Characterization of Spontaneous Inhibitory Synaptic Currents in Salamander Retinal Ganglion Cells

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Gao, Fan and Samuel M. Wu. Characterization of spontaneous postsynaptic responses in ganglion cells and amacrine cells: inhibitory synaptic currents in salamander retinal ganglion cells. are mediated by \( \alpha \)-amino-3-hydroxy-5-methyl-4-isoxazole-propionic acid (AMPA) and \( N \)-methyl-\( D \)-aspartate (NMDA) receptors (Coleman and Miller 1988; Hensley et al. 1993a; Massey and Miller 1988; Mittman et al. 1990; Slaughter and Miller 1983a,b). Amacrine cell output synapses are largely GABAergic or glycineric, and the postsynaptic responses of GABAergic inputs are mediated by \( \gamma \)-aminobutyric acid-A (GABA\(_ A \)) and perhaps GABA\(_ C \) receptors (Belgum et al. 1984, 1987; Lukasiewicz et al. 1997; Lukasiewicz and Werpion et al. 1994). Both bipolar cell and amacrine cell synapses made on ganglion cells contain synaptic vesicles (Dowling and Werpion 1969; Wong-Riley 1974). It was first shown by Fatt and Katz that discrete postsynaptic events due to spontaneous release of single vesicles of neurotransmitter, named “quanta,” occurred in the neuromuscular junction (Fatt and Katz 1952). In the retina, spontaneous excitatory postsynaptic current (sPSC) mediations are spontaneous vesicular release of glutamate from photoreceptors, and spontaneous inhibitory postsynaptic currents (iPSCs) mediated by vesicular release of glycine from amacrine cells and interplexiform cells, have been observed in bipolar cells (Maple et al. 1994; Maple and Wu 1998). Additionally, sPSCs presumably mediated by spontaneous vesicular release of glutamate from bipolar cell synaptic terminals have been observed in salamander retinal ganglion cells (Taylor et al. 1995). Each of these spontaneous postsynaptic current events may represent single quanta, or postsynaptic currents gated by neurotransmitters from a single vesicle. Alternatively, each event may be mediated by clusters of vesicles released synchronously from single or multiple releasing sites (Maple et al. 1994; Matthews 1996). In this study, we demonstrate discrete iPSCs, mediated by spontaneous release of synaptic vesicles from amacrine cells in salamander retinal ganglion cells. We isolated iPSCs in ganglion cells by blocking the ePSCs with glutamate receptor antagonists and examined the effects of GABA and glycine antagonists on these spontaneous events. We also studied the calcium dependence, tetrodotoxin (TTX) sensitivity, event interval distribution, event consistency over time, average peak amplitude, rise and decay kinetics of iPSC events, and voltage dependence of iPSC amplitude and frequency. Moreover, we estimated the number of iPSCs (number of vesicles released) during a light-evoked IPSC (leIPSC) in ON-OFF ganglion cells. Some of the results have been presented in an abstract (Gao et al. 1997).

Methods

Living retinal slices of the larval tiger salamanders (Amphioxus tigrinum) were used in this study. Detailed procedures for prepar-
ing retinal slices and recordings were described elsewhere (Wu 1987). Oxygenated Ringer solution was introduced continuously to the superfusion chamber, and the control Ringer contained (in mM) 118 NaCl, 2.5 KCl, 1.2 MgCl₂, 2 CaCl₂, and 5 N-2-hydroxyethylpiperazine-N’-2-ethanesulfonic acid (HEPES; adjusted at pH 7.7). For experiments using Ca²⁺ Ringer solution, calcium ions were replaced by cobalt. Retinal ganglion cells were stimulated by a photostimulator, and the intensity of unattenuated 500 nm light (log I = 0) is 2.05 × 10⁷ photons µm⁻² s⁻¹. The majority of ganglion cells in this study were stimulated by a 2.5-s 500-nm light step of −2 log unit attenuation, which gave saturated responses in ON-OFF ganglion cells in the tiger salamander retina (Hensley et al. 1993b).

Voltage-clamp recordings were made with an Axopatch 200A amplifier connected to a DigiData 1200 interface and pClamp 6.1 software (Axon Instruments, Foster City, CA). Patch electrodes of 3 MΩ tip resistance (series resistance <20 MΩ) when filled with internal solution containing (in mM) 118 Cs methanesulfonate, 12 CsCl, 5 ethylene glycol-bis(β-aminoethyl ether) -N,N,N’,N’- tetraacetic acid (EGTA), 0.5 CaCl₂, 4 ATP, 0.3 guanosine triphosphate (GTP), and 10 tris(hydroxymethyl)aminomethane (Tris), adjusted to pH 7.2 with CsOH were made with Narishige patch electrode pullers. The chloride equilibrium potential, Eₗ, with this internal solution is about −60 mV. We corrected voltages for the disappearance of the liquid junctional potential at the tips of the patch electrode when the seal was made. Correction varied from −9.2 to −9.6 mV for the electrode internal solution. For simplicity, we corrected all voltage measurements in this paper by −10 mV.

sIPSCs were analyzed by in-house software and the SigmaPlot (Jandel Scientific). Individual sIPSCs were detected by eye and by the computer with a detection threshold of ±5 pA from the center of the baseline noise, with monophasic rise phase (time-to-peak <10 ms) and exponential decays. The two methods generated nearly identical counts. For sIPSCs with multiple peaks (subsequent peaks occurred before the preceding peak returned to the baseline), subsequent peaks were counted as separate events only if the preceding peak had returned for >50% from its peak and the subsequent peak was >10 pA and a rise time <10 ms. We chose these criteria because they provided spontaneous postsynaptic current (sPSC) counts very close to the counts made by eye. sPSC peak amplitudes were measured at the computer at the rising phase of each event. Events were plotted against their peak amplitude to construct amplitude probability density histograms, and the cumulative probability function at a given amplitude was obtained by integration of the amplitude histogram from 0 to that amplitude (Bendat and Piersol 1986). The average sIPSC amplitude at each holding potential was calculated by the ratio of the sum of peak amplitudes to the total number of events. sIPSC events were counted, and the intervals between two events were measured by the same computer program. Frequency-voltage relations and event interval histograms were constructed.

**RESULTS**

**Light-evoked postsynaptic currents (lePSCs) and sPSCs in retinal ganglion cells**

In the tiger salamander retina, >80% of ganglion cells give rise to voltage responses at the onset and offset of light stimuli, and they are named ON-OFF ganglion cells (Hensley et al. 1993a; Miller 1979; Mittman et al. 1990). Under voltage-clamp conditions, these cells exhibit lePSCs at light onset and offset, and sPSCs (Gao et al. 1997; Mittman et al. 1990; Taylor et al. 1995). Figure 1 shows the current traces of an ON-OFF ganglion cell recorded under voltage-clamp conditions in a dark-adapted tiger salamander retinal slice. Currents were recorded at eight holding potentials (from −110 to 30 mV with 20-mV increments), and a 2.5-s light step (500 nm, −2 log unit attenuation) was delivered to the cell at each holding potential. At both light onset and offset, two components of lePSCs were observed. The early component (marked with ●) reversed at ~0 mV, and the late component (marked with ○) reversed near −50 mV. Because ganglion cells in the tiger salamander retina receive glutamatergic excitatory synaptic inputs from bipolar cells, and GABAergic and glycinergic inhibitory synaptic inputs from amacrine cells (Belgium et al. 1983, 1984; Frumkes et al. 1981; Hensley et al. 1993b; Maple and Wu 1998; Mittman et al. 1990), the early component is probably mediated by excitatory bipolar cell inputs that gate a cation conductance, and the late component is mediated by inhibitory amacrine cell inputs that gate chloride conductances (Belgium et al. 1982; Mittman et al. 1990). In addition to lePSCs, discrete sPSCs were observed in the ganglion cell at all holding potentials. The directions of sPSCs were the same as the directions of the lePSC. At holding potentials less than or equal to −70 mV, where lePSCs were inward, all sPSCs were seen as inward currents. At potentials between −50 and −10 mV, where the early component of the lePSCs were inward and the late components were outward, some sPSCs were inward, whereas others were outward (although their amplitudes were small because the driving forces were low). At potentials >10 mV, where lePSCs were outward, all sPSCs were outward. We recorded a total of 41 ON-OFF ganglion cells from dark-adapted salamander retinal slices, all of which showed similar lePSCs and sPSCs.

We next studied these spontaneous postsynaptic currents in darkness. Figure 2A shows voltage-clamp currents at various holding potentials from an ON-OFF ganglion cell without light stimuli. At all holding potentials, sPSCs occurred randomly (randomness of sPSCs is demonstrated later in this paper), sometimes in bursts, and with variable peak amplitudes. sPSCs at each holding potential were in the same direction as those shown in Fig. 1. This suggests that there may be two types of sPSCs, one with a reversal potential near −60 mV, the other with a reversal potential near 0 mV. One way to distinguish between the two types of sPSCs was to record currents at potentials between −60 and 0 mV, where the type reversed at −60 mV was outward and the type reversed at 0 mV was inward. However, because the driving forces for both types of sPSCs were small in this potential range, it was difficult to resolve individual sPSCs from the current noise. We therefore used pharmacological tools to separate the two types of sPSCs. Previous investigations have shown that glutamatergic excitatory inputs to salamander retinal ganglion cells can be blocked by 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX) [or 6,7-dinitroquinoxaline-2,3-dione (DNQX)] + 2-amino-7-phosphonoheptanoic acid (AP7) [or 2-amino-5-phosphonopentanoic acid (AP5)] (Hensley et al. 1993a; Mittman et al. 1990), and the inhibitory inputs can be blocked by picrotoxin + strychnine (Belgium et al. 1984; Lukasiewicz et al. 1995; Mittman et al. 1990). Figure 2B shows when 100 µM picrotoxin + 1 µM strychnine was applied to the ON-OFF ganglion cell shown in Fig. 2A, the frequencies of the sPSCs were greatly reduced, and all sPSCs reversed near 0 mV, consistent with the idea that they were mediated by glutamatergic excitatory synapses that gated a cation conductance in ganglion cells (Belgium et al. 1982; Mittman et al. 1990). This type of
sPSC is named sEPSCs, and it has been studied in a previous report (Taylor et al. 1995). We therefore focused the rest of the paper on the second type of sPSCs, the sIPSCs.

Figure 3 shows the sPSCs recorded from another ON-OFF ganglion cell in normal Ringer solution (A) and in the presence of 10 μM DNQX + 50 μM AP5 (B). sPSCs in Fig. 3A are very similar to those in Fig. 2A. In the presence of 10 μM DNQX + 50 μM AP5 (Fig. 3B), the frequencies of the sPSCs were greatly reduced (thus the events appeared more discrete), because DNQX and AP5 blocked the glutamatergic sEPSCs and reduced the rate of inhibitory transmitter release (sIPSCs) by hyperpolarizing amacrine cells (Dixon and Copenhagen 1992). All sPSCs in DNQX + AP5 reversed near −60 mV, the chloride equilibrium potential ($E_{Cl}$, which was set to be −60 mV by the chloride concentrations in the pipette and in the bath; see METHODS). Based
on their reversal potential and pharmacological properties (shown in the next section), these sIPSCs are likely to be the sIPSCs mediated by amacrine cells. In the rest of this paper, we routinely used 10 \mu M DNQX + 50 \mu M AP5 (DNQX + AP5) in the bath to isolate sIPSCs, even though we could have isolated sIPSCs by holding the voltage at 0 mV (equilibrium potential of sEPSCs). The DNQX + AP5 method had two advantages. First, it allowed us to study sIPSCs at all holding potentials so that voltage-dependent properties of sIPSCs could be determined. Second, DNQX + AP5 greatly reduced the frequency of sIPSCs so that individual sIPSCs were distinguishable without too much overlap (events with multiple peaks). This is important for accurate event counting and making kinetics measurements of sIPSC. In DNQX + AP5, events with multiple peaks account for <15% of the total sIPSC events.

**GABAergic and glycinergic sIPSCs**

In the tiger salamander retina, the majority of amacrine cells are either GABAergic or glycinergic (Li et al. 1990; Yang and Yazulla 1988a,b). Figure 4 shows the effects of 100 \mu M bicuculline (a GABA_A receptor antagonist) and 1 \mu M strychnine (a glycine receptor antagonist) on sIPSCs in ON-OFF ganglion cells. In ~70% of ON-OFF ganglion cells such as that in Fig. 4A (we named them type A ON-OFF ganglion cells), 100 \mu M bicuculline completely blocked sIPSCs. In the rest (30%) of the ON-OFF ganglion cells such as that in Fig. 4B (type B ON-OFF ganglion cells), bicuculline blocked 70–98% of the sIPSCs, and the remaining sIPSCs are completely blocked by the addition of 1 \mu M strychnine. The bicuculline-sensitive events are GABAergic sIPSCs (GABAIPSCs), and they account for ~95% of the total sIPSCs we recorded from ON-OFF ganglion cells in the presence of DNQX + AP5. The strychnine-sensitive events are glycineric sIPSCs (GLYsIPSCs), and they account for 5% of the total sIPSCs. All sIPSCs in Fig. 4 were recorded at a holding potential of 0 mV, which is near the reversal potential of the sEPSCs is near zero; therefore, if there are some properties of sIPSCs could be determined. Second, DNQX + AP5 greatly reduced the frequency of sIPSCs so that individual sIPSCs were distinguishable without too much overlap (events with multiple peaks). This is important for accurate event counting and making kinetics measurements of sIPSC. In DNQX + AP5, events with multiple peaks account for <15% of the total sIPSC events.

**Calcium dependence and TTX sensitivity of GABAIPSCs**

Anatomic and physiological evidence suggests that signal transmission from amacrine cells to ganglion cells are mediated by calcium-dependent vesicular synapses (Belgium et al. 1984; Frumkes et al. 1981; Wong-Riley 1974). Subpopulations of amacrine cells in the tiger salamander retina exhibit TTX-sensitive spikes (Eliasof et al. 1987; Miller and Dacheux 1976). We examined the effects of cobalt (a cal-

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**Fig. 4.** Effects of 100 \mu M bicuculline and 1 \mu M strychnine on sIPSCs in ON-OFF ganglion cells. A: in a type A ganglion cell, 100 \mu M bicuculline completely blocked sIPSCs. B: in a type B ganglion cell, bicuculline blocked ~70% of the sIPSCs, and the remaining sIPSCs were completely blocked by the addition of 1 \mu M strychnine. All sIPSCs in the figure were recorded at a holding potential of 0 mV, which is near the reversal potential of the sEPSCs (Fig. 2B).
cium channel blocker) (Weakly 1973) and TTX on GABA-
IPSCs in the presence of DNQX + AP5. Figure 5A shows
that the frequencies of GABAIPSCs in ON-OFF ganglion
cells were reversibly reduced when extracellular calcium
was replaced by 1 mM cobalt, consistent with the idea that
these GABAIPSCs are largely mediated by a calcium-de-
pendent mechanism (Belgium et al. 1984; Weakly 1973).
We repeated this experiment in seven other type A ON-OFF
ganglion cells, and 1 mM cobalt blocked 75–95% of the
GABAIPSCs in those cells. Figure 5B shows that 2 μM
TTX, a dose that suppresses action potentials in amacrine
cells (Eliasof et al. 1987), exerted no observable effects on
(DNQX + AP5)–isolated GABAIPSCs in type A ON-OFF
ganglion cells. In three other type A ON-OFF ganglion cells
in the presence of DNQX + AP5, we did not observe any
significant GABAIPSC frequency reduction caused by 2
μM TTX. However, 2 μM TTX substantially reduced the
frequency of sPSCs in normal Ringer solution, perhaps be-
cause amacrine cells were more depolarized in normal
Ringer solution than in DNQX + AP5 (Dixon and Copenha-
gen 1992), and thus they generate spontaneous spikes.
Because all GABAIPSCs we deal with in this paper were
recorded in DNQX + AP5, TTX effects in normal Ringer
solution are not shown.

GABAIPSCs occur randomly and persist over time

To determine whether GABAIPSCs occur randomly, we
constructed event interval histograms for long stretches of

\[
\int_{t_0}^{t} f(t) \, dt = \int_{\tau_i}^{\infty} f(t) \, dt = -\alpha \int_{\tau_i}^{\infty} e^{-\alpha t} \, dt = \frac{1}{\alpha} e^{-\alpha \tau_i}
\]

where \( f(t) \) is the number of GABAIPSC event intervals of
duration \( t \), \( \alpha \) is the number of intervals of very short dura-
tions (<40 ms; this limit is set by the average decay time of
individual GABAIPSCs and the criteria of event detection
described in METHODS), and \( \tau_i \) is the exponential constant.
For the GABAIPSC record in Fig. 6, \( \tau_i \) equal to 55.3 ms,
and the average interval was 83.3 ms. We repeated such
analysis on four other GABAIPSC records (3 122-s and 1
244-s continuous records) from three type A ON-OFF
ganglion cells; the histograms show that GABAIPSC event
intervals for all three cells were exponentially distributed
with average intervals of 112.5, 81.8, 167.6, and 203.3 ms.
The average of the five average intervals is 129.7
\( \pm \) 48.2 ms, and thus the average frequency of GABAIPSCs at
0 mV in DNQX + AP5 is \( (1/129.7 \, \text{ms}) = 7.71 \, \text{Hz} \). The
exponential interval distribution is consistent with the idea
that GABAIPSCs occur randomly with respect to time
(their occurrence is independent of each other) (Bendat
and Piersol 1986; Frerking et al. 1995; Johnston and Wu

The average number of GABAIPSC occurrences per sec-
ond (event frequency, \( F \)) varied from cell to cell. In a sample
of 23 type A ON-OFF ganglion cells in DNQX + AP5, \( F \)
ranged between 0.8 and 23 Hz. In any given cell, however,
the GABAIPSC frequency and amplitude persisted over
long recording periods. Figure 7 shows the time course of GABA IPSC frequency and amplitude over a period of 750 s (12.5 min). The frequency and amplitude data points were obtained by averaging the number and peak amplitudes of GABA IPSCs every 12.2 s, and they clearly indicated that neither the frequency nor the average amplitude of GABA IPSCs changed significantly with time. We repeated these measurements on GABA IPSCs from three other ganglion cells, all of which showed no change in GABA IPSC frequency and amplitude over 5–12.5 min. [Note: We believe that 12.5 min (the longest continuous recording period we had without changing the voltage or solution) is an underestimate of constant GABA IPSC recording. GABA IPSC records with several changes of voltage or solutions show that the GABA IPSC frequency and amplitude in ON-OFF ganglion cells persist over 30 min.] This suggests that GABA IPSCs in ON-OFF ganglion cells persist over at least 12.5 min without significant rundown. Thus changes of GABA IPSC amplitude and frequency in response to applications of pharmacological agents described in the previous section are unlikely to be caused by synaptic or cell rundown, because most of these experiments were carried out within periods shorter than 12.5 min.

**Kinetics and amplitude of GABA IPSCs**

To analyze the kinetics of GABA IPSCs, we selected GABA IPSCs with single peaks, monophasic rise time, and exponential decays. GABA IPSCs with multiple peaks (which account for <15% of total GABA IPSCs in DNQX + AP5) were considered as asynchronous multiples of single events, and their rise and decay time courses were not analyzed. Figure 8 shows the time course of six GABA IPSCs recorded at six holding potentials (−110, −90, −30, 10, and 30 mV) under voltage clamp in the presence of DNQX and AP5. The rise times (time-to-peak = τ₀) of the six GABA IPSCs in Fig. 8 varied from 3 to 6 ms. In a sample of 62 GABA IPSCs, the average value of τ₀ is 5.20 ± 2.05 ms. The decay time course of GABA IPSCs could be fitted with a double (sum of 2 singles) exponential function (shown as heavy lines in the decay phase of each GABA IPSC in Fig. 8)

\[
I(t) = A_1e^{-t/\tau_1} + A_2e^{-t/\tau_2}
\]

where \(I(t)\) is the GABA IPSC as a function of time in the
decay phase ($t = 0$ at the peak), $A_1$ and $A_2$ are amplitude scaling factors (in pA), and $\tau_{D1}$ and $\tau_{D2}$ are the fast and slow decay time constants respectively (in ms). The values of $\tau_{D1}$ and $\tau_{D2}$ of the six GABAsIPSCs in Fig. 8 varied from 4.6 to 20 ms for $\tau_{D1}$, and from 62.3 to 250 ms for $\tau_{D2}$. In a sample of 62 GABAsIPSCs, the average value $\pm$ SD for $\tau_{D1}$ = 10.49 $\pm$ 6.84 ms, and for $\tau_{D2}$ = 89.51 $\pm$ 64.32 ms. The 62 GABAsIPSCs were selected from records at 6 different holding potentials, and none of the 3 time constants appeared to be voltage dependent. Additionally, there was no correlation between the GABAsIPSC rise time and decay time, suggesting that dendritic filtering did not shape the time course of GABAsIPSCs.

Figure 9 shows that the time constants and peak amplitudes of GABAsIPSCs at the same holding potential differ substantially from one another. We selected six GABAsIPSCs at holding potential 30 mV in DNQX + AP5. $\tau_0$ varied from 3.0 to 6.0 ms, $\tau_{D1}$ varied from 2.8 to 16.7 ms, and $\tau_{D2}$ varied from 26.7 to 184.7 ms. The peak amplitude of the six GABAsIPSC events varied from 16.6 to 240 pA.

We next analyzed the amplitude distribution of the GABAsIPSCs in DNQX + AP5. The amplitude distribution histograms of GABAsIPSCs in continuous 122-s recordings from an ON-OFF ganglion cell held at 0 mV are shown in Fig. 10. There were totally 1,611 GABAsIPSC events, and the amplitude histogram shows that GABAsIPSCs are not normally distributed; instead, they are skewed toward smaller amplitude with an average peak GABAsIPSC amplitude of 75.3 pA. The cumulative probability, obtained by integrating the amplitude histograms, is given as the continuous curve in Fig. 10. The amplitude histogram or the cumulative probability function gives no indication of multiple peaks, a characteristic feature of quantal events (Edwards et al. 1990; Fatt and Katz 1952). We performed such amplitude distribution analysis on GABAsIPSCs from 16 other ON-OFF ganglion cells, the shapes of the amplitude histograms were similar to that shown in Fig. 10 (none showed amplitude histograms with multiple peaks), although the average peak GABAsIPSC amplitudes of these cells were smaller (we chose to show the ganglion cell with the largest GABAsIPSCs in Fig. 10). The average peak GABAsIPSC amplitude over all 17 ON-OFF ganglion cells in DNQX + AP5 held at 0 mV (totally 5,153 GABAsIPSC events) was 19.18 $\pm$ 15.17 (ranged from 6.8 to 75.3 pA; median, 30.20 $\pm$ 29.39 pA). Because the driving force of GABAsIPSCs is 60 mV [(V – $E_{Cl}$) = 0 mV – (–60 mV)], the average conductance change associated with a single GABAsIPSC event is (19.18 $\pm$ 15.17)/60 mV = 319.67 $\pm$ 252.83 pS.

**Current-voltage and frequency-voltage relations of GABAsIPSCs**

We next studied the voltage dependence of the average peak amplitude and frequency of GABAsIPSCs in the presence of DNQX + AP5. By using the same procedures described in the previous section, we first constructed the amplitude histograms of GABAsIPSCs at each holding potential, and then calculated the cumulative probability functions by integrating the amplitude histograms. Figure 11A shows examples of the peak amplitude histograms and cumulative probability functions of GABAsIPSCs in DNQX + AP5 at
10 mV. To save space, we only show the cumulative probability functions for other holding potentials in Fig. 11B, but not the amplitude histograms. It is clear from this figure that the amplitude distributions of sPSCs and GABAsIPSCs at potentials greater than or equal to −50 mV were positive (outward currents, increasingly skewed toward larger positive amplitude as the potential became more positive), and those at potentials less than or equal to −70 mV were negative (inward currents, increasingly skewed toward larger negative amplitude as potential became more negative). The average amplitudes of GABAsIPSCs at each potential were calculated by dividing the sum of peak current of individual events by the number of events. We performed such detailed amplitude distribution analysis of GABAsIPSCs at eight holding potentials on five other ON-OFF ganglion cells. In the sample of six cells, we calculated the average peak amplitudes (±SD) of GABAsIPSCs at each holding potential and plotted them in Fig. 11C. The current-voltage relation of the average GABAsIPSCs is approximately linear, with a reversal potential near −60 mV. This reversal potential is close to the chloride equilibrium potential ($E_{Cl}$), suggesting that GABAsIPSCs are associated with a chloride conductance increase in retinal ganglion cells. The slope of the average current-voltage relation was 330 pS, a value close
to the average GABAergic conductance change measured at a single voltage (0 mV) from 17 ganglion cells (319.67 ± 252.83 pS) described in the last section. We plotted in Fig. 11D the average frequency-voltage relation of GABAergic IPSCs in ON-OFF ganglion cells. Data points were averaged over GABAergic IPSC frequencies from the same six ganglion cells used in Fig. 11C. The average frequency-voltage relation of GABAergic IPSCs exhibited a large dip, indicating lower frequencies, between −30 and −90 mV. This dip, however, is probably artificial, because the membrane potentials in the dip are close to the reversal potential of the GABAergic IPSCs, and thus the driving force and the amplitude of the GABAergic IPSCs are small. The apparent low frequency at these potentials may result from missing counts of many small GABAergic IPSCs that are buried in the noise. The actual frequency of GABAergic IPSCs may be relatively constant at all potentials except for a slight elevation tilted toward the positive potentials. The average frequency of GABAergic IPSCs in DNXQ + AP5 is ~100/12.2 = 8.2 Hz near −110 mV and 130/12.2 = 10.7 Hz near 30 mV. Disregarding the dip, there is a slight voltage-dependent elevation of GABAergic IPSC frequency (dashed line). The implications of this voltage-dependent frequency elevation will be elaborated in the discussion.

Number of GABAergic synaptic vesicles released during leIPSCs in ON-OFF ganglion cells

Using the average amplitude and time course of the single GABAergic IPSC, and assuming linear summation, we estimated the number of synaptic vesicles released during individual leIPSCs in ON-OFF ganglion cells. Figure 12 shows the current response of an ON-OFF ganglion cell in normal Ringer solution (DNXQ + AP5 blocks light responses) to a 2.5-s light step (500 nm, −2 log unit attenuation, which elicited saturated light responses). The cell was held at 0 mV so that the excitatory inputs (leEPSCs) were eliminated. In the inset of Fig. 12, we show a simulated average GABAergic IPSC with the average peak amplitude and kinetics parameters described in the previous sections. The ratio of the area [charge (Q) = current × time, in pico-Coulomb (pC)] under the leIPSCs to the area under the single GABAergic IPSC gives the approximate number of synaptic vesicles required to produce the light response. For the cell showed in Fig. 12, the charge for the ON-response, QON = 157.6 pC, and the charge for the OFF-response, QOFF = 303.2 pC. Because the average charge of a single GABAergic IPSC is 1.06 pC, the number of vesicles released at the onset of the light response was ~149, and that at the light offset was ~286. We performed similar calculation on the light-evoked currents in 15 other ON-OFF ganglion cells. The average peak leIPSC (held at 0 mV) at the light onset is 509.0 ± 233.85 pA, and that at the light offset is 529.0 ± 339.88 pA. The average number of GABAergic vesicles released at the light onset is 118 ± 52 (n = 16, range 46–214), and that at the light offset is 132 ± 76 (n = 16, range 80–324).

Kinetics and average amplitude of GLYsIPSCs

The above sections present systematic studies on the pharmacological, statistical, kinetic and voltage-dependent properties of the GABAergic IPSCs. Because of the low event frequency (0.18 ± 0.56 Hz) of GLYsIPSCs, we were unable to carry out the same set of studies on glycinergic sIPSCs. We nevertheless analyzed the kinetics of individual glycinergic events and estimated the average peak conductance change associated with single GLYsIPSCs (by using the same procedures as for the GABAergic events). Figure 13 shows the time course of eight GLYsIPSCs recorded at two holding potentials (−110 and 10 mV) under voltage clamp in the presence of DNXQ + AP5 + bicuculline. The GLYs-IPSCs, like the GABAergic IPSCs, reversed at −60 mV. The rise times (τ0) of the eight sIPSCs in Fig. 13 varied from 1.5 to 9 ms. The decay time course of individual GLYsIPSCs can also be fitted by Eq. 2. The values of τD1 of the eight GLYsIPSCs in Fig. 13 varied from 4.7 to 18.2 ms, and the values of τD2 varied from 287 to 980 ms. In a sample of 21 GLYsIPSCs from 4 type B ganglion cells in the presence of DNXQ + AP5 + bicuculline, the average rise time τ0 is 3.54 ± 1.46 ms, the average decay time constants τD1 is 12.4 ± 3.27, and τD2 is 386.1 ± 241.2 ms. In a sample of 328 GLYsIPSCs from 12 type B ON-OFF ganglion cells, we estimated that the average peak conductance change associated with a single GLYsIPSCs is 301.68 ± 94.34 pS. We also simulated the average GLYsIPSC with the average peak amplitude and kinetics parameters given above. The charge
type B ganglion cell account for, for example, 5% of the The mean rise time of the GABA sIPSCs is 5.20 ms, a value very close to the transfer (\( Q \), the area under the average single GLY sIPSC) is 0.98 pC, a value very close to the \( Q \) value of the average GABA sIPSC (1.06 pS). Therefore, if GLY sIPSCs in a given type B ganglion cell account for, for example, 5% of the total sIPSCs (GABAergic and glycine), the number of glycineergic synaptic vesicles involved in mediating the inhibitory light-evoked currents will be \( \sim 5\% \), and the number of GABAergic synaptic vesicles will be close to 95%.

**DISCUSSION**

This study demonstrates the presence of sIPSCs in retinal ganglion cells. sIPSCs in all ON-OFF ganglion cells are completely blocked by bicuculline (or picrotoxin) + strychnine, indicating that they are mediated by GABAergic and glycineergic synaptic inputs. In \( \sim 70\% \) of ON-OFF ganglion cells (type A ganglion cells), bicuculline (or picrotoxin) completely blocks sIPSCs, suggesting all sIPSCs in these cells are mediated by GABAergic synaptic vesicles and GABA\(_A\) receptors (GABAergic sIPSCs, or GABA sIPSCs). In the rest 30% of ON-OFF ganglion cells (type B ganglion cells), bicuculline (or picrotoxin) blocks 70–98% of the sIPSCs, and the rest sIPSCs are blocked by strychnine (glycineergic sIPSCs, or GLY sIPSCs).

GABA sIPSCs occur randomly with an exponentially distributed interval probability density function, and they persist without noticeable rundown over time. The GABA sIPSC frequency is greatly reduced by cobalt (with zero calcium), consistent with the idea that they are largely mediated by calcium-dependent vesicular release. However, the cobalt blockade of GABA sIPSC was never complete, indicating that the spontaneous GABA release may not depend totally on calcium influx through calcium channels in the plasma membrane (Brown et al. 1979). GABA sIPSCs in DNQX + AP5 are TTX insensitive, suggesting that amacrine cells that release GABA under these conditions do not generate spontaneous action potentials.

**sIPSC amplitude, kinetics, and vesicular release**

The average GABA sIPSCs exhibited linear current-voltage relation with a reversal potential near the chloride equilibrium potential, and an average peak conductance of 319.67 ± 252.83 pS. This conductance value is close to that of single GABAergic miniature inhibitory postsynaptic currents (mIPSCs) recorded from granule cells in the hippocampus (140–400 pS) (Edwards et al. 1990; Otis and Mody 1992). It is worth noticing that the average current-voltage relation shown in Fig. 11C is approximately linear, with better fits for data points at potentials less than or equal to \(-90\) mV or greater than or equal to \(-10\) mV. As shown in the Fig. 11D, there is a dip of sIPSC frequency in potentials between \(-90\) and \(-30\) mV, caused by low driving force for the sIPSCs (also see discussion below). Therefore many small sIPSCs within this voltage range are buried in the noise and not included in the amplitude averaging process. For this reason, the average sIPSC amplitudes from \(-70\) to \(-30\) mV are not very accurate, and they are more likely to be larger than the true average amplitudes because some smaller sIPSC events are excluded. Because the straight line in Fig. 11C was drawn to fit data points at potentials less than or equal to \(-90\) mV or greater than or equal to \(-10\) mV, it avoids the problem created by the frequency dip and gives a reasonably accurate slope (average sIPSC conductance) for the sIPSCs.

The mean rise time of the GABA sIPSCs is 5.20 ± 2.05 ms, and the decay time course can be fitted by the sum of two exponentials. The fast component has a time constant of 10.49 ± 6.84 ms, and the slow component has a time constant of 89.51 ± 64.32 ms. For the GLY sIPSCs, the mean rise time is 3.54 ± 1.46 ms, the fast decay time constant is 12.4 ± 3.37 ms, and the slow decay time constant is 386 ± 241 ms. It is important to note that these values are approximate measures of the rise and decay times of sIPSCs in retinal ganglion cells. Our limited sample size and the possibility of insufficient space clamping of ganglion cells in the slice preparation may cause errors in the kinetics measurements. We therefore make no further attempts to correlate the kinetics of sIPSCs with the kinetics of GABA and glycine receptors.

It is reasonable to propose that each sIPSC in this study is mediated by GABA or glycine released from a single or synchronized multiples of synaptic vesicles. In the amplitude histograms of GABA sIPSCs, we do not observe multiple peaks, suggesting that larger events are not discrete multiples of elementary events, or quanta, of similar neurotransmitter contents, as in the neuromuscular junction (Fatt and Katz 1952). It has been shown that in retinal amacrine cells, the difference in neurotransmitter contents in individual GABAergic synaptic vesicles accounts for more than one-half of the variance in mIPSC amplitude, and the average amplitude and time courses of these mIPSCs are similar to the sIPSCs in this study (Frerking et al. 1995). Therefore the variation in GABA sIPSC and GLY sIPSC amplitudes in our study may reflect different GABA and glycine concentra-
tions in individual synaptic vesicles, although we cannot rule out the possibility that some larger events are mediated by synchronized release of multiple vesicles with variable neurotransmitter contents.

**GABAergic and glycinergic sIPSCs**

Our results demonstrate that sIPSCs can be completely blocked by 100 μM bicuculline (or picrotoxin) + 1 μM strychnine, suggesting that they are mediated by GABAergic and glycinergic synaptic vesicles released from amacrine cells. Because bicuculline and picrotoxin exert the same blocking actions, the GABAergic sIPSCs in ON-OFF ganglion cells are likely to be mediated by GABA<sub>Α</sub> receptors (Feigenspan et al. 1993; Qian and Dowling 1993, 1995).

In analyzing individual sIPSCs, we found that the reversal potential, average peak amplitude, and rise or decay time courses of the GABA<sub>IPSC</sub>s and GLY<sub>IPSC</sub>s are very close. The average charge transfer of a single GABA<sub>IPSC</sub> (1.06 pC) is only 7.5% higher than that of a single GLY<sub>IPSC</sub> (0.98 pC). This suggests that GABA and glycine released from each synaptic vesicle increase the chloride conductance, on average, by approximately the same amount. The unitary conductance of a single GABA<sub>Α</sub> receptor channel is ~27 pS in the retina (Feigenspan et al. 1993), and 14–30 pS in the brain (Dillon et al. 1995; Edwards et al. 1990; Otis et al. 1994). Therefore a GABAergic sIPSC in retinal ganglion cells represents the opening of, on average, 12 (319.67 pS/27 pS) GABA<sub>Α</sub>-gated channels. The unitary conductance of a glycine receptor channel is ~54 pS in the retina (Potti et al. 1997), and 32–96 pS in other parts of the nervous system (Virginio and Cherubini 1997; Walstrom and Hess 1994), and thus a glycinergic sIPSC in retinal ganglion cells represents the opening of, on average, six glycine-gated channels. These numbers are consistent with the numbers of open channels associated with individual GABAergic and glycinergic sIPSCs in other nervous tissues (Edwards et al. 1990; Feigenspan et al. 1993; Otis and Mody 1992).

It is not clear why the GLY<sub>IPSC</sub>s (~5%) are so much fewer than the GABA<sub>IPSC</sub>s (~95%) in the ON-OFF ganglion cells. Because the numbers of GABAergic and glycinergic amacrine cells in the salamander retina and the synaptic contacts made by the two types of amacrine cells on ganglion cells are similar (Li et al. 1990; Wong-Riley 1974; Yang and Yazulla 1988a,b), the difference in GABA<sub>IPSC</sub> and GLY<sub>IPSC</sub> frequency may not be caused by difference in synaptic contacts. One possibility is that glycinergic amacrine cells have a lower rate of spontaneous transmitter release than GABAergic amacrine cells in darkness. Another possibility is that DNQX + AP5 exerts stronger hyperpolarizing actions on glycinergic amacrine cells than on GABAergic amacrine cells. Therefore the GABAergic cells are more depolarized and hence have higher rates of transmitter release.

**sIPSC frequency in DNQX + AP5 and in normal Ringer solution**

Based on event interval histograms of GABA<sub>IPSC</sub>s from five ON-OFF ganglion cells, we estimate the average GABA<sub>IPSC</sub> frequency at 0 mV in DNQX + AP5 to be 7.71 ± 3.75 Hz. This is close to the value of sIPSC frequency at 0 mV in Fig. 11D, which is 9.67 (118/12.2). We argue that the dip between ~30 and ~90 mV in Fig. 11D is caused by missing counts of GABA<sub>IPSC</sub>s because the reversal potential of the sIPSC is near ~60 mV, and thus the driving forces and the amplitudes within this voltage range are low. Many of the small GABA<sub>IPSC</sub>s in this voltage range are buried in the noise. Although we do not have direct proof for this argument (because we cannot identify the small events either by eye or by the computer), we think it is reasonable for two reasons. First, the frequency of the observable events is roughly proportional to the driving force of the sIPSC in both directions of the voltage axis (Fig. 11D), with the lowest value near ~50 and ~60 mV. Second, even if the membrane voltage of a ganglion cell affects the amacrine cell neurotransmitter release rate through a feedback pathway, it is unlikely that the release rates are proportional to the chloride driving force in the ganglion cells, rather than a monotonic increase with membrane depolarization. The slight elevation of sIPSC frequency without the dip (dotted line in Fig. 11D), on the other hand, may suggest that membrane depolarization of the ganglion cells increases the neurotransmitter release of amacrine cells. In the catfish retina, it has been demonstrated that some ganglion cells are electrically coupled with amacrine cells (Naka and Christensen 1981). It is possible that depolarization in ganglion cells induces depolarization in adjacent amacrine cells through the gap junctions, and thus results in an increase of neurotransmitter release (higher GABA<sub>IPSC</sub> frequency).

Results in Fig. 3 show that the frequency of spontaneous postsynaptic currents in normal Ringer solution is much higher than that in DNQX + AP5. One obvious reason for this is that DNQX + AP5 suppresses sEPSCs by blocking glutamate receptors in ganglion cells, and thus lowers the sPSC frequency. Another reason is that DNQX + AP5 hyperpolarize the amacrine cells (Dixon and Copenhagen 1992), and decreases the rate of GABA release, and thus reduces the number of GABA<sub>IPSC</sub>s. The second action is evident when comparing the current traces at holding potentials near 0 mV (e.g., 10 and ~10 mV) in normal Ringer solution (Fig. 3A) and in DNQX + AP5 (Fig. 3B). At potentials near 0 mV (reversal potential for sPSCs), the sEPSCs become very small, and thus most observable sPSCs in normal Ringer solution are GABA<sub>IPSC</sub>s. The frequencies of GABA<sub>IPSC</sub>s in DNQX + AP5 at these potentials are much lower than that in normal Ringer solution. Therefore, under dark-adapted conditions in normal Ringer solution, the frequencies of GABA<sub>IPSC</sub>s are significantly higher than what we report in this paper, which was obtained in DNQX + AP5. We were unable to provide an accurate measure of the sIPSC frequency in normal Ringer because it is so high that individual GABA<sub>IPSC</sub>s fuse together, often forming large transient currents with sawtooth-shaped rise and decay time courses. It is difficult to clearly separate individual events under such conditions and to accurately count events either by eye or by the computer. For the same reason, we were unable to measure the amplitude and time constants of sPSCs in normal Ringer solution. In DNQX + AP5, however, the sIPSC frequency is much lower, ranging between 0.8 and 23 Hz. According to Fig. 6, the interval between two consecutive GABA<sub>IPSC</sub> events is, on average,
129.7 ms \((F = 7.71 \text{ Hz})\). Because the average decay constants of a sIPSC are \(10.49 \pm 6.84 \text{ ms}\) and \(89.51 \pm 64.32 \text{ ms}\), the majority of GABAA/IPSCs in DNQX + AP5 are separate events. As mentioned earlier, <15% of GABAA/IPSCs in DNQX + AP5 exhibit multiple peaks, and the majority of these peaks are separated by at least 45 ms. For two peaks separated by \(\leq 40 \text{ ms}\), we counted them as one GABAA/IPSC event and used the higher peak as the peak amplitude, but did not use them for averaging time constants. This selection rule undoubtedly causes errors in our measurements, but because of the low incidence of such multiple peak events in DNQX + AP5, the errors are estimated to be \(<10\%\).

Although it is difficult to make accurate measurements of sIPSC frequency in normal Ringer solution, we roughly estimated (by eye) that the frequency in normal Ringer is about two to three times higher than that in DNQX + AP5. This gives an average frequency 19.28 \((7.71 \times 2.5 \text{ Hz})\). We argue earlier in this section that the dip in the frequency-voltage relation in Fig. 11D is artificial and the sIPSC frequency near the sIPSC reversal potential is probably similar to that at other potentials. If this argument is true, then the average sIPSC frequency at the dark membrane potential of the ganglion cells \([\text{between } -60 \text{ and } -80 \text{ mV (Belgum et al. 1984; Diamond and Copenhagen 1995; Zhang et al. 1997)}]\) in normal Ringer solution will be \(\sim 20 \text{ Hz}\). From the Fig. 12, \(\text{inset}\), the average charge transfer mediated by a sIPSC at 0 mV is 1.06 pC. Therefore the total charge carried by the chloride currents gated by 20 GABAA/IPSCs is 21.8 pC, or 21.8 pA s\(^{-1}\). This gives a randomly occurring conductance increase of, on average, 21.8 pA/60 mV = 363.3 pS in the ganglion cells.

Because the dark membrane potential of the ganglion cells are very close to \(E_G\), the sIPSC-mediated random conductance increase generates small postsynaptic currents (due to small driving force). However, this conductance increase may contribute a substantial fraction of the input conductance (near 0.5–1.0 nS in most ON-OFF ganglion cells in salamander retinal slices) of the ganglion cells. Under physiological conditions in the dark, ganglion cells exhibit spontaneous action potentials (Belgum et al. 1984; Kuffler 1953), possibly mediated by spontaneous excitatory synaptic inputs (such as glutamatergic sEPSCs in Fig. 2B). The demonstration of sIPSCs in ON-OFF ganglion cells in this paper suggests that the spontaneous action potentials may be controlled not only by sEPSCs, but also by sIPSCs, which inhibit membrane depolarization by conductance shunting. A spontaneous action potential may occur when a large sEPSC is present and sIPSCs are not present.

**sIPSCs and lelEPSCs in retinal ganglion cells**

The same rule applies to lelEPSCs in ON-OFF ganglion cells. In Fig. 12, we estimated that the average peak lelEPSC (held at 0 mV) at the light onset is 509.0 ± 233.85 pA and that at the light offset is 529.0 ± 339.88 pA. This gives an average peak conductance increase at the light onset of 8.483 nS, and at the offset of 8.817 nS. The average light-evoked conductance increase mediated by the glutamatergic inputs in salamander ON-OFF ganglion cells is \(~2 \text{ nS (Mittman et al. 1990)}\). This suggests that although the lelEPSCs in these ganglion cells at the dark membrane potential are predominately excitatory (from bipolar cell glutamatergic synapses), due to the large driving force (60–80 mV), the light-evoked conductance increase mediated by the inhibitory (GABAergic and glycinergic amacrine cells) synapses is, however, more than four times higher than the excitatory conductance increase. Amacrine cells exert inhibitory actions on ganglion cells by shunting the depolarizing postsynaptic potentials generated either by spontaneous or light-evoked excitatory postsynaptic currents (sEPSCs or lelEPSCs) gated by glutamatergic vesicles from retinal bipolar cells.

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