Different Combinations of GABA\textsubscript{A} and GABA\textsubscript{C} Receptors Confer Distinct Temporal Properties to Retinal Synaptic Responses

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Lukasiewicz, Peter D. and Colleen R. Shields. Different combinations of GABA\textsubscript{A} and GABA\textsubscript{C} receptors confer distinct temporal properties to retinal synaptic responses. J. Neurophysiol. 79: 3157–3167, 1998. This study addresses how \textgamma-aminobutyric acid-A (GABA\textsubscript{A}) and GABA\textsubscript{C} receptors confer distinct temporal properties to neuronal synaptic responses. The retina is a model system for the study of postsynaptic contributions to synaptic responses because GABAergic amacrine cells synapse onto neurons, which have different combinations of GABA\textsubscript{A} and GABA\textsubscript{C} receptors. It is not known, however, how GABA\textsubscript{A} versus GABA\textsubscript{C} receptors influence the time course of retinal synaptic responses or what proportion of inhibitory input is mediated by each receptor type.

We examined the time courses of synaptic responses mediated by GABA receptors in ganglion and bipolar cells by recording currents evoked by activating amacrine cells with a stimulating electrode in the salamander retinal slice. The pharmacologically isolated, GABAergic synaptic currents were long-lasting in bipolar cells and relatively brief in ganglion cells. The receptors that mediated these temporally distinct synaptic responses exhibited different pharmacological properties. In ganglion cells, GABAergic synaptic currents were abolished by the GABA\textsubscript{A} receptor antagonists bicuculline or SR95531. In bipolar cells, the GABA\textsubscript{C} receptor antagonist 3-aminopropyl[methyl]phosphonic acid (3-APMPA) largely blocked GABAergic synaptic responses; the remaining response was blocked by bicuculline or SR95531. The GABA\textsubscript{A} receptor component of the bipolar cell response was relatively brief compared with the GABA\textsubscript{C} receptor component. Puffing GABA onto ganglion cell dendrites or bipolar cell terminals yielded similar pharmacological and kinetic results, indicating that transmitter release differences did not determine the response time courses. Moreover, the GABA\textsubscript{C} receptors on bipolar cells may be different from those reported in rat or fish retina because imidazole-4-acetic acid (14AA), which acts as an antagonist in these preparations, acts as an agonist in salamander. Our data show that the prolonged synaptic responses in bipolar cells were mediated predominantly by GABA\textsubscript{C} receptors, whereas transient synaptic responses in ganglion cells were mediated by GABA\textsubscript{A} receptors.

INTRODUCTION

In the CNS the inhibitory transmitter \textgamma-aminobutyric acid (GABA) interacts with two types of ionotropic receptors, GABA\textsubscript{A} and GABA\textsubscript{C}. The GABA receptor complement of specific neurons is important because there are differences in the properties of GABA\textsubscript{A} and GABA\textsubscript{C} receptors that may have significant functional consequences. These receptor types may determine the time course of synaptic responses. Native GABA\textsubscript{A} and GABA\textsubscript{C} receptors (Qian and Dowling 1993, 1995) as well as GABA\textsubscript{A} and \rho receptor subunits expressed in \textit{Xenopus} oocytes (Amin and Weiss 1994) show different time courses in response to GABA application. GABA\textsubscript{C} receptor–mediated responses show minimal desensitization and slow deactivation, whereas GABA\textsubscript{A} receptor–mediated responses show marked desensitization and rapid deactivation. However, it is not known whether these differences in receptor properties give rise to differences in time courses of synaptic responses.

GABA is an important inhibitory neurotransmitter in the vertebrate retina that modulates synaptic transmission at both the outer and inner plexiform layers (Chun and Wässle 1989; Lukasiewicz and Werblin 1994; Marc et al. 1978; Murakami et al. 1982; Pourcho and Owczarzak 1989; Wu 1991). There is an abundance of GABA\textsubscript{C} receptors in the retina, making it a good system for studying their synaptic function. In other parts of the CNS, the roles of GABA\textsubscript{C} receptors are poorly understood.

Because GABA\textsubscript{C} and GABA\textsubscript{A} receptors are differentially expressed on distinct classes of retinal neurons, we can determine how they might shape different types of synaptic responses. Electrophysiological studies indicate that GABA\textsubscript{C} receptors are found on horizontal cells of teleost fish (Dong et al. 1994; Qian and Dowling 1993) and on bipolar cells in both mammals and cold-blooded vertebrates (Feigenspan et al. 1993; Lukasiewicz et al. 1994; Lukasiewicz and Wong 1997; Matthews et al. 1994; Qian and Dowling 1995). In ganglion and amacrine cells, GABA-evoked responses appear to be mediated either predominately or exclusively by GABA\textsubscript{A} receptors (Dong et al. 1994; Feigenspan et al. 1993; Lukasiewicz et al. 1994; Lukasiewicz and Wong 1997). However, other studies report that ganglion cells in tiger salamander and turtle possess a minor component of GABA\textsubscript{C} receptors and a major component of GABA\textsubscript{A} receptors (Liu and Lasater 1994; Zhang and Slaughter 1995). In agreement with most of the physiological studies, immunocytochemical (Enz et al. 1996; Koulou et al. 1997) and in situ hybridization (Enz et al. 1995; Qian et al. 1997; but see Albrecht and Darlison 1995) studies show that \rho-subunits are found mainly on bipolar cells in mammals, fish, and birds.

In this study we have characterized the time courses of synaptic inhibition mediated primarily by GABA\textsubscript{A} receptors on bipolar cells and by GABA\textsubscript{A} receptors on ganglion cells in the tiger salamander retinal slice. We evoked synaptic responses that were mediated by GABA\textsubscript{A} and GABA\textsubscript{C} receptors by eliciting GABA release from amacrine cells with electrical stimulation in the inner plexiform layer (IPL). The GABA receptor–mediated components of the synaptic response were measured after blocking glutamate and glycine receptor function. Inhibitory synaptic responses in gan-
glion cells were relatively transient compared with those in bipolar cells. The results from our pharmacological studies indicated that the relatively prolonged synaptic responses in bipolar cells were mediated mainly by GABA<sub>C</sub> receptors. Small transient GABA<sub>A</sub> receptor–mediated responses were often seen in bipolar cells after GABA<sub>C</sub> receptor blockade. In addition, the salamander bipolar cell GABA<sub>C</sub> receptors were atypical in that they were activated by the GABA<sub>C</sub> receptor antagonist imidazole-4-acetic acid (I4AA) (Qian and Dowling 1994, 1995; Pan and Lipton 1995). Pharmacological results indicated that the transient GABAergic synaptic responses in ganglion cells, by contrast, were mediated exclusively by GABA<sub>C</sub> receptors. These results suggest that different GABA receptors contribute to the distinct time courses of inhibitory synaptic responses in bipolar and ganglion cells.

**METHODS**

**Whole cell patch recording in tiger salamander retinal slices**

Whole cell patch recordings (Hammill et al. 1981) were made from bipolar cells and ganglion cells in retinal slice preparations (Werblin 1978). The preparation of the retinal slices and the recording procedures have been described in detail previously (Lukasiewicz and Roeder 1995; Lukasiewicz and Werblin 1994). Tiger salamanders were obtained from C. D. Sullivan (Nashville, TN) and kept at 5°C on a 12 h light:12 h dark cycle. Experiments were performed at room temperature (19–22°C).

**Electrode and bath solutions**

The intracellular electrode solution consisted of (in mM) 95.25 cesium gluconate, 8 tetraethylammonium chloride (TEA), 0.4 magnesium chloride, 11 ethylen glycol-bis(β-aminoethanol ether)-N,N,N',N'-tetraacetic acid (EGTA), and 10 sodium N-2-hydroxyethylpiperazine-N'2-ethanesulfonic acid (HEPES), adjusted to pH 7.7 with cesium hydroxide. The bathing medium (salamander Ringer) contained (in mM) 112 sodium chloride, 2 potassium chloride, 2 calcium chloride, 1 magnesium chloride, 5 glucose, and 5 HEPES, adjusted to pH 7.8 with NaOH. The calculated EC<sub>50</sub> for these intra- and extracellular solutions was ~65 mM. Unless otherwise indicated, all chemicals were obtained from Sigma Chemical (St. Louis, MO). 6-Cyano-7-nitroquinoxaline-2,3-dione (CNQX), 2-(3-carboxypropyl)-3-amino-6-(4-methoxyphenyl)p- yridazinium bromide (SR-95531), and 3-amino-4-propyl[1(methyl)]- phosphonic acid (3-APMPA) were obtained from Research Biochemicals (Natick, MA), and 3-aminopropyl-[ methyl ]- phosphonic acid (d-AP5) was obtained from Precision Biochemicals (Vancouver, BC). In some cells, responses were recorded using the gramicidin, perforated-patch technique (Ebihara et al. 1995). The perforated-patch electrode solution consisted of the following (in mM): 107.5 cesium chloride and 10 HEPES, adjusted to pH 7.5 with Tris base. Gramicidin stock solution was prepared by dissolving 10 mg/ml in methanol and was diluted to its final concentration of 50 or 100 µg/ml with the electrode solution. The electrode tip was filled with gramicidin-free electrode solution to enhance seal formation. No differences in response were observed when whole cell versus perforated-patch techniques was used.

Membrane potential values given in this paper were corrected for junction potentials. Liquid junction potentials were determined for these intra- and extracellular solutions was ~65 mV. Unless otherwise indicated, all chemicals were obtained from Sigma Chemical (St. Louis, MO). 6-Cyano-7-nitroquinoxaline-2,3-dione (CNQX), 2-(3-carboxypropyl)-3-amino-6-(4-methoxyphenyl)p- yridazinium bromide (SR-95531), and 3-amino-4-propyl[1(methyl)]- phosphonic acid (3-APMPA) were obtained from Research Biochemicals (Natick, MA), and 3-aminopropyl-[ methyl ]- phosphonic acid (d-AP5) was obtained from Precision Biochemicals (Vancouver, BC). In some cells, responses were recorded using the gramicidin, perforated-patch technique (Ebihara et al. 1995). The perforated-patch electrode solution consisted of the following (in mM): 107.5 cesium chloride and 10 HEPES, adjusted to pH 7.5 with Tris base. Gramicidin stock solution was prepared by dissolving 10 mg/ml in methanol and was diluted to its final concentration of 50 or 100 µg/ml with the electrode solution. The electrode tip was filled with gramicidin-free electrode solution to enhance seal formation. No differences in response were observed when whole cell versus perforated-patch techniques was used.

Membrane potential values given in this paper were corrected for junction potentials. Liquid junction potentials were determined as described by Fenwick et al. (1982). They were typically ~11 and ~2 mV for the cesium gluconate and perforated-patch electrode solutions, respectively.

The control bathing solution used in our slice experiments was formulated to pharmacologically isolate the GABA receptor–mediated component of inhibitory postsynaptic currents (IPSCs). Glyceromic inhibitors of synaptic responses were blocked with strychnine (2 μM) and N-methyl-D-aspartate (NMDA), and non-NMDA receptor–mediated responses were blocked with d-AP5 (50 μM) and CNQX (10 μM), respectively (Belgum et al. 1984; Diamond and Copenhagen 1993; Mittman et al. 1990). Voltage-gated potassium currents were blocked by including cesium and TEA in the recording electrodes (Lukasiewicz and Werblin 1988). Antagonists were applied over a relatively large area of the slice under study (several mm in width) by a gravity-driven superfusion system as described previously (Lukasiewicz and Roeder 1995).

**Puffing agonists onto ganglion cell dendrites and bipolar cell terminals**

GABA agonists were puffed onto the dendrites of ganglion cells or the terminals of bipolar cells in the slice preparation with a Picospitzer (General Valve, Fairfield, NJ) at 45- or 60-s intervals. The puff pressure and/or duration were adjusted to give a half-maximal or smaller response to the GABA receptor agonists. Also, because the puff electrode could not be placed directly onto the cells’ processes and the slice was continuously superfused (diluting the puff), the GABA concentration at the receptors was most likely significantly less than the pipette concentration.

**Bipolar electrode stimulation of amacrine cell inputs**

Amacrine cell inputs to bipolar and ganglion cells were elicited by electrical stimulation in one of two ways. Synaptic inputs were elicited with an extracellular patch electrode filled with salamander Ringer (Zhang and Trussell 1994) that was placed in the IPL. A silver/silver chloride electrode (separate from the bath ground) placed near the slice served as return path for the stimulating current. In some cases, a custom concentric bipolar electrode (MCE-100, Rhodes Medical Instruments, Woodland Hills, CA), placed in the IPL, was used to elicit synaptic inputs. The distal part of the electrode shaft (70 mm) was 150-µm diam, and it tapered to a center contact of 25 µm with an outer contact of 100 µm. The stimuli were generated by either a Grass S48 or a Grass SD9 stimulator (West Warwick, RI) that was triggered by the data acquisition program. The duration and magnitude of the stimuli were adjusted to minimize synaptic fatigue and to elicit reproducible responses (typically 0.1–3 ms duration, 1–7 V). With the extracellular patch electrode, stimuli were typically shorter in duration (0.05–1 ms). Similar results were obtained with both methods. We could not test for temporal summation of synaptic GABA responses because interstimulus intervals of <90 s resulted in depression of the subsequent response, possibly due to presynaptic fatigue. Two observations indicated that the stimulation was confined to the IPL and was focal. First, we were never able to elicit responses from bipolar cells without axon terminals. (Some cells were filled with Lucifer yellow to image their terminals.) Second, the stimulating electrode had to be placed in the same IPL sublamina as the bipolar terminals to evoke a response. Responses were never elicited from presumed ON or OFF bipolar cells (as determined from the stratification of their terminal processes) when the stimulating electrode was placed in the OFF or ON sublamina, respectively (i.e., opposite IPL sublamina).

**Recording system**

The microscope system and patch-clamp apparatus used for this study were described in Lukasiewicz and Roeder (1995). Electrodes were pulled from borosilicate glass (TW150F-4, W.P.I., Sarasota, FL) with a Sachs-Flaming puller (Sutter In-
Instruments, Novato, CA) and had measured resistances of <5 MΩ. The measured series resistances were typically 15 MΩ (read from Axopatch 200B series resistance dial) and were usually compensated by 90%. Patchit software (Geo. Grant, Somerville, MA) was used to generate voltage command outputs, acquire data, and trigger the Picospritzer and the stimulator. The data were digitized and stored with a 486-PC using a Labmaster DMA data acquisition board (Scientific Solutions, Solon, OH). Responses were filtered at 1 kHz with the four-pole Bessel low-pass filter on the Axopatch 200B and sampled at 0.5–2 kHz. Data were analyzed using Tack software (Geo. Grant, Somerville, MA). Time constants for current decays (from 90% of peak current amplitude) were determined by fitting a three-parameter single exponential equation \( Y = A + Be^{-ct} \) to the response using the Marquardt-Levenberg algorithm. Results are expressed as means ± SE.

**RESULTS**

GABAergic IPSCs are brief in ganglion cells and long-lasting in bipolar cells.

Here we examine the time courses of synaptic responses thought to be mediated predominantly by either GABA\(_A\) or GABA\(_C\) receptors. Figure 1A shows a GABA receptor–mediated IPSC that was recorded from a ganglion cell in response to an electrical shock in the IPL. The GABA recep-

![Image](https://i.imgur.com/3QX.png)

**FIG. 1.** Time courses of \( \gamma \)-aminobutyric acid-C (GABA\(_C\)) and GABA\(_A\) receptor–mediated synaptic responses are distinct. A: GABAergic inhibitory postsynaptic currents (IPSCs) evoked by shocks in the inner plexiform layer (IPL) have different time courses. Responses in ganglion cells are brief; they rapidly rise and decay (see RESULTS: trace marked, ganglion cell). By contrast the responses in bipolar cells have a longer time-to-peak and decay more slowly (trace marked, bipolar cell). The bipolar response was scaled to the peak of the ganglion cell response for time course comparison (trace marked bipolar cell scaled). Open circles are the best fit single exponential (\( \tau = 1,351 \) ms) for the bipolar cell response decay. B: GABAergic IPSCs from ganglion cell in A plotted on faster time scale. Open circles are the best fit single exponential (\( \tau = 37 \) ms) for the ganglion cell response decay. C: puff-evoked GABA responses have different time courses. Similar to the IPSCs, the ganglion cell response is brief, and the bipolar cell response is prolonged. Open circles are the best fit single exponential (\( \tau = 872 \) ms) for the bipolar cell response decay. D: puff-evoked GABA responses from ganglion cell in C plotted on faster time scale. Open circles are the best fit single exponential (\( \tau = 77 \) ms) for the ganglion cell response decay. In this and in subsequent figures, the cells were held at 0 mV, glutamate and glycine receptors were blocked as indicated in METHODS, and each trace is the average of 2–5 current responses. In addition, the electrical stimulation is indicated by an inverted triangle above the current traces, and the timing of the puff stimulation is indicated by small bar above the current traces here and in subsequent figures.
The GABA puff results indicate that receptor complement mediated by blocking glycine and glutamate receptors as described in METHODS. Cells were voltage clamped to 0 mV to minimize the driving force for nonspecific cation responses and maximize the driving force for chloride-mediated, GABA responses (calculated $E_{Cl} = -65$ mV). In Fig. 1B the response is replotted on a shorter time scale. The ganglion cell IPSC reached its peak amplitude in 6 ms and then decayed rapidly. The falling phase of the response could be fit with a single exponential with a time constant of 37 ms. On average, the ganglion cell response time-to-peak amplitude was $5.0 \pm 0.3$ (SE) ms and decayed with a time constant of $41 \pm 4$ ms ($n = 13$). The same electrical shocks evoked IPSCs in bipolar cells that were much more prolonged than those measured in ganglion cells. Figure 1A also shows an example of a GABA receptor–mediated IPSC recorded in a bipolar cell. For comparison, the bipolar cell IPSC is scaled to the same amplitude as the ganglion cell IPSC. The bipolar cell synaptic response reached its peak amplitude in 245 ms, much more slowly than the ganglion cell response. In comparison to the ganglion cells, the bipolar cell responses decayed minimally over the time course of the recording. The decay of the bipolar response was fit with a single exponential with a time constant of 1,351 ms (Fig. 1A). On average, the bipolar cell response decayed with a time constant of 1,918 ± 163 ms, and its time-to-peak amplitude was 288 ± 44 ms ($n = 15$). The differences in response time courses could be due to either a difference in GABA receptor complements or to different time courses of GABA release onto ganglion and bipolar cells.

Responses evoked by puffs of GABA onto bipolar cells or ganglion cells show similar differences in time course (Fig. 1C). The ganglion cell puff-evoked responses were relatively transient, whereas the bipolar cell puff-evoked responses were more prolonged. The falling phase of the bipolar response to a GABA puff could be fit with a single exponential with a time constant of 872 ms (Fig. 1C). The average time constant for the bipolar cell response decay was $1,038 \pm 157$ ms ($n = 15$). The ganglion cell response to an identical GABA puff decayed more rapidly; its falling phase could be fit with a single exponential fit with a time constant of 77 ms (Fig. 1D). The average time constant for the ganglion cell response decay was $133 \pm 24$ ($n = 9$).

The GABA puff results indicate that receptor complement contributes to differences in IPSC time course independent of presynaptic GABA release mechanisms.

**Only GABA$_A$ receptors mediated GABAergic IPSCs in ganglion cells**

To determine the complement of GABA receptors that mediated IPSCs on ganglion cells, we evoked IPSCs in the presence or absence of the GABA$_A$ receptor antagonist bicuculline. The GABA receptor–mediated IPSC in the ganglion cell illustrated in Fig. 2A was completely blocked by bicuculline (100 µM). The responses in Fig. 2B, expressed as charge-transfer, were measured by integrating the currents. In all ganglion cells tested, the GABAergic IPSC was always completely ($1.6 \pm 0.9\%$ of control, $n = 14$) and reversibly blocked by bicuculline. These data indicate that GABAergic synaptic currents are mediated exclusively by GABA$_A$ receptors in ganglion cells. These results are in agreement with other studies showing that GABA-evoked currents in third-order retinal neurons are completely blocked by bicuculline (Dong et al. 1994; Feigenspan et al. 1993; Lukasiewicz and Wong 1997; Yeh et al. 1996).

**Pharmacological properties of GABA-evoked currents in ganglion cells**

It was recently reported that ~20% of the GABA-elicited current recorded in tiger salamander ganglion cells (the SR-95531–resistant component) was mediated by GABA$_C$ receptors (Zhang and Slaughter 1995). We puffed GABA onto the dendrites of ganglion cells (100–250 µM pipette concentration) after blocking glycine and glutamate receptors. Figure 3A shows that the GABA-elicited current in the ganglion cells was completely blocked by bicuculline, indicating that this response was mediated exclusively by GABA$_A$ receptors. We found that, at best, GABA$_C$ receptors underlie only a very minor part of the GABA-elicited response in tiger salamander ganglion cells. On average, the GABA-elicited charge transfer was reversibly reduced to $2.6 \pm 0.6\%$ ($n = 17$) of control by bicuculline (100–200 µM; Fig. 3B).

**FIG. 2.** GABAergic IPSCs in ganglion cells are mediated by GABA$_A$ receptors. A: IPSCs evoked by shocks in the IPL recorded in the absence (Control) or presence (Bicuculline) of bicuculline (100 µM). B: bar graph showing that GABAergic IPSCs were reduced by bicuculline to $1.6 \pm 0.9\%$ ($n = 14$) of control. On wash out of bicuculline, the responses recovered to $81 \pm 5\%$ of control levels (Recovery). The responses, obtained by integrating the currents, are expressed as normalized charge transfer here and in subsequent figures.
The lack of GABA<sub>C</sub> receptor-mediated responses in our ganglion cells may have been due to the wash out of an intracellular component essential for GABA<sub>C</sub> responses. Therefore we performed recordings using gramicidin-perforated patches. Results similar to our whole cell recordings were obtained using gramicidin-filled pipettes and SR-95531 (7 or 20 μM) to block GABA<sub>A</sub> receptors. The SR-95531-resistant component of the GABA-elicited currents com-

**FIG. 3.** Responses to GABA puffs onto ganglion cell dendrites are mediated by GABA<sub>A</sub> receptors. **A:** current responses to puffs of GABA (100–250 μM pipette concentration) were completely blocked by 100–200 μM bicuculline. The puff duration and pressure were always adjusted to give half-maximal or smaller responses here and in subsequent figures. **B:** bar graph indicating that GABA responses were reduced by bicuculline to 2.6 ± 0.6% (n = 17) of control levels (Bicuculline). On wash out of bicuculline, the responses recovered to 80 ± 6% of control levels (Recovery).

**FIG. 4.** GABAergic IPSCs in bipolar cells are mediated mainly by GABA<sub>C</sub> receptors. **A:** IPSCs evoked by shocks in the IPL recorded in the absence (Control) or presence of SR-95531 (15 μM) or 3-aminopropyl[methyl]phosphonic acid (3-APMPA; 500 μM) plus SR-95531 (SR & 3A). The current trace marked GABA<sub>A</sub> was obtained by subtracting the SR-95531 trace from the control trace. The decays of the GABA<sub>C</sub>- and SR-95531–resistant currents (○) were fit by single exponentials with time constants of 125 and 1,320 ms, respectively. **B:** IPSCs from another bipolar cell were significantly reduced by the GABA<sub>C</sub> receptor antagonist 3-APMPA (trace labeled 3-APMPA). The 3-APMPA–resistant component of the response was completely blocked by bicuculline (150 μM; trace labeled 3A & Bic). The decays of the control and 3-APMPA–resistant currents (○) were fit by single exponentials with time constants of 2,707 and 146 ms, respectively. **C:** bar graph showing that GABAergic IPSCs were reduced by bicuculline (bic) or SR-95531 (SR) to 79 ± 6% (n = 14) or 75 ± 11% (n = 4) of control, respectively. The addition of 3-APMPA to bicuculline or SR-95531 reduced the responses to 3 ± 1% (n = 8) or 2.4 ± 1.4% (n = 4) of control, respectively. On wash out of the GABA<sub>A</sub> and GABA<sub>C</sub> antagonists, the responses recovered to 82 ± 5% of control levels (Recovery). **D:** bar graph showing the effects of 3-APMPA on GABA-mediated synaptic charge transfer. Responses were reduced to 7.1 ± 1.5% (n = 12) of control by 3-APMPA. Bicuculline reduced the 3-APMPA–resistant component of the response to 1.3 ± 0.9% (n = 8) of control (3A & Bic). On wash out of the antagonists, the responses recovered to 80 ± 7% of control levels (Recovery).
Both GABA<sub>C</sub> and GABA<sub>A</sub> receptors mediated GABAergic IPSCs in bipolar cells

GABA-gated chloride currents in tiger salamander bipolar cells are mediated by both GABA<sub>C</sub> and GABA<sub>A</sub> receptors (Luksiewicz et al. 1994; Luksiewicz and Werblin 1994). Here we characterize the actions of the GABA<sub>C</sub> receptor antagonist 3-APMPA (Pan and Lipton 1995; Woodward et al. 1993) on GABAergic IPSCs and GABA-evoked currents.

To determine the contribution of GABA<sub>C</sub> receptors to IPSCs in bipolar cells, we evoked IPSCs in the presence or absence of GABA<sub>A</sub> receptor antagonists. Figure 4A shows a typical GABA receptor–mediated bipolar cell response evoked by synaptic activation of amacrine cells. Bath application of bicuculline or SR-95531 caused a small reduction of the synaptic response. On average, bicuculline (150 or 200 µM) or SR-95531 (15 µM) reduced the GABA<sub>A</sub> receptor–mediated synaptic charge transfer in bipolar cells to 79 ± 6% (n = 14) or 75 ± 11% (n = 4) of the control responses, respectively (Fig. 4C). These data are consistent with GABA<sub>C</sub> receptors mediating a major fraction of the bipolar cell IPSC.

In three bipolar cells, bicuculline increased the GABAergic IPSC charge transfer 1.40 ± 0.08-fold. The enhancement of the bipolar IPSC by bicuculline may have been due to disinhibition of GABAergic amacrine cells, which possess primarily GABA<sub>C</sub> receptors (Feigenspan et al. 1993; unpublished observations).

To confirm that the bicuculline-resistant component of the bipolar IPSC was indeed mediated by GABA<sub>C</sub> receptors, we determined whether or not this component was sensitive to the GABA<sub>C</sub> antagonist, 3-APMPA. Figure 4A shows that the SR-95531-resistant component of the IPSC was completely blocked by 3-APMPA (500 µM), confirming that this component of the response was mediated by GABA<sub>C</sub> receptors. In all bipolar cells tested, the GABA receptor–mediated IPSCs were completely blocked by the combination of 3-APMPA and bicuculline (3 ± 1% of control, n = 8) or SR-95531 (2.4 ± 1.4% of control, n = 4; Fig. 4C).

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**Fig. 5.** Responses to GABA puffs onto bipolar cell terminals are mediated by GABA<sub>C</sub> and GABA<sub>A</sub> receptors. A: current response to puffs of GABA (200 µM pipette concentration) is indicated by the trace marked Control. Bicuculline (100–150 µM) partially reduced the response to the GABA puff, indicated by the trace marked Bicuculline. The bicuculline-resistant component of the puff-evoked response was completely blocked by the addition of 3-APMPA (500 µM). This is indicated by the trace marked, Bicuculline & 3-APMPA. B: current responses to puffs of GABA from another bipolar cell. The GABA<sub>C</sub> receptor antagonist 3-APMPA dramatically reduced the response to the GABA puff (trace labeled 3-APMPA). The trace labeled GABA<sub>C</sub> was obtained by subtracting the current measured in 3-APMPA from the control current. The decays of the control and 3-APMPA–resistant currents (C) were fit by single exponentials with a time constants of 709 and 176 ms, respectively. C: bar graph showing the mean reduction in responses to GABA puffs by bicuculline or bicuculline plus 3-APMPA. Bicuculline reversibly reduced the response to 82.0 ± 4.5% (n = 7) of the control value (Bic). Bicuculline and 3-APMPA reversibly reduced the response to 2.1 ± 0.7% (n = 7) of the control value (Bic & 3APMPA). The response returned to 84.5 ± 3.7% of control after wash out of 3-APMPA (Bic) and 110 ± 8% of control after wash out of bicuculline (Recovery). D: bar graph showing the effects of 3-APMPA on GABA puff-mediated charge transfer. Responses were reduced to 22 ± 4% (n = 16) of control by 3-APMPA. Bicuculline reduced the 3-APMPA–resistant component of the response to 2.9 ± 0.9% (n = 16) of control (3A & Bic). On wash out of the antagonists, the responses recovered to 97 ± 10% of control levels (Recovery).
**Time course of the GABA<sub>A</sub> receptor component of bipolar cell IPSC was brief**

The majority of the GABAergic IPSC in bipolar cells was mediated by GABA<sub>C</sub> receptors, whereas the remaining minority of the response was mediated by GABA<sub>A</sub> receptors. Subtracting the bicuculline- or SR-95531-resistant component from the control response derived the GABA<sub>A</sub> receptor component of the bipolar cell IPSC. Figure 4A shows an example of a GABA<sub>A</sub> receptor-mediated IPSC determined by subtraction, which is compared with the GABA<sub>C</sub> (or SR-95531-resistant) component of the response. The GABA<sub>A</sub> component of the IPSC decayed more rapidly than the GABA<sub>C</sub> component and was fit by a single exponential decay with a time constant of 125 ms (169 ± 20 ms; n = 8). The decay time course of GABA<sub>C</sub> component (τ = 1,320 ms), measured in the presence of SR-95531, was similar to the time course of control bipolar cell IPSC noted above in Fig. 1.

Similar time course results were obtained when the GABA<sub>A</sub> receptor-mediated component of the bipolar cell IPSC was isolated by blocking GABA<sub>C</sub> receptors with 3-APMPA. Figure 4B shows a bipolar cell IPSC recorded in the absence or presence of 3-APMPA (500 μM). The response in this cell was almost completely blocked by 3-APMPA, indicating that the IPSC was mediated primarily by GABA<sub>C</sub> receptors. In all bipolar cells tested, 3-APMPA reduced the synaptic charge transfer to 7.1 ± 1.5% of control (n = 12). Bicuculline blocked the 3-APMPA-resistant component (1.3 ± 0.9% of control, n = 8), confirming that it was mediated by GABA<sub>A</sub> receptors (Fig. 4D). The 3-APMPA-resistant component of the response exhibited a relatively brief time course with a rapid time-to-peak of 12 ms (9 ± 2 ms, n = 5) and a relatively fast decay, which could be fit by a single exponential with a time constant of 146 ms (171 ± 42 ms, n = 5; Fig. 4B). The time courses of these responses were similar to those derived above by subtraction.

**Pharmacological properties of GABA-evoked currents in bipolar cells**

To determine the types of GABA receptors that mediated the bipolar cell response illustrated in Fig. 1C, GABA was puffed at the terminals of bipolar cells (200 μM pipette concentration) after glycine and glutamate receptors were blocked. Figure 5A shows that GABA-elicited currents had two components, a small bicuculline-sensitive current and a large bicuculline-insensitive current that are thought to be mediated by GABA<sub>A</sub> and GABA<sub>C</sub> receptors, respectively. The bicuculline-insensitive component of the response was on average 82 ± 4.5% (n = 7) of the control response (Fig. 5C), indicating that GABA<sub>A</sub> receptors mediated ~20% of the total response, confirming similar findings by others (Lukasiewicz et al. 1994; Wellis and Werblin 1995). In the presence of bicuculline (100 or 150 μM), 3-APMPA (500 μM) completely blocked the GABA-elicited current, confirming that the bicuculline-insensitive component of the

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**Figure 6.** Imidazole-4-acetic acid (I4AA) is an agonist at salamander GABA<sub>C</sub> receptors. A: current responses to I4AA puffs (500 μM pipette concentration) onto bipolar cell terminals in the absence (Control) or presence of 250 μM bicuculline (Bic, 250) or of 150 μM picrotoxin (Picro). B: bar graph summarizing the effects of GABA antagonists on I4AA responses in bipolar cells. Bicuculline (150–250 μM) reduced I4AA responses in bipolar cells to 94 ± 4% (n = 6) of control (Bic) and picrotoxin (150 μM) reduced responses to 3 ± 0.9% (n = 7) of control (Picro). Responses recovered to 85 ± 7% of control after wash out of picrotoxin (Rec). C: current responses to I4AA puffs onto ganglion cell dendrites in the absence (Control) or presence of bicuculline (100 or 250 μM; Bic 100, 250). D: bar graph summarizing the effects of GABA antagonists on I4AA responses in bipolar cells. Bicuculline (100 μM) reduced I4AA responses in ganglion cells to 3 ± 0.6% (n = 8) of control (Bic). Responses recovered to 65 ± 6% of control on bicuculline wash out (Rec).
response was mediated by GABA$_C$ receptors. In seven of seven bipolar cells, the coapplication of bicuculline and 3-APMPA completely and reversibly blocked (2.1 ± 0.7% of control charge transfer, $n = 7$) the GABA-elicited responses (Fig. 5C).

To examine the time course of the GABA$_{\lambda}$ receptor-mediated component of the GABA-evoked response, we blocked the GABA$_C$ receptors with 3-APMPA (500 $\mu$M). An example of a 3-APMPA-resistant, GABA$_{\lambda}$ receptor-mediated response is shown in Fig. 5B. GABA$_C$ receptors mediated most of the response in this cell, as evidenced by the large reduction by 3-APMPA. On average, 3-APMPA reduced the charge transfer to $22 \pm 4\%$ of control ($n = 16$; Fig. 5D). The 3-APMPA-resistant component of the response was blocked by bicuculline (to $2.9 \pm 0.9\%$ of control, $n = 16$), indicating that it was mediated by GABA$_{\lambda}$ receptors (Fig. 5D). The time course of the 3-APMPA-resistant, GABA$_{\lambda}$ receptor component decayed more rapidly than the control time course and was fit by a single exponential with a time constant of 176 ms (248 ± 22 ms, $n = 16$). The control time course, in contrast, was fit by a single exponential with a time constant of 709 ms, which was similar to the average control time constant noted above (1.038 ms). The GABA$_{\lambda}$ component of the puff-evoked response decayed somewhat more slowly than the puff-evoked response in ganglion cells (see Fig. 1D).

In telost horizontal cells (Qian and Dowling 1994) and in both rat and teleost bipolar cells (Pan and Lipton 1995; Qian and Dowling 1995), I4AA has been reported to reduce or block responses mediated by GABA$_C$ receptors. Because I4AA is also an agonist at GABA$_{\lambda}$ receptors, its effects at these receptors must be blocked with bicuculline before determining its actions at GABA$_C$ receptors. We found that in the presence of bicuculline, bath-applied I4AA elicited a relatively prolonged outward current when cells were held at 0 mV. The pharmacology of the receptors mediating the I4AA current was determined by puffing I4AA onto bipolar cell terminals in the presence or absence of GABA receptor antagonists. Figure 6, A and B, shows that the I4AA-elicited current was relatively insensitive to bicuculline antagonism. In the presence of bicuculline (150 or 250 $\mu$M), the I4AA response was 94 ± 4% ($n = 6$) of the control response charge transfer, indicating that the response was not mediated significantly by GABA$_{\lambda}$ receptors. Picrotoxin (150 $\mu$M), which acts at both GABA$_{\lambda}$ and GABA$_C$ receptors, by contrast, completely and reversibly blocked (3 ± 0.9% of control, $n = 7$) the I4AA-elicited current (Fig. 6, A and B). These data indicate that I4AA acts as an agonist, and not as an antagonist, at GABA$_{\lambda}$ receptors on the terminals of tiger salamander bipolar cells.

Puffs of I4AA (500 $\mu$M pipette concentration) also elicited responses in ganglion cells. In contrast to its effects at bipolar cells, I4AA-elicited currents were completely and reversibly blocked by bicuculline (100 $\mu$M; 3.0 ± 0.6% of control, $n = 8$) as shown in Fig. 6, C and D. This indicates that I4AA acts as an agonist at GABA$_{\lambda}$ receptors on ganglion cells and that the I4AA-sensitive, GABA$_C$ receptors found on bipolar cells are not present on ganglion cells.

**DISCUSSION**

**Do the properties of postsynaptic GABA receptors contribute to IPSC time courses?**

Studies with both isolated retinal neurons and with $\rho$-subunit receptor expression systems have shown that responses mediated by GABA$_{\lambda}$ and GABA$_C$ receptors have different time courses (Amin and Weiss 1994; Qian and Dowling 1993, 1995). Here, we show the first characterization of pharmacologically isolated, GABAergic synaptic responses in the vertebrate retina. Our results suggest that the time courses of ganglion cell and bipolar cell IPSCs are determined, in part, by different complements of GABA receptors. GABAergic synaptic responses in bipolar cells and ganglion cells were elicited by stimulating amacrine cell inputs with electrical shocks. Pharmacological analysis indicated that the GABA receptors mediating the IPSCs on these two neurons were different. Synaptic responses in bipolar cells were relatively prolonged and were largely bicuculline or SR-95531 insensitive, indicating that these responses were mediated mainly by GABA$_C$ receptors. By contrast, the synaptic responses in ganglion cells have a much more transient time course than the bipolar cell responses and were completely blocked by bicuculline or SR-95531, indicating that they were mediated exclusively by GABA$_{\lambda}$ receptors. Furthermore, current responses evoked by puffing GABA onto bipolar cell terminals or ganglion cell dendrites had time courses and pharmacological profiles that were similar to the synaptically evoked responses. Because puff-evoked responses are not determined by transmitter release kinetics, this result suggests that the properties of postsynaptic GABA receptors can, at least in part, shape the time course of IPSCs in bipolar and ganglion cells.

GABA$_C$ receptor $\rho$-subunit localization studies support our findings. Immunocytochemical studies in mammalian, bird, and fish retinas (Enz et al. 1996; Koulen et al. 1997) have shown that $\rho$-subunits are localized to bipolar cells but not amacrine or ganglion cells. Furthermore, most of the $\rho$-immunoreactive puncta are localized to the bipolar axon terminals where these cells receive synaptic input from amacrine cells, supporting the notion that GABA$_C$ receptors in the IPL are found primarily on bipolar cell axon terminals.

We found that bipolar cell and ganglion cell GABA$_{\lambda}$ receptor-mediated IPSCs had distinct time courses. GABA$_{\lambda}$ receptor-mediated synaptic responses in bipolar cells decayed approximately fourfold more slowly than those in ganglion cells. These differences may reflect different subunit compositions for GABA$_{\lambda}$ receptors on the different cell types. In rat retina, different classes of amacrine cells have particular GABA$_{\lambda}$ receptor subunit compositions, which are distinct from the rod bipolar cell GABA$_{\lambda}$ receptor subunit composition (Greferath et al. 1995). Mammalian cell lines transfected with cDNAs of different GABA$_{\lambda}$ receptor subunits have distinct response time courses (Dominguez-Perrot et al. 1996; Gingrich et al. 1995; Tia et al. 1996). The time courses of these GABA responses depended either on the subtype of $\alpha$-subunit expressed (Gingrich et al. 1995; Tia et al. 1996) or the presence or absence of an expressed $\gamma$-subunit (Dominguez-Perrot et al. 1996; Tia et al. 1996). Additional work is needed, however, to determine whether
differences in GABA<sub>A</sub> receptor subunit composition and/or other factors underlie the differences in GABA<sub>A</sub> receptor-mediated IPSC time courses.

The time courses of bipolar and ganglion cell responses may also be determined, in part, by differences in the rate of transmitter removal. The distinct patterns of immunolabeling for different subtypes of GABA transporters in the inner retina of rat (Johnson et al. 1996) and salamander (Yang et al. 1997) are consistent with this notion. It is possible that differences in the numbers of transporters or differences in the rates of different transporter subtypes could shape synaptic responses in the salamander retina. However, different GABA transporters probably do not play a major role in shaping responses in our studies. This is because synaptic or puff-evoked responses on the same bipolar cell, which were mediated by GABA<sub>A</sub> or GABA<sub>C</sub> receptors, had different time courses. Presumably, the rates of transmitter removal from the two types of GABA receptors were similar for a single bipolar cell. These results suggest that receptor properties were relatively more important than transmitter removal rates in determining synaptic response time courses mediated by GABA<sub>A</sub> and GABA<sub>C</sub> receptors.

The time courses of GABAergic synaptic responses in bipolar cells and ganglion cells may also be determined, in part, by different time courses of GABA release from distinct amacrine cells. For example, bipolar cells may receive inputs from sustained GABAergic amacrine cells, and ganglion cells may receive inputs from transient GABAergic amacrine cells. However, sustained, GABAergic amacrine cells have been shown to make synaptic contacts with bipolar cell axon terminals as well as with ganglion cell dendrites in goldfish retina (Muller and Marc 1990). Furthermore, ultrastructural evidence indicates that a single amacrine cell process can make synaptic contacts onto two postsynaptic processes in the tiger salamander IPL (Wong-Riley 1974). This anatomic evidence suggests that, in principal, a single GABAergic amacrine cell process can make synaptic contacts onto both bipolar cell terminals and ganglion cell dendrites. If this is indeed the case, then the time courses of synaptic responses of bipolar and ganglion cells may be determined by the complements of postsynaptic GABA receptors independent of presynaptic differences in transmitter release. We cannot rule out at this time that in addition to GABA receptor properties differences in transmitter release rates might also shape bipolar cell and ganglion cell GABAergic synaptic responses. However, our results clearly demonstrate that the properties of postsynaptic GABA receptors play an important role in shaping the time courses of these responses.

On the basis of the time course of the responses mediated by GABA<sub>A</sub> receptors, it might be expected that GABAergic responses in ganglion cells are always transient. This may not be the case, however, because sustained GABAergic synaptic responses have been reported in mudpuppy ganglion cells (Belgum et al. 1984). These sustained responses may be due to the temporal and spatial summation of presumed transient GABA<sub>A</sub> receptor-mediated synaptic responses. Similar findings have been reported for horizontal cells where transient responding α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) –type glutamate receptors may mediate sustained synaptic responses (Eliasof and Jahr 1997). For horizontal cell AMPA receptors, the recovery from desensitization is relatively rapid, allowing for temporal summation. Although our results indicate that synaptic responses mediated by GABA<sub>A</sub> receptors were relatively brief compared with those mediated by GABA<sub>C</sub> receptors, it is possible that sustained responses could be mediated by GABA<sub>A</sub> receptors in ganglion cells, provided that the transient responses can temporally and/or spatially summate. This suggests that neurons that possess GABA<sub>A</sub> receptors may have finer temporal resolution of inhibitory signals compared with neurons that possess mainly GABA<sub>C</sub> receptors.

**Do ganglion cells have GABA<sub>C</sub> receptors?**

Our results are in agreement with those reported for GABA-evoked currents in mammalian bipolar and ganglion cells (Feigenspan et al. 1993; Lukasiewicz and Wong 1997; Yeh et al. 1996), which showed that ganglion cells had exclusively GABA<sub>A</sub> receptors and bipolar cells had a combination of GABA<sub>C</sub> and GABA<sub>A</sub> receptors. In contrast to these, our studies, it has been reported that GABA<sub>C</sub> receptors may mediate a small, but significant (~20%), component of ganglion cell GABA-elicited responses in tiger salamander (Zhang and Slaughter 1995) and turtle (Liu and Lasater 1994). Messenger RNA for ρ2 and ρ3 subunits has been localized to the ganglion cells in rat retina (Ogurusu et al. 1997; Yeh et al. 1996), suggesting the presence of GABA<sub>C</sub> receptors on these cells. However, these messages are probably not expressed because only GABA<sub>A</sub>, and not GABA<sub>C</sub>, receptor-mediated currents were found in rat ganglion cells (Yeh et al. 1996). Also, the absence of ρ-subunit labeling suggests that these subunits are not expressed in amacrine and ganglion cells (Enz et al. 1996; Koulen et al. 1997). Furthermore, we never saw any evidence of a slowly decaying component of the synaptic response in ganglion cells as would be predicted if a portion of the response was mediated by GABA<sub>C</sub> receptors.

**Salamander bipolar cells may have a different subtype of GABA<sub>C</sub> receptor**

I4AA has previously been shown to act as a GABA<sub>C</sub> receptor antagonist in fish and rat retinas (Pan and Lipton 1995; Qian and Dowling 1995) and in ρ-subunits expressed in Xenopus oocytes (Kusama et al. 1993). In salamander, however, I4AA acts as a GABA<sub>C</sub> agonist, suggesting that GABA<sub>C</sub> receptors on salamander bipolar cells may be different from those previously described in other species. In contrast to the findings that I4AA is an antagonist at native GABA<sub>C</sub> receptors in white perch retina, Qian et al. (1997) recently reported that I4AA acted as an agonist at ρ-receptor subunits cloned from white perch retina and expressed in Xenopus oocytes. It is possible that a similar ρ-subunit is a component of salamander GABA<sub>C</sub> receptors.

**Roles of GABA<sub>A</sub> and GABA<sub>C</sub> receptors for inner retinal processing**

Presynaptic GABA<sub>C</sub> receptors on bipolar cell terminals control the excitatory output from bipolar cells (Lukasiewicz and Werblin 1994). These more sensitive, presynaptic GABA<sub>C</sub> receptors on bipolar cell terminals will be activated...
at lower synaptic GABA concentrations than the less sensitive GABA\textsubscript{A} receptors on both postsynaptic ganglion cell processes and bipolar cell terminals. Reciprocal synapses have been reported between bipolar terminals and amacrine cell processes in the salamander retina (Dowling and Werblin 1969; Wong-Riley 1974). Negative feedback could occur at a bipolar cell terminal contacting a GABAergic amacrine cell process. This feedback signal may lead to noise reduction as well as a reduction of synaptic gain at the bipolar cell output as Smith (1995) has proposed for feedback onto photoreceptor terminals. The presence of the more sensitive GABA\textsubscript{C} receptors on bipolar cell terminals suggests that feedback may occur at lower synaptic GABA concentrations than if only the less sensitive GABA\textsubscript{A} receptors were present. In addition, the presence of GABA\textsubscript{C} receptors suggests that feedback will be more prolonged than if only GABA\textsubscript{A} receptors were present.

The different functional properties of GABA\textsubscript{A} and GABA\textsubscript{C} receptors suggest that GABAergic signals in the IPL will have different effects on bipolar and ganglion cells. For example, weak inhibitory surround inputs mediated by amacrine cells may activate more sensitive GABA\textsubscript{C} receptors on the bipolar cell terminals, but not less sensitive GABA\textsubscript{A} receptors on ganglion cell dendrites. Previous work has shown that the properties of sustained antagonistic responses in salamander ganglion cells depend on surround intensity (Thibos and Werblin 1987). Dim surrounds cause a parallel rightward shift of the ganglion cell spot intensity-response function. This surround mechanism is consistent with presynaptic inhibition at the bipolar terminals (and/or at photoreceptor terminals) resulting in less transmitter release for a given spot stimulus, i.e., reduced synaptic gain. Brighter surrounds cause a further rightward shift of the spot intensity-response function and a decrease in the function maximum. This is consistent with direct inhibition of ganglion cells causing a decrease in maximal excitation, i.e., a reduced dynamic range. It is possible that the more sensitive GABA\textsubscript{C} receptors on bipolar cell terminals mediate presynaptic surround mechanisms at dim intensities, whereas the less sensitive GABA\textsubscript{A} receptors on ganglion cells mediate postsynaptic surround mechanisms at brighter intensities.

We thank Drs. Paul Cook and Rachel Wong for comments on the manuscript.

This work was supported by National Eye Institute Grants EY-08922 to P. D. Lukasiewicz, EY-07057 to C. R. Shields, and EY-02687, a Core Grant to the Department of Ophthalmology, and by the Research to Prevent Blindness.

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Received 12 August 1997; accepted in final form 6 March 1998.

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