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Amino acid neurotransmitters in the retina: a functional overview

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Abstract

Physiological and pharmacological mechanisms of glutamatergic, GABAergic and glycinergic synapses in the tiger salamander retina were studied. We used immunocytochemical and autoradiographic methods to study localizations of these neurotransmitters and their uptake transporters; and electrophysiological methods (intracellular, extracellular and whole cell patch electrode recordings) to study the light responses, miniature postsynaptic currents and neurotransmitter-induced postsynaptic currents in various retinal neurons. Our results are consistent with the following scheme: Glutamate is used by the photoreceptor and bipolar cell output synapses and the release of glutamate is largely mediated by calcium-dependent vesicular processes. The postsynaptic glutamate receptors in DBCs are L-AP4 receptors, in HBCs, HCs and ganglion cells are the kainate/AMPA and NMDA receptors. Subpopulations of HCs make GABAergic synapses on cones and gate chloride condunctance through $GABA_A$ receptors. GABAergic HCs do not make feedforward synapses on bipolar cell dendrites and the neurotransmitter identity of the HCs making feedforward synapses is unknown. Subpopulations of amacrine cells make GABAergic synapses on bipolar cell synaptic terminals, other amacrine cells and ganglion cells and GABA gates chloride conductances in theses cells. Glycinergic amacrine cells make synapses on bipolar cell synaptic terminals, other amacrine cells and ganglion cells and glycine opens postsynaptic chloride channels. Glycinergic interplexiform cells make synapses on bipolar cells in the outer retina and glycine released from these cells open chloride channels in bipolar cell dendrites.

Keywords: Glutamate; Y-aminobutyric acid (GABA); Glycine; Synaptic transmission; Bipolar cells

1. Introduction

In the vertebrate retina, three amino acids, L-glutamate, gamma-aminobutyric acid (GABA) and glycine are the principal neurotransmitters. Synapses containing these three neurotransmitters are found in the retinas of all vertebrates and they account for more than 90% of retinal synapses (Marc RE, personal communication). Although other neurotransmitters, such as acetylcholine, dopamine and serotonin, are used by retinal neurons and play key functional roles, they constitute a small fraction of all retinal synapses and are not ubiquitous in all vertebrate species [1,2].

A central feature of synaptic organization in the retina is the center-surround antagonistic receptive field (CSARF) [3–5]. Cells exhibiting CSARFs, such as bipolar cells and ganglion cells, receive central and lateral (surround) synaptic inputs of opposite polarities and the CSARFs exhibited by these cells are the fundamental units of spatial information encoding in the visual system [6,7]. The vast majority of synapses mediating center inputs to bipolar cells and ganglion cells are glutamatergic; and those mediating lateral synapses are GABAergic and glycinergic, although neurotransmitter identities of several lateral synapses are yet to be determined [8–12].

The three principal amino acid neurotransmitters appear to present a relatively simple synaptic organization in the retina. However, when one looks in detail at the molecular and physiological mechanisms of these amino acid synapses, a great deal of sophistication and complexity is apparent. In recent years, an explosion of new information has emerged that reveal molecular structures of individual receptors, channels and transporters in various neurotransmitter systems. For example, more than 30 genes and splicing variants for glutamate receptors have been identified [13] and they encode at least five physiological distinct classes of glutamate receptors, all of which have been found in

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vertebrate retinas [14–16]. There are at least three classes of GABA receptors in the retina, each exhibiting distinct pharmacological properties and physiological function [17–21]. The number of glycine receptor classes is uncertain, but recent studies have suggested at least two types of glycine receptors in the retina [22,23]. The molecular biology of neurotransmitter receptors in the retina is dealt with in other articles in this symposium. In this article, we present our recent results on the physiological properties of glutamatergic, GABAergic and glycinergic synapses that mediate central and lateral inputs of bipolar cells and ganglion cells using three different approaches. First, we used immunocytochemical and autoradiographic techniques to localize glutamatergic, GABAergic and glycinergic neurons and their uptake transporters in the retina. Secondly, we recorded miniature postsynaptic currents and used them to study mechanisms of transmitter release. Thirdly, we examined the effects of glutamate, GABA, glycine and their agonist and antagonists on the light-evoked responses and membrane conductances in bipolar cells and ganglion cells. These results provide a description of how amino acid neurotransmitter synapses mediate functional pathways such as the CSARFs in the retina. They also serve as a physiological guide for molecular biological studies of amino acid neurotransmitter systems in the retina.

2. Methods

Larval tiger salamanders (*Ambystoma tigrinum*) were used in this study. Detailed procedures of preparing flat-mounted isolated retinas and retinal slices, immunocytochemistry and autoradiography are described in previous publications [9,24–26]. For light response experiments, dissection and recordings were done under infrared illumination. The control Ringer's contained 108 mM NaCl, 2.5 mM KCl, 1.2 mM $MgCl_2$, 2 mM $CaCl_2$, 5 mM Hepes (adjusted to pH 7.7). All chemicals were dissolved in control Ringer's, except for those used in experiments with Co^{2+} Ringer's (in which all calcium ions were replaced by cobalt).

In voltage clamp experiments, patch electrodes of 5 MΩ tip resistance when filled with internal solution containing 69 mM Cs_2 SO_4, 15.3 CsCl, 2 mM EGTA, 1 mM $MgCl_2$, 0.1 mM $BaCl_2$, 1 mM ATP, 0.5 mM GTP, 1 mM Hepes, 0.8 mM Lucifer yellow, adjusted to pH 7.2 with CsOH were made with Narishige or Kopf patch electrode pullers. Axopatch 200 amplifiers were used and data aquisition and analysis were performed with the pClamp 6.1 software (Axon Instruments, Ca). Intracellular recordings were made with micropipettes drawn with a modified Livingston puller with Omega Dot tubing (1.0- and 0.5-mm i.d.). The micropipettes were filled with 2 M potassium acetate and had tip

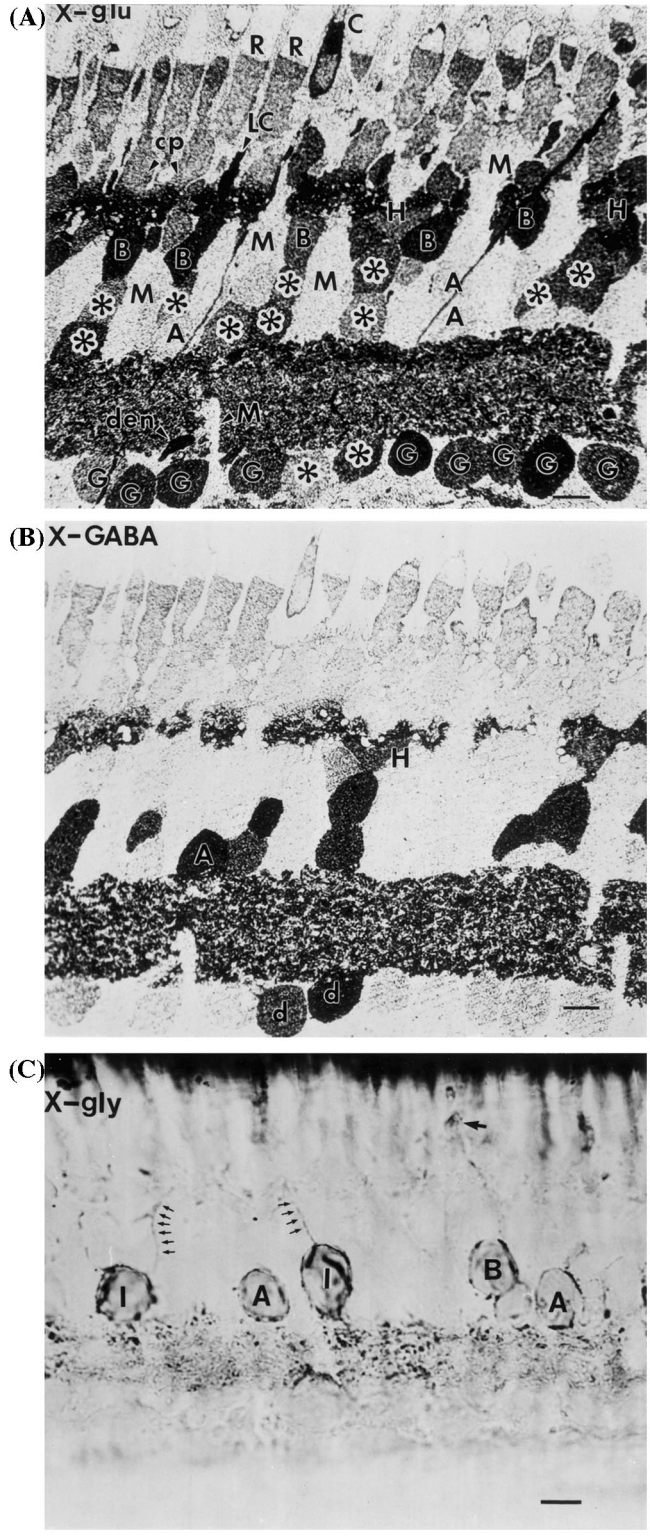

Fig. 1. A montage demonstrating glutamate (x-glu, A), GABA (x-GABA, B) and glycine (x-gly, C) immunoreactivity in the tiger salamander retina. A and B are two consecutive 0.5 μm sections and C is a 2 μm section from a different retina. R: rods; C: cones; cp: cone pedicle; LC: Landolt club; M: Muller cells; H: horizontal cells; B: bipolar cells; A: amacrine cells; I: interplexiform cells; G: ganglion cells; d: displaced amacrine cells or ganglion cells; *: GABAergic cells; den: ganglion cell dendrites; axons: bundles of ganglion cell axons. Calibration bars = 10 μm.

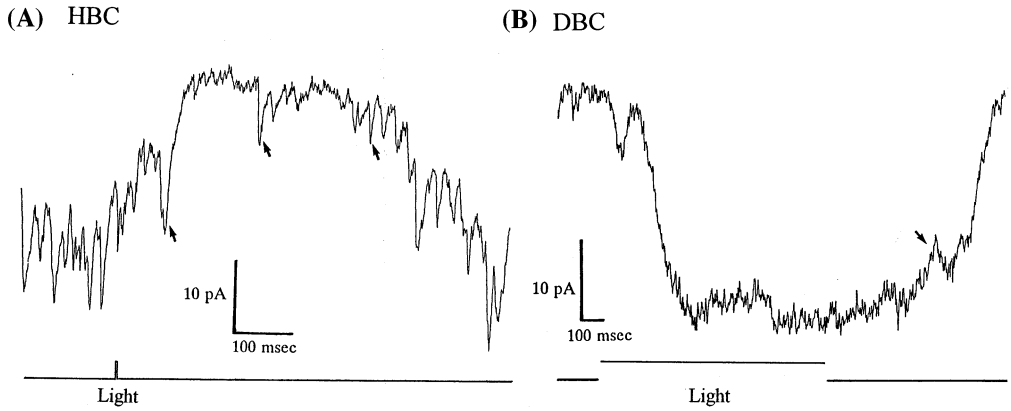

Fig. 2. Whole cell current responses of a HBC (A) and DBC (B) to light stimuli. The holding potential was set to E_{Cl}, which equals to -40 mV. Light elicited an outward current in the HBC and an inward current in the DBC. Arrows show discrete miniature postsynaptic currents.

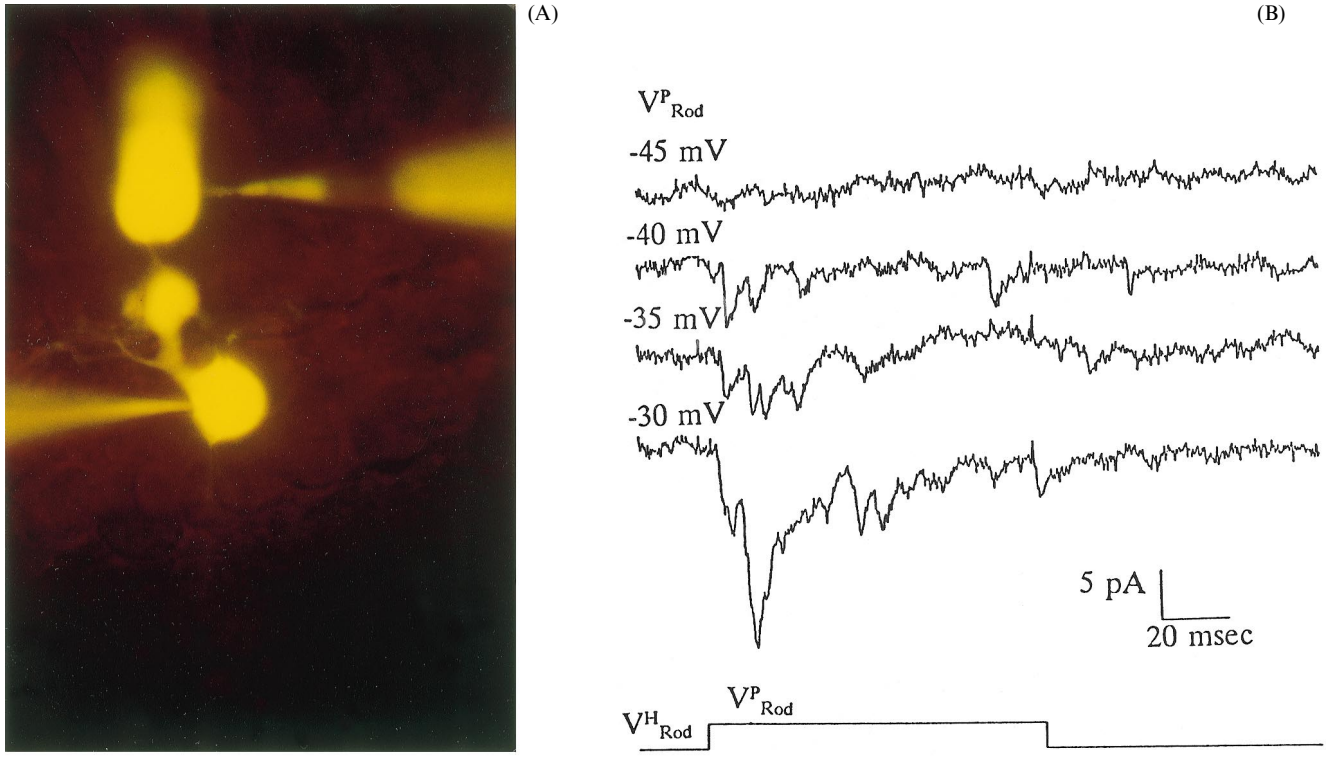

Fig. 3. (A) Simultaneous voltage clamp of a rod and a HBC with two electrodes filled with Lucifer Yellow. (B) Current responses of HBC to depolarizing voltage clamp steps in the rod. The HBC was voltage-clamped at -40 mV ($=E_{Cl}$) and the rod voltage was stepped from a holding voltage (V^H_{Rod}) of -50 mV to various pulse voltages (V^P_{Rod}). The probability of MEPC occurrance increased progressively as the rod was depolarized, suggesting MEPCs result from discrete packages of neurotransmitters from rods.

resistances measured in Ringer's solution of 100–600 MΩ. Voltage responses of retinal neurons were digitized and analyzed with the Biopak and Spike II computer A–D systems [27].

The flat-mounted isolated retina was stimulated with a dual-beam photostimulator. In most experiments described, large-field illumination (600–1200 μm in diameter) was used. For bipolar cells, the center stimulus was a 500 μm light spot and the surround stimulus was a light annulus of 650 μm inner diameter and 1200 μm outer diameter. The intensity of light sources was measured with a radiometric detector (United Detector Technology, Santa Monica, CA). The intensity of unattenuated 500 nm light (log I = 0) was 2.05×10^7 photons $\mu m^{-2}\ s^{-1}$.

3. Results

3.1. Localization of glutamatergic, GABAergic and glycinergic neurons in the retina

Fig. 1 shows glutamate (A), GABA (B) and glycine (C) immunoreactivity of the tiger salamander retina. A

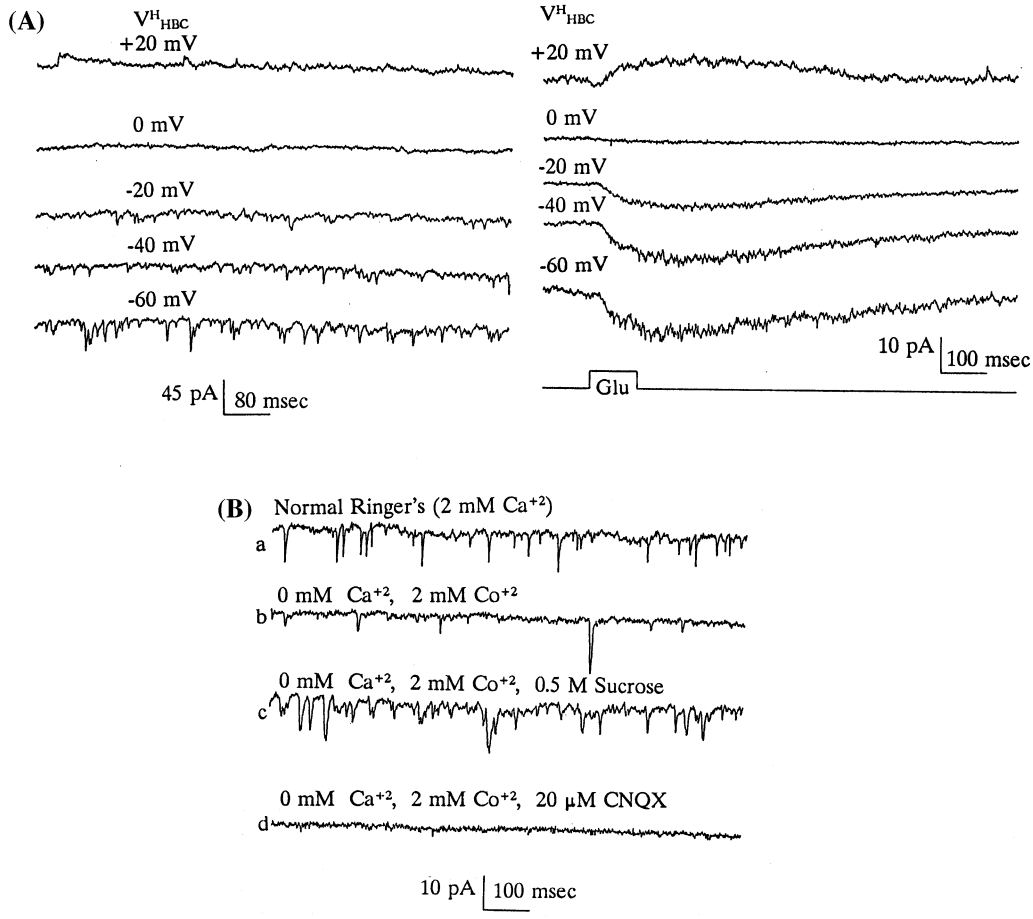

Fig. 4. (A left) Spontaneous MEPCs recorded from a HBC under voltage clamp conditions at various holding potentials (V^H_{HBC}). The MEPCs reversed at 0 mV. (A right) Current responses of a HBC to focal applications of glutamate (100 μM in a puff pippette) at the HBC dendritic region while the HBC was held at various potentials (V^H_{HBC}). The glutamate-induced currents also reversed at 0 mV. (B) MEPCs recorded from a HBC in (a) normal Ringer's; (b) Ringer's with Co^{2+} substituted for Ca^{2+}; (c) hyperosmotic Co^{2+} Ringer's and (d) Co^{2+} Ringer's with 20 μM CNQX, a kainate/AMPA glutamate receptor antagonist.

and B show two 0.5-μm consecutive sections and C is a 2-μm section from a different retina. Since neurons in the tiger salamander retina are typically 5–12 μm in diameter, the two consective sections in A and B are from the same cells. We studied glutamate and GABA immunoreactivity in the same cells because glutamate is a metabolic precursor of GABA and thus glutamate immunoreactivity is high in both glutamatergic and GABAergic neurons. For this reason, the location of glutamatergic cells was determined by subtracting GABA-immunoreactive cells in Fig. 1B from glutamate-immunoreactive cells in Fig. 1A (marked with asterisks). The result of such subtraction indicates that rods, cones, bipolar cells and ganglion cells are glutamatergic. GABA immunoreactivity is seen primarily in horizontal cells (H) and amacrine cells (* or A). The GABA immmunoreactive cells in the ganglion cell layer (d in Fig. 1B) are either displaced amacrine cells or GABAergic ganglion cells. The entire inner plexiform layer is stained. It is important to notice that not all HCs or amacrine cells are GABAergic, 30–50% of HCs and 40–60% of amacrine cells in the tiger salamander retina are not stained with GABA antibodies.

Fig. 1C shows that glycine immunoreactive cells include amacrine cells, interplexiform cells (with ascending processes from the somata, small arrows in Fig. 1C) and bipolar cells (with Landolt clubs, thick arrow in Fig. 1C). The entire inner plexiform layer is stained, but the pattern is much more sparse then the sections stained with the GABA antibody. This result suggests that subpopulation of amacrine cells and interplexiform cells in the tiger salamander retina are glycinergic. It is not clear if bipolar cells stained with glycine antibody actually use glycine as their neurotransmitter. Most, if not all, bipolar cell output synapses are excitatory. It is possible that glycine leaks into some bipolar cells through gap junctions with adjacent glycinergic amacrine cells [28,29].

In summary, immunocytochemical results shown above are in agreement with evidence obtained from other vertebrate retinas [30,18]: glutamate is the neurotransmitter for rod and cone photoreceptors, bipolar

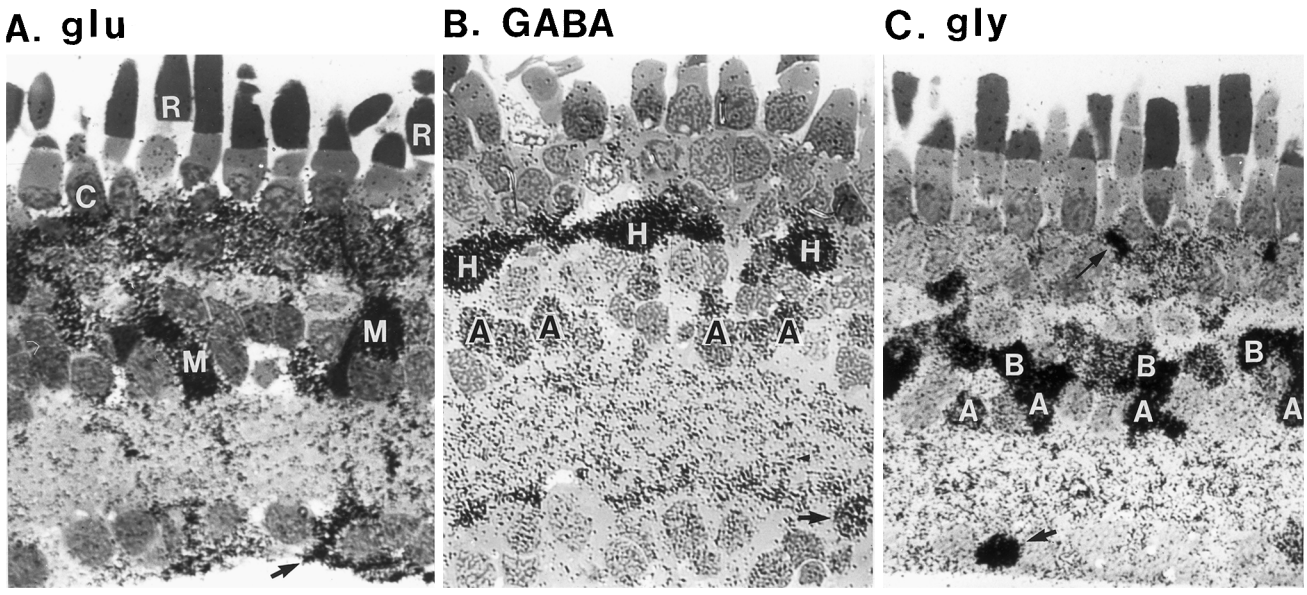

Fig. 5. Autoradiograms demonstrating localization of high-affinity uptake transporters of glutamate (A), GABA (B) and glycine (C) in the tiger salamander retina. Retinas were incubated with 15 μM [^{3}H]-glutamate, [^{3}H]-GABA or [^{3}H]-glycine. R: rods; C: cones; M: Muller cells; H: horizontal cells; A: amacrine cells; B: bipolar cells; thick arrows: displaced amacrine cells or ganglion cells; thin arrow: bipolar cell Landolt club. Calibration bars = 20 μm.

cells and ganglion cells; GABA is the neurotransmitter for a subpopulation of horizontal cells and some amacrine cells and perhaps a small population of ganglion cells; and glycine is the neurotransmitter for a subpopulation of amacrine cells and some interplexiform cells. It is important to point out that cells with these three neurotransmitters account for about 80–90% of the total neurons in the retina. Information processing in the vertebrate retina is predominantely mediated by synapses using these amino acids.

3.2. Mechanisms of neurotransmitter release and miniature postsynaptic currents

Retinal neurons that exhibit hyperpolarizing light responses, such as photoreceptors, HCs, HBCs and sustained off amacrine cells, release neurotransmitters in darkness [31,32]. Cells that give rise to depolarizing light responses, such as DBCs, sustained on amacrine cells, transient on–off amacrine cells and perhaps interplexiform cells, release neurotransmitters in light [33–35]. The majority of retinal synapses contain synaptic vesicles and neurotransmitter release appears calcium-dependent [36,37,32]. In the following set of experiments, we show that discrete miniature postsynaptic currents are observed in bipolar cells, supporting the idea of vesicular glutamate release from photoreceptors [32]. Each discrete miniature postsynaptic current results from openings (in the case of HBCs) or closings (in the case of DBCs) of cation channels gated by glutamate released from a cluster of one or more synaptic vesicles [37]. Fig. 2 shows light responses of a HBC and a DBC under voltage clamp conditions. Discrete miniature postsynaptic currents (arrows) were seen in both kinds of cells, but were more easily observed in HBCs. We therefore concentrated our study on the miniature excitatory postsynaptic currents (MEPCs) in HBCs. The effect of presynaptic depolarization on MEPCs were studied by simultaneously voltage clamping rods and HBCs with two patch electrodes in retinal slices (Fig. 3A). Fig. 3B shows the current responses of a HBC to depolarizing voltage clamp steps in a rod. The HBC responded to depolarization of the rod with an increase of the MEPC frequency, consistent with the idea of voltage-dependent vesicular neurotransmitter release from the rod. Fig. 4A shows that spontaneous MEPCs of the HBC reversed near 0 mV (left portion), the reversal potential for responses to focal application of 100 μM glutamate at the dendrites of the HBC (right portion). This suggests that theMEPCs represent a glutamatergic synaptic input from photoreceptors. In Fig. 4Bb, we show that the frequency of MEPCs decreased about 10-fold in the presence of Ringer's containing 0 Ca^{2+} and 2 mM Co^{2+}, suggesting that MEPCs are mediated by calcium-dependent mechanisms [38]. In Co^{2+} Ringer's the MEPC frequency increased about 4-fold when the osmolarity of the solution was increased by the addition of 0.5 M sucrose (Fig. 4Bc). Similar behavior was observed at the frog neuromuscular junction where hyperosmotic pressure increased the frequency of the spontaneous quantal release [39]. MEPCs were abolished by 20 μM CNQX, a kainate/AMPA receptor antagonist [40] (Fig. 4Bd). Together, these results support the idea that synaptic transmission

from photoreceptors to HBCs is mediated by calcium-dependent vesicular release of glutamate that activates cation channels (with a current reversal potential near 0 mV) in HBCs.

In addition to the MEPCs at the photoreceptor-bipolar cell synapses, we have also observed glutamatergic MEPCs in ganglion cells [41], glycinergic miniature inhibitory postsynaptic currents (MIPCs) in bipolar cells and ganglion cells and GABAergic MIPCs in ganglion cells [42,43]. All miniature postsynaptic currents are calcium-dependent, suggesting that many of the glutamatergic, GABAergic and glycinergic synapses in the retina release neurotransmitters in a quantal, vesicular manner.

3.3. Neurotransmitter uptake transporters

The actions of amino acid neurotransmitters in the synaptic cleft, unlike the actions of acetylcholine, are not terminated by enzymes that convert active transmitters into inactive molecules. They are removed from the synaptic cleft by uptake transporters [44]. The cellular location of the uptake transporters have been studied by autoradiography of [^{3}H] labeled neurotransmitters [8,45,11]. Fig. 5 shows autoradiographs of retinal sections of the tiger salamander incubated with [^{3}H]glutamate (A), [^{3}H]-GABA (B) and [^{3}H]glycine (C). Muller cells and photoreceptors contain uptake transporters for glutamate; subpopulations of HCs and amacrine cells contain uptake transporters for GABA; and subpopulations of amacrine cells and bipolar cells appear to contain uptake transporters for glycine.

It is important to notice that the cellular localization of GABA and glycine uptake transporters is very similar to that of GABA and glycine immuno-staining (Fig. 1B,C). This suggests that most GABAergic or glycinergic neurons also contain uptake transporters for their respective neurotransmitter. Glycinergic interplexiform cells may contain glycine uptake transporters, but they could not be identified by light microscope autoradiography. The cellular localization of glutamate transporters, on the other hand, is different from that of the glutamate immuno-staining (Fig. 1A). Although both were observed in rod and cone photoreceptors, glutamate transporters have not been seen in bipolar cells and ganglion cells that are glutamate immunoreactive. Muller cells, which are not glutamate immunoreactive, contain more glutamate transporters than any other cell in the retina. It seems that for GABAergic and glycinergic synapses in the retina, neurotransmitters are removed from the synaptic cleft by transport into the presynaptic neuron; whereas for glutamatergic synapses, neurotransmitters are removed from the synaptic cleft by transport either into the presynaptic neurons and/or into other surrounding cells.

Amino acid neurotransmitter transporters in the retina are largely electrogenic and they have been extensively studied by electrophysiological methods [46,47]. Recent studies have revealed that the transporter for each neurotransmitter can be divided into several subtypes (see Eliasof et. al. In this volume). The molecular structures of these transporter proteins are under investigation.

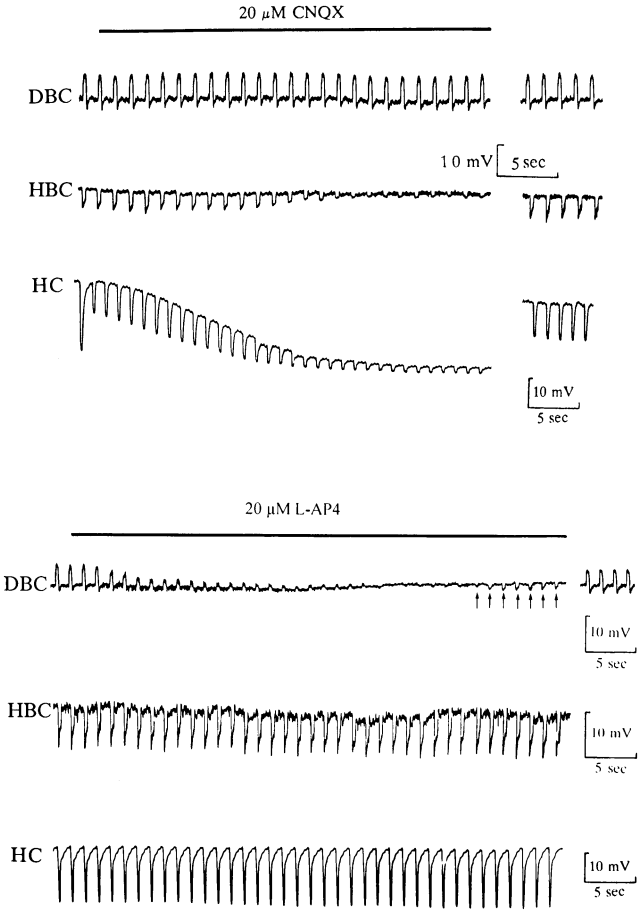

Fig. 6. Effects of 20 μM CNQX and 20 μM L-AP4 on light responses of DBC, HBC and HC. 20 μM CNQX suppressed the light reponses of the HBC and HC without affecting the DBC; and 20 μM L-AP4 suppressed the ligt responses of the DBC and converted them into hyperpolarizing responses (arrows) without affecting light responses of the HBC and HC.

3.4. Glutamatergic synapses

In darkness, glutamate is released continously from rods and cones and it depolarizes the horizontal cells (HCs) and hyperpolarizing bipolar cells (HBCs) by opening cation channels through AMPA/kainate receptors [48,49]. It hyperpolarizes the depolarizing bipolar cells (DBCs) by closing cation channels through L-AP4 receptors and a cGMP cascade [50–52]. This scheme is supported by our studies of the effect of glutamate receptor analogs on bipolar and horizontal cell light responses shown in Fig. 6. CNQX, a specific AMPA/kainate receptor antagonist, at 20 μM, suppresses the

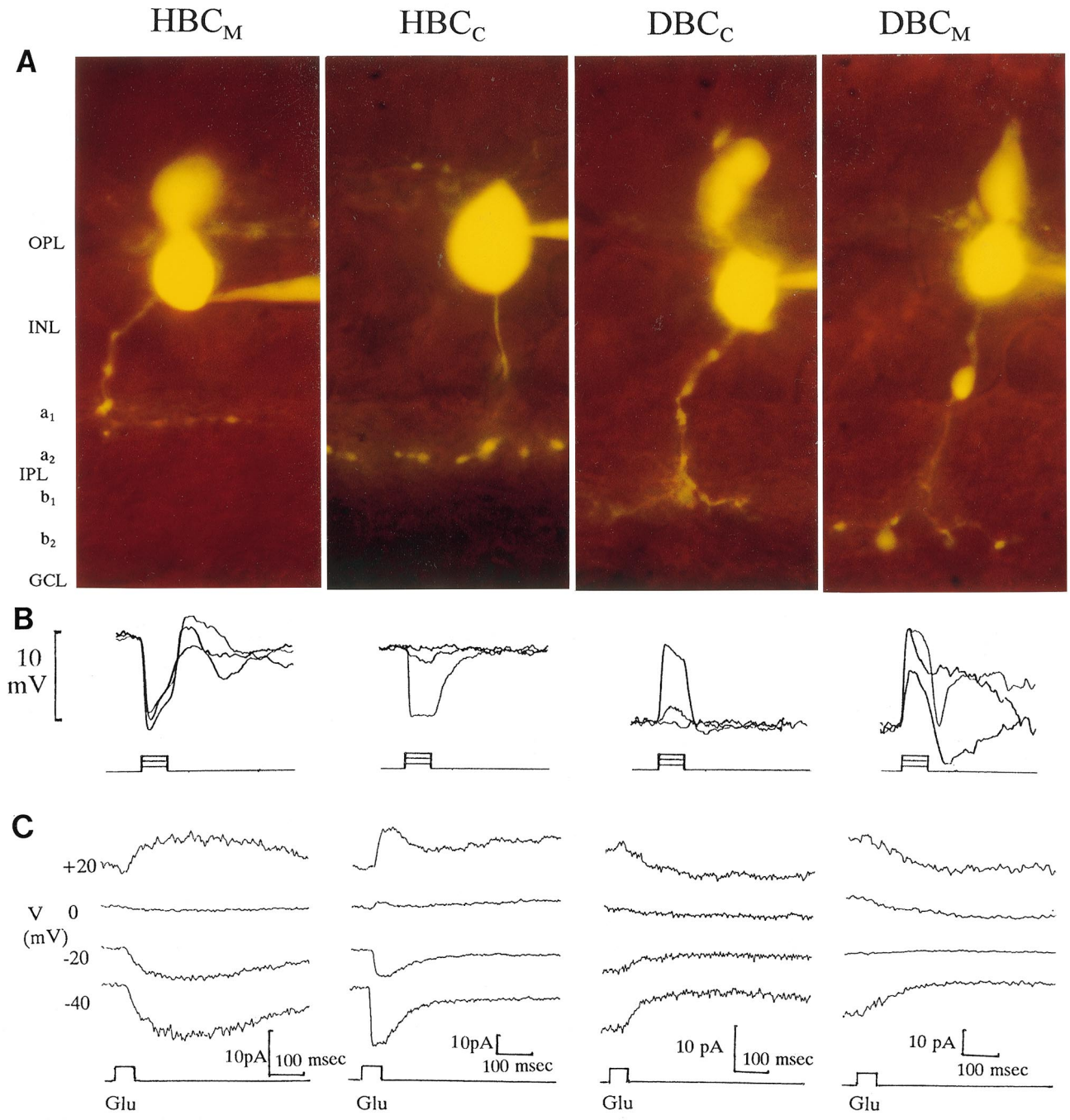

Fig. 7. (A) Lucifer Yellow-filled HBC$_M$, HBC$_C$, DBC$_C$ and DBC$_M$ in retinal slices of the tiger salamander. OPL: outer plexiform layer; INL: inner nuclear layer; IPL: inner plexiform layer; GCL: ganglion cell layer. Axon terminals of the HBC$_M$, HBC$_C$, DBC$_C$ and DBC$_M$ ramified at sublamina a1, a2, 21 and b2 respectively. (B) Voltage responses of HBC$_M$, HBC$_C$, DBC$_C$ and DBC$_M$ to 1 s 500 nm light steps of -6.3; (5.3 and -4.3 log unit attenuation. (C) Current responses to focal application of glutamate (100 μM in puff pippette) at bipolar cell dendrites recorded under voltage clamp conditions at various holding potentials from HBC$_M$, HBC$_C$, DBC$_C$ and DBC$_M$.

light responses of the HC and HBC, without affecting the DBC light responses; and 20 μM L-AP4 suppresses the light responses of the DBC, without affecting the HC and HBC.

Further studies examined the glutamate-induced postsynaptic currents in various types of bipolar cells in living slices of the tiger salamander retina. Bipolar cells were voltage clamped using patch electrodes filled with Lucifer yellow and their morphology is shown in Fig. 7A. We have shown in a previous study that bipolar cells, either the HBCs or the DBCs, could be divided into two types, one receiving mixed inputs from rods and cones and the other receiving only cone inputs. We named them HBC$_M$, HBC$_C$, DBC$_M$ and DBC$_C$ (Yang

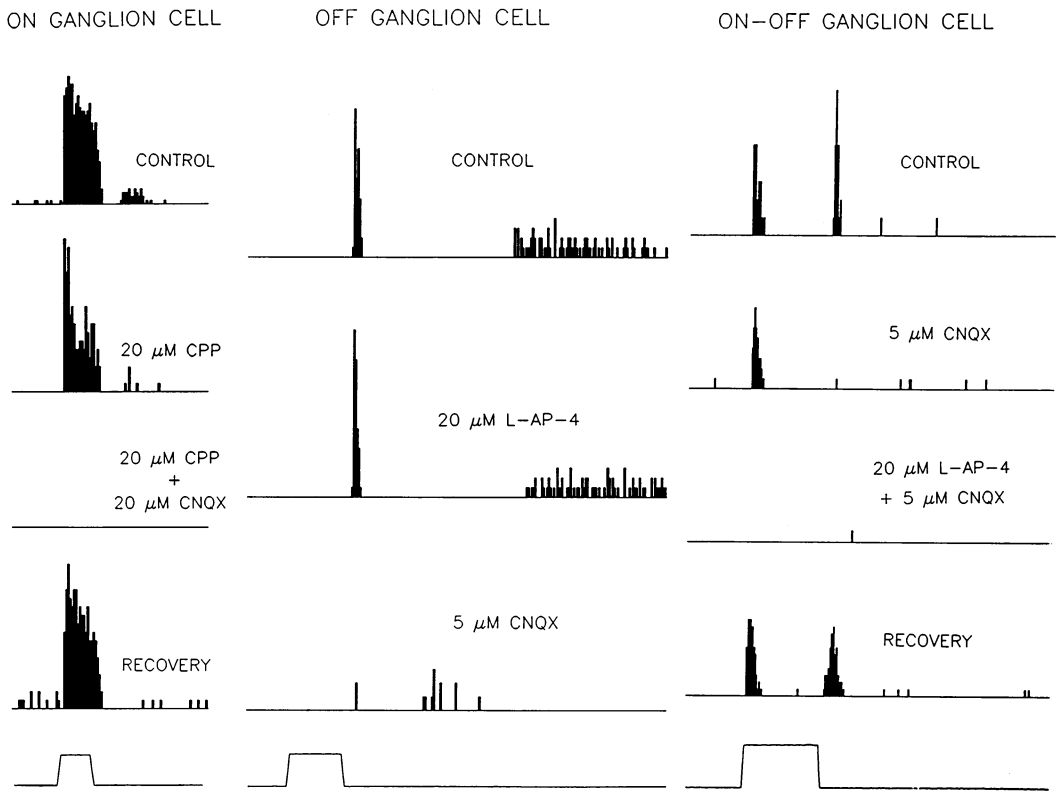

Fig. 8. Peristimulus histograms showing the effects of 20 μM CPP, 20 μM CPP + 20 μM CNQX on a sustained ON ganglion cell (left), the effects of 20 μM L-AP4 and 5 μM CNQX on a sustained OFF ganglion cell (middle) and the effects of 5 μM CNQX and 20 μM L-AP4 + 5 μM CNQX on a ON–OFF ganglion cell. Histograms are an average of 10 stimulus presentations and the binwidth of the histograms is 10 ms.

and Wu, in preparation). Under dark-adapted conditions, mixed bipolar cells were driven primarily by the rods and they exhibited much higher light sensitivity than cone-driven bipolar cells, as shown in Fig. 7B. Preliminary studies suggested these four types of bipolar cells had different morphology, as shown by the Lucifer yellow data in Fig. 7A. The axon telodendrites of the HBC_M s ramified in sublamina a1, those of the HBC_C s ramified in sublamina a2, those of the DBC_Ms ramified in sublamina b2, and those of the DBC_Cs ramified in sublamina b1 of the inner plexiform layer. In Fig. 3C, we show the current responses induced by focal application of glutamate at the dendrites of four bipolar cells exhibiting the four patterns of axon ramification in 3A. For both HBC_M and HBC_C, glutamate induced an inward current at negative potentials and it reversed around 0 mV, indicating that glutamate increased membrane conductance in these cells. For the DBC_M and DBC_C, glutamate induced an outward current associated with a conductance decrease and an average reversal potential about -11 mV.

In the inner retina, glutamate is released from bipolar cells and it activates AMPA/kainate and NMDA receptors in amacrine cells and ganglion cells [53,54]. There are three major types of ganglion cells in the tiger salamander retina, the sustained ON, the sustained OFF and the transient ON–OFF cells.

Fig. 8 shows the effects of various glutamate receptor antagonists and agonists on the single unit extracellular light responses of the three types of ganglion cells. The light offset responses of OFF and ON–OFF ganglion cells were completely blocked by concentrations of CNQX as low as 5 μM. The light onset responses of ON–OFF ganglion cells were blocked when the concentration of CNQX was raised to 20 μM. In addition, 20 μM CPP (a NMDA receptor antagonist) partially blocked the light onset responses of ON–OFF ganglion cells but had less effect on the light offset responses. LAP-4 at 20 μM blocked the light onset responses (because these responses were mediated by DBCs and LAP-4 blocked DBC light responses (Fig. 2B)) but had no effect on the light offset responses.

3.5. GABAergic synapses

Fig. 1B suggests that subpopulations of horizontal cells and amacrine cells in the tiger salamander retina use GABA as the neurotransmitter. In some bipolar cells, surround responses were blocked by application of GABA, although the exact percentage and subtypes of such cells are yet to be determined [8]. Fig. 9A shows a HBC whose surround response were suppressed by 2 mM GABA. This result is consistent with the idea that GABA is involved in the HC or/and amacrine cell

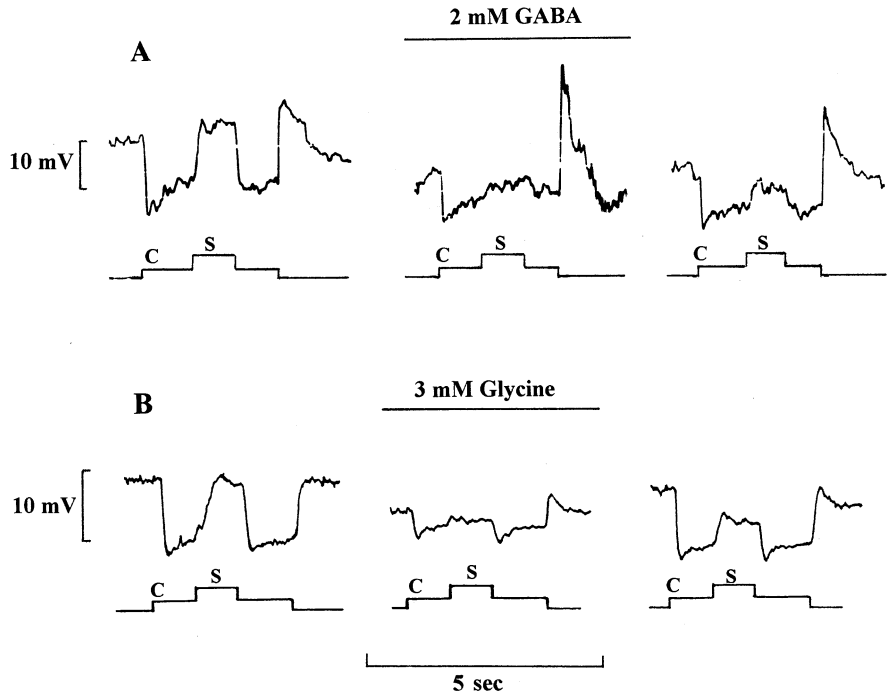

Fig. 9. Effects of 2 mM GABA (A) and 3 mM glycine (B) on the surround light responses of HBCs. Voltage responses of HBCs to a center light spot of 500 μm diameter (C) and light annulus of 650 μm inner diameter and 1200 μm outer diameter (S). Both GABA and glycine suppressed the surround responses of the HBCs.

synapses that mediate surround light responses of bipolar cells. Nevertheless, in many other HBCs or DBCs, GABA had no effect on the surround responses [55,8].

Horizontal cells make feedback synapses on cone photoreceptors [56,57] and feedforward synapses on bipolar cell dendrites [58,59]. By using the truncated cone preparation, we found that the feedback signal from HCs to cones was suppressed by 100 μM bicuculline, suggesting that the signal is mediated by $GABA_A$ receptors in cones. The feedback signal had a reversal potential near -65 mV, indicative of the involement of chloride conductance [56].

In order to determine whether GABAergic HCs are involved in feedforward synapses between HCs and bipolar cells, we studied the effects of focally applied GABA on bipolar cells in living retinal slices. Fig. 10A shows the postsynaptic currents recorded under voltage clamp conditions from a HBC and a DBC to focal applications of GABA on the dendrites (location 1) and axon terminals (location 2). These recordings were made from bipolar cells in Co^{2+} Ringer's (with 0 Ca^{2+}) so that presynaptic actions of GABA were blocked. GABA elicited no postsynaptic actions when applied to the bipolar dendrites, but it elicited large postsynaptic currents that reversed near the chloride equilibrium potential ($E_{Cl} = -50$ mV) when applied to the axon terminals. These data suggest that GABA receptors exist on bipolar cell axon terminals, but not on their dendrites. We therefore conclude that GABAergic HCs do not make feedforward synapses on bipolar cells and that GABAergic amacrine cells make feedback synapses on the axon terminals of most bipolar cells.

3.6. Glycinergic synapses

In about 40–60% bipolar cells, surround responses were blocked by application of glycine. Fig. 9B shows a HBC whose surround response were suppressed by 3 mM glycine. This result is consistent with the idea that glycine is involved in amacrine cell synapses that mediate the surround light responses of subpopulations of bipolar cells. Other HBCs or DBCs, were not glycine sensitive. We did not study the effects of GABA and glycine on surround responses of the same bipolar cells, so we do not know whether bipolar cells with GABA-sensitive surrounds also have a glycine-sensitive surround, or that the two types of bipolar cells are mutually exclusive.

Since subpopulations of amacrine cells and interplexiform cells in the tiger salamander retina are glycinergic, we examined whether glycinergic cells are involved in feedback synapses between amacrine cells and bipolar cells in the inner retina and the synapses between interplexiform cells and bipolar cells in the outer retina. Fig. 10B shows the postsynaptic currents recorded under voltage clamp conditions from a HBC and a DBC to focal applications of glycine on the dendrites (location 1) and axon terminals (location 2). These recordings were made from bipolar cells in Co^{2+} Ringer's

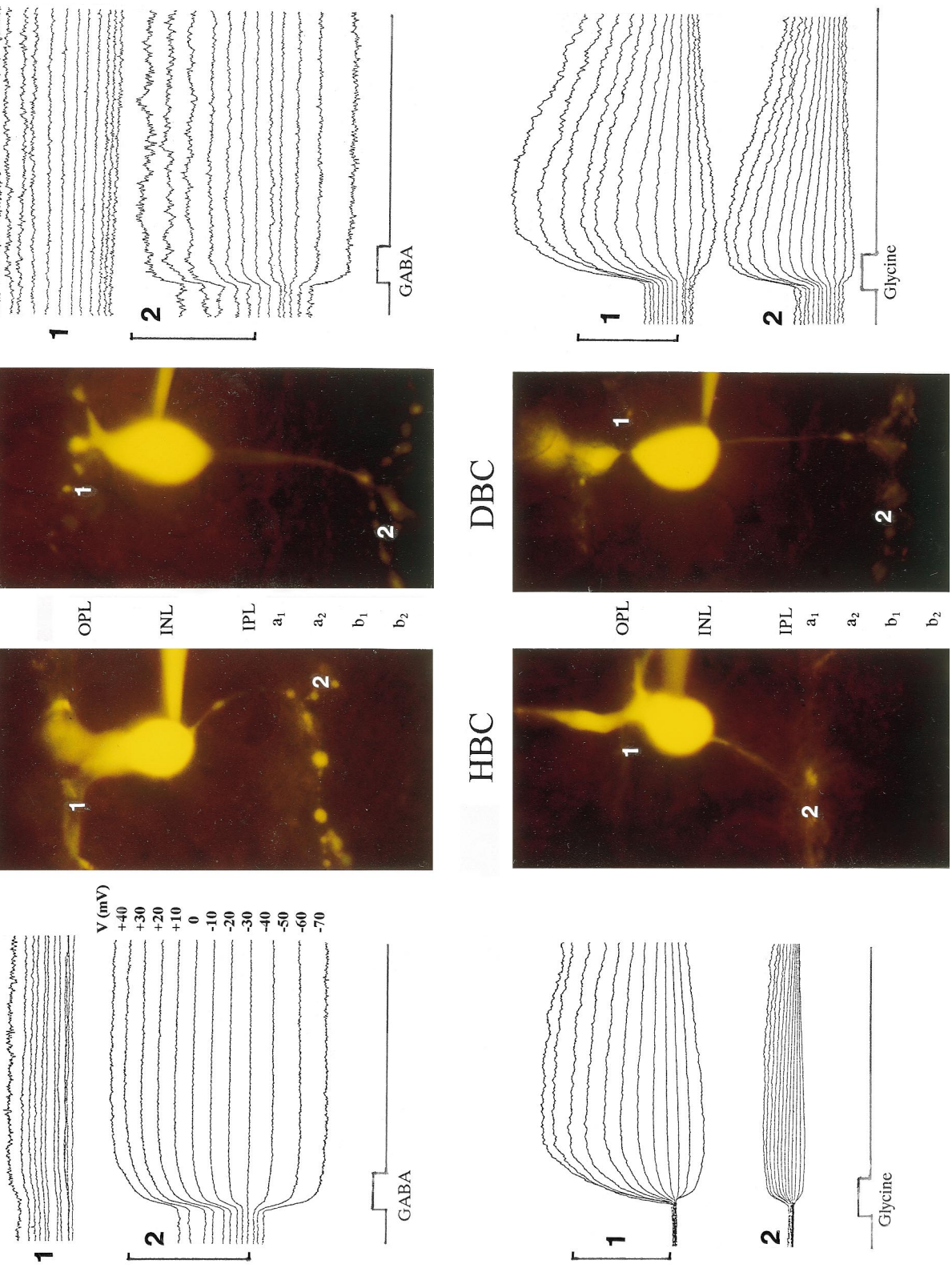

Fig. 10. Current responses to 100 m focal application of GABA (upper portion) and glycine (lower portion) (100 μM in puff pipettes) at the dendrites (location 1) and axon terminals (location 2) of the HBCs and DBCs. The cells were held at potentials from −70 to 40 mV with 10 mV increments under voltage clamp conditions with electrodes filled with Lucifer Yellow in Co^{2+} substituted Ringer's. GABA induced no current response at bipolar cell dendrites and elicited large current responses that reversed at E_{Cl} ($E_{Cl} = -50$ mV) when applied at the axon terminals. Glycine elicited current responses that reversed at E_{Cl} when applied at either the dendrites or axon terminals.

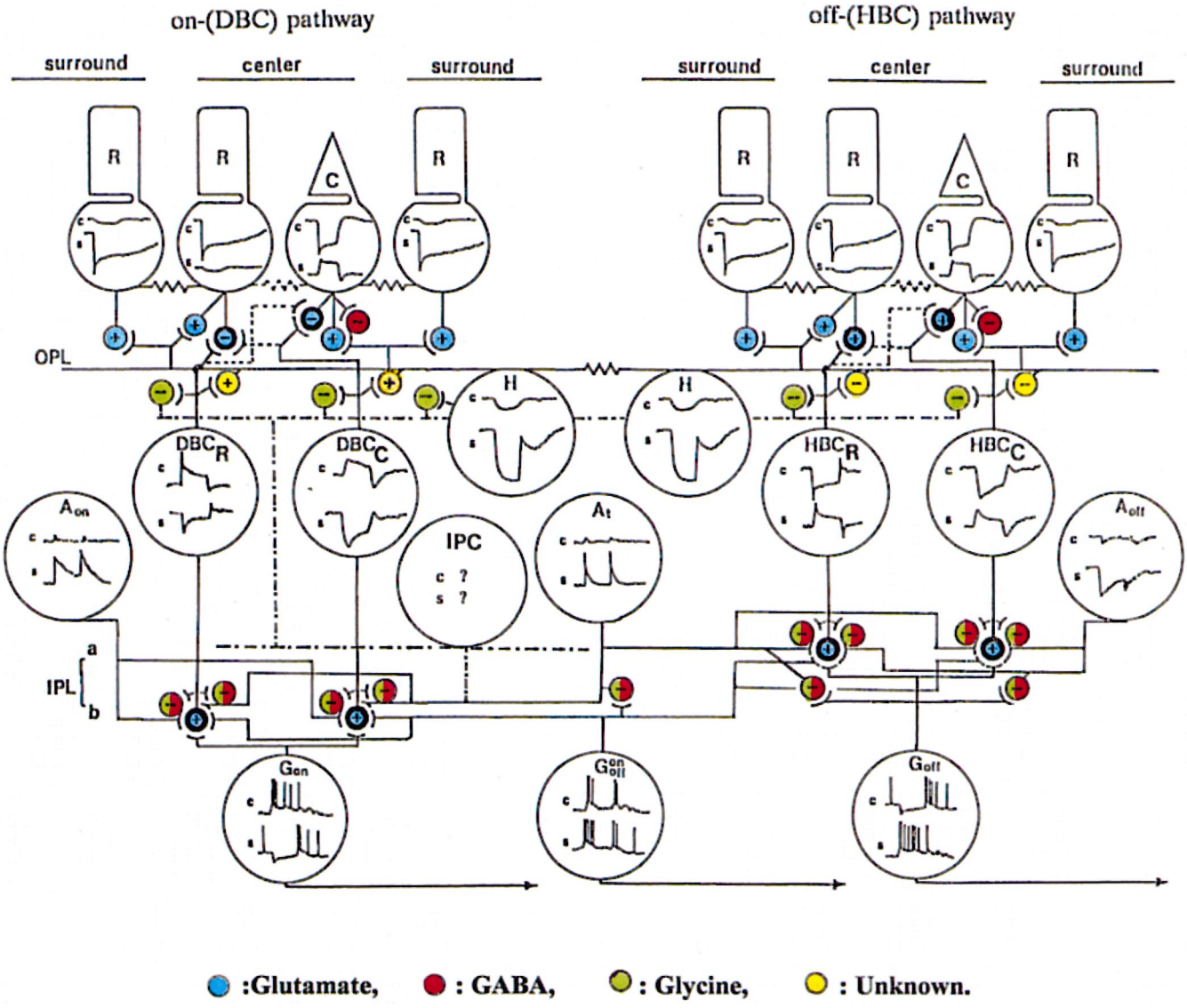

Fig. 11. Summary schematic diagram of major synapses that mediate CSARF of bipolar cells and ganglion cells in the retina. The left portion shows the on- or DBC- pathway and the right portion shows the off- or HBC-pathway. The upper trace in each cell is the voltage response to center illumination and the lower trace is the response to surround illumination. + are sign-preserving chemical synapses, − are sign-inverting chemical synapses and ∧ ∨ are electric synapses. Neurotransmitter color code: blue: glutamatergic, red: GABAergic, green: glycinergic and yellow: unknown. R: rod; C: cone; H: horizontal cell; HBC_M, HBC_C, DBC_C and DBC_M are mixed and cone-driven bipolar cells; Aon: sustained on amacrine cell; At: transient on–off amacrine cell; Aoff: sustained off amacrine cell; IPC: interplexiform cell; Gon: sustained ON ganglion cell; G on–off: ON–OFF ganglion cell; OPL: outer plexiform layer; IPL: inner plexiform layer.

(with 0 Ca^{2+}), so that presynaptic actions of glycine were blocked. Glycine elicited large postsynaptic currents that reversed near the chloride equilibrium potential ($E_{Cl} = -50$ mV) when applied tothe dendrites and to the axon terminals. These data suggest that glycine receptors exist on bipolar cell dendrites and axon terminals. The action at axon terminals is likely to be mediated by glycinergic amacrine cells that form feedback synapses with bipolar cells. In the outer retina, since there are no glycinergic HCs and the only glycine-containing neuronal elements are processes of the interplexiform cells, the action of glycine is probably mediated by interplexiform synapses.

4. Discussion

Our results show that glutamate is used by the photoreceptor and bipolar cell output synapses and the release of glutamate is largely mediated by calcium-dependent vesicular processes. The postsynaptic glutamate receptors in DBCs are L-AP4 receptors, in HBCs and HCs are kainate/AMPA receptors and in ganglion cells are the kainate/AMPA and NMDA receptors. Recent evidence suggests that the kainate/AMPA receptors in these cells are AMPA-preferring, because of their selectivity to glutamate desensitization agents [60–62]. Subpopulations of HCs make GABAergic synapses

on cones and gate chloride conductance through $GABA_A$ receptors. GABAergic HCs do not make feedforward synapses on bipolar cell dendrites and the neurotransmitter identity of the HCs making feedforward synapses is unknown. Subpopulations of amacrine cells make GABAergic synapses on bipolar cell synaptic terminals, other amacrine cells and ganglion cells. GABA gates chloride conductances and regulates intracellular calcium signals [63,64]. $GABA_A$,$GABA_B$ and $GABA_C$ receptors are involved in mediating various aspects of these postsynaptic actions [17,65–67]. It has been suggested GABA release from HCs is non-vesicular [31]. However, GABAergic MIPCs can be recorded from ganglion cells [42], indicative of vesicular release of GABA from amacrine cells. Glycinergic amacrine cells make synapses on bipolar cell synaptic terminals, other amacrine cells and ganglion cells and glycine opens postsynaptic chloride channels [68,10]. Glycinergic interplexiform cells make synapses on bipolar cells in the outer retina [68] and glycine released from these cells open chloride channels in bipolar cell dendrites. Glycine release from amacrine cells and interplexiform cells is mediated by calcium-dependent vesicular processes, as discrete strychnine-sensitive MIPCs can be observed in bipolar cell axon terminals and dendrites, as well as in ganglion cells [42,66].

A schematic diagram summarizing glutamatergic, GABAergic and glycinergic synapses in the retina is shown in Fig. 11. This diagram also illustrates the distribution of these synapses in the center-surround receptive field network. It is important to note that Fig. 11 is an oversimplification of the actual synaptic organization of the retina. Many neurons using other neurotransmitters (such as dopamine and acetylcholine) are not included. Moreover, for each of the three amino acid neurotransmitters discussed in this article, different subtypes of postsynaptic receptors are used by different postsynaptic cells. For each subtype of postsynaptic receptor, several molecular variants may exist to mediate different physiological functions [69,62]. For example, although both mixed HBC and cone-driven HBC use AMPA-preferring receptors, the single channel conductance and kinetics of the postsynaptic currents suggest that the glutamate receptors in the two HBCs are not identical (Maple and Wu, in preparation). It is crucial to study the detailed molecular structures of various neurotransmitter receptor/channels and transporters and to correlate the structure of single receptor/channel and transporter proteins with cellular, synaptic and network functions in the retina. This will lead to a comprehensive understanding of how molecular events in individual synapses mediate specific functional tasks and how these activities are integrated into the overall operation of the retina.

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