ryanodine. Based on differences in kinetics of the postsynaptic EPSC at −40 mV and −20 mV in Figure 4, it can be inferred that, at −40 mV, presynaptic release takes longer for onset and is slower in its rise. The early part of the EPSC at −40 mV is RyR dependent because, at this potential, evoked release requires a combination of Ca^{2+} influx and CICR from stores, hence the slower kinetics of the EPSC. At −20 mV, Ca^{2+} influx is sufficient for transmitter release. However, the stores are necessary for the late part of the EPSC, therefore producing a separation between influx-dependent and RyR-dependent release. Thus, RyRs play a role in exocytosis at the rod synapse at all levels of calcium channel activation, but RyRs are particularly crucial in the normal physiological voltage range of the rod. Although larger depolarizations (to −20 mV) produce sufficient transmembrane calcium influx to permit sizeable RyR-independent transmitter release, calcium influx alone seems insufficient to support transmitter release in the normal operating range of the rod, more negative than −40 mV.

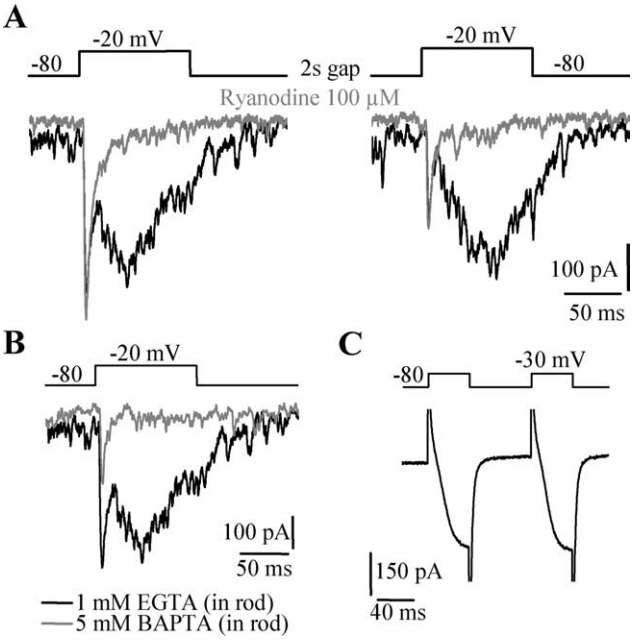

Figure 5. High-voltage-activated, ryanodine-insensitive release is susceptible to depression. ***A***, In dual recordings of a rod and a synaptically connected OFF bipolar cell, EPSCs were recorded in the OFF bipolar cell clamped at −70 mV while the rod was stimulated with paired pulses (−80 to −20 mV; 100 ms stimuli separated by 2 s). The early phase of the EPSCs exhibited paired-pulse depression, and the late phase recovered fully (dark trace). Ryanodine at 100 μM suppressed only the late, sustained component (gray trace) and did not alter the paired-pulse depression. Calcium buffering in the rod was reduced by using 1 mM EGTA in the recording pipette. ***B***, A similar dual-cell recording was performed but using 5 mM BAPTA in the rod recording pipette. An EPSC was recorded from an OFF bipolar cell voltage clamped at −70 mV while the connected presynaptic rod was stimulated with a step from −80 to −20 mV for 100 ms (gray trace). The trace is overlaid with the control trace from ***A*** for comparison. Buffering of the rod with 5 mM BAPTA results in suppression of the sustained component. ***C***, Recording of calcium currents in a rod depolarized by paired pulses (−80 to −30 mV for 40 ms, with an interpulse interval of 60 ms).

Paired-pulse stimulation indicates independence in the two forms of release

Stepping the rod to −20 mV was particularly informative because of the appearance of early transient and late sustained components that could be associated with calcium influx-dependent and ryanodine-sensitive release. Studies of vesicle release in goldfish retinal bipolar cells demonstrate two forms of release to a large step depolarization: a rapid release from the readily releasable pool and a sustained release (von Gersdorff and Matthews, 1994; Mennerick and Matthews, 1996; von Gersdorff et al., 1998; Tachibana, 1999). Distinct transient and sustained components of release also exist at supraphysiological levels of depolarization at the rod synapse, and ryanodine differentially affected them. To further investigate the properties of ryanodine-sensitive and -insensitive glutamate release, OFF bipolar cell EPSCs were compared during temporally paired stimulation of rods. Internal Ca^{2+} buffering in rods was reduced by lowering the calcium buffer in the rod recording pipette to 1 mM EGTA, which made ryanodine-sensitive release more prominent. Rods were held at −80 mV and depolarized by a pair of pulses to −20 mV for 100 ms, separated by 2 s. In an OFF bipolar cell held at −70 mV, transient and sustained EPSC components were observed during the first pulse stimulation of the rod (Fig. 5*A*). The second pulse, 2 s later, produced a smaller transient response, although the amplitude of the sustained EPSC was not reduced. The slow recovery of the transient response permitted a clear distinction between the two phases of release. Charge carried by the transient component of the EPSC during the second pulse was depressed by 67%. At shorter intervals, both transient and sustained EPSCs were reduced, but the transient was more suppressed. For example, with 1.5 s interpulse intervals, the transient EPSC was depressed by 83%, and the sustained ryanodine-sensitive component depressed by 23%. At longer interpulse intervals (>12 s), the transient component recovered completely.

Ryanodine did not reduce the transient EPSC in either pulse but strongly inhibited the late sustained EPSC in both the first and second pulse (Fig. 5*A*). The gray trace illustrates the effect of applying 100 μM ryanodine. Similar results were obtained in five other cells. Furthermore, when 5 mM BAPTA was substituted for 1 mM EGTA in the rod internal solution, it blocked the sustained EPSC release component while the transient component persisted, although it might have been reduced ($n = 2$). For comparison, Figure 5*B* shows an overlay of the control recording from Figure 5*A* with the EPSC in a different OFF bipolar cell produced with 5 mM BAPTA buffering of the rod. The RyR-sensitive component is much more sensitive to high presynaptic BAPTA.

Postsynaptic receptor desensitization probably does not account for the paired-pulse depression observed in the transient component because desensitization would be expected to depress both the transient and sustained components in the second pulse. The effects of 100 μM ryanodine indicate that the two components are independent of each other with respect to vesicle pool

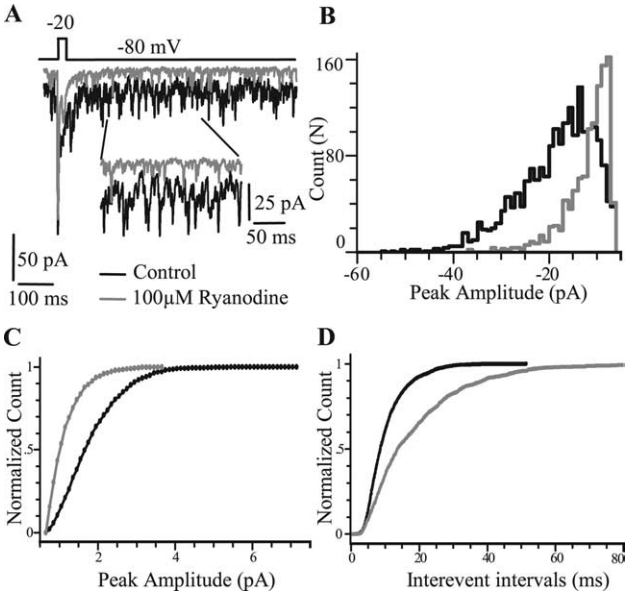

Figure 6. RyRs promote coordinated vesicle release in the rod. ***A***, High-frequency spontaneous EPSCs were observed in OFF bipolar cells voltage clamped at −70 mV in paired recordings with connected presynaptic rods (using 1 mM EGTA in the rod pipette). The rod was held at −80 mV and then depolarized to −20 mV for 25 ms (pulse at top of traces). Dark trace represents recording in control conditions, and gray trace is after treatment with 100 μM ryanodine, which suppresses observed events. ***B***, The amplitude histogram of high-frequency events detected under control conditions (dark trace) and after treatment with 100 μM ryanodine (data analyzed for 20 s in each condition). ***C***, ***D***, Normalized cumulative distributions of amplitude and interevent intervals in control conditions (dark) and after application of 100 μM ryanodine (gray).

sensitivity to different calcium domains or release mechanisms. The transient component is not ryanodine sensitive, is less BAPTA sensitive, is highly susceptible to paired-pulse depression, and probably represents fast release evoked directly by calcium influx via L-type channels. The onset of the fast EPSC in the OFF bipolar cell occurs in 1–3 ms, with a 10–90% rise time of 1.91 ± 0.14 ms when the rod is depolarized to −20 mV, which is likely before recruitment occurs in calcium stores at the rod terminal. The susceptibility to depression during the second pulse is not attributable to depression of the presynaptic calcium current. Calcium currents are not depressed by pulses of short duration at small interpulse intervals (Fig. 5*C*). The ryanodine-sensitive sustained EPSC is characterized by slower onset, which suggests that it depends on delayed amplification by internal calcium release. Because of its fast rate of recovery, the ryanodine-sensitive component is an attractive mechanism for constant glutamate release from the rod ribbon synapse in the dark.

Ryanodine receptors coordinate release when release probability is high

In all paired recordings of rods and their connected OFF bipolar cells, high-frequency spontaneous events occurred in addition to the EPSC evoked by stimulation of the rod (Fig. 6*A*). These high-frequency spontaneous events were never observed in OFF bipolar cell recordings, except in paired recordings with a connected rod. The events were similar to asynchronous release subsequent to stimulation, probably resulting from elevated intraterminal calcium in the presynaptic cell (Gleason et al., 1994; Goda and Stevens, 1994). Thus, the spontaneous events recorded from the OFF bipolar cell might be attributable to altered intracellular calcium handling in the presynaptic, recorded rod (rod recording pipette contained 1 or 5 mM EGTA).

The amplitude distribution of these high-frequency events spanned a larger range than spontaneous mEPSCs (compare with Fig. 3*C*), and their average rate of 88.8 Hz was far higher than for spontaneous mEPSCs (16 Hz). Thus, we can observe high-frequency spontaneous synaptic events arising from one rod under conditions of high release probability. The high-frequency spontaneous EPSCs (hf-EPSCs) were similar to mEPSCs with respect to the shape of individual events (compare hf-EPSCs presented in Fig. 6 with mEPSCs shown in Fig. 3) (hf-EPSC time-to-peak of 1.8 ± 0.01 ms and half-width of 3.2 ± 0.02 ms; mEPSC time-to-peak of 1.52 ± 0.01 ms and half-width of 2.77 ± 0.02 ms). Treatment with 100 μM ryanodine reduced the hf-EPSC frequency in this case from 97 to 53 Hz. Although ryanodine almost halved the frequency of events, they were still far more frequent than mEPSCs, indicating that the rod synapse has a high release probability in these experiments.

In contrast to its effect on mEPSCs, ryanodine did suppress the mean amplitude of hf-EPSCs. Mean amplitude of hf-EPSCs under control conditions was −18.43 ± 0.17 pA and, in 100 μM ryanodine, reduced to −11.75 ± 0.14 pA. Significantly, large events were preferentially suppressed by 100 μM ryanodine. There was a concomitant increase in the number of observed small-amplitude events. This indicates that ryanodine was reducing the number of vesicles present in each hf-EPSCs. Normalized cumulative interevent interval and amplitude histograms illustrate the reduction in amplitudes and dispersal of interevent intervals (Fig. 6*B–D*). Similar results were observed for three other rod-driven OFF bipolar cells that were analyzed during paired recordings with rods. Summing the data from all four cells, 100 μM ryanodine reduced mean event frequency from 88.8 ± 15.9 to 47.8 ± 12.3 Hz ($n = 4$; $p = 0.007$) and mean event amplitude from −15.7 ± 2.13 to −10.8 ± 1.9 pA ($n = 4$; $p = 0.03$). The waveform of the events was not changed (control vs ryanodine; time-to-peak, 2.02 ± 0.3 vs 2.0 ± 0.3 ms; half-width, 3.4 ± 0.6 vs 3.3 ± 0.5 ms; $p = 0.68$). The normalized cumulative amplitude and interevent distributions followed a similar pattern in all four cells. The frequency of occurrence of the large events and the similarity of their waveform to spontaneously occurring mEPSCs suggests that they represent coordinated release of multiple vesicles in conditions of high release probability. That ryanodine application suppresses the large-amplitude events preferentially while reducing frequency of event occurrence is indicative of a role for the RyRs in coordinating vesicle release.

Discussion

RyRs are necessary for normal rod synaptic transmission

Rods release transmitter steadily in the dark, a state in which their resting potential activates a small fraction of the calcium channels at the rod terminal. Ryanodine reduced the rod-driven EPSCs in second-order neurons. Furthermore, ryanodine blocked the light responses in ON and OFF bipolar cells with opposite polarity effects, yet did not alter the current elicited by exogenous glutamate at the bipolar cell membrane. Ryanodine also suppressed the frequency but not the amplitude of mEPSCs. All of these observations indicate that ryanodine disrupts synaptic communication by suppressing rod glutamate release. Presynaptically, ryanodine did not alter photoreceptor light responses nor the calcium currents that initiate transmitter release. Other agents that influence calcium stores, thapsigargin and CPA, acted like ryanodine to reduce transmission at the rod synapse. These results indicate that the action of ryanodine is based on inhibition of internal calcium release in rods and that this release is a necessary step in normal rod synaptic transmission. Caffeine, an ago-

nist at RyRs, produced glutamate release even when voltage-gated calcium channels were blocked. Thus, internal calcium release via RyRs can initiate glutamate release in the absence of calcium influx. This is the first demonstration of an obligate role for internal calcium stores in synaptic transmission.

Why would the rod require an internal calcium amplification mechanism via RyRs to support glutamate release? Rods rest near −40 mV in darkness, and, at this potential, the calcium currents are at the foot of their activation curve. Transmembrane calcium influx could be increased by having rods operate at more depolarized voltages, such as −20 mV. However, the extracellular space at the rod synapse is confined, and a constant, high influx of calcium could deplete extracellular calcium (Rabl and Thoreson, 2002). This might not only reduce calcium current but also alter the calcium channel voltage sensitivity attributable to surface charge effects (Baldridge et al., 1998). Moreover, the rod voltage, calcium current, and transmitter release are linked linearly (Witkovsky et al., 1997). This would not be true at −20 mV because delayed rectifier potassium channels begin to open at approximately −25 mV (Mao et al., 2003). In addition, the I_h channel is important in the normal physiology of rods, and I_h currents become small and reverse direction at −30 mV (Hestrin, 1987). Thus, the voltage sensitivity of the various rod channels may dictate that a second-messenger calcium system be used to maintain linearity.

RyRs facilitate spontaneous vesicle release

Spontaneous mEPSCs occur in rod-dominated OFF bipolar cell recordings. The majority of mEPSCs are <10–15 pA in size, the average event being near 7 pA. Application of 100 μM ryanodine reduced frequency of events, but there was no change in the amplitude distribution or the waveform of the mEPSC. Thus, RyR-dependent CICR mediates at least some of these spontaneous events. Presumably, stochastic opening of a voltage-gated calcium channel allows calcium influx, leading to amplification of the calcium signal by ryanodine receptors, followed by vesicle fusion. A similar association of L-type calcium channels and ryanodine receptors produces stochastic activation of BK channels in amacrine cells (Mitra and Slaughter, 2002). Membrane calcium channels are clearly the initiators of mEPSCs because blocking Ca^{2+} channels with cadmium completely eliminates them. Ryanodine application greatly reduces the frequency of miniature events, signifying that, whereas calcium influx initiates spontaneous events, ryanodine-sensitive stores provide the amplification necessary to increase release probability.

RyRs mediate slow sustained transmitter release from the rod

Studies on retinal bipolar cell ribbon synapses describe phasic and tonic modes of transmitter release (Heidelberger et al., 1994; von Gersdorff and Matthews, 1994; Mennerick and Matthews, 1996; von Gersdorff et al., 1998; Tachibana, 1999; Singer and Diamond, 2003). We found that the two components are most apparent when rods are depolarized to −20 mV and buffered with 1 mM EGTA. The peak of the phasic EPSC occurs within 2 ms and is ryanodine insensitive. This is probably because voltage-gated calcium channels are maximally active at this voltage and the peak current occurs before significant recruitment of internal calcium stores. In addition to transient exocytosis, ribbon synapses support tonic exocytosis (von Gersdorff et al., 1998; Tachibana, 1999; Singer and Diamond, 2003) and continuous secretion of neurotransmitter that persists for prolonged periods (Lagnado et al., 1996). This is the more physiologically relevant release and requires RyRs.

The appearance of tonic and phasic components are attributed to the presence of two pools, a rapidly releasable and a secondary pool (Tachibana, 1999). The sustained component may occur after delayed mobilization of vesicles from the reserve pool with help from heightened internal calcium (von Gersdorff et al., 1998). We found that, when two pulses to −20 mV were paired, the fast and slow components showed distinctly different recovery rates. Under our experimental conditions, the recovery of the phasic ryanodine-insensitive component was prolonged. Inhibition of the ryanodine-sensitive component did not reduce the paired-pulse depression in transient release, suggesting that the phasic component in the rod synapse is not a result of delayed vesicle mobilization. One reason for greater depression in the transient component, representing the readily releasable vesicle pool, might be because its refilling is very sensitive to Mg-ATP (Heidelberger, 1998). Although this is an attractive explanation, addition of Mg-ATP to the rod did not alter the decay of the transient component.

The differential effects of ryanodine on the transient and sustained release suggest that ryanodine can alter release at a pool separate from the readily releasable pool. In the process of vesicle cycling, it appears that, before attachment at sites in close association with calcium channels, vesicles exist in another pool that is release competent (Zenisek et al., 2000; Sakaba et al., 2005). That is, vesicles are docked and ready to release but not yet linked to sites of high calcium channel density. Presumably, this explains why it is often observed that the rapidly releasable pool is slower to recover than the slow release pool. Thus, sustained release may represent a second pool of vesicles that are regulated by ryanodine receptors. If two pools exist, then ryanodine plays a role in both processes during the normal physiology of the rod.

Another interpretation is that there are two calcium sensors at the synapse: a low-affinity sensor mediating phasic release and a high-affinity sensor located at a different site that would account for tonic and hf-EPSC release, similar to the interpretations for the source of asynchronous release compared with transient exocytosis in hippocampal neurons (Geppert et al., 1994; Goda and Stevens, 1994; Neher and Penner, 1994)

RyRs can coordinate multivesicular release

Dual recording of rods and OFF bipolar cells revealed that rods are capable of high-frequency release. High-frequency events (hf-EPSCs) were only observed in OFF bipolar cells in paired recordings with a presynaptic rod. Thus, these large high-frequency events are the product of a single presynaptic rod. The hf-EPSCs, indicative of high P_r at the synapse, might be the result of reduced calcium buffering in the stimulated rods. Under low P_r, calcium entry is far less likely to produce a release event (mEPSC frequency is ~10-fold lower than hf-EPSCs, and the mEPSCs derive from more than one rod). The mean amplitude of hf-EPSCs is more than twice the amplitude of the average mEPSC, but they are quite similar to the mEPSCs in waveform. In the presence of 100 μM ryanodine, the mean amplitude of hf-EPSCs declined because of a decrease in the large-amplitude events, yet the mean time-to-peak and half-width of the events did not change. This indicates a reduction of synchronized events. Coordinated multivesicular events have been documented in hair cells (Glowatzki and Fuchs, 2002) and at the rod-bipolar–AII amacrine cell synapse (Singer et al., 2004). Large- and small-amplitude asynchronous events are suppressed by reducing external calcium, suggesting a role for calcium in synchronizing vesicle release (Singer et al., 2004). In our experiments, 100 μM ryanodine suppressed large events, causing a concomitant increase in small events. This leads to the interpretation that RyRs support the simultaneous

release of multiple vesicles. RyRs have been shown to be involved in the generation of large-amplitude IPSCs through multivesicular release at a central synapse (Llano et al., 2000). Assuming that the hf-EPSCs are composed of superimposed mEPSCs that occur within 1 ms, then the 88.8 Hz mean frequency of hf-EPSCs represents ~218 mEPSCs per second, which is reduced by 100 μM ryanodine to 81 mEPSCs per second. Based on Poisson statistics, under control conditions, there would be ~20 events per second with twice the amplitude of mEPSC and one event per second that was three times the mEPSC amplitude. However, in OFF bipolar cells under control conditions, ~40% of the events equal or exceed three times the mean amplitude of mEPSCs. This is far in excess of a chance occurrence, indicating that they are coordinated.

In summary, internal calcium release through ryanodine-sensitive channels is essential for physiological transmitter release in rods, they amplify stochastic events in voltage-gated channels leading to spontaneous vesicle release, and they can coordinate multivesicular release under conditions that raise release probability at the rod synaptic terminal.

References

Attwell D, Borges S, Wu SM, Wilson M (1987) Signal clipping by the rod output synapse. Nature 328:522–524.

Awatramani GB, Slaughter MM (2000) Origin of transient and sustained responses in ganglion cells of the retina. J Neurosci 20:7087–7095.

Baldridge WH, Kurennyi DE, Barnes S (1998) Calcium-sensitive calcium influx in photoreceptor inner segments. J Neurophysiol 79:3012–3018.

Bouchard R, Pattarini R, Geiger JD (2003) Presence and functional significance of presynaptic ryanodine receptors. Prog Neurobiol 69:391–418.

Dacheux RF, Miller RF (1976) Photoreceptor-bipolar cell transmission in the perfused retina eyecup of the mudpuppy. Science 191:963–964.

Dowling JE, Ripps H (1973) Effect of magnesium on horizontal cell activity in the skate retina. Nature 242:101–103.

Emptage NJ, Reid CA, Fine A (2001) Calcium stores in hippocampal synaptic boutons mediate short-term plasticity, store-operated Ca^{2+} entry, and spontaneous transmitter release. Neuron 29:197–208.

Galante M, Marty A (2003) Presynaptic ryanodine-sensitive calcium stores contribute to evoked neurotransmitter release at the basket cell–Purkinje cell synapse. J Neurosci 23:11229–11234.

Geppert M, Goda Y, Hammer RE, Li C, Rosahl TW, Stevens CF, Sudhof TC (1994) Synaptotagmin I: a major Ca^{2+} sensor for transmitter release at a central synapse. Cell 79:717–727.

Gleason E, Borges S, Wilson M (1994) Control of transmitter release from retinal amacrine cells by Ca^{2+} influx and efflux. Neuron 13:1109–1117.

Glowatzki E, Fuchs PA (2002) Transmitter release at the hair cell ribbon synapse. Nat Neurosci 5:147–154.

Goda Y, Stevens CF (1994) Two components of transmitter release at a central synapse. Proc Natl Acad Sci USA 91:12942–12946.

Heidelberger R (1998) Adenosine triphosphate and the late steps in calcium-dependent exocytosis at a ribbon synapse. J Gen Physiol 111:225–241.

Heidelberger R, Heinemann C, Neher E, Matthews G (1994) Calcium dependence of the rate of exocytosis in a synaptic terminal. Nature 371:513–515.

Hestrin S (1987) The properties and function of inward rectification in rod photoreceptors of the tiger salamander. J Physiol (Lond) 390:319–333.

Hua SY, Nohmi M, Kuba K (1993) Characteristics of Ca^{2+} release induced by Ca^{2+} influx in cultured bullfrog sympathetic neurones. J Physiol (Lond) 464:245–272.

Katz B, Miledi R (1967) The timing of calcium action during neuromuscular transmission. J Physiol (Lond) 189:535–544.

Kennedy HJ, Meech RW (2002) Fast Ca^{2+} signals at mouse inner hair cell synapse: a role for Ca^{2+}-induced Ca^{2+} release. J Physiol (Lond) 539:15–23.

Krizaj D, Bao JX, Schmitz Y, Witkovsky P, Copenhagen DR (1999) Caffeine-sensitive calcium stores regulate synaptic transmission from retinal rod photoreceptors. J Neurosci 19:7249–7261.

Krizaj D, Lai FA, Copenhagen DR (2003) Ryanodine stores and calcium regulation in the inner segments of salamander rods and cones. J Physiol (Lond) 547:761–774.

Lagnado L, Gomis A, Job C (1996) Continuous vesicle cycling in the synaptic terminal of retinal bipolar cells. Neuron 17:957–967.

Llano I, DiPolo R, Marty A (1994) Calcium-induced calcium release in cerebellar Purkinje cells. Neuron 12:663–673.

Llano I, Gonzalez J, Caputo C, Lai FA, Blayney LM, Tan YP, Marty A (2000) Presynaptic calcium stores underlie large-amplitude miniature IPSCs and spontaneous calcium transients. Nat Neurosci 3:1256–1265.

Llinas R, Sugimori M, Silver RB (1992) Microdomains of high calcium concentration in a presynaptic terminal. Science 256:677–679.

Mao BQ, MacLeish PR, Victor JD (2003) Role of hyperpolarization-activated currents for the intrinsic dynamics of isolated retinal neurons. Biophys J 84:2756–2767.

Maple BR, Werblin FS, Wu SM (1994) Miniature excitatory postsynaptic currents in bipolar cells of the tiger salamander retina. Vision Res 34:2357–2362.

Maple BR, Gao F, Wu SM (1999) Glutamate receptors differ in rod- and cone-dominated off-center bipolar cells. NeuroReport 10:3605–3610.

Mennerick S, Matthews G (1996) Ultrafast exocytosis elicited by calcium current in synaptic terminals of retinal bipolar neurons. Neuron 17:1241–1249.

Mitra P, Slaughter MM (2002) Mechanism of generation of spontaneous miniature outward currents (SMOCs) in retinal amacrine cells. J Gen Physiol 119:355–372.

Neering IR, McBurney RN (1984) Role for microsomal Ca storage in mammalian neurones? Nature 309:158–160.

Neher E, Penner R (1994) Mice sans synaptotagmin. Nature 372:316–317.

Pang JJ, Gao F, Wu SM (2004) Stratum-by-stratum projection of light response attributes by retinal bipolar cells of Ambystoma. J Physiol (Lond) 558:249–262.

Peng YW, Sharp AH, Snyder SH, Yau KW (1991) Localization of the inositol 1,4,5-trisphosphate receptor in synaptic terminals in the vertebrate retina. Neuron 6:525–531.

Rabl K, Thoreson WB (2002) Calcium-dependent inactivation and depletion of synaptic cleft calcium ions combine to regulate rod calcium currents under physiological conditions. Eur J Neurosci 16:2070–2077.

Rieke F, Schwartz EA (1994) A cGMP-gated current can control exocytosis at cone synapses. Neuron 13:863–873.

Sakaba T, Stein A, Jahn R, Neher E (2005) Distinct kinetic changes in neurotransmitter release after SNARE protein cleavage. Science 309:491–494.

Schwartz EA (1986) Synaptic transmission in amphibian retinae during conditions unfavourable for calcium entry into presynaptic terminals. J Physiol (Lond) 376:411–428.

Singer JH, Diamond JS (2003) Sustained Ca^{2+} entry elicits transient postsynaptic currents at a retinal ribbon synapse. J Neurosci 23:10923–10933.

Singer JH, Lassova L, Vardi N, Diamond JS (2004) Coordinated multivesicular release at a mammalian ribbon synapse. Nat Neurosci 7:826–833.

Slaughter MM, Miller RF (1981) 2-Amino-4-phosphonobutyric acid: a new pharmacological tool for retina research. Science 211:182–185.

Tachibana M (1999) Regulation of transmitter release from retinal bipolar cells. Prog Biophys Mol Biol 72:109–133.

Treiman M, Caspersen C, Christensen SB (1998) A tool coming of age: thapsigargin as an inhibitor of sarco-endoplasmic reticulum Ca^{2+}-ATPases. Trends Pharmacol Sci 19:131–135.

Trifonov IU (1968) Study of synaptic transmission between photoreceptor and horizontal cell by electric stimulations of the retina. Biofizika 13:809–817.

von Gersdorff H (2001) Synaptic ribbons: versatile signal transducers. Neuron 29:7–10.

von Gersdorff H, Matthews G (1994) Dynamics of synaptic vesicle fusion and membrane retrieval in synaptic terminals. Nature 367:735–739.

von Gersdorff H, Sakaba T, Berglund K, Tachibana M (1998) Submillisecond kinetics of glutamate release from a sensory synapse. Neuron 21:1177–1188.

Waterhouse AL, Pessah IN, Francini AO, Casida JE (1987) Structural aspects of ryanodine action and selectivity. J Med Chem 30:710–716.

Witkovsky P, Schmitz Y, Akopian A, Krizaj D, Tranchina D (1997) Gain of rod to horizontal cell synaptic transfer: relation to glutamate release and a dihydropyridine-sensitive calcium current. J Neurosci 17:7297–7306.

Zenisek D, Steyer JA, Almers W (2000) Transport, capture and exocytosis of single synaptic vesicles at active zones. Nature 406:849–854.