GABA-Mediated Inhibition Between Amacrine Cells in the Goldfish Retina

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Received 29 July 1999; accepted in final form 26 June 2000

Watanabe, Shu-Ichi, Amane Koizumi, Shinya Matsunaga, Jonathan W. Stocker, and Akimichi Kaneko. GABA-mediated inhibition between amacrine cells in the goldfish retina. J Neurophysiol 84: 1826–1834, 2000. Retinal amacrine cells have abundant dendro-dendritic inhibitory networks between neighboring amacrine cells. Therefore an amacrine cell has both presynaptic and postsynaptic aspects. To understand these synaptic interactions in the amacrine cell, we recorded from amacrine cells in the goldfish retina slice preparation with perforated- and whole cell-patch clamp techniques. As the presynaptic element, 19% of the cells recorded (15 of 78 cells) showed spontaneous tetrodotoxin (TTX)-sensitive action potentials. As the postsynaptic element, all amacrine cells (n = 9) were found to have GABA-evoked responses and, under perforated patch clamp, 50 μM GABA hyperpolarized amacrine cells by activating a Cl⁻ conductance. Bicuculline-sensitive spontaneous postsynaptic currents, carried by Cl⁻, were observed in 82% of the cells (64 of 78 cells). Since the source of GABA in the inner plexiform layer is amacrine cells alone, these events are likely to be inhibitory postsynaptic currents (IPSCs) caused by GABA spontaneously released from neighboring amacrine cells. IPSCs were classified into three groups. Large amplitude IPSCs were suppressed by TTX (1 μM), indicating that presynaptic action potentials triggered GABA release. Medium amplitude IPSCs were also TTX sensitive. Small amplitude IPSCs were TTX insensitive (miniature IPSCs; n = 26). All of spike-induced, medium amplitude, and miniature IPSCs were Ca²⁺ dependent and blocked by Co²⁺. Blocking of glutamatergic inputs by α,2-aminophosphonoheptanoate (AP7; 30 μM) and 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX; 2 μM) had almost no effect on spontaneous GABA release from presynaptic amacrine cells. We suggest that these dendro-dendritic inhibitory networks contribute to receptive field spatiotemporal properties.

INTRODUCTION

The amacrine cells are a group of third-order neurons that exhibit a wide variety of light-evoked responses and morphological subtypes (Chan and Naka 1976; Hidaka et al. 1993; Kaneko 1973; Kolb 1997; Kujiiraoka et al. 1988; MacNeil and Masland 1998; Murakami and Shimoda 1977; Terniishi et al. 1987; Vaney 1990; Wagner and Wagner 1988; Watanabe and Murakami 1985). GABAergic cells constitute the majority of the amacrine cell population (Marc 1992, 1997; Muller and Marc 1990; Yazulla 1986) and are generally believed to mediate lateral inhibitory interactions between vertical excitatory channels composed of bipolar and ganglion cells. In fact, different GABA receptor types have been identified in axon terminals of bipolar cells (Grefeath et al. 1993; Lin and Yazulla 1994) and in ganglion cell dendrites (Lin and Yazulla 1994; Wässele et al. 1998), and GABA-mediated inhibition has been demonstrated in bipolar cells (Euler and Wässle 1998; Karschin and Wässle 1990; Kondo and Toyoda 1983; Qian et al. 1997; Suzuki et al. 1990; Tachibana and Kaneko 1988) and ganglion cells (Cohen et al. 1989; Gao and Wu 1998; Protti et al. 1997).

A unique feature of amacrine cells is the abundance of serial conventional synapses found between the dendrites of amacrine cells (Dowling 1987). In spite of their numerical dominance in the inner plexiform layer, there have been no direct systematic physiological assessments of synapses between amacrine cells. There have been some suggestions from recordings of ganglion cells that GABAergic inhibitory synapses between amacrine cells operate (Roska et al. 1998; Zhang et al. 1997). However, direct recording of GABAergic input to amacrine cells was reported only from a type of amacrine cells in the tiger salamander, the wide-field amacrine cell (Roska et al. 1998). Another feature of some amacrine cells is generation of action potentials superimposed on light-evoked slow potentials (Barnes and Werblin 1986; Feigenspan et al. 1998; Miller and Dacheux 1976; Murakami and Shimoda 1977). The action potentials are shown to be due to transmitter release from amacrine cells, but its contribution is reported to be only partial (Bieda and Copenhagen 1999; Hartveit 1999; Protti et al. 1997). Cook and McReynolds (1998) suggested that surround inhibition observed in ganglion cells was mediated by action potentials generated in amacrine cells. It is the aim of the present study to obtain direct evidence on the physiological properties of GABAergic serial synapses between retinal amacrine cells and contribution of action potentials in release of GABA from amacrine cells. We studied amacrine cells in the slice preparation of the goldfish retina using patch-clamp techniques, and found 1) an extensive inhibitory network mediated by GABAergic synapses, 2) a large release of GABA triggered by action potentials generated in presynaptic amacrine cells, and 3) spontaneous release of GABA from presynaptic amacrine cells independent of the glutamatergic input to them.
Methods

Preparation

All experiments were performed on retinal slices prepared from goldfish. The care of the animals was in accordance with the Guidelines for the Care and Use of Laboratory Animals of Keio University School of Medicine, and our experiments have been approved by the University Animal Welfare Committee. Each goldfish was dark adapted for more than 2 h, rapidly decapitated, double pithed, and eyes enucleated and hemisected. To liquify the vitreous humor the eyecup was soaked for 10 min in hyaluronidase (0.07 mg/ml, Type I-S, 300 units/mg, Sigma)–containing Ringer solution composed of (in mM) 125 NaCl, 2.6 KCl, 2.5 CaCl₂, 1.0 MgCl₂, 10 HEPES, and 16 glucose, pH 7.8. The eyecup was then rinsed with the Ringer solution without hyaluronidase. The retina was carefully removed from the pigmented epithelium and placed photoreceptor side up on a round filter paper (cellulose nitrate, pore size, 0.2 μm, 13 mm diam, Advantec Toyo, Tokyo, Japan). The liquefied vitreous humor was first absorbed from the back of the filter paper with tissue paper, then the filter paper with the retina was placed on a syringe filter holder (SX0001300, Millipore) with the retina side up. Suction was applied so that the remaining vitreous humor was more thoroughly removed and the retina became firmly attached to the filter paper. The retina was cut together with the filter paper in 150-μm slices by a made-to-order slicer. Slices were fixed to the glass bottom of the recording chamber (cut surface parallel to the bottom) with a small amount of silicone grease (Dow Corning) at both ends. All experiments were performed under normal room illumination and room temperature of 20–25°C.

Recording procedure

Slices were continuously perfused with solutions by gravity feed and viewed using a microscope (Optiphoto-2 Nikon or BHS-RFC Olympus) equipped with a water-immersion lens (WI-40 Nikon or WPlanFL40XUV Olympus), epifluorescence illumination and a camera (FX-35AUFX-II Nikon or PM-20 Olympus). Patch and pressure ejection pipettes were pulled (P-87 Sutter, or PP-83 Narishige) from borosilicate filament tubing (1.5 mm OD; 0.87 mm ID; Hilgenberg, Malsfeld, Germany). The tip diameter of the patch pipette was 1–2 μm giving a resistance of approximately 3–7 MΩ when filled with the pipette solution and measured in Ringer solution. The tip of the patch pipette was coated with Apcizeon wax (Apcizeon Products) or dental wax (GC corporation, Tokyo, Japan) to reduce stray capacitance. Several kinds of pipette solution were used according to the type of experiment. The pipette solution for perforated patch clamp was composed of (in mM) 120 KCl, 10 NaCl, 5 EGTA, and 10 HEPES, pH 7.3, and contained 15 μg/ml gramicidin D. The gramicidin D ionophore is thought to be impermeable to Cl⁻ (Akaike 1996). Gramicidin was first dissolved in dimethyl sulfoxide at 37.5 mg/ml and then dissolved into the pipette solution to make the final concentration. The tip of the patch pipette was filled with gramicidin D-free pipette solution, and gramicidin D pipette solution was then back filled immediately before use. The pipette solution for the whole cell clamp (Hamill et al. 1981) contained equimolar CsCl instead of KCl to block outward currents. A part of Cl⁻ of the pipette solution was replaced with equimolar gluconate or methansulfonate ions when used in experiments to measure the reversal potential of the GABA-induced current and the spontaneous postsynaptic current. All pipette solutions contained 0.2% Lucifer yellow to visualize cells under the epifluorescence microscope.

The recording pipette was connected to a patch-clamp amplifier (LM EPC7 List, Darmstadt, Germany or CEZ-2300 Nihon Kohden, Tokyo, Japan). An Ag-AgCl electrode was connected to the superfusate via ceramic- or agarose-bridge and served as an indifferent electrode. Signals were filtered with a bandwidth of 0–2 or 3 kHz by a built-in filter of the patch-clamp amplifier, recorded on a thermal array recorder (WR7600 or WR7700 Graphitec, Yokohama, Japan; a bandwidth, 0–5 kHz), stored on magnetic tape (A-45 or PC204A Sony, Tokyo, Japan; bandwidth, 0–10 kHz), and digitized by a 12-bit A/D converter. Command voltages were generated by a personal computer (DeskproXE466 Compaq or PC-9801RX NEC, Tokyo, Japan). In whole cell voltage-clamp recordings, capacitance (approximately 10–50 pF) was electrically compensated as much as possible, but complete elimination was unachievable. The series resistance varied from about 10 to 50 MΩ. As the voltage error produced by the series resistance was <10%, it was not compensated.

Pharmacology

Sources of chemicals were as follows: gramicidin D, bicineulline methochloride, and strychnine were purchased from Sigma; 6-cyano-7-nitroquinoline-2,3-dione (CNQX) and 1,2-amino-phosphonooxymethane (AP7) from Research Biochemicals; tetrodotoxin (TTX) from Sankyo (Tokyo, Japan); and Bay K8644 from Funakoshi (Tokyo, Japan). Solution containing Ca²⁺ did not contain Ca²⁺. CNQX and AP7 were mixed directly into the Ringer solution. Pharmacologic agents were applied either through a puffer pipette (2–5 μm diam) by pressure or Y-tube by gravity.

Cell identification

Cells were classified as ON, OFF, or ON-OFF type by dendritic structure (ON type, dendrite in sublamina b; OFF type, dendrite in sublamina a; ON-OFF type, dendrite in both sublamina a and b). As we used the slice preparation, our recordings were exclusively from pyriform amacrine cells and not from fusiform amacrine cells according to the morphological classification of the carp amacrine cells by Teranishi et al. (1987). Although Teranishi et al. (1987) reported Lucifer yellow coupling within the same type of the carp amacrine cell, dye coupling between amacrine cells was not observed in the present study.

Data analysis

As the properties of GABA-induced currents and spontaneous postsynaptic currents were almost identical in all amacrine cells of different morphological subtypes, we pooled all data without subtype classification in the following analyses.

AMPLITUDE OF GABA-INDUCED CURRENT. The current-voltage relation of GABA-induced current was obtained by measuring the response peak amplitudes. Then, the reversal potential was determined.

QUANTITATIVE ANALYSIS OF THE SPONTANEOUS POSTSYNAPTIC CURRENTS. As the spontaneous events occurred randomly, we used mean current of the postsynaptic current. The zero current level was determined by eye on the computer monitor. The current integral was divided by the time of the recording segment. Both methods gave very similar values (mostly method 1 was used and method 2 was applied to relatively short records). Then,
the current-voltage relation of the mean current was obtained for each cell and the reversal potential was determined. For analysis of reversal potentials, ionic activity was used instead of concentration and liquid junction potential was corrected.

AMPLITUDE HISTOGRAM OF THE SPONTANEOUS POSTSYNAPTIC CURRENTS. From the digitized record each spontaneous postsynaptic event was identified by eye and peak amplitude (from the onset to peak) was measured on the computer monitor, using pCLAMP software (Axon Instruments). Records from cells that did not show a clear onset and peak of the event were not used for amplitude histogram analysis.

Data are reported as means ± SE.

RESULTS

Effect of GABA is inhibitory on amacrine cells

Application of exogenous GABA (50 μM, applied by a puffer pipette) induced hyperpolarization in all amacrine cells (n = 9; 4 ON-type cells, 2 OFF-type cells, 3 ON-OFF-type cells) when recorded by a gramicidin perforated patch, indicating that GABA is functioning as an inhibitory transmitter in all amacrine cell types (Fig. 1A). The resting potential was −57 ± 3 (SE) mV, almost the same voltage as previously reported for light-responding amacrine cells in the intact retina recorded by intracellular microelectrode (Djamgoz et al. 1996; Watanabe and Murakami 1985). Hyperpolarization induced by 50 μM GABA measured 9 ± 2 mV (Fig. 1E). Spontaneous action potentials (see the next section) were suppressed by the GABA-induced hyperpolarization (Fig. 1A). The reversal potential of the GABA-induced current estimated under voltage clamp was −76 ± 4 mV (n = 6; 4 ON-type cells, 1 OFF-type cell, 1 ON-OFF-type cell, Fig. 1B).

The reversal potential of the GABA-induced current was dependent on the intracellular Cl− concentration. When the whole cell configuration was established in the cell shown in Fig. 1A by breaking the perforated patch membrane, GABA induced a tonic depolarization in accordance with an increase of intracellular Cl− concentration by the intracellular dialysis with the pipette solution ([Cl−], 32 mM, Fig. 1C). Under this condition, GABA-induced current reversed its polarity at about −30 mV (Fig. 1D; calculated equilibrium potential for Cl−, $E_{Cl}$ was −32 mV). The relation between GABA-induced current and the voltage was almost linear (Fig. 1F), and the reversal potential was nearly identical to $E_{Cl}$ calculated by the Nernst equation (Fig. 1G). These observations indicate that

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**FIG. 1.** Inhibitory effect of GABA on the amacrine cell. In a perforated patch recording, application of 50 μM GABA by a puffer pipette induced hyperpolarization and suppressed action potential generation in an amacrine cell when recorded in current-clamp mode (A). In voltage-clamp mode, the GABA-induced current reversed at about −75 mV (B). When patch membrane was broken to obtain whole cell recording, GABA evoked a depolarization in current-clamp mode (C). Pipette solution contained 32 mM Cl−. In whole cell recording, the reversal potential of the GABA-induced current was about −30 mV (D). This cell showed spontaneous action potential generation even under “voltage-clamped” condition (B, −57 mV; D, −47 mV). E: summary of effect of GABA on membrane potential in perforated patch current-clamp recording. GABA caused hyperpolarization by 9 ± 2 mV (n = 9) in all of the recorded cells (different symbols represent different cells). F: peak GABA-induced current-voltage relation in voltage-clamp recording. Each point is an average of 2–8 cells. The reversal potential was about −75 mV in perforated patch recording. In whole cell recordings reversal potential was dependent on [Cl−]. ⬤, perforated patch-clamp ([Cl−] was 10 or 32 mM). Open symbols are data from whole cell recordings. [Cl−], (in mM): ⬤, 10; ⬤, 32; ⬤, 130. Inset shows peak current-voltage relation of GABA-induced current recorded from cells that had lost their dendrite. Each curve consists of data from a single cell. ⬤, [Cl−] = 20 mM. G: relationship between [Cl−] and the reversal potential of GABA-induced current obtained by whole cell recording. The solid line was drawn according to the Nernst equation. Number of experiments are indicated in parentheses.
GABA selectively opens a Cl\(^-\)-permeable channel. The intracellular Cl\(^-\) concentration in amacrine cells was estimated to be lower than 10 mM from the reversal potential of the GABA-induced current obtained in the perforated patch recordings. The GABA-induced current was suppressed by 100 \(\mu\)M bicuculline (data not shown), suggesting that it was mediated by GABA\(_A\) receptors.

The effect of GABA was identical for all amacrine cells, regardless of morphological subtype. Similar results were obtained from all cells that showed fluorescence only from the soma, possibly their dendrites lost in the preparation process \((n = 3, \text{Fig. 1F, inset}). These findings indicate that GABA receptors are widely distributed over the cell surface, the soma, and dendrite, of various types of amacrine cells.

**Action potential of amacrine cells**

The spontaneous action potentials seen in current-clamp recordings (see Fig. 1A) were also seen even when the cell was “voltage clamped” (Figs. 1, B and D, and 2). These activities were identified as the current due to regenerative action potentials (“spike currents”) because 1) the amplitudes were large (>100 pA) and almost constant, and they had a rapid time course (Fig. 2A, top and bottom traces with expanded time scale, *), 2) the current was inward and seen at the holding potential between approximately –60 and –40 mV (Fig. 1, B and D; see also Fig. 2B). They were observed in 19% of cells recorded (15/78) including all morphological subtypes (ON-type cell, 5/17; OFF-type cell, 5/23; ON-OFF-type cell, 5/38). Furthermore, under the voltage-clamp condition, depolarization evoked a transient inward current in all spiking and some nonspiking amacrine cells (ON-type cell, 9/17; OFF-type cell, 13/23; ON-OFF-type cell, 8/38). Both spike currents and the transient inward currents evoked by depolarization were suppressed by 1 \(\mu\)M TTX (not shown). Under voltage clamp, the action potential might be generated somewhere remote from the soma where the membrane voltage escaped from the holding voltage due to an incomplete space clamp.

**GABA\(_A\) receptor-mediated spontaneous postsynaptic currents in amacrine cells**

In addition to the spike current, other spontaneous events were seen in whole cell voltage-clamp recordings. These events were smaller in amplitude and slower in the time course than the spike current. The amplitude varied from one event to another, and they were reversibly suppressed by 100 \(\mu\)M bicuculline (Fig. 2A, top trace and bottom traces with expanded time scale), suggesting that they were mediated by GABA\(_A\) receptors. These events, interpreted as spontaneous postsynaptic currents, were observed in 82% of cells recorded (64/78) including all morphological subtypes (ON-type cell, 14/17; OFF-type cell, 21/23; ON-OFF-type cell, 29/38).

We also tested strychnine (2 \(\mu\)M), CNQX (5 \(\mu\)M), and AP7 (50 \(\mu\)M) to determine whether glycine, \(\alpha\)-amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA)/kainate (KA) or \(N\) -methyl-\(d\)-aspartate (NMDA) receptors significantly contributed to the generation of the spontaneous postsynaptic currents (cf. Dixon and Copenhagen 1992). The spontaneous postsynaptic currents (determined as mean currents, see METHODS) were largely suppressed by bicuculline (by 78 \(\pm\) 7%, \(n = 8\), but slightly by other antagonists (7 \(\pm\) 3% by strychnine, \(n = 5\); 13 \(\pm\) 7% by CNQX, \(n = 9\); 8 \(\pm\) 3% by AP7, \(n = 9\), Fig. 3). Despite nonuniform coverage of dendritic fields by puffer-applied antagonists, there was an obvious difference among the potencies.

The reversal potentials of spontaneous postsynaptic currents were dependent on \([\text{Cl}^-]\) (Fig. 4). The current was inward at membrane voltages more negative than \(E_{\text{Cl}}\) (about –30 mV, \([\text{Cl}^-]\), 32 mM), and outward at membrane voltages more positive than \(E_{\text{Cl}}\) in a cell shown in Fig. 4A. At the voltage slightly more positive than \(E_{\text{Cl}}\), spontaneous inward currents (–27 mV, Fig. 4A, \(\Delta\)) were seen together with the reversed outward currents (\(\nabla\)). It seems likely that these inward currents represent cation-mediated postsynaptic events, most likely evoked by spontaneous glutamate release from bipolar cells (Gao and Wu 1999; Taylor et al. 1995; Tian et al. 1998).

Actually, application of 50 \(\mu\)M glutamate evoked currents in all amacrine cells (data not shown). The current-voltage relation of the mean current was almost linear (Fig. 4B), and the reversal potential measured with various \([\text{Cl}^-]\) was close to \(E_{\text{Cl}}\) (Fig. 4C). Slight deviations from \(E_{\text{Cl}}\) (solid line) to the
Depolarizing direction may reflect a small contamination of cation currents, possibly glutamatergic inputs from bipolar cells. The reversal potential followed well when $[\text{Cl}^-]_o$ was changed (Fig. 4, D and E). All these data strongly suggest that the spontaneous postsynaptic currents in amacrine cells are mostly mediated by GABA$_A$ receptors, and that they represent IPSCs. Spontaneous postsynaptic currents with identical properties were also observed in some cells that seemed to have lost their dendrites (3 of 5 cells recorded; Fig. 4B, inset), suggesting that GABAergic synapses are also located on the soma (see Fig. 1F, inset).

Since amacrine cells are the primary and perhaps only sources of GABA in the inner plexiform layer, it is reasonable to assume that most of the spontaneous postsynaptic currents were IPSCs evoked by GABA released from amacrine cells. We further conclude that there is a physiologically relevant network within the inner plexiform layer based on GABA-mediated inhibition between amacrine cells.

**Control of GABA release in presynaptic amacrine cells**

**CONTRIBUTIONS OF SPONTANEOUS ACTION POTENTIALS TO GABA RELEASE FROM AMACRINE CELLS.** Spontaneous postsynaptic currents consisted of two groups: a TTX-sensitive group and a TTX-insensitive group (Fig. 5). The amplitude of TTX-sensitive spontaneous postsynaptic current was large (approximately $>50$ pA at holding potential of $-61$ or $+59$ mV, or $>67$ pA)

**FIG. 5.** Large and medium spontaneous postsynaptic currents were suppressed by TTX. A: effect of $1 \mu M$ TTX on spontaneous postsynaptic currents recorded in an amacrine cell. TTX suppressed large and medium events. Holding potential was $-61$ mV. $[\text{Cl}^-]_o$ 130 mM. B: amplitude histogram of spontaneous postsynaptic currents in the Ringer solution and in TTX solution.

FIG. 4. The spontaneous postsynaptic current was carried mainly by Cl$^-$ ions. A: spontaneous postsynaptic currents recorded at various holding potentials in an amacrine cell. At $-27$ mV (slightly more positive than $E_{\text{Cl}}$, about $-30$ mV), spontaneous inward currents were seen together with the reversed outward currents. $[\text{Cl}^-]_i$ 32 mM. B: mean current-voltage relation at various $[\text{Cl}^-]_i$. Each point shows an average of 2–6 cells. Reversal potential was dependent on $[\text{Cl}^-]_i$; $\ominus$, $32$ mM; $\odot$, $130$ mM. Inset shows an example of mean current-voltage relation from a cell that had lost its dendrite ($[\text{Cl}^-]_i$, $32$ mM). C: relation between $[\text{Cl}^-]_i$ and the reversal potential. The solid line was drawn according to the Nernst equation. Number of experiments are indicated in parentheses. Data from cells that showed spike currents were not included.
FIG. 6. Release of GABA from amacrine cells was Ca$^{2+}$-dependent. A: the spontaneous postsynaptic currents were suppressed reversibly by bath application of 4 mM Co$^{2+}$. Holding potential, –61 mV. [Cl$^{-}$], 130 mM. B: summary of effect of Co$^{2+}$. Mean current in Co$^{2+}$ solution are expressed relative to values in the normal Ringer solution (n = 5).

Release of GABA by the presynaptic amacrine cell is Ca$^{2+}$-dependent. All of the TTX-sensitive large spontaneous postsynaptic currents, medium-sized TTX-sensitive spontaneous postsynaptic currents, and the TTX-insensitive spontaneous postsynaptic current were Ca$^{2+}$-dependent. Application of 4 mM Co$^{2+}$ to the bath suppressed spontaneous postsynaptic currents almost completely (the mean postsynaptic current was suppressed by 91 ± 3%, n = 5, Fig. 6). On the other hand, the frequency and amplitude of the spontaneous postsynaptic currents were enhanced by an application of 10 μM Bay K8644 (data not shown). Thus it is likely that GABA is released by presynaptic amacrine cells in a Ca$^{2+}$-dependent manner. Perhaps GABA release is triggered by Ca$^{2+}$ entering into the presynaptic amacrine cell via L-type Ca$^{2+}$ channels.

Spontaneous release of GABA from the presynaptic amacrine cell is independent of glutamatergic input to the presynaptic cell. As a small fraction of the spontaneous postsynaptic currents are thought to be carried by cation (Fig. 4A), we examined whether spontaneous release of GABA is driven by depolarization of presynaptic amacrine cells induced by the spontaneous glutamatergic input from bipolar cells to the presynaptic cell. In the following experiment, no clear hint suggesting the glutamatergic drive was obtained. Bath application of AP7 (30 μM) and CNQX (2 μM), together with strychnine (2 μM) did not affect the current significantly at –81 or +79 mV, [Cl$^{-}$], 130 mM; Figs. 5, 6, and 8). When 1 μM TTX was perfused, postsynaptic events larger than 50 pA were suppressed (Fig. 5). In addition, medium-sized events (amplitude about 20–50 pA) seemed to be suppressed slightly. Small events remained relatively unaffected. These results suggest 1) that the TTX-sensitive large spontaneous postsynaptic current was generated by the release of a large amount of GABA, driven by an action potential in the presynaptic amacrine cells, 2) that the TTX-sensitive medium spontaneous postsynaptic current was generated by spontaneous GABA release independent of action potentials from the presynaptic amacrine cell, but by mechanism sensitive to TTX (Watanabe et al. 2000), or by dendritic small amplitude action potential (Miller and Dacheux 1976), and 3) that the TTX-insensitive spontaneous postsynaptic current (miniature IPSCs) was generated by spontaneous GABA release (cf. Bieda and Copenhagen 1999). Similar results were obtained in 10 other cells.

Blocking of glutamatergic inputs did not suppress spontaneous GABA release from the presynaptic amacrine cell. To isolate GABA-mediated components, currents were recorded under the bath application of 2 μM strychnine using low Cl$^{-}$ pipette solution. Under this condition, when the holding potential was set at 0 mV, only GABA-mediated spontaneous postsynaptic currents were recorded as the outward current. [Cl$^{-}$] was 10 mM. Bath application of 30 μM AP7 and 2 μM CNQX in the presence of 2 μM strychnine did not block postsynaptic currents (n = 8, Fig. 7). It is therefore likely that the membrane potential of the presynaptic GABAergic amacrine cell fluctuates spontaneously without glutamatergic influence. Spontaneous generation of action potentials in the absence of synaptic inputs was reported in dopaminergic interplexiform amacrine cells dissociated from the mouse retina, and this activity was abolished by GABA or glycine (Feigenspan et al. 1998; Gustinich et al. 1997).

The amplitudes of the small spontaneous postsynaptic currents seemed to be integer multiples of a presumed single quantum amplitude in most cells (69%, 18 of 26 cells). In a cell shown in Fig. 8 the presumed single quantum amplitude was about 16 pA (▲, 16 pA; △, 32 pA; ▲, 48 pA; holding potential, –81 mV). Therefore these events can be miniature IPSCs, reflecting quantal release of GABA. However, we did not perform amplitude histogram analysis in more detail, since these spontaneous currents can be contaminated by glutamatergic and glycinergic inputs in the absence of their specific blockers.

FIG. 7. Blocking of glutamatergic inputs did not suppress spontaneous GABA release from the presynaptic amacrine cell. To isolate GABA-mediated components, currents were recorded under the bath application of 2 μM strychnine using low Cl$^{-}$ pipette solution. Under this condition, when the holding potential was set at 0 mV, only GABA-mediated spontaneous postsynaptic currents were recorded as the outward current. [Cl$^{-}$] was 10 mM. Bath application of 30 μM AP7 and 2 μM CNQX in the presence of 2 μM strychnine did not block postsynaptic currents.

FIG. 8. An example of presumed quantal release of GABA from the presynaptic amacrine cell. Spontaneous postsynaptic currents recorded from an amacrine cell (holding potential, –81 mV). [Cl$^{-}$], 130 mM. Dashed lines are drawn with distances of 16 pA. Amplitudes of the relatively small postsynaptic events seem to be approximately 16 pA or multiples of 16 pA (▲, 16 pA; △, 32 pA; ▲, 48 pA).


**DISCUSSION**

**Postsynaptic inhibition in the amacrine cell**

Anatomical studies have shown that GABAergic cells dominate the amacrine cell population in the goldfish retina (Marc 1992; Marc et al. 1978; Yazulla 1986). Presence of GABA<sub>A</sub> receptors in amacrine cells has been also shown by immunocytochemistry (Lin and Yazulla 1994). Inhibitory GABAergic synapses between amacrine cells have been suggested indirectly by recordings from ganglion cells and directly in some type of amacrine cells (Roska et al. 1998; Zhang et al. 1997). We have demonstrated directly that goldfish amacrine cells not only use GABA as a transmitter, but that most and perhaps all also receive GABAergic input. In the present study we obtained evidence that GABA mediates inhibitory signals between amacrine cells. We recorded GABA-induced currents in amacrine cells of the goldfish retinal slice, and a spontaneous postsynaptic activity that is also mediated by GABA. We infer that both of these responses were mediated by GABA<sub>A</sub> receptors as the currents, carried by Cl⁻, were inhibited by bicuculline. Perforated patch experiments demonstrated that GABA produces hyperpolarizing voltage changes in amacrine cells. In fact, GABA suppressed spontaneous action potentials. Our results demonstrate that GABAergic synapses between amacrine cells likely function in key circuit processes in the goldfish retina, providing a physiological role for anatomically ubiquitous serial synapses.

To understand the role of this postsynaptic inhibition of the amacrine cells in information processing, it will be important to know the distribution of GABA<sub>A</sub> receptors on amacrine cell dendrites and somas. Although the slice preparation is inappropriate to examine the spatial distribution of GABA receptors, we obtained direct evidence that GABA<sub>A</sub> receptors are present not only on the dendritic membrane, but also in the region close to the soma as has been reported in the salamander (Cook and Werblin 1994; Maguire 1999). We recorded GABA-induced and spontaneous postsynaptic currents from cells that had apparently lost their dendrites in the slice preparation. One of the imaginable functions of GABA receptors in the primary dendritic stem or on the soma is to shunt currents generated in the peripheral dendrites, thus isolating every dendrite as an independent site of signal processing. Functioning GABAergic synapses between amacrine cells have been suggested by recordings from ganglion cells in the retina (Roska et al. 1998; Zhang et al. 1997) and reported in a wide-field amacrine cells of the tiger salamander (Roska et al. 1998). Such synapses have been also reported by Gleason et al. (1993) in cultured amacrine cells of the chick retina. We show here that functioning GABAergic synapses are common in the intact retina.

**Control of GABA release from the amacrine cells**

GABA release from presynaptic amacrine cells, as assayed both TTX-sensitive and TTX-insensitive IPSCs, is dependent on Ca<sup>2+</sup> entry, possibly through L-type Ca<sup>2+</sup> channels, as shown for cultured chick amacrine cells (Gleason et al. 1994). Spontaneous release might occur when membrane potential of the presynaptic amacrine cell is depolarized to the activation level of the L-type Ca<sup>2+</sup> channel. In the chick amacrine cell, GABA release was observed only when the presynaptic voltage was depolarized to −40 mV or more under voltage clamp (Gleason et al. 1993). Threshold voltage of L-type Ca<sup>2+</sup> channel is between −60 and −50 mV both in the chick (Fig. 1A of Gleason et al. 1994) and goldfish amacrine cells (unpublished data). As the resting membrane potential of the goldfish amacrine cell was about −60 mV in the present study, depolarization of only a few millivolts would activate L-type Ca<sup>2+</sup> channels. Recently, we reported that goldfish amacrine cells also possess TTX-sensitive persistent current that seemed to be activated about −50 mV (Watanabe et al. 2000). The TTX-sensitive persistent current might contribute to TTX-sensitive medium-sized, action potential–dependent spontaneous postsynaptic currents. There was a CNOX-sensitive component in the spontaneous postsynaptic current (Fig. 3) that might be mediated by cations (inward currents at −27 mV in Fig. 4A). However, it is likely that these spontaneous glutamatergic inputs do not trigger spontaneous release of GABA from presynaptic amacrine cells, because blocking of glutamatergic inputs did not suppress the spontaneous GABA-mediated postsynaptic currents significantly (Fig. 7). Therefore some intrinsic depolarizing mechanism should exist in the presynaptic amacrine cell.

**Role of the action potential in the amacrine cell**

It has been suggested that amacrine cells produce dendritic spikes (Miller and Dacheux 1976) and that the properties of action potentials of amacrine cells are different from those of ganglion cells (Barnes and Werblin 1986). Recently action potential propagation in dendrites has been reported in neocortical pyramidal cells (Stuart and Sakmann 1994), hippocampal pyramidal neurons (Colbert et al. 1997), mitral cells of the olfactory bulb (Bischofberger and Jonas 1997), and rabbit ganglion cells (Velte and Masland 1999). However, the functional significance of dendritic spikes in amacrine cells has not been identified, although there have been several reports that used simulation-based models (Smith and Vardi 1995; Velte and Miller 1997). A recent report by Cook and McReynolds (1998) demonstrated that TTX-suppressible action potentials contribute long distance lateral inhibition. We demonstrated here that spontaneous action potentials contributed to the release of large quantities of GABA from presynaptic amacrine cells to postsynaptic amacrine cells. The release of large quantities of GABA from amacrine cells has also been suggested by responses recorded from ganglion cells in the rat retinal slice (Protti et al. 1997; Tian et al. 1998). Spontaneous action potentials in presynaptic amacrine cells might be evoked by spontaneous depolarization of presynaptic amacrine cells as reported in dopaminergic interplexiform amacrine cells (Feigenspan et al. 1998; Gustiniech et al. 1997) and in some case by spontaneous glutamatergic inputs from bipolar cells (Gao and Wu 1999; Taylor et al. 1995) (see also Fig. 4A).

**Gap junctions between amacrine cells**

Amacrine cells of the identical subtype are shown to be dye coupled (Hidaka et al. 1993; Teranishi et al. 1987; Vaney 1991). Gap junctions and reciprocal GABAergic synapses are also shown between amacrine cells cultured from the chick embryo (Gleason et al. 1993). In our experiments, dye coupling was not observed. Lack of detectable dye coupling suggests...
that coupling may be weak, although it is likely that a smaller probe such as neurobiotin will diffuse into neighboring amacrine cells as reported by Vaney (1991). Through the putative gap junction, action potentials generated in neighboring amacrine cells may invade the recorded cell. This does not necessarily mean that the action potential we recorded in an amacrine cell spread from its neighbors. It is possible, however, that the tonic spread of neighboring action potential helps triggering spontaneous action potentials in the recorded cell.

Another effect of putative gap junctions is to make space clamp incomplete, resulting in incorrect measurement of the reversal potential. In our recording condition, the frequency and amplitude of spontaneous postsynaptic currents were almost the same at the voltages either positive or negative from the reversal voltage in equal amount (cf. Fig. 4A). This finding indicates that most of the postsynaptic membrane was nearly equally polarized from the reversal voltage. We therefore did not account a significant contribution of gap junctions in the present study.

Functional significance of the inhibitory network among amacrine cells

GABAergic input was observed in most amacrine cells recorded, regardless of their morphology. This kind of GABAergic interaction was also observed in mouse amacrine cells in the slice preparation (Kaneda and Kaneko 1998). Therefore it is likely that the GABAergic inhibition system is a common motif in amacrine cell circuits. Similar inhibitory interactions between neurons have been reported in perigeniculate neurons in the dorsal lateral geniculate nucleus (Sanchez-Vives et al. 1997). There are several functional aspects of inhibitory interactions between amacrine cells, possibly common features of interneurons in general, which should be further investigated. 1) It should be determined whether GABAergic inputs between amacrine cells restrict the spread of excitatory information from the bipolar cell, perhaps attenuating propagation of dendritic action potentials in amacrine cells.

2) Interneuronal reciprocal inhibition mediated by GABA is reported to be involved in oscillation mechanisms (Zhang et al. 1998). In the frog retina, Ishikane et al. (1999) reported that oscillation and synchronization of the ganglion cell firings were affected by bicuculline.

3) Amacrine cell inhibitory networks may be subject to modulation, perhaps by activation of metabotropic glutamate receptors (mGlur) as reported for olfactory neurons (Hayashi et al. 1993; Isaacman and Strowbridge 1998), because several types of mGlur have been reported to be located on amacrine cells (Brandstätter et al. 1998).

To fully understand the functional roles of amacrine cells, we have to accumulate further information on the distributions of excitatory and inhibitory inputs, sodium channels, and other ion channels within dendrites and the soma, as well as defining the release sites of various transmitters. However, it is clear that GABAergic serial synapses could have a profound influence on the operations of amacrine cells as lateral interneurons.

We thank Dr. R. Marc for commenting on the manuscript.

This work was supported in part by a grant from Keio Gijuku Academic Development Funds and a Research Grant for Sciences and Medicine from Keio University Medical Fund to S.-I. Watanabe, by a grant from Keio Health Counseling Center Foundation and a Keio University Grant-in-Aid for En-