Analysis of Excitatory and Inhibitory Spontaneous Synaptic Activity in Mouse Retinal Ganglion Cells

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Tian, Ning, Thomas N. Hwang, and David R. Copenhagen. Analysis of excitatory and inhibitory spontaneous synaptic activity in mouse retinal ganglion cells. J. Neurophysiol. 80: 1327–1340, 1998. Spontaneous inhibitory and excitatory post-synaptic currents (sIPSCs and sEPSCs) were identified and characterized with whole cell and perforated patch voltage-clamp recordings in adult mouse retinal ganglion cells. Pharmacological dissection revealed that all cells were driven by spontaneous synaptic inputs mediated by glutamate and γ-aminobutyric acid-A (GABA_A) receptors. One-half (7/14) of the cells also received glycinergic spontaneous synaptic inputs. Both GABA_A and glycine receptor–mediated sIPSCs had rise times (10–90%) of <1 ms. The decay times of the GABA_A receptor–mediated sIPSCs were comparable with those of the glycine receptor–mediated sIPSCs. The average decay time constant for monoexponentially fitted sIPSCs was 63.2 ± 74.1 ms (mean ± SD, n = 3278). Glutamate receptor–mediated sEPSCs had an average rise time of 0.50 ± 0.20 ms (n = 109) and an average monoexponential decay time constant of 5.9 ± 8.6 ms (n = 2705). Slightly more than two-thirds of the spontaneous synaptic events were monoexponential (68% for sIPSCs and 76% for sEPSCs). The remainder of the events was biexponential. The amplitudes of the spontaneous synaptic events were not correlated with rise times, suggesting that the electrotonic filtering properties of the neurons and/or differences in the spatial location of synaptic inputs could not account for the difference between the decay time constants of the glutamate and GABA_A/glycine receptor–mediated spontaneous synaptic events. The amplitudes of sEPSCs were similar to those recorded in tetrodotoxin (TTX), consistent with the events measured in control saline being the response to the release of a single quantum of transmitter. The range of the sIPSC amplitudes in control saline was wider than that recorded in TTX, consistent with some sIPSCs being evoked by presynaptic spikes having an average quantal size greater than one. The rates of sIPSCs and sEPSCs were determined under equivalent conditions by recording with perforated patch electrodes at potentials at which both types of event could be identified. Two groups of ganglion cell were observed; one group had an average sEPSCs/sIPSCs frequency ratio of 0.96 ± 0.77 (n = 28) and another group had an average ratio of 6.63 ± 0.82 (n = 7). These findings suggest that a subset of cells is driven much more strongly by excitatory synaptic inputs. We propose that this subset of cells could be OFF ganglion cells, consistent with the higher frequency of spontaneous action potentials found in OFF ganglion cells in other studies.

INTRODUCTION

The processing of visual information in retina requires an interplay between neurons relaying inhibitory and excitatory signals. The kinetics of light-evoked responses and the receptive field organization underlying center-surround and directionally selective responses in retinal ganglion cells result from interactions between excitatory glutamatergic inputs and inhibitory GABAergic and glycinergic inputs (see review by Massey and Redburn 1987). An understanding of excitatory and inhibitory signal processing at the single cell level requires knowledge of the specific synaptic connections between pre- and postsynaptic neurons, the nature of the neurotransmitters and their postsynaptic receptors, and the kinetic properties of the quantal events underlying signal transfer at each type of synapse. As an initial step in elucidating the signal processing, we sought to obtain information on the type, rate, and kinetic properties of spontaneous synaptic events in this study.

In mammalian retina, it is known that bipolar and amacrine cells, the neurons presynaptic to ganglion cells, accumulate glutamate, γ-aminobutyric acid (GABA), and glycine (Agardh et al. 1986; Chun and Wässle 1989; Kalloniatis et al. 1996; Koontz and Hendrickson 1990; Marc et al. 1990, 1995; Marc and Liu 1985; Moxon and Hendrickson 1990; Moxon and Yazulla 1985, 1987; Osborne et al. 1986; Vaney and Young 1988a,b; Wässle and Chun 1989). Immunohistochemical evidence indicates that ganglion cells express glutamate receptors as well as various subtypes of GABA_A and glycine receptors (Greferath et al. 1994a,b, 1995; Grünert and Wässle 1993, 1996; Hughes et al. 1989, 1991; Jojich and Pourcho 1996; Koulou et al. 1996; Muller et al. 1992; Pourcho and Owczarzak 1991; Qin and Pourcho 1996). Consistent with the neurotransmitter and receptor localization studies, electrophysiological recordings from isolated ganglion cells and from ganglion cells in slice preparations demonstrate that virtually all of the ganglion cells respond to exogenous glutamate, GABA_A, and glycine agonists (Cohen et al. 1989; Ishida 1992; Ishida and Cohen 1988; Röger and Grantyn 1993; Tauck et al. 1988; Zhou et al. 1994). However, recordings of spontaneous excitatory and inhibitory synaptic currents (sEPSCs and sIPSCs) from rat retinal ganglion cells reveal that amino acid–mediated synaptic inputs to every ganglion cell may not be ubiquitous, suggesting that presynaptic circuitry may provide more selective inputs to individual ganglion cells. In young rats (postnatal day 5), only 3% of the tested ganglion cells displayed sIPSCs, whereas 23% of the cells displayed sEPSCs. All cells, however, responded to exogenously applied GABA, glycine, and glutamate agonists (Röger and Grantyn 1993). In adult rats, all of the tested ganglion cells exhibited sIPSCs, but in 68% the sIPSCs were exclusively driven by GABA_A receptors, and in 16% the sIPSCs were exclusively driven by glycine receptors. These results reveal that there is a disparity be-
between presynaptic pathways inferred from drug applications and those inferred from an analysis of spontaneous synaptic inputs. We sought to determine if mouse ganglion cells displayed neurotransmitter-selective spontaneously active inputs similar to those in rat.

In rat retina, Protti et al. (1997) found that GABA_A receptor-mediated sIPSCs had shorter decay times than did glycine receptor-mediated sIPSCs. This difference is opposed to the report that in rat spinal cord GABA_A receptor-mediated sIPSCs had longer decay times than glycine sIPSCs (Yoshimura and Nishi 1995). We investigated whether glycine receptor-mediated sIPSCs in mouse retinal ganglion cells also have longer decay times as in rat, which, if true, might suggest that retina-specific isoforms of the GABA and glycine receptors might be expressed in mammals.

The integration of signals in ganglion cells depends not only on the receptor subtypes but also on the relative numbers of synaptic release sites and their activity. A comparison of the relative frequency of sEPSCs and sIPSCs provides one measure of the weighting of these inputs and gives an indication of tonically active inputs to the ganglion cells.

This comparison has not been made in a mammalian retina; measuring these rates was one of the principal goals of our study. Some of the results were presented in two abstracts (Tian et al. 1996, 1997).

**METHODS**

**Retinal slice preparation**

Retinas were obtained from adult [postnatal day (P) 30–P152] C57BL/6 mice (The Jackson Laboratory, Bar Harbor, ME). The handling and maintenance of animals and tissue preparation met the National Institute of Health guidelines and were approved by the University of California, San Francisco, Committee on Animal Research. After cervical dislocation the eyes were enucleated and hemisected at the ora serata. The slice procedures were similar to the preparation of amphibian retinal slices (Mittman et al. 1990; Tian and Slaughter 1994; Werblin 1978; Wu 1987). Briefly, the cornea, iris, lens, and vitreous were removed from an eye that was immersed in a Petri dish filled with oxygenated extracellular solution. Next, the retina was detached from the eye cup mechanically and put on a piece of Millipore filter paper (0.22 μm) with photoreceptors facing the filter paper. The retina as well as the filter paper were cut into 200 μm thick slices. Preparation of the retinal slice in this way allows better access to the ganglion cells than the more commonly utilized retinal slice preparation in which the ganglion cell surface of the retina faces the filter paper. All slices were incubated continuously in oxygenated extracellular solution. Single slices were mounted in a superfused recording chamber for each experiment and continuously perfused during the experiments. All of the procedures were performed at room temperature (21°C) and in normal room light. No light responses were recorded from these cells presumably because of the bleaching of the photopigment in the photoreceptors. Preliminary experiments done in darkness and at higher temperature revealed light-evoked activity in the ganglion cells, demonstrating that the dissection and tissue preparation did not permanently compromise the synaptic circuitry.

**Solutions and drug applications**

The extracellular solution contained (in mM) 137 NaCl, 5 KCl, 2.5 CaCl_2, 1 MgCl_2, 28 glucose, and 10 N-2-hydroxyethylpiperazine-N′-2-ethanesulfonic acid (HEPES), pH 7.4. Osmolarity was 318 mOsm. The pipette solution for perforated patch-clamp experiments contained (in mM) 120 CsCl, 5 CaCl_2, 3 MgCl_2, 20 HEPES, and 28 glucose; pH was balanced to 7.2 with the use of CsOH. Osmolarity was 314 mOsm. The pipette solution for whole cell patch-clamp experiments contained (in mM) 120 CsCl, 120 d-gluconic acid, 1 CaCl_2, 2 MgCl_2, 10 HEPES (hemisodium salt), 11 ethylene glycol-bis-(β-aminomethyl)ether)-N,N,N′,N′-tetraacetic acid (EGTA), and 30 glucose, pH 7.2. Osmolarity was 327 mOsm. The stock solution of gramicidin (gramicidin D, Dubos, Sigma, St. Louis, MO) was prepared by dissolving 25 mg gramicidin into 500 μl dimethylsulfoxide (DMSO). This solution was aliquoted and stored frozen. Each day before an experiment, 2 μl of stock solution was added to 1 ml of pipette solution. The final concentration of gramicidin in the pipette solution was 100 μg/ml (0.2% of DMSO). All of the neurotransmitter antagonists and channel blockers, such as DL-2-amino-7-phosphonoheptanoic acid (AP7), 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX), (−)-bicuculline methiodide (bicuculline), picrotoxin (PTX), and strychnine, were dissolved into the extracellular solution and bath applied by perfusion from a multibottle array to the recording chamber by a gravity superfusion system at a rate of 1 ml/min. Maximum drug effects were obtained in 40–60 s. CNQX was purchased from Tocris Cookson (Ballwin, MO). All other chemicals were obtained from Sigma (St. Louis, MO).

**Membrane current recordings**

The recording chamber was mounted on the stage of an upright microscope (Zeiss Axioskop, Carl Zeiss, Oberkochen, Germany). A ×40 water immersion objective lens was used for visualizing the retina and the recording pipettes with the use of Nomarski DIC optics. We recorded from visually identified cells in the ganglion cell layer. To bias our sampling toward ganglion cell-like neurons we correlated spiking activity and sodium currents. We found in a series of control experiments that all the cells in the ganglion cell layer (17/17) that exhibited continuous random spiking activity when we recorded in cell-attached configuration had sodium currents >200 pA when they were subsequently recorded in whole cell mode. In contrast, all of the cells (12/12) recorded from the proximal margin of the inner nuclear layer, where the majority of the amacrine cells are found, had peak sodium currents well below 200 pA and a much reduced frequency of spontaneous synaptic activity (Tian et al. 1996). The cells in the ganglion cell layer with sodium currents <200 pA, in response to steps in holding potential from −90 to −30 mV, were tentatively classified as displaced amacrine cells and were not included in this study. Although we realize that some of the cells in this study could be displaced amacrine cells with large sodium currents, we will refer to the cells as ‘‘ganglion cells’’ in the remaining text.

Patch pipettes were made of borosilicate glass (type 7052, Garner Glass, Claremont, CA) on a Brown-Flaming horizontal puller (model P-80/PC, Sutter Instruments, CA). The pipette-to-bath resistance ranged from 3 to 15 MΩ. For perforated patch clamp experiments, the electrodes were tip-filled with a small volume (300–500 μm in length) of gramicidin-free pipette solution and then backfilled with pipette solution containing gramicidin to avoid interference of gramicidin with seal formation. In the experiments, cells were held at −70 mV once the GΩ seal was formed. Access resistance and cell conductance were monitored every 5–10 min during the entire course of each experiment. Recordings began after the access resistance reached a stable plateau (≥100 MΩ), which generally took 20–30 min. Electrode capacitance was compensated by 80–90% after the GΩ seal; cell capacitance was not compensated. Membrane currents were recorded with an Axopatch 1D amplifier (Axon Instruments, Burlingame, CA), and data were collected with a Macintosh-based interface (ITC-16 Mac computer interface, Instutech, Great Neck, NY) run by HEKA software (Pulse+PulseFit, HEKA elektronik GmbH, Lambrecht, Ger-
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FIG. 1. Recording and analysis of spontaneous synaptic events. A: 1-min recording of ganglion cell membrane current at the holding potential of 0 mV with the use of a whole cell patch electrode. Recording was done in control saline. *Inset A1*: event (solid line) fitted with a biexponential curve (dashed line) and *Inset A2* shows an event (solid line) fitted with a monoexponential curve (dashed line), respectively. *Inset A3* shows an example in which one event overlaps another temporally. The amplitudes of the three insets are normalized.

B: histogram of the decay time constants of the monoexponentially fitted spontaneous synaptic events (10-min recording); binwidth is 1 ms.

C: cumulative distribution of the decay time constants as shown in Fig. 1B.

D: histograms of the amplitudes of the spontaneous synaptic events (open bars) and an all-points plot of event-free recording (filled bars) to show the baseline noise. Binwidth of the histograms is 1 pA for spontaneous synaptic events and 0.2 pA for the baseline noise. Figure 1E shows the cumulative distribution of the amplitudes of spontaneous synaptic event amplitudes.

Data analysis

Off-line data analysis was carried out on a Macintosh Power PC computer using Igor (WaveMetrics, Lake Oswego, OR). We wrote a series of macro programs to detect and analyze the spontaneous synaptic events. This program makes two passes through a continuous current recording to detect and analyze events. During the first pass, it uses the first derivative and the second derivative to locate local maxima and local minima. For outward events, each local maximum is the peak of a putative event, whose start point is identified as the local minimum immediately preceding the peak. The approximate amplitude of that event is the difference between the current at the start and peak. All events <4 pA (or 5 pA in the rise-time analysis) are ignored in the first pass. During the second pass, the program averages the current in the 10 ms preceding each start point to more accurately determine the baseline and then recalculates the event amplitude. During the decay phase of an event, the program calculates the “effective” end of the event by detecting when it decays to the baseline value. Events that last <5 ms or longer than 1,000 ms as well as overlapping events are automatically discarded.

The decay phase of each of the remaining events is fitted with a monoexponential and/or a biexponential curve. The macro program uses the $R^2$ and the $F$ statistic as measures of goodness-of-fit. It uses the adjusted $R^2$ statistic, which takes into account the degrees of freedom, to compare whether a biexponential fit is statistically better than a monoexponential fit (Glantz and Slinker 1990). Events that do not fit well ($R^2 < 50\%$ or $P > 0.01$ for $F$ test) are discarded. Finally, the rise time is defined as the time required to travel from 10 to 90% of the amplitude on the rising phase of the event. Figure 1 shows an example of the data analysis. Figure 1A shows a 1-min recording of membrane current at the holding potential of 0 mV with a whole cell patch electrode. At this voltage, spontaneous synaptic events were presumably inhibitory neurotransmitter-mediated sIPSCs because the driving force for excitatory synaptic current was zero (see RESULTS). Figure 1A1 is an example of an event fitted with a biexponential decay (dashed line), and Fig. 1A2 is an event fitted with a monoexponential decay. Spontaneous synaptic events that overlapped temporally were utilized for the calculation of event frequency but were disregarded for amplitude, rising time, and decay time constant calculations. Figure 1A3 shows an example of overlapping events; Fig. 1B shows the histogram of the decay time constants from a monoexponential fitting of every event. These decay time constants follow a unitary distribution with a mode value of 21 ms. The mean ± SD was 29 ± 25 ms ($n = 274$). Figure 1C shows the cumulative distribution of the decay time constants as in Fig. 1B.

Cumulative distributions minimize any distortions of histograms resulting from binwidth selection. The dashed line indicates the median of the decay time constants, which was 22 ms for this cell. Figure 1D shows the histograms of the amplitudes of the spontaneous synaptic events (open bars) and the all-points plot of event-free recording (filled bars, 2 s, 4,000 points). The binwidth of the histogram was 1 pA for synaptic events and 0.2 pA for the baseline noise. Figure 1E shows the cumulative distribution of the amplitudes from Fig. 1D.

The Kolmogorov-Smirnov (K-S) test was used to quantify the statistical significance of the differences among the distributions of spontaneous synaptic event amplitudes or decay time constants. This test does not require one to assume that the data being analyzed are normally distributed (Press et al. 1986; Van der Kloot 1991) and was used previously for the same purposes (Cohen et al. 1992; Manabe et al. 1992; Otis et al. 1991). The Student’s $t$-test was used to examine the difference between two means. These two tests were carried out with the software StatView (Abacus Concepts, Berkeley, CA).

RESULTS

Identification and characterization of both GABA$_A$ and glycine receptor–mediated sIPSCs in ganglion cells

Previous pharmacological studies using exogenously applied agonists showed that virtually every mammalian retinal
ganglion cell that was tested expressed functional GABA_A and glycine receptors (Cohen et al. 1994; Tauc et al. 1988; Zhou et al. 1994). However, Protti et al. (1997), who recorded sIPSCs in rat retina, demonstrated that some ganglion cells may exhibit spontaneous events mediated by only one type of inhibitory receptor. This suggests that, in terms of identifying the tonic spontaneous inputs, and possibly even the light-evoked synaptic inputs, an analysis of spontaneous events can be informative and provide a rudimentary idea of the likely synaptic pathways driving ganglion cells. Accordingly, we recorded and characterized sIPSCs under voltage clamp with whole cell patch pipettes held at 0 mV, the reversal potential for excitatory synaptic inputs. Bicuculline and strychnine were used to antagonize sIPSCs mediated by GABA_A and glycine receptors, respectively. Figure 2 shows an example of a ganglion cell that exhibited both GABA_A and glycine receptor-mediated sIPSCs. Figure 2A shows 60-s segments of membrane current recorded from a ganglion cell in control saline, during bicuculline, and during bicuculline plus strychnine application. Bath application of 50 μM bicuculline decreased the frequency of sIPSCs larger than 4 pA from 25 to 15 events/min (sample length = 10 min). Bath application of 50 μM bicuculline with 1 μM strychnine completely blocked the sIPSCs. Figure 2B shows 60-s segments of membrane current recorded from another ganglion cell in which the order of antagonist application was reversed. The frequency of sIPSCs was 37 events/min in control and decreased to 7 events/min during 500 nM strychnine application (sample length = 10 min). Bath application of 500 nM strychnine with 50 μM bicuculline completely blocked the sIPSCs. These results indicate that both GABA_A and glycine receptors can directly generate sIPSCs in mouse ganglion cells.

However, of the 14 ganglion cells tested, only 7 cells required both bicuculline and strychnine to completely eliminate the sIPSCs. In the other seven cells, bicuculline alone completely blocked the sIPSCs. Strychnine alone could not completely eliminate the sIPSCs in any of the cells. Therefore approximately one-half of the ganglion cells received exclusively GABA_A-mediated sIPSCs, and the other one-half received both GABA_A and glycine-mediated inputs. In contrast, rat retina had a larger percentage (25/37) of ganglion cells that received exclusively GABA_A-mediated synaptic inputs and a smaller percentage (6/37) of cells that received both GABAergic and glycineric synaptic inputs (Protti et al. 1997).

We quantified the kinetics of the sIPSCs by fitting the decay phase of each individual sIPSC with both a monoexponential and a biexponential curve. A biexponential fit was significantly better than a monoexponential fit in only 32% of the events and provided no significant improvement in the remaining 68% of the events (n = 3,907, 15 cells) (see METHODS for details of fitting). The average decay time constant of all sIPSCs fitted with a monoexponential curve was 92.4 ± 13.5 ms (n = 2,661) with a mode value of 21 ms. For biexponential events, the average fast decay time constant was 19 ± 4.5 ms (n = 1,246) with a mode value of 3 ms, and the average slow decay time constant was 137.5 ± 135.9 ms with a mode value of 36 ms. When the average sIPSC waveforms, rather than individual events, were fitted, 14 of 15 cells were better fitted with a biexponential. The significance of why some events were better fitted by biexponential decays is not readily evident. Biexponential decays could reflect more complex channel opening kinetics, or they might reflect differences in the diffusion of neurotransmitter from the cleft. Interestingly, a greater proportion...
of the events fitted with biexponentials had larger amplitudes. Figure 2C plots the cumulative distribution curves of the amplitudes of the mono- and biexponential fitted events. The K-S test indicated that the difference between these two distributions was highly significant ($P = 0.0001$).

We tested whether the properties of GABA$_A$ and glycine receptor–mediated sIPSCs were different by comparing the amplitudes and decay time constants of monoexponentially fitted sIPSCs recorded in either control or during strychnine. Figure 2D shows the cumulative distribution curves of the amplitudes of the sIPSCs recorded in control and during strychnine application. Although the frequency of sIPSCs was decreased by strychnine, demonstrating the glycinergic input to this cell, these two curves overlapped considerably. The K-S test indicates that the difference between these two distributions is not statistically significant ($P = 0.22$), consistent with glycine and GABA$_A$ receptor–mediated sIPSCs having similar amplitudes. Moreover, the difference between the average amplitude in control (6.4 ± 2.2 pA, 10 min, $n = 302$) and in strychnine (6.5 ± 2.7 pA, 10 min, $n = 71$) is not significantly statistically ($P > 0.05$). In terms of kinetics, a previous study in adult rat retina reported that GABA$_A$ receptor–mediated events had shorter decay time constants than those of glycine receptor–mediated events (Protti et al. 1997). However, our data showed that the two classes of events had similar decay times. Figure 2E shows the cumulative distribution curves of the decay time constants of the sIPSCs recorded in control and during strychnine application. These two cumulative distribution curves again appear to overlap each other. The K-S test indicated that the difference between these two distribution curves was not significant ($P = 0.34$). We also tested whether the monoexponential or biexponential fitting pattern reflected a preponderance of either GABA$_A$ or glycine receptor–mediated events. Figure 2F shows that the difference between the percentage of monoexponential events in control (60%, $n = 1832$, 7 cells) and in strychnine (58%, $n = 362$) was not significant ($P > 0.1$). We interpret this finding as evidence that comparable percentages of both glycinergic and GABAergic events were monoexponential. These results also reveal that the GABA$_A$ and glycine receptor–mediated sIPSCs have similar kinetic properties. The similarity of the kinetic properties of GABA$_A$ and glycine receptor–mediated sIPSCs most likely reflects the similarity of GABA$_A$ and glycine receptor single channel kinetic properties (Bormann et al. 1987).

Comparison of sIPSC and sEPSC kinetics

Previous studies have shown that mammalian retinal ganglion cells express ionotropic glutamate receptors and that these receptors can mediate spontaneous synaptic events (Cohen et al. 1994; Rörg and Grantly 1993; Tauck et al. 1988; Zhou et al. 1994). In vertebrate retina, as well as in other neural systems, glutamate receptor–mediated sEPSCs have much faster decay time constants than those of GABA$_A$ receptor–mediated sIPSCs (Edwards et al. 1990; Jonas et al. 1993; Rörg and Grantly 1993; Salin and Prince 1996; Stern et al. 1992; Taylor et al. 1995). We sought to determine the kinetics of sEPSCs and then investigate whether the sEPSCs and sIPSCs in mouse retinal ganglion cells could be readily distinguished by their decay time constants. Figure 3 shows membrane currents recorded with the use of a whole cell patch pipette at holding potentials of 0, −30, and −70 mV. $E_{Cl}$ was adjusted to −70 mV and $E_{static}$ was 0 mV. Under these conditions, sEPSCs and sIPSCs were well differentiated by their reversal potentials. At 0 mV (Fig. 3, A and B, top traces), only slower outward spontaneous synaptic currents were observed (traces in Fig. 3B were on expanded time scale). At −30 mV (Fig. 3, A and B, middle traces), both slower outward-going current events and faster inward-going current events were observed. At −70 mV (Fig. 3, A and B, bottom traces) $E_{Cl}$ for this cell, only the faster inward-going events, which we postulate were the sEPSCs, were observed. The slower outward-going events were eliminated by the chloride channel blocker PTX, whereas the faster inward-going events were eliminated by the glutamate receptor antagonists CNQX and AP7 (data not shown).

The decay time courses of the events were quantified to determine how well the faster sEPSCs and slower sIPSCs could be reliably distinguished from one another kinetically. As with the sIPSCs, the decay phase of each sEPSC was also fitted with both a monoexponential and a biexponential curve. We found that a biexponential curve described the decay of only 24% of the sEPSCs, significantly better than a monoexponential curve, and provided no significant improvement in the remaining 76% of the events ($n = 2,660$, 15 cells). The average decay time constant of the monoexponential fits was $14.3 ± 32.3$ ms ($n = 629$) with a mode value of 2 ms. The average fast decay time constant of the biexponential fits was $2.4 ± 4.6$ ms ($n = 629$) with a mode value of 2 ms, and the average slow decay time constant was $37.2 ± 47.1$ ms with a mode value of 17 ms. Similar to the sIPSCs, when the averaged sEPSC waveform were fitted, 13 of 15 cells were better fitted with biexponential curves. However, the relative percentage of monoexponential versus biexponential sEPSCs was not related to the amplitude of the events ($P = 0.29$).

One goal of this study was to determine how well sEPSCs could be distinguished from sIPSCs on the basis of the decay kinetics. The distinction of sEPSCs and sIPSCs based on their kinetics alone will be useful under conditions where one wants to compare the relative frequency of spontaneous synaptic events but does not want to alter the membrane potential or the receptor activity on presynaptic neurons with antagonists. To facilitate comparisons, we fitted all of the sIPSCs and sEPSCs with a monoexponential curve, although 24–32% of the events were biexponential. Figure 3C shows the histograms of the decay time constants of the sEPSCs and sIPSCs recorded at the holding potentials of −70 mV (filled bars) and 0 mV (open bars) from a ganglion cell, respectively. The mode value of decay time constants of the sEPSCs was 2 ms (4.3 ± 8.4 ms, $n = 196$). The mode value of decay time constants of the sIPSCs was 21 ms (29 ± 24.6 ms, $n = 274$). These histograms show a clear demarcation between the decay time constants of sEPSCs and sIPSCs at 6 ms. When 6 ms was used as a ‘‘cutoff’’ decay time constant, only 24 of 196 (12%) sEPSCs had decay time constants <6 ms, and 2 of 274 (0.7%) sIPSCs had decay time constants <6 ms. Figure 3D shows the cumulative distribution curves of the decay time constants of the sEPSCs and sIPSCs. The difference between
FIG. 3. Spontaneous excitatory post-synaptic currents (sEPSCs) and sIPSCs have different decay time constants. A: membrane currents recorded with whole cell patch electrode at holding potentials of 0 mV (top trace), −30 mV (middle trace), and −70 mV (bottom trace). B: overlapped segments of membrane current recordings as in A plotted in expanded time scale to show the decay time courses of glutamate and GABA$_A$/glycine receptor–mediated spontaneous synaptic events. C and D: histograms and cumulative distribution curves of the decay time constants of the sEPSCs recorded at −70 mV holding potential and sIPSCs recorded at 0 mV holding potential from the same cell as for A and B. E and F: histograms and cumulative distribution curves of the decay time constants of the sEPSCs and sIPSCs summarized from 15 cells. Binwidth in C and E is 1 ms.

these two distributions was highly significant statistically (P = 0.0001).

Figure 3E shows the histograms of the decay time constants of sEPSCs (filled bars) and sIPSCs (open bars) summarized from 15 cells. The x-axis was truncated to 100 ms to emphasize the demarcation of the decay time constants of sEPSCs and sIPSCs. The mode value of decay time constants of the sEPSCs was 2 ms (median = 3.2 ms, 5.9 ± 8.6 ms, n = 2,705). The mean decay time constant (5.9 ms) was a little longer than the decay time constants of glutamate receptor–mediated sEPSCs reported from young rat retinal ganglion cells (2 ms) (Rörg and Grantyn 1993), salamander retinal ganglion cells (3.75 ms) (Taylor et al. 1995), neurons in rat primary visual cortex (2.39 ms) (Stern et al. 1992), and rat CNS neurons (4.8 ms) (Jonas et al. 1993). By comparison, the mode value of decay time constants of the sIPSCs was 12 ms (median = 37.4 ms, 63.2 ± 74.1 ms, n = 3,278). This mean decay time constant (63.2 ms) was also longer than the slow time constants of GABA receptor-activated elementary conductance recorded from goldfish retinal ganglion cells (30 ms) (Ishida and Cohen 1988) and the decay time constants of GABA$_A$ receptor–mediated sIPSCs in young rat retinal ganglion cells (22.7 ms) (Rörg and Grantyn 1993). A clear demarcation between the decay time constant histograms of summarized sEPSCs and sIPSCs was evident at 5 ms. When 5 ms was used as a cutoff decay time constant, 31% of the sEPSCs were longer than the cutoff value, and 2.1% of the sIPSCs were shorter than the cutoff. However, if the cutoff decay time constant was at 8 ms, only 18% of the sEPSCs were out of the range and 5.5% of the sIPSCs were faster than the cutoff. These results indicate that a highly significant number of the events >8 ms would be GABA/glycine receptor–mediated sIPSCs. Similarly most of events <8 ms would be glutamate receptor–mediated sEPSCs. Figure 3F plots the cumulative distribution curves of the decay time constants of the summarized sEPSCs and sIPSCs. The difference between these two distributions proved to be highly significant (P < 0.001). On this basis we felt ourselves justified with the use of monoexponential fits to differentiate sEPSCs from sIPSCs.

Recording of sEPSCs and sIPSCs at −70 mV

One of our principal goals of this study was to compare the relative frequency of excitatory and inhibitory spontaneous synaptic inputs. A simple comparison of the rates obtained at $E_C$ versus those at $E_{cation}$, however, proved to be misleading. Our own preliminary evidence and that reported by Gao et al. (1997) showed that the frequency of ganglion cell sIPSCs increased with depolarization of the holding potential in the recorded cells. This effect might be caused by electronic coupling from ganglion cells to amacrine cells (Dacey and Brace 1992; Jacoby et al. 1996; Penn et al. 1994; Xin and Bloomfield 1997). Nonetheless, this effect obscures
brane current recorded at a holding potential of ±70 mV synaptic events with decay time constants ±70 mV in control and during application of GABA A /glycine receptor antagonists. Figure 4A shows ganglion cell membrane current recorded at a holding potential of ±70 mV in control, during application of glutamate receptor antagonists, and finally during application of both glutamate and GABA A /glycine receptor antagonists. In control, both fast and slow events were recorded. As mentioned earlier, the decays of all events were fitted with a monoexponential curve for comparison purposes. There were 299 fast events and 58 slow events identified in a 5-min recording with 7 ms as the demarcation time. The mean decay time constant was 2.1 ± 0.9 ms for fast events and 29.2 ± 39.7 ms for slow events. Blockage of the glutamate receptor–mediated events were selectively blocked by GABA A /glycine receptor antagonists completely blocked all of the spontaneous synaptic events. The slow spontaneous synaptic events were distinguishable with perforated patch recordings. A: 1-min recordings of membrane current from a ganglion cell using a gramicidin perforated patch electrode at a holding potential of ~70 mV in control, during DL-2-amino-7-phosphonoheptanoic acid (AP7) and 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX) application, and during CNQX, AP7, strychnine, and picrotoxin (PTX) application. B: 1-min recordings of membrane current from another ganglion cell at a holding potential of ~70 mV in control and during strychnine and PTX. C: cumulative distribution curves of monoexponentially fitted decay time constants of spontaneous synaptic events in control (5-min recording, n = 357) and during CNQX and AP7 application (2 min, n = 8). D: cumulative distribution curves of decay time constants of the spontaneous synaptic events in control (10-min recording, n = 62) and during strychnine and PTX (5-min recording, n = 16).
have a high ratio (5.5±8, 6.63 ± 0.82, n = 7). Therefore for most cells the ratio of glutamate to GABA/glycine synaptic events is ~1, but for 20% of the cells, the rate of glutamate-mediated events is much higher.

Effects of TTX on sIPSCs and sEPSCs

TTX reduced the frequency of sIPSCs, which suggests that some of the events result from action potential-mediated release of neurotransmitter in the presynaptic neurons, confirming the results of Protti et al. (1997). The remaining TTX-insensitive miniature IPSCs (mIPSCs) most likely represent the events caused by the release of a single quantum of transmitter. We sought to determine the quantal content of the spike-evoked sIPSCs. In rat hippocampus the quantal content varies from 3 to 10 (Edwards et al. 1990; Raastad et al. 1992). We compared the amplitude distribution of sIPSCs recorded in control with that of mIPSCs recorded during TTX and Cd application. Figure 6A shows the cumulative distribution curves of the amplitudes of the sIPSCs recorded in control and the amplitudes of the mIPSCs recorded during 1 μM TTX and 50 μM Cd application. These data summarize the results from eight ganglion cells. It can be seen that a greater number of larger events are observed in control saline with the largest event of 47.2 pA, whereas the largest event in the presence of TTX and Cd is 15.4 pA. The mean sIPSCs amplitude is 7.3 ± 4.5 pA (n = 799), and the mean mIPSCs amplitude is 5.9 ± 1.9 pA (n = 365).
The K-S test indicates that the difference between these two distributions was highly significant ($P = 0.007$). The average quantal content is 1.2. Although it is difficult to determine rigorously a number for quantal content because both sIPSCs and mIPSCs were included in the control cases, these results indicate that the quantal content of many individual events is certainly greater than 1 and varies in range from 1 to 8, which is close to the 3–10 range found in hippocampus.

Similar analysis of sEPSCs and mEPSCs showed that TTX and Cd made no difference on the event amplitudes. Figure 6B shows the cumulative distribution curves of the amplitudes of the sEPSCs recorded in control and the mEPSCs recorded during application of 1 $\mu$M TTX and 50 $\mu$M Cd from the same eight ganglion cells as in Fig. 6A. The mean amplitude was 6.1 ± 2.5 pA ($n = 613$) for sEPSCs and 5.9 ± 1.8 pA ($n = 339$) for mEPSCs. The difference between these two distributions was not significant statistically ($P = 0.191$). In hippocampus a similar finding was interpreted as evidence that each action potential releases, on average, a single vesicle containing glutamate (Raastad et al. 1992). Because the bipolar cells that release glutamate in retina are nonspiking neurons, we have to interpret our findings somewhat differently. Basically, our results confirm the idea that there is no functional difference between an sEPSC and an mEPSC. To a first-order approximation, every sEPSC event measured at the ganglion cell represents the release of a single quantum of transmitter. Although we cannot rigorously exclude the possibility that more than one vesicle is released per quantal event, it is clear that none of these events is triggered by an action potential.

Decay time constants of neither sEPSCs nor sIPSCs were affected by membrane filtering

In the foregoing sections the differences in the decay time constants of events were used to distinguish sEPSCs from sIPSCs. To rule out the likelihood that electrotonic filtering-induced artifacts produced different decay times, we examined the correlations between amplitudes and rise times and between the amplitudes and decay time constants of the larger spontaneous synaptic events (>5 pA) (Rall 1969). One will expect the more electrotonically distant inputs to be attenuated and also broadened by dendritic conduction; therefore the rise time should increase with decreasing amplitude. Figure 7 shows histograms of 10–90% rise times of sEPSCs (Fig. 7A) and sIPSCs (Fig. 7B). The sEPSCs and sIPSCs were recorded at holding potentials of −70 and 0 mV with whole cell patch electrodes, respectively. It can be seen that both of the 10–90% rise-time distributions were unimodal. The mode value of 10–90% rise times of sEPSCs was 0.7 ms with a mean of 0.64 ± 0.27 ms ($n = 116$, 3 cells). This value was slightly shorter than the mean rise time of sEPSCs obtained from neonatal rat retinal ganglion cells (0.93 ms) (Rööig and Grantyn 1993) and tiger salamander retinal ganglion cells (1.3 ms) (Taylor et al. 1995) but similar to the rise time reported by Jonas et al. (1993) for rat CNS neurons (0.6 ms). The mode value of the rise times of sIPSCs was 0.8 ms with a mean of 0.80 ± 0.28 ms ($n = 81$, 3 cells). This was longer than the 20–80% rise times of sIPSCs obtained from rat CNS neurons (0.2 ms) (Stern et al. 1992) but similar to the 10–90% rise times obtained from adult rat somatosensory neurons (0.9 ms) (Salin and Prince 1996). These recordings (Fig. 7, A and B) also showed that the difference between the means of the rise times of sEPSCs and sIPSCs was not significant statistically ($P > 0.05$). In addition, the 10–90% rise times of sEPSCs and sIPSCs were plotted against their peak amplitudes as shown in Fig. 7, C and D, respectively. These plots were fitted with a least-square, linear-regression line, and the correlation coefficients were 0.12 for sEPSCs and 0.103 for sIPSCs. These results indicate that the rise times of the spontaneous synaptic events were not amplitude dependent. The decay time constants of sEPSCs and sIPSCs were also plotted against their amplitudes in Fig. 7, E and F. Linear regression yielded correlation coefficients of 0.035 and 0.074. In summary, the analysis indicates that the rise times were no longer than 1 ms. Because we could easily see events with rise time <1 ms, these results provide strong evidence that the much longer decay time constants of the spontaneous synaptic events were not significantly affected by the electrotonic filtering of the cells.

Because the increased access resistance of perforated patch electrodes could distort the recorded kinetics of the spontaneous synaptic events, we also examined the correlation between amplitudes and rise times and the correlation between the amplitudes and decay time constants of spontaneous synaptic events recorded with these electrodes. Figure 8, A and B, shows histograms of 10–90% rise times of sEPSCs and sIPSCs, respectively. The sEPSCs and sIPSCs were separated by a demarcation decay time constant at 5 ms in this cell. It can be seen that both of the distributions of 10–90% rise times were unimodal. The mode value of the 10–90% rise times of sEPSCs was 0.4 ms (0.50 ± 0.20 ms, $n = 109$). The mode value of the rise times of sIPSCs was 0.6 ms (0.54 ± 0.24 ms, $n = 42$). Figure 8C shows the plot of 10–90% rise times of the spontaneous synaptic events (both sEPSCs and sIPSCs) against their peak amplitudes. The correlation coefficient of the least-square, linear-regression fitting was −0.03. The decay time constants of spontaneous synaptic events were still easily discernible in the perforated patch measurements and that the higher access resistance of the perforated patch electrodes did not obscure the kinetic difference between the sIPSCs and sEPSCs.

D I S C U S S I O N

A principal goal of this study was to determine the relative weighting of spontaneous synaptic inputs in mouse retinal ganglion cells mediated by excitatory neurotransmitters (glutamate and acetylcholine) and inhibitory neurotransmitters (GABA and glycine). Whole cell recording at $E_{Cl}$, the imposed reversal potential for chloride, revealed faster decaying events that were eliminated by ionotropic glutamate receptor antagonists (Fig. 3). Whole cell recording at 0 mV, the reversal potential for excitatory events, revealed slower decaying events that were eliminated by GABA$_A$/glycine receptor antagonists (Figs. 2 and 3). These findings indicate
that glutamatergic, glycinergic, and GABAergic presynaptic neurons provide inputs to the ganglion cells. All sIPSCs and sEPSCs were blocked by a combination of glutamate and GABA<sub>A</sub>/glycine receptor antagonists, indicating that these ganglion cell did not receive spontaneous synaptic inputs from cholinergic presynaptic neurons. Perforated patch recordings demonstrated that the sEPSCs and sIPSCs could be readily distinguished on the basis of their different decay times at the same holding potential. This allowed a pharmacologically and voltage-independent method of comparing the frequency of the spontaneous synaptic inputs from glutamatergic and GABAergic/glycinergic presynaptic neurons. A major group of ganglion cells exhibited sEPSC:sIPSC frequency ratios of ~1. A smaller group had ratios of sEPSC:sIPSC ~7.

**Characteristics of sIPSCs in rodent retinal ganglion cells**

In previous studies of adult mammalian retina ganglion cells, direct application of exogenous neurotransmitter agonists revealed that virtually every cell expresses functional glutamate, GABA<sub>A</sub>, and glycine receptors (Cohen et al. 1989; Ishida 1992; Ishida and Cohen 1988; Rörg and Grantyn 1993; Tauck et al. 1988; Zhou et al. 1994). Interestingly, analyses of spontaneous synaptic events of retinal ganglion cells, which presumably reflect the release of neurotransmitters onto receptors at their synaptic sites and not the random activation of individual receptors by residual agonists in the extracellular fluid, demonstrated much more selective activation patterns. We found that all the mouse retinal ganglion cells had both GABA<sub>A</sub> and glutamate receptor–mediated spontaneous synaptic events. However, only one-half (7/14 cells tested) had glycine receptor–mediated spontaneous synaptic events. By comparison, in rat retina, Protti et al. (1997) found that 6 of 37 cells had sIPSCs mediated exclusively by glycine receptors. Twenty-five had GABA<sub>A</sub> receptor–mediated sIPSCs exclusively, and only 6 of 37 cells had both GABA<sub>A</sub> and glycine receptor–mediated events. The reasons behind the mouse/rat differences are unclear. Further studies in rodent and other mammalian retina should provide insight as to whether these differences reflect variations in the selectivity by which presynaptic neurons contact the ganglion cells or simply variations in the spontaneous release of specific types of presynaptic neurons. In either case, excitation, inhibition, and likely light-evoked responses will be affected.

Differences between the decay times of sIPSCs in mouse and rat suggest differences in the subunit composition of GABA<sub>A</sub> and glycine receptors in these two species. We found that the decay times of GABA<sub>A</sub> and glycine receptor–mediated events were very similar, and comparable percentages of the events were well fitted with a monoexponential versus biexponential curve. In rat retina, most sIPSCs were reportedly best fitted with biexponential curves (Protti et al. 1997); the average of the slower component of rat glycine sIPSCs was much longer than that of GABA<sub>A</sub> events (147 vs. 40.3 ms). Because glycine receptor–mediated events were longer than GABA<sub>A</sub> receptor–mediated events in rat retina (opposite to the case in rat spinal cord), it was conceivable that this reflected a retinal-specific expression pattern of slower glycine receptor channels. Given that mouse retinal GABA/glycine receptor–mediated events have simi-
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mediated synaptic activity of retinal ganglion cells increases with age. In young rat retina (P5) only 3% of the ganglion cells displayed sIPSCs, whereas 23% of the cells displayed sEPSCs (Rörig and Grantyn 1993). In adult rat (Protti et al. 1997) and mouse (shown here) sIPSCs were observed in every cell. Our own preliminary data (Tian et al. 1997) in mouse showed that the frequency of sIPSCs increases from neonatal to adult ages.

Characteristics of sEPSCs

sEPSCs that are eliminated by ionotropic glutamate receptor antagonists are observed across ages from P5 to adult in rodent retina (Rörig and Grantyn 1993; Protti et al. 1997) and in tiger salamander retinal ganglion cells (Taylor et al. 1995). These events have recorded rise times of ~1 ms and monoexponentially fitted decay times averaging between 2 and 4.8 ms. The sEPSCs recorded from mouse retina in our study were similar to those recorded from rat ganglion cells but were shorter than those from tiger salamander retina and rat CNS neurons (Jonas et al. 1993; Stern et al. 1992; Taschenberger et al. 1995; Taylor et al. 1995).

In all the retina studies, sEPSCs had the decay times consistent with activation of \( \alpha \)-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) rather than \( N \)-methyl-\( D \)-aspartate (NMDA) receptors that are known to have much longer decay times (Barnes-Davies and Forsythe 1995; Taschenberger et al. 1995). In tiger salamander, extensive efforts were made to unmask an NMDA component of the sEPSCs because the light responses exhibit a prominent NMDA component (Taylor et al. 1995). The absence of an NMDA component in the tiger salamander (Taylor et al. 1995) sEPSCs led to the suggestion that AMPA and NMDA receptors were not colocalized adjacent to the presynaptic release site. In a similar manner, rat retinal ganglion cells express agonist-evoked NMDA responses (Taschenberger et al. 1995), yet show no obvious NMDA receptor–mediated spontaneous synaptic events recorded from cells synaptically coupled in culture. This suggests that AMPA and NMDA receptors may be similarly separated at postsynaptic sites and there is no discernible spontaneous release of glutamate at NMDA sites in mouse and rat retinal ganglion cells, as hypothesized for tiger salamander retina.

Relative frequency of sEPSCs and sIPSCs

The relative responsiveness of ganglion cells to light-evoked excitatory and inhibitory inputs will reflect the weighting of selective synaptic inputs. The temporal patterns of light responses and the receptive field patterns result in part from interaction between the excitatory and inhibitory presynaptic neurons that have their own receptive field structures. To date, different approaches yielded somewhat different conclusions as to the weighting of excitatory and inhibitory inputs. Virtually all ganglion cells appear to express glutamate, glycine, and GABA\( \_A \) receptors that can be activated by exogenous agonist application (Cohen et al. 1989; Ishida 1992; Ishida and Cohen 1988; Protti et al. 1997; Tauck et al. 1988; Zhou et al. 1994). On the basis of these findings, one would conclude that all ganglion cells receive synaptic inputs from all three classes of neuron. This conclusion is
at odds with the results from the analysis of sIPSCs, which shows that cells are selectively activated by either GABA or glycine inputs (Protti et al. 1997). The next important step in elucidating the retinal circuitry will be to determine whether the light-evoked responses are mediated by all of the three classes of amino acid neurotransmitters or whether they are mediated by a subset of these receptors, similar to that of the spontaneous inputs.

Although one can isolate and identify sIPSCs and sEPSCs with the use of voltage-clamp approaches, two additional obstacles arise when trying to determine the relative frequency of these events. The rate of sIPSCs is voltage dependent; depolarization increases the rate of sIPSCs as mentioned in the RESULTS. Pharmacological agents block not only neurotransmitter receptors on the ganglion cells but similar receptors on amacrine cells. Thus using antagonists to isolate relative weighting of synaptic inputs can be misleading. Because of these problems, we adapted perforated-patch techniques that enabled us to quantify the rate of sIPSCs and sEPSCs without altering the holding potential or using antagonists.

This method yields the least error-prone approach to identifying and characterizing the relative weight of spontaneous synaptic inputs to individual ganglion cells. With this approach we found all ganglion cells were driven by GABA\(_\text{A}\) and glutamate (probably AMPA) receptors. Roughly one-half was also driven by glycinegic neurons. We also found that there were two distinct classes of cells, one with comparable rates of sIPSCs and sEPSCs and one with a much higher ratio of sEPSCs to sIPSCs. We hypothesize that the group of cells having a higher ratio of sEPSCs to sIPSCs are OFF-type cells. This conjecture is based on two correlations. First, the group of cells with higher sEPSCs/sIPSCs ratios comprises ~20% of the cells tested, which is the percentage of OFF ganglion cells identified in whole-mount mouse retina with extracellular recordings (Nirenberg and Meister 1997). Second, in ferret retina, Wong and Oakley (1996) showed that after P21, the OFF cells have a much higher rate of spontaneous action potentials than do the ON cells. This spontaneous action potential activity of OFF cells presumably reflects an increased ratio of excitatory to inhibitory synaptic inputs. Further study incorporating physiological and immunohistochemical identification of mouse ganglion cells and the recording of spontaneous action potential rate will be used to test this hypothesis directly.

The accuracy of our measurements of the relative frequency of sEPSCs and sIPSCs is affected by some methodological limitations in this study. It is known that the frequency and the decay kinetics of spontaneous synaptic events of mammalian CNS neurons are temperature dependent (Otis and Mody 1992). One would expect that the optimum temperature to assess physiological in vivo parameters would be at normal body temperature. However, we did all the experiments at room temperature for three principal reasons. First, the only two previous studies of mammalian retinal ganglion cell spontaneous synaptic activity were done at room temperature (Protti et al. 1997; Röig and Grantyn 1993). We felt that it was simply more convenient to perform our experiments at the same temperature to compare our results with the previous ones. Second, at higher temperature, the correspondingly higher rate of spontaneous synaptic activity makes it more difficult to accurately isolate individual events. Overlap of the spontaneous synaptic events makes the fitting of the decay phase of the events difficult and the identification of the events by their kinetic properties more uncertain. Finally, although the decay time constant of spontaneous synaptic events will be shorter at higher temperature, the magnitude of the change in decay time constant as estimated from Otis and Mody’s (1992) study should not obscure our ability to distinguish sEPSCs from sIPSCs by their decay kinetics. Moreover, we found no published evidence to suggest that a change in temperature will preferentially affect excitatory or inhibitory spontaneous synaptic activity.

Another potential limitation of this study is that the dendritic branches of the ganglion cells are not likely to be fully intact in the slice preparation. For each individual cell, the ratio of excitatory to inhibitory synaptic inputs represents the glutamate and GABA/glycine inputs from the remaining dendritic branches. Most of the cells in this study had their somata located close to the cut surface. Assuming the dendritic trees are symmetric, approximately one-half of the dendritic branches would remain. If the excitatory and inhibitory synaptic inputs are distributed uniformly, cutting off one-half of the dendritic branches close to the soma will decrease the number of synaptic inputs by 50% but will not change the ratio of excitatory to inhibitory very much. For the cells with asymmetric dendritic branches, the relationship between our measured ratios and the in vivo measurement will be more complicated and more difficult to predict. A previous study showed that mouse retinal ganglion cells have both symmetric and asymmetric dendritic trees and the diameters of their dendritic trees are <200 μm (Doi et al. 1995). Nonetheless, when the ratios of the excitatory to inhibitory synaptic inputs are evaluated as a group of cells, the effects of slicing on the spatial distribution on the ratios of excitatory to inhibitory synaptic inputs should be reduced because the slices were cut in a random fashion. However, the potential problem of distorting results by the cutting of the dendritic trees of the cells in our preparation is one that must be kept in mind.

**Elementary events**

Analysis of the amplitude distributions of sIPSCs in control and during TTX enabled us to estimate the quantal content of spike-induced synaptic events. Figure 6 showed that in control more of the larger amplitude events were observed. The difference in the distribution of amplitudes in control and during TTX was highly significant (\(P = 0.001\)). The mean amplitude of sIPSCs recorded in eight cells was 7.3 pA in control and 5.9 pA in TTX. These findings indicate that presynaptic spikes can cause the release of >1 quanta of neurotransmitter. In control the larger sIPSCs (amplitude >18 pA) could be accounted for by the synchronous release of three to eight vesicles of transmitter. This quantal content is close to the 3–10 range for the quantal content estimated for dentate gyrus cells in hippocampus (Edwards et al. 1990). There was no significant difference between the amplitude distributions of sEPSCs recorded in control and in TTX (Fig. 6). This is to be expected given that the bipolar cells, the glutamatergic neurons presynaptic to ganglion cells, do not fire action potentials.
With the use of estimates of receptor channel conductance from other studies we can tentatively conclude that the neurotransmitter released by a single quanta, a mIPSC or miniature EPSC (µEPSC), opens ~3–10 receptor channels at both the GABA<sub>A</sub>/glycine and glutamate synaptic sites. In cultured spinal neurons, Bormann et al. (1987) found multiple conductance states for glycine-activated (29, 18, and 10 pS) and GABA-activated (28, 17, and 10 pS) channels. The most frequently observed conductance states were 29 and 17 pS for glycine and GABA receptors, respectively. In isolated goldfish retinal ganglion cells, Cohen et al. (1989) reported the most frequent conductance state for GABA<sub>A</sub> receptor channels was 16 pS, close to that reported by Bormann et al. (1987). These values suggest that the GABA<sub>A</sub>-mediated mIPSCs reflect the concurrent opening of five to nine channels. Glycine mIPSCs, having similar peak amplitudes but larger single channel conductance, would represent the opening of three to five channels. These estimates are about one-half the number of channel openings thought to underlie mIPSCs in rat hippocampus (Edwards et al. 1990).

Similar determinations for sEPSCs that use a single-channel conductance of 8.5–10 pS (Cull-Candy and Usowicz 1989; Jonas et al. 1993) and an average peak amplitude of 84 pS (5.9 pA/70 mV) suggest that each µEPSC reflects the simultaneous opening of 8–10 channels. These estimates are roughly one-half mts/m for AMPA-mediated mEPSCs in guinea-pig thalamic neurons (16) (Paulsen and Heggelund 1996) and in stellate cells of rat visual cortex (10–20) (Stern et al. 1992), but closer to the estimate for mEPSCs in tiger salamander retinal ganglion cells (13) (Taylor et al. 1995).

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