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N-type and L-type calcium channels mediate glycinergic synaptic inputs to retinal ganglion cells of tiger salamanders

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Abstract

Synaptically localized calcium channels shape the timecourse of synaptic release, are a prominent site for neuromodulation, and have been implicated in genetic disease. In retina, it is well established that L-type calcium channels play a major role in mediating release of glutamate from the photoreceptors and bipolar cells. However, little is known about which calcium channels are coupled to synaptic exocytosis of glycine, which is primarily released by amacrine cells. A recent report indicates that glycine release from spiking AII amacrine cells relies exclusively upon L-type calcium channels. To identify calcium channel types controlling neurotransmitter release from the population of glycinergic neurons that drive retinal ganglion cells, we recorded electrical and potassium evoked inhibitory synaptic currents (IPSCs) from these postsynaptic neurons in retinal slices from tiger salamanders. The L-channel antagonist nifedipine strongly inhibited release and FPL64176, an L-channel agonist, greatly enhanced it, indicating a significant role for L-channels. ω -Conotoxin MVIIC, an N/P/Q-channel antagonist, strongly inhibited release, indicating an important role for non-L channels. While the P/Q-channel blocker ω -Aga IVA produced only small effects, the N-channel blocker ω -conotoxin GVIA strongly inhibited release. Hence, N-type and L-type calcium channels appear to play major roles, overall, in mediating synaptic release of glycine onto retinal ganglion cells.

Keywords

Amacrine cell; Retina; Glycine; Exocytosis; Calcium channel

Introduction

The properties of synaptically localized calcium channels play a large role in determining the kinetics and magnitude of synaptic transmission. The biophysical properties of calcium channels can shape the timecourse of neurotransmitter release (Mennerick & Matthews, 1996; von Gersdorff & Matthews, 1996, von Gersdorff et al., 1998). Neuromodulation of different subtypes of synaptically localized calcium channels profoundly influences synaptic output from neurons (Wu & Saggau, 1997). Furthermore, mutations in calcium channel subtypes can produce critical functional deficits (Burgess & Noebels, 1999). For example, mutations in the L-type calcium channel in photoreceptors produces a form of congenital stationary night blindness in which photoreceptor to bipolar cell synaptic transmission is lost (Strom et al., 1998).

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As a general functional pattern, mature spiking neurons rely on non-L (N/P/Q/R) calcium channels to trigger fast-acting neurotransmitter release while graded-potential neurons predominantly use L-channels (Tachibana et al., 1993; Dunlap et al., 1995; Schmitz & Witkovsky, 1997). However, in retina it has recently been shown that some bipolar cells, which are graded potential neurons, use both L- and T-type calcium channels to mediate release (Pan et al., 2001). On the other hand, AII amacrine cells, which produce action potentials, apparently use only L-type channels for release (Habermann et al., 2003).

Glycine is a major neurotransmitter used by inhibitory interneurons in mammalian and nonmammalian retinæ. Glycinergic inhibition plays a large role in shaping the output of the retina (Dowling, 1987; Bieda & Copenhagen, 2000). Glycinergic amacrine cells are a subset of a larger, morphologically, physiologically, and neurochemically diverse set of inhibitory neurons in the inner retina (Dowling, 1987). In spite of strong evidence for glycinergic synaptic inputs from amacrine cells to both bipolar cells and ganglion cells (for review, Dowling, 1987), there is little information on calcium channels coupled to glycine release in the retina, aside from a recent report that AII amacrine cells in rat apparently use L-type channels exclusively to mediate glycine release (Habermann et al., 2003).

A variety of calcium channels are expressed in the larger population of amacrine cells; however, it is difficult to draw general conclusions about which types are coupled to neurotransmitter release. *In situ* hybridization reveals the presence of a large number of calcium channel subunits (alpha-1a to alpha-1f) in the inner nuclear layer of the retina, suggesting that amacrine cells may possess a variety of calcium channel types (Kamphuis & Hendriksen, 1998; Strom et al., 1998). Electrophysiological studies, using pharmacological blockers, show amacrine cells in a variety of species (e.g. chick, rat, tiger salamander) under a variety of experimental conditions (e.g. neurons in slices, cultured neurons, acutely dissociated cells) generally express both L and non-L calcium channels (Gleason et al., 1994; Taschenberger & Grantyn, 1995; Maguire, 1999; Koizumi et al., 2001; Vigh et al., 2003; but see Habermann et al., 2003). However, it is important to note that the presence of a calcium channel type in a cell does not demonstrate that it is used for neurotransmitter release. For example, while L-type channels are a prominent calcium channel type expressed in many spiking neurons (e.g. hippocampal CA3 pyramidal cells), these channels generally do not play a role in fast neurotransmitter release in these cells (for review, Dunlap et al., 1995). For γ -aminobutyric acid (GABA) release, there are contradictory results between studies using cultured GABAergic chick amacrine cells (L-but not N-channels) (Gleason et al., 1994) and cultured GABAergic rat amacrine cells (N-but not L-channels) (Taschenberger & Grantyn, 1995). While the existence of only L-type calcium channels in AII amacrine cells implies that these channels must regulate glycine release in this cell type, there have been no studies, to our knowledge, that directly measure synaptic release of glycine in relation to roles of calcium channel subtypes.

We therefore sought to identify which calcium channels play the most prominent roles in mediating glycinergic inputs into retinal ganglion cells. Given that glycine release from AII amacrine cells appears to rely exclusively on L-type calcium channels (Habermann et al., 2003), we were particularly interested in testing whether non-L channels appeared to play a significant role. The optimum method to answer these questions is to record from single identified glycinergic amacrine cells and a synaptically connected postsynaptic cell. However, performing these paired recordings in an intact retina is technically difficult and, furthermore, interpretation of data from this approach is confounded by electrical and synaptic coupling between amacrine cells. Accordingly, we took the approach of stimulating glycine release from a group of amacrine cells while applying blockers of calcium channels. The methodologies that we employed—electrical and high-potassium stimulation—are standard approaches for studying calcium channel coupling to neurotransmitter release (for review, Dunlap et al., 1995), and have been used in cases where the presynaptic neurons are very diverse, such as in

the case of hippocampal inhibitory interneurons (Doze et al., 1995) and spinal cord inhibitory interneurons (Takahashi & Momiyama, 1993). The disadvantage of this methodology is that it will not identify calcium channel coupling in single types of amacrine cells or differentiate between amacrine cells with potentially disparate uses of channels. However, one can identify the major types of calcium channels mediating glycine release using this approach.

Materials and methods

Retinal slices from tiger salamanders (*Ambystoma tigrinum*) were prepared as previously described (Bieda & Copenhagen, 1999, 2000); all experiments were performed in ambient room light. Animals were sacrificed using decapitation followed by immediate pithing of the brain; in accordance with all relevant NIH and UCSF animal care guidelines. Recordings from putative retinal ganglion cells were from neurons in the ganglion cell layer. Recordings of amacrine cell resting potentials were from inner nuclear layer cells possessing a putative fast voltage-gated sodium current (i.e. a fast-activating and inactivating inward current; in other experiments, this current was blocked by 1 μ M tetrodotoxin). In all cases, bath application of antagonists was used. For eIPSC experiments, the extracellular solution was (in mM) 112 NaCl, 2-5 KCl, 2-4 CaCl₂, 1 MgCl₂, 5 hemisodium HEPES, and 25 glucose, pH 7.6 w/NaOH. For high potassium experiments, the extracellular solution was (in mM) 94 NaCl, 15 or 20 KCl, 2 CaCl₂, 1 MgCl₂, 20 glucose, and 5 hemisodium HEPES, pH 7.6 w/NaOH; a few experiments used a combination of 0.5 mM CaCl₂ and 2.5 mM MgCl₂. The majority of synaptic experiments used a CsMES-based internal solution (in mM): 101 CsMES, 6 TEACl, 3 MgCl₂, 5 EGTA, 5 HEPES, 2 disodium ATP, and 0.5 sodium-GTP. In a few experiments, the CsMES concentration was changed to 83 mM and the TEACl concentration was increased to 24 mM; or a CsMES/CsF solution was used (in mM): 45 CsMES, 40 CsF, 6 TEACl, 10 TEA-acetate, 0.5 MgCl₂, 10 EGTA, 5 BAPTA, and 5 HEPES; or a CsCl-based internal solution was used (in mM): 70 CsCl, 30 CsF, 3 MgCl₂, 10 EGTA, and 10 hemisodium HEPES. Measurements of amacrine cell resting potentials employed (in mM) 110 Kgluconate, 3 MgCl₂, 5 EGTA, 5 HEPES, 2 Na-ATP, and 0.5 Na-GTP. pHs of internal solutions was adjusted to 7.4 using CsOH for cesium-based solutions and using KOH for the Kgluconate-based solution. These various solutions were used in an attempt to obtain a higher rate of recordings with good responses (<10% of cells showed good responses to electrically evoked stimuli). In terms of the pharmacological modulation of evoked currents, no discernible difference was found using the different intracellular solutions. All external solutions included antagonists of NMDA receptors (100 μ M APV or 100 μ M AP7), of AMPA/Kainate receptors (CNQX, 2-4 μ M), of GABA receptors (10 μ M SR95531 or 100 μ M picrotoxin or 100 μ M bicuculline) and, in some experiments, of nicotinic acetylcholine receptors (100 μ M hexamethonium). Electrical stimuli (interstimulus interval of 20-45 s) were provided through an electrode filled with normal extracellular solution positioned in the inner retina; the ganglion cells were clamped at 0 mV. Strychnine-insensitive components were also found to be insensitive to cobalt and cadmium and therefore considered stimulation artifacts (see figures). Values are presented as mean $\pm$ SEM, with the two-tailed Student's *t* test used to evaluate significance. For analysis of eIPSCs, we measured the integrated charge during a period beginning slightly after the stimulus artifact to the end of the event and subtracted the charge measured in strychnine at the end of the experiment. Because individual IPSCs were difficult to separate in high potassium saline, we used the integrated charge as a measure of synaptic activity. For the electrical stimulation recordings, we only counted cells in which the evoked responses were >4 times the noise amplitude. For high-K⁺ experiments, acceptable responses exhibited clear synaptic events. Stability was assessed during a 10-min or longer control baseline period; if there was any discernible rundown or runup of response, the recording was rejected. In addition, if the response varied >30% from the mean, even if the mean was stable, the recording was rejected.

We used the well-known cooperative relationship of calcium to release (e.g. Wu & Saggau, 1997) to estimate the relation between the block of calcium influx and synaptic transmission [eqn. (1)]:

$$Ca_b = 1 - (1 - S_b)^{1/k}, \quad (1)$$

in which Ca_b = fractional block of calcium influx, S_b = fractional block of synaptic transmission, and k is the cooperativity of calcium for release. This equation has been used extensively to estimate calcium channel contributions to release in electrical stimulation experiments similar to the ones described in this report (Takahashi & Momiyama, 1993; Dunlap et al., 1995; Wu & Saggau 1997).

Nifedipine and FPL64176 stocks were made fresh daily in 95% or 100% ethanol (final ethanol concentration of 0.05-0.1%). For experiments with peptide toxins, cytochrome c (0.2-1 mg/ml) was present in all solutions to block nonspecific binding sites, and ~90% of the tubing was made from teflon. All compounds were purchased from Sigma (St. Louis, MO)/RBI (Natick, MA), except some peptide toxins from Bachem (Torrance, CA).

Results

Electrically evoked glycinergic inhibitory synaptic currents (eIP-SCs) induced by a stimulation electrode in the inner retina were recorded from ganglion cells. Most recordings (~90%) were rejected due to small and/or unstable responses. To identify the calcium channels mediating glycine release, we applied well-characterized antagonists of calcium channel subtypes in the presence of GABA and glutamate receptor blockers (see Methods).

Application of the L-type channel antagonist nifedipine led to strong, mostly reversible suppression of the eIPSC (Fig. 1A). To overcome poor penetrance of nifedipine into retinal slices and low-affinity nifedipine block of some retinal L-channels (Wilkinson & Barnes, 1996; Schmitz & Witkovsky, 1997), we used relatively high concentrations of nifedipine. Overall, nifedipine (20 or 40 μ M) reduced eIPSC charge by an average of $82 \pm 7\%$ ($P < 0.01$; $n = 5$ cells). These results imply a significant role for L-channels in mediating glycine release.

However, these findings do not eliminate an alternative hypothesis involving a nonsynaptic role for L-channels. It is theoretically possible that L-channels are not coupled to release, but rather an L-channel-dependent depolarization is required for activation of non-L calcium channels, which are coupled to release. To address similar considerations, Doze et al. (1995) used continuous application of 15-20 mM K^+ external saline to stimulate release. With this approach, current flow through calcium channels is no longer expected to affect the membrane potential *per se*, and release is triggered by the direct, constant potassium-mediated depolarization of the terminal. We found that application of 20 mM K^+ saline depolarized amacrine cells from $-51 \text{ mV} \pm 3.0 \text{ mV}$ to $-29 \text{ mV} \pm 1.2 \text{ mV}$ ($n = 5$; $P < 0.001$). In a separate set of experiments, we found that cadmium (75 μ M) had no effect ($V_m = -29 \pm 1.5 \text{ mV}$ in 20 mM K^+ ; $V_m = -29 \pm 1.5 \text{ mV}$ in 20 mM K^+ saline with cadmium; $n = 4$). Hence, using this protocol, cells are depolarized to a potential at which calcium channels are active and blockade of calcium channels does not affect this depolarization, *per se*. If L-channels acted solely by depolarizing amacrine cells, then, in high potassium saline, nifedipine should not affect glycine release. Because high potassium saline induced very high rates of release (e.g. Fig. 1B), we used the integrated charge as a measure of synaptic activity (see Methods).

Nifedipine (50 μ M) strongly suppressed glycinergic synaptic inputs produced by high potassium (15-20 mM K^+) saline (HK-sIPSCs) (Fig. 1B). Additive application of cadmium led to further suppression. On average, nifedipine (50 or 60 μ M) induced $82 \pm 1\%$ ($n = 4$ cells; $P < 0.01$) inhibition of release. In all cells, there was a clear nifedipine-insensitive release

component that was blocked by cadmium and strychnine. These results add additional evidence that calcium influx directly *via* L-channels is coupled to release of glycine.

In studies of other cell types, the role of L-channels in synaptic transmission has also been probed using compounds (BayK8644 and FPL64176) which selectively enhance L-type calcium currents (Gleason et al., 1994; Wheeler et al., 1994; Schmitz & Witkovsky, 1997; von Gersdorff et al., 1998). FPL64176 (2 μ M) induced a large increase in both the eIPSC and basal synaptic release that was partially reversible (Fig. 1C). In a set of four cells, FPL64176 (2 μ M) enhanced the eIPSC charge to $776 \pm 321\%$ ($P < 0.05$) of control and the baseline spontaneous synaptic charge transfer to $1310 \pm 747\%$ ($P < 0.05$) of control. These results provide further evidence that calcium influx *via* L-type channels can trigger release. The small nifedipine-insensitive/cadmium-sensitive component of release (Figs. 1A & 1B), however, suggests that another type of calcium channel may contribute to release.

The N/P/Q-channel antagonist ω -conotoxin MVIIC produced a large, essentially irreversible block of the eIPSC (Fig. 2A). In a set of four cells, ω -conotoxin MVIIC (5 μ M) reduced the charge of the eIPSC by $86 \pm 6\%$ ($P < 0.05$). Similarly, it strongly suppressed HK-sIPSCs, with the remaining inputs almost entirely suppressed by additive application of cadmium (100 μ M) (Fig. 2B). On average in five cells, ω -conotoxin MVIIC (5 μ M) reduced the charge transfer by HK-sIPSCs by $54 \pm 13\%$ ($P < 0.05$). The essentially irreversible nature of the inhibition by ω -conotoxin MVIIC is consistent with previous studies (Wheeler et al., 1994). These results indicate that calcium influx through N- and/or P/Q-channels can also induce release.

The P/Q-channel antagonist Aga-IVA (200 nM) suppressed the eIPSC only slightly (Fig. 3A); on average, a suppression of $12 \pm 11\%$ ($n = 4$ cells; $P > 0.3$; NS). For HK-sIPSCs (Fig. 3B), Aga-IVA effects were slightly larger, with an average suppression of $24 \pm 7\%$ ($P < 0.05$; $n = 5$ cells). Hence, these results suggest a relatively minor role for P/Q-channels, implying that the MVIIC effects are primarily due to blockade of N-channels.

To directly test the role of N-channels, we employed the N-channel antagonist ω -conotoxin GVIA. In the displayed experiment (Fig. 4A), for clarity, the electrical artifact has been mostly removed from these traces by digitally subtracting the average waveform recorded in strychnine at the end of the experiment. On average, ω -conotoxin GVIA (1 or 2 μ M) reduced eIPSC charge by $95 \pm 5\%$ ($n = 4$ cells; $P < 0.05$). For HK-sIPSCs, the displayed events (Fig. 4B) are inward because a high-chloride solution was used in the pipette and the cell was voltage clamped at -60 mV. On average, ω -conotoxin GVIA (2 μ M) reduced HK-sIPSC charge transfer by $77 \pm 10\%$ ($n = 4$ cells; $P < 0.05$). The essentially irreversible inhibition by ω -conotoxin GVIA is consistent with previous studies (Wheeler et al., 1994). These results suggest that N-channels play a major role in mediating synaptic glycine release.

Discussion

The principal finding of this study is that, overall, N- and L-channels primarily regulate glycine release, with a much smaller role for P/Q-channels.

Our results are consistent with previous studies demonstrating the presence of L-(α 1-C,D) and N-type (α 1-B) calcium channel subunits in the inner nuclear layer of the retina (Kamphuis & Hendriksen, 1998) and the finding that L-channels contribute ~50% of the calcium current recorded from amacrine cells in tiger salamander slices (Maguire 1999; Bieda & Copenhagen, unpublished observations). Our data indicating a large role for L-channels is also consistent with a recent report that glycinergic AII amacrine cells appear to use only L-channels for release (Habermann et al., 2003). Our results demonstrating a role for N-channels in glycine release contrast with some previous studies of GABA release from amacrine cells,

but agree with others. L- but not N- or P/Q-channels are coupled to GABA release in cultured chick amacrine cells (Gleason et al., 1994). N- but not L- or P/Q-channels are coupled to GABA release in cultured rat amacrine cells (Taschenberger & Grantyn, 1995). The disparate results may be due to differences in neurotransmitter studied, species, or in use of cultured versus acute slice neurons. Because we have found that most tiger salamander retinal ganglion cells which are recorded in our whole-cell experiments are transient ON-OFF cells (e.g. Bieda & Copenhagen, 2000), our results probably reflect primarily this population. It would be of interest to investigate whether N- and L-channels mediate the majority of glycinergic input to ON and OFF cells as well.

Our data indicate that both selective L-channel blockade and selective N-channel blockade separately induce an ~80-90% reduction in release, which seemingly implies an impossible >150% inhibition of release with toxin coapplication. This phenomenon has been termed “supra-additivity” and previous studies in other synapses have found similar “supra-additivity” with N- and P/Q-channel block (e.g. Takahashi & Momiyama, 1993). These previous studies have rationalized these results with models (Takahashi & Momiyama, 1993; Dunlap et al., 1995; Wu & Saggau, 1997) featuring moderate to high calcium cooperativity and calcium influx *via* two or more types of calcium channel contributing to release. If we interpret our data within this framework, then, at some release sites in some types of glycinergic amacrine cell, both L- and N-channels control release and calcium may act with cooperativity greater than one. However, this extrapolation from our data will need to be verified with studies of single types of amacrine cells, and it seems highly likely that different types of amacrine cells will have differing contributions of L- and N-channels to release [or just use one channel subtype, as with AII amacrine cells, which apparently only use L-channels (Habermann et al., 2003)] and probably different values for cooperativity, or even no cooperativity [i.e. $k = 1$ in eqn. (1)]. Still, our data do strongly suggest that, at some release sites for some types of glycinergic amacrine cells, both L- and N-channels are used, and that calcium acts with cooperativity greater than one. For electrical stimulation experiments, interactions between amacrine cells could distort interpretation of results; however, this problem should be minimized in the high potassium experiments, in which amacrine cells are “locked” at a depolarized potential that is not affected by calcium channel blockade.

Possible confounding nonselective nifedipine actions do not appear to qualitatively affect our conclusions. Nifedipine block of sodium or potassium channels would be expected to enhance or have no effect on HK-sIPSCs, but nifedipine produced strong suppression, implicating L-channels. Also, it is unlikely that our nifedipine results are due to blockade of T-type calcium channels, which play a role in retinal bipolar cell exocytosis (Pan et al., 2001), for several reasons: (1) T-channels do not appear to be present in tiger salamander amacrine cells (Barnes & Werblin, 1986; Maguire 1999; Bieda & Copenhagen, unpublished observations); (2) at our measured amacrine cell resting potentials (-50 mV for eIPSC experiments and -30 mV for 20 mM K⁺), most neuronal T-channels, particularly those linked to exocytosis (Pan, 2000; Pan et al., 2001), would be largely inactivated; (3) enhancement of release by the L-channel agonist FPL64176 and the cadmium (75-100 μ M) block of ω -conotoxin MVIIC/Aga-insensitive transmission are pharmacologically consistent with L-channels, but not T-channels. Therefore, our results with nifedipine are most consistent with an action on L-channels not T-channels. However, these data do not directly exclude a role for T-channels; in particular, given the large variety of amacrine cells, many cells may deviate from the ~-50 mV resting potential and in these cells in particular T-channels could conceivably play a role.

Our conclusion that N-channels play a role in release is based on our results with ω -conotoxin GVIA, ω -conotoxin MVIIC, and Aga. ω -conotoxin GVIA produces a slight block (15%) of L-type channels in tiger salamander photoreceptors at the concentrations used in our experiments (Wilkinson & Barnes, 1996). However, (1) the ~90% block of transmission by

ω -conotoxin GVIA is not consistent with a 15% block of L-channels and (2) the large reduction in release by ω -conotoxin MVIIC, which does not appear to affect L-channels (e.g. Wilkinson & Barnes, 1996), coupled with the small effects of the P/Q blocker Aga, strongly implicate N-channels. Hence, ω -conotoxin GVIA effects are most likely due to effects on N-channels.

The large percentage block of transmission by both nifedipine and ω -conotoxin GVIA separately appear to leave a relatively smaller role, overall, for R-channels (alpha-1e subunit), even with a cooperativity of 2-4 overall in the population. This claim is based on the idea that if we assume a cooperativity model with $k = 2-4$, then this model [using eqn. (1)] indicates that N-channels, with about ~90% block of synaptic transmission, account for ~44% ($k = 4$) to ~68% ($k = 2$) of calcium influx, L-channels, with about ~80% block of release, account for ~33% ($k = 4$) to ~55% ($k = 2$), and P/Q-channels, assuming ~25% block of release, from ~7% ($k = 4$) to ~13% ($k = 2$). Hence, even with $k = 4$, N-channel block accounts for ~44% of calcium relevant to release and L-channel block accounts for ~33% of calcium influx relevant to release; this is a total of 77%. Hence, R-channels appear to play a smaller role; if cooperativity of the population is less than 4, R-channel role may be very small. Our attempts to test for R-channels with nickel (200 μ M; Wu et al., 1998) led to a nonspecific block of nearly all release ($n = 3$; data not shown), probably due to powerful effects of nickel on L-channels (Zamponi et al., 1996). It is important to note that R-channels, and other non-L and non-N channels, may play large roles for certain types of glycinergic amacrine cells. Our methods, because they average over a population, would not observe roles for these channels if they are critical for only small numbers of glycinergic cells. Future studies using paired recordings, which are very difficult in this system, will probably be necessary to better dissect release from the various types of glycinergic amacrine cells.

There are several possible rationales for the existence of both N- and L-channel coupling to release in the population of glycinergic amacrine cells. Action potentials have an important role in mediating glycinergic inhibition; however, graded potentials also play a role (Cook et al., 1998; Bieda & Copenhagen, 1999). Recent work indicates that L-channels may generally be only weakly activated by action potentials, while graded potentials may generally efficiently activate both L and non-L channels (Mermelstein et al., 2000). Hence, for amacrine cells relying primarily on action potentials for release, N-channels may be critical, while for amacrine cells using graded potentials, L-channels may play a larger role. In addition, sustained activation of L-channels may establish residual calcium levels, which can control asynchronous (post-depolarization) exocytosis (Gleason et al., 1994). Finally, the presence of both N- and L-channels may allow differential modulation of synaptic release among different types of amacrine cells (Wu & Saggau, 1997).

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References

- Barnes S, Werblin F. Gated currents generate single spike activity in amacrine cells of the tiger salamander retina. *Proceedings of the National Academy of Sciences of the U.S.A* 1986;83:1509–1512.
- Bieda MC, Copenhagen DR. Sodium action potentials are not required for light-evoked release of GABA or glycine from retinal amacrine cells. *Journal of Neurophysiology* 1999;81:3092–3095. [PubMed: 10368424]
- Bieda MC, Copenhagen DR. Inhibition is not required for the production of transient spiking responses from retinal ganglion cells. *Visual Neuroscience* 2000;17:243–254. [PubMed: 10824678]

- Burgess DL, Noebels JL. Voltage-dependent calcium channel mutations in neurological disease. *Annals of the New York Academy of Sciences* 1999;868:199–212. [PubMed: 10414295]
- Cook PB, Lukasiewicz PD, McReynolds JS. Action potentials are required for the lateral transmission of glycinergic transient inhibition in the amphibian retina. *Journal of Neuroscience* 1998;18:2301–2308. [PubMed: 9482814]
- Dowling, JE. *The Retina: An Approachable Part of the Brain*. Harvard University Press; Cambridge, Massachusetts: 1987.
- Doze VA, Cohen GA, Madison DV. Calcium channel involvement in GABAB receptor-mediated inhibition of GABA release in area CA1 of the rat hippocampus. *Journal of Neurophysiology* 1995;74:43–53. [PubMed: 7472344]
- Dunlap K, Luebke JI, Turner TJ. Exocytotic Ca^{2+} channels in mammalian central neurons. *Trends in Neuroscience* 1995;18:89–98.
- Gleason E, Borges S, Wilson M. Control of transmitter release from retinal amacrine cells by Ca^{2+} influx and efflux. *Neuron* 1994;13:1109–1117. [PubMed: 7524563]
- Habermann CJ, O'Brien BJ, Wassle H, Protti DA. All amacrine cells express L-type calcium channels at their output synapses. *Journal of Neuroscience* 2003;23:6904–6913. [PubMed: 12890785]
- Kamphuis W, Hendriksen H. Expression patterns of voltage-dependent calcium channel alpha 1 subunits (alpha 1A-alpha 1E) mRNA in rat retina. *Brain Research Molecular Brain Research* 1998;55:209–220. [PubMed: 9582423]
- Koizumi A, Watanabe I, Kaneko A. Persistent Na^{+} current and Ca^{2+} current boost graded depolarization of rat retinal amacrine cells in culture. *Journal of Neurophysiology* 2001;86:1006–1016. [PubMed: 11495968]
- Maguire G. Spatial heterogeneity and function of voltage- and ligand-gated ion channels in retinal amacrine neurons. *Proceedings of the Royal Society B (London)* 1999;266:987–992.
- Mennerick S, Matthews G. Ultrafast exocytosis elicited by calcium current in synaptic terminals of retinal bipolar neurons. *Neuron* 1996;17:1241–1249. [PubMed: 8982170]
- Mermelstein PG, Bito H, Deisseroth K, Tsien RW. Critical dependence of cAMP response element-binding protein phosphorylation on L-type calcium channels supports a selective response to EPSPs in preference to action potentials. *Journal of Neuroscience* 2000;20:266–273. [PubMed: 10627604]
- Pan Z-H. Differential expression of high- and two types of low-voltage-activated calcium currents in rod and cone bipolar cells of the rat retina. *Journal of Neurophysiology* 2000;83:513–527. [PubMed: 10634892]
- Pan Z-H, Hu H-J, Perring P, Andrade R. T-Type Ca^{2+} channels mediate neurotransmitter release in retinal bipolar cells. *Neuron* 2001;32:89–98. [PubMed: 11604141]
- Schmitz Y, Witkovsky P. Dependence of photoreceptor glutamate release on a dihydropyridine-sensitive calcium channel. *Neuroscience* 1997;78:1209–1216. [PubMed: 9174087]
- Strom TM, Nyakatura G, Apfelstedt-Sylla E, Hellebrand H, Lorenz B, Weber BH, Wutz K, Gutwillinger N, Reuther K, Drescher B, Sauer C, Zrenner E, Meitinger T, Rosenthal A, Meindl A. An L-type calcium-channel gene mutated in incomplete X-linked congenital stationary night blindness. *Nature Genetics* 1998;19:260–263. [PubMed: 9662399]
- Tachibana M, Okada T, Arimura T, Kobayashi K, Piccolino M. Dihydropyridine-sensitive calcium current mediates neurotransmitter release from bipolar cells of the goldfish retina. *Journal of Neuroscience* 1993;13:2898–2909. [PubMed: 7687280]
- Takahashi T, Momiyama A. Different types of calcium channels mediate central synaptic transmission. *Nature* 1993;366:156–158. [PubMed: 7901765]
- Taschenberger H, Grantyn R. Several types of Ca^{2+} channels mediate glutamatergic synaptic responses to activation of single Thy-1-immunolabeled rat retinal ganglion neurons. *Journal of Neuroscience* 1995;15:2240–2254. [PubMed: 7891163]
- Vigh J, Solessio E, Morgans CW, Lasater EM. Ionic mechanisms mediating oscillatory membrane potentials in wide-field retinal amacrine cells. *Journal of Neurophysiology* 2003;90:431–443. [PubMed: 12649310]
- von Gersdorff H, Matthews G. Calcium-dependent inactivation of calcium current in synaptic terminals of retinal bipolar neurons. *Journal of Neuroscience* 1996;16:115–122. [PubMed: 8613777]

- von Gersdorff H, Sakaba T, Berglund K, Tachibana M. Submillisecond kinetics of glutamate release from a sensory synapse. *Neuron* 1998;21:1177–1188. [PubMed: 9856472]
- Wheeler DB, Randall A, Tsien RW. Roles of N-type and Q-type Ca^{2+} channels in supporting hippocampal synaptic transmission. *Science* 1994;264:107–111. [PubMed: 7832825]
- Wilkinson MF, Barnes S. The dihydropyridine-sensitive calcium channel subtype in cone photoreceptors. *Journal of General Physiology* 1996;107:621–630. [PubMed: 8740375]
- Wu LG, Saggau P. Presynaptic inhibition of elicited neurotransmitter release. *Trends in Neuroscience* 1997;20:204–212.
- Wu LG, Borst JG, Sakmann B. R-type Ca^{2+} currents evoke transmitter release at a rat central synapse. *Proceedings of the National Academy of Sciences of the U.S.A* 1998;95:4720–4725.
- Zamponi GW, Bourinet E, Snutch TP. Nickel block of a family of neuronal calcium channels: Subtype- and subunit-dependent action at multiple sites. *Journal of Membrane Biology* 1996;151:77–90. [PubMed: 8661496]

Fig. 1. L-channels mediate release of glycine. A: Nifedipine (20 μ M) strongly suppresses the electrically evoked IPSC (eIPSC) in a mostly reversible manner. Traces are mean of three consecutive evoked responses per condition. B: Nifedipine (50 μ M) suppresses release induced by continuous application of 20 mM K⁺-saline (HK-sIPSCs). Cadmium (100 μ M) suppresses almost all nifedipine-insensitive release. Raw sweeps (6 s) are shown. C: FPL64176 (2 μ M), a L-channel agonist, enhances the eIPSC and baseline transmission in a partially reversible manner. Traces are mean of four consecutive responses per condition. Scale bars: A = 30 pA, 300 ms; B = 20 pA, 500 ms; and C = 30 pA, 500 ms. *Con*: control; *Nif*: nifedipine; *Nif + Cd*: Nifedipine + cadmium; *Wash*: post-drug washout; and *Strych*: strychnine (10 μ M); and *FPL*: FPL64176.

Fig. 2.
 ω -conotoxin MVIIC (5 μ M), an N/P/Q antagonist, suppresses release of glycine. A: MVIIC suppresses the majority of glycine release. Traces are mean of 3/condition. B: MVIIC suppresses release induced by continuous application of 20 mM K⁺-saline. MVIIC-resistant release is cadmium (100 μ M) sensitive. Traces are raw 60-s sweeps. Scale bars: A = 30 pA, 500 ms; and B = 25 pA, 10 s. MVIIC: ω -conotoxin MVIIC (5 μ M); others are as in Fig. 1.

Fig. 3. P/Q channels play a small role in mediating glycine release. A: ω -Aga IVa (200 nM) has little effect on eIPSC. Traces are mean of 3/condition. B: ω -Aga IVa (200 nM) has small effects on release induced by continuous application of 20 mM K^+ -saline. Cadmium (75 μ M) mostly reversibly blocks release. Traces are raw 60-s sweeps. Scale bars: A = 40 pA, 800 ms; and B = 15 pA, 10 s. Aga: ω -Aga IVa; others are as in Fig. 1.

Fig. 4.

N-channels play a major role in release of glycine. A: ω -conotoxin GVIA ($1 \mu\text{M}$) strongly suppresses eIPSC. Traces shown have strychnine-insensitive electrical artifact digitally subtracted for clarity (see Results). Traces are mean of 3/condition. B: GVIA ($2 \mu\text{M}$) strongly suppresses release induced by continuous application of 20 mM K^+ -saline. Cell was voltage clamped at -60 mV with high chloride internal solution. Events are inward (downward) currents. In this recording, cadmium ($100 \mu\text{M}$) and strychnine ($10 \mu\text{M}$) induce little additional block. Traces are raw 60-s sweeps. Scale bars: A = 20 pA , 800 ms ; and B = 30 pA , 10 s . GVIA: ω -conotoxin GVIA; others are as in Fig. 1.